EFFICACY OF SELECTED MODERN PHYTO- CHEMICALS AND *Trichoderma harzianum* AGAINST THE PATHOGEN OF SCLEROTIUM ROOT ROT DISEASE RESPONSIBLE FOR SUGARBEET DECLINE

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Dedicated
To
My Beloved
Parents

CERTIFICATE

This is to certify that the thesis entitled **"EFFICACY OF SELECTED MODERN PHYTO- CHEMICALS AND *Trichoderma harzianum* AGAINST THE PATHOGEN OF SCLEROTIUM ROOT ROT DISEASE RESPONSIBLE FOR SUGARBEET DECLINE"** *submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in PLANT PATHOLOGY****, embodies the result of a piece of bona-fide research work carried out by* **A.K.M. ARIF-UZ-ZAMAN, REGISTRATION NO. 18-09100** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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:
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ABSTRACT

A field experiment on management of sclerotium root rot disease of sugarbeet was conducted in the central farm of Sher-e-Bangla Agricultural University, Dhaka during November, 2019 to March, 2020. The prime aim of the study was to manage the sclerotium root rot disease of sugarbeet using seven modern phyto- chemicals and bio-agent, *Trichoderma harzianum.* BSRI released tropical sugarbeet line HI 0044 was used as a planting material and seven modern phyto-chemicals viz., Amister Top 325 SC, Acibin, Tilt 250 EC, Ridomil Gold, Dithane M-45, Cabrio Top, Autostin were used in the experiment as a codified treatments. The sugarbeet was grown for sugar production and natural inoculums were relied upon for the infection of sclerotium root rot. Growth parameters, yield attributes and physiological features were significantly affected by the application of selected modern phyto-chemicals and bio- agent. Percent disease incidence and severity of sclerotium root rot was varied among the treatments. The lowest disease incidence (16.67, 13.89 and 16.67%) was found in Amister Top 325 SC, Acibin and Tilt 250 EC treated plots. The highest disease incidence (56.94%) was found in control treatment. The lowest disease severity (4.63, 4.63 and 5.40%) was found in Amister Top 325 SC, Acibin and Tilt 250 EC treated plots. The highest disease severity (14.20, 13.43 and 11.57%) was found in control treatment, Autostin and Dithane M-45 treated plots. Although the Acibin and Tilt 250 EC showed the better performance regarding the percent disease incidence and severity, but other considering parameters were not found at satisfactory level in Acibin and Tilt 250 EC treated plots due to the chemical effects. Plants were severely affected and showed the stunted and shrinked symptoms. The highest individual beet diameter, weight and yield per plot was obtained in Amister Top 325 SC, Dithane M-45, Cabrio Top and Autostin treated plots (41 cm, 1.02 kg, 24.40 kg; 32.28 cm, 0.78 kg, 18.80 kg; 33.38 cm, 0.85 kg, 20.40 kg and 32.58 cm, 0.85 kg, 20.40 kg respectively). The lowest individual beet diameter (19.75 cm), weight (0.51 kg) and yield per plot (12.16 kg) was obtained in control treatment. The highest brix (%) and sucrose/pol (%) was also measured in Amister Top 325 SC, Dithane M-45 and Cabrio Top treated plots (17.8 %, 12.7%; 16.9%, 12.62% and 16.9%, 12.62% respectively). The lowest brix (11.5%) and sucrose/pol (6.53%) was obtained in control treatment. However, from the findings of the study, it may be concluded that among the selected phyto- chemicals, Amister Top 325 SC and Cabrio Top showed the promising performance for management of sclerotium root rot disease of sugarbeet and gave better yield and yield attributes. On the other hand, bio- agent *Trichoderma harzianum* showed non-satisfactory results regarding the all considering parameters.

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ABBREVIATIONS AND ACRONYMS

AEZ= Agro-Ecological Zone

BARC = Bangladesh Agricultural Research Council

BBS = Bangladesh Bureau of Statistics

BSRI = Bangladesh Sugarcrop Research Institute

cm = Centimeter

CV % = Percent Coefficient of Variation

DAS = Days After Sowing

RCBD = Randomized Complete Block Design

et al., = And others

e.g. = exempli gratia (L), for example

etc. = Etcetera

g = Gram (s)

i.e. = id est (L), that is

Kg = Kilogram (s)

LSD = Least Significant Difference

m^2 = Meter squares

mL = Milliliter

M.S. = Master of Science

No. = Number

SAU = Sher-e-Bangla Agricultural University

var. = Variety

^oC = Degree Celceous

% = Percentage

INTRODUCTION

Sugarbeet (*Beta vulgaris* L. ssp. *vulgaris*), member of the family Chenopodiaceae and order Caryophylalles, is a plant whose root contains a high concentration of sucrose. It is grown commercially for sugar production mostly in temperate countries (Rashid, 1999). The European Union, the United States, and Russia are the world three largest sugarbeet producers in the world. This crop is additionally a promising crop for the production of ethanol (BSRI, 2005). Sugarbeet is a specialized biennial crop. Although it is grown in temperate region, but now it can also be grown successfully in a wide range of climates on different soils between latitudes of 30^0 and 60^0 N, as winter and winter/summer crops. More recently it's been grown as a winter crop within the warm regions of the temperate zones, including parts of South America, Africa, the center East, and Southern Europe. It is grown in 57 countries mainly produced in Europe and, to a lesser extent, in Asia and North America (Kumar and Pathak, 2013). Top fifteen sugarbeet producing countries are Russian Federation, Ukraine, United States of America, Germany, France, Turkey, China, Poland, Egypt, United Kingdom, Iran (Islamic Republic), Belarus, Netherlands, Italy and Belgium (Rahman *et al*., 2016).

In India, research has shown that the successful cultivation of the crop is possible as a supplementary sugar crop, particularly in northwestern India. During 1972-1998, the crop was commercially grown in the Sriganganagar

area of Rajasthan and was processed for sugar production during April and May every year with a good recovery of sugar (Srivastava, 2000).

Recently, some tropical sugarbeet varieties have been developed which can be grown in tropical as well as subtropical region of the world. In Bangladesh, sugarbeet is a new crop and few farmers are growing in limited areas for vegetable purpose. The weather condition of Bangladesh is suitable for sugarbeet farming, its sweetness and yield rate are much higher than sugarcane and the crop can be harvested in within five months than a year in case of sugarcane (Rashid, 1999). Production of sugarbeet has got many benefits compared to sugarcane production. It is short duration crop (5-6 months) with high sucrose contents (14- 20%) while sugarcane is a long duration crop (12-14 months) with low sucrose (10-12%) contents (Anon, 2004). Since sugarcane is long duration crop thus farmers are moving to grow short duration crop for higher profit. In Bangladesh, most of the sugar mills remain idle for a particular period of time due to acute shortage of sugarcane availability. So, sugarbeet might be an excellent alternative of sugarcane if processing facilities are developed in the sugar mills. Before that, feasibility study for sugarbeet cultivation in Bangladesh need to be assessed properly and in this regard, no systematic research work has so far been done in Bangladesh.

So, we can say that sugarbeet may be miracle for us if we can cultivate it properly with proper agronomical and pathological practices with latest agro-technology. To meet increasing needs of sugar, all aspects of the way to produce this crop efficiently has been widely researched, and work continues rapidly in all

countries where they're grown. Bangladesh has immense potentiality for sugarbeet cultivation particularly northern part of the country which can take the production of sugar to a satisfactory level.

From the earliest time of history, sugarbeet has formed a basic food for man, animal and plant. It is an important part of the human diet, providing energy to maintain body temperature. it is also widely used as a sweetener and preservative for other foods like beverages, confectionaries, canned foods and pharmaceuticals. Composition wise a raw sugarbeet is 88% water, 10% carbohydrates, 2% protein, and less than 1% fat. The young leaves are often added raw to salads, whilst the mature leaves are most frequently served boiled or steamed, during which case they have a taste and texture similar to spinach. (Kumar and Pathak, 2013).

Generally the main constraints in sugarbeet cultivation around the world is insect infestation and disease infection. The introduction of a temperate crop in a tropical and sub-tropical climate poses many important pathological problems due to prevailing high temperature. The conditions suitable for growth and development of the crop and the succulent nature of its foliage and roots are also favorable for quick development, proliferation and spread of the diseases. The major deterrents in culturing are diseases caused by fungi, of which seedling afflictions and root rot within the plains, leaf spots and nematode disorders both in the plains and the hills are most destructive. Fortunately, the extent of the damage caused by bacteria and viruses are negligible, while fungi and nematodes are proving limiting factors in the profitable cultivation of the crop in the country

(Srivastava, 2000). About 16-20% of the crop is destroyed by diseases every year. The diseases of the sugarbeet have played an enormously important role within the current distribution of the beet sugar industry and sugarbeet crops in most of the sugarbeet growing countries (Duffus and Ruppel, 1993). The crop is subject to attack by these diseases from the time of seed-sowing, until the harvest of the crop. All parts of the sugarbeet plant (seeds, seedlings, roots and foliage) are susceptible to attack by a number of diseases which reduce the quantity and quality of roots and seed. World-wide over 50 diseases are known to affect sugarbeets, of which nearly 20 are of economic importance (Mukhopadhyay, 1987). With the expansion in the area under sugarbeet production world-wide, the diseases have increased in number and severity.

Some of the common diseases that affect sugarbeet include Cercospora leaf spot, Alternaria leaf spot, Powdery mildew, Colletotrichum leaf spot, Phoma leaf spot, Dry root rot, Fusarium, Sclerotium, Rhizoctonia crown and root rot etc (Alam *et al.*, 2017).

The sclerotial root rot due to *Sclerotium rolfsii* commonly known as "Southern stem and root rot" is of great economic importance causing much damage in the tropics and sub-tropics in sugarbeet. However, crop losses are greater in the tropics than in the sub-tropics. The disease is a limiting factor in the cultivation of sugarbeet crop in Southern U.S.A., in warmer, humid areas of Europe, Middle east, India and Asia (Srivastava, 2000).

For the management of the disease, no single control is effective. Prevention of the disease is far more important and better than to check it when the plants have

been infected in field. Therefore, integrated disease management (IDM) system involving cultural, chemical, biological and host resistance may be employed to manage the disease (Srivastava, 2000). Sanitary measures like uprooting and burning of diseased plants, particularly at early stage of infection should be followed. After the harvesting of the crop, all the diseased plants also along with crop debris of other plants should be burnt completely in the field. Avoidance of sugarbeet sowing in infected fields can minimize the effect of disease on yield. It is desirable to determine the distribution and intensity of infestation of *S. rolfsii* within the field before sowing, that various techniques are reported (Punja *et al.*, 1985; Srivastava *et al.*, 1987). However, rotation with less susceptible crops like maize or wheat may result in less disease incidence in subsequent years by lowering the inoculum levels. Nitrogenous fertilizers, like calcium nitrate, urea, calcium, and ammonium sulphate have been found effective in reducing root rot incidence under field conditions (Mukhopadhyay, 1987). The effect of nitrogen has been attributed partly due to stimulation of a biological control agent, *Trichoderma harzianum* (Mukhopadhyay and Upadhyay, 1981).

Biological control through *Trichoderma harzianum* and *T. viride* have been found effective in reducing root rot incidence both under glass house and field conditions (Ciccarese, *et al.*, 1992; Mukhopadhyay and Upadhyay, 1983; Srivastava and Tripathi, 1996; Upadhyay and Mukhopadhyay, 1986). Soil application of *Trichoderma harzianum* combined with seed pelleting gave effective control of seedling as well as root rot of adult sugarbeet plant (Srivastava and Tripathi, 1996).

Several fungicides, like carboxin (Vitavax), benomyl (Benlate), quintozine, Demosan (chloroneb), Dithane M-45, Calixin, Bavisitn, Thiram and Brassicol (PCNB) have been evaluated as soil drench to manage the sclerotial root rot in fields (Mukhopadhyay and Thakur, 1971; Sharma *et al.*, 1990; Singh *et al.*, 1974; Waraitch *et al.*, 1986).

The purpose of the proposed study was to manage sclerotium root rot disease by using the suitable modern phyto-chemicals and *Trichoderma harzianum*.

OBJECTIVES

This study was carried out to achieve the following specific objectives-

- To evaluate the efficacy of selected modern phyto- chemicals and bio-agent against the sclerotium root rot disease in field condition.
- To determine the pathogen potentiality through pathogenicity test.

REVIEW OF LITERATURE

Sugarbeet (*Beta vulgaris*) suffers from many diseases of which sclerotium root rot disease causes severe damage to the crop. Available literatures on various aspects of this disease so far have been presented in this chapter.

2.1 Sugarbeet

Rashid (1999) reported that Sugar beet (*Beta. vulgaris* L. ssp. *vulgaris*), member of the family *Chenopodiaceae* and order Caryophylalles, is a plant whose root contains a high concentration of sucrose.

BSRI (2005) reported that the European union, the United States, and Russia are the world three largest sugarbeet producers in the world. This crop is also a promising alternative energy crop for the production of ethanol.

Kumar and Pathak (2013) reported that Sugarbeet is grown in 57 countries mainly produced in Europe, and, to a lesser extent, in Asia and North America.

Rahman *et al*., (2016) reported that Top fifteen sugarbeet producing countries are Russian Federation, Ukraine, United States of America, Germany, France, Turkey, China, Poland, Egypt, United Kingdom, Iran (Islamic Republic of), Belarus, Netherlands, Italy and Belgium.

During 1972-1998, the crop was commercially grown within the Sriganganagar area of Rajasthan and was processed for sugar production during April and should per annum with a good recovery of sugar (Srivastava, 2000).

The weather of Bangladesh is suitable for sugar beet farming, its sweetness and yield rate are much above sugarcane and therefore the crop are often harvested in just five months than a year in case of sugarcane (Rashid, 1999).

Production of sugarbeet has got many benefits compared to sugarcane production. It is short duration crop (5-6 months) with high sucrose contents (14-20%) while sugarcane is a long duration crop (12-14 months) with low sucrose (10-12%) contents (Anon., 2004).

Srivastava (2000) reported that globally contribute about 36% of the entire centrifugal sugar produced, with the remaining 64% from sugarcane.

Kumar and Pathak (2013) said that composition wise a raw sugarbeet is 88% water, 10% carbohydrates, 2% protein, and less than 1% fat. The young leaves are often added raw to salads, whilst the mature leaves are most ordinarily served boiled or steamed, during which case they need a taste and texture similar to spinach.

Alam *et al*., (2017) reported that generally the main constraints in sugarbeet cultivation around the world is insect infestation and disease infection. Sugarbeet is usually attacked by beet caterpillar, beet aphid etc.

Duffus and Ruppel (1993) said that about 16-20% of the crop is destroyed by diseases every year. The diseases of the sugarbeet have played a particularly important role within the current distribution of the beet sugar industry and sugarbeet crops in most of the sugarbeet growing countries.

Mukhopadhay (1987) reported that world-wide over 50 diseases are known to affect sugarbeets, of which nearly 20 are of economic importance.

Alam *et al*., (2017) reported that some of the common diseases that affect sugarbeet include Cercospora leaf spot, Alternaria leaf spot, Powdery mildew,

Colletotrichum leaf spot, Phoma leaf spot, Dry root rot, Fusarium, Sclerotium, Rhizoctonia crown and root rot etc.

