EVALUATION OF RESIDENT *Trichoderma* isolates AGAINST *Sclerotium rolfsii* CAUSING DAMPING OFF OF MUNG BEAN SEEDLINGS

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Dedicated to
my beloved parents
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CERTIFICATE

This is to certify that the thesis entitled, **"EVALUATION OF RESIDENT *Trichoderma* isolates AGAINST *Sclerotium rolfsii* CAUSING DAMPING OFF OF MUNG BEAN SEEDLINGS "** *submitted to the Department of Plant pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* **MD. SAJEDUZZAMAN** *bearing Registration No.* **13-05574** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma elsewhere in the country or abroad.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 30 September, 2020

Place: Dhaka, Bangladesh

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION/SYMBOL	ELABORATION
%	Percentage
°C	Degree Centigrade
AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
CFU	Colony Forming Unit
Cm	Centimeter
CMI	Commonwealth Mycological Institute
CV	Coefficient of Variance
DAI	Days After Inoculation
Ed.	Edited
Eds.	Edition
et al.	And others
etc.	Et cetera (and so forth)
G	Gram
ha^{-1}	Per hectare
Kg	Kilogram
LSD	Least Significant Difference
Ml	Milliliter
Mm	Millimeter
MT	Metric Ton
PDA	Potato Dextrose Agar
Psi	Pound per square inch
SAU	Sher-e-Bangla Agricultural University
T	Ton
T	Treatment
V	Volume
viz.	Videlicet (namely)
W	Weight
CWDEs	Cell wall degrading enzymes

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The Author

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ABSTRACT

An investigation was carried out to find out the most effective native *Trichoderma* isolate against *Sclerotium rolfsii* causing damping off disease of mung bean seedlings. The experiments were carried out at Mycology Laboratory and Net house of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka, during June 2019 - July 2020. All together 13 *Trichoderma* isolates collected from seven different districts in Bangladesh were explored in the investigation. The isolate JaGot.T was found to be promising in respect of mycelial growth, sporulation and antagonistic effect on *Sclerotium rolfsii*. The isolate JaGot.T was recorded as the fastest growing antagonistic fungus that covered 89.33 mm of the culture plate by 3 days. The 2nd highest (86.00mm) and the 3rd highest (85.333mm) mycelial growth rate was recorded in case of isolate GaJoy.T and GaJoy.T, respectively. The isolate JaGot.T and GaJoy.B were found to sporulate within 2 days producing 6.8433×10^4 and 6.0767×10^4 spores/mm^2, respectively. JaGot.T inhibited 50.35% growth of *S. rolfsii* followed by CuDeb.B (44.17%) and GaJoy.B (44.05 %) in dual culture method. The resident *Trichoderma* isolate JaGot.T showed better performance against *Sclerotium rolfsii* causing damping off of mung bean next to Autostin and formulated *Trichoderma* based biopesticide 'Bioderma'. JaGot.T yielded 76.66% seedling emergence which was statistically similar to 'Bioderma' (76.66%) and Autostin (78.0%). The lowest pre-emergence damping off (1.34%) and post-emergence damping off (5.367%) were recorded in case of resident *Trichoderma* isolate JaGot.T. The *Trichoderma* isolate JaGot.T also showed better performance in increasing dry matter, shoot length, root length and seedling vigor index of mung bean. The highest seedling vigor index was recorded in case of isolate JaGot.T (2336.88) which was statistically similar to Autostin and Bioderma. The isolate JaGot.T was noted as the best resident isolate of *Trichoderma* against *Sclerotium rolfsii* causing damping off of mung bean seedlings among the isolates tested.

CHAPTER I

INTRODUCTION

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Pulses have been consumed for at least 10000 years and are the most extensively used foods in the world. Pulse crop is one of the most important protein sources for the majority of the people in Bangladesh as well as in the world. Pulses are considered as the protein sources of the poor people as these are less expensive than animal proteins which makes it essential components of the daily diets of the people. It contains protein about twice as much as cereals. Pulses also provide fibers as well as a significant number of vitamins and minerals, such as iron, zinc, folate and magnesium in significant amount. Pulse is an important source of an essential amino acid lysine, which is generally absent in cereal grains (Elias, 1986). Consuming half a cup of beans or peas per day can enhance diet quality by increasing intake of other micronutrients. In addition, pulses possess the phytochemicals, saponins, and tannins that contains antioxidants and anti-carcinogenic properties, indicating that pulses may have significant anti-cancer effects. Pulse consumption also improves serum lipid profiles and positively affects several other cardiovascular disease risk factors, such as blood pressure, platelet activity, and inflammation. Pulses have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels. Pulse bran is also used as quality protein source in formulation of poultry, animal and fish feeds. Moreover, pulse crops are a very logical component to ensure sustainability in agricultural practices. Their ability to fix nitrogen and add organic matter to the soil are important factors in maintaining soil fertility by natural means (Senanayake *et al*., 1987 and Zapata *et al*., 1987). Pulses facilitate the growth of bacteria living in specialized pockets in their roots to ‘fix’ the nitrogen so that it stays in the soil in a readily available form for the plants. Pulses’ residues leaving in the soil after harvesting of the crop, left nitrogen which lowers the fertilizer doses for the following crops (Sharma and Prasad, 1999). Among the food legumes grown in Bangladesh, lentil, grass pea, black gram, chickpea and mung bean are the major

ones which cover more than 95% of the total pulse production in the country (Rahman, 1998).

Among the pulse species, mung bean (*Vigna radiata*) is an important pulse crop seeking global economic importance as dietary ingredients of regular food. This is one of the best plant-based sources of protein which is rich in essential amino acids, such as phenylalanine, leucine, isoleucine, valine, lysine, arginine etc. (Mubarak, 2004). Mung bean seeds are rich in about 24% digestible protein, fiber, antioxidants, and phytonutrients (Itoh *et al*., 2006). Mung bean grains contain 19.5% - 28.5% protein (AVRDC, 1988). Fried mung bean seed is a palatable snack popularly eaten by young people. Young seedlings may be used as vegetable. The green plant is used as nitrogen containing fodder in many areas. It is also a good green manuring crop. The dried stems and pod walls remaining after threshing are also used as cattle feed as a source of nitrogen.

Among the widely grown major pulses, mung bean is considered to be in the 4th place based on its production covering 102,109 acres of land with total production of 33,951 MT (BBS, 2019). Being a short duration crop, mung bean can fit in between two major cropping seasons Rabi and Kharif-1in Bangladesh. It contributes 20.95% of total pulse production in the country (Krishi diary, 2012).

A recent trend of drastically decreasing in mung bean cultivation has been noticed. Survey shows that a good amount of mung bean area has been replaced by cereals (Abedin *et al*., 1991). Biotic and abiotic stresses are considered to be the major factors for decreasing mung bean production. Among the biotic factors, extreme pressure of disease and insect infestation, deterioration of genetic stability, high pressure of weed infestation etc. are noticeable. Mung bean has been proved to be infected as a host of different groups of pathogens including fungi, bacteria**,** viruses and mycoplasma (Pandey *et al.*, 2018). A total of twenty diseases of mung bean have been recorded in Bangladesh (Bakr and Rashid, 2007). The majority of mung bean diseases are caused by fungi, of which

damping off of seedlings caused by *Sclerotium roflsii* is the most devastating one (Nene, 1973).

Sclerotium rolfsii is a noxious soil-borne pathogen in warm and moist climates worldwide, which attacks more than 500 plant species (Ahmed *et al.*, 1984). It causes damping-off, foot and root rot, collar rot and wilts of mung bean (Lichtenzveig *et al*., 2006). The plant stands are seriously affected by pre-emergence and post emergence damping off by this soil borne fungus (Horst, 2013).

Management of *Sclerotium rolfsii* is difficult because this pathogen can survive in soil by resting spores like sclerotia and chlamydospores in adverse situation. This specialty favors *Sclerotium rolfsii* to perpetuate in soil year after year which has made them challenging to control (Mondal *et al.,* 1996 and Agrios, 1997).

Chemical pesticides are proven not so effective in controlling this soil inhabitant. Besides, use of chemical pesticides in the soil is not cost effective (Talukder *et al*., 2007). Chemicals such as methyl bromide and a fungicide named Bavistin are being frequently used to control the pathogen. However, use of chemicals is expensive and hazardous for environment as well as human health. On the other hand, indiscriminate use of chemical pesticides is responsible for inducing resistance in pathogens to that pesticides. Thus, search for an efficient alternative approach for its management is urgent demand. Biocontrol agents (BCAs) may be the cost effective and eco-friendly alternative options for the management of the pathogen. Biocontrol agents inhibit or reduce inoculum potentials of the pathogens. A number of biocontrol agents are now being used to suppress the plant pathogens in the world. The most commonly used fungal species as BCA are *Trichoderma* species (Singh *et al*., 2013). *Trichoderma* based bio-pesticides are proven to be the most promising BCA specially against soil borne pathogens (Chet and Inbar, 1994). It has some antimicrobial properties as well as properties for agricultural applications. That is why, *Trichoderma* has attracted the focus of research interest around the globe.

Trichoderma is a filamentous, asexual spore producing Ascomycetic fungi belonging to the class Deuteromycetes. *Trichoderma* spp. are characterized by faster growth, mostly bright green conidia and with a profusely branched conidiophore structure (Gams and Bissett, 1998). *Trichoderma* spp. are ubiquitous colonizers of cellulosic materials in decaying plant (Kubicek *et al*., 2008 and Jaklitsch, 2009) as well as in the rhizosphere (Harman, 2000).

Trichoderma species are opportunistic, avirulent plant symbionts. The strains of this antagonists provide biocontrol against fungal phytopathogens by competition, mycoparasitism and antibiosis. Root colonization by *Trichoderma* strains frequently enhances root growth, soil fertility status, crop productivity, resistance to abiotic stresses and the uptake and use of plant nutrients (Arora *et al*., 1992). Antibiosis occurs during interactions involving low-molecular-weight diffusible compounds or antibiotics excreted by *Trichoderma* spp. that inhibit the growth of other microorganisms. It is a very complex process involving a series of sequential events, including recognition, attack and subsequent penetration and killing of the host pathogen. *Trichoderma* spp. may exert direct biocontrol by parasitizing a range of fungi, detecting other fungi and growing towards them. The remote sensing is partially due to the sequential expression of CWDEs, mostly chitinases, glucanases and proteases (Harman *et al*., 2004).

Research on biological control agent is running in the world for searching new *Trichoderma* spp. that is more efficient against particular pathogenic strains. Continued commercial success of *Trichoderma* would depend on identification of novel strains adapted to local conditions. Since the diversity of *Trichoderma* is profound on the above-ground, the success of novel strains would be fruitful if the new isolates are found naturally myco-parasitic for the specific locality with specific plant pathogen (Mukherjee *et al*., 2014). For this reason, research is going on in different regions of the world in searching of native strains of *Trichoderma* which will be the best option as biocontrol agent (BCA) against target pathogen.

Considering all the above facts, the present experiment was conducted to achieve the following objectives:

1. To isolate *Trichoderma* isolates from the rhizosphere of different location of the country,
2. To identify effective *Trichoderma* isolate against *Sclerotium rolfsii* as BCA,
3. To formulate *Trichoderma* based biopesticide with the effective *Trichoderma* isolate, and
4. To evaluate formulated *Trichoderma* isolate against *Sclerotium rolfsii* causing damping off of mung bean seedlings in net house.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Damping off is one of the most devastating diseases of pulse crops in Bangladesh caused by *Sclerotium rolfsii,* a soil borne pathogen. It is one of the major constraints for the production of pulses in the country. The present piece of research aims to manage this disease by eco-friendly approach using native *Trichoderma* strain, an effective bio-control agent (BCA). In this chapter, attempt is taken to review the former research works regarding this piece of research.

Agrios (1997) reported that *Sclerotium rolfsii* and *Rhizoctonia solani* are responsible in developing of damping-off of seedlings, stem canker, crown blight, root-rot, crown rot, bulb, tuber and fruit rot diseases of different crops. They frequently affect a variety of plants, including cereals, legumes, vegetables, flowers and forage plants. Druzhinina *et al.* (2011) described that soil is the most common and natural habitat of most of these members of fungal genus.

Lichtenzveig *et al*. (2006) observed that the soil-borne fungal phytopathogens cause various noxious diseases of economic importance such as damping-off, foot rot, root rot, seedling blight and vascular wilts.

Pandya *et al.* (2011) reported that soil-borne fungal pathogens cause economic losses due to being the most aggressive organisms. Several phytopathogenic fungi such as *Pythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Fusarium* widely spread during the last few years due to change of intensive farming and environmental degradation.

Meah (2007) carried out an experiment to test the pathogenicity of *Sclerotium rolfsii* on eggplant (var. Dohazari) by using 10 isolates. The experiment showed result affecting seed germination, pre-emergence death, damping off, foot rot and plant stand significantly by all the isolates of *S. rolfsii*.

Meah (2003) prepared a list of a number of diseases of eggplant caused by fungi, bacteria, virus, nematode and mycoplasma. Among them, collar rot caused by *Sclerotium rolfsii* is severe in damaging the crop. Crop rotations with non-host crops and drenching with some copper fungicides before planting were found somewhat helpful in controlling this disease.

Chet *et al.* (1994) reported that *Sclerotium rolfsii* Sacc. causes the disease known as southern blight in a wide variety of crops. They also reported that *Sclerotium rolfsii* in the form of brownish sclerotia can survive in the soil for longer period of time.

According to an investigation of Singh (1970), *Sclerotium rolfsii* attacks at the base of stems developing a fan like structure of silky white mycelium and round mustard seed like sclerotia which are initially white but become darken over time.

Punja *et al.* (1988) stated that root-rot disease caused by *Sclerotium rolfsii* was one of the most important diseases of crops.

According to Ahmed and Hossain (1985), *Sclerotium rolfsii* causing collar rot, foot and root rot diseases was responsible in leading considerable damage both in seedling and adult stages of Indian spinach. They also reported that variation in disease incidence was found in different parts of Bangladesh.

Punja *et al.* (1988) reported that temperature is the main limiting factor in the geographic distribution of *Sclerotium roifsii.* The disease rarely occurs in those area where average daily minimum winter temperature remains below freezing (0^0 C). 25^0-35^0 C temperature is optimum for mycelial growth as well as maximum disease occurrence in field condition. Germination of *sclerotia* also ranges at 25^0-35^0 C temperature.

According to Bertus (1929), *Sclerotium rolfsii* possesses the ability to induce damping off disease in seedlings of certain plants when the pathogen was brought in contact with stem of these plants at soil level. High temperature and

high humidity were appeared to be necessary for the fungus while existing in the upper four inches of the soil. It causes collar rot of a number of hosts like chilly, tomato, groundnut etc.

Farr *et al.* (1989) reported that the fungus *Sclerotium roifsii* attacks all plant parts including stems, roots, and fruits remaining at contact with soil under favorable environmental condition.

Aycock (1966) reported that *Sclerotium rolfsii* belongs to a very wide host range and includes not only many important horticultural crops but also many economic agronomic crops. He also stated that this soil borne phytopathogenic fungus, *Sclerotium rolfsii,* is responsible to attack more than 500 species of plants belonging to over 100 families.

According to Aycock (1966), stem rot disease popularly known as *Sclerotium* wilt, *Sclerotium* blight and white mold affect all the plant parts at any growth stage of crop life cycle. The first characteristics symptom of these disease is development of deep brown lesions around the meristem beneath the soil surface. Radiating mycelia would cover the lesions that would encircle the affected portion of the stem which would lead the development of yellowing and wilting of the whole or part of the plant.

Choudhury (1967) reported *Sclerotium* sp. being responsible for foot rot disease in brinjal. Minute mustard seed like structures known as sclerotia adhere to the stem at soil level from which mycelia is germinated to enter the stem and choke the vessels. Irrigation water is one of the principle routes of spreading this disease from one plant to the others as well as from one plot to other plots. Controlling of this disease is nightmarishly difficult as the fungus persist in the soil in many forms for a long time.

