SCLEROTIUM ROOT ROT OF SUGARBEET (*Beta vulgaris L.*): INCIDENCE, SEVERITY AND ITS *IN-VITRO* CONTROL THROUGH CHEMICAL, BOTANICAL AND BIOLOGICAL MEANS

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ABSTRACT

Incidence and severity of sclerotium root rot disease of sugarbeet was investigated at Sher-e-Bangla Agricultural University (SAU) and Bangladesh Sugarcrop Research Institute (BSRI) campus during January to March 2019. From the survey study it revealed that, the highest disease incidence (21.6%) was observed at BSRI farm, Iswardi in compared to SAU field (19.4%) and the disease severity was higher at BSRI campus (7.73%) compare to SAU campus (5.51%). The lab experiment was conducted for *in-vitro* management of sclerotium root rot disease of sugarbeet through chemical, botanical and biological means during April to december 2019. Seven fungicides viz. Amister Top 325 SC, Acibin 28 SC, Tilt 250 EC, Ridomil Gold, Dithane M-45, Cabrio Top 55 WG, Benda 50 WP, one plant extract (Neem leaf) and two bio- agents (*Trichoderma harzianum* and *Pseudomonus fluorescens*) were evaluated for their efficacy against *Sclerotium rolfsii*. The selected chemical fungicides and neem leaf extract were evaluated following by cup method and two bio-agents were evaluated through dual culture method. Among the tested chemical fungicides, Cabrio Top 55 WG and Acibin 28 SC gave the better performance in controlling the radial mycelial growth of *Sclerotium rolfsii*. Amister top showed better Performance in reduction of radial mycelial growth (inhibition 70% over control) followed by Tilt 250 EC, Benda, Ridomil Gold, and Dithane M-45 (inhibition 64.78%, 64.11%, 18.56%, 14.44% over control respectively) at 4 days after inoculation. Neem leef extract was not found satisfactory result in reducing the growth of fungus in *in-vitro*. Between two bio-agents *Trichoderma harzianum* (% inhibition 42.77% over control) showed better performance in controlling Sclerotium root rot disease of sugarbeet compared to *Pseudomonus fluorescens* (% inhibition 27.60 % over control)in reduction of radial mycelial growth of *S. rolfsii*. In this study, the potentiality to cause sugarbeet root rot disease caused by *Sclerotium rolfsii* was also confirmed through pathogenicity test. However, from the findings of the in-vitro study, it may be concluded that among the selected chemical fungicides, Acibin 28 SC, Cabrio top 55WG and bio-agents *Trichoderma harzianum* can be suggested for field trail for management of sclerotium root rot disease of sugarbeet.

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ABBREVIATIONS AND ACRONYMS

DI	=	Disease Incidence
DS	=	Disease Severity
DSI	=	Disease Severity Index
BSRI	=	Bangladesh Sugarcrop Research Institute
BSMRAU	=	Bangabandhu Sheikh Mujibur Rahman Agricultural University
SAU	=	Sher-e-Bangla Agricultural University
FAO	=	Food and Agricultural Organization
Ppm	=	Parts Per Million
P.S.I	=	pound per square inch
et al.	=	And Others
PDA	=	Potato Dextrose Agar
CRD	=	Completely Randomized Design
DAI	=	Days after inoculation
G	=	Gram (s)
Kg	=	Kilogram
No.	=	Number
Cm	=	Centimeter
N.A	=	Nutrient Agar

LSD	=	Least Significant Difference
^0C	=	Degree Celsius
Mm	=	Millimeter
%	=	Percent
cv.	=	Cultivar
CV%	=	Percentage of coefficient of variance
Hr	=	Hour
viz.	=	Videlicet (namely)

CERTIFICATE

*This is to certify that the thesis entitled '**SCLEROTIUM ROOT ROT OF SUGARBEET (Beta vulgaris L.): INCIDENCE, SEVERITY AND ITS IN-VITRO CONTROL THROUGH CHEMICAL, BOTANICAL AND BIOLOGICAL MEANS'**. 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 results of a piece of bona fide research work carried out by Registration No.18-09160 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:
Dhaka Bangladesh

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INTRODUCTION

Sugarbeet (*Beta vulgaris L.*) belongs to the family Chenopodiaceae, is considered as the second important sugarcrop all over the world after sugar cane (*Sacchurum officinarum L.*). The crop is believed to originate from Asia. Sugarbeet is a biennial crop but for sugar production beets are harvested in the first year and flowering occurs during the second year. The crop is grown under rainfed conditions but also widely under irrigation in the subtropics where the crop is known for its high tolerance to saline and alkali soils. Sugarbeet has been selectively bred since the early nineteenth century with the principle objective to develop varieties with the maximum tap root and sucrose yield potential at the lowest economic and environmental costs possible (Richardson, 2010).Sugarbeet is grown in 57 countries in the world. 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. Sugarbeet is mainly produced in Europe and, to a lesser extent, in Asia and North America (Kumar and Pathak, 2013).

Russian Federation is the top sugarbeet producing country in the world. As of 2018, sugarbeet production in Russian Federation was 42.1 million tones that accounts for 15.30% of the world's sugarbeet production. The top 5 countries (others are France, the United States of America, Germany, and Turkey) account for 57.04% of it. The world's total sugarbeet production was estimated at 274 million tons in 2018 (FAO, 2019). Bangladesh Sugarcrop Research Institute (BSRI) has been conducting

research since 2002 to find the possibility of producing sugarbeet as an alternate crop to sugarcane for sugar production in the country. It has been found that sugarbeet can be grown in 5-6 months. The yield of different varieties ranged from 88 to 133 tons per hectare. The pol (sugar content) percentage of sugarbeet in Bangladesh has found 14-15%, which was 12-13% in sugarcane. This crop can cultivate in saline soil and it reduce the soil salinity. Therefore, sugarbeet can be the only crop not only to grow in the vast coastal area of Bangladesh successfully but also can be the salinity reducer crop in Bangladesh. The by-products of the sugarbeet, such as pulp and molasses, give an added value of up to 10% of the value of the sugar and it also can be used as cattle feed and fertilizer. Also ethanol can be produced from sugarbeet (BSRI, 2005), which can be used as bio-fuel by mixing with diesel and petrol.

In Bangladesh, about 25% sugar demand meeting domestically from sugarcane and rest 75% sugar demand is fulfilled by importation (Rahman *et al.*, 2016). Sugarbeet matures within 5 to 6 months and its tap root contains 16-19% sucrose with a recovery of 12-14%. Sugarcane is a long duration crop thus farmers are discouraged to continue its production and moving towards short duration crop like maize and vegetables for higher profit. In this regard, sugarbeet might be an excellent alternative of sugarcane if processing facilities are developed in the sugar mills. In Bangladesh, most of the sugar mills remain idle for a particular period due to acute shortage of sugarcane. On the other hand, sugarbeet crop matures in March-April when the crushing season of sugarcane is nearly over in our country. In this

situation sugarbeet is can be the best alternative of sugarcane for production of sugar and ethanol. Feasibility of sugarbeet cultivation in Bangladesh is under trial although some people are growing low sucrose containing genotype as salad and vegetable purposes. Thus, it is necessary for selection of suitable genotype to promote sugarbeet as supplementary sugar based cropping system in Bangladesh.

There are several limiting factors for sugarbeet cultivation in Bangladesh. Disease is one of the most important known limiting factors of sugarbeet especially in tropical countries like Bangladesh, India, and Pakistan etc. The sugarbeet is susceptible to diseases, pests and some natural calamities. Among the diseases of sugarbeet, Sclerotium root rot disease caused by *Sclerotium rolfsii* is the most overwhelming disease which decreases the production of sugarbeet to a great extent.