For the management of the disease, no single control is effective. Prevention of the disease is way more important and better than to see it when the plants are infected in field. Therefore, integrated disease management (IDM) system involving cultural, chemical, biological and host resistance could also be employed to manage the disease (Srivastava, 2000).

2.2 Sclerotium root rot disease

Sclerotium rolfsii is a soil-borne plant pathogen of worldwide occurrence that infects quite 500 plant species (Aycock, 1966 and Punja, 1985).

According to Aycock (1966), Mostly *Sclerotium rolfsii* diseases have been reported on dicotyledonous hosts, but several monocotyledonous species have also been infected.

Punja *et al*., (1985) reported that the large number of sclerotia produced by *S. rolfsii* and their ability to continue the soil for several years, also because the profuse rate of growth of the fungus make it well compatible facultative parasite and a pathogen of major importance throughout the world.

Mukhopadhayay (1971), Sharma and Pathak (1994) and Waraitch *et al.*, (1986) reported that it causes 14-59% loss in root yield and reduces sugar content up to 20% in certain varieties under favorable conditions in sugarbeet.

2.3 Occurrence and symptoms of sclerotium root rot disease

The first visible symptoms within the field include yellowing and wilting of leaves followed by rotting of roots of affected plants. White cottony mycelium develops on rotted basal portions of roots and causes gradual semi-watery decay. As the mycelial growth advances, the affected leaves turn yellow and wither pre-maturely. At later stage mycelial growth becomes more profuse and almost covers the major portions of the fleshy root. Decomposition gives a distorted appearance to the roots. Such affected roots become unfit for sugar extraction also feeding animals. On rotted roots, innumerable small, light to dark brown sclerotia of mustard seed size develop on the mycelium. These hyphal strands and sclerotia are also found in the soil, radiating outwards from affected roots. Such affected plants Topple down on the ground. The diseased plants can easily be pulled out due to massive damage to the tap root system as a result of rotting. The fungus also causes seedling blight of sugarbeet resulting in a poor stand of crop (Srivastava, 2000).

Backman and Rodriguez-Kabana (1972) reported that the disease is caused by fungus *Sclerotium rolfsii* (imperfect stage). The perfect stage is also known as *Pellicularia rolfsii* (Syn. *Corticium rolfsii, Athelia rolfsii*). The imperfect stage consists of mycelium and sclerotia. It can be easily grown on potato dextrose agar (PDA) medium under laboratory conditions.

Srivastava (2000) found that the fungus survives in soil from one season to another by means of sclerotia formed abundantly on affected roots, crop debris, adjoining soil and other suitable substrates. Under favorable conditions, these

sclerotia germinate and give rise to vegetative mycelium and a pathogenic phase. The fungal mycelium first grow near roots and form a network of strands in surrounding soil. As the strands extend through soil, they infect healthy roots and continue their destruction.

Duffus and Ruppel (1993) reported that Sclerotia are the principal means of survival of *S. rolfsii* in soil even within the absence of suitable hosts or conditions favouring its active growth. Thus sclerotia which persist for long periods in soil serve as the source of primary infection. These are spread *via* cultivation and irrigation water for secondary infection from one location to another. Disease severity is influenced by the population of viable sclerotia (inoculum density) within the beet field and therefore the longevity of sclerotia in soil.

Sen *et al.*, (1979) reported that inoculation of sugarbeet in first week of February using 750 g of sand maize-meal inoculum causes the very best and most uniform mortality of roots. Temperature influences root rot incidence of sugarbeet. The maximum disease development occurs at temperatures approximately favorable for the growth of the pathogen in culture, *i.e.* 30-35^{0} C. Disease incidence and severity gradually reduces as the temperature decreases. Minimum disease severity was noted at or below 15^{O} C.

Moisture also influences the root rot development. It has been observed that fields receiving 16 irrigations during crop season show minimum root rot incidence and maximum root yield as compared to 12, 8 and 4 irrigations, respectively (Singh *et al.*, 1986).

Similar observations have also been recorded by (Maiti *et al*., 2000). Saturated soil moisture conditions at higher frequency level of irrigations may cause lysis of hyphae and sclerotia of *S. rolfsii* which in turn reduces disease incidence. The disease occurs in many sorts of soil, but it's often severe on light sandy soils followed by sandy loam or loam soils (Mukhopadhyay, 1987; Srivastava, 2000).

2.4 Biological control of sclerotium root rot disease of sugarbeet

Biological control through *Trichoderma harzianum* and *T. viride* are found effective in reducing sclerotium root rot incidence both under glass house and field conditions (Ciccarese, *et al.*, 1992; Mukhopadhyay and Upadhyay, 1983; Srivastava and Tripathi, 1996; Upadhyay and Mukhopadhyay, 1986).

Mukhopadhyay (1994) stated that biological control represents a natural and ecological approach in controlling diseases that reduces chemical inputs and their effects.

Chet and Inber (1994) reported that *Trichoderma* based biopesticides have gained considerable recognition as biological agent. Several strains of *Trichoderma* have been found to be effective as bio-control agent of various soil borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoctonia* etc.

Ferrata and D'Ambra (1985) observed that *Trichoderma harzianum* isolate shows low ability in coiling round hyphae of *Sclerotium rolfsii*, but it is very effective in penetrating or growing inside them. *T. harzianum* adversely affect them even without penetration.

Wells *et al.* (1972) stated that *T. harzianum* effectively controlled *S. rolfsii* on blue lupines, tomatoes and peanuts. Under natural field conditions, one to three

applications of *T. harzianum* inoculums applied over the plants onto the soil surface was highly effective in reducing *S. rolfsii* damage to tomato.

Backman and Rodriguez (1975) observed a diatomaceous earth granule impregnated with 10% of molasses solution was found suitable for growth and delivery of *T. harzianum* to peanut fields. *Trichoderma* was grown on sterile earth granules for 4 days and applied to 70 and 100 days after planting. Significant reduction in *S. rolfsii* in peanut fields was recorded over the 3-years test period.

Mehrotra and Tiwari (1976) showed that dipping of cutting in a *Trichoderma viride* cell suspension effectively reduced the foot rot disease of betel vine.

Chet *et al*. (1979) stated that, when applied in the form of wheat bran culture to soil infested with *R. solani* and *S. rolfsii* in the greenhouse, *T. harzianum* effectively controlled damping off diseases of peanuts, beans, and eggplants caused by soil borne plant pathogens. Field experiments were carried out and a significant reduction in disease incidence was obtained. Application of PCNB at sub inhibitory doses improved control of disease when applied together with the *T. harzianum.*

Grinstien *et al.* (1979) demonstrated a wheat-bran preparation of an antagonistic fungus, *T. harzianum* Rifaiaggr. applied to fields at rates of 500-1500 kg/ha, reduced the incidence of diseases caused by *S. rolfsii* and *R. solani* and this control lead to increase yield in various crops.

Elad *et al.* (1980) reported that incorporation of a wheat bran inoculums of *T. harzianum*in pathogen infested soil significantly reduced bean (*Phaseolus vulgaris*) disease caused by *S. rolfsii* and *R. solani* under glasshouse condition.

Almeida and Landim (1981) reported that an isolate of *Trichoderma spp*. was hyper parasitic on *S. rolfsii* of cowpea under field and laboratory condition.

Echeverria *et al.* (1982) studied the antagonistic effect of *Trichoderma* spp. to control *S. rolfsii* on *Phaseolus vulgaris* under field and laboratory conditions and found antagonism between *Trichoderma* spp. and *S. rolfsii*.

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

Mukhopadhyay (1987) stated that, application of wheat bran saw dust preparation of *T. harzianum* and *T. koningii* brought an excellent control of damping-off of tomato and eggplant, wilt and foot rot of lentil (caused by *S. rolfsii*) under field conditions.

Abada (1994) reported that, the *T. harzianum* caused a great reduction in the infection level of damping off and root rot disease of sugar beet caused by *R. solani, S. rolfsii, Fusarium sp., Mucor sp., Alternaria sp., Pythium debaryanum* and resulted in increased root weight both in pot and field experiments.

Kay and Stewart (1994) found that, *Trichoderma viride* and *T. harzianum* were capable of reducing the incidence of onion white rot caused by *S. rolfsii.* Reduction was observed when the test fungi were applied as seed coating or incorporated into alginate pellets.

Muthamilan and Jeyarajan (1996) reported that, *T. harzianum* reduced groundnut root rot caused by *S. rolfsii*. Maximum number of plants survived when the antagonist was applied as seed treatment prior to sowing.

Rjurkar *et al.* (1998) reported the antagonistic effect of *Trichoderma* spp. on the wilt causal organism *S. rolfsii.*

2.5 Chemical control of sclerotium root rot disease of sugarbeet

Several fungicides, like carboxin (Vitavax), benomyl (Benlate), quintozine, Demosan (chloroneb), Dithane M-45, Calixin, Bavisitn, Thiram and Brassicol (PCNB) are evaluated as soil drench to manage the sclerotiam root rot disease in fields of sugarbeet (Mukhopadhyay and Thakur, 1971; Sharma *et al.*, 1990; Singh *et al.*, 1974; Waraitch *et al.*, 1986).

Mukhopadhyay and Thakur (1971) reported that ridge soil drenching with PCNB or Demosan (15 kg/ha) or Vitavax (2 kg/ha) during mid- February minimized the disease and also increased root yield.

Sharma *et al.* (1990) reported that PCNB (10 kg/ha) as soil drench controlled the disease and slightly improved the sucrose content. Combined application of mustard or groundnut cakes at 50 q/ha (15 days before sowing) and PCNB at 15 kg/ha (as soil drench in mid- February) also reduced the incidence of root rot (Sen *et al.*, 1973).

Sen *et al.* (1974) found that, *Sclerotium rolfsii* on wheat might be controlled by seed treatment with 5g PCNB [Quintozene] per kg seed.

Agnihorti *et al*. (1975) screened a number of fungicides in controlling root rot of sugarbeet incited by *Sclerotium rolfsii* both *in vivo* and *in vitro.* In *In vivo*

Vitavax and Quintozene showed fungicidal and fungi static effect, while *in vitro.* Vitavax, Demosan and PCNB were found effective in inhibiting the expansion of *Sclerotium rolfsii.*

Dutta (1975) found that, soil application of fungicides such Bavistin (0.5%), Brassicol (0.1%), 3 times at 20 days interval has been effective in controlling foot and tuber rot disease of tuberose.

Dhamnikar and Peshney (1982) evaluated twenty fungicides against *Sclerotium rolfsii* on peanut by different methods *in vivo.* Rovral, Vitavax, Brassicol, Captaf and Dithane M-45 controlled the disease effectively as dry seed dresser. As soil drench, Vitavax-200 was the most effective followed by Rovral and Brassicol controlling the disease.

Patil and Rane (1982) observed Vitavax, Ceresan wet proved to be effective in inhibiting the growth of the pathogen as well as affecting germination of sclerotia. These fungicides were also proved effective in reducing the incidence of seed borne and soil borne infection by seed and soil treatments.

Punja *et al*. (1982) found that, eruptive and hyphal germination of dried seed sclerotia of two isolates of *Sclerotium rolfsii* at 1% Noble and Bacto water agar was totally inhibited by Carboxin, Cycloheximide, Oxycarboxin and experimental fungicides CGA-64251 within the agar @ 100 and 200 µg a.i /ml.

Fahim *et al*. (1984) observed, seed treating agent Vitavax-200, Homai80, Orthocide-75 and Captan @3g/kg seed by dusting or glutting (modification or pelleting method) to reduce pre-emergence damping-off of sugar beet (*Beta*

vulgaris) caused by *Sclerotium rolfsii* in infested soil. Post-emergence damping-off was greatly reduced in soil infested before sowing after seed germination.

Patil *et al*. (1986) reported that, in field trials against the foot rot disease of *Piper betle* caused by *Sclerotium rolfsii* were control by soil drenches with Copper oxychloride, Cupravit, Dithane M-45, Difolatan and Bordeaux mixture were very effective.

Pan and Sen (1987) demonstrated soil drenches with Benodanil and seed treatments with Campogram M were also highly effective in reducing wheat seedling mortality caused by *Sclerotium rolfsii.*

Shahid *et al.* (1990) evaluated ten fungicides *in vitro* test and found Ridomil [Metalaxyl] was the most effective in inhibiting mycelia growth and sclerotial production of *Sclerotium rolfsii.* Benlate [Benamyl] and Metalaxyl inhibited germination of sclerotia most effectively. Metalaxyl and Benomyl at 500 ppm applied as seed treatment and soil drench, respectively gave 100% control of collar rot lentil seedlings.

Rahman *et al*. (1994) demonstrated that the effect of Vitavax-200, ApronTZ, Dithane M-45, Thiram, Captan and Baytan 100-S [Triadimeno] on foot and root rot disease on cowpea (*Vigna unguiculata*) caused by *Corticium rolfsii*. Seeds of a susceptible variety were treated before sowing. Vitavax-200 was the best fungicides in respect to controlling seedling mortality.

Rondon *et al.* (1995) used Copper Oxychloride, Vinclozolin (as Ronilan), Iprodione (as Rovral), Metalaxyl (as Ridomyl), Chlorothalanil (as Daconil), PCNB [Quintozene], Captan, Benomyl, Carboxin + Thiram and Thiabendazole

at five concentrations against the growth and sclerotia formation of *Sclerotium rolfsii*. Carboxin + Thiram, Copper Oxychloride and Quintozene were found to be most effective, both in inhibiting mycelia growth and sclerotia formation at low concentration.

2.6 Sucrose (%) or Pol (%) and Total soluble solids (TSS) or Brix (%)

Brix (%), pol (%), purity (%) and recoverable sucrose (%) in sugarbeet juice were recorded at harvest. Brix (%) refers to the total soluble solids while pol (%) refers to percentage of sucrose content in beet juice. Purity (%) refers to ratio of sucrose content (pol %) to the total soluble solids (brix %) in juice. Five beets were selected from each plot at random and was crushed with a mini power crusher for juice extraction. The collected juice was poured in to a glass cylinder and the brix (%) was determined by brix hydrometer. The same juice was clarified with basic lead sub-acetate and after filtration it was poured in 200 mm polarimeter tube for determination of pol (%) of beet (Khan and Minhas, 2006). Sucrose (%) was estimated in fresh samples of sugarbeet roots, polarimeterically on a lead acetate extract of fresh macerated root according to Le Docte (1927).

MATERIALS AND METHODS

The present study regarding efficacy of phyto- chemicals against sclerotium root rot disease has been conducted during November 2019 to March 2020 at the central farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. Required materials used and methodology followed are described below under the following headings and subheadings.

3.1 Experimental site

The experiment was conducted at the central farm of Sher-e-Bangla Agricultural University, Dhaka. The site was 22°46'N and 90°22' E Latitude and at Altitude of 9 m from the sea level. The location of the experiment field had been shown in Appendix-I.

3.2 Characteristics of soil

The soil of the experimental site is a medium high land belonging to the Modhupur Tract under the Agro Ecological Zone (AEZ) 28. The soil texture was silty loam with a pH 6.7. Soil samples of the experimental plot was collected from a depth of 0 to 30 cm before conducting the experiment and analyzed in the Soil Resource Development Institute (SRDI), Farmgate, Dhaka. Details of the mechanical analysis of soil sample have been shown in Appendix-II.

