ECO-FRIENDLY APPROACHES FOR THE MANAGEMENT OF COLLAR ROT OF MUNGBEAN CAUSED BY *Sclerotium rolfsii*

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CERTIFICATE

This is to certify that the thesis entitled, **"ECO-FRIENDLY APPROACHES FOR THE MANAGEMENT OF COLLAR ROT OF MUNGBEAN CAUSED BY *Sclerotium rolfsii*"** *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* **SANZIDA MOKARROMA** *Registration No.* **18-09033** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma elsewhere in country or abroad.*

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

Dated: 15 September, 2020

Place: Dhaka, Bangladesh

(Dr. Md. Rafiqul Islam)
Professor
Department of Plant Pathology
Supervisor

Dedicated

To

My Beloved Parents & Teachers

List of Abbreviations of Technical Symbols and Terms

Full Word	**Abbreviation/ Symbol**
Agricultural	Agril.
Agriculture	Agric.
Analysis of Variance	ANOVA
And	&
And Others	*et al.*
Bangladesh Agricultural Research Institute	BARI
Centimeter	Cm
Commonwealth Mycological Institute	CMI
Coefficient of Variance	CV
Complete Randomized Design	CRD
Days After Inoculation	DAI
Days After Sowing	DAS
Degree Centigrade	℃
Gram	G
Integrated Disease Management	IDM
Journal	*J.*
Least Significant Difference	LSD
Litre	L
Millimeter	Mm
Namely	viz.
Negative Logarithm of Hydrogen Ion Conc.	pH
Percentage	%
Percentage of Disease Incidence	% DI
Potato Dextrose Agar	PDA
Randomized Complete Block Design	RCBD
Sher-e-Bangla Agricultural University	SAU
Sodium Hypochlorite	NaClO

(Cont'd)

That Is	i.e.
Ton	T
Wettable Powder	WP
Water Dispersible Granule	WDG

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The Author

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ABSTRACT

Mungbean is a highly nutritive and protein rich crop. Collar rot caused by *Sclerotium rolfsii* is the major disease of mungbean causes a considerable yield loss in Bangladesh. Frequent use of chemical fungicides to control the disease is causing serious environmental hazards. The *in vitro* experiment was conducted at Plant Pathology Laboratory located in Wazed Miah Central Laboratory and field experiment was conducted in Central farm of Sher-e-Bangla Agricultural University during January, 19−January, 2020. The experiment was conducted to isolate, identify and to develop eco-friendly management of *Sclerotium rolfsii*. The isolated organism causing collar rot of mungbean identified as *Sclerotium rolfsii* based on its key characteristics (CMI Description No. 410). For management of the disease, a bioagent (*Trichoderma harzianum*), two botanicals (allamanda and neem leaf extract), three soil amendments (mustard oil cake, Krishibid organic fertilizer, virmicompost), two fungicides (Autostin 50WDG and Goldton 50WP) were evaluated in laboratory as well as in field and integration of those components was done in field. Seed treatment with *Trichoderma harzianum* and soil drenching with allamanda leaf extract was found most effective in reducing 91.31% disease incidence and increasing 183.01% yield over control. Soil drenching with *Trichoderma harzianum* suspension and seed treatment with Autostin 50 WDG reduced disease incidence 91.05% and increased yield 169.81% over control. *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost, *Trichoderma harzianum* suspension + Goldton 50 WP, *Trichoderma harzianum* suspension, Krishibid organic fertilizer, vermicompst, mustard oil cake was found effective and increased 156.60%, 118.87%, 116.87%, 113.01%, 88.67%, 88.6%, 37.7% yield over control, respectively. The highest BCR value was recorded in combined application of *Trichoderma harzianum* and allamanda leaf extract.

CHAPTER I

INTRODUCTION

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INTRODUCTION

Pulse crops (mungbean, lentil, pea, chick pea etc.) considered as the most versatile and diverse crops in the world. These are one nutrient and protein dense food crops having many beneficial effects on human health (Mudryj, 2019). Pulses are considered as staple source of protein in several countries of Asia and Africa as the people of those countries meet up their protein demand by consuming pulses instead of meat or fish (Asaduzzaman *et al.,* 2008).

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the important members of pulse crops, belongs to the botanical family Fabaceae. The protein percentage of mungbean grain is between 19.5-28.5%. It is also the richest sources of iron (5.9-7.6 mg 100 g^{-1} grain), vitamins and minerals. Carotinoids, polyphenols and fibers present in mungbean are also highly beneficial for human health (Dahiya *et al*., 2015).

Mungbean is a short duration crop. Globally, 7.3-million-hectare lands are under mungbean cultivation with 721 kg ha^{-1} average yield (FAO, 2017). Asia region is the main area of mungbean cultivation. Only the Indian sub-continent contributes 60% of total global production. India, Myanmar, China, Indonesia, Bangladesh, Thailand, Kenya and Tanzania are designated as larger producer countries of mungbean (Nair and Schreinemachers, 2020).

Mungbean is a day neutral crop and is grown in all regions of Bangladesh in all cropping seasons. In 2016-2017, the total production mungbean was 34783 MT covering 102311 acres of land but in 2017-2018, it covered 92655 acres with 34102 metric ton yield (BBS, 2017-2018). These data are the indication of gradual decreasing of mungbean cultivation as well as production in Bangladesh. There are so many reasons behind the deterioration of mungbean production in Bangladesh. Both the biotic and abiotic constraints influence mungbean production. Biotic stresses caused by viral, bacterial, and fungal

pathogens result massive declination of mungbean yield and cause huge economic loss. Among all biotic stresses, fungal disease only can reduce 40-60 % yield (Kaur *et al*., 2011).

Major fungal diseases of mungbean reported worldwide are powdery mildew, anthracnose, Cercospora leaf spot, Fusarium wilt, Rhioctonia root rot, Collar rot, Macrophomina charcoal rot, dry root rot or blight etc. (Panday, *et al*., 2018). In Bangladesh, foot and collar rot, Seed and seedling rot, Mungbean yellow mosaic virus, Cercospora leaf spot, Sclerotinia white mould, and puffy pod disorder are the most commonly occurred diseases of mungbean (Azad *et al*., 2017). Among all, collar rot caused by *Sclerotium rolfsii* is one of the most noxious disease of mungbean that can cause up to 80% yield loss (Ayub *et al*., 1989).

Collar rot caused by *Sclerotium rolfsii* occurs at very early stage at the collar zone of plant and collapses root function. In consequence, plant cannot uptake water and nutrients, gradually and eventually died at seedling stage. The first signs of infection are dark brown lesions on the stem or just beneath the soil level or collar zone. Yellowing and wilting of leaves are the visible symptom of the disease. Gradually the fungus produces abundant white, fluffy mycelium on infected tissues and the soil. In adverse environment the mycelium aggregated and form sclerotia which can survive in soil for year after year without host (Agrios, 2005).

Sclerotium rolfsii is a soil inhabiting fungi with a wide host range. It can attack near about 500 plant species (agronomic and horticultural) having economic importance and so, has been referred as an omnivorous pathogen (Nair *et al*., 2019). This fungal pathogen can survive in the soil for many years in the absence of a host plant by forming resting structures viz. microsclerotia, sclerotia, chlamydospore or oospores. Consequently, these pathogens are practically difficult to detect, diagnose and control (Aycock, 1966).

The above facts have made the fungi, one of the devastating pant pathogens in the world.

Chemical fungicides are always the major tools for management of plant diseases. However, chemicals have harmful residual effects on man, animal and environment. Health hazards such as skin problem, inhalation problem, irritation of eyes and digestion problem are observed in human due to adverse effect of chemicals. Phytotoxicity can also be occurred in case of many other plants (Faruq, 2018)

Indiscriminate and frequent use of chemicals induce resistance of pathogen to the particular chemical. Excessive use of fungicide also makes our environment hazardous and cause depletion of atmospheric ozone layer. These problems generated in agricultural productions made it clear that dependence on chemical fungicides for disease management should be avoided. Different approaches have been tested in the past to overcome these problems. Due to environmental concerns, researchers have focused on finding alternatives to chemical pesticides for suppression of plant pathogens. In this context, alternative approaches including use of organic soil amendment, botanicals, bioagents, chemicals in an ecofriendly approach draw considerable interest among scientists and agricultural producers (Larkin *et al*., 1998).

Integrated Disease management (IDM) is the best solution to control *Sclerotium rolfsii*. The soil borne diseases of crops incited by species of *Sclerotium* are difficult to be managed through single approach (Anahosur, 2001).

IDM involves the need-based use of chemical fungicides only when the disease incidence reaches Economic Threshold Level. IDM promotes the buildup of many eco-friendly (IDM) components in the crop ecosystem. In this experiment, eco-friendly management of collar rot of mungbean was explored by selecting IDM components to achieve following objectives.

Objectives:

1. To isolate and identify the causal organism of foot and collar rot of mungbean;
2. To evaluate the IDM components in *in vitro* against *Sclerotium rolfsii* causing foot and collar rot of mungbean; and
3. To integrate the selected IDM components for the management of foot and collar rot of mungbean.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mungbean (*Vigna radiate* L.) is prone to attack of many diseases at all stages of growth, especially at seedling stage. Foot and collar rot of mungbean is a destructive disease causes even 100% yield loss. Foot and collar rot of mungbean has been studied in Bangladesh to a limited extent. *Sclerotium rolfsii* Sacc. is a devastating soil borne pathogenic fungus with a wide host range of agricultural and horticultural crops and very much difficult to control. It is a facultative saprophyte and can maintain continuity of generation under adverse situation by the formation of sclerotia. The available literature about foot and collar rot of mungbean and its ecofriendly management approaches have been reviewed in this chapter.

2.1. Mungbean

Khan *et al.* (1982) reported that mungbean (*Vigna radiata* L.) is an important pulse crop belonging to the family Leguminosae. It is a rich source of vegetable protein. For the nutritional value, mungbean perhaps the best of all pulses.

Bashir and Malik (1988) reported that mungbean is affected by several diseases caused by fungi bacteria, viruses and nematodes. The main diseases of mungbean are yellow mosaic virus (YMV), leaf crinkle virus (LCV), cercospora leaf spot and bacterial blight & foot rot.

Tang *et al.* (2014) reported the nutritional value of mungbean. Mungbean contains about 20%–24% protein. Globulin and albumin are the main storage proteins found in mungbean seeds. Globulin make up over 60% and albumin make up over 25% of the total mung bean protein. The seeds of mung bean contain abundant nutrients. Flavonoids, phenolic acids, organic acids, amino acids, carbohydrates and lipids present in mungbean.

Moreover, mungbean shows antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antihypertensive, lipid metabolism accommodation and antitumor effects.

2.2. Pathogen and Symptomology of Collar Rot of Mungbean

Rolfs (1892) first discovered *Sclerotium rolfsii* Sacc.from Florida in U.S.A. the causal organism of tomato blight caused 70% loss. Itis polyphagous, ubiquitous, omnivorous and most destructive soil borne fungus. Saccardo (1911) named the fungus as *Sclerotium* sp.

Aycock (1966) found that *Sclerotium rolfsii* is soil borne pathogen commonly occurs in the tropics and sub-tropics regions of the world causing root and foot rot of many crops including mungbean.

Ahmed (1980) reported that the fungus *S. rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of sclerotia.

Fakir (1983) described that different phyto pathogenic soil borne as well as seed borne fungi are responsible for disease development of pulses, which attack plants during seedling to maturity stages and are more destructive at the seedling stage.

Ahmed (1985) found that foot rot (caused by *Sclerotium rolfsii*) is considered as an important and destructive disease of pulses in almost all legume-growing countries of the world.

Agrios (2005) described that the pathogens of sclerotial diseases cause damping-off of seedlings, stem canker, crown blight, root-rot, crown rot, bulb, tuber and fruit rots. Sclerotial diseases frequently affect a wide variety of plants, including most vegetables, flowers, legumes, cereals, forage plants and weeds.

Meah and Khan (2003) as well as Talukder (1974) described that, *Sclerotium rolfsii* frequently causes disease in different crops in Bangladesh. Typical symptoms are water soaked lesion near the soil with profuse mycelial growth.

Nagamma and Nagaraja (2015) stated that *Sclerotium rolfsii* is a soil borne plant pathogen causing root rot, stem rot, collar rot, and foot rot diseases on more than 500 plant species of agricultural and horticultural crops throughout the world. Most of the first symptoms associated with *Sclerotium rolfsii* are usually yellowing and wilting of leaves following collar rot infections.

Khalequzzaman (2016) reported that foot and root rot of lentil caused by *Sclerotium rolfsii* is a soil borne pathogenic fungi. The fungi can attack the crop during any time from seedling to flowering stage and are comparatively more destructive at the seedling stage.

Dwivedi and Prasad (2016) described that the hyphae of *Sclerotium rolfsii* grew upward on the surface of the infected plant covered with a cottony white mass of mycelium, scattered inside and outside of infected stem nearby the soil surface. The fungus produced numerous small rounds, white sclerotia of uniform size when immature and dark brown at mature stage.