Sclerotium rolfsii is a serious soil borne pathogenic fungus and harmful to many crops which are economically valuable in the most of the tropical and subtropical region of the world (Aycock, 1966). It has a wide host range and it has been referred as an almost omnipathogenic organism (Talukdar, 1974). The fungus *Sclerotium rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of sclerotia. As the fungus *Sclerotium rolfsii* is soil borne and omnipathogenic, it is very difficult to control even by the use of chemical fungicide. Some fungicides such as, Cupravit, Dithane M-45, Copper oxychloride, Difolatan and

Bordeaux mixture were found very effective against *Sclerotium rolfsii* (Patil *et al.*1986). At present diseases are mainly managed by fungicides. The continuous and indiscriminate uses of chemicals to manage crop disease result in accumulation of harmful chemicals residues in the soil, water and plants. In a third world country like Bangladesh, farmers are illiterate and they seldom follow the appropriate methods in handling chemicals, which created health hazards. The indiscriminate use of chemicals not only hazardous to living being but also break the natural ecological balance by killing the beneficial and antagonists microorganisms. The continuous and spontaneous chemical application also induced the development of resistant isolates of the pathogens, which sometimes become more virulent. Hence, efforts have to be made to retain pathogen activity below economic threshold level by choosing methods alternative to of chemicals only. Biological control could be successful alternative to control the pathogens.Biological control of soil borne pathogens offer environmentally safe, durable and cost effective alternative to chemicals (Papavizas and Lumsden, 1980; Mukhopadhyay, 1994). Many species of fungi and bacteria are reported to be effective bio-control agents against soil borne plant pathogens (Papavizas, 1985; Mukhopadhyay, 1994 ;). *Trichoderma spp*. are known antagonists to plant pathogenic fungi and have been shown to be very potential bio-control agents of several soil borne plant pathogenic fungi under both greenhouse and field conditions. Especially, *Trichoderma spp*. was found to be effective against different sclerotia forming fungi including *Rhizoctonia solani* and *Sclerotium rolfsii* (Hadar *et al*. 1979).

Trichoderma spp. has the ability to stimulate the growth of the different plant (Iqbal *et al*. 1995). Botanical extracts are biodegradable and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazards .Research on the active ingredients, fungicide preparation, application rate and environmental impact of botanical fungicides is a prerequisite for sustainable agriculture (Buss and Park, 2002). Botanical fungicides are unique because they can be produced easily by the farmers and small industries. Few works have been done by using tobacco, neem, garlic, and some other plant extracts to control some other fungi. Different natural biocides also used separately or in combination with plant extracts to control some other fungi by the farmers. Antifungul activities of garlic, neem, allamonda, have been reported by many researchers (Islam, 2005 and Arun *et al.* 1995).

So, the present experiment was undertaken to study the incidence and severity of Sclerotium root rot disease of sugarbeet and the effect of some chemical fungicides, bio-agents and botanical extracts on the growth of *Sclerotium rolfsii in vitro*.

Objectives:

The research work was carried out to achieve the following specific objectives:

- To determine the disease incidence and severity of Sclerotium root rot disease of sugarbeet at BSRI and SAU campus.

- To evaluate *in-vitro* efficacy of some selected fungicides, plant extracts and bio-agents against the sclerotium root rot disease of sugarbeet.

REVIEW OF LITERATURE

The pathogen, *Sclerotium rolfsii*, is a soilborne fungus widely distributed in the southern U.S., as well as the warmer parts of the world. The fungus has a very broad host range, causing disease in over a thousand species of dicotyledonous plants which resulted millions of dollars loss, on a variety of crops, every year.

2.1 Occurrence and distribution

Sclerotium rolfsii has a wide host range with more than 500 plant species (Aycock 1966). They consist of mono and di-cotyledons. Until now, no worldwide compilation of host genera has been published, however, more than 270 host genera have been reported in the USA. These include agricultural crops such as sweet potato (*Ipomoea batatas*), pumpkin (*Cucurbita pepo*), corn (*Zea mays*), wheat (*Triticum vulgare*), groundnut (*Arachis hypogeae*), and some horticultural crops such as Narcissus (*Narcissus spp*.), Iris (*Iris spp*.), Lilium (*Lilium spp*.), Zinnia (*Zinnia spp*.), and Chrysanthemum (*Chrysanthemum spp*.) (Farr *et al*. 1989). In Vietnam, many crops are infected by *S. rolfsii* including groundnut (*Arachis hypogaea*), mungbean (*Vigna radiata*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*) and taro (*Colocasia esculenta*) (Le 1977; Do 2001).

2.2 Chemical control of *Sclerotium rolfsii*

There are several published reports in controlling *Sclerotium rolfsii* of different crops by the application of fungicides. Some of the important ones are listed here. Khare *et al.* (1974) conducted an experiment with thirteen fungicides to control *Sclerotium rolfsii* from wilted lentil plant. Five fungicides namely Benlate, Thiram, Dithane-M 45, Captan and Phaltan complex reported cease the growth of *Sclerotium rolfsii.*

Sen *et al*. (1974) found that, *Sclerotium rolfsii* on wheat could 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- vitro. and in- vivo*. 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*. Dutta (1975) found that, soil application of fungicides such as Bavistin (0.5- o.7%), Brassicol (0.1%), three times at 20 days interval has been effective in controlling foot and tuber rot disease of tuberose. Reddy *et al*. (1976) demonstrated Plantvax [Oxycarboxin] and Vitavax [Carboxin] as seed treatments at 2g/kg gave effective control of *Sclerotium rolfsii* on wheat up to 35 days after seeding. Diomande and Beute (1977) used a soil plate method to evaluate seven fungicides for control of *Sclerotium rolfsii* in laboratory tests. In all tests 7 Carboxin and Tryphenyltin hydroxide were effective in preventing mycelia growth of *Sclerotium rolfsii*.

Kulkarni (1980) found that, in field trials foot rot of wheat caused by *Sclerotium rolfsii* was controlled effectively by seed treatment with Panoram, Brassicol, Panoctine-35 [Guazatine]. Vitavax and Calixin [Tridemorph] were less effective. Seed treatment with 0.2% of these chemicals protects wheat seedlings for up to 35 days even in heavily infested soil.

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 in the agar @ 100 and 200 µg a.i /ml. Fahim *et al*. (1984) observed, seed treating agent Vitavax-200, Homai 80, Orthocide-75 and Captan @3g/kg seed by dusting or glutting (modification or pelleting method) to reduce pre-emergence damping-off of sugarbeet (*Beta vulgaris*) caused by *Sclerotium rolfsii* in infested 8 soil samples. 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% contrl 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 the most effective, both in inhibiting mycelia growth and scierotia formation at low concentration.

2.3 Evaluation of botanical extracts against *Sclerotium rolfsii*

Dutta and Deb (1986) studied the effect of organic and inorganic amendments on the soil and Rhizosphere microflora in relation to the biology and control of *Sclerotium rolfsii*. They reported that, leaf extract of Eupatorium adenophorum reduced the pathogen population in the rhizosphere.

Singh and Dwivedi (1987) observed that, hyphal dry weight and sclerotial production of *Sclerotium rolfsii* were significantly reduced by bark extracts of *Acacia arabica*. They also tried with bulb and leaf extracts of garlic and onion, leaf extracts of *Rauvolfia serpentine*, *Lawsoni alba*, *Datura stramonium*, *Solanum xanthicarpum*, *Calotropis procera*, *Eucalyptus globus* and fruit and leaf extracts of *Azadirachta indica* and rhizome extracts of turmeric and ginger applied against *S. rolfsii* and found that those extracts were more or less effective in inhibiting the fungus.

Sivakadacham (1988) reported that, leaf extracts of *Adatho davasica* and *Cullen corylifolium* suppressed the mycelial growth of *Sclerotium rolfsii.* Singh *et al.* (1989) reported that, out of six tested plant oils against *S. rolfsii*, leaf oil of *Azadirachta indica* was found most effective followed by that from *Eucalyptus globules* and *Ocimum canum*.

Singh and Dwivedi (1990) reported that, the viability of sclerotia was reduced when treated with neem oil. Dayaram and Tewari (1994) found that, the soil application

of green leaves of *Adatho davasica*, *Aegle marchelos*, *Anisomele sovata*, *Azadirchta indica, Cymbopogon flexuous,* rhizomes of *Curcuma amada* and resin of *Ferula foetida* at 2 to 5 per cent concentration reduced both pre and post emergence collar rot of chickpea caused by *Sclerotium rolfsii*. Five percent *Ferula foetida* resin applied 48 hours before sowing of seeds in artificial inoculation of soil provided nearly 100 per cent protection.

Arun *et al*. (1995) observed that the extracts of garlic bulb were effective in suppressing radial growth of the pathogen *Fusarium spp*. and *S. rolfsii* and was more effective when added after sterilization.