Chakravarty and Bhowmik (1983) carried out an experiment on symptoms and techniques of inducing collar-rot disease in sunflower caused by *Sclerotium rolfsii* Sacc. The fungus was found responsible in causing pre-emergence as well

as post- emergence damping off of sunflower seedling and collar rot of adult plants. Disease incidence was found to be highest with maximum rotting in internal tissues when the plants were 60 days old.

Yasmin-Ahmed *et al.* (1988) found *Sclerotium rolfsii* causing collar rot disease in maize. They isolated the pathogen from infected maize crop. Maize cv. Shaheen was subsequently inoculated with pure culture of the pathogen by sowing seeds in soil infested with the pathogen. Sclerotia were seen to develop on the soil surface surrounding the seedlings within 15 days of emergence. Seedlings were found to lead to death within 10-15 days.

According to Sugha *et al.* (1991), *Sclerotium rolfsii* caused collar rot disease in chickpea. A total of 210 lines and cultivars of chickpea were tested by putting single wheat grain fully covered with mycelium of *S. rolfsii* at the collar region of 7 days old seedling in pot of sterilized garden soil. All the seedlings were found to be susceptible to collar rot disease.

Alexander and Stewart (1994) experimented on *Sclerotium rolfsii* (Teleomorph; *Athelia rolfsii)* and found it causing serious root and stem rot of a wide range of economically important fruit and vegetable crops. Sclerotia were noted to be the important propagules for the survival of this pathogen. Under favorable conditions, sclerotia may germinate and develop infections at or just below the soil surface showing symptoms including yellowing, browning and wilting of entire plants.

Yaqub and Shahzad (2005) carried out a pot experiment to test the pathogenicity of *Sclerotium rolfsii* on a variety of crops. They found evidence of *Sclerotium rolfsii* to be highly pathogenic to sunflower; mildly pathogenic on tomato, lentil, sweet pumpkin and cabbage and non-pathogenic on cauliflower. Increase in inoculum density of *Sclerotium rolfsii* indicated gradual relation in growth parameters of sunflower and mungbean. A positive correlation was proved between root colonization and population of *Sclerotium rolfsii* in rhizosphere soil.

According to Harman *et al*. (2004), amongst the range of antagonists, different species of *Trichoderma* of phylum Ascomycota are the most useful and frequently isolated soil fungi that exist in rhizosphere soil of plants. These fungi are opportunistic, avirulent plant symbionts, as well as being parasites of other phytopathogenic fungi.

According to Sharma (2011), many *Trichoderma* species are regarded as growth promoter of plants. They can influence the increasing of fresh weight, height and flowering of plants while potentially inhibiting pathogenic growth.

According to Manczinger *et al*. (2002), *Trichoderma* species are very wide spread in nature with high population densities in soils and plant litters. They are also filamentous, saprophytic, quick growing and easy to culture in artificial means. They can produce large number of propagules with long shelf life.

Eziashi *et al*. (2006) conducted an experiment on the effect of metabolites produced by *Trichoderma* species and have found *Trichoderma* spp. as an effective biological control agent against many soil-borne pathogens.

Sanchez *et al*. (2006) conducted an experiment aiming at *in vitro* antagonistic behavior of *Trichoderma* spp. The experiment show that *Trichoderma* controls pathogens in an indirect way by producing several groups of antibiotics that inhibit the growth of the pathogen. Apart from that, there are direct methods showing antagonism against the pathogen which is called mycoparasitism. *Trichoderma* species can also inhibit or reduce the growth of plant pathogens especially fungi, through competition for space, enzyme substrates, nutrients, and or oxygen.

While working on biological control of *Rhizoctonia solani* in strawberry field by using *Trichoderma harzianum*, Elad *et al*. (1980) reported that Bio-control with *Trichoderma* was found to be effective against different sclerotia forming fungi including *Sclerotium rolfsii*.

Faruk and Rahman (2016) conducted an *in-vitro* evaluation of *Trichoderma harzianum* against soil-borne fungi of economic importance at the lab of plant pathology division of Bangladesh Agricultural Research Institute (BARI). They found *T. harzianum* as a strong antagonistic agent against three different soil borne pathogenic fungi viz. *S. rolfsii, R. solani* and *F. oxysporum* in dual culture assay on PDA media.

Atanosova *et al.* (2013), Hojos-Carvajal and Bissett (2011), and Schuster and Schmoll (2010) recognized the members of the fungal genus *Trichoderma* as green spored ascomycetes which includes more than 200 species and can be found in different geographical regions and climatic zones of the world.

Hojos-Carvajal and Bissett (2011) described the habit of *Trichoderma* spp. According to their opinion, *Trichoderma* spp. are ubiquitous colonizers of cellulosic materials. Thus, they can be found where decaying plant material is available as well as in the rhizosphere of plants. The members of this fungal genus are characterized by rapid growth, mostly bright green conidia and a repetitively branched conidiophore. They can also be characterized by the ability to assimilate a diverse array of substrates and production of a range of antimicrobials.

According to Schuster and Schmoll (2010) *Trichoderma* spp. are highly successful colonizers of their habitats, which is reflected both by their efficient utilization of the substrate at hand as well as their secretion capacity for antibiotic metabolites and enzymes. Under all the conditions, they respond to their environment by regulation of growth, conidiation, enzyme production, and hence adjust their lifestyle to current conditions, which can be exploited for the benefit of mankind.

Monte (2001) and Benitez *et al.* (2004) reported that *Trichoderma* spp. are able to control ascomycetes, basidiomycetes, and oomycetes.

Likewise, Sivasithamparam and Ghisalberti (1998) found that *Trichoderma* secretes not only potential antibiotics but also mycotoxins for suppression of other fungi. They found more than 100 metabolites with antibiotic activity including polyketides, pyrones, terpenes, metabolites derived from amino acids, and polypeptides in *Trichoderma* spp.

Morgan (2011) reported *Trichoderma* as the most intensively studied among the biological control agents.

Vinale *et al*. (2007) reported that *Trichoderma* species are the most frequently isolated soil-borne fungi commonly found in plant root ecosystem.

Harman *et al*. (2004) describe *Trichoderma* as opportunistic, avirulent plant symbionts which are antagonistic towards many phytopathogenic fungi. They found proof of *Trichoderma* to be effective in improving root and plant growth, as well as inducing resistance in plants by the application depending upon different strains.

Gams and Bissett (2002) described *Trichoderma* spp. as the successful colonizers of their habitats because of their diverse metabolic capability and aggressively competitive nature.

Benítez *et a*l. (2004) studied on the habit and survival of *Trichoderma.* They described *Trichoderm*a species as relatively good antagonists against pathogenic fungi. They found that *Trichoderm*a are able to survive under extreme competitive conditions. They are able to overcome fungistatic effects.

Harman *et al.* (2004) studied the reason of extreme survival capacity of *Trichoderma.* They found that *Trichoderma* species are resistant against many toxic compounds, metabolites produced by soil microflora and plants, herbicides, fungicides and antibiotics. They described that these abilities might be due to the presence of ATP-binding cassette (ABC) transporter. The increased expression of these ABC-transporter genes inhibits toxicant accumulation in the

cells which allows them to survive under extreme conditions and become more competitive than other soil fungi.

According to Benítez *et al*. (2004) *Trichoderma* species are good in mobilizing and uptaking of nutrients compared to other soil organisms.

The study of Harman *et al.* (2004), Benitez *et al*. (2004) and Vinale *et al*. (2007) revealed the same result that presence of *Trichoderma* species at the root ecosystems enhances plant root development which, in turn, increases drought tolerance of the plants and may improve the resistance of plants towards compacted soils.

According to a report of Kumar (2013), *Trichoderma* is an asexually reproducing fungal genus which can be present in all types of soil. Recent reports describe them opportunistic, avirulent plant symbionts, as well as being parasites on other fungi. A number of successful biocontrol products based on different species of *Trichoderma* have been commercially formulated in India, USA and elsewhere in the world.

Kashem *et al*. (2011) conducted a series of experiment to study the effect of 14 isolates of *Trichoderma* spp. (*T. harzianum* and *T. viride*) to control foot and root rot of lentil caused by *Fusarium oxysporum*. The pathogenicity of 12 isolates of *F. oxysporum* and the mass production of an isolate of *T. harzianum* on 25 substrates were also studied. The study revealed the result showing *Trichoderma* isolates inhibiting the growth of *F. oxysporum* up to 92.07 % on agar media.

Pandya *et al.* (2011) reported that *Trichoderma* as a biological control agent (BCAs) is well known for their extremely high reproductive capacity. They also show strong antagonism against phytopathogenic fungi and some degree of efficiency in promoting plant growth and defense mechanisms.

Amin *et al.* (2010) conducted a study on six isolates of *Trichoderma* spp. to identify their ability to inhibit soil borne pathogens of different vegetables *viz.*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* isolated from

tomato under *in vitro* conditions. *Trichoderma viride* showed the best result inhibiting 65.71% of mycelial growth of *Rhizoctonia solani* over control in dual culture of pathogens and *Trichoderma* spp. In case of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, *T. viride* was proved to be potential to inhibit mycelial growth and development.

Islam (2005) was working on controlling of seedling diseases of eggplant. He reported that *Trichoderma harzianum* T_{22} showed effective control of damping off disease of seedlings.

According to Meah *et al*. (2004), *Trichoderma harzianum* cp and *Trichoderma harzianum* T_{22} which were grown on peat soil and black gram bran-based substrate were found effective against nursery diseases like damping off, seedling blight and tip over of eggplant seedlings. A strong proof in favor of using *T. harzianum* cp and *T. harzianum* T_{22} was found to promote seed germination and seedling vigor.

Sultana *et al*. (2001) experimented on growth and storability of *T. harzianum* and its effect on germination of egg plant seeds. The study showed that seeds treated with *Trichoderma* resulted up to 48.62% germination than that of control (untreated).

Jacobs and Kamoen (1986) reported that *Trichoderma harzianum* produces cell wall degrading lysine enzymes which was chiefly responsible to be antagonistic against plant pathogens and to improve biological controlling capacity.

Spiegel and Chet (1998), Navazio *et al.* (2007) and Vinale *et al.* (2009) studied on the defence mechanism of *Trichoderma*. They found the defense mechanisms comprised by both enzymatic and chemical weapons, that make Trichoderma spp. Efficient mycoparasites, antagonists, as well as biocontrol agents. These characteristics or the metabolites secreted by these fungi can be exploited by Trichoderma spp. to fight plant diseases caused by pathogenic fungi.

Dababat *et al.* (2006), Kyalo *et al.* (2007) and Goswami *et al.* (2008) found a strong effect of Trichoderma on nematode suppression.

Vinale *et al*. (2008) reported that in order to survive and compete in their ecological niche, fungi apply not only enzymatic weapons but also have a potent arsenal for chemical warfare at their disposal.

Vinale *et al.* (2007) studied on control mechanism of *Trichoderma* species. They found *Trichoderma* possessing of several control mechanisms to fight against phytopathogenic organisms. These biocontrol mechanisms are competition with plant pathogens, mycoparasitism, antibiosis, production of lytic enzymes and secretion of secondary metabolites.

Vinale *et al*. (2007) conducted an experiment on mechanism of *Trichoderma* to suppress plant pathogens. They found *Trichoderma* to compete for nutrients, growth factors and space with plant pathogens in the soil. They also found that *Trichoderma* species can parasitize other fungi. Under normal conditions, they always secrete low level of cell wall degrading enzymes (CWDEs) *viz.* chitinases and glucanases. In the presence of pathogenic fungi, CWDEs lyses the cell wall of pathogens and release cell wall oligomers from pathogens. The degraded products from pathogens further induce the expression of mycoparasitic gene expression. Thus, *Trichoderma* species grow towards plant pathogens.

Harman *et al.* (2004) described the parasitizing mechanism of *Trichoderma*. While coming into contact with pathogenic fungi, *Trichoderma* species attach and coil around the pathogens. Then, a specialized pressing organ known as appressoria will be formed to puncher the pathogenic structures. Holes can be produced at entrances of appressoria and *Trichoderma* hyphae enter into the target fungi resulting in killing the pathogenic fungi.

According to Ghisalberti and Sivasithamparam (1991), *Trichoderma* can produce secondary metabolites and these can be classified into three categories:

(i) volatile antibiotics such as 6-pentyl-α-pyrone (6PP), (ii) water soluble compounds such as heptelidic acid and (iii) peptaibols which are classified under a class of linear oligopeptides.

According to Harman *et al*. (2004), *Trichoderma* species are capable in controlling deleterious microbes at rhizosphere soil. These microbes reduce root development. *Trichoderma* species are resistant to the cyanide produced by such type of microbes as well as able to remove them from the root zone through mycoparasitic effects. Thus, the interactions between *Trichoderma* and plant are always positive and associated with improvements in plant yield and biomass. For example, maize treated with *Trichoderma* strain T-22 had shown about 5% increase in average yield.

According to Kannangara *et al*. (2017), Biological control of plant pathogens using various microorganisms has been accepted as a more natural and an environmentally sound alternative to the chemical control methods.

Eziashi *et al*. (2006) found *Trichoderma* spp. as an effective BCA against many soil- borne pathogens.

Papavizas (1985), Chet and Inbar (1994), Desai and Schlosser (1999) and Iqbal *et al*. (1995) revealed their experimental results on controlling of damping off disease of seedlings. They mentioned several methods for controlling damping off disease of seedling. Among them, *Trichoderma* spp. had played a considerable role as biocontrol agent. and was recognized as an effective biocontrol agent against soil-borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoctonia* etc. *Trichoderma* significantly destroys the sclerotia of *S. rolfsii*, overlaps the pathogen and suppresses their growth as well.

Tvetdyukov (1994) reported that *Trichoderma* species produce chemicals called trichodermin which is responsible for its antagonistic properties.

According to Cruz *et al.* (1995), it is important to isolate *Trichoderma* spp. having high potentiality to secrete extra cellular lytic enzymes *viz*. chitinase and β -1,3-glucanase. These lytic enzymes break down cell wall polysaccharides into short oligomers and by this way facilitate the hyper parasitism to penetrate into the cytoplasm of the target pathogenic fungi.

On the other hand, Bosah *et al.* (2010) reported that chitinase and β-1, 3 glucanase are well known fungus-controlling enzymes to break down two essential cell wall components: chitin and β-1, 3 glucans. That is why these chemicals are frequently used in many chemical fungicides to control the plant diseases.

Ilius *et al*. (2005) reported that *Trichoderma* species are able to degrade domestic wastage material relatively quickly without emitting bad odours.

Rahman *et al.* (2011) conducted an experiment on the biodegradative potential of *Trichoderma* isolates on kitchen solid waste *in vitro*. They found using *Trichoderma* as a nonhazardous, environmentally friendly, sustainable technique involving bioconversion of domestic waste, so found Molla et al. (2002).

Papavizas (1985) and Rifai (1969) reported that different *Trichoderma* strains produce metabolites depending on different ecological factors, and that is why different strains show varying effects on plant pathogens.

Ghisalberti (1991) and Lynch *et al*. (1990) isolated some of these metabolites from sporulating and mycelial cultures. Surprisingly they found that sub-culturing was responsible for the decrease of production of the peptide antibiotics.

Mukhopadhyay (1995) conducted an extensive experiment on two bio-agents *viz.*, *Gliocladium virens* and *Trichoderma harzianum* in controlling a wide range soil borne plant pathogens *viz.*, *Sclerotium rolfsii*, *Rhizoctonia solani Fusarium oxysporum*, and *Pythium spp.* He applied the antagonists individually on the crops as seed treatment and also integrated them with chemical fungicides which

were insensitive to these bioagents. The integrated treatments were found to be highly effective against the phytopathogens and resulted in enhanced crop performance compared with biological or chemical treatments alone.