3.3 Weather conditions during the experiment

The weather condition of the experimental site was under the sub-tropical monsoon climate, which is characterized by heavy rainfall during Kharif season (April to September) and in the Rabi season (November to March), low rainfall associated with moderately low temperature, low humidity and short day. There

was little rain during the month of November, January and March. Rabi is the more favorable for vegetable production. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the study period were collected from Bangladesh Meteorological Department, Agargaon, Dhaka-1207, Dhaka and have been presented in Appendix-III.

3.4 Plant material

The tropical sugarbeet line "HI 0044" was used in this study (Figure 1). This is a high yielding sugarbeet line developed by Bangladesh Sugarcrop Research Institute, Ishurdi, Pabna.

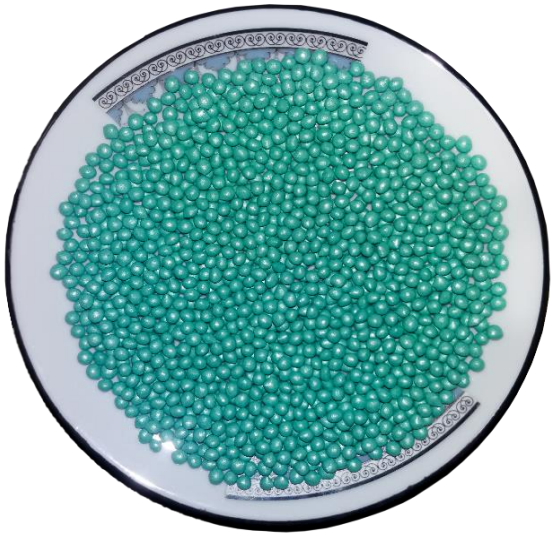


Figure 1. Sugarbeet seed (Line 'HI 0044')

3.5 Collection of modern phyto-chemicals and *Trichoderma harzianum*

Seven fungicides namely Amister Top 325 SC, Acibin, Tilt 250 EC, Ridomil Gold, Dithane M-45, Cabrio Top, Autostin were collected from local market (presented in tabulated form with trade name, formulation and active ingredient in Table 1). Pure culture of bio- control agent *Trichoderma harzianum* (Figure 2) was collected from the Plant Disease Diagnostic Clinic, Department of Plant

Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur-1706.

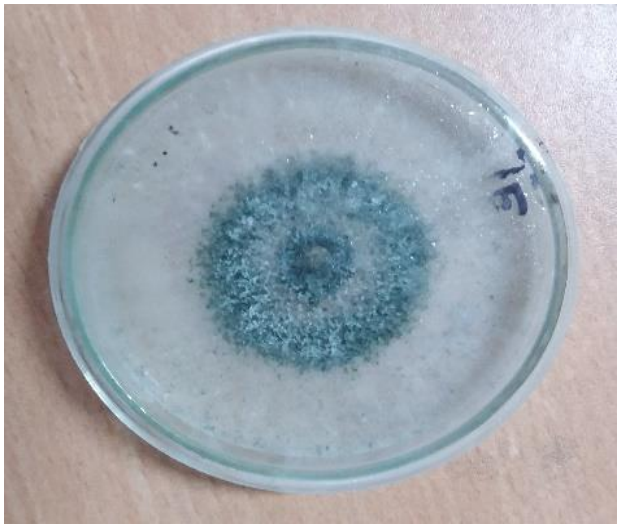


Figure 2. Culture media of *Trichoderma harzianum*

Table 1: List of fungicides used in this study

SL. No.	Trade name	Company name	Active ingredient
1	Amister Top 325 SC	Syngenta Bangladesh Ltd.	Azoxystrobin + Difenoconazole
2	Acibin 28 SC	ACI Crop Care Ltd.	Azoxystrobin + Ciproconazole
3	Tilt 250 EC	Syngenta Bangladesh Ltd.	Propiconazole
4	Ridomil Gold MZ 68 WG	Syngenta Bangladesh Ltd.	Mancozeb + Metalaxyl
5	Dithane M-45 WP	Bayer Crop Science Limited	Mancozeb
6	Cabrio Top	BASF Bangladesh Limited	Pyraclostrobin +Metiram
7	Autostin 50 WDG	Auto Crop Care Ltd.	Carbendazim

3.6 Treatments of the Experiment

In total nine (9) treatments were considered in this experiment. These were as follows:

T_0= Control

T_1= Amister Top 325 SC

T_2= Acibin 28 SC

T_3= Tilt 250 EC

T_4= Ridomil Gold

T_5= Dithane M-45

T_6= Cabrio Top

T_7= Autostin

T_8= *Trichoderma harzianum*

3.7 Layout and design

The experiment comprised 9 treatments and laid out in Randomized Complete Block Design (RCBD) with three replications. The whole experimental plot was divided into three equal blocks and each block consisted of 9 plots, altogether 27 unit plots. Each plot was 4 m^2 (2 m × 2 m) in size. The distance between plot to plot was 50 cm and plant to plant was 50 cm, and line to line was 50 cm. (Figure 3)

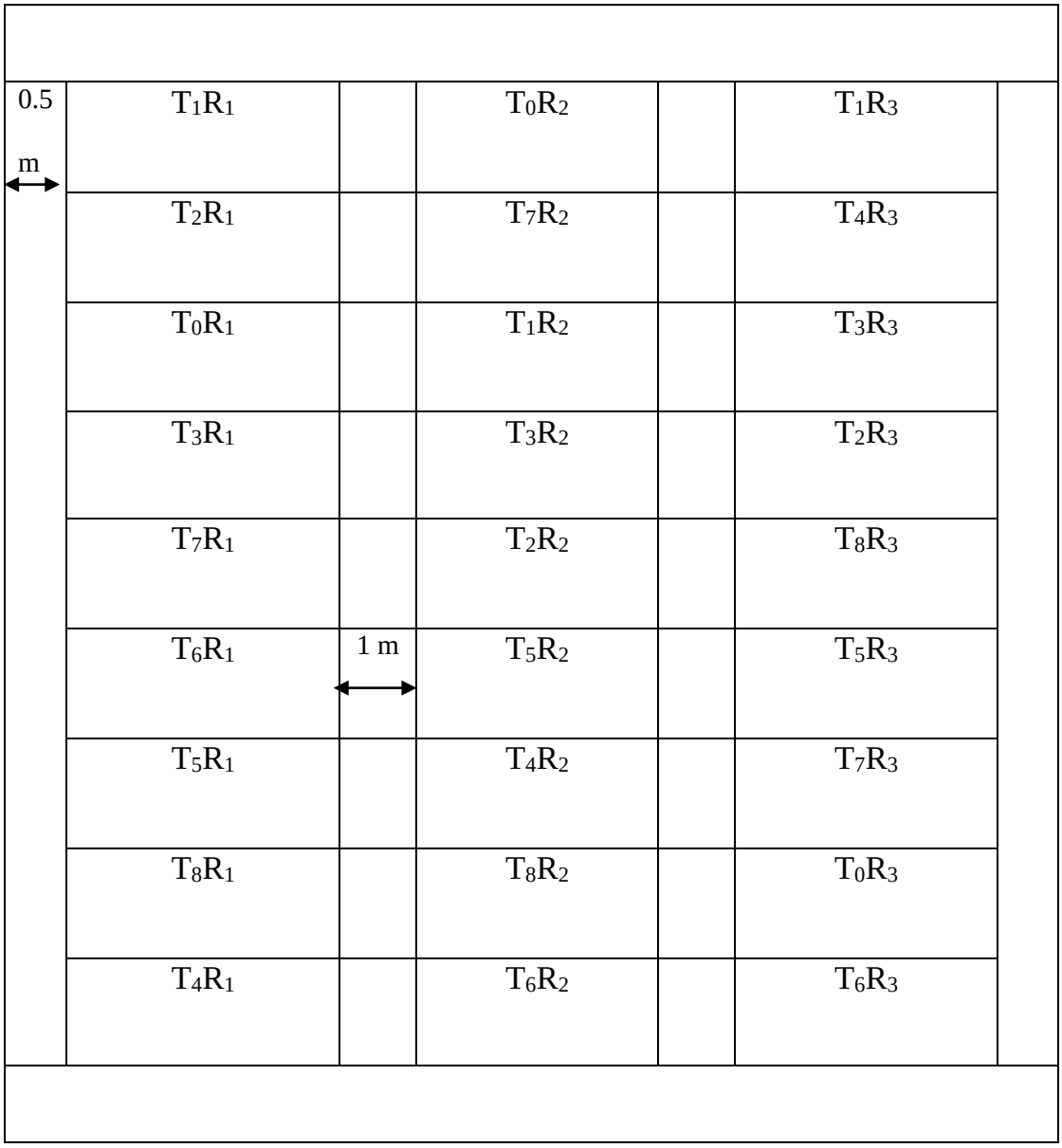


Figure 3. Layout and design

T_0= Control, T_1= Amister Top 325 SC, T_2= Acibin, T_3= Tilt 250 EC, T_4= Ridomil Gold, T_5= Dithane M-45, T_6= Cabrio Top, $T_{7=}$ Autostin, T_8= *Trichoderma harzianum*

3.8 Manure and fertilizer application

The following doses of manure and fertilizers were used (BARC- 2012).

Manure/fertilizer	Dose/ha
Cowdung	250 kg
Urea	6 kg
TSP	3 kg
MP	6 kg
Gypsum	3 kg
Zinc Sulphate	0.25 kg
Boric Acid	0.5 kg

3.9 Preparation of *Trichoderma harzianum* suspension

At first a jar was taken and filled with sufficient amount of water, then half of the radial growth of *Trichoderma harzianum* culture was cut with a sharp sterilized knife and put into the jar. Proper suspension was formed with shaker, then filtered it with a strainer. The suspension was poured into a hand sprayer for application to the collar region of the tested plants.

3.10 Cultivation of sugarbeet

The plants were always kept under close observation. Necessary intercultural operations were done throughout the cropping season to obtain proper growth and development of the sugarbeet plants.

3.10.1 Land preparation

The selected land for the experiment was first opened in October, 2019 by power tiller and expose to the sun for a week. After one week the land was ploughed

and cross-ploughed several times with a power tiller and laddering was done to obtain good tilth. Weeds and stubbles were removed and the large clods were broken into smaller pieces to obtain a desirable tilth of soil for sowing of seeds. After removal of the weeds, stubbles and dead roots, the land was leveled and the experimental plot was separated into the unit plots. Cowdung was applied as per production technology of sugarbeet before land preparation and one-third of the Urea and MP fertilizer were applied during land preparation. The remaining fertilizers were applied during earthing up as a form of top dressing.

3.10.2 Sowing of seeds

Sugarbeet crops were raised in ridges situated on a relatively high. The area was well prepared with spade and made into loose, friable and dried mass to obtain fine tilth. All weeds and stubbles were removed and the soil was mixed with well decomposed cow dung. The seeds were sown on 5 November 2019, maintaining line to line and plant to plant distance 50 cm and 50 cm, respectively. There were 6 pits in a line and 3 seeds were sown in a pit (Figure 4). After sowing, the seeds were covered with light soil to a depth of about 0.6 cm. Flooded irrigation was given on the next day by using a long pipe in order to trigger the germination process. Complete germination of the seeds happened within 4-6 days of sowing. Weeding, thinning and irrigation were done from time to time as and when needed. In order to gap filling and to check the border effect, some extra seeds were also sown around the border area of the experimental field.

A

B

Figure 4. Field layout (A) and Sowing of seed (B)

3.10.3 Intercultural operations

The following intercultural operations were done during the period of the experiment.

3.10.3.1 Thinning

Thinning was done at 30 DAS. Keeping one plant in one pit is important for proper growth and getting desirable yield. It is also important for proper collection of data.

3.10.3.2 Gap filling

Gap filling was done in place of dead or wilted seedlings in the field using healthy seedlings of the same stock previously planted in the border area.

3.10.3.3 Weeding

Weeding was accomplished as and whenever necessary to keep the crop free from weeds, for better soil aeration and to break the soil crust. Four subsequence weeding were done manually at 15, 30, 45 and 60 DAS to keep the plots free from weeds.

3.10.3.4 Top dressing and earthing up

In sugarbeet field, first Urea and MP top dressing and earthing up were done at 30 DAS and last were done at 60 DAS (Figure 5). Have to be careful that the soil should not cover the collar region of the plant during earthing up. After top dressing and earthing up, light irrigation was applied in order to mix the fertilizer throughly with soil.

Figure 5. Earthing up

3.10.4 Irrigation

Flooded and light irrigations were given throughout the growing season as and when necessary. (Figure 6)

Figure 6. Flooded irrigation

3.10.5 Spraying

Spraying selected modern phyto-chemicals and *Trichoderma harzianum* for 3 times at 30, 45 and 60 days respectively. (Figure 7)

Figure 7. Spraying of phyto-chemicals and *Trichoderma harzianum*

3.11 Identification and estimation of disease incidence (%) and disease severity (%)

Identification of the sclerotium root rot disease was done on the basis of visual observation of typical symptoms like yellowing and wilting of leaves followed by rotting of roots of affected plants. On rotted roots, innumerable small, light to mustard seed like dark brown sclerotia develop on the mycelia (Mukhopadhayay, 1971; Sharma and Pathak, 1994; Waraitch *et al.*, 1986). The incidence of sclerotium root rot disease was calculated by counting the infected plants at 65, 80 and 95 and 110 DAS on the basis of the appearance of typical symptoms.

The following formulas were used to calculate the percent disease incidence,

$$\text{Disease incidence (\%)} = \frac{Xi}{X} \times 100$$

X= Total number of plants in a unit

Xi= Number of infected plants in a unit plot

Disease severity was calculated by Townsend- Heuberger (2004) formula:

$$\text{Disease severity (\%)} = \frac{n \times V}{Z \times N} \times 100$$

Where,

n = Number of selected plants with different disease severity in the scale

V = Scale value

Z = The highest scale value

N = Observed total number of plants

For calculation of disease severity, the following disease severity scale was used (Weiland and Koch, 2004):

0 – Whole plant is healthy

1 – Onset of disease; Appearance of first strains on outer leaves

2 – Increase in number of strains on outer leaves

3 – The strains also appeared on the intermediate leaves outside the central leaves

4 – Spots coming together apparently

5 – Large dead areas on the leaves

6 – Large dead areas on the leaves

7- Dead parts in at least half or more of the palms of the outer leaves

8- Dead leaves in almost all of the outer leaves and large dead areas in the middle leaves

9 – Forming new leaves in plants

3.12 Parameter assessed

The following parameters were recorded-

- Disease incidence (%)
- Disease severity (%)
- Individual beet diameter (cm)
- Individual beet weight (kg)
- Yield per plot (kg)
- Brix (%)
- Sucrose/Pol (%)

3.13 Isolation of causal organism

3.13.1 Collection of sclerotia

Mustard seed like brown sclerotia were collected from the collar region of infected sugarbeet plant. Collected sclerotia were put in tissue paper immediately after collection to protect them from drying. (Figure 8)

Figure 8. Diseased collar region of a sugarbeet plant

3.13.2 Sterilization of materials and equipment's

Liquid materials, like media and water were sterilized in an autoclave 121^{o}C and 15 pound per square inch (p.s.i.) for 20 min. At first plant parts and soil were separated from collected sclerotia. Then the sclerotia were surface sterilized with 1% chlorox for 5 minutes, and rinsed with sterilized water for 3 times. Rectified spirit was used for other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

3.13.3 Transfer of sterilized sclerotia on PDA media for isolation

Sterilized sclerotia were plated on autoclaved PDA media in 90 mm petridishes and incubated at room temperature followed by of 25±1° C for 7-10 days and examined daily for any fungal growth. A mycelial block (5 mm dia) was transferred to another PDA plate and incubated in same incubation condition. After 10-15 days, mustard seed like brown sclerotia were formed.

