MANAGEMENT OF FOOT AND ROOT ROT DISEASE OF BETEL VINE CAUSED BY *Sclerotium rolfsii*

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MANAGEMENT OF FOOT AND ROOT ROT DISEASE OF BETEL VINE CAUSED BY *Sclerotium rolfsii*

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CERTIFICATE

This is to certify that thesis entitled **"MANAGEMENT OF FOOT AND ROOT ROT DISEASE OF BETEL VINE CAUSED BY *Sclerotium rolfsii*"** submitted to the faculty of agriculture, 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 **Rabeya Parvin, Registration No. 11-04700** 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.

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Place: Dhaka, Bangladesh

Dedicated To

My Parents

Who Laid the Foundation of My Success

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The Author

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ABSTRACT

Betel vine *Piper betle* L. is largely grown as an important cash crop throughout the tropical and subtropical regions in Bangladesh. Disease damage to the crop is one of several known limiting factors. Foot and root rot of betel vine caused by *Sclerotium rolfsii* is the most overwhelming disease which decreases the production of betel leaf to a great extent. Management of foot and root rot disease of betel vine caused by *Sclerotium rolfsii* was studied during cropping season 2012-2013. The experiment was conducted in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka. The field experiments were conducted in the field of Malonchi upazila in Pabna district under natural condition. The experiments were laid out in a Randomized Complete Block Design (RCBD) with 3(three) replications. Initially 6 fungicides, 5 plant extracts and 2 bio-agents were evaluated for their efficacy against *Sclerotium rolfsii* by an *in vitro* test. In *in vitro* experiment (cup method) Bavistin proved to be the best in controlling the radial mycelum growth of *Sclerotium rolfsii*. Among them 3 fungicides, 2 plant extracts and 1 bio-agent were found promising and selected for field evaluation against foot and root rot disease of betel vine. Treatment of betel vine stem followed by spraying with 7 treatments comprising Bavistin, Topgan, Tilt 250 EC, Garlic clove extracts, Neem leaf extracts, *Trichoderma harzianum* based BAU-biofungicides and control were explored in the experiment. A remarkable reduction of the severity of foot and root rot was achieved by treating with fungicides. The lowest severity was found in the Bavistin (0.71%) followed by Topgan (0.94%) whereas the highest disease severity was recorded under the untreated control treatment (2.87%). The highest yield was found in case of Bavistin which was 68.46% increased over untreated control followed by Topgan (49.05%). Stem treated with Bavistin by spraying at 7 days interval after inoculation minimized disease incidence, severity and increased yield followed by Topgan. Garlic clove extract and *Trichoderma harzianum* showed better performance in controlling foot and root rot disease of betel vine compared to control. Among the ecofriendly approach *T. harzianum* based BAU-Biofungicide increased 30.15% yield over control

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LIST OF ACRONYMS

AEZ	=	Agro-Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
Ppm	=	Parts per million
et al.	=	And others
N	=	Nitrogen
TSP	=	Triple Super Phosphate
MP	=	Murate of Potash
RCBD	=	Randomized complete block design
DAI	=	Days after inoculation
ha^{-1}	=	Per hectare
G	=	gram (s)
Kg	=	Kilogram
DAT	=	Days after transplanting
SAU	=	Sher-e-Bangla Agricultural University
No.	=	Number
Wt.	=	Weight

LSD	=	Least Significant Difference
^{0}C	=	Degree Celsius
NS	=	Not significant
Mm	=	Millimeter
Max	=	Maximum
Min	=	Minimum
%	=	Percent
cv.	=	Cultivar
CV%	=	Percentage of coefficient of variance
Hr	=	Hour
T	=	Ton
viz.	=	Videlicet (namely)

INTRODUCTION

The betel leaf familiarly known as 'pan' (*Piper betle* L.) is a perennial climber cultivated largely for its leaves, which is an important cash crop of Bangladesh and used as a masticatory. The betel vine thrives cultivate under tropical conditions having a cool, shade, considerable humidity and a good supply of soil moisture. Geographically it belongs to the region bounded by 68° E to 118° W longitudes and 30° N to 12° S latitudes. It is grown from sea level to an altitude of about 900 m (Chaurasia, 2001).

It is a climbing plant with shiny, green heart-shaped leaves. The stem is climbing by many short adventitious roots (Hassan and Shahadat, 2005). Leaves of betel vine are chewed along with areca nut as a masticator. Usually the people of South-Asia, Southeast Asia, Gulf States and Pacific islands chew betel leaves. All classes of people of Bangladesh chew betel vine not only as a habit but also as an item of rituals, etiquette and manners.