Haran (1996) and Rifai (1969) described *Trichoderma* species as a possible biocontrol agent resulted from the ability of growth in a wide range of temperatures, capability of antagonizing plant pathogens, using lignocellulosic materials for growth and both antibiosis and hyper parasitism.

Samuels J Gary (2006) described *Trichoderma* as a very useful fungus for industry and as a biocontrol agent, showing little host specificity, colonizing most plants, widely studied fungi and most commonly used biological control agents in agriculture and being its products alternatives to synthetic agrochemicals.

Omorusi *et al.* (2007) studied on *in vitro* assessment of biological control of white root rot of rubber (*Hevea brasiliensis*) by antagonistic fungi. They found that the use of *Trichoderma spp.* as biological control agent had provided a very appealing substitute and less dangerous method in plant disease management. They stated biocontrol of plant pathogens as a potential non-chemical mean for plant disease management which could serve as a substitute for costly chemical treatment.

Yaqub and Saleem (2010) studied on competitive colonization of wheat straw by *Trichoderma* species and *Sclerotium rolfsii*. On the other hand, Doley K and Jite PK (2012) studied on *in vitro* efficacy of *Trichoderma viride* against *Sclerotium rolfsii*. Both of them reached the same conclusion *i,e,* different species of *Trichoderma* are known to be extremely efficient against various pathogenic fungi.

Rekha *et al.* (2012) experimented on *in vitro* screening of native *Trichoderma* isolates against *Sclerotium rolfsii* causing collar rot of ground nut. They found

that *Trichoderma* spp. are able to produce unpredictable antibiotics in agar medium and their culture filtrates can also be used for controlling of fungi.

Bosah *et al.* (2010) experimented on *in vitro* microbial control of pathogenic *Sclerotium rolfsii*. They recommended biocontrol of plant pathogens as an impressive way especially with hyper parasitizing possibilities of antagonists on pathogenic fungi. They also noticed that biocontrol agents might create competition against pathogens and induce resistance in plant by producing different hydrolytic enzymes.

Djonovic *et al.* (2007) studied on a proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* which is required for induced systemic resistance in maize. They found *Trichoderma* species to promote plant growth and induce biotic and abiotic stress resistance in plants.

Verma *et al.* (2007) found the ability of *Trichoderma* species to sense, invade and destroy other fungi as the major driving force behind their commercial success as bio-pesticidal formulations. They also stated that more than 60% of all registered biopesticides are *Trichoderma*-based.

Hossain and Hossain (2010) formulated a *Trichoderma* based bio-fungicide named 'BAU-bio fungicide' which was found very effective against several tikka diseases of groundnut, foot and root rot of pulses and diseases of some vegetable crops. BAU-bio fungicide was also proven helpful to control seed borne mycoflora, increasing seed germination and seedling vigor of some vegetables.

Tran (2010) carried out a number of surveys on food crops, vegetable crops, fruit crops and industrial crops in the north and south of Vietnam. According to the outcome of those surveys, *Trichoderma* can be isolated easily from rhizosphere soil, root and plant organic matters. Both laboratory and field trials showed that *Trichoderma* species have the ability to suppress growth and development of fungal phytopathogens and enhance plant growth and development. He reported

that several *Trichoderma* based bio-products had been commercially formulated by several companies, institutes and universities such as: BIMA, Trico-DHCT, Promot Plus WP, Vi DK, NLU-Tri, Bio-Humaxin etc. and were frequently available in market. *Trichoderma* based products can be used in the field in many ways including seed treatment, direct application to the soil before planting and added organic fertilizers.

According to Meah and Islam (2005), *Trichoderma* based bio-fungicide can control Phomopsis fruit rot, foot/collar and root rot of eggplant and wilt of some vegetables very effectively.

According to a report of Howlader (2003), *Trichoderma harzianum* cp showed good result in controlling phomopsis blight and foot rot of eggplant in the field.

According to Chowdhury *et al.* (2000), seeds treated with *Trichoderma harzianum* and *Gliocladium viride,* against *Sclerotium rolfsii,* resulted up to 21.61% and 48.43% respectively increase in germination in mungbean, pigeon pea, black gram and tomato. It also showed good effect on controlling seed born mycoflora. Moreover, seeds treated with antagonists resulted into a significant enhanced growth in mungbean, black gram and tomato. These antagonists were found effective against *Sclerotium rolfsii*.

Sultana and Hossain (1999) experimented on *Trichoderma harzianum* in controlling of foot and root rot disease (caused by *Fusarium oxysporum* and *Sclerotium rolfsii*) of lentil cv. BARI Masur-1 under field condition. Seeds treated with *Trichoderma harzianum* @ 2×10^{6} conidia/seed contributed 47.85% to 112.49% reduction of foot and root rot disease over control. *Trichoderma harzianum* treated seeds increased germination up to 13.37% and showed up to 3.69% more field emergence over control. *Trichoderma harzianum* treated seeds of lentil yielded up to 1783.33 kg/ha that resulted 81.60% higher seed yield.

Begum (1997) investigated on 4 *Trichoderma* spp. and evaluated their antagonistic potentiality against the major soil-borne phytopathogens *viz. Sclerotium rolfsii, Fusarium oxysporum* and *Macrophomina phaseolina.* Two induced mutants of *Trichoderma* spp. showed better performances over control strain in reduction of seedling mortality of chickpea and lentil caused by *Fusarium oxysporum* and *Sclerotium rolfsii* under greenhouse condition.

Roberti *et al*. (1996) studied on the effect of *Trichoderma harzianum* on bean (*Phaseolus vulgaris*) rot caused by *Scelorotium rolfsii. T. harzianum* was applied to seeds as seed treatment. *Trichoderma* strains were effective in controlling bean root rot by ensuring inhibition of *Scelorotium rolfsii*. *Trichoderma harzianum* showed evidence to reduce the growth of *Scelorotium rolfsii* and parasitize its hyphae by direct contact as well as forming coils, short contact branches and hook-shaped hyphal tips.

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

According to Sugha *et al*. (1993), seeds coated with conidia of the antagonistic *Trichoderma harzianum* and *T. viride* significantly decrease seedling mortality caused by *Sclerotium rolfsii* from 47% to 65% compared with controls which were not treated.

Xu *et al.* (1993) reported that two isolates, *Trichoderma* T_{82} and *Trichoderma* NF_9, inhibited hyphal growth of *Sclerotium rolfsii, Rhizoctonia solani, Pythium aphanidermatum, P. spinosum* and *Fusarium oxysporum.* In greenhouse experiments, soil treated with *Trichoderma* T_{82} @ 0.6 % (w/w) on bran-based mass culture (1×10^7CFU /g) after 20 days of inoculation with the pathogens reduced disease incidence caused by *S. rolfsii, R. solani* and *P. aphanidermatum* by 46.5%, 28.4% and 81.2% respectively. Seed treatment with spore suspension of *Trichoderma* T_{82} and *Trichoderma* NF_9 @ 1×10^8 CFU /ml at 11 days after

inoculation with *S. rolfsii* increased emergence of cucumber seedlings by 14% and 20%, respectively.

Monaco *et al*. (1991) isolated *Trichoderma harzianum*, *T. koningii* and *T. aureoviride* from naturally infected tomato fields with *Fusarium* spp. and *Sclerotium (Corticum) rolfsii* in the renowned horticultural area of Laplanta, Argentina. They reported all these species of *Trichoderma* to be effective against *Fusarium* spp. and *Sclerotium rolfsii in vitro* as well as in subsequent field trials. They used these antagonists as seed treatment in the field. Seeds treated with *Trichoderma harzianum* showed significant increase in seedling emergence in soil infected with the pathogens. They also reported each of these three *Trichoderma* spp. to be effective against *S. rolfsii*.

Kumar and Khare (1990) reported that *Trichoderma harzianum* showed some antagonistic effect against *Sclerotium rolfsii* while soybean seeds treating with *Trichoderma harzianum*, *Gliocladium virens*, *Bacillus subtilis* and *Streptomyces* spp. They also reported that *Fusarium* infection in sunflower was reduced by *Trichoderma harzianum*.

Krishnamoorthy and Bhoskaran (1990) ensured that soil inoculated with *Trichoderma harzianum* and *T. viride* resulted in significant control of *Sclerotium rolfsii*. Their investigation revealed that treated pots with these antagonists gave 78.2% and 72.2% germination of eggplant seeds, respectively, compared to 19.3% seed germination in control (untreated).

Shin *et al.* (1987) reported that soil treated with *Trichoderma viride* decreased damping off disease of sesame seedlings. Seedlings in the treated beds with the antagonist grew better than seedlings in untreated beds. They also reported that both soil and seeds treated with *T. viride* resulted in reduced infection of *S. sclerotium* and *B. cinerea* in sunflower grown in glasshouse as well as in the field.

Agrawal *et al*. (1977) found evidence of inhibition of the growth of *Sclerotium rolfsii* due to use of filtrates of *Trichoderma* on PDA. Pot trial revealed significant control of seedling mortality due to use this antagonist. They also reported that application on seed was more effective rather than on soil.

According to the investigation of Wells *et al*. (1972), *Trichoderma harzianum* was pathogenic to *Sclerotium rolfsii* in agar medium. They found *T. harzianum* to control *Sclerotium rolfsii* effectively on peanuts, tomatoes and blue lupins under greenhouse condition as well as field condition while applying 1-3 times over the plants on to the soil surface.

According to Agrios (1997) and Mondal *et al.* (1996), control of soil-borne fungal phytopathogens is very challenging due to their years together existence in the soil in various form viz. oospore, conidia, sclerotia, chlamydospores or others.

Several researchers found successful results to control soil borne phytopathogens such as *Sclerotium rolfsii* and *Rhizoctonia solani* using *Trichoderma* based integrations using appropriate fungicides and organic amendments. Khan （2003) conducted an experiment on integrated management of seedling mortality of chickpea caused by *Sclerotium rolfsii* at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh. Raihan *et al.* (2003) set an experiment aiming at finding the efficiency of integration of an antagonist and fungicide to suppress seedling mortality of peanut caused by *Rhizoctonia solani* and *Sclerotium rolfsii*. Begum and Bhuiyan (2006) conducted an experiment on integrated control of seedling mortality of lentil caused by *Sclerotium rolfsii*. Bhuiyan and Sen (2013) experimented on integrated management of Southern Blight disease of Carrot caused by *Sclerotium rolfsii*. Islam and Bhuiyan (2006) conducted an experiment on Integrated management of foot and tube rot of tuberose (*Polianthes tuberosa*) caused by *Sclerotium rolfsii*. All the above researchers made the same conclusion that *Trichoderma*

harzianum based composts are effective to control soil borne plant diseases as well as to enhance the growth of plants.

Rubayet and Bhuiyan (2016) studied on Integrated management of stem rot of potato caused by *Sclerotium rolfsii* at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Dhaka, Bangladesh. They found the integration of *Trichoderma harzianum*, mustard oilcake @ 60 g/m2 and Provax-200 @ 0.02% effective to control *Sclerotium rolfsii* causing pre and post emergence mortality of potato. They also reported a significant increase in the yield of potato.

Chandrasehar *et al.* (2005) conducted lab and green house experiments to measure the antagonistic effect of *T. harzianum* against *S. rolfsii* which causes collar rot disease in tomato plants. The experiment revealed the result that *T. harzianum* in *in vitro* condition was able to complete suppression of the growth of *S. rolfsii*. On the other hand, the survival percentage of seedlings treated with seed treatment and soil drenching was high in pot culture in green house condition.

Inber *et al*. (1994) applied *Trichoderma harzianum* to cucumber seedlings as a peat-bran based preparation by incorporating with the propagating mixture in a commercial seedling production nursery. 23.8% of increase in seedling height and 96.1% of increase in leaf area were recorded. They also noticed that *Trichoderma*-treated seedlings were more developed, grew more vigorously and contained higher levels of chlorophyll over control but no significant differences were found in N, P or K content among treatments. *Trichoderma* treated plants resulted in more resistant to damping off diseases caused by *Pythium* spp. and *Rizoctnia solani*. 4% of the non-treated plants died compared with *Trichoderma* treated plants where no damping-off was occurred. During the second growing cycle, significant reductions in damping-off by 67% and 52% were obtained in middle and border beds, respectively, compared with untreated controls.

Kaur and Mukhapadhyay (1992) investigated on integrated use of *Trichoderma harzianum* with fungicidal seed treatments. The study showed significant reduction in the incidence of chickpea wilt complex and increased crop yield in field condition. Seed treatment alone with fungicide like Vitavax-200 (Carboxin + Thiram) or Ziram resulted in 29.9% reduction in disease. When *Trichoderma harzianum* was integrated with these fungicides, the control of disease increased to 63.3%.

Elad *et al*. (1982) experimented on management of plant infection by biological means. *Trichoderma harzianum* isolated from the soil was grown on cell walls of the pathogens which showed antibiotic activity against *Sclerotium rolfsii*. *T. harzianum* showed evidence to produce extra cellular β (1-3) glucose and chitinase while applying it multiplicated on wheat bran to the soil infested with *Sclerotium rolfsii* in the glass house. According to their report, *Trichoderma harzianum* could effectively control damping off disease of eggplant seedlings.

Lievens *et al.* (2004) observed a severe rot and collar rot or foot rot in two-month old wilted tomato *(Lycopersicum esculentum)* plants in a large-scale commercial greenhouse in Belgium. Symptom development was found to be confined to lower parts of plant with severe rotting of the whole root system and dark lesions surrounding the stem base.

Babar (1999) applied 10 g of colonized dried oat grains and drenched surrounding soil near plant base with fungal suspension for inoculation and kept covered with moist cotton. Older plants (60-90 days old) showed quicker onset of infections (within 8-10 days) and larger sized lesions than that in younger ones (10-45 days old).

Bell *et al*. (1982) and Henis (1984) reported dual culture method as the most common one to select optimal antagonists with a test in petri dishes.

Kashem (2005) conducted an experiment by using soil infestation technique for inoculation of *S. rolfsii*. He found the result that soil inoculated with grain culture @ 0.1% in weight basis of dry soil before sowing seeds caused heavy infestation.

Islam *et al*. (2002) carried out an experiment to evaluate 9 organic substrates for mass culture of an isolate (GT-1) of *T. harzianum*, a potential biocontrol agent. They concluded that maize meal was the most suitable substrate for maximum spore production. Larger colony diameter and faster mycelial growth were found in maize meal compared to others.

Shamarao *et al*. (1998) carried out an experiment on mass multiplication and sporulation of *Trichoderma viride* using different types of substrates such as wheat bran, farmyard manure, poultry manure, cowdung, jaggery, oil cake, groundnut cake, neem cake and pongamia. Wheat bran was identified as the most suitable substrate for sporulation of *T. viride.*

Sangeetha *et al*. (1993) conducted an experiment to evaluate some substrates on which *Trichoderma harzianum* and *Trichoderma viride* were formulated. They identified farmyard manures as the best substrate for formulation followed by wheat and rice bran. Peat soil alone and rice straw were reported as poor substrates.

Islam (2008) performed soil inoculation method to induce infection in eggplant by using barley culture of the pathogen, *Sclerotium rolfsii.* He found all the varieties infected ranging from 66.66 to 100%. The experiment also showed that the varieties varied in percent mortality.

Kashem (2005) performed soil infestation technique for inoculation of *S. rolfsii.* He observed heavy infestation when soil was inoculated with grain culture before sowing seeds at the rate of 0.1% in weight basis of dry soil.

Hiremath *et al.* (1998) conducted an experiment to compare 5 inoculation techniques of *S. rolfsii.* Soil infestation method was the most effective for

inducing infection in seedlings by *Sclerotium rolfsii.* Incorporation of 2% of inoculum in soil was sufficient to produce disease in high level. Incidence of disease on plants inoculated at 30 DAS and 60 DAS by toothpick method increased with plant age.