Kazmi *et al* (1995) conducted an experiment to see the effect of neem oil on *in-vitro* growth of root infecting fungi. The effect of neem oil on the growth of the root infecting fungi *Marcophomina phaseolina, S. rolfsii, R. solani* and *Fusarium moniliformae* was examined.

Neem showed greater suppression of growth of *S. rolfsii, R. solani* and *Fusarium moniliformae*.

Pani and Patra (1997) utilized some phyto-extracts for controlling *S. rolfsii* during paddy straw mushroom (*Volvariella volvacea*) cultivation. *In- vitro* and *in- vivo* studied were conducted to determine the effect of extracts of *Azadirachta indica, Psidium guagava, Lantana camara, Sopindus trifoliate, Cynodon dactylon, Tamarindus indica, Echhornia crassipes, Adhatoda vasica, Pongamia glabraand Tagetese rectaon* the mycelia growth of *Volvariella volvacea* and *S. rolfsii*. Paddy straw mushroom inoculated with *S. rolfsii* and treated with *Tamarindus indica* leaf

extract resulted in the highest sporophore yield followed by *Sopindus trifoliate* seed extract and *Moring gaoleifera* root extract.

Enikuomehin *et al*. (1998) worked on the evaluation of ash from some tropical plants of Nigeria for the control of *S. rolfsii*. On wheat (*Trichum aestivum L*.). Nine tropical plants were screened for their abilities to inhibit mycelial growth and sclerotial germination of Nigerian isolate of *Corticium rolfsii* on agar and in soil. Of the 11 samples tested 10 showed some activity against mycelial growth of *C. rolfsii in- vitro.*

Morteza and Mohammed (2001) applied some plants products to control some soil borne fungal pathogens. More than 15 plants species were tested for their antifungal effects on radial growth and spore germination of *Fusarium oxysporum f.sp cumini* causing cumin wilt and *Fusarium equisetii* causing dry rot of potato tubers and *Rhizoctonia solani* causing sugarbeet root rot. In this experiment seed extract of *Trachyspermum copticum*, leaf extract of *Lavandula angustifolia* and flower extract of *Rhjeumribes* effectively inhibit the radial growth and spore germination of these fungi by using filter paper and poisoned food methods.

Seshakiran (2002) reported that, *Eupatorium odoralum L., C. occidentalis* and *Azadrachta indica* were highly antifungal to mycelial growth of *S. rolfsii*. He also observed that the root extract of *Pathenium hysterophorus L.* exhibited maximum inhibition of mycelium growth of *S. rolfsii*.

2.4 Effect of bio agents on *Sclerotium rolfsii in-vitro*

Homer *et al.* (1971) showed that *Trichoderma harzianum* effectively controlled *S. rolfsii* on blue lupins, tomatoes and peanuts. Under natural field conditions one to three applications of *T. harzianum* inoculum applied over the plants onto the soil surface was highly effective in reducing *S. rolfsii* of the transplanted tomato.

Harder and Troll (1973) tested the antagonism of *Trichoderma spp*. to sclerotia of *Typhulain carnata* and observed that several *Trichoderma spp*. parasitized the sclerotia in culture on artificial media and on soil, greatly reducing the viability of the sclerotia.

Mathur and Sarbhoy (1978) observed that the comparative effectiveness of *T. viride* and *T. harzianum* under both *in-vitro* and glasshouse conditions against root rot of sugarbeet caused by *S. rolfsii*. Both species of *Trichoderma* appeared to be strongly antagonistic, causing 88% and 86% inhibition of the growth of *S. rolfsii* by *T. viride* and *T. harzianum*, respectively. While tested under glasshouse condition, S. rolfsii caused only 13.3% and 20% infection in presence of *T. viride* and *T. harzianum*, respectively compared with 100% infection recorded in absence of any of the antagonists. Arora and dwivedi (1979) found that, *T. harzianum* significantly reduced the growth of *S. rolfsii*, the causal organism of root disease of lentil (*Lens esculenta*) on agar.

Almedia and Landim (1981) reported that an isolate of *Trichoderma spp*. was hyper parasite of *S. rolfsii* on PDA culture and found to be most effective in contrillings *S. rolfsii* on cowpea in green house.

Elad *et al*. (1983) studied the parasitism of *Trichoderma harzianum* to the soil borne plant pathogen, *S. rolfsi* .They observed that hyphae of the parasites contact with their host either producing appressorium like bodies or coiling around the hyphae, enzymatically digest host cell walls.

Henis *et al.* (1983) reported that *Trichoderma* produced volatile and non-volatile antibiotics which are active against *S. rolfsii* and also inhibited the sclerotial germination. D' Ambra and Chamswarng and Sangkaha (1988) collected 147 isolates of *Trichoderma* and *Gliocladium* group. In- vitro test of bio-control potential of all isolates indicate that 123 were antagonistic to *S. rolfsii*.

Ikotun and Adekunle (1990) isolated *T. harzianum* from soils grown to cassava plants and observed that *T. harzianum* was an active hyper parasite which attacked the mycelia of target organisms (*S. rolfsii*) and prevented their continued growth.

Lim and Teh (1990) reported that isolates of *T.harzianum*, inhibited the growth of *S.rolfsii* up to 67% in dual culture on malt agar and up to 100% using a cellophane overlay technique at 20 ± 1.5˚ C. Growth of the test organism was inhibited by the production of both diffusible and volatile metabolites and various hyphal interactions were observed; hyphal coiling, appressoria and hooks were produced by the *Trichoderma spp*. and host cells exhibited vacuolations, granulation, coagulation, disintegration and lysis.

Iqbal *et al*. (1995) tested the micro-organisms for antagonism to *Sclerotium rolfsii*. All the organisms viz., *Trichoderma harzianum, Trichoderma koningii, Trichoderma viride, Gliocladium virens Miller, Aspergillus canditus Link,*

Paecilomyces lilacinus (Thom) Samson and Bacillus spp. significantly inhibited the mycelial growth of *S. rolfsii, Trichoderma harzianum, Trichoderma koningii and Trichoderma viride* overlapped the pathogen and suppressed growth by 63.6 %, 54.9 % and 51.89 % respectively.

Mukherjee *et al.* (1995) compared antagonistic properties of *T. harzianum* and *Gliocladium virens* in suppressing *S.rolfsii* and *Rhizoctonia solani* in *in-vitro*. They observed that *T. harzianum* was less effective than *G.virens*. Only *T. harzianum* parasitized the hyphae of *S.rolfsii* and the two antagonists were comparable in respect to antibiosis on the test pathogens. Muthamilan and Jeyarajan (1996) reported that, 67.4 percent reduction of sclerotial production in *Sclerotium rolfsii* was observed in the presence of *Trichoderma viride*. Mature sclerotia from each dual culture plate were smaller than the control plate.

Virupaksha *et al*. (1997) tested the antagonistic organisms against *Sclerotium rolfsii*. Among them, *Trichoderma harzianum* and *Trichoderma viride* were found to be effective in inhibiting the mycelial growth and reducing production of sclerotial bodies irrespective of inoculation periods. They also observed inhibition zone and reduction in size of sclerotial bodies in presence of antagonists.

Desai and Schlosser (1999) collected 44 isolates of *Trichoderma* belonging to eight species were tested for their ability to infect, macerate and kill the sclerotia of *S.rolfsii*. Of them 14 isolates infected and killed the sclerotia of *S.rolfsii*.

Mondal (1999) tested 55 isolates of *T. harzianum*, isolate TF-24 showed 93% inhibition of mycelia growth of *S. rolfsii* on PDA. Bari *et al*. (2000) reported

significant reduction on radial growth of *S.rolfsii* by *Trichoderma spp*. in dual culture on PDA plate.

Biswas and Sen (2000) reported the dual culture of the 11 isolates of *T.harzianum* viz. T8, T10 and T12 were effective against *S. rolfsii* and they over grew the pathogen up to 92%, 85% and 79% respectively *in-vitro*. Both the T8 and T10 isolates reduced stem rot incidence significantly when delivered as seed dressing or soil application in the pot trials of groundnut. Disease reduction through seed dressing by the isolates T8 andT10 were 33% and 50%, respectively while disease reduction through soil dressing were 72% and 83%, respectively over control.