The scientific name of betel vine is *Piper betle* L. It belongs to the family Piperaceae. The vine is a dioecious (male and female plants are different), shade loving perennial root climber. There are about 100 varieties of betel vine in the world, of which about 40 are found in India and 30 in West Bengal (Guha, 1997; Maity, 1989; Samanta, 1994). Desi Bangla, Bangla, Kali Bangla, Jhali, Sanchi, Goyeshi, Bhabna, Mitha, Geso, Bonhoogly etc. betel vine cultivars are found in Bangladesh. The most probable place of origin of betel vine is Malaysia.

It is also used as a special item offered to the guests in order to show respect and for such traditional use of betel leaf in the Indian society, the leaf really stands alone without any parallel even today (Guha, 1997;

Mehrotra, 1981). In fact, this edible leaf has achieved an esteemed position in the human society right from the dawn of civilization, particularly in the countries like Bangladesh, Burma, China, India, Indonesia, Malaysia, Nepal, Pakistan, Phili- pines, South Africa, Sri Lanka, Thailand etc. (Jana, 1996; Khoshoo, 1981; Samanta, 1994; Sharma *et al*., 1996), where leaves are traditionally used for chewing in their natural raw condition along with many other ingredients like sliced areca nut, slaked lime, coriander, aniseed, clove, cardamom, sweetener, coconut scrapings, ashes of diamond, pearl, gold and silver (Ayurvedic preparations), jelly, pepper mint, flavouring agent, fruit pulp etc. (CSIR, 1969).

Bangladesh is the second largest grower of betel vine on about 14000 hectares. Total annual production of the crop in Bangladesh is about 72,500 tons. The average yield is 2.27 ton per acre (Anonymous, 2006). But the acreage of betel vine is decreasing fast because of some physical and socioeconomic barriers like unavailability of credit facilities, uncontrolled marketing system and infestation of disease and pest (Islam, 2005). The extent of losses varies from 5-90 percent (Dasgupta and Sen, 1999; Dasgupta *et al*. 2005).

Disease damage to the crop is one of several known limiting factors. The betel vine is highly susceptible to diseases, pests some natural calamities (Sayeeduzzaman, 1988). Humid and moist shaded conditions are favorable for betel vine growth and also favor a variety of root and foliage disease development (Goswami *et al*. 2002). Among the diseases of betel vine foot and root rot caused by *Sclerotium rolfsii* is the most overwhelming disease which decreases the production of betel leaf to a great extent. Farmers growing *Piper betle* in three upazilas of Rajshahi

incurred a huge loss as foot rot disease damaged about 60% of the cultivation in the year of 2004 (Islam, 2005).

Sclerotium rolfsii Sacc. is a serious soil borne pathogenic fungus and harmful to many crops which are economically valuable in 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 (Ahmed, 1980). 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 very effective to control foot rot disease on *Piper betle* caused by *Sclerotium rolfsii* (Patil *et al.*1986). At present diseases are mainly managed by the use of chemicals such as 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/or 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 of 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).

Plant growth promoting fungi (PGPF) isolated from rhizosphere and rhizoplane of different crops suppress the pathogen and protect the plant by promoting plant growth, antagonistic activity including systematic resistance of the plant (Meera *et al*. 1993 and Hyakumachi, 1994). *Trichoderma* spp. has the ability to stimulate the growth of the different plant (Inbar *et al*. 1994).

Botanical extracts are biodegradable (Devlin and Zettel, 1999) and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazards (Grange and Ahmed, 1988). 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 (Roy *et al*. 2005). 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 effect of some chemical fungicides, bio-agents and botanical extracts on the growth and sclerotia formation of *Sclerotium rolfsii* in *vitro*. and also to control the foot rot disease of betel vine caused by *Sclerotium rolfsii.*

Considering the above facts, the present study has therefore, been undertaken with the following objectives:

-To isolate and identify the causal pathogen of foot and root rot disease of betel vine.

- Screening of selected chemicals, plant extracts and bio-agents against the pathogen.

-To find out the effective methods to control foot and root rot disease of betel vine in the field condition.

REVIEW OF LITERATURE

2.1. 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 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.*

Dutta (1975) found that, soil application of fungicides such 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

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 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.

Wokocha and Ebeneb (1986) used six fungicides Aatopam-N, Aldrex-T, Calixin-M, PCNB [Quintozene], Captan and Captafol at 200 mg a.i. litter in green house tests against *Sclerotium rolfsii* on tomato completely suppressed the disease when applied as soil drenches up to 4 d before inoculation, but only Quintozene was effective when applied 10 d before and post-inoculation treatments were all 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, Apron-TZ, 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 scierotia formation at low concentration.

2.2. 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 bulb and leaf extracts of garlic and onion, leaf extracts of *Rauvolfia serpentine, Lawsoni alba, Datura stramonium, Solanum xanthicarpum, Calotropis procera, Eucalyptus globus* and *Azadirachta indica* fruit and leaf extracts, *Emblica officinalis* and rhizome extracts of turmeric, ginger extracts 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 mycelium growth of *Sclerotium rolfsii*.