Siddique *et al*. (2018) reported that *Sclerotium rolfsii* is a necrotrophic, soil borne fungal plant pathogen. That can be very destructive to numerous vegetables, pulse crops and fruit crops, especially in mungbean, lentil, eggplant, tomato, pepper, melon, and watermelon. Signs of infection include the development of coarse white strands of mycelium. Mycelium are grown on stem at the soil level in a fan-shaped pattern. Under dry conditions, at least a trace of white mycelium on the surface of the stem is evident of infection. In some cases, mycelium was found only underground. Mycelial coagulation forms sclerotia which is tan to brown in color and mustard seed like structure. Sclerotia can survive in soil from 6 to 10 years. Disease infection usually restricted to plant parts in contact with the soil. Early symptoms consist of water-soaked lesions on lower part of the stem. The disease was recognized by

the yellowing and wilting of foliage, followed by a complete collapse of the plant. Vascular bundles became brown in the infected stem.

2.3. Epidemiology of Collar Rot of Mungbean

Epps *et al.* (1951) observed no growth of fungus through sand from infected wheat seeds when the moisture content was 0.93 per cent or less, but good growth occurred at 1.02 per cent. They further reported that, *S. rolfsii* is capable of growing from inoculum through soil at a moisture level much below that is required for seed germination of rice and soybean.

Gondo (1962) reported that the optimum soil temperature was 30^0C for mycelia development and 25^0C for sclerotia development by *S. rolfsii*.

Sulladmath *et al*. (1977) studied variation in requirement of temperature by different isolates and found that all isolates grew well between 23^0 and 25^0C. The optimum temperature for groundnut isolate was 25^0C and 30^0C for tobacco and potato but 35^0C for rest of the isolates.

According to Punja *et al*. (1988), temperature is the principal limiting factor in the geographic distribution of *Sclerotium roifsii*. The disease rarely occurs where average daily minimum winter temperatures are below freezing (0^0C). Maximum disease occurs at 25-35^0C which is also optimum range for mycelia growth and sclerotia germination of the fungus.

Hari *et al*. (1988) reported that optimum temperature was 26^0C for growth of *Sclerotium rolfsii*. Further, they observed maximum growth of *Sclerotium rolfsii* at 30^0C.

Farr *et al*. (1989) found that, fungus *S. roifsii* attacks all plant parts in the contact with the soil under favorable environmental conditions including stems, roots, and fruits.

Mitchell *et al*. (1990) and Alexander and Stewart (1994) showed more rapid sclerotial degradation and reduced survival in soil with higher clay content and

relatively low pH (less than 6), and lower survival in clay loam than in sandy loam.

Hari *et al.* (1991) reported the radial growth of *Sclerotium rolfsii* causing collar rot of groundnut at pH range of 2.0 to 9.0 but the maximum growth was at pH 6.0.

Tu *et al.* (1991) reported that the percentage germination of sclerotia increased when the sclerotia were incubated under dry condition for 3 days at 20ºC and then remoistened and placed at 28ºC. They found that the sclerotia germinated best at 20-28ºC on soil plates.

Alexander and Stewart (1994) attributed lower survival in clay loam with greater water holding capacity, which affected drying and wetting of soil, resulting in greater microbial activity. Factors such as drying, wetting, and heating that increase activity of soil microorganisms near sclerotia and predispose sclerotia to antagonism may accelerate their mortality rate.

Kulkarni and Kulkarni (1998) found the maximum saprophytic activity of *Sclerotium rolfsii* at pH level of 6.0 followed by 5.5, 6.0 and 8.4.

Pinheiro (2010) described that, *Sclerotium rolfsii* develops at intermediate soil moisture level (70%) of field capacity and at temperature range between 25^0C to 30^0C. Sclerotium blight caused by *Sclerotium rolfsii* Sacc.in soybean reduces the crop yield but in certain situations, significant yield losses can also occur due to the monoculture or short rotation of soybean with another plant susceptible to the pathogen.

Kator *et al.* (2010) reported that *Sclerotium rolfsii* is spread by the wind, water, animal and soil.

2.4. Yield loss due to Collar rot of Mungbean

Ayub *et al.* (1989) reported that foot and collar rot of mungbean caused by *Sclerotium rolfsii* can cause 80% yield loss.

Richard *et al.* (2002) described that, pulses are very prone to attack of *Sclerotium rolfsii*. Due to this pathogen 50-53.4% yield loss occurred in all pulses.

2.5. Biological control of Collar rot of Mungbean

Chet (1975) studied that *Sclerotium rolfsii* form brownish sclerotia that survive in soil for long period tolerate biological and chemical degradation due to the presence of melanin pigment in the outer membrane of *Sclerotium.*

Lewis and Papavizas (1991) reported that *Tricoderma harzianum* inhibit *Sclerotium rolfsii* caused southern stem blight of soybean.

Haran *et al.* (1996) revealed that southern stem blight of soybean caused by *Sclerotium rolfsii* effectively controlled by *Trichoderma sp*

Biswas and Sen (2000) reported that *Tricoderma harzianum* inhibit stem-rot of groundnut caused by *Sclerotium rolfsii*.

Hermosa *et al.* (2000) revealed that *Sclerotium rolfsii* causing southern stem blight disease of soybean completely inhibited by *Trichoderma* species.

Anahosur (2001) has studied fungal antagonists viz., *Trichoderma harzianum, Trichoderma viride, Gliocladium virens, Penicillium sp., Paeocilomyces lilacinus, Bacillus subtilis, Pseudomonas fluorescens* and mycorrhizae; which inhibit root rot, foot rot, wilts, and damping-off diseases caused by *Sclerotium rolfsii, Macrophomina phaseolina, Rhizoctonia solani, Fusarium* sp. and *Pythium debarianum*.

Abd-Allah (2005) revealed that Bacillus subtilis control *Sclerotium rolfsii* by 92% under greenhouse condition in peanut.

Radawan *et al*. (2006) reported that *Trichoderma harzianum* and *Trichoderma hamatum* were most effective against *Sclerotium rolfsii* and inhibited the mycelial growth by 79%.

Karthikeyan *et al.* (2006) reported that *Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens* were inhibitory bioagents against the growth of *Sclerotium rolfsii* (Sacc.) in stem-rot of groundnut. *T. viride* inhibited the mycelial growth of the pathogen by 69.40%, while *P. fluorescens* inhibited 64.40%.

Verma *et al*. (2007) evaluated that *Trichoderma* species have also been used in commercial enzyme productions like cellulases, hemicellulases, proteases and β-1, 3-glucanase. Those components have direct effect on disease resistance of plant.

Eziashi *et al.* (2007) described that the combination of *Rhizobium* and *T. harzianum* were significantly effective against *S. rolfsii* which caused stem rot disease and promote the plant growth and increase seed production of groundnut.

Ghildiyal and Pandey (2008) studied that *Trichoderma harzianum* has antagonistic activity against *Sclerotium rolfsii* as it produces antibiotics substance such as Viridin, gliotoxin, glioviridin, dermin and trichodermin.

Hooda *et al.* (2008) described that *Trichoderma viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* have been reported as biocontrol agent against *Sclerotium rolfsii* responsible for causing southern blight disease in *Zea mays*.

Anand and Reddy (2009) evaluated that *Trichoderma* sp. has been reported to have antagonistic activity against *S. rolfsii* and *Fusarium cicer* under *in vivo* condition.

Bosah *et al.* (2010) revealed that *Trichoderma, Penicillium* and *Aspergillus* species significantly inhibited the growth of *S. rolfsii* under *in vitro* condition.

Ramanujam *et al.* (2010) described that *Trichoderma* has gained maximum attention as biocontrol agent due to the fact that it is effective against a large number of soil-borne plant pathogenic fungi without adversely affecting beneficial microbes and capable of promoting growth of certain crops. There are two major methods of inoculum production of *Trichoderma sp.* solid fermentation, the fungus is grown on various cereal grains, agricultural wastes and byproducts and liquid state fermentation, *Trichoderma* is grown in inexpensive media like molasses and yeast medium on a commercial scale. These products are used mainly for direct soil application in nurseries or main fields to suppress the soil-borne inoculum. Both formulations are effective against *Sclerotium rolfsii*.

Pastor *et al.* (2010) observed that *Pseudomonas* sp. showed more potent antagonistic activity against *Sclerotium rolfsii* in the rhizospheric soil of groundnut.

Bhuiyan *et al.* (2012) revealed that the arbuscular mycorrhizal fungus suppress the harmful effect of stem rot disease caused by *Sclerotium. Trichoderma harzianum* showed the highest inhibition (83.06%) of mycelial growth of *Sclerotium rolfsii*.

Rekha (2012) reported that *Trichoderma* sp. inhibited the mycelial growth and formation of sclerotial bodies of *S. rolfsii*.

Hannan *et al.* (2012) evaluated that the efficacy of BAU-biofungicide, cowdung and BINA-biofertilizer alone or in combination for controlling foot rot disease of lentil caused by *Sclerotium rolfsii*. BAU-biofungicide which is black gram bran-based *Trichoderma harzianum*. BAU-biofungicide as the seed treating agent resulted in higher plant stand. When seed treated with *Trichoderma harzianum* resulting 81.33% germination, and reduced 17% post

emergence death of plant over control. Mycelial colonization of *Trichoderma harzianum* in the root zone of the plant inhibit the post emergence death of plant.

Samsuzzaman *et al*. (2012) reported that *Trichoderma harzianum* reduced the mortality in tomato plants inoculated with *S. rolfsii* in soil and increase height and production of tomato, so biofungicide inhibit growth of *S. rolfsii* causes collar rot disease of tomato and no risk of environmental pollution than chemical control.

Podmaja *et al.* (2013) revealed that *Trichoderma* species have been reported as a biocontrol agent against *S. rolfsii*.
Patro and Madhuri (2013) reported that *T. harzianum* inhibits mycelial growth of *S. rolfsii* under *in vitro* condition which cause foot rot in finger millet.

Khalid (2013) isolated four bioagents viz, *Bacillus subtilis, Pseudomonas fluorescens*, yeast and *Trichoderma viride* which inhibited damping-off disease of bean caused by *S. rolfsii. Bacillus subtilis, T. viride,* and *P. fluorescens* control 88.7%, 83.7% and 86.3% respectively as bioagents against *S. rolfsii* and plants survived by 90.3%, 86.1% and 87.6% respectively.

Valle *et al.* (2013) described that *Trichoderma, asperellum* increased glucanase, chitinase and peroxidase activity in bulbs, roots and leaves of onion and it also inhibits the growth of *Sclerotium rolfsii.*

Mohiddin and khan (2013) selected *Trichoderma viride, T. harzianum, Pochoniachl amydosporia, Pseudomonas fluorescens* and *Bacillus subtilis*. Those have been reported as biocontrol agent against *Sclerotium rolfsii* responsible for causing southern blight disease in *Zea mays*.

Darvin *et al*. (2013) selected three species of Trichoderma (*T. viride, T. harzianum* and *T. longibrachiatum)* for inhibition of radial growth of *S. rolfsii*. *T. viride* and *T. harzianum* have highest radial growth inhibition and *T.*

longibrachiatum has lowest radial growth inhibition of *S. rolfsii* using dual culture technique under *in vitro* condition.

Mahato and Mondal (2014) reported that the plaster of Paris, Azotobacter, vermicompost, *Pseudomonas*, FYM and *Trichoderma viride* were highly effective against *S. rolfsii*.

Muthukumar and Venkatesh (2014) evaluated that *Trichoderma* and P*seudomonas* showed highest inhibitory activity (68.28% and 74.25%, respectively) against *Sclerotium rolfsii* under *in vitro* condition.

Sab *et al.* (2014) studied eight bio-agents tested against *S. rolfsii* in dual culture technique, all bio-agents inhibit growth of *S. rolfsii* causal agent of collar rot of chickpea, but *Trichoderma harzianum* recorded maximum growth inhibition of *S. rolfsii*, compared to *Pseudomonas fluorescens* and *Bacillus subtilis*.

Majdah *et al.* (2015) recorded that *Trichoderma harzianum* and *Trichoderma viride* act as biocontrol agent and inhibits 53.8% to 83.1% growth of *Sclerotium rolfsii* and *Rhizoctonia solani*.

Basumatary *et al*. (2015) recorded six fungal species viz. *Penicillium* sp., *Aspergillus niger*, *Curvularia* sp., *Trichoderma harzianum*, *Trichoderma viride* and *Fusarium* species retards the growth of *Sclerotium rolfsii* under *in vitro* condition in dual culture technique.

Swathi *et al*. (2015) reported that *T. harzianum* and *T. virens* were more active against *S. rolfsii* with 100% inhibition under *in vitro* condition.

Nagamma and Nagaraja (2015) described that biological management of the disease through antagonists is an eco-friendly approach apart from better alternative to the use of chemicals. Maximum inhibition of mycelial growth (71.67%) was noticed in *Trichoderma harzianum* which was followed by *Trichoderma viride* (63.33%). The results indicated that the application of these

micro-organisms successfully decreases the stem rot incidence and also increases the growth of the plants.