MATERIALS AND METHODS

The present study was conducted at Plant Pathology Laboratory under the division of Pathology, Bangladesh Sugarcrop Research Institute (BSRI), Ishwardi, Pabna, and at Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka1207 during January 2019 to April 2020. The samples were collected from the field of BSRI and SAU. The details of the materials required and the methodology adopted during the study are described in this chapter. This chapter deals with three experiments throughout the study period in order to study the Sugarbeet diseases. The experiments were as follows:

I. Determination of disease incidence (DI) and disease severity index (DSI) of sclerotium root rot disease of sugarbeet in two selected locations.
II. Evaluation of some selected chemicals, botanical extract and bio-agents against identified *Sclerotium rolfcii* isolate.
III. Performed pathogenicity test.

3.1. Survey location and period

The survey study was conducted at central research field of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka1207 and BSRI farm, Ishwardi,Pabna. Survey study was made during the period of January-March, 2019.

Plate 1. Survey field-1: BSRI farm

Plate 2. Survey field-2: Central research field of SAU


White mycelial mat grown at the base of the plant


Yellowing of leaves

Round brown sclerotia formed at the base of the plant

Plate 3. Symptoms of Sclerotium root rot disease of sugerbeet found in Survey area

3.1.1. Survey data collection

Survey data were collected from selected research field during sugarbeet cultivation to observe disease incidence and disease severity. Data were collected from the selected locations according to typical symptoms of sclerotium root rot. Symptoms of the sugarbeet diseases were studied by visual observation.

3.1.2. Calculation of disease incidence

For calculation of Disease incidence (%) in total 5 plots were selected randomly from each of the selected fields.

Total plant were counted from selected plot and among them disease infested plant were counted to calculate percent disease incidence. The disease incidence was calculated using the following formula.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

3.1.3. Calculation of disease severity index (DSI)

The plants were scored for disease class on a scale of 0 to 4.After recording the disease class for each control and treatment, the Disease Severity Index (DSI) was calculated using the following formula:

$$\text{Disease Severity Index (DSI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of plants assessed} \times \text{maximum disease grade}} \times 100$$

For calculation of disease severity, the following disease severity scale was used:

followed by, Abdullah *et al*. (2003) and K. Athira. (2017).

Disease class	Signs and symptoms of infection
0	Healthy plants with green leaves without appearance of fungal mycelium on any part of plants
1	Appearance of white fungal mass on any part of plants , with or without chlorotic leaves
2	Appearance of fungal mass/mycelium on any part of plants with chlorotic leaves (1-3)
3	Appearance of fungal mass / mycelium on any part of plants with
4	Formation of well-developed sclerotia and plants dried /wilted

3.2. Collection of diseased specimens

Diseased root samples of sugarbeet were collected from BSRI field. Collected samples were put in polyethylene bags immediately after collection to protect them from drying. Then the samples were preserved at 4^{o}C in refrigerator for isolation of *Sclerotium rolfsii.*

3.2.1. Sterilization of materials and equipments

Liquid materials, such as media and distilled water were sterilized in an autoclave 121^{o}C and 15 pound per square inch (p.s.i.) for 30 minutes. For surface sterilization 0.1% sodium hypochlorite (NaOCl) was used, and rectified spirit used for other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

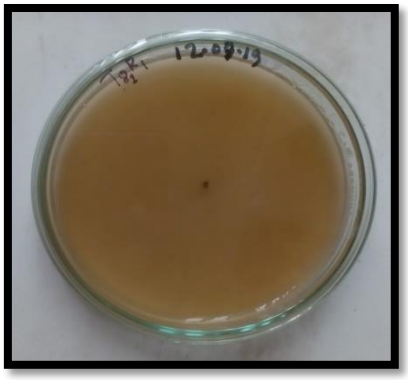
3.2.2. Isolation of causal organism

The pathogens associated with the sclerotium root rot of sugarbeet was isolated following tissue planting method (Tuite 1969, Mian 1995).

At first the diseased plant parts (root) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into small pieces (5 mm) from advancing end of the lesions. The cut portion were surface sterilized with 0.1% sodium hypochloride (NaOCl) for 5 minutes, and rinsed with sterilized water for 3 times. Surface sterilized plant pieces were plated on PDA media in 90 mm petridishes and incubated at 25 ± 1^{o} 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 at same conditioons. After 10-15 days of inoculation mycelia as well as mustard seed like brown sclerotia are formed.

A

B



C

D

Plate 4. A. Sclerotium root rot disease sample of Sugarbeet, B. Inoculation of pathogen inoculum in PDA media C. Pure culture of *S. rolfsii* showing immature sclerotia, D. Pure culture of *S. rolfsii* showing mature sclerotia.

3.2.3. Identification, multiplication and preservation of the pathogen

Pure culture of the isolates were prepared by using mature sclerotia (Tuite 1969, Mian, 1995) and subsequently transferred to fresh PDA media in petridishes. Pure culture of *Sclerotium rolfsii* was stored at 4^{o}C.

3.3. 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, a pathogen free viable seed of BSRI Sugarbeet-1 variety was collected. Then some medium size pots were prepared for sowing sugarbeet seed.

3.3.1. Pot preparation for pathogenicity test

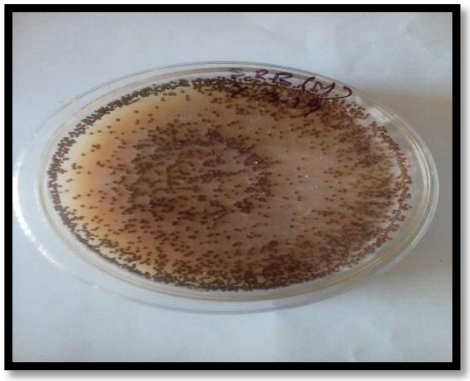
Cow dung 1 kg /5 kg soil and urea 200 g /5 kg soil were mixed properly with sun dry soil.Two seed per pot was sown. Everyday water was provided on the tobs to keep soil always moist.

3.3.2. Artificial inoculation

After 1 month of seed sowing when the seedlings were at four to five leaf stage than inoculation of fungus in the plant was done artificially. Inoculation was done by spreading sclerotia on soil around the plant root zone. Each plant was inoculated with the test pathogen isolate. For maintaining environment that is needed for successful infection large plastic bag was used for each pot. After inoculation plant was wrapped with a large plastic bag to seal moisture. Nearly 95% humidity for 24 hours gives better inoculation (Sreenivasaprasad *etal*: 2005). The pots containing inoculated plant provided nearly 98% humidity. Then waited for development of

Sclerotium root rot symptoms on the leaves and roots. It was replicated three times for each of two isolates. The temperature in the net house during the study period were 24±5ºC in late January. After that the plants were taken care for 30 days for necessary study.

Collected sample



Pure culture of *Sclerotium rolfsii*

Growing seeding in pot

Inoculation with *Sclerotium rolfsii* suspension

Covered with polythene bag for suitable environment

Replacement in natural condition for disease development

Plate 5. Steps involved in pathogenicity test for *Sclerotium rolfsii*

3.4. Evaluation of suitable management strategies for controlling Sclerotium root rot disease of Sugarbeet caused by *Sclerotium rolfsii*

Followng treatments were considered to conduct the management study

T_o =Untreated control

T_1 = Ridomil gold MZ 68 WP

T_2 = Cabriotop 55WG

T_3 = Amistar top 325 SC

T_4 =Dithane M-45

T_5 = Benda 50WP

T_6 = Acibin 28 SC

T_7 = Tilt 250 EC

T_8 == Neem leafextract

T_9 =*Trichoderma harzianum*

T_{10} = *Pseudomonas fluorescens*

3.4.1. Selection of fungicides

Seven fungicides namely Ridomil gold, Cabrio top, Benda 50WP, Dithane M-45, Amister top, Acibin, Tilt 250 EC were tested following poisoned food technique *in-vitro* to evaluate their efficacy on colony growth and sclerotia formation of *Sclerotium rolfsii*. The details of the fungicides are presented in Table 1.