Singh *et al*. (1989) reported that, out of six plant oils tested 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 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, Echhorniacrassipes, Adhatodavasica, Pongamiaglabra*and *Tagetese recta*on 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 sugar beet 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*. However, root extract of *Pathenium hysterophorus* L. exhibited maximum inhibition of mycelium growth of *S. rolfsii.*

2.3. Effect of bio agents on *Sclerotium rolfsii* in *in vitro*

Homer *et al.* (1971) showed that *T. 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 sugar beet 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 Ferrata (1984) observed the reduction of mycelial growth, sclerotial formation, sclerotial germination and number of sclerotia of *Sclerotium rolfsii* when inoculated with different inoculum concentration of *Trichoderma harzianum.*

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.

2.4. Effect of bio agents on *Sclerotium rolfsii* in *in vivo*

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.

Farzana *et al.* (1991) observed that, infection of 30 and 60 days old soybean plants by root infecting fungi (*S.rolfsii, R. solani,* and *Fusarium* spp.) was significantly reduced following seed treatment with *T. harzianum.*

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, Mucor, Alternaria, Pythium debaryanum* and resulted in increased root weight both in pot and field experiments.

Jagadeesh and Geeta (1994) reported that, a wheat bran and biogas manure mixture (1:1) stimulated growth and multiplication of the biological control agent *T. harzianum,* which suppressed *S. rolfsii* and increased seedling emergence of groundnut.

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.

Silveria *et al.* (1994) reported the antagonistic potential of 14 *Trichoderma* isolates collected from 7 beans and cowpea growing areas of Brazil and tested against *S. rolfsii.* Isolates TN-50, TN-21 and TN-52 gave the best inhibition of mycelial growth at 76.8%, 73.6% and 71.6%, respectively. TN-21.TN-50 and TN-1 gave the best control of sclerotia production by 93.4%, 83.6% and 82.8%, respectively. Isolates TN-21 and TN-16 showed the greatest hyper parasitic activity towards sclerotia of the pathogen, with values of 79.2% and 75%, respectively. Further they also demonstrated the antagonists as seed and soil treatment under greenhouse condition and the best results were achieved by treating the soil with the selected isolates. Among tested isolates TN-21 reduced disease severity by 35.15 and graded as the best antagonist against *S. rolfsii.*

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.

Ellil *et al*. (1998) stated that, *T. harzianum* reduced root rot infection by 6.7-45.0% in bean. *Trichoderma* spp. obviously antagonized the effects caused by the pathogen, *S. rolfsii* and *Fusarium solani*.

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

MATERIALS AND METHODS

3.1. Laboratory experiment

3.1.1. Experimental site

The experiment was conducted at the Laboratory, Department of Plant Pathology, Sher-E-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka- 1207.

3.1.2. Experimental period

The laboratory experiments were conducted from June 2012 to December 2012.

3.1.3. Collection of diseased specimens

Diseased stem samples of betel vine (*Piper betle* L.) were collected from different "boroj" in Pabna district. 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.*

3.1.4. Sterilization of materials and equipment's

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 20 min. For surface sterilization 0.1% sodium hypochlorite (NaOCl) was used for plant materials such as leaf, stem, seed etc., and rectified spirit used for other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

3.1.5. Isolation of causal organism

The pathogens associated with the foot rot disease of betel vine were isolated following tissue planting method (Tuite 1969, Mian1995).

At first the diseased plant parts (stem) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into small pieces (5 mm) from advancing end of the lesions. The cut portion were surface sterilized with 1% chlorox (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 room temperature of 22 ± 20^{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. After 10-15 days of inoculation mycelia as well as mustard seed like brown sclerotia are formed.

3.1.6. Identification, multiplication and preservation of the pathogen

Pure culture of the isolates were prepared following hyphal tip methods (Tuite 1969, Mian, 1995) and subsequently transferred to fresh PDA slants in test-tubes and petridishes. Petridishes and test tube slants containing pure culture of *Sclerotium rolfsii* were stored at 4^{o} C.