Aanuoluwa *et al*. (2015) studied that *Trichoderma viride* contain antagonistic activity at optimum temperature 37 ^{0}C and pH 6.0 against *Sclerotium rolfsii*.

Basumatary *et al.* (2015) have selected six fungal species viz. P*enicillium* sp, *Curvularia* sp, *Aspergillus niger, Trichoderma harzianum, Trichoderma viride* and *Fusarium* spp as bio-agents against *Sclerotium rolfsii*. The maximum percentage of growth inhibition of *Sclerotium rolfsii* was observed with *Trichoderma harzianum* (77.39%) and *Trichoderma viride* (76.54%), while *Penicillium* sp (29.05%), *Aspergillus niger* (30.48%), *Curvularia* sp (13.57%) and *Fusarium* sp. inhibited the growth by 3.02% under *in vitro* condition. *Trichoderma* species produced β-xylosidase, α-glycosidase, β-glucosidase, cellobiohydrolase, trypsin, chymotrypsin and chymoelastase-like proteases and Nacetyl β-glucosaminidase which are responsible for the biocontrol activity of *Sclerotium rolfsii*.

Elshahawy *et al.* (2016) reported that ten *Trichoderma* species and seven fungicides were effective against *Fusarium solani, F. oxysporum, Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotium species*.

Singh *et al.* (2016) studied that *Trichoderma harzianum* and *T. atroviride* inhibited the growth of *S. rolfsii* but the mutant parent were more efficient compared to parent bio-agent against collar rot disease of chickpea caused by *S. rolfsii*. Mutant strain *T. harzianum* and *T. atroviride* successfully inhibited the excessive growth of *S. rolfsii* by 82.9%.

Ramzan *et al*. (2016) studied 15 bio-agents against *S. rolfsii* responsible for root rot of mungbean in which *Trichoderma harzianum, Bacillus cereus, B. subtilis, T. virens, Pseudomonas fluorescens* and *Micrococcus varians* were effective bio-agents but Bacillus subtilis and Trichoderma harzianum were more effective.

Khalequzzaman (2016) evaluated that *Trichoderma harzianum* and *Trichoderma viridie* have strong biofungicidal effect on *Sclerotium rolfsii* caused foot and root rot of lentil. Disease reduction rate over control is 35% and 21%, respectively.

2.6. Botanicals used in controlling Collar rot of mungbean

Organic amendment of soil with neem/castor oil cake can influence the pathogenicity of *Sclerotium rolfsii*. (Gautam and Kotle, 1979)

Islam (2005) mentioned antifungul activities of garlic, neem, allamanda against plant pathogens. Soil borne pathogens can be controlled by these botanical extracts.

Masuduzzaman *et al.* (2008) conducted an experiment to determine their inhibitory efficiency of Allamanda leaf extract against *Sclerotium rolfsii*. Higher concentration (1:1, 1:2) completely inhibited *Sclerotium rolfsii* whereas lower concentration (1:3, 1:4) arrested its growth to some extent.
Islam and Faruq (2012) studied garlic clove, alamonda leaf, ginger rhizome, neem leaf, kalijira seed, turmeric rhizome, bel leaf, katamehedi leaf and onion bulb were effective against damping-off of tomato but neem leaf extract was most efficient than other extract.

Amin *et al.* (2013) selected different plants, rhizome ginger, neem leaf, tobacco leaf, rhizome of turmeric, and cow's urine. All plant extracts inhibited the growth of *Sclerotium rolfsii* at only higher concentration, while rhizome of turmeric inhibited the growth at a low level.

Parvin *et al.* (2016) conducted an experiment to determine the effect of botanicals on radial mycelial growth of *S. rolfsii in-vitro*. Garlic extracts showed profound and significant effect on reduction of radial mycelial growth of the fungus. The performance of Garlic in reduction of radial mycelial growth was the best followed by Onion, Ginger, Neem, Allamanda.

Butt *et al.* (2016) reported that two important indigenous plants like *Alstonia scholaris* and *Azadirachta indica* leaf extract were more effective against *S. rolfsii* under *in vitro* condition at different concentrations (1%, 2%, 3%, 4% and 5%).

Yasmin (2016) conducted an experiment to investigate the effectiveness of three botanical extracts namely garlic, ginger and neem at different concentrations to reduce the mycelial growth of *Bipolaris sorokiniana, Fusarium oxysporum* and *Sclerotium rolfsii*. The different botanical extracts inhibited the mycelial growth of fungi significantly ($p<0.01$).

Siddique *et al.* (2018) evaluated the efficacy of plant extracts against foot and root rot disease of eggplant caused by *Sclerotium rolfsii*. Plant extracts were sprayed at the base of each plant and adjacent soil. The effect of neem extract on reduction of disease incidence and disease severity reduction over control on eggplant was recorded 60% and 58.64%. Similarly, the effect of alamanda extract on disease incidence reduction and disease severity reduction over control on eggplant was recorded 60% and 44.39%.

2.7. Chemical control of Collar rot of Mungbean

Yaqub and Shahzad (2006) reported that six fungicides viz. benomyl, sancozeb, thiovit, dithane M-45, carbendazim, and topsin-M were effective against *Sclerotium rolfsii*. No fungicide inhibited the growth of *Sclerotium rolfsii* at low concentration while high concentration of dithane M-45 and mancozeb significantly reduced the growth of *Sclerotium rolfsii.*

Toorray *et al*. (2007) evaluated seven fungicides at different concentration against *S. rolfsii* under in-vitro condition. Complete inhibition of growth of *S. rolfsii* was recorded under Captan, Thiram, Mancozeb, Hinosan (edifenphos) and Antracol where as Cholrothalonil showed partial inhibition at low concentration.

According to Arunasri *et al*. (2011) Crossandra, an important flower plant affected by collar rot disease by *Sclerotium rolfsii* was controlled by four fungicides, viz., captan 50% WP, thiophanate-methyl 70% WP, propiconazole 25% EC and thiram 75% SD at five different concentrations. The fungicide propiconazole, thiram and captan significantly reduced the mycelial growth of *S. rolfsii*.

Manu *et al*. (2012) reported that the fungicides hexaconazole, propiconazole, difenoconazole; combi product, avatar (hexaconazole 4% + zineb 68%), nativo (tebuconazole 50%+ trifloxystrobin25%) and vitavax powder (thiram 37.5%+carboxin 37.5%) and bioagent *Trichoderma harzianum* inhibits *Sclerotium rolfsii* under field condition.

Tariq *et al*. (2012) studied that captan, carbendazim, metalaxyl and carboxin were efficient against wilt pathogen under *in vitro* condition.

Khan and Javaid (2015) reported that four fungicides tegula (tebuconazole), thiophanate methyl, ridomil gold (metalaxyl+mancozeb) and mancozeb significantly inhibits the radial growth of *S. rolfsii* under in vitro condition. Besides it, two fungicides thiophanate methyl and mancozeb substantially control the growth of *S. rolfsii* under *in vivo* condition responsible for causing collar rot disease in Chickpea

Khalequzzaman (2016) evaluated the effect of chemical, botanicals, biocontrol agents and healthy-looking seeds against foot and root rot of lentil caused by *Sclerotium rolfsii*. Provax 200 showed the best result in disease control. Provax 200FF is a world leading fungicide for seed treatment which is combination of Carboxin and Thiram.

Puri (2016) described that Soil fumigant such as methyl bromide, methane sodium and chloropicrin inhibit the growth of mycelium of *S. rolfsii*.

Parvin *et al*. (2016) conducted an experiment to find out the effect of fungicides on radial mycelial growth of *S. rolfsii in-vitro*. All tested fungicides

significantly reduced radial mycelial growth of the fungus. Bavistin was found promising in reducing the growth of the fungus in the laboratory followed by Ridomil Gold.

2.8. Soil amendments for controlling Collar rot of Mungbean

Punja *et al*. (2002) evaluated the disease suppression potential of three composts (greenhouse waste, windrow dairy solids, and vermicompost dairy solids) and commercially available biological control agents (BCA) to reduce foot and root rot disease caused by some soil borne fungi. They reported that all three composts reduced root and stem rots to some degree, and autoclaved compost lost its suppression effect suggesting the microbial antagonism.

Shlevin *et al*. (2003) reported that *Fusarium oxysporum* and *S. rolfsii* during solarization in an attempt to develop a dynamic model for expressing the thermal inactivation of the pathogens. After 20 days of exposure, the population of *F. oxysporum*, and *S. rolfsii* were reduced by 69 to 95% and by 47.5 to 100%, respectively.

Ersahin *et al*. (2009) reported that disease suppressiveness of vermicompost produced from agricultural wastes consisting of cattle manure, tree bark (Salix spp.), potato culls, and apples was assayed on damping-off of cucumber seedlings infected by *Rhizoctonia solani*. Suppression effect was assessed at the rates of 0, 10, 20 and 30% (v/v) vermicompost either blended with *Trichoderma harzianum*, amended with potting mixtures consisting of sand and garden soil (1:1, v/v). Vermicomposts not blended with *T. harzianum* effectively controlled damping-off of cucumber by *R. solani* (AG-4) at the rate of 20% and 30%. Vermicompost not blended with *T. harzianum* improved plant growth as well as that blended with *T. harzianum*.

Ahamed *et al*. (2012) conducted an experiment to evaluate the effect of manuring on root rot disease caused by *Fusarium solani* and agronomic characters of groundnut. Poultry manure, farmyard manure, cattle manure,

Brassica campestris straw and *Cicer ariantum* straw used as soil amendments. In the *Fusarium* inoculated control, disease incidence and plant mortality were 85 and 22.2%, respectively whereas, disease incidence and plant mortality were both 0% in non-inoculated control. All the manuring treatments managed the disease to variable extent and influenced agronomic characters of groundnut. Poultry manure was the most effective in disease management followed by cattle manure.

Khalequzzaman (2015) reported that *Rhizobium* biofertilizer was more effective to control foot and root-rot of Chickpea.

2.9. Integrated approach for controlling Collar rot of mungbean

Madhavi *et al.* (2011) conducted an experiment on dry root rot disease caused by *Sclerotium rolfsii* (Sacc.) of Chillies under rainfed conditions. Integration of different treatments including seedling dip with carbendazim+mancozeb, addition of vermicompost, drenching with fungicide and application of *T. harzianum* (7%) were found to be effective in management of disease in comparison with individual treatments.

Appana *et al.* (2011) reported the efficacy of integrated application of biocontrol agent, chemical fungicide and organic amendments against S. rolfsii and Bradyrhizobium sp. The experiment revealed that integration of *Pseudomonas florescens* FPD-15, *Trichoderma harzianum*, Neem cake, Captan gave better result than the single application of those treatments.

Kumari *et al*. (2012) reported that the integrated management of the disease with biocontrol agents, fungicides, herbal oils, plant extracts and organic manure as well as their combinations was found effective as an environment friendly approach (2012).

Hannan *et al*. (2012) found that, application of cowdung in the soil as an organic source of nutrient resulted in higher shoot and root lengths, and dry shoot and root weights of lentil, significantly increased the number of nodules

per plant and nodules weight of lentil. Soil amendment with cowdung increased the biomass production of lentil up to 75.56% over the control. BAU-biofungicide as black gram bran-based *Trichoderma harzianum* formulation was compatible with cowdung and BINA-biofertilizer which was (peat soil-based *Rhizobium leguminosarum).* Black gram bran-based *Trichoderma harzianum* has single as well as combined effects in controlling the pathogenic fungi *Sclerotium rolfsii*. BAU-biofungicide resulted in highest biomass production of lentil by 75.56% in comparison with the control and also beneficial for nodule formation.

Khalequzzaman (2016) found that Provax 200 treated plot shows the best result in yield contributing characters. Number of pods/plant, number of seeds/plant, weight of seeds/plant and yield of lentil under different treatments varied significantly from one to another. The second-best result shows *Trichoderma harzianun*.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This section includes the detail of the materials that have been used to conduct the experiment and the procedures that have been followed to carry out the experiment concerned.

3.1 Experimental Site

***In vitro* or lab experiment**: The *in vitro* experimental works viz. Isolation, identification, culturing of organism and bioassay was conducted in Plant Pathology Laboratory, Wazed Miah Central Laboratory, Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka- 1207, Bangladesh.

***In vivo* or field experiment:** The evaluation of eco-friendly components in field condition was performed in the central farm of Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka- 1207, Bangladesh.

3.2 Experimental Period

Both lab and field experiments were conducted during January, 2019 to January, 2020.

3.3. Design of the experiment

The lab experiments were laid out in Complete Randomized Design (CRD) and the field experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

3.4. Sample Collection

Mungbean seedlings showing typical symptoms like water soaked lesion in collar region were collected from the agronomy field of Sher-e-Bangla Agricultural University (SAU), Dhaka- 1207. Water soaked, dark lesion at the collar symptom showing plant with adjacent soil were collected and were kept

in polyethylene bag immediately after collection to reduce water loss. After collecting the samples in the laboratory were preserved in refrigerator at 4 °C for further use.