Table1. Fungicides used in the Bio –assay against *Sclerotium rolfsi*

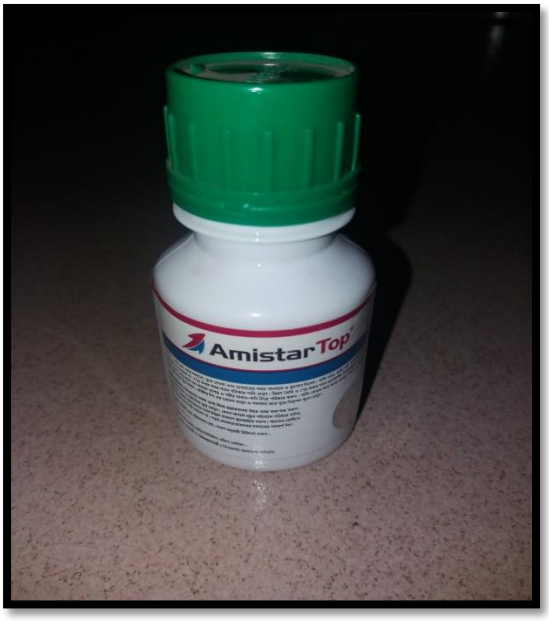
Trade name	Common name	Active ingredient	Application dose
Ridomil gold MZ 68 WP	Metalaxyl+Mancozeb	68% Metalaxyl	2g / L
Cabrio top 55WG	Pyraclostrobin+Metriam	55%Metrium	1.5g / L
Benda 50WP	Carbendazim	50% Carbendazim	1g/L
Dithane M-45	Manganous-ethylene bisdithio carbamate-ion	80% Mancozeb	2.5g / L
Amister top 325 SC	Azoxystrobin+Difenoconazole	Azoxystrobin 20% +Difenoconazole 12.5%	0.5 ml/ L
Acibin 28 SC	Azoxytrobin+Cyproconazole	Azoxytrobin 20%+Cyproconazole 8%	o.2 ml / L
Till 250 EC	1-[2-(2,4-Dichlorophenl-4-propyle-1,3-dioxalan]-2	250 ml/ Litre Propiconazole	1ml /L

Ridomil Gold

Cabrio Top

Dithane M-45

Acibin



Amistar Top **Tilt 250 EC**

Plate 6. Different chemicals used to test antifungal activity against *Sclerotium rolfsii.*

3.4.2. Collection of botanical

For this experiment Neem leaves were used as botanical treatment. Neem leaves was collected from Bangladesh Sugar Crop Research Institute (BSRI), Ishwardi, Pabna.

Plate 7. Neem leaf (*Azadirachta indica*) used to test antifungal activity against *Sclerotium rolfsii.*

3.4.3. Preparation of plant extract

The extracts were prepared following the method of Ashrafuzzaman and Hossain (1992). For preparation of extracts, collected leaves were weighted in an electric balance and then washed in the water. After washing the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended in an electric blender and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:2 (w/v) ratio 200 ml of distilled water was added with 100g neem leaves.

3.4.4. Effect of bio-agent against *Sclerotium rolfsii*

Two bio-agents *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated against *S. rolfsii* following dual culture method.

3.4.5. Isolation / collection of biocontrol agents

Biocontrol agents *Trichoderma harzianum* were collected from Laboratory of Sher-e-Bangla Agricultural University department of Plant Pathology and *Pseudomonas fluorescens* were collected from Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) department of Plant Pathology.The fungal antagonists were cultured in Potato Dextrose Agar (PDA) medium and the bacteria in Nutrient Agar (N.A) medium.

A **B**

Plate 8. Bio-agents used to test antifungal activity against *Sclerotium rolfsii* A. Pure culture of *Trichoderma harzianum* and B. Pure culture of *Pseudomonas fluorescens*

3.5. Bio-assay of selected treatments

3.5.1. Bioassay following growth inhibition technique using fungicides and plant extracts

Groove/ Cup method: From a PDA plate three 5 mm discs of the medium were scooped from three places maintaining an equal distance from the centre by a sterilized disc cutter. One milliliter of plant extract was put into each hole and the plates were stored overnight in refrigerator for diffusion of the input in the medium around the hole before resumption of fungal growth. The next day, one 5-mm block of 7 days old fungal culture (pathogen) cut by sterilized disc cutter and was placed at the centre of the plate. The linear growth (cm) of mycelium of *S. rolfsii* was recorded at 24 hr. interval until the control plates were filled in (Nene and Thaplial, 1979).

3.5.2. Dual culture method for bio-assay of bio-agents against *Sclerotium rolfsii*

PDA media was prepared and sterilized in an autoclave at 121°C for 15 minutes then the medium (20 ml) was poured into sterilized petri-plate (90 mm diameter) the medium is in lukewarm state and allow it to solidify at room temperature. The culture discs (7 days old) of the bio agents and pathogen was cut separately with the help of sterilized cork bores (5 mm). The culture discs of pathogen and bio agent aseptically was transferred and place them at periphery of the petriplate containing the medium (Care should be taken to place the both discs of pathogen and bio agent at equidistance i.e. 2 to 3 cm apart from the periphery from the petri plate in opposite direction). Inoculate with culture disc of the pathogen alone in the petri plates containing PDA, which serves as control. The inoculated petri plates was transferred into the incubator and incubate at 25°C. The growth of the pathogen was observed periodically and antagonist in petri plates and measure the colony growth (diameter) in each petri plate. The percent inhibition of the pathogen was calculated by the bio-agent when the growth of the pathogen is full in the control plates.

3.6. Measurement of radial growth (cm) and determination of percent inhibition

After 60 hours of incubation, radial growth (cm) of *S. rolfsii* in petridishes was recorded. The radial growth (cm) of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony and then these plates were kept for 30 days for sclerotia formation.

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

$$Percent\ inhibition = \frac{X - Y}{X} \times 100$$

X= Average radial growth (cm) of *S. rolfsii* in control petridishes.

Y= Average radial growth (cm) of *S. rolfsii* in each fungicides, plant extracts and bio-agent treated petridishes.

3.7. Statistical analysis of data :

The survey data and data from lab experiment were analyzed by using Statistix-10 software. Treatment means were compared by LSD at 0.05 level of significance. The data obtained in the present investigation for various parameters were subjected to ANOVA for a completely randomized design for *in -vitro* studies in lab.

RESULTS

This chapter includes the experimental results i.e. disease incidence and severity of Sclerotium root rot of sugarbeet in selected locations and effect of the treatments in controlling Sclerotium root rot disease of sugarbeet caused by *Sclorotium rolfsii* was assessed *in vitro* method. The results were compiled based on the percent disease incidence, percent disease severity and inhibition of radial mycelial growth.

4.1 Disease symptoms of the Sclerotium root rot of sugarbeet :

Symptoms of this disease can be severe. The initial symptom of infection is wilting of the leaves (a). Observation of the root reveals the presence of a white cottony growth on its surface (b). This white growth is called mycelium which is the vegetative growth of the fungus. As the infection proceeds, the mycelia will eventually cover the entire surface of the root. The tissues of the root become soft and have a water-soaked appearance that is evident when cut open (c). Many spherical, dark brown structures may be observed on or in the infected tissue, or in the surrounding soil (d).

These structures resemble like mustard seeds and are called sclerotia, the survival form of the fungus. These structures will remain in the soil over the winter and be available to cause infection during following years.

a **b**

c **d**

Plate 9. Symptoms of Sclerotium root rot disease of sugerbeet

In the fall of the year symptoms are confusing. Beets may have symptoms of Sclerotium root rot, but without signs of the fungus. This is due to changing environmental parameters. As temperatures cool, other soil microorganisms become more competitive and attack the pathogen, the mycelia may be destroyed and the sclerotia may not develop.

4.2 Survey study

The survey was conducted in two selected areas in Bangladesh viz. SAU field and BSRI farm. Data were collected in random sampling procedures. From each fields, 5 untreated plots were selected for calculating disease incidence (DI) and disease severity index (DSI).

4.2.1 Disease incidence (%) of Sclerotium root rot disease of sugarbeet

The location wise disease incidence data was recorded at 60 days, 75 days and 90 days after seed sowing in the field. The highest disease incidence was observed by 21.6% in 90 days after sowing at BSRI field followed by 60 days and 75 days as 14.4% and 18.4 %, respectively. At SAU the highest disease incidence was found by 19.4% in 90 days after sowing followed by 60 days and 75 days as 9.6% and 15.2 % respectively. However, Sclerotium root rot disease incidence was lower at SAU field than BSRI farm at 60, 75 and 90 days after sowing. Results are shown in Figure 1.