3.14 Measurement of Brix percentage

Brix (%) refers to the total soluble solids in beet juice. Five beets were collected from each plot randomly. Then the beets were washed throughly by tap water and then kept for 30 minutes for drying. After drying, brix (%) was measured by hand refractometer manually. Total procedures are presented in figure 9.

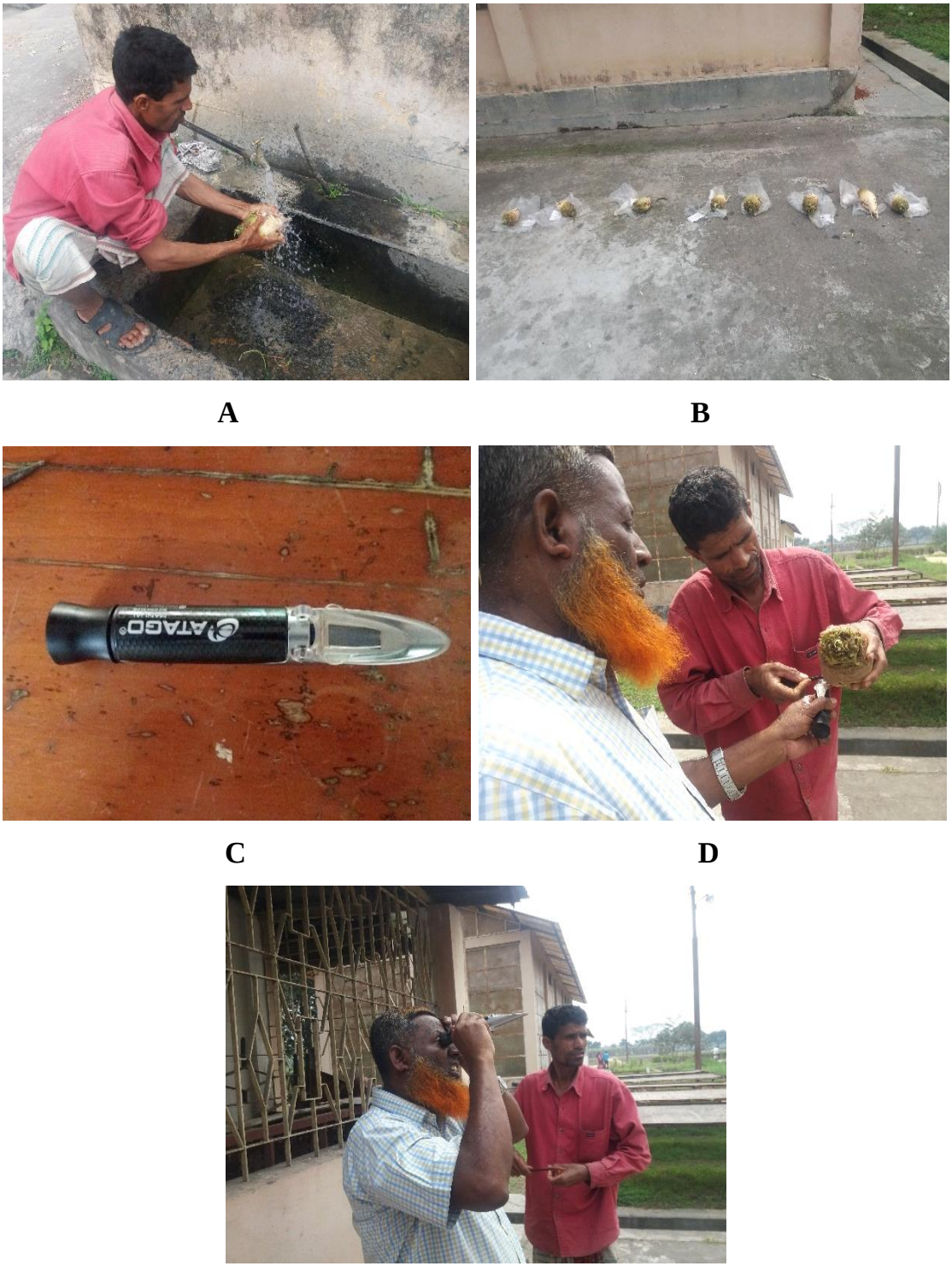


Figure 9. Washing of Sugarbeet (A). Sugarbeet kept for drying (B). Hand refractometer (C). Collection of beet juice (D). Observing the brix reading through refractometer lens (E)

3.15 Measurement of Sucrose/Pol percentage

Sucrose/Pol (%) refers to percentage of sucrose content in beet juice. It had been determined by using Automatic Polarimeter (Model: ATAGO AP-300) standardized at 20°C by Horne's dry lead method at Physiology and Sugar Chemistry Division of BSRI, Ishurdi, Pabna. Sucrose (%) was estimated in fresh samples of sugarbeet roots, polarimeterically on a lead acetate extract of fresh macerated root according to Le Docte (1927). Five beets were collected from each plot randomly and was crushed with a mini power crusher for juice extraction. The juice was then clarified with basic lead sub-acetate and after filtration it had been poured in 200 mm polarimeter tube for determination of pol (%) of beet (Anon., 1970). Total procedures are presented in figure 10.

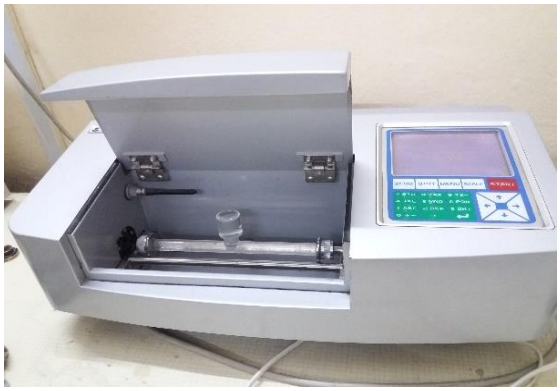
A

B

C

D

E



F

Figure 10. Slicing of sugarbeet pieces through mini sugarbeet slicer (A). Blending of sugarbeet slices to make beet juice (B). Mixing basic lead acetate into beet juice (C). Filtered the solution through whatman no. 1 (D). ATAGO AP-300 Atomatic Polarimeter (E). Filtered solution was transferred to 200mm pol tube and placed inside the polarimeter to measure the sugar content (F)

3.16 Pathogenicity test

Pathogenicity test was done in the net house of Department of Plant Pathology, Sher-e- Bangla Agricultural University, Dhaka-1207. For this test, Pots were filled with sun dried soil. Then seeds were sown in each pot and sufficient amount of water was given after sowing in order to facilitate germination process. After germination, every 3 days, sufficient water was provided. After 45 days of sowing, when the seedlings were at three to four leaf stage then inoculation was done with sclerotia. Each plant was inoculated with the test pathogen isolate. Just after inoculation, the pots were wrapped with polythene bag for successful infection as well as to seal moisture. Nearly 95% humidity for 24 hours give better inoculation (Sreenivasaprasad *et al*., 2005). After 3 days, the polythene bags were removed. Then waited for disease symptoms on the leaves and sclerotia on the roots. Total steps are clearly shown in figure 11.

a. Diseased portion of plant

b. Pure culture of *Sclerotium rolfsii*

c. Wrapped with polythene bag after inoculation

d. Diseased plant

Figure 11: Steps involved in pathogenicity test for *Sclerotium rolfsii*

3.17 Statistical Analysis

The field trials were conducted following Randomized Complete Block Design (RCBD). The replicated data generated from different experiments were tabulated and analyzed by using STATISTIX-10 software. Treatment means were compared by LSD range test at 5% level of significance.

RESULTS

The experiment was conducted to manage sugarbeet against sclerotium root rot disease using different suitable phyto- chemicals viz. Amister Top 325 SC, Acibin, Tilt 250 EC, Ridomil Gold, Dithane M-45, Cabrio Top, Autostin and a bio- control agent *Trichoderma harzianum* under field condition. Results were compiled based on disease incidence and severity, morphological and physiological parameters at 65, 80, 95 and 110 days after sowing (DAS) are presented in this chapter.

4.1 Effect of selected modern phyto- chemicals and *Trichoderma harzianum* on disease incidence (%) of sclerotium root rot disease in sugarbeet at 65, 80, 95 and 110 days after sowing (DAS)

Disease incidence was recorded per plot after application of different treatments viz., T_0 (Control), T_1 (Amister Top 325 SC), T_2 (Acibin), T_3 (Tilt 250 EC), T_4 (Ridomil Gold), T_5 (Dithane M-45), T_6 (Cabrio Top), T_7 (Autostin), T_8 (Trichoderma) in Sugarbeet line HI 0044 against sclerotium root rot disease. At 65 DAS, the highest disease incidence (22.22%) was recorded in T_0 (Control) followed by T_8 (*Trichoderma harzianum*), T_5 (Dithane M-45), T_6 (Cabrio Top), T_7 (Autostin) and T_1 (Amister Top 325 SC) that were 19.45%, 18.06%, 18.05%, 18.05% and 15.28% respectively, which were statistically similar with each other. The lowest disease incidence (12.50%) was estimated in T_2 (Acibin) which was statistically different from all the treatments. Among the selected treatments,

treatment T_3 (Tilt 250 EC) and T_4 (Ridomil Gold) showed moderate disease incidence that was 13.89% which were statistically similar with each other.

At 80 DAS, the highest disease incidence (26.39%) was recorded in T_0 (Control) followed by T_8 (*Trichoderma harzianum*) and T_6 (Cabrio Top) that was 26.39% and 22.22% respectively, which were statistically similar with each other. The lowest disease incidence (13.89%) was estimated in T_2 (Acibin) preceded by T_1 (Amister Top 325 SC) and T_3 (Tilt 250 EC) that were 15.28%, which were statistically similar with each other. Among the selected treatments, treatment T_4 (Ridomil Gold), T_5 (Dithane M-45) and T_7 (Autostin) showed moderate disease incidence that were 19.45%, 20.83%, 20.84% respectively, which were statistically similar with each other.

At 95 DAS, the highest disease incidence (52.78%) was found in T_0 (Control) which was statistically different from all the treatments. The lowest disease incidence (13.89%) was recorded in T_2 (Acibin) preceded by T_1 (Amister Top 325 SC) and T_3 (Tilt 250 EC) that were 13.89%, 15.28% and 16.67% respectively, which were statistically similar with each other. Among the selected treatments, Treatment T_5 (Dithane M-45), T_6 (Cabrio Top), T_4 (Ridomil Gold), T_7 (Autostin) and T_8 (*Trichoderma harzianum*) showed moderate disease incidence that were 25.00%, 27.78%, 27.78%, 33.34% and 33.34% respectively, which were statistically similar with each other.

At 110 DAS, the highest disease incidence (56.94%) was recorded in T_0 (Control) which was statistically different from all the treatments. The lowest disease incidence (13.89%) was found in T_2 (Acibin) preceded by T_1 (Amister

Top 325 SC) and T_3 (Tilt 250 EC) that was 16.67%, which were statistically similar with each other. Among the selected treatments, treatment T_5 (Dithane M-45), T_6 (Cabrio Top), T_4 (Ridomil Gold), T_7 (Autostin) and T_8 (*Trichoderma harzianum*) showed moderate disease incidence that were 26.39%, 27.78%, 29.17%, 34.72% and 36.11% repectively, which were statistically similar with each other. All results are presented in table 2 and figure 12 and 13.

A

B

Figure 12. Disease Incidence (%) in Control plot at 95 DAS (A) and 110 DAS (B)

A

B

Figure 13: Disease Incidence (%) in Amister Top 325 SC treated plot at 95 DAS (A) and 110 DAS (B)

Table 2: Disease Incidence (%) of sclerotium root rot disease in sugarbeet under different treatments at 65, 80, 95 and 110 DAS

Treatments	Disease Incidence (%) of Sclerotium root rot disease at			
	65 DAS	**80 DAS**	**95 DAS**	**110 DAS**
T_0	22.22 a	26.39 a	52.78 a	56.94 a
T_1	15.28 ab	15.28 bc	15.28 d	16.67 cd
T_2	12.50 b	13.89 c	13.89 d	13.89 d
T_3	13.89 ab	15.28 bc	16.67 cd	16.67 cd
T_4	13.89 ab	19.45 abc	27.78 bc	29.17 b
T_5	18.06 ab	20.83 abc	25.00 bcd	26.39 bc
T_6	18.05 ab	22.22 ab	27.78 bc	27.78 bc
T_7	18.05 ab	20.84 abc	33.34 b	34.72 b
T_8	19.45 ab	26.39 a	33.34 b	36.11 b
CV (%)	31.81	22.12	25.68	22.89

T_0= Control, T_1= Amister Top 325 SC, T_2= Acibin, T_3= Tilt 250 EC, T_4= Ridomil Gold, T_5= Dithane M-45, T_6= Cabrio Top, T_7= Autostin, T_8= *Trichoderma harzianum*

In the present study considering the disease incidence (%), it was observed that among the selected phyto-chemicals, Acibin and Tilt 250 EC gave the best result in controlling the sclerotium root rot disease of sugarbeet in field condition, but both chemicals greatly affected in growth, yield and yield contributing characters. Almost all plants were become stunted and the leaves become shrinked as clearly shown in figure 14.

A

B

Figure 14. Stunted plants in Acibin treated plot (A) and Tilt 250 EC treated plot (B)

4.2 Effect of selected modern phyto- chemicals and *Trichoderma harzianum* on disease severity (%) of sclerotium root rot disease in sugarbeet at 65, 80, 95 and 110 days after sowing (DAS)

Disease severity was recorded per plot after application of different treatments viz., T_0 (Control), T_1 (Amister Top 325 SC), T_2 (Acibin), T_3 (Tilt 250 EC), T_4 (Ridomil Gold), T_5 (Dithane M-45), T_6 (Cabrio Top), T_7 (Autostin), T_8 (Trichoderma) in Sugarbeet line HI 0044 against sclerotium root rot disease. At 65 DAS, the highest disease severity (7.10%) was recorded in T_5 (Dithane M-45) and T_7 (Autostin) followed by, T_6 (Cabrio Top), T_0 (Control), T_8 (*Trichoderma harzianum*) and T_3 (Tilt 250 EC) that were 6.02%, 5.86%, 5.09% and 4.32% respectively, which were statistically similar with each other. The lowest disease severity (2.93%) was estimated in T_2 (Acibin) preceded by T_1 (Amister Top 325 SC) and T_4 (Ridomil Gold) that were 3.52% and 3.55% respectively, which were statistically similar with each other.