3.5. Sterilization of equipment

Oven was used for sterilization of glass wares viz. petri dishes, conical flask, glass tube, glass rod etc. 0.1 % sodium hypochlorite (NaOCl), 70% ethanol were used for surface sterilization of plant materials such as leaf, stem, seed etc. Hexisol (alcohol based sanitizer) was used for hand sanitization. Rectified spirit was used in lamp for burning other equipment like inoculation-needle tip, forceps. Formaldehyde was used for sterilization of inoculation chamber by fumigation.

3.6. Lab experiment

3.6.1. Identification and Isolation of Causal Organism

To identify and isolate causal organism of collar rot of mungbean moist blotter method and tissue planting method were used.

3.6.1.1. Moist blotter method

The pathogen associated with the diseased plant parts collar of mungbean were cut into several pieces by scissors and placed on the wet moist filter paper (Whatman no.1). Three pieces of filter paper were dipped in sterile water and were placed in each of the petridishes. The petridishes were incubated at 23±1 °C in alternating cycles of light and darkness in the incubation chamber of the Plant Pathology Laboratory of Sher-e-Bangla Agricultural University (SAU) for 7-10 days. After completion of incubation, the plates were examined under stereomicroscope for primary identification of the organism.

3.6.1.2 Tissue Planting Method

3.6.1.2.1 Preparation of potato dextrose agar (PDA) media

Standard potato dextrose agar medium is needed for tissue planting method. Ingredients used for preparing 1 liter of PDA media (Ricker and Ricker,1936) is presented in table 1.

Table 1. Composition of PDA media

Ingredients	Quantity
Peeled potato	200 g
Dextrose powder	20 g
Agar powder	20 g
Distilled water	1 L

Firstly, diseased free potato was collected and rinsed under running water. Cleaned potatoes were peeled and cut into pieces to boil. Potato pieces were taken in a clean vessel and added water and boiled using hot plate. After boiling 30 minutes potato extract was taken in a conical flask. Other ingredients were added to the potato extract in the conical flask and shake gently. The conical flask was sealed with cotton plug and then wrapped with aluminum foil paper. After that, the conical flask was placed in autoclave to sterilized for 30 minutes at 121^0 C temperature under 15 PSI pressure. Lactic acid was added to the media to make it acidic for culturing fungi. Then 15-20 ml the media transferred in to 9 cm petri dishes for culturing organism.

3.6.1.2.3. Tissue planting in PDA media

At first the diseased plant parts (stem) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into small pieces (5 mm) from advancing end of the lesions. The cut portion was surface sterilized with 0.1% NaOCl for 1 minute and rinsed with sterilized water for 3 times. Surface sterilized plant pieces were placed on PDA media in 9 cm petri dishes.

3.6.1.2.4. Incubation

The petri dishes were incubated at 22± 2 °C for 7-10 days. During incubation plant pieces were examined daily to investigate fungal growth. After incubation, the inoculated plates were observed with naked eye and under compound microscope. The organism was pure cultured following tip culture method.

3.6.1.2.5. Identification of causal organism

After incubation of several days the organism was identified based on the key characteristics of CMI description 410.

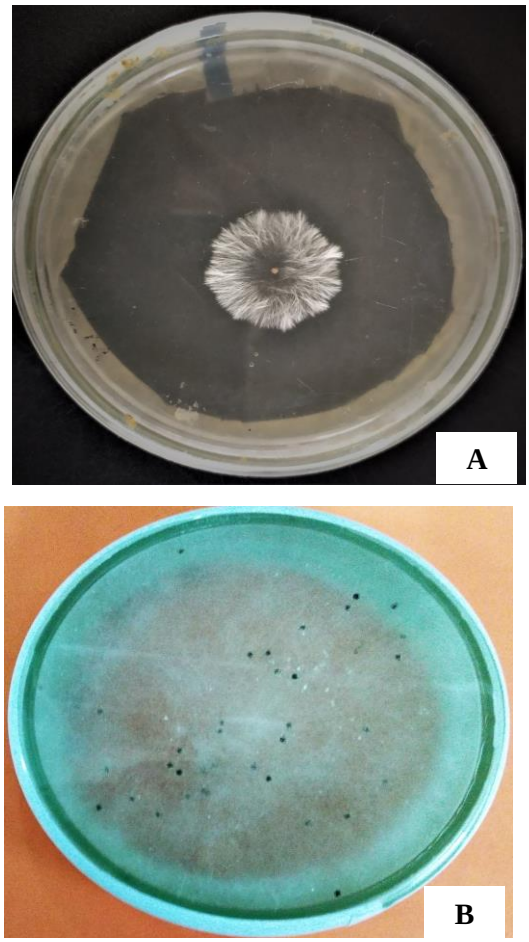


Plate 1. Identification of causal organism. (A) Culturing organism in PDA media (B) Pure culture of causal organism

3.7. Field Experiment

Field experiment was performed to evaluate the efficiency of eco-friendly management components in field condition.

3.7.1. Field preparation

Well drained loamy and sandy loam, slightly acidic soil was selected for cultivation of mungbean. Soil characteristics briefly described in appendix 1. Weeds, small bricks, stone, trashes were removed from the field. Two or three ploughing followed by cross ploughing and laddering was performed to make to soil loose and friable. Sevin 85 SP was used to remove ants and termite from soil. The land was levelled properly. Seed beds were prepared. Lines for seed showing was made. The treatments which were needed to apply during land preparation was added. Mustard oil cake, krishibid organic fertilizer and vermicompost were added in specific bed.

3.7.2. Layout

After preparing the land a suitable layout was made. The land was divided into three equal blocks. In every block 10 beds were prepared. In every bed three lines were made. Seeds were sown in lines maintaining plant to plant distance. Measurements are given bellow and appendix 2.

Length of the land = 20 m
Breadth of the land = 10m
Block = 3
Bed = 30
Length of the bed = 2.5 m
Breadth of the bed = 0.9 m
Line to line distance = 3 cm
Plant to plant distance = 5 cm

3.7.3. Treatments

Eco-friendly management components along with control were selected as treatment. Those are given bellow:

T_1 = *Trichoderma harzianum* suspension (Biotech Care *Trichoderma* suspension)

T_2 = *Trichoderma harzianum* suspension + Allamanda leaf extract

T_3 = *Trichoderma harzianum* suspension + Neem leaf extract

T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG

T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP

T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic fertilizer + vermicompost

T_7 = Mustard oil cake

T_8 = Krishibid organic fertilizer

T_9 = Vermicompost

T_{10} = Control

3.7.4. Preparation of treatments

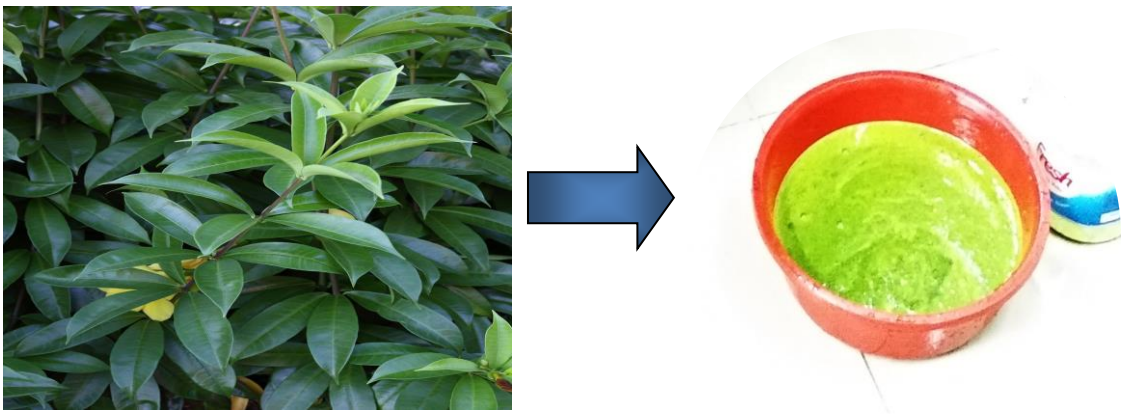
3.7.4.1. Preparation of botanical extract

Table 2. Botanical extracts used in the experiment

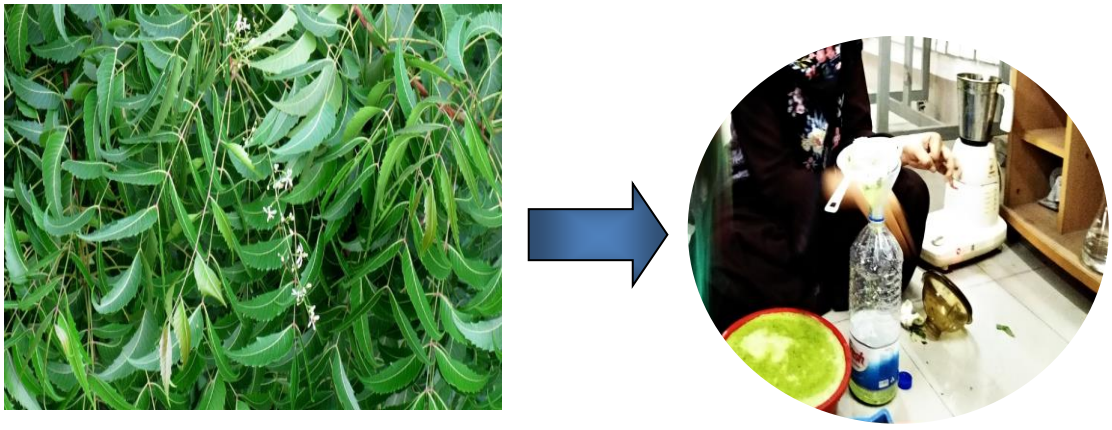
Common name	Scientific name	Plant part used	concentration
Allamanda	*Allamanda cathertica*	Leaf	1:2
Neem	*Azadiracta indica*	Leaf	1:2

For preparing botanical pesticides, fresh, green, mature neem and allamanda leaves were collected. Leaves were rinsed in running tap water. Neem and allamanda leaf paste were made separately by using blender. After that both pastes were sieved and separate the extracts. Distilled water was added to the

extract in proportion of 1:2 extract and water. These botanicals were used freshly for getting better result.



(A) Preparation of allamanda leaf extract



(B) Preparation of neem leaf extract

Plate 2. Preparation of botanical extract

3.7.4.2. Preparation of chemical fungicide solution

Table 3. Chemical fungicide used in the experiment

Trade name	Common name	Concentration
Autostin 50 WDG	Carbendazim	0.3%
Goldton 50 WP	Copper oxychloride	0.5%

2g and 5g of Autostin 50 WDG and Goldton 50 WP, respectively were added to 1L of distilled water. Applied immediately after preparation.

3.7.4.3. Preparation of bioagent

Bioagent *Trichoderma harzianum*

Formulation Suspension

Trade name Biotech care *Trichoderma* suspension

Biotech Care Trichoderma Suspension 3ml was added to 1 liter of water.

Plate 3. Chemical fungicides and bioagents used in the experiment

(A)Autostin 50 WDG

(B) Goldton 50 WP

(C) Biotech care *Trichoderma* suspension

3.7.4. Application of treatments

Trichoderma harzianum suspension was applied for soil drenching and seed treatment in $T_{1.}$ *Trichoderma harzianum* suspension was applied for soil drenching and allamanda leaf extract used for seed treatment in T_2. Soil drenching with *Trichoderma harzianum* suspension was also performed in $T_{3,}$ T_4, and T_5. Seed treatment with neem leaf extract, Autostin 50 WDG, Goldton 50 WP was done in T_3, T_4 and $T_{5,}$ respectively. Combination of mustard oil cake, krishibid organic fertilizer and vermicompost were applied to soil during land preparation and seeds were treated with *Trichoderma harzianum* suspension in $T_{6.}$ For soil amendment, mustard oil cake, krishibid organic fertilizer and vermicompost were applied in the soil during land preparation in case of T_7, T_8 and T_9, respectively.