Waraitch *et al.* (1986) used soil mixing technique to inoculate *S. rolfsii.* The inoculum of *S. rolfsii* was multiplied on sterilized sorghum seeds overnight pre-soaked in 2% of sucrose solution. The inoculum was mixed in the rhizosphere soil of the plants @ 3× 500 ml conical flask per 100 m of area.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

For conducting the present piece of research, different materials were used and methodologies were followed. Those are purposely described in this chapter. The *in vitro* (Laboratory) and Pot experiments (net house) were conducted to achieve the objectives of the study.

3.1. *In vitro* experiment

3.1.1. Experimental site

The *in vitro* experiments were conducted at the Mycology Laboratory of Plant Pathology Department, M Wazed Miah Central Laboratory Building, Sher-e-Bangla Agricultural University, Dhaka, 1207.

3.1.2. Duration of the experiment

The experiment was carried out during the period from January to September 2019.

3.1.3. Collection of soil sample and isolation of *Trichoderma*

Trichoderma isolates were isolated from rhizosphere soil which were collected from seven different districts viz. Meherpur, Jashore, Panchagor, Bogura, Manikgonj, Gazipur and Cumilla, Bangladesh and isolated by "Dilution Plate Technique" as described by Dhingra and Sinclair (1985).

Plate 1. Collection of soil sample

3.1.4. Dilution Plate Technique

3.1.4.1. Disinfection of working area

The surface of the working area was disinfected with cotton soaked in methyl alcohol @ 70% at v/v basis. The hands and equipment were disinfected by the same process. The glass wares (test tubes, petri dishes, pipettes, beakers etc.) were sterilized in electric dry oven.

3.1.4.2. Preparation of working samples

All the samples were collected in polythene made zipper bags so that moisture could not evaporate from the soil sample. Immediate after collection of individual samples, a composite sample was made from which working samples were prepared.

3.1.4.3. Making of suspension (soil dilution)

a. 1g of soil from the working sample was taken in a test tube containing 9 ml of sterile water and stirred thoroughly for few minutes so that a uniform 1:10 soil suspension could be obtained. This suspension was used as stock solution.

b. 1ml of that 1:10 stock solution was taken into the 2^{nd} test tube containing 9 ml sterile water with the help of sterile pipette and shaken vigorously to make 10^{-1} dilution.

c. 1ml of the 10^{-1} dilution was transferred to the 3^{rd} test tube containing 9 ml sterile water by sterile pipette to make 10^{-2} dilution. By following the same technique, dilution was made up to 10^{-4}.

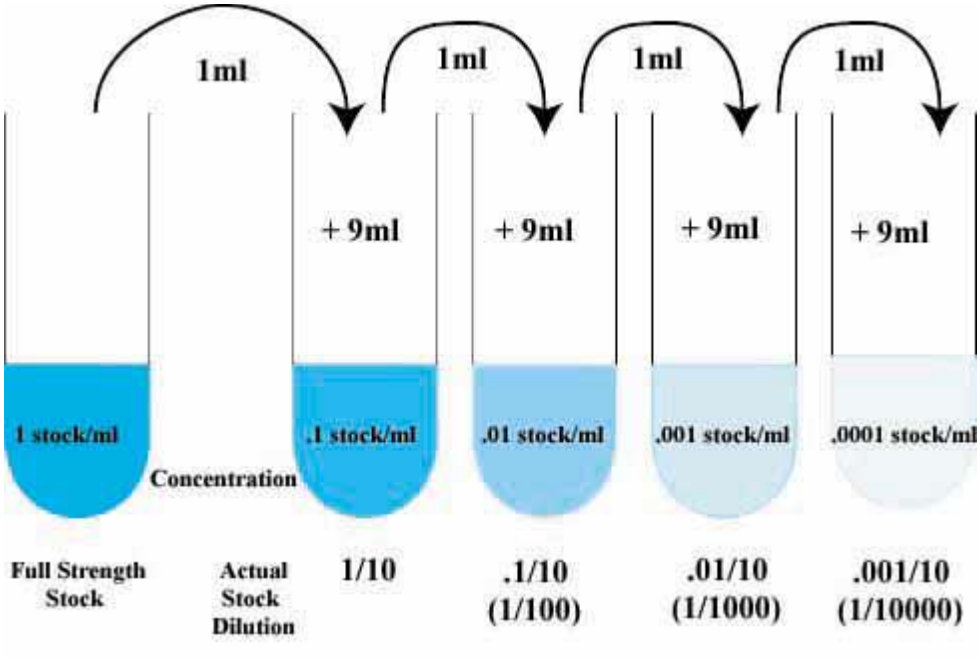


Figure 1. Preparation of dilution series of soil sample

3.1.5. Isolation and identification of micro-organisms (*Trichoderma* isolates)

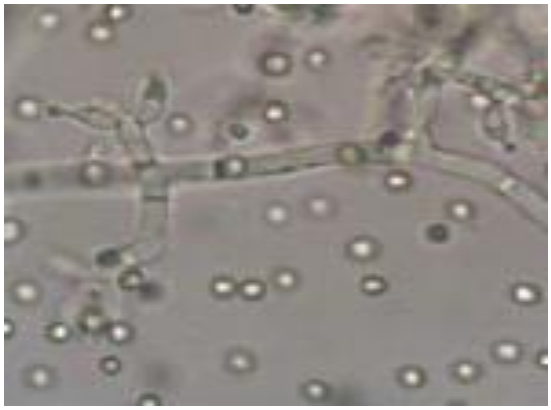
a. 20 ml of warm (approx. 45^{o}C) melted PDA media was poured in each sterile petri-dish.

b. 1 ml of diluted soil sample (10^{-4}) was placed at the center of the petri-plate containing PDA and spread it along the media surface. Thus, three petri-dishes were inoculated with 1 ml of each diluted sample.

c. The inoculated PDA plates were incubated at 25±2^{o}C temperature for 7-10 days.

d. After 3-5 days of incubation, the colonies grown out on PDA were observed under compound microscope (Model: Olympus CH2i) and was identified by CMI description. Subcultures were done by transferring a small colony to a new petri-dish. Further re-cultures were performed by hyphal tip culture method to obtain pure culture of *Trichoderma* isolates. The pure cultures were preserved at 4±0.5°C temperature for further use. The contaminated PDA plates were discarded.

A. Isolation of *Trichoderma* isolates from diluted plate



B. Conidia of *Trichoderma* isolate observed under compound microscope (40X)

C. Hyphal tip culture of *Trichoderma* isolate

Plate 2. Isolation, identification and purification of *Trichoderma* isolates

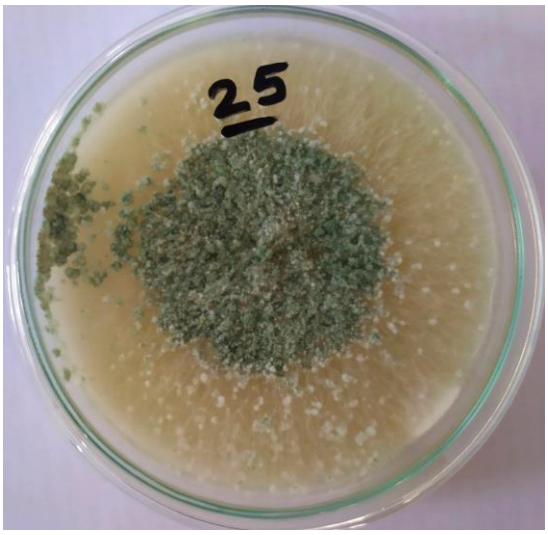
3.1.6. Nomenclature of the *Trichoderma* isolates

All together 13 isolates of *Trichoderma* were obtained. The nomenclature of the *Trichoderma* isolates was performed by following the rule based on location and source crop as described by Aminuzzaman *et al.* (2010). For example, an isolate named MeGan.B represents that it was isolated from the rhizosphere soil of brinjal at Gangni upazila under Meherpur district.

Table 1. List of *Trichoderma* isolates with nomenclature

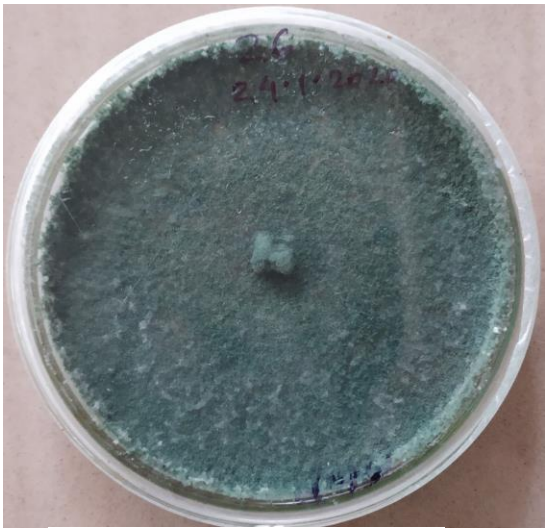
Sl. no.	*Trichoderma* isolates	Area of collection & Host plant		
		District	Upazila/ Village	Host plant
01	CuChn.T	Cumilla	Chandina	Tomato
02	CuChn.B	Cumilla	Chandina	Brinjal
03	CuChn.P	Cumilla	Chandina	Potato
04	CuDeb.B	Cumilla	Debidwar	Brinjal
05	CuDeb.T	Cumilla	Debidwar	Tomato
06	MnMah.B	Manikganj	Maheshpur	Brinjal
07	BoMah.B	Bogura	Mahasthangor	Brinjal
08	PnDeb.P	Panchagor	Debigonj	Potato
09	PnDeb.C	Panchagor	Debigonj	Chilli
10	MeGan.B	Meherpur	Gangni	Brinjal
11	GaJoy.B	Gazipur	Joydevpur	Brinjal
12	GaJoy.T	Gazipur	Joydevpur	Tomato
13	JaGot.T	Jashore	Gotkhali	Tomato

CuChn.T

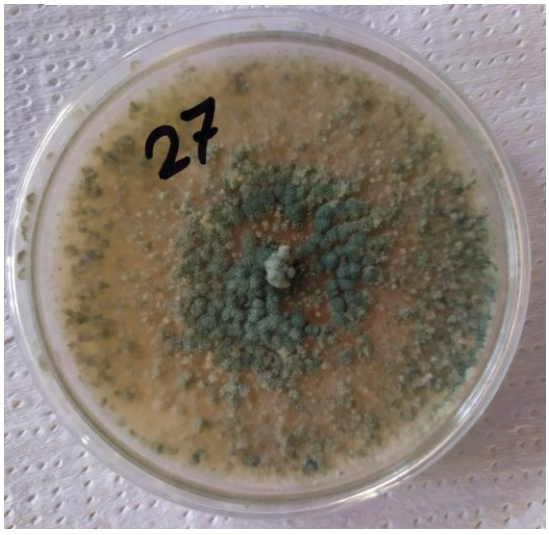


CuChn.B

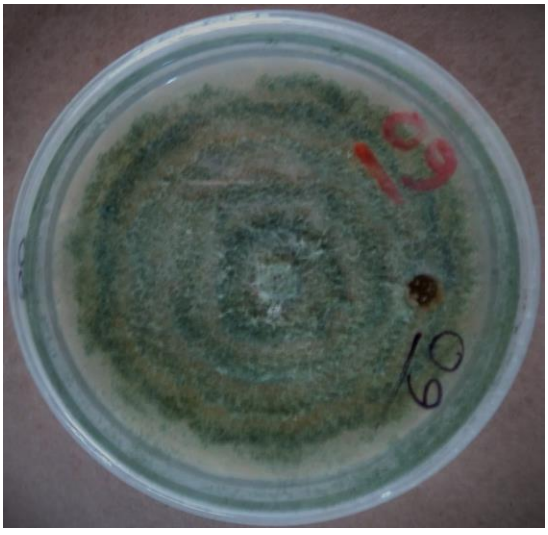
CuChn.P



CuDeb.B

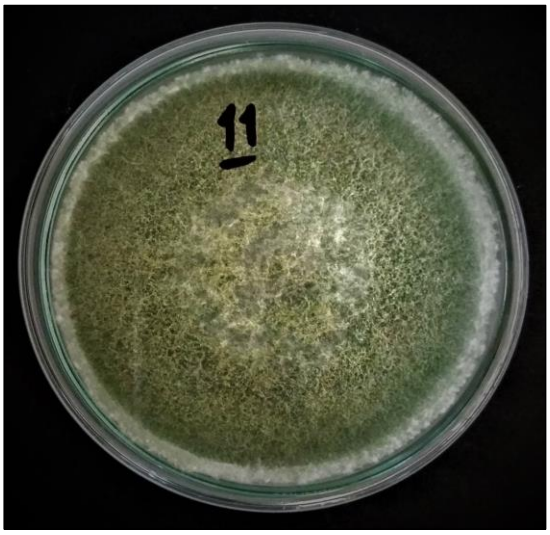


CuDeb.T

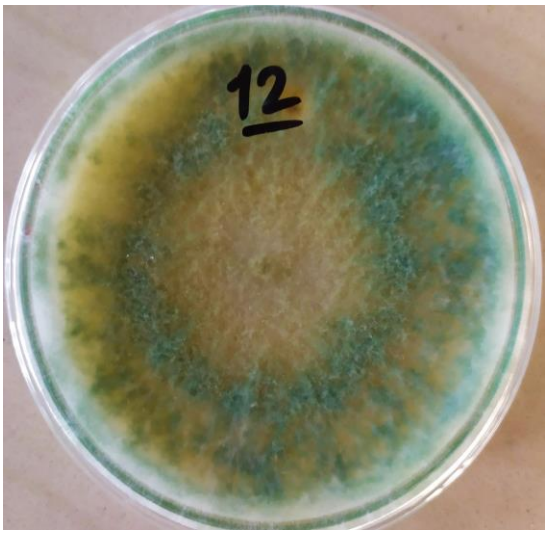


MnMah.B

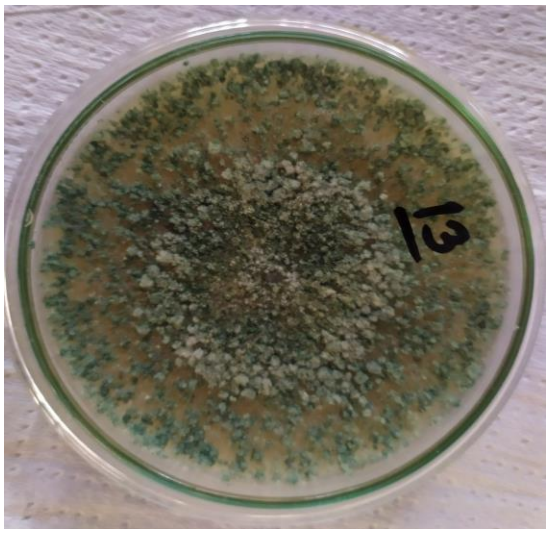
Plate 3. Resident *Trichoderma* isolates obtained from rhizosphere of different locations of Bangladesh