At 80 DAS, the highest disease severity (10.50%) was found in T_7 (Autostin) followed by T_5 (Dithane M-45), T_0 (Control), T_8 (*Trichoderma harzianum*) and T_6 (Cabrio Top) that were 8.64%, 8.18%, 7.87% and 7.41% which were statistically similar with each other. The lowest disease severity (3.70%) was estimated in T_2 (Acibin) preceded by T_1 (Amister Top 325 SC), T_3 (Tilt 250 EC) and T_4 (Ridomil Gold) that were 4.01%, 4.63% and 5.40% respectively, which were statistically similar with each other.

At 95 DAS, the highest disease severity (12.65%) was recorded in T_7 (Autostin) followed by T_0 (Control) and T_5 (Dithane M-45) and T_8 (*Trichoderma*

harzianum) that were 12.04%, 9.57% and 8.80% respectively, which were statistically similar with each other. The lowest disease severity (4.01%) was estimated in T_1 (Amister Top 325 SC) preceded by T_2 (Acibin) and T_3 (Tilt 250 EC) that were 4.17% and 4.63% respectively, which were statistically similar with each other. Among the selected treatments, treatment T_6 (Cabrio Top) and T_4 (Ridomil Gold) showed moderate disease severity that were 7.72% and 7.41% respectively, which were statistically similar with each other.

At 110 DAS, the highest disease severity (14.20%) was estimated in T_0 (Control) followed by T_7 (Autostin) and T_5 (Dithane M-45) that were 13.43%, 11.57% respectively, which were statistically similar with each. The lowest disease severity (4.63%) was found in T_1 (Amister Top 325 SC) and T_2 (Acibin) followed by T_3 (Tilt 250 EC) that was 5.40% which were statistically similar with each other. Among the selected treatments, treatment T_6 (Cabrio Top), T_8 (*Trichoderma harzianum*) and T_4 (Dithane M-45) showed moderate disease severity that were 8.33%, 9.57% and 9.72% respectively, which were statistically similar with each other. All results are presented in Table 3.

Table 3: Disease Severity (%) of sclerotium root rot disease in sugarbeet under different treatments at 65, 80, 95 and 110 DAS

Treatments	Disease Severity (%) of Sclerotium root rot disease at			
	65 DAS	**80 DAS**	**95 DAS**	**110 DAS**
T_0	5.86 ab	8.18 ab	12.04 ab	14.20 a
T_1	3.52 b	4.01 de	4.01 e	4.63 d
T_2	2.93 b	3.70 e	4.17 de	4.63 d
T_3	4.32 ab	4.63 cde	4.63 de	5.40 cd
T_4	3.55 b	5.40 bcde	7.41 cde	9.72 b
T_5	7.10 a	8.64 ab	9.57 abc	11.57 ab
T_6	6.02 ab	7.41 abcd	7.72 cd	8.33 bc
T_7	7.10 a	10.50 a	12.65 a	13.43 a
T_8	5.09 ab	7.87 abc	8.80 bc	9.57 b
CV (%)	39.75	30.02	27.11	24.54

T_0= Control, T_1= Amister Top 325 SC, T_2= Acibin, T_3= Tilt 250 EC, T_4= Ridomil Gold, T_5= Dithane M-45, T_6= Cabrio Top, $T_{7=}$ Autostin, T_8= *Trichoderma harzianum*

In the present study considering the disease severity (%), it was observed that among the selected phyto-chemicals, Acibin and Tilt 250 EC gave the best result in controlling the sclerotium root rot disease of sugarbeet in field condition, but both chemicals greatly affected in growth, yield and yield contributing characters. Almost all plants were become stunted and the leaves become shrinked as clearly shown in figure 15.

A

B

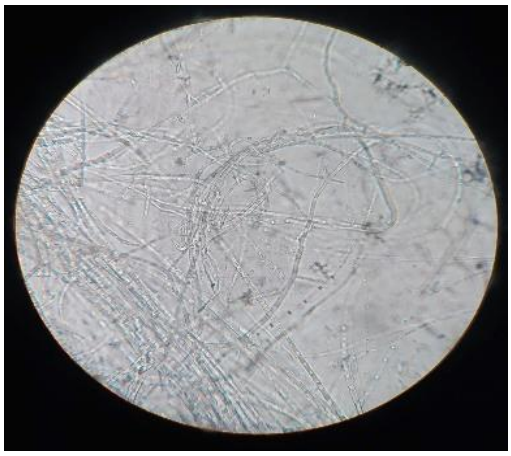
Figure 15. Stunted plant on Acibin treated plot (A) and Tilt 250 EC treated plot (B)

4.3 Isolation, identification and characterization of *Sclerotium rolfsii*

For isolation of causal organism of sclerotium root rot disease of sugarbeet, mature sclerotia were collected from infected plant and transfer on PDA media. After the mycelial growth, the cultural characteristics were studied; white cottony thread like mycelia were formed on PDA media after 10-15 days. At first sclerotia were cottony in color that was turned dark brown to black while mature as shown in figure 16.

A

B



C

Figure 16: Sclerotia formed at the collar region (A). Pure culture of *S. rolfsii* showing mature sclerotia (B). Microscopic view of *Sclerotium rolfsii* (C)

4.4 Individual beet diameter (cm), weight (kg) and yield per plot (kg)

The highest individual beet diameter (41 cm) was obtained in T_1 (Amister Top 325 SC) followed by T_6 (Cabrio Top), T_7 (Autostin) and T_5 (Dithane M-45) that were 33.58 cm, 32.58 cm and 32.28 cm respectively, these were statistically similar with each other but statistically different from T_1 (Amister Top 325 SC) treatment. The lowest beet diameter (19.75%) was obtained in T_0 (Control) treatment preceded by T_2 (Acibin), T_3 (Tilt 250 EC) and T_8 (*Trichoderma harzianum*) that were 24.94 cm, 25.87 cm and 25.99 cm respectively, these were statistically similar with each other but different from T_0 (Control) treatment. The moderate individual beet diameter (29.58 cm) was found in T_4 (Ridomil Gold).

The highest individual beet weight (1.02 kg) was recorded in T_1 (Amister Top 325 SC) followed by T_6 (Cabrio Top), T_7 (Autostin) that was 0.85 kg these were statistically similar with each other but different from T_1 (Amister Top 325 SC). The lowest individual beet weight (0.51 kg) was found in T_0 (Control) treatment preceded by T_2 (Acibin), T_3 (Tilt 250 EC) and T_8 (*Trichoderma harzianum*) that were 0.63 kg, 0.67 kg and 0.69 kg respectively, these were statistically similar with each other but different from T_0 (Control) treatment. The moderate individual beet weight was found in T_4 (Ridomil Gold) and T_5 (Dithane M-45) treatment that were 0.76 kg and 0.78 kg respectively, these were statistically similar with each other.

The highest yield per plot (24.40 kg) was recorded in T_1 (Amister Top 325 SC) followed by T_6 (Cabrio Top), T_7 (Autostin) that was 20.40 kg these were statistically similar with each other but different from T_1 (Amister Top 325 SC)

treatment. The lowest yield per plot (12.16 kg) was found in T_0 (Control) treatment preceded by T_2 (Acibin), T_3 (Tilt 250 EC) and T_8 (*Trichoderma harzianum*) that were 15.20 kg, 16.00 kg and 16.56 kg respectively, these were statistically similar with each other but different from T_0 (Control) treatment. The moderate yield per plot was obtained in T_4 (Ridomil Gold) and T_5 (Dithane M-45) that were 18.16 kg and 18.80 kg respectively, these were statistically similar with each other. All results are presented in Table 4.

Table 4: Individual beet diameter, weight and yield per plot

Treatment	Diameter (cm)	Weight (kg)	Yield (kg)
T_0	19.75 e	0.51 e	12.16 e
T_1	41.00 a	1.02 a	24.40 a
T_2	24.94 d	0.63 d	15.20 d
T_3	25.87 d	0.67 d	16.00 d
T_4	29.58 c	0.76 c	18.16 c
T_5	32.28 b	0.78 c	18.80 c
T_6	33.28 b	0.85 b	20.40 b
T_7	32.58 b	0.85 b	20.40 b
T_8	25.99 d	0.69 d	16.56 d
CV (%)	3.27	4.84	4.84

T_0= Control, T_1= Amister Top 325 SC, T_2= Acibin, T_3= Tilt 250 EC, T_4= Ridomil Gold, T_5= Dithane M-45, T_6= Cabrio Top, T_7= Autostin, T_8= *Trichoderma harzianum*

Although the percent disease incidence and severity were low in Amister Top 325 SC, Acibin and Tilt 250 EC treated plot, but the individual beet weight and total yield per plot was not satisfactory in Acibin and Tilt 250 EC treated plot as clearly shown in figure 17,18 and 19.

Figure 17. Randomly taken five plants from Amister Top 325 SC treated plot

Figure 18. Randomly taken five plants from Acibin treated plot

Figure 19. Randomly taken five plants from Tilt 250 EC treated plot

4.5 Effect of selected modern phyto- chemicals and *Trichoderma harzianum* on brix (%) and sucrose/pol (%) of beet per plot

The highest brix (17.8%) was found in T_1 (Amister Top 325 SC) followed by T_5 (Dithane M-45) and T_6 (Cabrio Top) that was 16.9%. The lowest brix (11.5%) was found in T_0 (Control) treatment. Almost similar brix (%) was measured in the remaining treatments.

The highest pol (12.7%) was found in T_1 (Amister Top 325 SC) followed by T_5 (Dithane M-45), T_6 (Cabrio Top), T_4 (Ridomil Gold) and T_6 (Autostin) that were 12.68%, 12.62%, 12.45% and 12.05% respectively. The lowest pol (6.53%) was found in T_0 (Control) treatment. Almost similar pol (%) was measured in the remaining treatments. All results are graphically presented in figure 20.

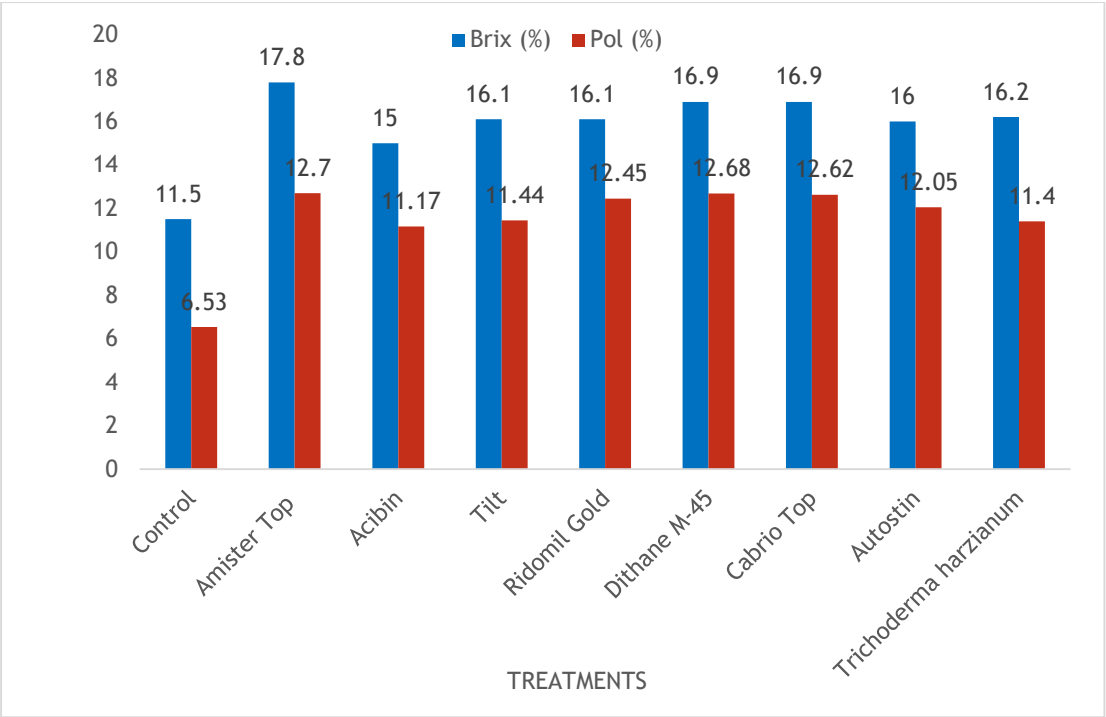


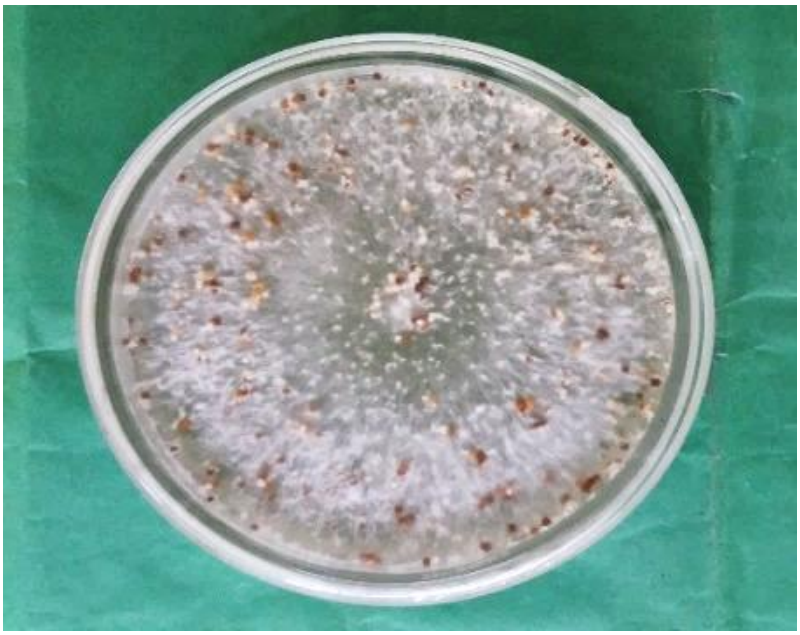
Figure 20: Graphical presentation of brix (%) and sucrose/pol (%) of beet per plot

4.6 Pathogenicity test

In order to prove the pathogenic nature of *Sclerotium rolfsii* producing sclerotium root rot disease in sugarbeet, Koch's postulates test was performed. After 7/8 days of inoculation, first visible symptoms include yellowing and wilting of leaves followed by rotting of roots of affected plants. White cottony mycelium developed on rotted basal portions of roots and causes gradual semi-watery decay. As the mycelial growth advances, the affected leaves turned yellow and wither pre-maturely. At later stage mycelial growth becomes more profuse and almost covers the key portions of the fleshy root. On rotted roots, innumerable small, light to dark brown sclerotia of mustard seed like developed on the mycelium. The sclerotia sample was placed in PDA media and kept in aseptic condition. It developed white cottony mycelia in the plate and observed

under light microscope. So, the re-isolated pathogen from the net house infected plants produced similar type of growth and spore characteristics that was found in first isolation of the pathogen *in vitro*. The results are shown in figure 21.

A



B

Figure 21. Visible sclerotia at the collar region in inoculated sugarbeet plant (A). Re-isolated *Sclerotium rolfsii* from inoculated infected plant showing mature sclerotia (B)

4.7 Relationship between individual beet diameter (cm) and Disease Incidence (%)

Different treatments that were used in the present study regarding individual beet diameter (cm) and disease incidence (%), it was revealed that individual beet diameter (cm) was increased with the decreased of disease incidence (%). The highest individual beet diameter (41 cm) was found in T_1 (Amister Top 325 SC) where control plot showed the lowest beet diameter that was 19.75 cm as depicted in the figure 22.