A **B** **C** **D** **E**

Plate 4. Mungbean seed Treatment with (A) 0.3% Autostin 50 WDG (B) 50% Neem extract (C) 50% Alamanda extract (D) 0.3% Biotech Care Trichoderma suspention (E) 0.5% Goldton 50 WP

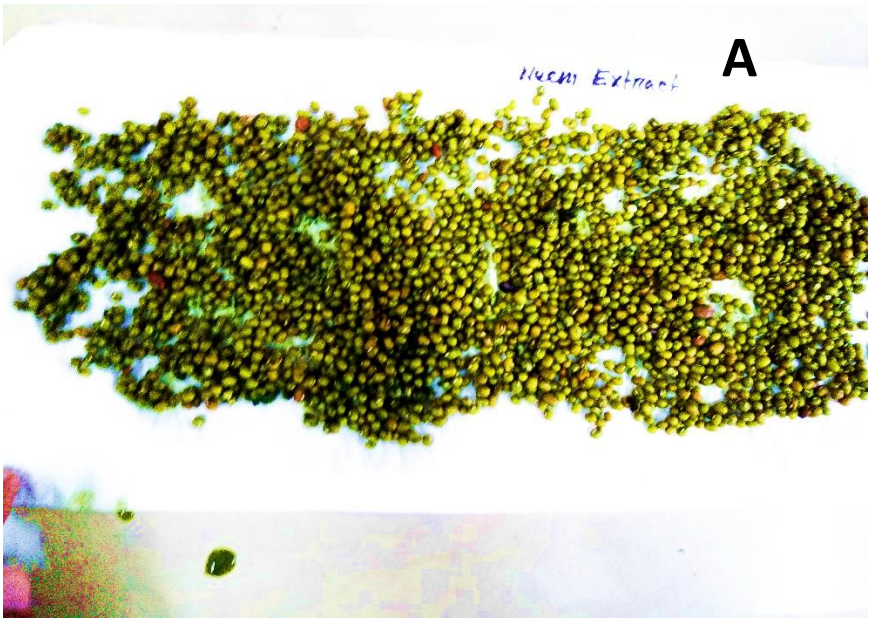


Plate 5. Application of treatment in experimental plot (A) + (B) Seed treatment and drying in air (C) Spraying after 10 days of seed sowing (D) Spraying after 17 days of seed sowing

3.7.5. Fertilization

Application of fertilizer and manure in the soil enhance nutrient supply to the plant which results very quick recovery from disease and disease resistance of plant. Mungbean is generally grown on the basic fertility of soil. Fertilizer dose according to Fertilizer recommendation Guide was applied to the soil is given bellow:

Manur and fertilizer	**Quantity (kg/ha)**
Cow dung	10ton
Urea	40
TSP	105
MoP	40.5
Boron	10
Zink	10
Gypsum	14

All fertilizers were added to the soil during land preparation.

3.7.6. Seed rate and sowing

Seed rate: 20 Kg/ha

Seeds were sown after 7 days of land preparation in 08 March, 2019. Seeds were selected randomly for every treatment. Seeds were placed in 5 cm deep furrow and 5 cm apart from seed to seed. Furrows were covered with loose soil following a slight irrigation.

3.7.7. Water management

5 irrigations were applied. First and light irrigation was applied after seed sowing. After 15 days after seed sowing second irrigation applied. Third irrigation was applied during pod formation stage 25 days after seed sowing. Other two was applied as per requirement. But no water was applied during full bloom stage. Stagnant water due to heavy rain was drained out.

3.7.8. Weeding

First weeding was done after 20 days of seed sowing before pod formation stage. After that 2 times weeding was done as needed.

3.7.9. Thinning and gap filling

Thinning and gap feeling was done with first weeding 20 days after seed sowing.

3.7.10. Netting and tagging

Tags were added to every treatment. Netting was done to protect the seeds from birds.

3.7.11. Harvesting, threshing and weighing

Pods were harvested when 80% of pods were matured. Pods were threshed by beating pods. Seeds were dried in sun to obtain 10-11% moisture and stored in air tight container for further use.

Figure 1. Netting and tagging in field

3.7.12. Data collection on

No. of Infected seedling,

no. of healthy seedling

plant height (cm)

no. of leaves/plant

no. of pod/plant

seeds/pod

Thousand seed weight (g)

yield (t/ha)

Percent disease incidence was calculated using the following formula (Islam, 2005):

1. (%) disease Incidence = $\frac{\text{No.of diseased plants}}{\text{No.of total plant}} \times 100$
2. % Inhibition over control = $\frac{x-y}{x} \times 100$

   x = Finding of control

   y = Finding of treatments
3. % Increased over control = $\frac{y-x}{x} \times 100$

   x = Finding of control

   y = Finding of treatments

3.7.12.1. Analysis of cost and production

Costing of application of treatments for management of collar rot of mungbean was done based on the current market price of inputs, rate of labour wage and agricultural equipment. Price of the gross return was determined on the basis of current market price of 2019 (Appendices 3, 4 and 5). Estimation of Cost-Benefit Ratio (BCR) was done according to (Islam et al., 2005) using the following formula:

Benefit Cost Ratio (BCR) = $\frac{\text{gross return in (tk/h)}}{\text{tota cost of production(tk/h)}}$

3.8. Bioassay in laboratory

Bioassay is the process to determine activity or potency of a substance (fungicide or bio agents) by testing its effect on the growth of the organism. There are many types of bioassay. To determine the efficacy of treatments on *Sclerotium rolfsii* by cup method was used.

3.8.1. Cup method

PDA media was poured in petri dishes. Three blocks at same distance were placed in the media by using block cutter. Treatments applied in same concentration as used in field experiment. 0.5% and 0.3% concentration of Goldton 50 WP and Autostin 50 WDG, 0.3% concentration of Biotech Care *Trichoderma* Suspension, neem and allamanda leaf extract were applied in the blocks of the media separately. 5 drops of solutions were added to each block of petri dishes and preserved in refrigerator at 4^0 C overnight. Newly sub cultured *Sclerotium rolfsii* was placed in the middle of the petri dishes by using block cutter. Then petri dishes kept in incubation chamber for 7 days at 25^0 C and checked the growth in every 6 hours. Inhibition of growth was observed clearly in comparison to control.

3.8.2. Measurement of radial growth

After 70 hours of incubation, radial growth (mm) of S. rolfsii in petri dishes was measured. The radial growth (mm) of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony and then plates were kept for 30 days for sclerotia formation.

Inhibition of radial growth was computed based on colony diameter on control plate using the following formula:

$$\% \text{ growth inhibition } = \frac{x-y}{x} \times 100$$

X= Average radial mycelium growth (cm) of S. rolfsii in control plate.

Y= Average radial growth (cm) of S. rolfsii in treated petri dishes.

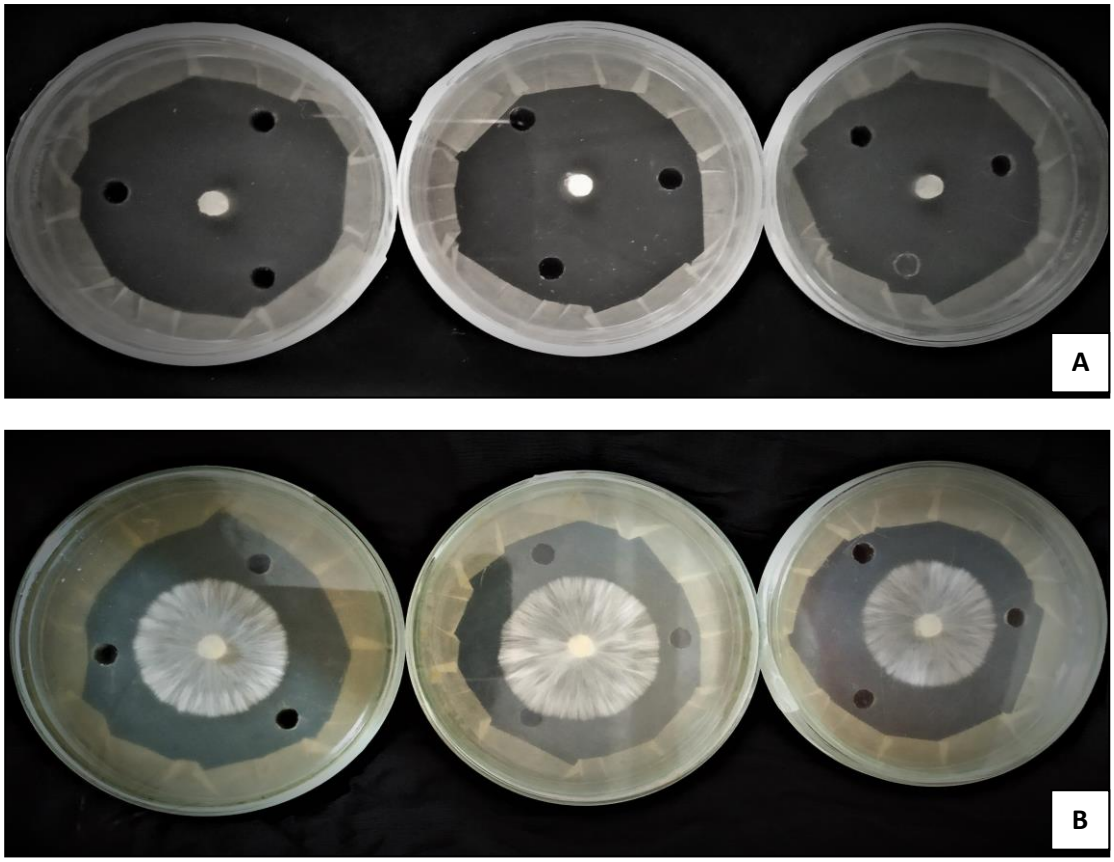


Plate 6. Preparation of bioassay (A) application of treatment and placement of *Sclerotium rolfsii* block in cup method (B) growth at 2 days after inoculation in control

3.9. Analysis of data

The relevant data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments by Statistics 10 software.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Isolation and identification of *Sclerotium rolfsii* causing collar rot of mungbean

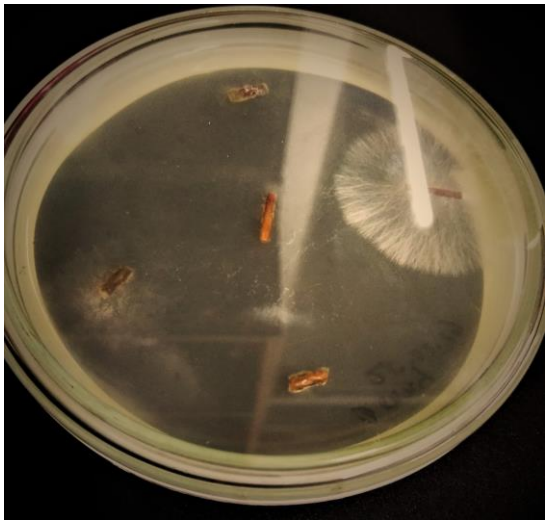
4.1.1. Isolation and identification

The fungus was isolated from infected collar zone of mungbean by tissue planting method, pure cultured and identified as *Sclerotium rolfsii* based on key characteristics (CMI description: 410).

Sclerotium rolfsii is a fast growing fungus. White, fluffy, cottony mycelium was found to grow from the first day of inoculation even at 30-35^0 C. Within 4 days, it covered full PDA plates (90mm). Microscopic examination of the fungal culture showed hyaline, thin walled, septate, profusely branched mycelium with clamp connections. Mycelial constriction was observed.

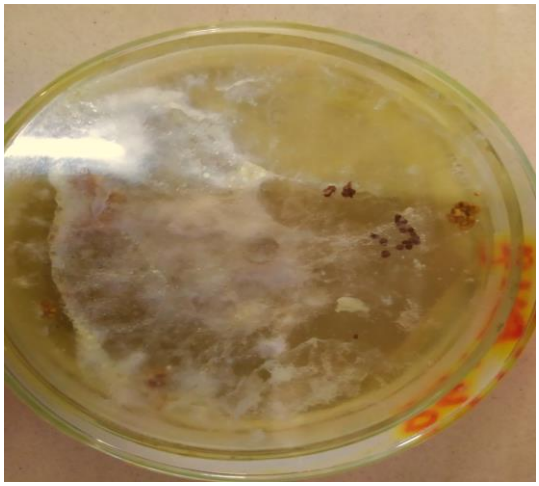
When the mycelial colony attained maturity (within 9 - 15 days of culture), small mycelial knots were found to form, which were turned to mustard seed like sclerotia. The colour of fully matured sclerotia varied as light brown, brown and dark brown. The shape of sclerotia was round to spherical. The number of sclerotia was varied from 20-80/ petri plate (90mm).

These findings of the present study are in agreement with the findings of Dwivedi and Prasad (2016) who described that the hyphae of *Sclerotium rolfsii* grew upward on the surface of the infected plant covered with a cottony white mass of mycelium, scattered inside and outside of infected stem nearby the soil surface. The fungus produced numerous small, round, white sclerotia of uniform size when immature and sclerotia turned dark brown at mature stage.