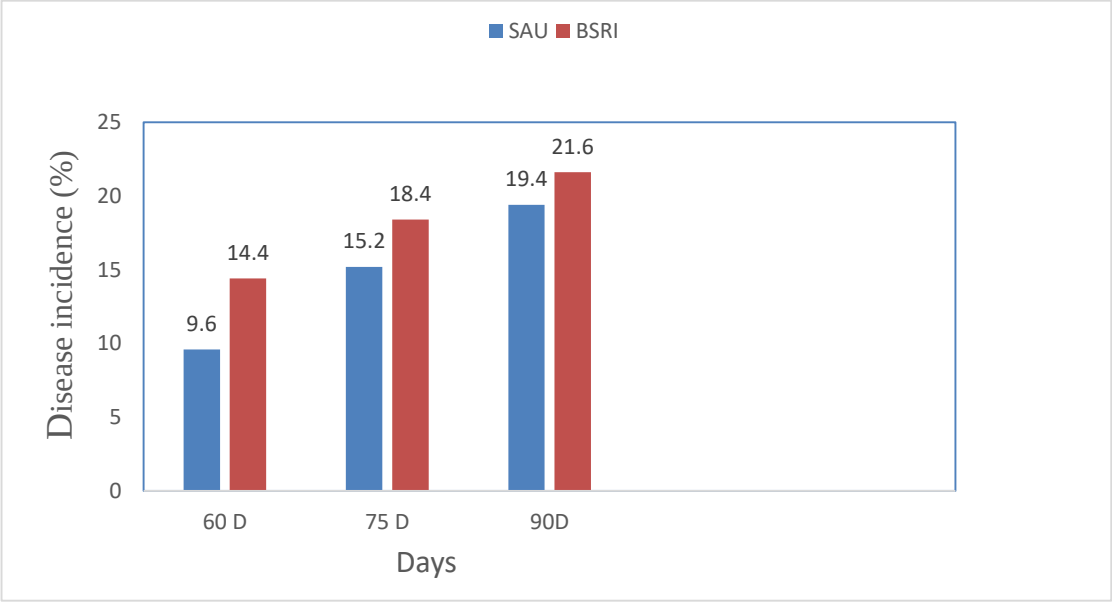


Figure 1. Disease incidence (%) of Sclerotium root rot disease of sugarbeet at SAU field and BSRI farm

4.2.2. Disease severity of Sclerotium root rot of Sugarbeet

The location wise disease severity data was recorded at 60, 75 and 90 days after seed sowing in field. The highest disease severity was observed by 7.73% in 90 days after sowing at BSRI farm followed by 60 and 75 days as 5.51 % and 7.11 % respectively. At SAU field the highest disease severity was found by 5.51% at 90 days after sowing followed by 60 and 75 days as 3.2% and 4.53% respectively. However, Sclerotium root rot disease severity was lower at SAU field than BSRI farm at 60, 75 and 90 days after sowing. Results are sown in figure 2.

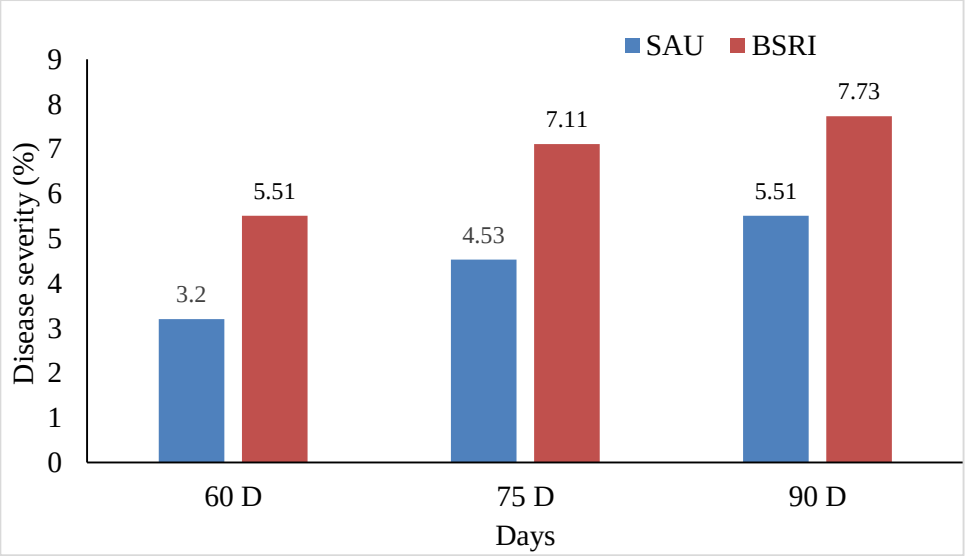


Figure 2. Disease severity (%) of Sclerotium root rot disease of sugarbeet at SAU field and BSRI farm

4.3. Pathogenicity test for *Sclerotium rolfsii* pathogen

In order to prove the pathogenic nature of *Sclerotium rolfcii* pathogen producing Sclerotium root rot disease, Koch's postulates test was conducted on leaves and root side of the sugarbeet plants. After 10 days of inoculation, the initial symptom of infection is wilting of the leaves started and Observation of the root reveals the presence of a white cottony growth on its surface.

This symptom was similar in appearance with the Sclertium root rot disease found in the field. The root sample with developed fungal mycelia were placed in PDA media and kept in aseptic condition. It developed white mycelial growth and mustared seed like sclerotia was observed. So, there-isolated pathogen from the developed symptoms in the net house was producing similar type of growth and mycelia characteristics which was found in first isolation of the pathogen *in vitro*. In this way Koch's postulate was established.

Plate 10. Visible symptom on infected plant

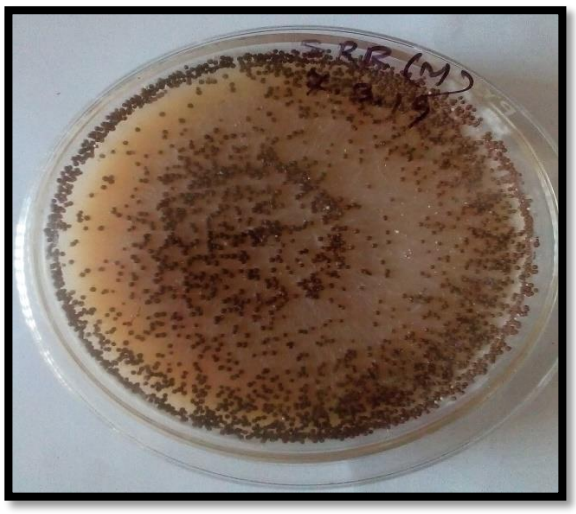


Plate 11. Re-isolated *Sclerotium rolfsii* fungal mycelia

4.4. Laboratory experiment

4.4.1. *In vitro* efficacy of fungicides and plant extracts in inhibition of mycelial growth of *Sclerotium rolfsii* in poisoned food technique

Fungicides showed profound effect on reduction of radial mycelial growth of fungus. All the tested fungicides reduced the radial mycelial growth of the fungus significantly which ranged from 0 cm to 9.00 cm recorded after inoculation of 4 days. No radial mycelial growth (0.00cm) was recorded in case of Cabriotop and Acibin at 1 , 2 , 3 , 4 days after inoculation. Radial mycelial growth were recorded in case of Amistar top 1.37, 2.23, 2.47 and 2.70 cm at 1, 2, 3 and 4 days after inoculation respectively.

On the other hand, the highest radial mycelial growth was found by 9.00 cm within 4 days after inoculation of untreated control preceded by Dithane M-45 (7.70 cm), Ridomil Gold (7.19 cm), Benda (3.23 cm) and Tilt 250 EC (3.17 cm) at 4 days after inoculation. Cabrio top and Acbin were found promising in reducing the growth of the fungus in the laboratory followed by Amistar top.

In botanical, Plant extracts have profound effect on reduction of radial mycelial growth of the fungus. Radial mycelial growth 1.73 cm, 3.4 cm, 5.43 cm, 7.10cm of *Sclerotium rolfsii* were recorded in case of neem leaf extracts at 1, 2, 3 and 4 days after inoculation respectively.(table 2. and plate 12).