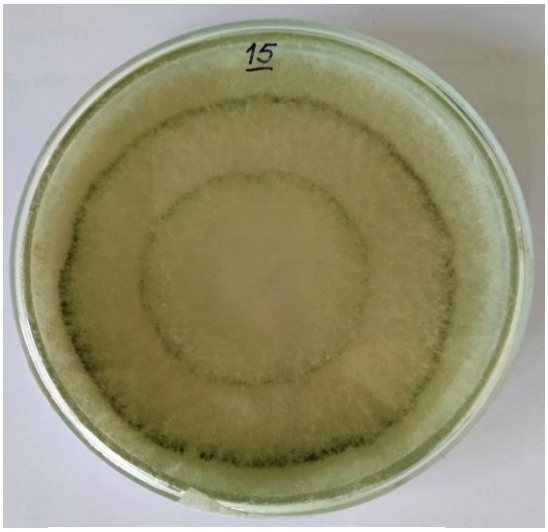
BoMah.B



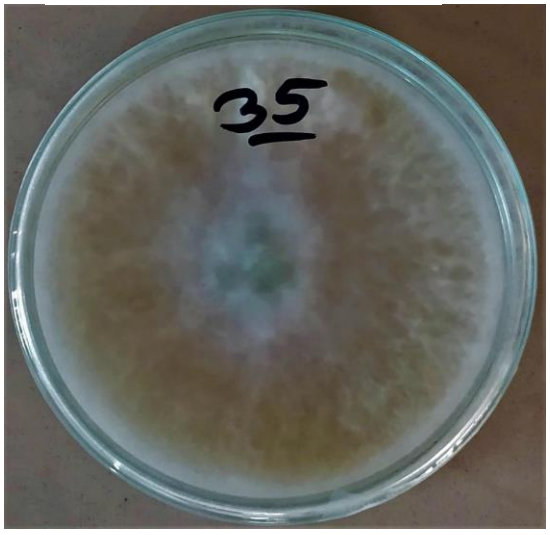
PnDeb.P



PnDeb.C



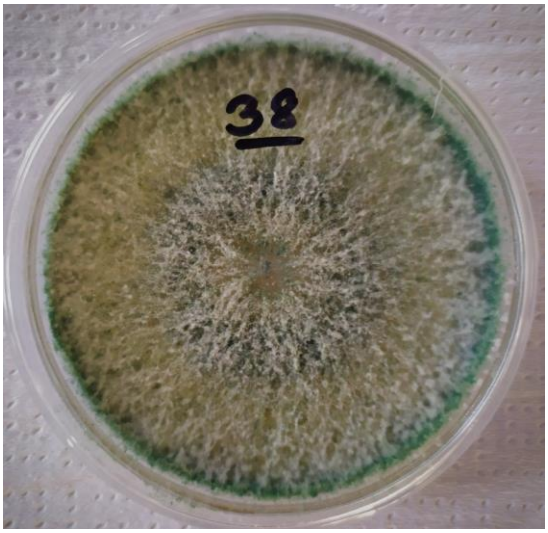
MeGan.B



GaJoy.B

GaJoy.T

Plate 3. Resident *Trichoderma* isolates obtained from rhizosphere of different locations of Bangladesh

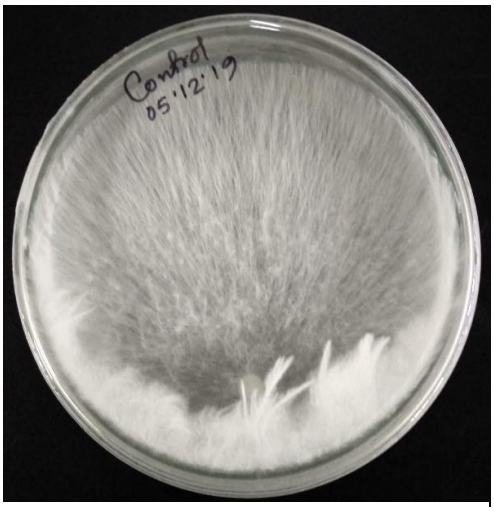


JaGot.T

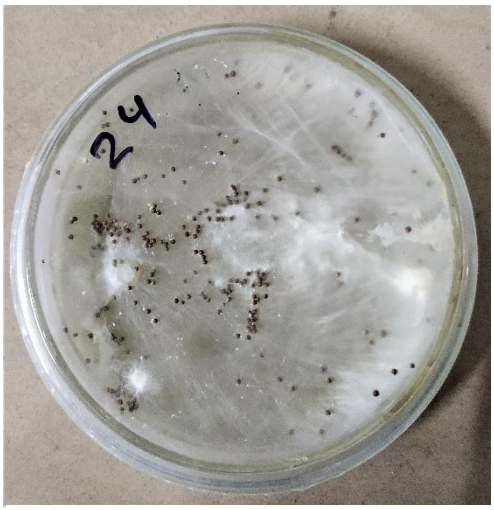
Plate 3. Resident *Trichoderma* isolates obtained from rhizosphere of different locations of Bangladesh

3.1.7. Collection, isolation and maintenance of *Sclerotium rolfsii*

The pathogen was obtained from naturally infected mung bean plants grown in the experimental field of the Department of Plant Pathology, SAU, Dhaka. The typical collar rot symptoms of mung bean plant showed a rot with dry black to brown black lesions around the stem at collar region. The plant was still alive with pale green, and reduced sized leaves. A number of round and brown to black sclerotia with typical fan shaped whitish mycelia were found around the stem of the infected plant. Both sclerotia and infected tissues were collected. The sclerotia were surface sterilized with 10% Clorox for 30 seconds followed by three times rinsing with distilled water. Then a single sclerotium was placed on PDA media acidified with one drop of 5% lactic acid and incubated at 25± 2ºC for 7days. After incubation, typical fan shaped white mycelia as well as sclerotia were found (Plate 4). The pathogen was further purified and multiplied through hyphal tip culture on PDA for further use.



Pure culture of *S. rolfsii*



Formation of sclerotia

Plate 4: Mycelial growth and sclerotia formation of *S. rolfsii* on PDA media

3.1.8. Treatments of the experiment

All together 13 *Trichoderma* isolates along with a control were used in this experiment stated below:

1. CuChn.T+ *Sclerotium rolfsii*
2. CuChn.B+ *Sclerotium rolfsii*
3. CuChn.P+ *Sclerotium rolfsii*
4. CuDeb.B+ *Sclerotium rolfsii*
5. CuDeb.T+ *Sclerotium rolfsii*
6. MnMah.B+ *Sclerotium rolfsii*
7. BoMah.B+ *Sclerotium rolfsii*
8. PnDeb.P+ *Sclerotium rolfsii*
9. PnDeb.C+ *Sclerotium rolfsii*
10. MeGan.B+ *Sclerotium rolfsii*
11. GaJoy.B+ *Sclerotium rolfsii*
12. GaJoy.T+ *Sclerotium rolfsii*
13. JaGot.T+ *Sclerotium rolfsii*
14. *Sclerotium rolfsii* (as Control)

3.1.9. Determination of sporulation rate

15-20µl of cell suspension was placed between the hemocytometer and cover glass using a P-20 pipette (Figure 2). The number of cells in all four outer squares were counted and divided by four (the mean number of cells/square). The number of cells per square $\times 10^4$ = the number of cells/ml of suspension (Ashrafuzzaman, 1976).

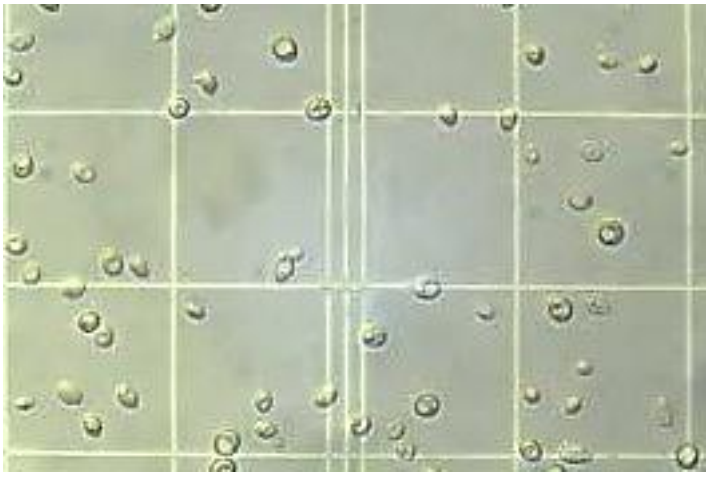


Figure 2. *Trichoderma* conidia counting on hemocytometer

3.1.10. Performing of Dual culture *in vitro*

An agar disc (5 mm dia.) was taken from 4-day-old *Trichoderma* culture plate of each isolates and placed at the periphery of a fresh PDA plate (90 mm). Another agar disc of the same size of *S. rolfsii* was also placed at the periphery but on the opposite end of the same petri-dish (Figure 3). As a control, *S. rolfsii* was placed in a similar way at the periphery on a fresh PDA plate (Figure 3). All inoculated PDA plates were incubated at 28°C for 4 days (Dennis and Webster 1971a). Then antagonistic activity was tested by measuring the radial growth of the *S. rolfsii* colony (R_2) to the direction of the antagonist colony and the radius of the *S. rolfsii* colony in the control plate (R_1). The two readings were transformed into percent inhibition of radial growth using the formula developed by Skidmore and Dickinson (1976).

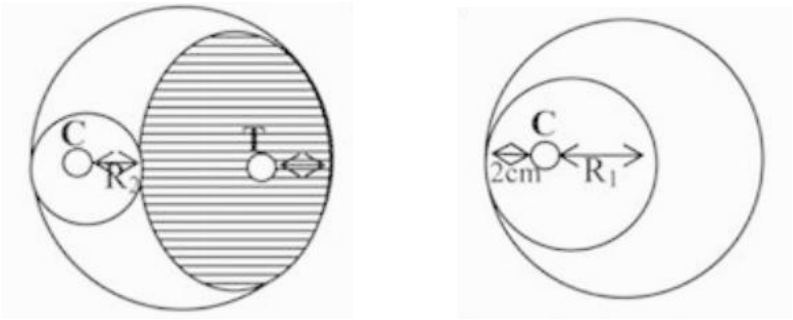


Figure 3. Showing A) Radial growth of pathogen in dual culture plate B) Radial growth of pathogen in control plate

$$\%\text{Inhibition} = \frac{R1-R2}{R1}\times 100$$

Here,

R_1 = Radial growth of pathogen in control plate

R_2 = Radial growth of pathogen in dual culture plate

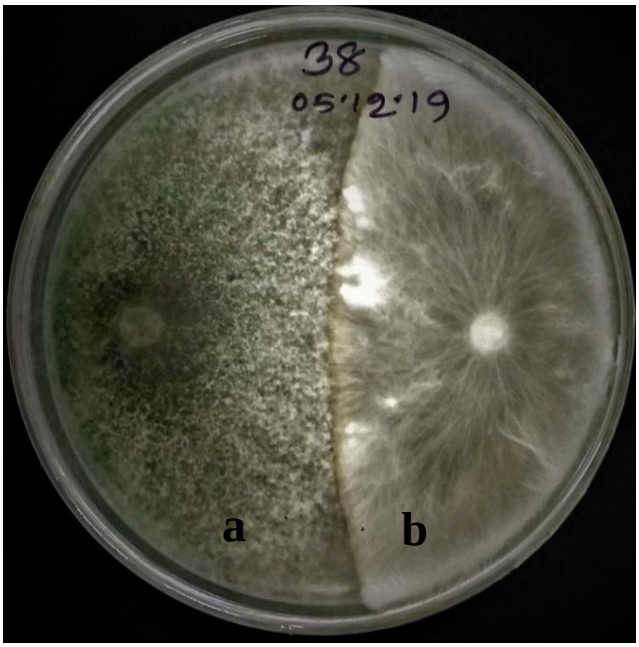


Plate 5: Dual culture of *Trichoderma* isolate (a) and *S. rolfsii* (b)

3.1.11. Data collection

Data were collected on the following parameters:

i. Mycelial growth of *Trichoderma* isolates

ii. Time required for sporulation

iii. Rate of sporulation of *Trichoderma* isolates

iv. % Inhibition of mycelial growth of *S. rolfsii*

3.1.12. Statistical analysis of data

The collected data obtained for various parameters were compiled, coded, simulated and analyzed by STATISTIX 10 computer package.

3.2. Pot Experiment

3.2.1. Experimental site

Pot experiment was conducted at the net house of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka-1207. Each of the 14 inches sized earthen pot was used as unit plot.

3.2.2. Experimental duration

The experiment was carried out during the period from October 2019 to March 2020.

3.2.3. Collection of peat soil

Peat soil with characteristics color was collected from Tungipara, Gopalgonj, Bangladesh.

3.2.4. Collection of substrates

Culturing substrates for *Trichoderma* isolates and *Sclerotium rolfsii* viz. black gram bran, barley etc. were collected from a farmer's house at Betbaria village of Gangni upazila under Meherpur district. Substrates were kept in 4^0C after collection until use.

3.2.5. Collection of earthen pots

The standard sized earthen pots (tobs) were collected from the local market at Rayer Bazar, Mohammadpur, Dhaka.

3.2.6. Collection of seeds

Mung bean seeds were collected from Siddik Bazar, Gulistan, Dhaka.

3.2.7. Variety used

The "BARI Mug-6" variety was used in the experiment.

3.2.8. Treatments of the experiment

The treatments used in pot experiment were:

1. CuChn.B + Sc.
2. CuDeb.B + Sc.
3. GaJoy.T + Sc
4. JaGot.T + Sc.
5. Autostin + Sc.
6. Bioderma +Sc.
7. CuChn.B + S_0
8. CuDeb.B + S_0
9. GaJoy.T + S_0
10. JaGot.T + S_0
11. T_0 + Sc. (control-I)
12. T_0 + S_0 (control- II)

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

3.2.9. Sterilization of substrates and inoculation of *Trichoderma* for multiplication

Required amount of peat soil, black gram bran and water @ ratio of 1:1:2 were thoroughly mixed in 1000 ml Erlenmeyer flasks and autoclaved at 121^0C for 15 minutes for sterilization. The flasks with sterilized substrate were allowed to cool down and then inoculated with 10-15 mycelial discs of 5 mm dia. of 7 days old *Trichoderma* isolates of each of the culture found promising in dual culture *in vitro*. Inoculated flasks were then incubated at room temperature $(25\pm2)^0$ C for 25 days (Adan *et al.*, 2015).

3.2.10. Formulation of *Trichoderma* based biopesticide

After incubation for a duration of 25 days, the substrates were taken out from the flasks, kept in laminar airflow cabinet for drying and grinded in a blender. The grinded materials were the ultimate formulated *Trichoderma* (Adan *et al*., 2015)

Plate 6: Formulations of biopesticide using promising *Trichoderma* isolates

3.2.11. Preparation of inocula of *Sclerotium rolfsii*

For multiplication and preparation of inocula of *Sclerotium rolfsii,* "Barley Culture Method" was followed. Required amount of barley grains collected from farmer's house were thoroughly washed in water and kept soaked in 2% of glucose solution for 24hrs. After decantation, barley grains were grinded in a blender and taken in 500 ml Erlenmeyer flasks at the rate of 200g in each. The flasks were plugged with cotton followed by wrapping the mouth with foil paper. The flasks containing grinded moist barley grains were sterilized in autoclave at 121ºC for 15 minutes under pressure of 15 psi. The sterilized materials in the flask were allowed to cool down. Then the sterilized materials were aseptically inoculated with 7-10 mycelial blocks of 5mm dia. of pure culture of *Sclerotium rolfsii* cultured in PDA media and incubated at room temperature for 2 weeks. The flasks were periodically shaken with hand for proper distribution of pathogen mycelium throughout the entire mass of the inoculated barley grains in the flasks. The mycelial growth of the fungus covered entire barley mass in the flask and small round whitish sclerotia started to develop. After fifteen days of incubation, the entire mass was taken out of the flasks and spread in laminar air flow cabinet for drying. The colonized dried barley grains were used as inocula for inoculation of pot soil (Babar, 1999).

Plate 7: Preparation of the inocula of *Sclerotium rolfsii*

3.2.12. Preparation of soil

Soil, sand and cow dung were mixed at 2:1:1 ratio and kept for 2 weeks. Then the soil was sterilized with 5 ml formalin (40% of Formaldehyde) diluted in 20 ml water for 4 kg of soil (Dasgupta, 1988) and the prepared soil was heaped covering with polyethylene sheet for 72 hrs. After 72 hrs the heap was uncovered and the next day, earthen pots were filled up with the sterilized soil @ 20 kg/pot (Adan *et al.*, 2015).

Mixing of soil, sand and cow dung

Mixing of formalin with soil **Covering of sterilized soil**

Plate 8. Preparation and sterilization of soil

3.2.13. Application of formulated *Trichoderma* and *Sclerotium rolfsii* to the pot soil

Trichoderma formulations were incorporated with the pot soil of each earthen pot (except control) @ of 20g/kg soil. Then it was incubated for 7 days maintaining proper soil moisture. After that, the pot soil containing *Trichoderma* formulations was inoculated with mass culture of *Sclerotium rolfsii* @ 20g/kg of soil. Inoculated soil was incubated for another 7 days maintaining soil moisture properly (Adan *et al.*, 2015).