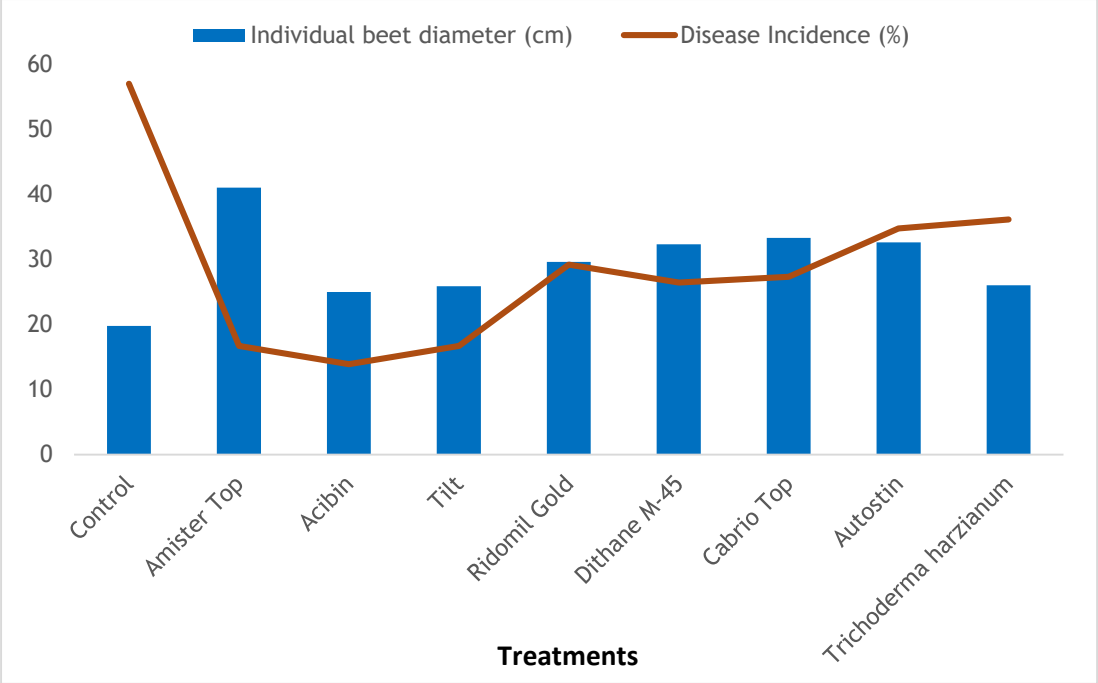


Figure 22: Relationship between individual beet diameter (cm) and Disease Incidence (%)

4.8 Relationship between individual beet diameter (cm) and Disease Severity (%)

Different treatments that were used in the present study regarding individual beet diameter (cm) and disease severity (%), it was revealed that individual beet diameter (cm) was increased with the decreased of disease severity (%). The highest individual beet diameter (41 cm) was found in T_1 (Amister Top 325 SC) where control plot showed the lowest beet diameter that was 19.75 cm as depicted in the figure 23.

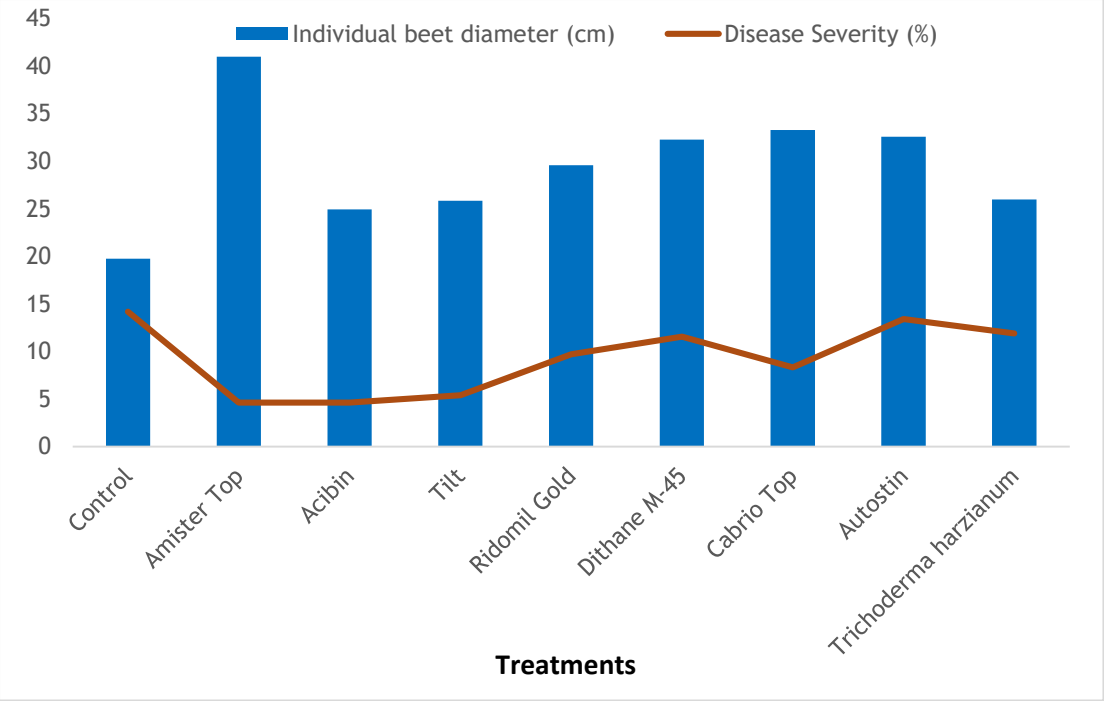


Figure 23: Relationship between individual beet diameter (cm) and Disease Severity (%)

4.9 Relationship between individual beet weight (kg) and Disease Incidence (%)

Different treatments that were used in the present study regarding individual beet weight (kg) and disease incidence (%), it was revealed that individual beet weight (kg) was increased with the decreased of disease incidence (%). The highest individual beet weight (1.02 kg) was found in T_1 (Amister Top 325 SC) where control plot showed lowest beet weight that was 0.51 kg as depicted in the figure 24.

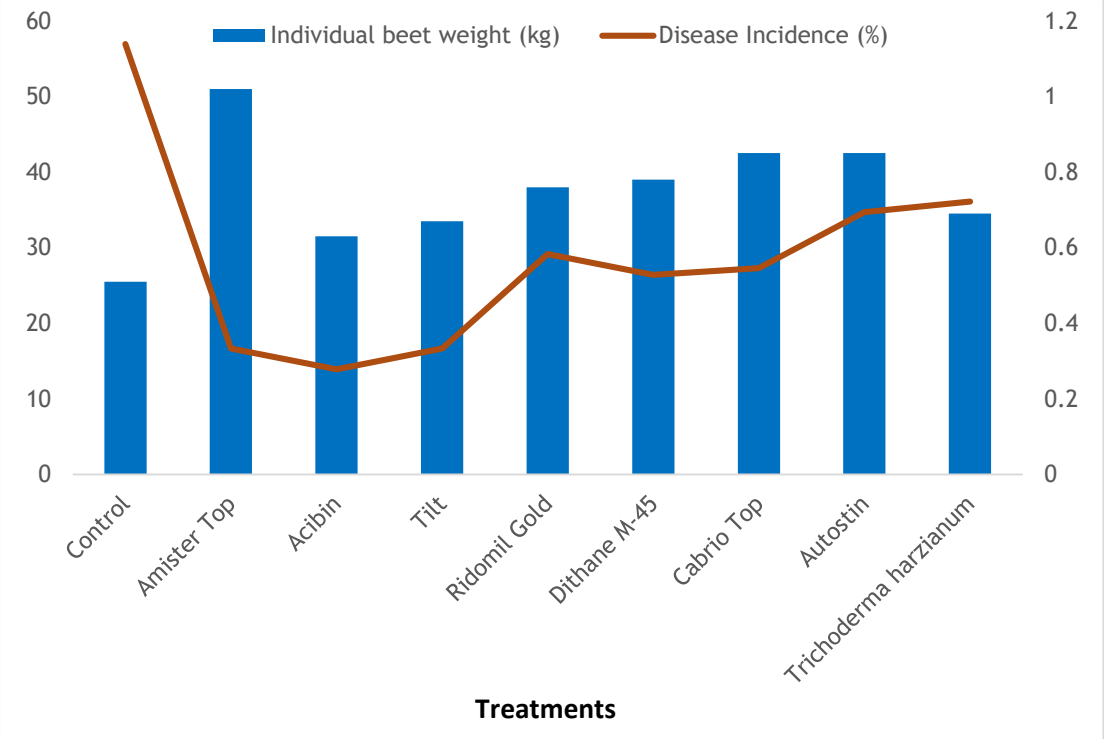


Figure 24: Relationship between individual beet weight (kg) and Disease Incidence (%)

4.10 Relationship between individual beet weight (kg) and Disease Severity (%)

Different treatments that were used in the present study regarding individual beet weight (kg) and disease severity (%), it was revealed that individual beet weight (kg) was increased with the decreased of disease severity (%). The highest individual beet weight (1.02 kg) was found in T_1 (Amister Top 325 SC) where control plot showed lowest beet weight that was 0.51 kg as depicted in the figure 25.

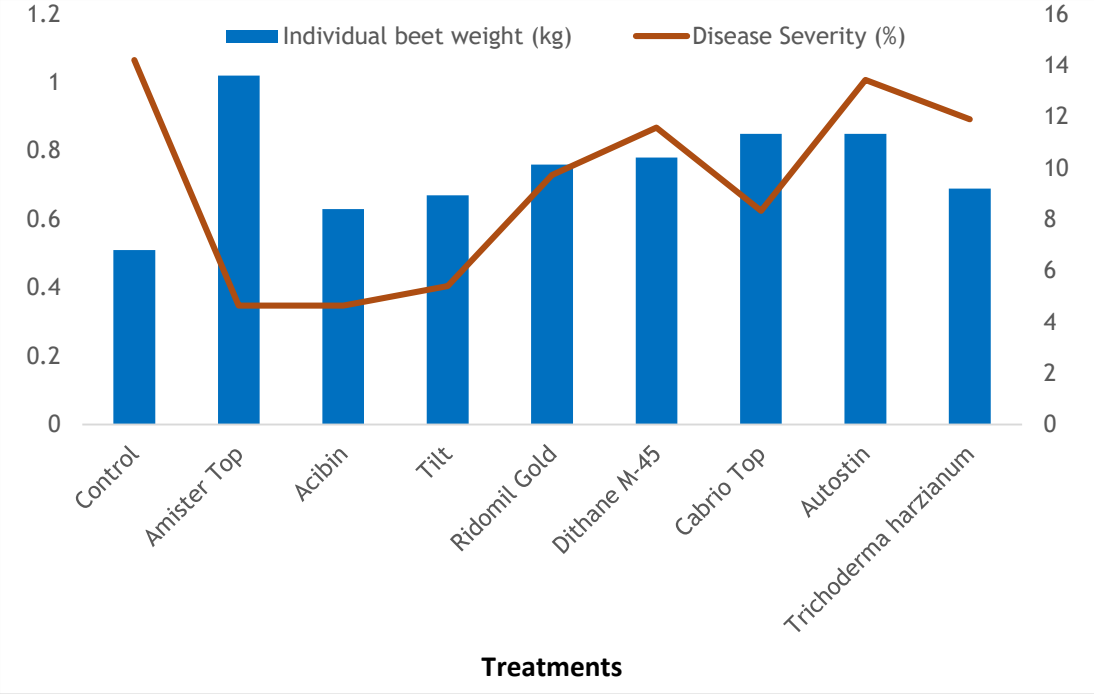


Figure 25: Relationship between individual beet weight (kg) and Disease Severity (%)

4.11 Relationship between yield per plot (kg) and Disease Incidence (%)

Different treatments that were used in the present study regarding yield per plot (kg) and disease incidence (%), it was revealed that yield per plot (kg) was increased with the decreased of disease incidence (%). The highest yield per plot (24.40 kg) was found in T_1 (Amister Top 325 SC) where control plot showed lowest yield that was 12.16 kg as depicted in the figure 26.

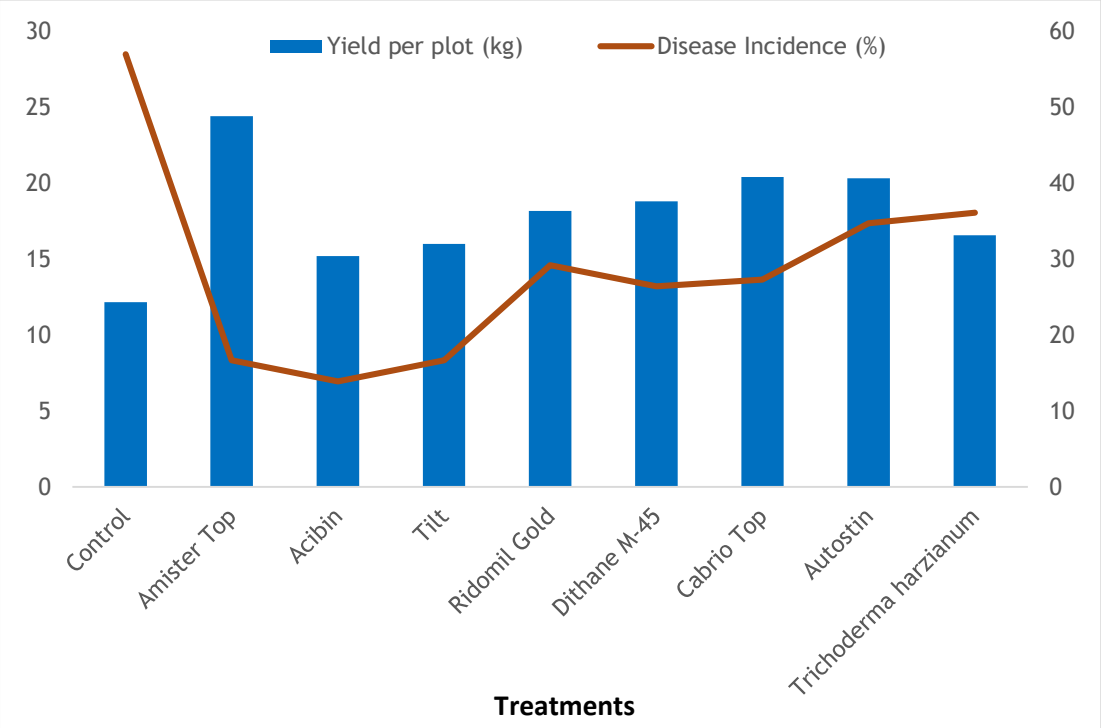


Figure 26. Relationship between yield per plot (kg) and Disease Incidence (%)

4.12 Relationship between yield per plot (kg) and Disease Severity (%)

Different treatments that were used in the present study regarding yield per plot (kg) and disease severity (%), it was revealed that yield per plot (kg) was increased with the decreased of disease severity (%). The highest yield per plot (24.40 kg) was found in T_1 (Amister Top 325 SC) where control plot showed lowest yield that was 12.16 kg as depicted in the figure 27.