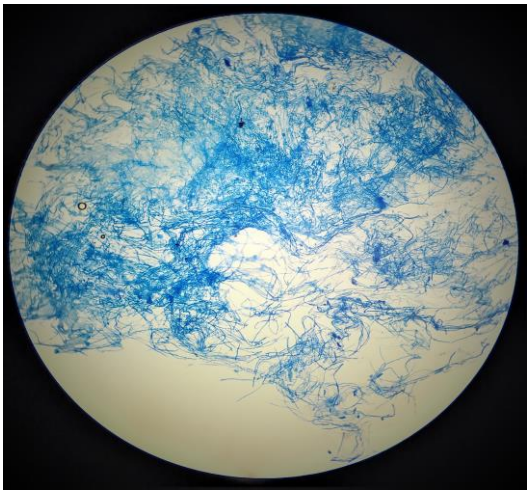


A. Isolation from diseased plant part

B. White fluffy massive mycelial growth



C. Formation of sclerotia



D. Microscopic view of *Sclerotium rolfsii*

Plate 7. Isolation and identification of causal organism of collar rot mungbean

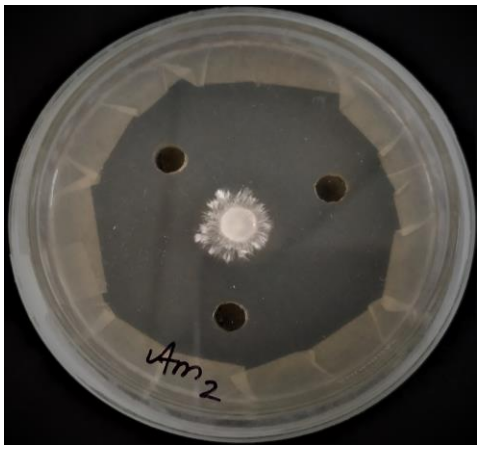
4.2. Bioassay of botanicals, fungicides and bioagent as IPM components against *Sclerotium rolfsii* causing collar rot of mungbean under *in vitro* condition

Bioassay of Autostin 50 WDG, Goldton 50 WP, Neem, Allamanda, *Trichoderma harzianum* in cup method was performed and the results are presented in table 4. Autostin 50 WDG showed the lowest mycelial growth followed by Goldton 50 WP. The highest mycelial growth was observed in control. Allamanda leaf extract showed comparatively better performance in curving the mycelial growth (7.33mm) than neem leaf extract (15.00mm). Lower mycelial growth (6.00) was recorded in case of *Trichoderma harzianum* suspension. The highest inhibition of mycelial growth (96.3%) was recorded in Autostin 50 WDG followed by Goldton 50 WP (95.18%), *Trichoderma harzianum* (93.33%), allamanda leaf extract (92.67%) and neem leaf extract (83.33%) over control.

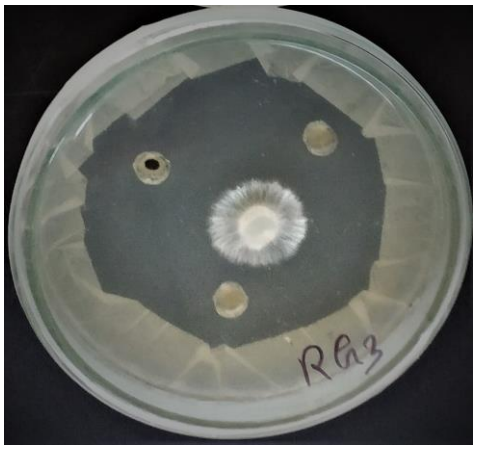
Yaqub and Shahzad (2006) reported that bioassay with carbendazim against *Sclerotium rolfsii* resulted 90- 100% inhibition of mycelial growth. Wavare *et al*. (2017) reported complete inhibition of mycelial weight *S. rolfsii* by carbendazim 0.1%. Siddique *et al*. (2016) also reported that Bavistin 50 WP and Topgan 50 WP remarkably reduced sclerotia production of *Sclerotium rofsii*.

Manu *et al.* (2012) reported that *Trichoderma harzianum* inhibited 81.11-100% mycelial growth of *Sclerotium rolfsii*. This result was in accordance with the results of Kulkarni (1998).

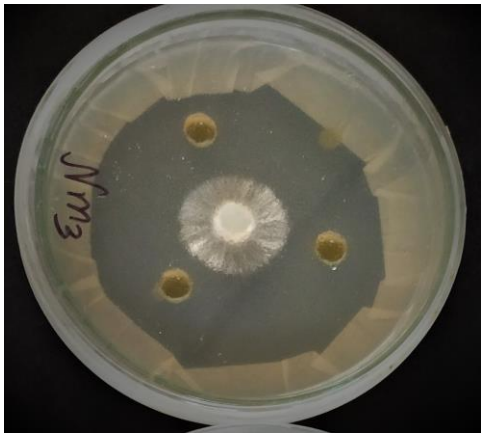
Masuduzzaman *et al.* (2008) reported that mycelial growth of *Sclerotium rolfsii* was completely controlled at high concentration (1:1, 1:2) of allamanda leaf extract.



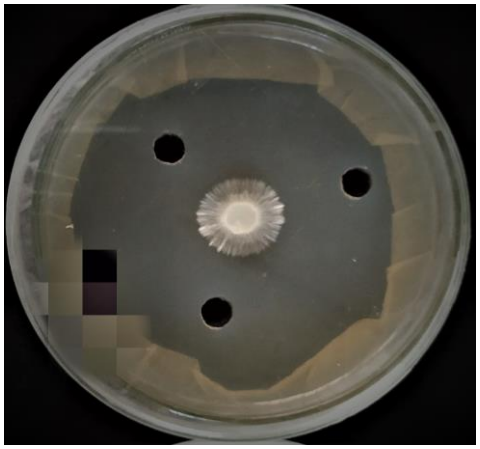
A. Autostin 50 WDG



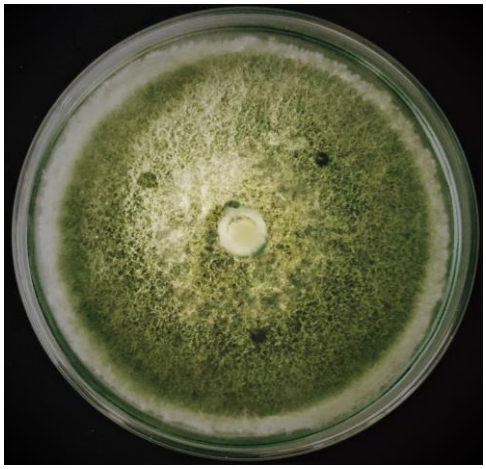
B. Goldton 50WP



D. Neem leaf extract



C. Allamanda leaf extract



D. *Trichoderma harzianum* suspension

E. Control plate

Plate 9. Bioassay of *Sclerotium rolfsii* by cup method

Table 4. Evaluation of different treatments on inhibition of mycelial growth (mm) of *Sclerotium rolfsii* in *in vitro* condition

Sl. No	Treatments	Mycelial growth (mm)	% inhibition of mycelial growth over control
1.	Autostin 50WDG	3.33 f	96.30
2.	Goldton 50 WP	4.33 e	95.18
3.	Neem leaf extract	15.00 b	83.33
4.	Allamanda leaf extract.	7.33 c	92.67
5.	*Trichoderma harzianum*	6.00 d	93.33
6.	Control	90.00 a	
7.	LSD (0.05)	1.25	
8.	% CV	3.39	

4.3. Evaluation of different treatments against collar rot of mungbean in field condition

4.3.1. Disease incidence of collar rot of mungbean

Evaluation of different treatments on disease incidence of collar rot of mungbean is presented in Table 5. Highest disease incidence (76.66%) observed in control. Among the selected IDM components, the lowest disease incidence (6.75%) was recorded in T_4 (*Trichoderma harzianum* + Autostin 50 WDG) where 91.05% inhibition was counted over control. This result is statistically similar to T_2 (*Trichoderma harziaum* suspension + Allamanda) and T_6 (*Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost). Disease incidence was recorded in case of T_2 and T_6 6.66% and 7.34%; and reduction of disease incidence were 91.31% and 90.116%, respectively over control.

T_1 and T_5 were statistically same and showed 11.33% and 11.35% disease incidence with 85.95% and 84.04% reduction of disease incidence over control. Disease incidence of T_3 and T_8 were 16.00% and 18.35% with reduction of disease incidence 79.12% and 76.06% over control, respectively. Treatment T_7 yielded 32.37% disease incidence with 57.77% reduction preceded by T_9 (20%, 73.91%).

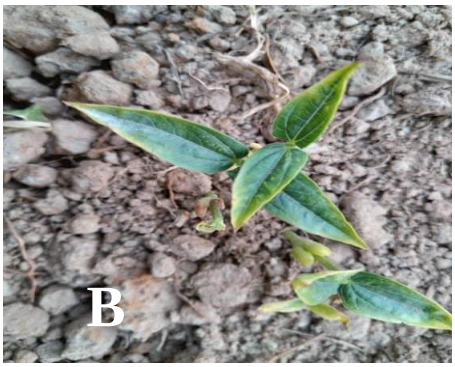


Plate 10. A view of field experiment

A. **Infected mungbean seedling at collar zone**
B. **Disease free healthy seedling from treated plot**
C. **Affected damping off seedling collected from control plot**
D. **Damping off of seedling from control plot**

Table 5. Evaluation of different treatments on reduction of Disease Incidence of collar rot of Mungbean

Sl. No	Treatments	Disease Incidence	% reduction of DI over control
1.	T_1 = *Trichoderma harzianum* suspension	11.33 e	85.95
2.	T_2 = *Trichoderma harziaum* suspension + Allamanda leaf extract	6.66 f	91.31
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	16.00 d	79.12
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	6.75 f	91.05
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	11.35 e	84.04
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic fertizer + vermicompost	7.34 f	90.116
7.	T_7 = Mustard oil cake	32.37 b	57.77
8.	T_8 = Krishibid organic fertilizer	18.35 d	76.06
9.	T_9 = Vermicompost	20.00 c	73.91
10.	T_{10} = Control	76.66 a	00.0
LSD (0.05)		3.88	
CV (%)		10.78	

4.3.2. Days to appear disease in field condition

Days required to appear damping off disease in field condition are presented in Table 6. Disease incidence appeared very early (3.33 Days After Sowing) in control. Disease appeared very late in T_2 (17.33 DAS). T_6 (14 DAS) and T_4 (13.66 DAS) were statistically similar to T_2. T_3 and T_1 required 10.34 DAS and 7.33 DAS. T_9 (6.00 DAS) required second lowest time (6.00 DAS) followed by T_7 (6.33 DAS) and T_5 (7.00 DAS) and their performance in delayed disease were statistically significant.

The findings of the present experiment are supported by findings of Rahman (2017) who reported that the durations required for appearance of visible symptoms of the disease in inoculated as well as treated pot soils were 95, 76, 86, 47, 91, 70, 44, 29, 87 and 10 DAI under treatments with Provax 200, Tilt-250 EC, Score 250 EC, Pencozeb 80 WP, Garlic clove extract, Allamanda leaf extract, Vermicompost, Poultry manure and T. harzianum, respectively to control foot and root rot of betel vine caused by *Sclerotium rolfsii.*

Table 6. Evaluation of different treatments against collar rot on days required to appear Disease in field condition

Sl. No	Treatments	Days to appear disease
1.	T_1 = *Trichoderma harzianum* suspension	7.33 d
2.	T_2 = *Trichoderma harzianum* suspension + Allamanda leaf extract	17.33 a
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	10.34 c
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	13.66 b
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	7.00 d
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost	14.00 b
7.	T_7 = Mustard oil cake	5.66de
8.	T_8 = Krishibid organic fertilizer	6.33 d
9.	T_9 = Vermicompost	6.00 d
10.	T_{10} = Control	3.33 e
LSD (0.05)		2.52
CV (%)		16.15

4.4. Effect of treatments against collar rot of mungbean on yieldcontributing characters and yield of mungbean

Yield contributing characters like plant height, number of leaves/plants, number of pods/plants, number of seeds/ pods, thousand seed weight were significantly influenced by the treatments with some extent.