Plate 9. Application of *Trichoderma* based bioformulation and *S. rolfsii* to the pot soil

3.2.14. Sowing of seeds in earthen pots

Twenty-five seeds of BARI mug-6 were sown by dibbling method in each earthen pot by maintaining equal distance.

Plate 10. Sowing of mung bean seeds in earthen pots

3.2.15. Data collection

Data was collected on the following parameters-

i. Seedling emergence

ii. Pre-emergence damping off (PEDO)

iii. Post-emergence damping off (PoEDO)

iv. Root length (cm)

v. Shoot length (cm)

vi. Seedling vigor index

vii. Seedling fresh weight (g)

viii. Seedling dry weight (g)

Seedling emergence, pre-emergence damping off, post-emergence damping off were calculated by following formula described by Kataria and Grover (1967) and seedling vigor index was calculated by following the formula described by Vashisth and Nagarajan (2010).

$$\% \text{ Seedling emergence} = \frac{\text{Number of emerged seedling per pot}}{\text{Total number of seeds sown per pot}} \times 100$$

% PEDO (due to *S. rolfsii*)

$$= \frac{\text{Emerged seedlings in control} - \text{Emerged seedlings in treated pot}}{\text{Total seeds sown in tha pot}} \times 100$$

Here,

PEDO = Pre-emergence damping off.

$$\%\text{PoEDO} = \frac{\text{Number of damping off of seedlings after emergence}}{\text{Total number of emerged seedlings}} \times 100$$

Here,

PoEDO = Post-emergence damping off

Seedling vigor index = % Germination × Mean seedling length (root + shoot)

$$\% \text{ Increase seedling emergence} = \frac{C-T}{C} \times 100$$

Here,

C = Seedlings emergence in *Trichoderma* treated pot

T = Seedlings emergence in control/ check pot

$$\% \text{ Reduction of PEDO} = \frac{\% \text{ PEDO in control/check pot} - \% \text{ PEDO in treated pot}}{\% \text{ PEDO in control/check pot}} \times 100$$

3.2.16. Measurement of pre-emergence damping off

The seedling emergence obtained in each pot was deducted from the seedling emergence obtained in control pot where both *Trichoderma* and *S. rolfsii* were absent. The mean difference between these two seedling emergences was documented as pre-emergence damping off.

3.2.17. Statistical analysis of data

The collected data obtained from various parameters were coded, tabulated and analyzed by STATISTIX10. Treatment means were compared by Duncan's New Multiple Range Test (DMRT).

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV
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The present investigation was carried out by *in vitro* experiment and pot experiment. The *Trichoderma* isolates were tested based on various parameters *in vitro* condition. The isolates were varied significantly in respect of the parameters tried. Taking the promising isolates of *Trichoderma*, the next step of pot experiment was conducted.

4.1. Mycelial growth of different *Trichoderma* isolates

The *Trichoderma* isolates significantly differed in respect of mycelial growth on PDA culture media. Data was recorded at different days after inoculation (DAI) and incubated at 28^0 C (Figure 4).

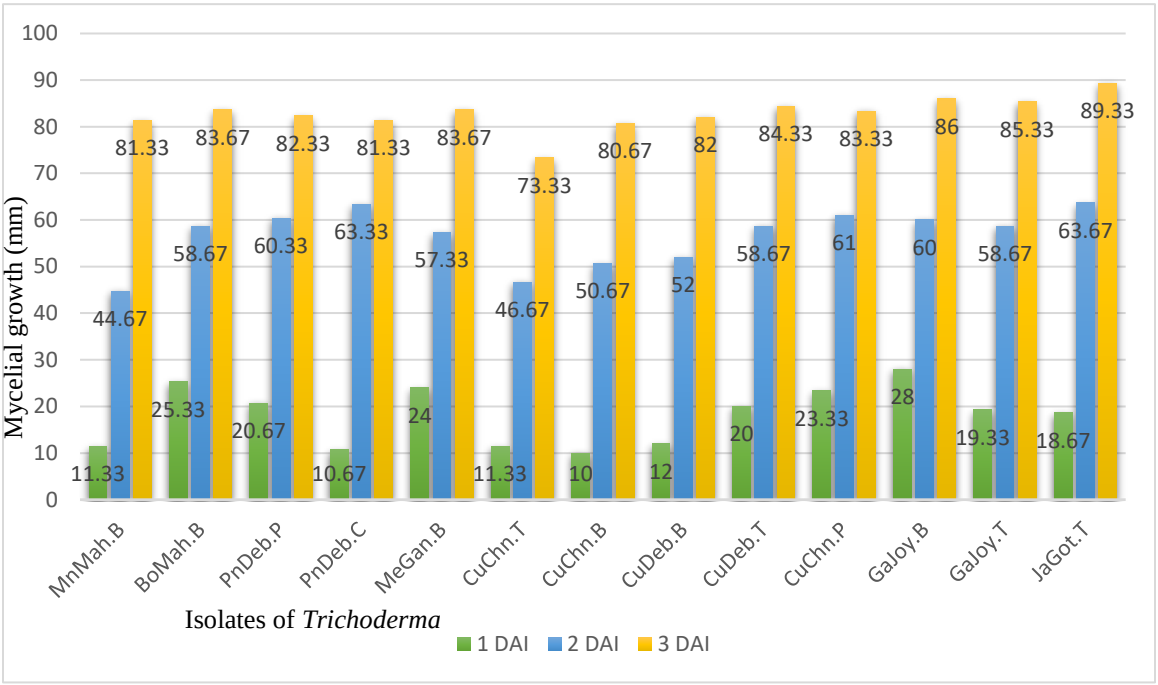

Figure 4. Mycelial growth of different *Trichoderma* isolates

The mycelial growth of different *Trichoderma* isolates was recorded from the day 1 to 3rd days after inoculation until the culture plate were full grown. At 3 DAI, the highest growth (89.33 mm) was found in case of the isolate JaGot.T that was collected from rhizosphere soil of tomato at Gotkhali area, Jashore followed by the isolate GaJoy.B (86.0 mm). The 3rd highest mycelial growth (85.33mm) was recorded in case of the isolate GaJoy.T. The lowest growth (73.33mm) was recorded in case of the isolate CuChn.T preceded by isolate CuChn.B (80.667 mm).

It was observed that *Trichoderma* is a very first growing fungus. It was also observed that the mycelial growth of different isolates was not distinctly differed in PDA media at the beginning but with the progress of time the mycelial growth was found to differ distinctly among the isolates. At 3 days after inoculation, it was found to cover up the entire petri-plate of 90 mm diameter by some of the vigorously grown isolates.

The present findings indicate the similarities to the findings of previous research reports. Sharma and Singh (2014) opined that mycelial growth of *Trichoderma* was the highest in the temperature ranged from 25^0 C to 30^0 C and reduced growth was noticed above 35^0 C. They reported that all the isolates of *Trichoderma* found fast growing and reached to the highest growth (full grown petri plate) within 72 h of incubation at 25^0 C on PDA media.

4.2. Time of sporulation of different *Trichoderma* isolates

The time required for starting sporulation by the *Trichoderma* isolates were distinctly differed among them. The result data were presented in Table 2. At initial growth stage, *Trichoderma* culture remains white in PDA media. It changes its color with the progress of time due to sporulation. The culture plates of all isolates recorded white at 1st day of inoculation i.e., they did not start sporulation. At 2nd day of inoculation, the isolates GaJoy.T and JaGot.T, began to turn green at the central part of the plate that means sporulation started. At 3rd day of inoculation, all the

isolates except CuChn.T turned into white to greenish or yellowish green color. Isolate MeGan.B formed greenish concentric rings on the culture media. In some cases, white powdery postules were found at the central parts of the culture plates that seemed to begin of sporulation profusely.

The results showed that most of the isolates started sporulation on 3rd day of inoculation but in case of isolate CuChn.T, sporulation started at 7 days of inoculation.

Table 2. Time of sporulation of different *Trichoderma* isolates

***Trichoderma* isolates**	**Sporulation occurred on the day**
MnMah.B	3
BoMah.B	3
PnDeb.P	3
PnDeb.C	3
MeGan.B	3
CuChn.T	7
CuChn.B	3
CuDeb.B	3
CuDeb.T	3
CuChn.P	3
GaJoy.B	3
GaJoy.T	2
JaGot.T	2

The present study revealed that *Trichoderma* isolates were very fast sporulating fungus. Earlier studies support the results of the present experiment. Sharma and Singh (2014), reported that some isolates of *Trichoderma* initially produced flat pustules in concentric rings. The pustules appeared powdery due to dense conidiation. They also noticed that the conidial color changed from white to varying shades of green and conidia were formed by 48 hours incubation and turned green within 72 hours. They also stated that conidiation in the *T. harzianum* isolates was predominantly effuse covering the entire surface of the plates.

4.3. Number of spores produced by different *Trichoderma* isolates

Number of spores recorded in case of different *Trichoderma* isolates was presented in Table 3. The *Trichderma* isolates differed significantly in respect of the number of spore/mm^2. The highest number of spore/mm^2 was observed 6.84×10^4 in the case of isolate JaGot.T followed by 6.07×10^4 in isolate GaJoy.T. The 3rd highest (5.83×10^4) spores produced by GaJoy.B. The lowest number of spore/mm^2 was recorded 4.92×10^4 in case of the isolate MnMah.B.

Table 3. Number of spores produced by different *Trichoderma* isolates per mm^2

Trichoderma isolates	No. of spore/mm^2 ($\times10^4$)
MnMah.B	4.92 i
BoMah.B	5.12 gh
PnDeb.P	5.07 h
PnDeb.C	5.20 g
MeGan.B	5.52 ef
CuChn.T	5.80 c
CuChn.B	5.59 de
CuDeb.B	5.69 d
CuDeb.T	5.46 f
CuChn.P	5.20 g
GaJoy.B	5.83 c
GaJoy.T	6.07 b
JaGot.T	6.84 a
LSD (0.05)	0.10
CV (%)	1.06

Earlier study on sporulation of resident *Trichoderma* strain is not available in literature. However, some studies have been found supporting the present findings. Adan *et al.* (2015) found 6.42×10^4 spores/mm^2 in case of *Trichoderma harzianum* isolate collected from Joydebpur, Gazipur while 5.30×10^4 spores/ mm^2 from the isolate collected from Chandina, Cumilla and 5.13×10^4 spores/mm^2 from the isolate collected from Taragonj, Rangpur. Sriram and Savitha (2011), counted spores by hemocytometer to contain 2.15×10^8 conidia per gm soil.

4.4. Antagonistic effect of *Trichoderma* isolates against *S. rolfsii*

Dual culture test showed significant differences among the *Trichoderma* isolates in terms of antagonism. The antagonistic effect of the test fungus against the pathogen concerned was expressed as % inhibition of the test pathogen. The result of interaction among *Trichoderma* isolates and *S. rolfsii* is represented in the Table 4.

Table 4. Mycelial growth inhibition of *S. rolfsii* by *Trichoderma* isolates in dual culture

Trichoderma isolates	% Inhibition
MnMah.B	18.05 e
BoMah.B	25.14 de
PnDeb.P	36.96 bc
PnDeb.C	40.05 b
MeGan.B	25.93 de
CuChn.T	30.65 cd
CuChn.B	21.20 e
CuDeb.B	44.11 ab
CuDeb.T	22.77 de
CuChn.P	24.35 de
GaJoy.B	36.95 bc
GaJoy.T	44.17 ab
JaGot.T	50.35 a
LSD (0.05)	8.28
CV (%)	15.25

The highest inhibition (50.35%) was recorded in case of the isolate JaGot.T while the 2nd highest inhibition (44.17%) was observed in the isolate GaJoy.T which is statistically similar to the isolate JaGot.T. The isolate CuDeb.B showed the 3rd highest inhibition (44.11 %) against the test pathogen. On the contrary, the lowest inhibition was recorded 18.05 % in case of MnMah.B isolate preceded by CuChn.B (21.2 %) and they were statistically similar.

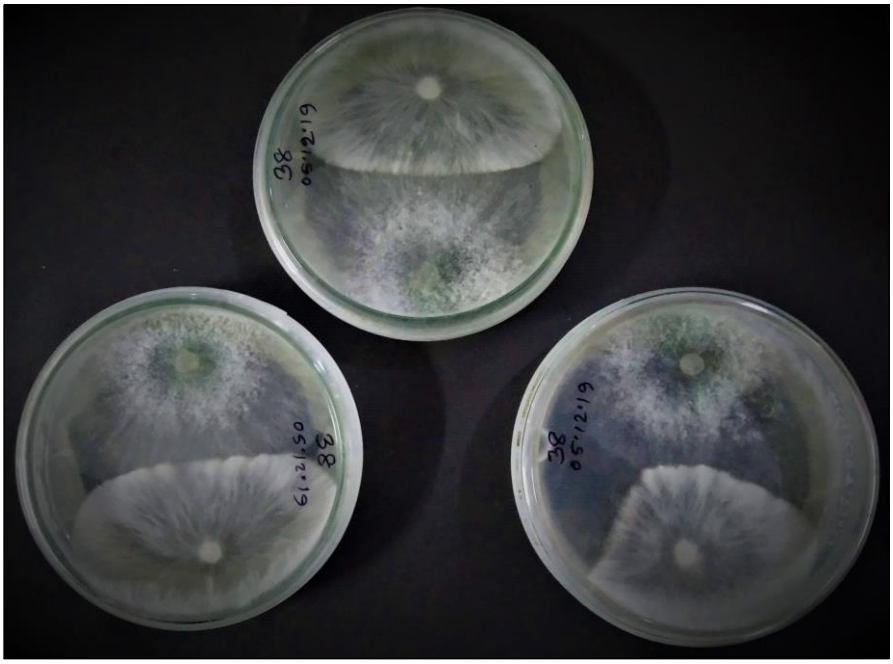

Plate 11. Growth inhibition of *S. rolfsii* in dual culture

The present study revealed that each of the *Trichoderma* isolate has some potential to suppress the test pathogen *S. rolfsii* that supports the findings of Wells *et al*. (1972), Agrawal *et al*. (1977), Amin *et al*. (2010), Nagamma and Nagaraja (2015) and Faruk and Rahman (2016). Mukherjee and Raghu (1997) reported that all the test isolates of *Trichoderma* parasitized *S. rolfsii* in dual culture plates.

Faruk and Rahman (2016) reported that most of the *T. harzianum* isolates showed better performance in reducing the growth of *S. rolfsii* that indicated the antagonistic

effect of *Trichoderma* on the radial growth of the target pathogen. The *T. harzianum* isolates reduced 19.42 – 42.72 % radial growth of *S. rolfsii* at 48 hrs of incubation. This result is close to the outcome of the present study.

The variation of antagonistic potential between *Trichoderma* isolates was due to the variation in mycelium-coiling rate, sporulation, fungitoxic metabolites and induced growth response (Barkat *et al*., 2006). Microscopic observation has revealed the formation of coils around the hyphae of *S. rolfsii* by *Trichoderma* isolates causes lysis of the hyphal walls of the pathogenic fungus (Mukherjee and Raghu, 1997).

4.5. Pot Experiment

The isolates those showed the promising performances regarding mycelial growth, sporulation time & rate and inhibition of the test pathogen were subjected to further evaluation by pot experiment in net house to identify the most effective resident *Trichoiderma* isolate against *Sclerotium rolfsii* causing damping off of mung bean. The promising isolates JaGot.T, GaJoy.T, CuDeb.B along with the below graded isolate CuChn.B were considered to evaluate in the the pot experiment. Two check treatments viz. 'Bioderma' (a commercially formulated *Trichoderma* based biopesticide) by Ispahani Agro Limited and Autostin (Carbendazim- a chemical pesticide) were used to compare the inhibitory effect of resident *Trichoderma* isolates.