All the tested fungicides have strong effect to produce percent inhibition against *Sclerotium rolfsii* in culture media. The highest percent inhibition (100%) was recorded in case of Cabriotop and Acibin preceded by Amistar top (70%), Tilt 250 EC (64.78 %), Benda (64.11%), Rdomil gold (18.56%) and Dithane m-45 (14.44%) at 4 days after inoculation. The percent inhibition 21.11% was recorded in case of neem leaf extract at 4 days after inoculation. No growth inhibition was found in case of untreated control treatment.

Table 2. *In vitro* efficacy of fungicides and plant extracts in inhibition of mycelial growth of *Sclerotium rolfsii* in poisoned food technique

Treatments	Radial mycelial growth (cm)				% Inhibition of mycelial growth (4DAI)
	1 DAI	2 DAI	3 DAI	4 DAI	
Control	2.10 a	6.07 a	8.47 a	9.00 a	---
Ridomil Gold	1.57 b	4.33 b	5.23 b	7.33 b	18.56
Cabriotop	0.00 d	0.00 d	0.00 e	0.00 e	100
Amistar top	1.37 c	2.23 c	2.47 d	2.70 d	70
Dithane M-45	1.7 b	4.37 b	5.37 b	7.70 b	14.44
Benda	1.57 bc	2.47 c	2.70 cd	3.23 c	64.11
Acibin	0.00 d	0.00 d	0.00 e	0.00 e	100
Tilt 250 EC	1.53 bc	2.53 c	2.87 c	3.17 c	64.78
Neem leaf extracts	1.73 b	3.4 c	5.43 b	7.10 b	21.11
CV (%)	7.79	4.81	3.07	3.19	

In a column, DAI = Days after inoculation

A. Control

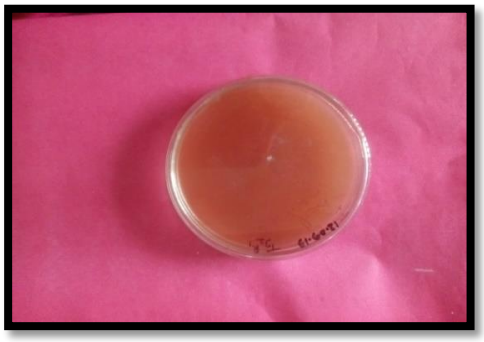
B. Ridomil Gold

C. **Cabriotop**

D. Amistar Top

E. Dithane M-45

F.Benda



G. Acibin

H. Tilt 250 EC

I. Neem leaf extract

Plate 12. Radial mycelial growth of *S. rolfsii* against A. Control, B. Ridomil Gold, C. Cabrio Top, D. Amister Top, E. Dithane M-45, F. Benda G. Acibin, H. Tilt 250 EC I. Neem leaf extract after 4 days of inoculation.

4.4.2. *In vitro* efficacy of bio-agents in inhibition of mycelial growth of *Sclerotium rolfsii* in dual culture method

Efficacy of bio-agents on radial mycelial growth of *Sclerotium rolfsii* is shown in table 3. and plate 13). Bio-agents have significant effect on reduction of radial mycelial growth of the fungus. Radial mycelium growth of *Sclerotium rolfsii* against all the tested bio-agents ranged from 5.15 cm to 9.00 cm recorded after inoculation of 4 days. The lowest radial mycelial growth 1.16 cm, 2.15 cm, 3.81 cm and 5.15 cm of *Sclerotium rolfsii* was recorded in case of *Trichoderma harzianum* at 1 day, 2 days, 3 days and 4 days after inoculation respectively. The performance of *Trichoderma harzianum* in reduction of radial mycelial growth was the best followed by *Pseudomonus fluorescens* irrespective of days after inoculation. The highest radial mycelium growth 9.00 cm was recorded in untreated control preceded by *Pseudomonus fluorescens* (6.51cm) and *Trichiderma harzianum* (5.15) at 4 days after inoculation. *Trichoderma harzianum* is better than *Pseudomonus fluorescens* in reduction of radial mycelial growth of *S. rolfsii* in dual culture.

All the tested bio-agents have strong effect to produce percent growth inhibition against *Sclerotium rolfsii* in culture media. The highest percent inhibition (42.77%) was recorded in case of *Trichoderma harzianum* preceded by *Pseudomonuss fluorescens* (27.66%) at 4 days after inoculation. No percent inhibition was found in case of untreated control treatment.

Table 3. *In vitro* efficacy of bio-agents in inhibition of mycelial growth of *Sclerotium rolfsii* in dual culture method

Treatments	Radial mycelial growth (cm)				% Inhibition of mycelial growth (4DAI)
	1DAI	2DAI	3DAI	4DAI	
Trichoderma harzianum	1.16 c	2.15 c	3.81 c	5.15 c	42.77
Pseudomonas fluorescens	1.17 b	2.38 b	4.34 b	6.51 b	27.66
Control	2.10 a	6.07 a	8.47 a	9.00 a	-
LSD (0.05)	0.0006	0.0893	0.0006	0.0006	-

In a column, DAI = Days after inoculation

A. *Trichoderma harzianum*

B. *Pseudomonas fluorescens*

C. Control

Plate 13. Radial mycelial growth of *S. rolfsii* against A. *Trichoderma harzianum*, B. *Pseudomonas fluorescens* and C. Control after 4 days of inoculation.

DISCUSSION

Sclerotium root rot disease of sugarbeet is a soil borne disease. So far very few research on sugarbeet disease have been conducted in Bangladesh. This experiment has been done on the incidence and severity of Sclerotium root rot disease of sugarbeet and their control by using some chemicals, botanical extract and bio-agents. Symptoms of Sclerotium root rot of sugarbeet as observed in the present investigation conformed to those reported by Abada (1994) and Waraitch *et al.* (1986). The causal organism of Sclerotium root rot of sugarbeet identified as *Sclerotium rolfsii* has earlier been reported by many workers (Abada, 1994; Upadhyay and Mukhopadhyay, 1986; Chaluat, *et al*. 1981;). Incidence of Sclerotium root rot of sugarbeet was observed at two locations at SAU fields, Dhaka and at BSRI farm, Ishwardi, Pabna during January to March, 2019. The lowest disease incidence was found at SAU (9.6%) in 60 days after sowing as the highest disease incidence (21.5 %) was observed in 90 days after seed sowing at BSRI farm (figure 1). The lowest disease severity was found at SAU (3.2 %) in 60 days after sowing and the highest disease severity (7.73 %) was observed in 90 days after seed sowing at BSRI farm (figure 2). However, these findings are in agreement with the statement of Waraitch *et al*. (1986) who showed Sclerotium root rot disease incidence in sugarbeet upto 50% at 25-30^{o}C with high soil moisture. The prime aim of this study was to find out the effective management options to control Sclerotium root rot disease of sugarbeet *in-vitro*. Effect of the treatments in controlling Sclerotium root rot of sugarbeet caused by *Sclerotium rolfsii* was assessed based on the result of laboratory experiment. Discussions on results of laboratory experiment has

presented in this chapter as well. Chemical fungicides are not thought of as a long term solution to crop health management. Requirement for repeated application, residue problems, health and environmental hazards and development of fungicide resistance in the pathogen are the major problems associated with the use and overuse of chemical fungicides (Mukhopadhyay and Mukherjee, 1996). In recent years, boi-control based management also practices as well as chemical fungicides. In this study, the *in-vitro* efficacy of chemical fungicides, neem leaf extracts and bio-agents were evaluated against *Sclerotium rolfsii*. The radial growth of the mycelia was recorded four times at 1,2,3,4 DAI. The inhibitory efficacy of Acibin 28 SC (Azoxytrobin +Cyproconazole) and Cabriotop 55 WG (Pyraclostrobin +Metriam) have strong effect to inhibit mycelial growth of *Sclerotium rolfsii* in culture media. The present findings were well supported by the reports of Rondon *et al.* (1995) where they 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 mycelial growth and sclerotia formation at low concentration. In the present study, between two bio-agents it has been also found that *Trichoderma harzianum* (% inhibition 42.77% over control) have strong effect to inhibit mycelial growth of *Sclerotium rolfsii* in culture media compared to *Pseudomonus florescence* (% inhibition 27.60% over control). The findings of the present studies were well supported by Lim and Teh (1990). They

reported that isolates of *T. harzianum* inhibited the growth of *S. rolfsii* up to 67% in dual culture on malt agar and up to 100% using a cellophane overlay technique at 20 ± 1.5˚C. Biswas and Sen (2000) reported the dual culture of the 11 isolates of *T. harzianum* where isolates of T8, T10 and T12 were effective against *S. rolfsii* and they over grew the pathogen up to 92%, 85% and 79% respectively *in- vitro*.