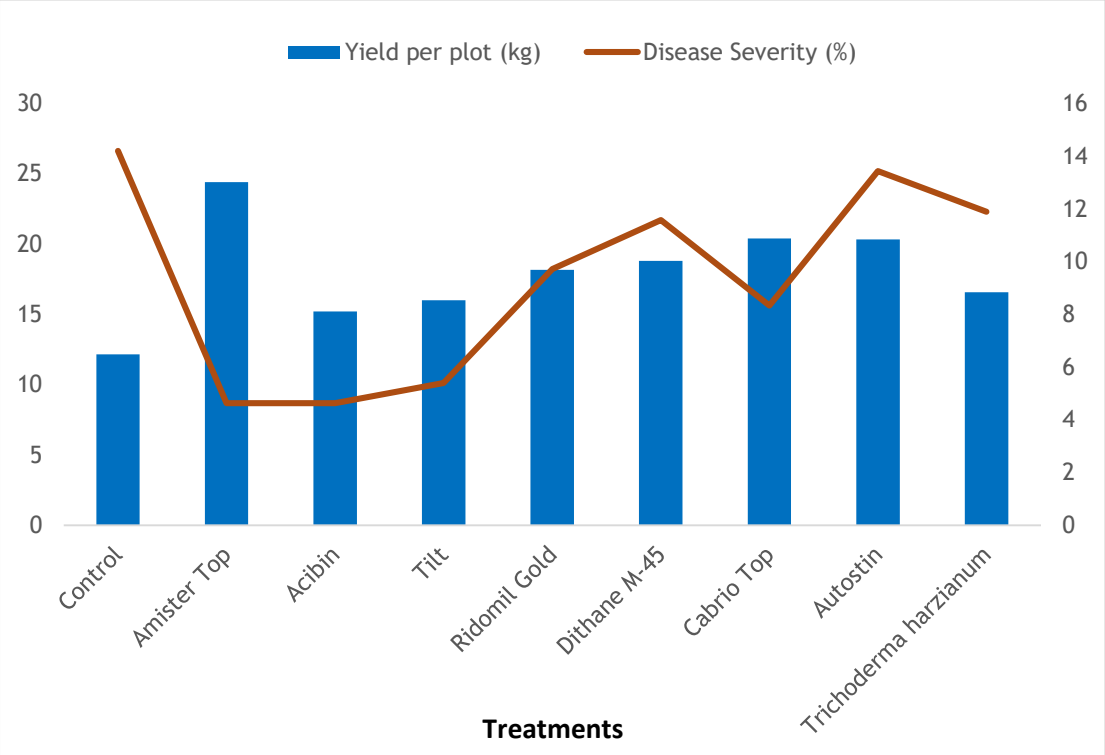


Figure 27. Relationship between yield per plot (kg) and Disease Severity (%)

DISCUSSION

Sugar beet *(Beta vulgaris L.*), belongs to the member of the Chenopodiaceae family is the second source of sugar in all over the world. It is a temperate crop whose root contains high concentration of sucrose and is successfully grown on a commercial scale for sugar production. The sugarbeet plant consists of the root and a rosette of leaves. In addition to sugar production, it can also be used as a vegetable. In Bangladesh, about 25% sugar demand meeting domestically from sugarcane and rest 75% sugar demand is fulfilled by importation (Rahman *et al.*, 2016). It matures within 5 to 6 months and its root contains 16-19% sucrose with a recovery of 12-14%. But the soil borne fungal pathogen *Sclerotium rolfsii* attacks sugarbeet roots a couple of weeks before harvest, causing up to 50% - 80% losses in crop yield and quality (Khettabi *et al*., 2004). It is difficult to manage root rot disease caused by *Sclerotium rolfsii* because of its wide host range and pervasive presence of sclerotia in tropical to subtropical areas. The objectives of the present study was to manage sclerotium root rot disease of sugarbeet through selected eight modern phyto-chemicals and one bio- agent, *Trichoderma harzianum*.

4.13 Disease Incidence and Severity

The disease incidence due to sclerotium root rot disease as affected by spraying modern phyto-chemicals and *Trichoderma harzianum* were estimated. From the present study, it was revealed that the highest disease incidence was found when no phyto- chemicals and bio- agent applied, and the lowest disease incidence was found in Amister Top 325 SC, Acibin and Tilt 250 EC treated plots in all

observations. It was also observed that the highest yield was found in Amister Top 325 SC treated plot, but yield was not found at satisfactory level in Acibin and Tilt 250 EC treated plots due to the chemical effects. Plants were severely affected and showed the stunted and shrinked symptoms. The lowest yield was was recorded in control treatment followed by *Trichoderma harzianum* treated plots. The moderate disease incidence was found in Ridomil Gold, Dithane M-45, Cabrio Top, Autostin and *Trichoderma harzianum* treated plots. In case of disease severity, the highest disease severity was found when no phyto-chemicals and bio-agent applied, as well as in Dithane M-45 and Autostin treated plots at 110 DAS. The lowest disease severity was found in Amister Top 325 SC, Acibin and Tilt 250 EC treated plots. These results regarding the percent disease incidence and severity are agreement with some previous studies. Mukhopadhyay and Thakur, (1971) reported that ridge soil drenching with Demosan (15 kg/ha) or Vitavax (2 kg/ha) can be minimized the percent disease incidence and severity and also increased root yield. Sharma *et al.* (1990) also reported that PCNB (10 kg/ha) as soil drench controlled the percent disease incidence and severity, and slightly improved the sucrose content. It is also reported that combined application of mustard or groundnut cakes at 50 q/ha (15 days prior to sowing) and PCNB at 15 kg/ha (as soil drench) also reduced the disease incidence of root rot of sugarbeet (Sen *et al.*, 1973). Agnihorti *et al.* (1975) screened a number of fungicides in controlling root rot of sugarbeet incited by *Sclerotium rolfsii* both *in vivo* and *in vitro.* In *In vivo,* Vitavax and Quintozene showed fungicidal and fungi static effect, while *in vitro,* Vitavax,

Demosan and PCNB were found effective in inhibiting the growth of *Sclerotium rolfsii.*

Sharma *et al.,* (1990) reported that Dithane M-45 has been evaluated as soil drench to manage the sclerotial root rot in fields of sugarbeet. But in this study, it was observed that Dithane M-45 showed the moderate disease incidence and the highest disease severity.

In this study, bio- agent, *Trichiderma harzianum* was used as a treatment and applied as soil drenching. From this study it was found that *Trichoderma harzianum* was not given the satisfactory result in field condition. Although the different previous study reported that *Trichoderma harzianum* gave the best result in controlling the sclerotium root rot disease in sugarbeet where they used the *Trichoderma harzianum* as a biofortified. (Grinstien *et al.* 1979; Srivastava and Tripathi, 1996; Chet and Inber 1994)

4.14 Individual beet diameter (cm), brix (%) and sucrose/pol (%)

In this study, individual beet diameter (cm), brix (%) and sucrose/pol (%) were also recorded using slide calipers, hand refractometer and automatic polarimeter (Model: ATAGO AP-300), respectively. The highest individual beet diameter was obtained from Amister Top 325 SC, Dithane M-45, Cabrio Top and Autostin treated plots. The lowest beet diameter was got in control treatment, Acibin, Tilt 250 EC and *Trichoderma harzianum* treated plots. The highest brix (%) was found in beets collected from Amister Top 325 SC, Dithane M-45 and Cabrio Top treated plots. The lowest brix (%) was found in beets that collected from control treatment. In case of sucrose/pol (%) of beet, the highest sucrose/pol (%)

was recorded from the beet which were collected from the Amister Top 325 SC, Ridomil Gold, Dithane M-45, Cabrio Top and Autostin treated plots. The lowest sucrose/pol (%) was got in beets collected from control treatment. One previous study that was conducted by Khan and Minhas (2006) and they also recorded the brix (%), pol (%), purity (%) and recoverable sucrose (%) in sugarbeet juice after harvest. Brix (%) refers to the total soluble solids while pol (%) refers to percentage of sucrose content in beet juice. Purity (%) refers to ratio of sucrose content (pol %) to the total soluble solids (brix %) in juice. In their study, five beets were selected from each plot at random and was crushed with a mini power crusher for juice extraction. The collected juice was poured into a glass cylinder and the brix (%) was determined by brix hydrometer. The same juice was clarified with basic lead sub-acetate and after filtration it was poured in 200 mm polarimeter tube for determination of pol (%) of beet. Sucrose (%) was estimated in fresh samples of sugarbeet roots, polarimeterically on a lead acetate extract of fresh macerated root according to Le Docte (1927).

4.15 Confirmation of causative agent through pathogenicity test

For confirmation of the causative agent, pathogen was isolated and pathogenicity test was performed. For isolation of causal organism, mature sclerotia were collected from infected plant and transfer on PDA media. After the mycelial growth, the cultural characteristics were studied that were white cottony thread like mycelia were formed on PDA media after 10-15 days and mycelia was become aggregated to form the sclerotia. Sclerotia were cottony in color in the young stage but dark brown to black while mature. The mature sclerotia were

used for artificial inoculation in pot culture and pathogen was re- isolated from the artificially infected sugarbeet plant. It was observed that the characteristic symptoms in artificially inoculated sugarbeet plant was similar to naturally infected sugarbeet plant. It was also revealed that the pathogenic structures that found from the re- isolation similar to the pathogenic structures that was isolated from naturally infected sugarbeet plants in the experimental field. (Sreenivasaprasad *et al*., 2005)

SUMMARY AND CONCLUSION

The present piece of research work was carried out in the field allotted for the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, during the period from November 2019 to March 2020. The study was conducted to evaluate the effect of eight selected modern phyto- chemicals and *Trichoderma harzianum* on disease incidence and severity of sclerotium root rot disease of sugarbeet. Yield and yield contributing characters of sugarbeet plant that changes due to the disease infection which cause serious damages of sugarbeet production was also the part of this study. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

From the study, it has been observed that the highest disease incidence was found when no phyto- chemicals and bio- agent applied and the lowest disease incidence was found in Amister Top 325 SC, Acibin and Tilt 250 EC treated plots. In case of disease severity, it was also observed that the highest disease severity was found in control treatment, and Dithane M-45 and Autostin treated plots. The lowest disease severity was found in Amister Top 325 SC, Acibin and Tilt 250 EC treated plots.

In case of beet diameter, the highest individual beet diameter was found in Amister Top, Dithane M-45, Cabrio Top and Autostin treated plots. The lowest beet diameter was found in control treatment, Acibin, Tilt 250 EC and *Trichoderma harzianum* treated plots.

It was also observed that the highest yield per plot was recorded in Amister Top 325 SC, Cabrio Top and Autostin treated plots. The lowest yield per plot was

obtained in control treatment, Acibin, Tilt 250 EC and *Trichoderma harzianum* treated plots.

The highest brix (%) was measured in beets collected from Amister Top 325 SC, Dithane M-45 and Cabrio Top treated plots. The lowest brix (%) was measured in beets that collected from control treatment.

In polarimetric analysis, the highest sucrose/pol (%) was recorded in the beet which were collected from Amister Top 325 SC, Ridomil Gold, Dithane M-45, Cabrio Top and Autostin treated plots. The lowest sucrose/pol (%) was estimated in beets collected from control treatment.

From the relationship study between disease incidence and severity with individual beet diameter, beet weight and yield per plot, it was revealed that individual beet diameter was increased with the decreased of disease incidence and severity. Same trend was found in relationship study between disease incidence and severity with individual beet weight and yield per plot.

From the study, it may be concluded that among the selected phyto- chemicals Amister Top 325 SC and Cabrio Top showed promising performance for management of sclerotium root rot disease of sugarbeet and gave better yield and yield attributes. However, further study need to be carried out for a consecutive years including more options as management practices in different Agro-ecological zones (AEZs) of the country. After that it may be recommended to use these phyto- chemicals by the farmers for the management of sclerotium root rot disease of sugarbeet.

REFERENCES

Abada, K.A. (1994). Fungi causing damping off and root rot on sugarbeet and their biological control with *Trichoderma harzianum*. *Agriculture-Ecosystem-and-Environment*. **51**(3): 333-337.

Agnihotri, V.P., Sen, C. and Srivastava, S.N. (1975). Role of fungitoxicants in the control of Sclerotium root rot disease of sugarbeet (*Beta vulgaris* L.). *Indian Journal of Experimental Biology*. **13**: 89-91.

Alam, M.J., Rahman, M.S., Islam, A.K.M.R., Sohel, A.T., Roy, H.M. (2017). Tropical Sugarbeet Production Technology in Bangladesh. 3rd edition, Bangladesh Sugarcrop Research Institute, Ishurdi, Pabna, Bangladesh.

Almeida, R.T. and Landim, C.M.U. (1981). Preliminary studies on the biological control of *Sclerotium rolfsii* causal agent of sclerotia wilt of chickpea (*Vigna unguiculata*) *Walp. Fitossanidade*. **5**(1): 15-20.

Anonymous. (2004). Sugarbeet cultivation in Bangladesh. Syngenta Bangladesh Ltd. p. 4.

Aycock, R. (1966). Stem rot and other diseases caused by *Sclerotium rolfsii*. *North Carolina Agricultural experiment Station Technical Bulletin.* **2**: 174-202.

Backman, P.A. and Rodriguez Kabana, R. (1972). Development of selective medium for isolation of *Sclerotium rolfsii*. *PhyTopathology*. **62**: 744-745.

Backman, P.A. and Rodriguez-Kabana, R. (1975). A system for the growth and delivery of biological control agents to the soil. *PhyTopathology*. **65**:819-821.

BSRI. (2005). Sugar beet cultivation in Bangladesh. 5th edition, Bangladesh Sugarcrop Research Institute, Ishurdi, Pabna, Bangladesh.

Chet, I., Hadar, Y., Katan, J. and Henis, Y. (1979). Biological control of soil borne plant pathogens by *Trichoderma harzianum.* **In**: Soil- Borne Plant Pathogens. B. Schippers and W. Gams, (ed.). London Academic Press, London, UK. pp. 585-592.

Ciccarese, F., Frisullo, S., Amenduni, M. and Cirulli, M. (1992). Use in the open field of *Trichoderma harzianum* Rifai in the biological control of sugarbeet root rot caused by *Sclerotium rolfsii* Sac. *Informatore FiTopathologico*. **42**: 63-64.

Diffus, J.D. and Ruppel, E.G. (1993). Diseases of Sugarbeet. **In**: The sugarbeet crop: Science into Practice. D.A. Cooke and R.K. Scott, (ed.). Chapman and Hall, London, UK. p. 675.

Dutta, A.K. (1975). Sclerotium wilt of *Polyanthes* and *Caladium* and their control. *Sci. and Cult*. **41**: 424.

Echeverria, E., Gonzales, A.M. and Marrero, H. (1982). Two methods of controlling the fungus causing southern blight of beans. *Cienciasde-La-Agricultura*. **12**: 11-16.

Elad, Y., Chet, I. and Khan, I. (1980). *Trichoderma harzianium* a bicontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *PhyTopathology*. **70**(2): 119-121.

Fahim, M.M., Kararah, M.A., Gharabawi, A.A. and Abada, K.A.M. (1984). Chemical control of damping off of sugar beet (*Beta vulgaris*) caused by *Sclerotium rolfsii*. *Egyptian. J. PhyTopathol*. **16**(1-2): 35-44.