4.6. Effects of *Trichoderma* isolates on seedling emergence of mung bean

The *Trichoderma* isolates showed promising results in increasing seedling emergence of mung bean while the pot soil was inoculated with *Sclerotium rolfsii*. The control pot (control-I) yielded only 39.00% seedling emergence. The resident isolate JaGot.T showed better performance (76.66%) next to formulated 'Bioderma' (76.66%) and chemical fungicide Autostin (78.00%). The *Trichoderma* isolates increased seedling emergence ranged from 57.25 – 100% over control-I. The increase of seedling emergence (96.56%) was recorded in case of resident

Trichoderma isolate which was statistically similar to Autostin (100%) and formulated 'Bioderma' (96.56%) treated pots. Among the resident *Trichoderma* isolates, all the isolates tested showed potential results except CuChn.B which showed poor performance in Lab trial**.** The control-II yielded 72.66% seedling emergence where pot soil was not inoculated with *Sclerotium rolfsii*. In that case, the seedling emergence was slightly influenced by the action of *Trichoderma* isolates. This is might be due to the inhibitory action of *Trichoderma* against the seed borne microflora other than *Sclerotium rolfsii.*

Table 5. Effects of *Trichoderma* isolates on emergence of mung bean seedlings

Treatments	% Emergence of seedlings	% Increased emergence over control – I	% Increased emergence over control – II
1. CuChn.B + Sc.	61.33 d	57.25	-
2. CuDeb.B + Sc.	68.00 c	74.35	-
3. GaJoy.T + Sc	72.33 bc	85.46	-
4. JaGot.T + Sc.	76.66 ab	96.56	-
5. Autostin + Sc.	78.00 ab	100	-
6. Bioderma + Sc.	76.66 ab	96.56	-
7. CuChn.B + S_0	78.00 ab	-	0.00
8. CuDeb.B + S_0	79.33 a	-	1.71
9. GaJoy.T + S_0	79.00 a	-	1.28
10. JaGot.T + S_0	79.66 a	-	2.13
11. T_0+Sc. (control – I)	39.00 e	-	-
12. T_0+ S_0 (control – II)	78.00 ab	-	-
LSD (0.05)	6.46	-	-
CV (%)	5.25	-	-

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

Plate 12. Effect of *Trichoderma* isolates on seedling emergence

The present experiment revealed that *Trichoderma* isolates enhanced field emergence to some extent. The pot soil amended with only *Trichoderma* isolates showed more seedling emergence over control-II. When only *S. rolfsii* was added to the pot soil (control-I), the seedling emergence were reduced to 39.00 %.

The results of present study show close similarities to the reports of many previous researchers (Chowdhury *et al.*, 2000, Sultana and Hossain, 1999, Krishnamoorthy and Bhoskaran, 1990, Adan *et al*., 2015). They observed the enhancement of seed germination and seedling emergence due to application of *Trichoderma harzianum*. Krishnamoorthy and Bhoskaran (1990) reported that soil inoculated with *Trichoderma harzianum* and *Trichoderma viride* gave good control of *Sclerotium rolfsii* and in treated pots gave 78.2% and 72.2% seed germination, respectively compared to 19.3% in the control. Sultana and Hossain (1999) reported that *Trichoderma harzianum* treated seeds increased germination of chick pea seeds up to 13.37% and resulted up to 3.69% more field emergence over control. Chowdhury *et al.* (2000) reported that seeds treated with *Trichoderma harzianum* against *Sclerotium rolfsii*, resulted up to 21.61% increase in seed germination in mung bean.

Shamsuzzaman (2003a) observed that seed treatment with *Trichoderma harzianum* resulted up to 16.66% higher seed germination in black gram.

4.7. Effects of *Trichoderma* isolates on pre-emergence damping off of mung bean seedlings

The treatments had profound effect on pre-emergence damping off and reduction of pre-emergence damping off disease. The highest pre-emergence damping off (39%) was recorded in control-I where the pot soil was inoculated with *S. rolfsii.* Statistically, the lowest pre-emergence damping off was recorded in case of treatment-4 (JaGot.T+ Sc.), treatment-5 (Autostin + Sc.) and treatment-6 (Bioderma+Sc.) where resident *Trichoderma* isolate JaGot.T, Autostin and formulated *Trichoderma* based biopesticide 'Bioderma' were applied, respectively, in inoculated pot soil with *S. rolfsii* and their effects were statistically similar. The *Trichoderma* isolate CuChn.B yielded 16.67% pre-emergence damping off followed by CuDeb.B (10.00%) and GaJoy.T (5.67%). The rest of the treatments didn't produce any pre-emergence damping off of mung bean.

The result revealed that highest reduction of pre-emergence damping off over control-I was 100% in case of application of Autostin while 96.56% reduction of pre-emergence damping off was recorded in case of resident *Trichoderma* isolate JaGot.T and formulated Bioderma. The rest of the resident *Trichoderma* isolates also showed promising inhibitory effect against pre-emergence damping off ranged from 57.26 - 85.46%.

The present study has similarities with previous research works. Adan *et al*. (2015) reported that the pot soil amended with *Trichoderma* based formulation showed the lowest pre- emergence damping off (2.33%) which is closely similar to the present study.

Table 6. Effects of *Trichoderma* isolates on pre- emergence damping off of mung bean seeds

Treatments	% Pre-emergence damping off	% Decrease of pre-emergence damping off over control-1
1. CuChn.B + Sc.	16.67 b	57.26
2. CuDeb.B + Sc.	10.00 c	74.36
3. GaJoy.T + Sc	5.67 cd	85.46
4. JaGot.T + Sc.	1.34 d	96.56
5. Autostin + Sc.	Nil	100.00
6. Bioderma +Sc.	1.34 d	96.56
7. CuChn.B + S_0	Nil	-
8. CuDeb.B + S_0	Nil	-
9. GaJoy.T + S_0	Nil	-
10. JaGot.T + S_0	Nil	-
11. T_0+Sc. (control-I)	39.00 a	-
12. T_0+ S_0 (control- II)	-	-
LSD (0.05)	6.18	-
CV (%)	59.25	-

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

4.8. Effects of *Trichoderma* isolates on post-emergence damping off of mung bean seedlings

The effect of treatments on post-emergence damping off of mung bean seedlings caused by *S. rolfsii* was found to show similar trend of results as on recorded in case of pre-emergence damping off. The treatments where the pot soil was inoculated with *S. rolfsii* showed higher post-emergence damping off (5.67-45.33%) than the treatments where the soil was not inoculated with *S. rolfsii*. The very few post-emergences damping off (0.66-1.33%) was recorded in case of treatments where the pot soil was not inoculated with *S. rolfsii*.

Among the treatments inoculated with *S. rolfsii*, the lowest post-emergence damping off (5.367%) was counted in case of application of Autostin which was statistically similar to 'Bioderma' (6.52%) and resident *Trichoderma* isolate JaGot.T (6.67%) where the highest post-emergence damping off (45.33%) was counted in case of control-I. the rest of the resident *Trichoderma* isolates yielded post-emergence damping off ranged from 17.33-22.67%.

From the results, it was revealed that application of Autostin showed the highest performance in reduction (87.49%) of post-emergence damping off followed by Bioderma (85.62%) and the resident *Trichoderma* isolate JaGot.T (85.29%).

It was noted that the pot soil where *S. rolfsii* was not inoculated, showed some post-emergence damping off. This might be due to the presence of seed borne microflora. It was also noted that the application of resident *Trichoderma* isolates was found to reduce such post-emergence damping off compared to control-II. In that case, the highest reduction of post-emergence damping off was counted in case of the resident *Trichoderma* isolate JaGot.T (50.38%) followed by CuChn.B (24.81%).

Table 7. Effects of *Trichoderma* isolates on post-emergence damping off of mung bean seedlings

Treatments	% Post emergence damping off	Reduced % post emergence damping off over control-1	% Reduction of post emergence damping off over control-2
1. CuChn.B+Sc.	22.67 b	59.99	-
2. CuDeb.B+Sc.	21.33 b	52.95	-
3. GaJoy.T+Sc	17.33 bc	61.77	-
4. JaGot.T+Sc.	6.67 c	85.29	-
5. Autostin + Sc.	5.67 c	87.49	-
6. Bioderma.+Sc.	6.52 c	85.62	-
7. CuChn.B+ S_0	1.00 d	-	24.81
8. CuDeb.B+ S_0	1.33 d	-	0.00
9. GaJoy.T+ S_0	1.33 d	-	0.00
10. JaGot.T+ S_0	0.66 d	-	50.38
11. T_0+Sc.(control-I)	45.33 a	-	-
12. T_0+ S_0 (control-II)	1.33 d	-	-
LSD (0.05)	6.97	-	-
CV (%)	34.08	-	-

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

Plate 13. Post-emergence effect of *S. rolfsii* on mung bean seedlings

Many previous researchers described *Trichoderma* as an antagonist against *S. rolfsii* as was found in the present study. Some recent research works revealed *Trichoderma* as the most effective biocontrol agent against *S. rolfsii*. Faruk and Rahman (2016), Agarwal *et al.* (1977), Sugha *et al*. (1993) reported that *T. harzianum* and *T. viride* conidia coated seeds significantly decreased seedling mortality caused by *Sclerotium rolfsii* from 47% to 65% compared to controls which were not treated. *Trichoderma* was found to control seedling death in pot trial (Agrawal *et al*., 1977). Seeds of lentil treated with *Trichoderma harzianum* contributed 47.85% to 112.49% reduction of damping off disease over control (Sultana and Hossain, 1999). Adan *et al*. (2015) found 6.33% post-emergence damping off and 78.90% reduction in post emergence damping off of eggplant seedlings due to application of *Trichoderma* based bioformulation. These results of the previous works favor the present study with close similarities.

4.9. Effect of *Trichoderma* isolates on fresh weight of mung bean seedlings

Application of *Trichoderma* based treatments showed a significant influence on fresh weight of mung bean seedlings. The seedlings where the pot soil was inoculated with *Trichoderma* isolates showed higher fresh weight (3.24g -2.75g) than the seedlings where *S. rolfsii* was inoculated. The highest seedling fresh weight (3.24g) was recorded in case of resident *Trichoderma* isolate JaGot.T followed by GaJoy.T (2.90g) where *S. rolfsii* was absent. The lowest fresh weight of seedling (1.38g) was recorded in case of control-I where *Trichoderma* was absent. Among the treatments inoculated with both *Trichoderma* isolate and *S. rolfsii*, the highest seedling fresh weight (2.33g) was recorded in case of Autostin followed by resident *Trichoderma* isolate JaGot.T (2.27g) and Bioderma (2.24g) while the lowest one (1.52g) was recorded in case of CuChn.B.

It was noticed that application of resident *Trichoderma* isolates enhanced seedling fresh weight over control-II and that was highest (21.8%) in case of JaGot.T and lowest (3.38%) in case of CuDeb.B. Again, treatment-5 (Autostin + Sc.), treatment-4 (JaGot.T+Sc.) and treatment-6 (Bioderma + Sc.) showed 68.84%, 64.49% and 62.32% increased fresh weight over control-I where only *S. rolfsii* was present.

Table 8. Effect of *Trichoderma* isolates on fresh weight of mung bean seedlings

Treatments	Fresh weight (g)	Increase in %fresh wt. over control- I	Increase in %fresh wt. over control- II
1. CuChn.B + Sc.	1.52 gh	10.14	-
2. CuDeb.B + Sc.	1.72 gh	24.63	-
3. GaJoy.T + Sc	1.94 fg	40.57	-
4. JaGot.T + Sc.	2.27 d-f	64.49	-
5. Autostin + Sc.	2.33 c-f	68.84	-
6. Bioderma + Sc.	2.24 ef	62.32	-
7. CuChn.B + S_0	2.82 a-c	-	6.02
8. CuDeb.B + S_0	2.75 a-d	-	3.38
9. GaJoy.T + S_0	2.90 ab	-	9.02
10. JaGot.T + S_0	3.24 a	-	21.80
11. T_0 + Sc.(control-I)	1.38 h	-	-
12. T_0 + S_0 (control-II)	2.66 b-e	-	-
LSD (0.05)	0.50	-	-
CV (%)	13.04	-	-

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

Many previous researchers opined in favor of *Trichoderma* to increase fresh weight of seedlings which favors the present study. Shamsuzzaman (2003) reported 266.33% higher fresh shoot weight and 157.14% higher fresh root weight due to seed treatment *Trichoderma harzianum* in cucurbits.

4.10. Effect of *Trichoderma* isolates on dry weight of mung bean seedlings

Application of *Trichoderma* based treatments showed a significant influence in case of dry matter production of mung bean seedlings. The seedlings where the pot soil was inoculated with *Trichoderma* isolates showed higher dry matter (1.08g-0.82g) than the seedlings where *S. rolfsii* was inoculated. The highest dry matter (1.08g) was recorded in case of resident *Trichoderma* isolate JaGot.T followed by GaJoy.T (0.95g) where *S. rolfsii* was absent. The lowest dry matter of seedling (0.39g) was recorded in case of control-I where *Trichoderma* was absent. Among the treatments inoculated with both *Trichoderma* isolate and *S. rolfsii*, the highest seedling dry matter (0.92g) was recorded in case of Autostin followed by Bioderma (0.91g) and resident *Trichoderma* isolate JaGot.T (0.78g) while the lowest one (0.63g) was recorded in case of CuDeb.B.

It was observed that application of resident *Trichoderma* isolates influenced dry matter of mung bean seedlings over control-II and that was highest (50.00%) in case of JaGot.T and lowest (7.2%) in case of CuChn.B. Treatment-6 (Bioderma + Sc.), treatment-5 (Autostin + Sc.) and Treatment-4 (JaGot.T+Sc.) showed 135.90% and 133.33% and 100.00% increased dry matter production over control-I where only *S. rolfsii* was present.

Plate 14. Showing A) fresh and B) dry weight of mung bean seedlings

Table 9. Effect of *Trichoderma* isolates on dry weight of mung bean seedlings

Treatments	Dry weight (g)	% Increased dry matter over control- I	% Increased dry matter over control- II
1. CuChn.B + Sc.	0.69 bc	76.92	-
2. CuDeb.B + Sc.	0.63 cd	61.54	-
3. GaJoy.T + Sc	0.72 bc	84.62	-
4. JaGot.T + Sc.	0.78 bc	100	-
5. Autostin + Sc.	0.92 ab	135.90	-
6. Bioderma + Sc.	0.91 ab	133.33	-
7. CuChn.B + S_0	0.82 a-c	-	7.2
8. CuDeb.B + S_0	0.87 a-c	-	20.83
9. GaJoy.T + S_0	0.95 ab	-	31.94
10. JaGot.T + S_0	1.08 a	-	50.00
11. T_0+Sc. (control- I)	0.39 d	-	-
12. T_0 + S_0 (control- II)	0.72 bc	-	-
LSD (0.05)	0.25	-	-
CV (%)	19.38	-	-

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

The result of the present study is supported by previous researchers' works. Shahid and Khan (2016) stated that mung bean plants produced greatest dry matter when were applied with *T. harzianum* that resulted in 12-17% increase in the dry weight of shoot and root over uninoculated control.

4.11. Effect of *Trichoderma* isolates on seedling length and seedling vigor index

Application of *Trichoderma* based treatments resulted in significant influence on root length, shoot length and seedling vigor index. Significantly, the highest root length and shoot length of seedlings were recorded in case of application of resident *Trichoderma* isolate JaGot.T, Autostin and Bioderma which contribute a lot in increasing the seedling vigor. Among the treatments inoculated with *S. rolfsii*, the highest seedling vigor index was recorded in case of Autostin (2336.88) followed by Bioderma (2239.24) and the resident *Trichoderma* isolate JaGot.T (2229.27) application. The lowest vigor index was calculated in control-I (721.836) preceded by CuChn.B (1562.08), CuDeb.B (1789.76) and GaJoy.T (2042.60) application. Among the non-inoculated treatments with *S. rolfsii*, the highest seedling vigor index was found in case of resident *Trichoderma* isolate JaGot.T (2543.54) followed by GaJoy.T (2462.43), CuChn.B (2310.36) and CuDeb.B (2307.33) compared to control-II (2299.44).