In-vitro efficacy of a Botanical extract (neem leaf) has been evaluated against *Sclerotium rolfsii as well*. Neem leaf extract inhibit the mycelial growth of *Sclerotium* over control. However, neem leaf extract was not found satisfactory results in reducing the radial mycelial growth. The plants and its derivatives are of great use in agriculture, public health, medicines, cosmetics, etc. Plant extracts are effective against plant pathogens as they have unique antimicrobial properties that act in a holistic manner due to presence of certain secondary metabolites, viz., alkaloids, terpenoids, glycocides and phenolic acids (Srivastava *et al*., 1994; Singh *et al*., 1999). The present findings were well supported by the reports of Arun *et al.* (1995). They observed that extracts of Garlic bulb were effective in suppressing radial growth of the pathogen *Fusarium* spp. and *S. rolfsii* and was more effective when added after sterilization. Singh *et al*. (1989) reported that, out of six plant oils tested against *S. rolfsii,* leaf oil of *Azadirachta indica* (Neem) was found most effective followed by that from *Eucalyptus globules* and *Ocimum canum*. Singh and Dwivedi (1989) reported that, the viability of sclerotia was reduced when treated with neem oil.In comparison of three types of treatments for inhibition efficacy, it is stated that chemical treatments give the highest percent inhibition than bio-agent and botanical extract (neem leaf).

Finally, it may be stated that Sclerotium root rot disease of Sugarbeet cannot be kept under control with just a single management strategy. In the present *in-vitro* study, different treatments like chemicals and bio-agents showed in a desirable level of inhibition against Sclerotium root rot disease of sugarbeet , neem leaf extracts can not able to inhibit mycelial growth at satisfactory level. However, more investigation need to persue including more fungicides, plant extracts and bio-agents for consecutive year to confirm the result.

In this study, the potentiality to cause sugarbeet root rot disease caused by *Sclerotium rolfsii* was also confirmed through pathogenicity test.The pathogen was isolated from the infected root sample from the field in PDA media. The isolated pathogen was inoculated in the healthy plants. Same type of symptom was noticed. It was re-isolated again in the same media and observed the mycelial growth and sclerotia were similar to the earlier growth in the media. In this way Koch's postulates was established. Pathogenicity test was conducted for *Sclerotium rolfsii* by Perello *et al.,* (2015). Artificially inoculated Plants were incubated in a moist condition at 22°C and 100% RH . Inoculated plants gave symptoms which were similar to those observed in the field. Dutta (2017) was conducted koch’s postulates for *Sclerotium* for the conformity of the Pathogen, and found the plant that was found in natural condition.

SUMMARY AND CONCLUSION

The sugarbeet is the strategic and energetic crop, which can multiply (by the best way) the invested energy. Sugar crops are improving the soil fertility and the growing of sugarbeet increasing the yield of crops produced after the sugar beet within the crops rotation cycle. This crop can reduce the soil salinity and the soil turn into other crop production friendly.Sugarbeet is not only raw material for food industry. It is used for food production (white sugar, alcohol), it is also used as a renewable source of energy (dehydrated alcohol, raw material for biogas units), feed materials (fresh beet pulp and granulated beet pulp, distiller's grains), fertilizers (green parts, carbonation lime) and CO2 (liquid carbon dioxide for both alcoholic and nonalcoholic beverages production). Sclerotium root rot caused by *Sclerotium rolfsii* is a limiting factor of Sugarbeet production in Bangladesh. The fungus not only reduces the yield but also decline the sugar content. The research program were undertaken to study the incidence and severity of Sclerotium root rot of sugarbeet in survey area and its in -vitro control through selected fungicides, plant extract and bio-agents. The experiment was carried out at the Laboratory, division of Pathology, Bangladesh Sugarcrop Research Institute (BSRI), Ishwardi,Pabna; and at the field of Sher-e-Bangla Agricultural University, Dhaka 1207 and the field of BSRI.

In survey study, the lowest incidence was found at SAU field (9.6%) in 60 days after sowing as the highest disease incidence (21.5%) was observed in 90 days after seed sowing at BSRI farm. The lowest disease severity was found at SAU (3.2%) in 60 days after sowing as the highest disease severity (7.73 %) was observed in 90 days after seed

sowing at BSRI farm.The *in vitro* effect of the fungicides, plant extracts and bio-agents were compiled based on inhibition of mycelial growth. In *in vitro* assay, Acibin 28 SC and Cabriotop 55 WG performed the best result for inhibition of mycelial growth of *Sclerotium rolfsii* followed by Amistar top 325 SC. In botanical,neem leaf extracts showed better performance than untreated control in inhibition of mycelial growth but it was not in desired level. Between the two bio-agents *Trichoderma harzianum* showed better performance than *Pseudomonus fluorescens* in inhibition of mycelial growth of *Sclerotium rolfsii.* From the findings of the present investigation it may be concluded that Acibin 28 SC (Azoxytrobin + Cyproconazole) and Cabriotop 55 WG (Pyraclostrobin + Metriam) had a promising effect in reducing the disease incidence and severity of Sclerotium rot disease of Sugarbeet. Amistar top 325 SC (Azoxytrobin + Difenoconazol) also showed the second highest performance in suppressing the disease. Although *Trichoderma harzianum* showed significantly better performances but the neem leaf extracts failed to give desirable result . Therefore, from the present study it can be concluded that the chemicals viz Acibin 28 SC (Azoxytrobin + Cyproconazole), Cabrio top 55 WG (Pyraclostrobin + Metriam) and bio-agent *Trichoderma harzianum* is suggested to conduct *in-vivo* experiments to observed their efficacy in field condition against Sclerotium root rot disease of sugarbeet.

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APPENDICES

Appendix -I. Survey questioner

Structural questioner for survey study of Sclerotium Root Rot disease

Survey on Sclerotium root rot disease of sugarbeet in Bangladesh

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Name of the District:
Name of the upazilla:
Type of the field:
Name of the Owner:
Area of the field:
Total No. of plant:
No. of plant Observed:

Table : Disease scale for Sclerotium Root Rot 0-4 (Abdullah *et al*. 2003;Ilias 2000)

Disease class	Signs and symptoms of infection
0	Healthy plants with green leaves without appearance of fungal mycelium on any part of plants
1	Appearance of white fungal mass on any part of plants , with or without chlorotic leaves
2	Appearance of fungal mass/mycelium on any part of plants with chlorotic leaves (1-3)
3	Appearance of fungal mass / mycelium on any part of plants
4	Formation of well-developed Sclerotia and plants dried /wilted

%D.I= **%D.S=**

Cultural practices:

1. **Cleaning**
2. **…..**
3. **……**
4. **…….**
5. **……**

Use of fertilizer

1. **Chemical: No**

   ……..

   ……

   ……

2. **Biological: Household waste**

   ……..

   ……..

   ……..

Other information:

Type of soil of the area:

GPS of the area:

Soil pH:

Problems faced by the cultivar:

Very unconscious about the agricultural practices.

Comments after survey

……

……

……

Survey held by

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Appendix - II.

Table: Disease Incidence (DI) and Disease Severity (DS) at SAU campus and BSRI campus

Days	DI		DS	
	BSRI	SAU	SAU	BSRI
60	14.40 b	9.60 c	3.20 b	5.51 b
75	18.40 ab	15.20 b	4.53 ab	7.11 ab
90	21.60 a	19.4 a	5.51 a	7.73 a

Appendix - III.

Media preparation

Preparation of PDA media

Sterilization of media

Dedicated to…

My beloved
parents and the farmers
who feed the nation

Plagiarism Detection Center

Original Document Information

Title	SCLEROTIUM ROOT ROT OF SUGARBEET (Beta vulgaris L.): INCIDENCE, SEVERITY AND ITS IN-VITRO CONTROL THROUGH CHEMICAL, BOTANICAL AND BIOLOGICAL MEANS
Author	SABJANA AKTER
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University	Sher-e-Bangla Agricultural University
Year	2017

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