FRG. (2012). Fertilizer Recommendation Guide, Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka 1215. p.274

Grinstien, A., Elad, Y., Katan, J. and Chet, I. (1979). Control of *Sclerotium rolfsii* by means of herbicide and *Trichoderma harzianum*. *Plant Dis. Reporter*. **63**: 823-826.

Kay, S.J. and Stewart, A. (1994). Evaluation of fungal antagonists for control of onion white rot in soil box trials. *Plant Pathol*. **43**(2): 371-377.

Khan, N.S. and Minhas, S. (2006). Selection and development of sugarbeet varieties for tonnage, pol% and sugar yield. *Pak. Sugar J.* **21**(4): 10-13.

Kumar, R. and Pathak, A.D. (2013). Recent trend of sugarbeet in world. Souvenir- IISR- Industry Interface on Research and Development Initiatives for Sugarbeet in India. May. 28-29, Sugarbeet Breeding Outpost of IISR IVRI Campus, Mukteswar, Nainital, India, pp. 46-48.

Le-Docte, A. (1927). Commercial determinations of sugar in the beet root using the sacks. Le-Docte process. *Int. Sugar. J.* **29**: 488-492.

Maiti, D., Das, S., Mandal, B. and Chakraborty, P.B. (2000). Influence of irrigation on growth and disease incidence of sugarbeet (*Beta vulgaris* L.). *Indian Agriculturist*. **44**: 63-69.

Mehrotra, R.S. and Tiwari, D.P. (1976). Organic amendments and control of foot rot of *Piper betle* caused by *Phytophthora parasitica* var. *piperina*. *Annal. Microbial*. **27**: 415-421.

Mian, I.H. (1995). Methods in plant pathology. *IPSA-JICA Project Publication*. **24**:100.

Mukhopadhyay, A.N. (1971). Sclerotium root rot disease of sugarbeet in India. *Mycopathologia et Mycologia Applicata*. **44**: 265-270.

Mukhopadhyay, A.N. (1987). Hand book on diseases of sugarbeet. Volume I and II. CRC Press, Boca Raton, Florida, U.S.A.

Mukhopadhyay, A.N. and Thakur, R.P. (1971). Control of Sclerotium root rot disease of sugarbeet with systemic fungicides. *Plant Disease Reporter*. **55**: 630-634.

Mukhopadhyay, A.N. and Upadhyay, J.P. (1981). Mechanism of reduction of Sclerotium root rot disease of sugarbeet through nitrogenous amendments and exploitation of *Trichoderma harzianum* as a potential biological agent. Proc. 3, Int. Symp. on plant pathology, Dec. 14-18, New Delhi, India, p. 110.

Mukhopadhyay, A.N. and Upadhyay, J.P. (1983). Control of *Sclerotium rolfsii* by *Trichoderma harazianum* in sugarbeet. Proc. 3, Int. Cong. of plant protection, Nov. 20-24, Brighten, England, pp. 50-52.

Muthamilan, M. and Jeyarajan, R. (1996). Integrated management of Sclerotium root rot disease of groundnut involving *Trichoderma harzianum, Rhizobium* and Carbendazim. *Indian J. of Mycol. And Plant Path*. **26**(2): 204-209.

Pan, S. and Sen, C. (1987). Chemical control of foot rot of wheat caused by *Sclerotium rolfsii*. *Indian Science Academy*. **23**: 416-422.

Patil, M.B. and Rane, M.S. (1982). Incidence and control of Sclerotium wilt of groundnut. *Pesticides*. **16:** 23-24.

Punja, Z.K., Grogan, R.G. and Unruh, T. (1982). Chemical control of *Sclerotium rolfsii* on golf greens in Northern California. *Plant Disease*. **66**:108-111.

Punja, Z.K., Smith, V.L., Campbell, C., Lee and Jenkins, S.F. (1985). Procedure to estimate numbers, spatial pattern and temporal distribution of sclerotia of *Sclerotium rolfsii* in soil. *Plant Disease*. **69**: 467-473

Rahman, M.K., Kabir, R.L., Alam, M.J., Hossain, M.S. and Islam, A.K.M.R. (2006). Sugarbeet Cultivation in Bangladesh. 2nd edition, Bangladesh Sugarcrop Research Institute, Ishurdi, Pabna, Bangladesh.

Rahman, M.L., Haque, M.S., Muqit, A., Alam, K.B., and Ali, S. (1994). Response of *Phytophthora parasitica* to different fungicides. *Bangladesh Journal of Plant Pathology*. **10**:1-2, 35-36.

Rashid, M.M. (1999). SabjiBiggan (in Bengali). Rashid Publishing House, 94 Old DOHS, Dhaka. p. 455

Rjurkar, R.B., Gade, R.M., Paslawar, A.N. and Chauke, R.P. (1998). Management of betel vine wilt through cultural and biological methods. *J. of Soils and Crops*. **8**(2): 176-178.

Rondon, A., Flores, Y., Soto, E. and Mujica, Y. (1995). Chemical control *in vitro* and in the greenhouse of the fungus causing white rot. *Revista-de-la Facultad-de-Agronomia, Universidad-del-Zulia*. **12**(1): 1-13.

Sen, C., Srivastava, S.N. and Agnihotri, V.P. (1974). Seedling diseases of sugarbeet and their chemical control. *Indian PhyTopathology*. **27**: 596-602.

Shahid, M.A., Mukhtar, A., Khan, M.A. and Ahmed, M. (1990). Chemical control of collar rot of lentil caused by *Sclerotium rolfsii*. *Sarhad-J. Agriculture*. **6**(5): 503-597.

Sharma, B.S. and Pathak, V.N. (1994). Yield and sucrose losses in sugarbeet due to root rot. *Indian PhyTopathology*. **147**: 408-411.

Sharma, B.S., Pathak, V.N. and Bhatnagar, K. (1990). Fungicidal management of root rot of sugarbeet induced by *Sclerotium rolfsii* Sacc. *Indian Journal Mycology Plant Pathology*. **20**: 207-210.

Singh, K., Agnihotri, V.P., Srivastava, S.N. and Misra, S.R. (1974). Factors affecting growth and production of sclerotia by *Rhizoctonia bataticola*. *Indian PhyTopathology*. **27**:85-90.

Singh, K., Sen, C. and Srivastava, S.N. (1974). Sugarbeet (*Beta vulgaris* L.): *Sclerotium rolfsii*. *Fungicide and Nematicide Test Results*. **30**: 102.

Singh, K., Srivastava, S.N. and Misra, S.R. (1986). Irrigation and potassium application in relation to sclerotial root rot of sugarbeet. *Indian Journal Sugarcane Technology*. **3**: 121-125.

Sreenivasa prasad, S., Takan, J.P., Mgonja, M.A., Manyasa, E.O., Kaloki, P., Wanyera, N.M., Okwadi, J., Muthumeenakshi, S., Brown, A.E. and Lenné, J.M. (2005). Enhancing finger millet production and utilization in East Africa through improved blast management and stakeholder connectivity. Pathways out of Poverty, *Aspects of Applied Biology*. **75**(2):11-22.

Srivastava, S.N. (1998). Diseases of Sugarbeet and their Management. **In**: Diseases of field crops and their management. T.S. Thind, (ed.). NATIC, Ludhiana, India. pp. 327-348.

Srivastava, S.N. (2000). Management of Sugarbeet Diseases in India. **In**: Advances in Plant Disease Management. Udit Narain, Kumud Kumar and Mukesh Srivastava, (ed.). Advance Publishing Concept, New Delhi, India. pp. 123-148.

Srivastava, S.N. and Tripathi, R.C. (1996a). Management of root rot of Sugarbeet (*Beta vulgaris* L.) through *Trichoderma harzianum* Rifai. *Indian Journal of Sugarcane Technology*. **11**: 45-48.

Srivastava, S.N., Agnihotri, V.P. and Singh, K. (1987). Factors influencing competitive saprophytic ability of *Sclerotium rolfsii* in soil. *Indian PhyTopathology*. **40**: 495-499.

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

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

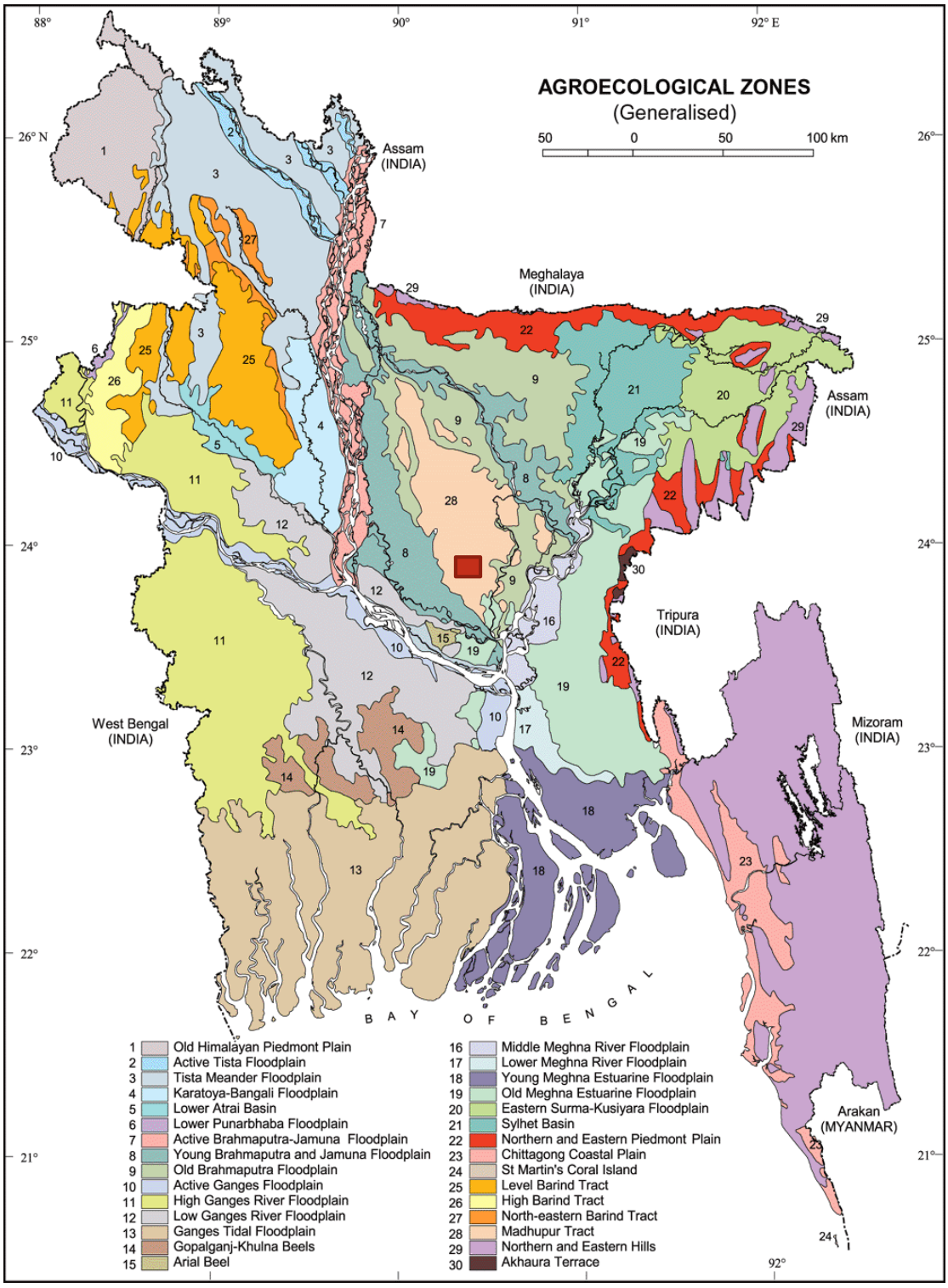
Waraitch, K.S., Kanwar, R.S. and Kumar, B. (1986). Fungicidal control of Sclerotium root rot disease of sugarbeet (*Beta vulgaris* L.) caused by *Sclerotium rolfsii*. *Indian PhyTopathology*. **39**: 100-102.

Weiland J and Koch G. (2004). Sugar Beet Leaf Spot Disease (*Cercospora beticola Sacc*). *Molecular Plant Pathology*. **5** (3): 157-166.

Wells, H.D., Bell, D.K. and Jaworski, C.A. (1972). Efficacy of *Trichoderma harzianum* as a bio- control for *Sclerotium rolfsii*. *PhyTopathology*. **62**: 442-447.

APPENDICES

Appendix I. Agro- Ecological Zone of Bangladesh showing the experimental location



Experimental site

Appendix II. Characteristics of experimental soil analyzed at Soil Resources Development Institute (SRDI), Farmgate, Dhaka.

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Central Farm, SAU, Dhaka
AEZ	Modhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained
Cropping pattern	Not Applicable

Source: Soil Resource Development Institute (SRDI)

B. Physical and chemical properties of the initial soil

Characteristics	Value
Particle size analysis % Sand	27
% Silt	43
% Clay	30
Textural class	Silty Clay Loam (ISSS)
pH	6.7
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.03
Available P (ppm)	20
Exchangeable K (me/100 g soil)	0.1
Available S (ppm)	45

Source: Soil Resource Development Institute (SRDI)

Appendix III. Monthly records of air temperature, relative humidity and rainfall during the period from November 2019 to March 2020

Month	RH (%)	Air Temperature (^{0}C) (Mean)	Rainfall (mm)
November, 2019	74	24.9	37.0
December, 2019	74	19.3	5.0
January, 2020	76	18.5	21.0
February, 2020	59	21.6	1.0
March, 2020	57	26.4	30.0

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka-1207.

Appendix IV Effect of selected modern phyto- chemicals and *Trichoderma harzianum* on brix (%) of beet per plot

Brix (%) of beet per plot

Treatment	Brix (%)
T_0	11.50 c
T_1	17.80 a
T_2	15.00 b
T_3	16.10 ab
T_4	16.10 ab
T_5	16.90 ab
T_6	16.90 ab
T_7	16.00 ab
T_8	16.20 ab
CV (%)	8.57

T_0= Control, T_1= Amister Top 325 SC, T_2= Acibin, T_3= Tilt 250 EC, T_4= Ridomil Gold, T_5= Dithane M-45, T_6= Cabrio Top, $T_{7=}$ Autostin, T_8= *Trichoderma harzianum*

Appendix V Effect of selected modern phyto- chemicals and *Trichoderma harzianum* on sucrose/pol (%) of beet per plot

Sucrose/Pol (%) of beet per plot

Treatment	Sucrose/Pol (%)
T_0	6.53 b
T_1	12.70 a
T_2	11.17 a
T_3	11.44 a
T_4	12.45 a
T_5	12.68 a
T_6	12.62 a
T_7	12.05 a
T_8	11.40 a
CV (%)	12.05

T_0= Control, T_1= Amister Top 325 SC, T_2= Acibin, T_3= Tilt 250 EC, T_4= Ridomil Gold, T_5= Dithane M-45, T_6= Cabrio Top, T_7= Autostin, T_8= *Trichoderma harzianum*

Appendix VI A view of the experimental field at early stage

Appendix VII A view of the experimental field at later stage

Appendix VIII. Signboard