Table 10. Effect of *Trichoderma* isolates on root length, shoot length and seedling vigor index

Treatments	Root length (cm) on 21 DAI	Shoot length (cm) at 21 DAI	Seedling vigor index
1. CuChn.B+Sc.	5.34 ef	20.13 e	1562.08 g
2. CuDeb.B+Sc.	5.34 f	20.98 de	1789.76 f
3. GaJoy.T+Sc	5.47 ef	22.77 b-d	2042.60 e
4. JaGot.T+Sc.	6.59 a-d	22.49 cd	2229.27 c-e
5. Autostin + Sc.	6.91 ab	23.05 abc	2336.88 bc
6. Bioderma +Sc.	6.65 abc	22.56 cd	2239.24 cd
7. CuChn.B+ S_0	6.03 cde	23.59 abc	2310.36 b-d
8. CuDeb.B+ S_0	6.01 cdef	20.98 de	2307.33 de
9. GaJoy.T+ S_0	6.57 bcd	24.60 ab	2462.43 ab
10. JaGot.T+ S_0	7.27 a	24.66 a	2543.54 a
11. T_0+Sc.(control-I)	3.47 g	15.04 f	721.83 h
12. T_0+ S_0 (control- II)	5.95 def	23.53 abc	2299.44 b-d
LSD (0.05)	0.68	1.87	
CV (%)	6.84	5.01	

*Sc. = *Sclerotium rolfsii*; *S_0 = *Sclerotium rolfsii* Nil; *T_0 = *Trichoderma* Nil

*CuChn.B= *Trichoderma* isolate from Chandina, Cumilla (brinjal)

*CuDeb.B= *Trichoderma* isolate from Debidwar, Cumilla (brinjal)

*GaJoy.T= *Trichoderma* isolate from, Joydebpur, Gazipur (tomato)

*JaGot.T= *Trichoderma* isolate from Gotkhali,Jashore (tomato)

Many previous researchers opined in favor of the present study. A number of researchers - Chowdhury *et al.* (2000), Harman *et al*. (2004), Vinale *et al*. (2008) and Pandya *et al*. (2011) opined *Trichoderma* to promote seedling growth as well as seedling vigor index. Seeds treated with *Trichoderma harzianum* showed good effect on controlling *Sclerotium rolfsii* and seeds treated with this antagonist resulted in a significant enhanced growth in mung bean (Chowdhury *et al.,* 2000). Application of biocontrol agents in uninoculated pots influenced the growth of mung bean plants (Vinale *et al*., 2008). Shamsuzzaman (2003) reported that seed treated with *Trichoderma* grown on black gram resulted up to 98.55 more vigor

index of cucurbits over control. *Trichoderma* enhanced uptake of nutrients due to promotion of root growth (Harman *et al*., 2004). Inber *et al*. (1994) reported 23.8% increased height of seedlings that was treated with *Trichoderma*. They also noticed that *Trichoderma* treated seedlings were more developed, grew more vigorously and contained higher levels of chlorophyll over control. Treatment with *T. harzianum* was found superior over other biocontrol agents and increased the shoot and root length by 4% and 5%, respectively, over uninoculated control (Shahid and Khan, 2016).

Trichoderma spp. have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity (Harman *et al.*, 2004; Vinale *et al.*, 2008). Possible explanations of this mechanisms include: control of minor population of pathogens leading to stronger root growth and nutrient uptake (Harman, 2000; Yedidia *et al.*, 2001), secretion of plant growth regulatory factors such as phytohormones (Celar and Valic, 2005; Muthukumar *et al.*, 2005) and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil (Ousley *et al.*, 1994). Moreover, recent studies have indicated that these fungi also induce localized or systemic resistance systems in plants (Yedidia *et al.*, 1999; Howell). Thus, the variety of effects indicate that these beneficial fungi have multiple modes of action. *Trichoderma* spp. parasitize on a broad spectrum of fungi including *Sclerotium rolfsii*. Once the fungi come into contact, whenever to connect to the host can coil around it and form appressoria on the host surface. Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to locate on the target fungus. Another mechanism of the mode of action of the genus *Trichoderma* is producing several fungi-toxic cell wall-degrading enzymes and probably also a peptaibol antibiotic. These compounds with combined activities can result in parasitism of the target fungus and dissolution of the cell walls. At the sites of the appressoria, holes are produced in the target fungus that causes direct entry of *Trichoderma* hyphae into the lumen of the target fungus

(Harman *et al.,* 2004). Pandya *et al*. (2011) reported that *Trichoderma* as a bio-control agent (BCA) is well recognized due to their high reproductive capability and show strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms. *Trichoderma* spp., produces plant growth promoting factors and secondary metabolites which may act as auxin like compound due to which enhancement of plant growth occurs (Vinale *et al*., 2008). The *Trichoderma* spp. excrete one or more of the following antibiotics (metabolites) *viz*. Viridine, Trichodermine, pachybascine and gliotoxins, which have the strong role in inhibiting the pathogenic fungi (Papavizas, 1985). As well as some cell walls degrading enzymes such as chitinases, glucances that break down polysaccharides, chitins and glucanase, thereby destroying the cell wall integrity. Possible mechanisms of antagonism employed by *Trichoderma* spp. include, nutrient and niche competitions, antibiosis by producing volatile components and non-volatile antibiotics that are inhibitory against soil borne fungi, *Sclerotium rolfsii* (Akrami *et al.,* 2011).

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V
SUMMARY AND CONCLUSION

The present investigation was carried out to identify the resident *Trichoderma* isolate from rhizosphere of different crop growing areas of Bangladesh effective against *Sclerotium rolfsii* causing damping off of mung bean seedlings. The experiments were conducted in the central Plant Pathology Laboratory and in net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. All together thirteen isolates of *Trichoderma* were assayed on various parameters in *in vitro* condition. Among them the isolate JaGot.T was found to be promising in respect of mycelial growth, sporulation and antagonistic effect on *Sclerotium rolfsii*. The isolate JaGot.T was recorded as the first growing antagonistic fungus that covered 89.33 mm of the culture plate by 3 days. The 2nd highest (86.00mm) and the 3rd highest (85.333mm) mycelial growth rate was recorded in case of isolate GaJoy.T and GaJoy.T, respectively. The isolate CuChn.T was the slowest one which covered only 73.33mm of the culture plate by 3 days. The isolate JaGot.T and GaJoy.B were recorded to sporulate in 2 days while the isolate CuChn.T took 7 days to sporulate. The isolate JaGot.T produced the highest number of spores (6.84×10^4) per mm^2 followed by isolate GaJoy.B (6.07×10^4 spores/mm^2) and GaJoy.B (5.83×10^4 spores/mm^2). The isolate MnMah.B was recorded to produce the lowest (4.92×10^4spores/mm^2) number of spores. In case of antagonism, the isolate JaGot.T was recorded to inhibit 50.35 % of the test pathogen followed by the isolate CuDeb.B (44.17%) and GaJoy.B (44.05 %) in dual culture. The isolate MnMah.B showed only 18.05 % inhibition of the test pathogen. The best performing resident *Trichoderma* isolate was JaGot.T along with two other promising isolates GaJoy.T and CuDeb.B and a below graded performing isolate CuChn.B were considered to perform in the pot experiment in net house.

In case of seedling emergence, the resident *Trichoderma* isolate JaGot.T showed better performance (76.66%) next to commercially formulated *Trichoderma* based bio-pesticide ‘Bioderma’ (76.66%) and chemical fungicide Autostin

(78.0%) compared to control-I (39.0%) where the pot soil was inoculated with *S. rolfsii.* The highest increase of seedling emergence (96.56%) was recorded in case of resident *Trichoderma* isolate JaGot.T which was statistically similar to Autostin (100%) and formulated 'Bioderma' (96.56%) treated pots. Among the resident *Trichoderma* isolates, all the isolates tested showed increased seedling emergence compared to control-II where both *Trichoderma* and *S. rolfsii* were absent. The highest pre-emergence damping off (39%) was recorded in control-I where the pot soil was inoculated with *S. rolfsii*. The lowest pre-emergence damping off was recorded in case of chemical fungicide Autostin (Nil), resident *Trichoderma* isolate JaGot.T (1.34%) and formulated *Trichoderma* based biopesticide 'Bioderma' (1.34%) being statistically similar. The resident *Trichoderma* isolates were found to decrease pre-emergence damping off ranged from 96.56 - 57.26% over Control-I where pot soil was inoculated with *S. rolfsii.* Among the treatments inoculated with *S. rolfsii*, the lowest post-emergence damping off (5.367%) was recorded in case of application of Autostin which was statistically similar to 'Bioderma' (6.52%) and resident *Trichoderma* isolate JaGot.T (6.67%) while the highest post-emergence damping off (45.33%) was found in case of control-I (T_0 + Sc.). The resident *Trichoderma* isolate were found to decrease post emergence damping off ranged from 0.0 - 50.38 % over control-II (T_0+$Sc._0$) and 52.95 - 85.29 % over control-I. The highest seedling fresh weight (3.24g) and the lowest (1.38g) were recorded in case of resident *Trichoderma* isolate JaGot.T and control-I, respectively. Among the inoculated treatments with both *Trichoderma* isolate and *S. rolfsii*, the highest seedling fresh weight (2.33g) was recorded in case of Autostin followed by resident *Trichoderma* isolate JaGot.T (2.27g) and Bioderma (2.24g) while the lowest one (1.52g) was recorded in case of isolate CuChn.B. The highest increase of fresh weight (64.49 %) was recorded in case of resident *Trichoderma* isolate JaGot.T next to Autostin (68.84 %) and Bioderma (62.32 %) and the lowest (10.14 %) in case of isolate CuChn.B over control-I.

The highest (1.08g) and the lowest (0.39g) dry matter of seedlings were recorded in case of resident *Trichoderma* isolate JaGot.T and control-I, respectively. Among the treatments inoculated with both *Trichoderma* isolate and *S. rolfsii*, the highest dry matter (0.92g) was recorded in case of Autostin followed by Bioderma (0.91g) and resident *Trichoderma* isolate JaGot.T (0.78g) while the lowest (0.63g) was recorded in case of isolate CuDeb.B.

The highest increase of dry matter (100%) was recorded in case of resident *Trichoderma* isolate JaGot.T next to Autostin (68.84 %) and Bioderma (133.33 %) and the lowest (61.54%) in case of isolate CuChn.B over control-I. The highest root length (7.27 cm) and shoot length (24.66 cm) of seedlings were recorded in case of application of resident *Trichoderma* isolate JaGot.T in the non-inoculated soil with *S. rolfsii.* The lowest root length (3.47 cm) and shoot length (15.04 cm) were recorded in case of control where the pot soil was inoculated with *S. rolfsii* only. Among the treatments inoculated with *S. rolfsii*, the highest seedling vigor index was recorded in case of application of Autostin (2336.88) followed by Bioderma (2239.24) and the resident *Trichoderma* isolate JaGot.T (2229.27). The lowest seedling vigor index was recorded in control-I (721.83). Among the non-inoculated treatments with *S. rolfsii*, the highest seedling vigor index (2543.54) was found in case of resident *Trichoderma* isolate JaGot.T and the lowest (2307.33) was recorded in case of isolate CuDeb.B compared to control-II (2299.44).

The results of the present study revealed that the resident *Trichoderma* isolate JaGot.T collected from the rhizosphere of tomato at Jashore district can be recommended for formulation of *Trichoderma* based biopesticide to use for the management of damping off of mung bean seedlings caused by *Sclerotium rolfsii* at farmer's level. Further extensive study is recommended taking more soil samples from every AEZ of the country to find out the more effective resident *Trichoderma* isolate (s), if any, against *Sclerotium rolfsii* causing damping off disease of mung bean seedlings.

CHAPTER VI

REFERENCES

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CHAPTER VII

APPENDICES

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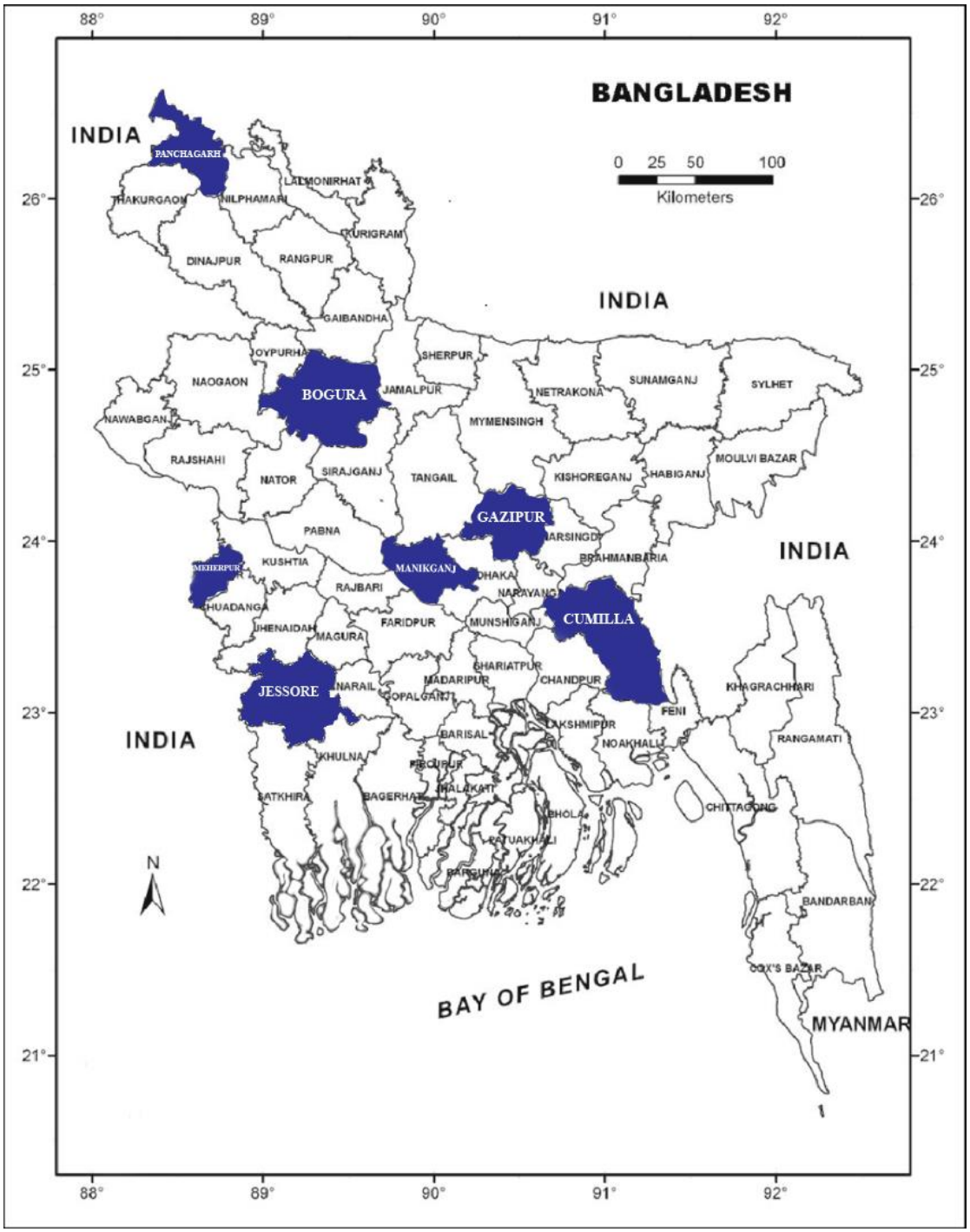
APPENDICES

Appendix I: Map showing the regions of sample collection



Appendix II: Composition of PDA media

Ingredients	Amount
Potato (Peeled and sliced)	200g
Dextrose	20g
Agar	20g
Water	1000ml