4.4.1. Plant height (cm)

The highest plant height was recorded in T_2 (44.34 cm) which was significantly similar to T_4 (43.40), T_6 (43.00), T_1 (40.00) and T_5 (38.33). are statistically same and showed the best result. 15-27% increased of plant height over control was recorded for these five treatments. The lowest plant height was recorded in control (32.33) preceded by T_7 (33.00), T_9 (35.66), T_8 (36.33), T_3 (37.00). The treatments were showed 2.07% to 37.14% increase of plant height over control. (Table 7)

4.4.2. Number of leaves per plant

Maximum number of leaves was recorded in T_2 (19.33) followed by T_4 (18.33), T_6 (18.00), T_5 (16.00), T_1 (16.00) and T_8 (16.00) which was statistically significant and increased number of leaves per plant 141.62%, 129.12%, 125%, 100%, 100% and 100% over control, respectively. The lowest number of leaves found in control (8.00) preceded by T_7 (11.33) and T_9 (14.33) which increased number of leaf/plant 41.62% and 79.12% over control. (Table 7)

Table 7. Evaluation of different treatments against collar rot of mungbean on Plant Height (cm) and leaf per plant of Mungbean

Sl. no	Treatments	Plant Height		Leaf per plant	
		Plant height (cm)	% increase of plant height over control	Number of Leaf per plant	% increase of no. of leaf per plant over control
1.	T_1 = *Trichoderma harzianum* suspension	40.00 a-c	23.72	16.00 ab	100
2.	T_2 = *Trichoderma harzianum* suspension + Allamanda leaf extract	44.34 a	37.14	19.33 a	141.62
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	37.00 b-d	14.44	13.667bc	70.08
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	43.40 ab	34.24	19.33 a	129.12
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	38.33 a-d	18.55	16.00 ab	100
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost	43.00 ab	33.00	19.00 a	125
7.	T_7 = Mustard oil cake	33.00 d	2.07	11.33 cd	41.62
8.	T_8 = Krishibid organic fertilizer	36.33 cd	12.37	16.00 ab	100
9.	T_9 = Vermicompost	35.66 cd	10.30	14.33 bc	79.12
10.	T_{10} = Control	32.33 d		8.00 d	
LSD (0.05)		6.40		3.63	
CV (%)		9.74		14.03	

4.4.3. Pods per plant

The highest number of pods per plant was recorded in T_2 (18.00), T_4 (16.66), T_4 (16.00), T_1 (15.33), T_5 (15.00) and T_3 (14.66) these treatments are statistically identical. The Lowest number of pods per plant was recorded in control (6.66) preceded by T_7 (11.00), T_8 (13.00), T_9 (15.00). The increase of number of pods per plant ranges from 65.16% to 170.27% over control was observed for applying treatments. (Table 8)

4.4.4. Seeds per pod

The highest number of seeds per pod was recorded for T_2 (12.00) followed by T_4 (11.33), T_6 (11.00), T_1 (10.66), T_3 (10.00), T_5 (9.66) and T_8 (8.66) were statistically identical. The Lowest number of seeds per pod was recorded in control (3.00) preceded by T_7 (6.33) and T_9 (7.00). Treatments were showed 111%-300% increase of seeds per plant over control. (table 8)

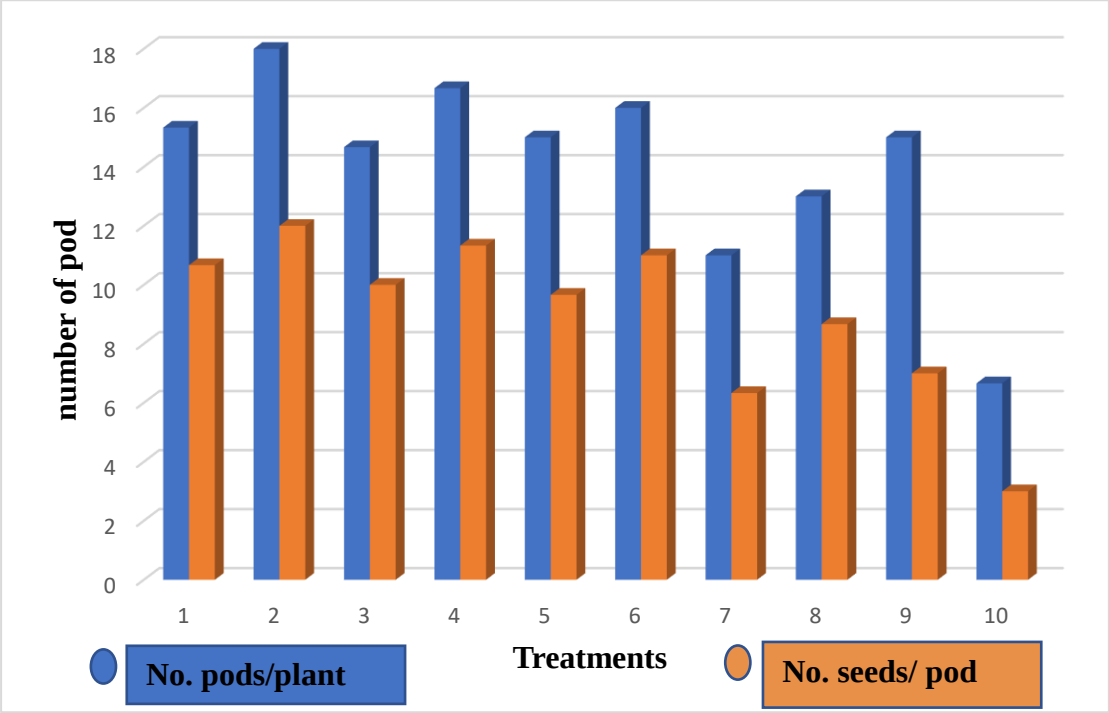


Figure 2. Graphical representation of no. of pods/plant and no. of seeds per pod against treatments

Table 8. Evaluation of different treatments against collar rot of mungbean on pod per plant and seed per pod

Sl. no	Treatments	Pod per plant		Seed per pod	
		Number of Pod per plant	% increase of number of pods per plant over control	Seed/pod	% increase of no. of seed per pod over control
1.	T_1 = *Trichoderma harzianum* suspension	15.33 ab	130.18	10.66 a	255.56
2.	T_2 = *Trichoderma harzianum* suspension + Allamanda leaf extract	18.00 a	170.27	12.00 a	300
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	14.66ab	120.12	10.00 a-c	233.33
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	16.66 ab	150.25	11.33 a	277.66
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	15.00 ab	125.22	9.66 a-c	222
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost	16.00 ab	140.24	11.00 a	266.66
7.	T_7 = Mustard oil cake	11.00 bc	65.16	6.33 cd	111
8.	T_8 = Krishibid organic fertilizer	13.00 ab	95.19	8.667 a-c	188.90
9.	T_9 = Vermicompost	15.00 ab	125.22	7.00 bc	133.33
10.	T_{10} = Control	6.66 c		3.00 d	
LSD (0.05)		5.5624		3.5356	
CV (%)		22.89		22.99	

4.4.5. Thousand Seed weight

Thousand seed weight was maximum in T_2 (341.67g) which was statistically significant. Statistically similar results showed by T_4 (336.67g) and T_6 (330.00g), T_1 (316.67g), T_3 (316.67g) and T_5 (300g). The lowest TSW was obtained from control (193.33). The second lowest weight counted T_7 (226.67g) followed by T_9 (266.33) and T_8 (276.67g). T_8 and T_9 are statistically similar. 17.24-76.72% increased TSW was recorded for these treatments over control. (Table 9)

4.4.6. Yield of mungbean

The treatment applied in the experiment showed profound effect on increase of seed yield of mungbean over control. Statistically the highest yield (1.5 t/h) was recorded in treatment T_2 followed by T_4 (1.46) and T_6 (1.36 t/ha) which were statistically similar. Among the treatment other than control, the lowest yield was recorded in case of treatment T_7 (0.73 t/h) preceded by T_8 (1.00 t/h), T_9 (1.00 t/h), T_3 (1.13 t/h), T_1 (1.16) and T_5 (1.15 t/ha). The effect of T_8 and T_9 as well as T_1 and T_5 statistically similar in seed yield. Based on performance of the treatments, it is revealed that 183.01% seed yield was increased by T_2 over control where *Trichoderma harzianum* suspension and allamanda leaf extract were applied combinedly. The second highest yield increased was counted in case of treatment T_4 (169.81%) where *Trichoderma harzianum* was combinedly applied with Autostin 50 WDG. All the treatment showed remarkable contribution in increasing seed yield over control. (Table 9)

Table 9. Evaluation of different treatments against collar rot of mungbean on Thousand seed weight and yield

Sl. no	Treatments	Thousand seed weight		Yield	
		Thousand seed weight (gm)	% increase of Thousand seed weight over control	Yield (t/ha)	% Increase of yield over control
1.	T_1 = *Trichoderma harzianum* suspension	316.67 a-c	63.76	1.16 b-d	118.87
2.	T_2 = *Trichoderma harzianum* suspension + Allamanda leaf extract	341.67 a	76.72	1.50 a	183.01
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	316.67 a-c	63.76	1.13 cd	113.20
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	336.67 a	74.14	1.46 ab	169.81
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	300.00 a-c	55.17	1.15 b-d	116.87
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost	330.00 ab	70.69	1.36 a-c	156.60
7.	T_7 = Mustard oil cake	226.67 de	17.24	0.73 e	37.7
8.	T_8 = Krishibid organic fertilizer	276.67 b-d	43.10	1.0 de	88.67
9.	T_9 = Vermicompost	266.33 cd	37.75	1.0 de	88.67
10.	T_{10} = Control	193.33 e		0.53 f	
LSD (0.05)		5.795		0.325	
CV (%)		11.51		17.12	

4.5. Relationship between disease incidence (%DI) and yield (t/h)

Correlation and regression analysis between % DI and yield (t/h) of mungbean revealed that seed yield of mungbean is negatively correlated with disease incidence. It means seed yield of mungbean was decreased with the increase of % disease incidence. The regression equation is y= -0.0196x + 1.4577 and value of R^2 is 0.9464.

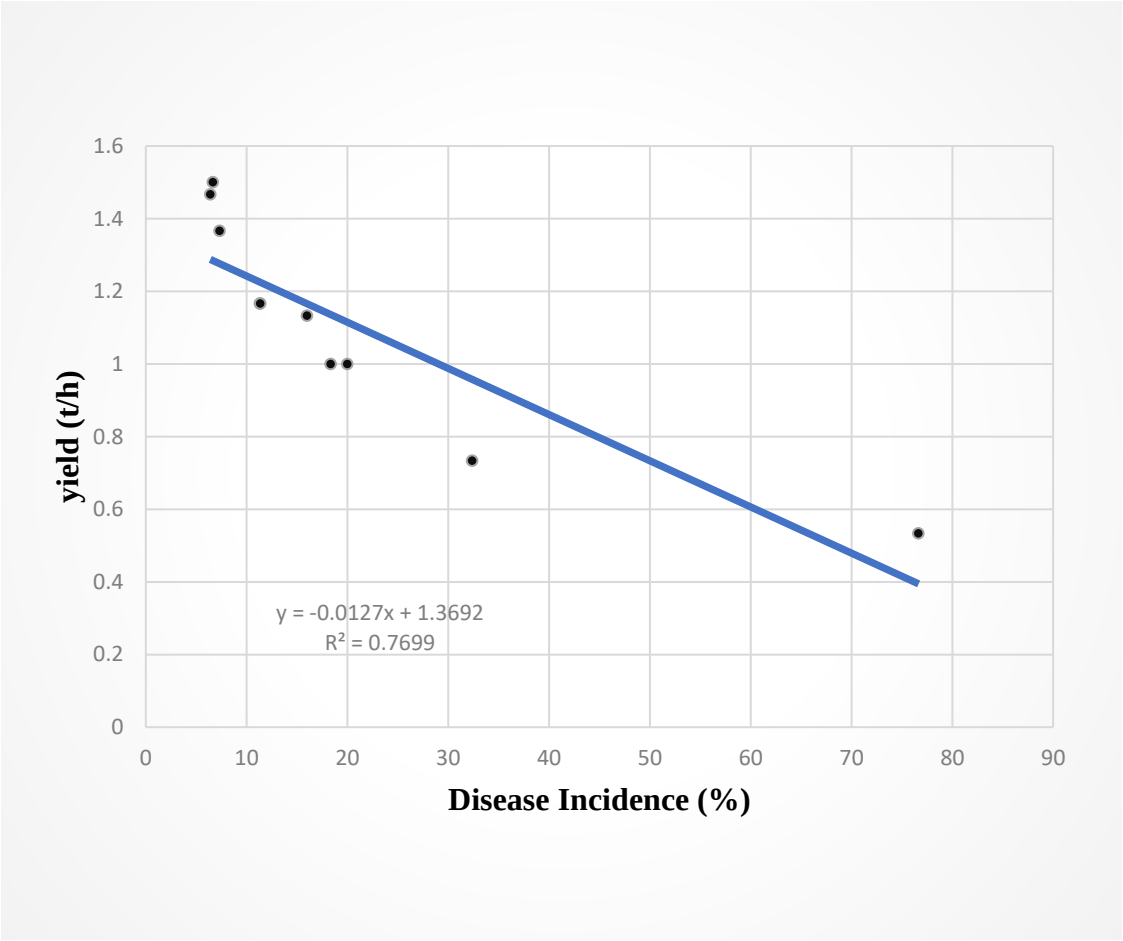


Figure 3. Relationship between Disease Incidence (%) and yield (t/ha) of mungbean as affected by collar rot disease

4.6. Cost analysis

The combined application of *Trichoderma harzianum* suspension and Allamanda leaf extract (T_2) was found very cost effective. Benefit cost ratio (BCR) was counted 1.94 which was the highest among the treatments. In case of seed yield T_6 and T_4 were statistically identical to T_2 but cost of production was higher. BCR of T_6 and T_4 was 1.64 and 1.86. the lowest BCR was found in T_7 (1.01) other than control preceded by T_8 (1.36), T_7 (1.38), T_3 (1.45), T_5 (1.46) and T_1 (1.48).

Table 10. Benefit Cost Ratio for different treatments of foot collar rot of mungbean

Sl. No	Treatments	BCR
1.	T_1 = *Trichoderma harzianum* suspension	1.48
2.	T_2 = *Trichoderma harzianum* suspension + Alamanda leaf extract	1.94
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	1.45
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	1.86
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	1.46
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost	1.64
7.	T_7 = Mustard oil cake	1.01
8.	T_8 = Krishibid organic fertilizer	1.36
9.	T_9 = Vermicompost	1.37

The findings of the present experiment are supported by the findings of researchers.

Siddique *et al*., (2016) reported that the reduction in disease incidence of collar rot of eggplant was found promising by application of Bavistin 50 WP (carbendazim) and Topgan 50 WP (copper oxychloride).

Wavare *et al.,* (2017) reported that maximum collar rot incidence (56.33%) was observed in control treatment at 30 DAS, followed by 46.33% in Metalaxyl, 0.2% and Carbendazim 0.1% exhibited minimum collar rot incidence (16.33%) at 60 DAS and which were significantly superior over control.

Organic amendments increase the availability of nutrients by improving physical condition of soil. Thus, increased the yield and reduced the soil-borne diseases. Organic soil amendments have also been reported to be effective in controlling the soil borne pathogen. The superiority of botanicals may be due to release of some inhibitory substances like nimbicidin, nimbin or azadirachtin on the decomposition, affecting the population of pathogen. The organic amendments may have a possible role in enhancing the host growth and vigour, increasing antagonistic microbial activity and enabling them to resist the attack of pathogen. (Bhagat, 2014)

CHAPTER V

SUMMARY AND CONCLUSION

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Mungbean is highly nutritive and protein rich crop which is a supplement of animal protein in Bangladesh perspective. The crop is attacked by various diseases and caused massive yield loss. It is one of the main reasons of farmer's unwillingness to cultivate mungbean. Among all diseases of mungbean collar rot caused by *Sclerotium rolfsii* is the most devastating one which causes considerable losses of the crop in the country. It is one of noxious plant pathogens which can survive in soil for year after year by forming sclerotia in adverse condition. it is very difficult to control due to growing habit and survival technique of the pathogen.

Chemical fungicides are always the major tools for management of the disease. However, chemicals have harmful residual effects on man, animal and environment. Health hazards such as skin problem, inhalation problem, irritation of eyes and digestion problem are observed in human due to adverse effect of chemicals. Phytotoxicity can also be occurred in case of many other plants.

The experiment was conducted to isolate, identify and to develop eco-friendly management of *Sclerotium rolfsii*. The isolated organism causing collar rot of mungbean identified as *Sclerotium rolfsii* based on its key characteristics (CMI Description No. 410). The eco-friendly components to manage the disease were selected based on previous promising results, a bioagent (*Trichoderma hrzianum* suspension from Biotech care, BAU, Mymansing), two botanicals (allamanda and neem leaf extract), three soil amendments (mustard oil cake, Krishibid organic fertilizer, virmicompost), two chemical fungicide (Autostin 50 WDG and Goldton 50 WP).

Efficacy of Autostin 50 WDG, Goldton 50 WP, neem leaf extract, allamanda leaf extract and *Trichoderma harzianum* suspension was evaluated by

performing bioassay (cup method) and was found 96.3%, 95.18%, 83.33%, 92.667% and 93.33% reduction of mycelial growth over control, respectively.

Integrated application of selected components was also performed in field condition. Seed treatment with *Trichoderma hrzianum* and soil treatment with allamanda leaf extract was found most effective in controlling disease incidence 91.31% over control and increasing yield 183.01% over control. *Trichoderma harzianum* suspension + Autostin 50 WDG reduced disease incidence 91.05% and increased yield 169.81% over control. *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic manure + vermicompost, *Trichoderma harzianum* suspension + Goldton 50 WP, *Trichoderma harzianum* suspension, Krishibid organic fertilizer, vermicompst and mustard oil cake were found effective and increased 156.60%, 118.87%, 116.87%, 113.01%, 88.67%, 88.6%, 37.7% yield over control, respectively.

Trichoderma harzianum suspension combined with other IDM components was found more effective than single application of *Trichoderma harzianum* suspension in terms of lowering disease incidence and all yield contributing characteristics. Though single effect of Krishibid organic fertilizer, vermicompst, mustard oil cake was not satisfactory but in combined application with *Trichoderma harzianum* suspension was found very effective. But in this case cost of production remained very high compared to other treatments. The highest BCR value (1.9) was recorded in combined application of *Trichoderma hrzianum* and allamanda leaf extract.

It can be concluded that ***Trichoderma harzianum*** suspension (Biotech Care, BAU, Mymansing) for seed treatment followed by drenching of rhizosphere with **Allamanda leaf** extract can be used to manage collar rot of mungbean.

CHAPTER VI

REFERENCES

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CHAPTER VII

APPENDICES

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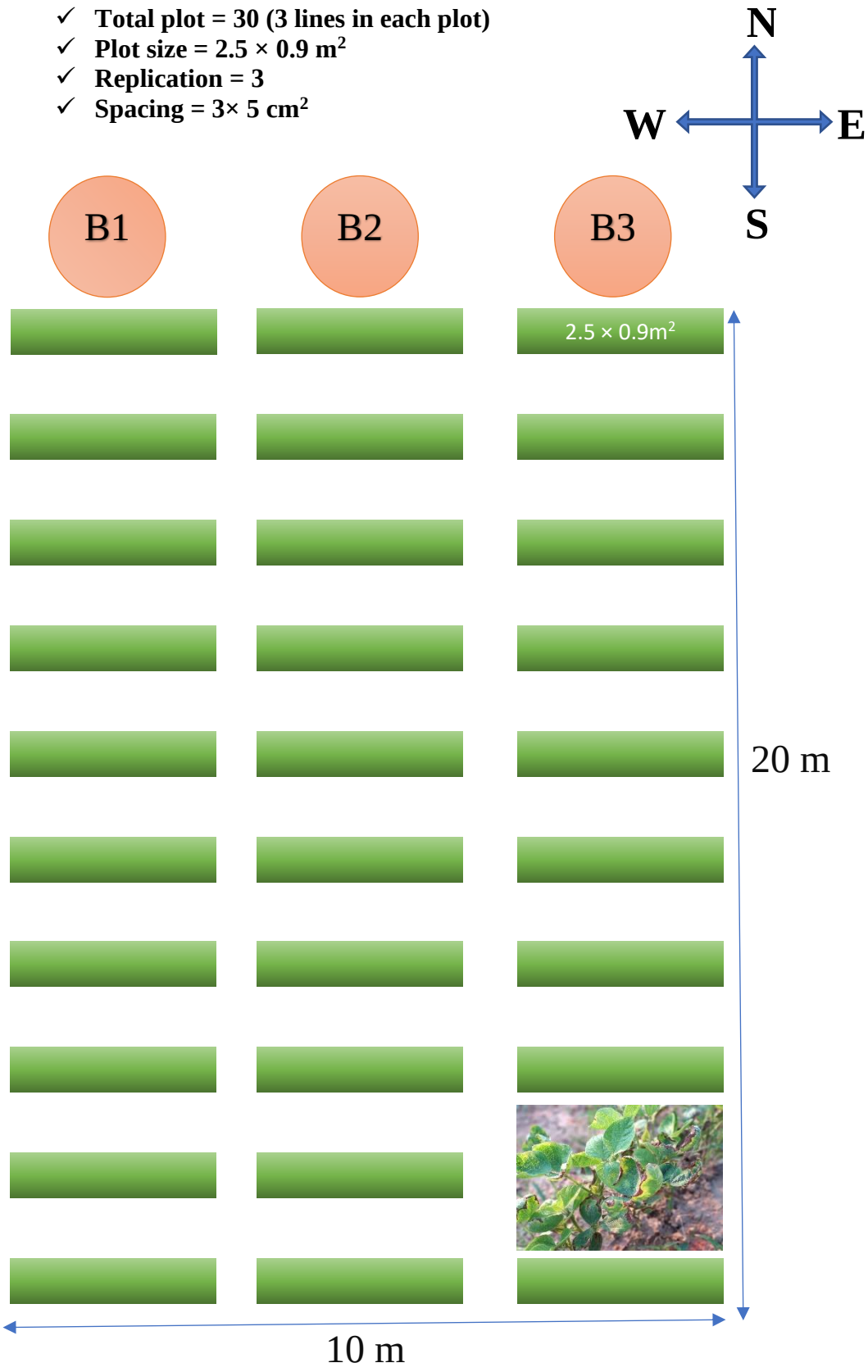
Appendix 1. Soil type of experimental plot

Agro-ecological region: Madhupur Tract (AEZ-28).

Land Type	Medium high land.
General soil type	Non-Calcareous Dark gray floodplain
Soil series	Tejgaon Topography
Topography	Up land
Elevation	8.45 Location
Location	SAU Farm, Dhaka.
Field level	Above flood level.
Drainage	Fairly good
Firmness (consistency)	Compact to friable when dry.

Appendix 2. Layout of field experiment

- ✓ **Total plot = 30 (3 lines in each plot)**
- ✓ **Plot size = 2.5 × 0.9 m^2**
- ✓ **Replication = 3**
- ✓ **Spacing = 3× 5 cm^2**



Appendix 3.Details of cost of production of mungbean excluding treatment cost

Sl. No.	Cost item	Unit	Quantity	Cost per unit (tk)	Times	Per hectare cost in TK
1.	Seed	kg	30	200	1	6000
2.	Land preparation					
	Hired human labour	Man/day	61	500	1	30500
	Mechanical power	Per day	-	6050	1	6050
3.	Fertilizer					
	Cow dung	ton	10	4000		40000
	Urea	Kg	40	20		800
	TSP	Kg	105	19		2000
	MoP	Kg	40.5	21		850
	Boron	Kg	10	50		500
	Zink	Kg	10	30		300
	Gypsum	Kg	14	15		200
4.	Irrigation					
	Cost for water	-	-	-		2000
	Hired human labour	Man/day	10	500	3	15000
5.	Weeding					
	Hired human labour	Man/day	10	500	3	15000
6.	Miscellaneous cost					10800
Total cost						**130000**

*Calculated on the basis of market price of 2019

Appendix 4. Cost of application of treatments for production of mungbean

Sl. No.	treatment	Cost item	Unit	Quantity	Cost/ Unit (tk)	times	Cost/hin tk.
1.	T_1	*T. harzianum* suspension	L	3	1200	3	10800
		Human labour	M/D	5	500	3	15000
		Total cost (a)					**25800**
2.	T_2	*T. harzianum* suspension	L	3	1200	2	7200
		Alamanda leaf	kg	90	-	1	2000
		Human labour	M/D	10	500	3	15000
		Total cost (b)					**24200**
3.	T_3	*T. harzianum* suspension	L	3	1200	2	7200
		Neem leaf	kg	100	-	1	2000
		Human labour	M/D	12	500	3	16000
		Total cost (c)					**25200**
4.	T_4	*T. harzianum* suspension	L	3	1200	2	7200
		Autostin 50 WDG	kg	2	1000	2	4000
		Human labour	M/D	10	500	3	15000
		Total cost (d)					**26200**
5.	T_5	*T. harzianum* suspension	L	3	1200	2	7200
		Goldton 50 WP	kg	2.88	1000	2	5760
		Human labour	M/D	10	500	3	15000
		Total cost (e)					**27960**
6.	T_6	*T. harzianum* suspension	L	3	1200	2	7200
		Mustard oil cake	kg	10	150	1	1500
		Krishibid organic fertilizer	kg	10	200	1	2000
		Vermicompost	kg	10	220	1	2200
		Human labour	M/D	15	500	3	22500
		Total cost (f)					**35400**
7.	T_7	Mustard oil cake	kg	30	150	1	4500
		Human labour	M/D	20	500	1	10000
		Total cost (g)					**14500**
8.	T_8	Krishibid organic fertilizer	kg	30	200	1	6000
		Human labour	M/D	20	500	1	10000
		Total cost (h)					**16000**
9.	T_9	Vermicompost	kg	30	220	1	6600
		Human labour	M/D	20	500	1	10000
		Total cost (i)					**16600**

*Calculated on the basis of market price of 2019
M/D= Man per day

Appendix 5. Gross Return of mungbean against different treatments

Sl. no	Treatments	gross return × 1000 (Tk/ha)
1.	T_1 = *Trichoderma harzianum* suspension	232
2.	T_2 = *Trichoderma harzianum* suspension + Alamanda leaf extract	300
3.	T_3 = *Trichoderma harzianum* suspension + Neem leaf extract	226
4.	T_4 = *Trichoderma harzianum* suspension + Autostin 50 WDG	292
5.	T_5 = *Trichoderma harzianum* suspension + Goldton 50 WP	232
6.	T_6 = *Trichoderma harzianum* suspension + Mustard oil cake + Krishibid organic fertilizer + vermicompost	272
7.	T_7 = Mustard oil cake	146
8.	T_8 = Krishibid organic fertilizer	200
9.	T_9 = Vermicompost	200
10.	T_{10} = Control	106

APPENDIX 6. Some photographs of the experiment

A. Land preparation

B. View of experimental plot

C. Working inside of laminar air flow cabinet