A B

C

Plate 1. A. Foot rot disease sample of betel vine, B. Infected vine segment on moist blotter and C. Pure culture of *S. rolfsii* showing immature sclerotia

3.1.7. Evaluation of suitable management strategies for controlling foot and root rot disease of betel vine caused by *Sclerotium rolfsii*

Treatments

T_1 = Bavistin 50 WP

T_2 = Topgan

T_3 = Tilt 250 EC

T_4 = Ridomil gold

T_5 = Rovral 50 WP

T_6 = Dithane M-45

T_7 = Garlic clove extract

T_8 = Onion bulb extract

T_9 = Ginger rhizome extract

T_{10} = Neem leaf extract

T_{11} = Allamonda leaf extract

T_{12} = *Trichoderma harzianum*

T_{13} = Pseudomonas *fluorescens*

T_{14}= Untreated control

3.1.7.1. Screening of fungicides, plant extracts and bio-agents against *Sclerotium rolfsii*

3.1.7.2. Selection of fungicides

Six fungicides namely Bavistin 50 WP, Topgan, Tilt 250 EC, Ridomil gold, Rovral 50WP, and Dithane M-45 were tested following poisoned food technique *in-vitro* to evaluate their effect on colony growth and sclerotia formation of *Sclerotium rolfsii*. The details of the fungicides are presented in the Table 1

Table 1. Fungicides used in the Bio –assay against *Sclerotium rolfsi*

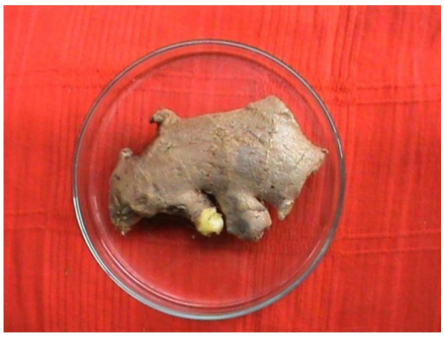
Trade name	**Common name**	**Active ingredient**	**Conc. Used**
Bavistin 50 WP	Mythyl—Benzimidazole Carbamate	50% Carbendazim	2g / L
Topgan	Copper-oxychloride	50% Copper-oxychloride	2g / L
Tilt 250 EC	1-[2-(2,4-Dichlorophenyl)-4-propyle-1,3-dioxalane-2	250 ml/ Litre Propiconazole	2ml /L
Ridomil gold MZ 68 WP	Metalaxyl+Mancozeb	68% Metalaxyl	5g / L
Rovral-50WP	3-(3,5-dichlorophenyl)-N-(1-(methyl)-2,4-dioxo-1-imidazolidine-carboxamide	50% Iprodione	2g / L
Dithane M-45	Manganous ethylene bisdithiocarbamate-ion	80% Mancozeb	2g / L

3.1.7.3. Collection of botanicals

Botanicals were collected from different places (Plate.2). Garlic, ginger, onion were collected from the Agargoan market, Tejgoan, Dhaka. Leaves of Neem and Allamanda were collected from Sher-e-Bangla Agricultural University campus.

A

B



C

D

E

Plate 2. Plant parts used to test antifungal activity against *Sclerotium rolfsii* A. Garlic (*Allium sativum*), B. Onion (*Allium cepa*), C. Ginger (*Zingiber officinale)* D. Neem (*Azadirachta indica*) and E. Allamanda (*Allamanda cathertica*)

Table 2. The particulars of plant species used in this study

Common name	**English name**	**Scientific name**	**Plant parts used**
Garlic	Garlic	*Allium sativum*	Clove
Onion	Onion	*Allium cepa*	Bulb
Ginger	Ginger	*Zingber officinale*	Rhizome
Neem	Margosa tree	*Azadirachta indica*	Leaf
Allamanda	Allamanda	*Allamanda cathertica* L.	Leaf

3.1.7.4. Preparation of plant extracts

The extracts were prepared by using 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 plant parts. The particulars of the botanicals used for the experiment are listed in Table. 2.

3.1.7.5. 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

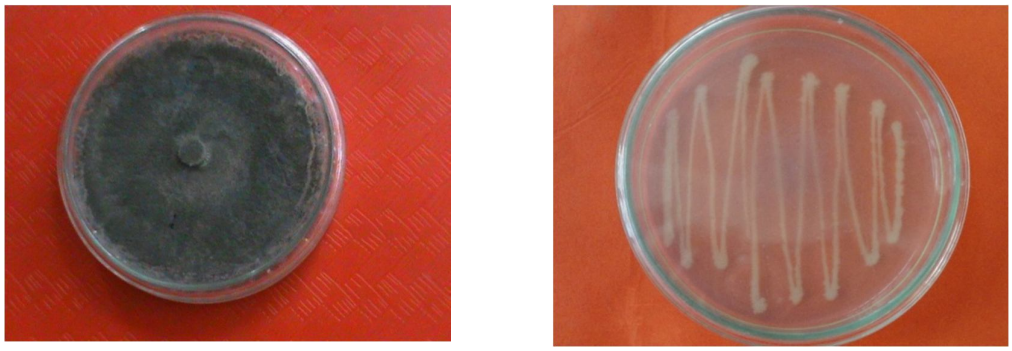
from the centre by a sterilized disc cutter. One milliliter of plant extract was put into each hole and the plates were stored overnight in refrigerator for diffusion of the input in the medium around the hole before resumption of fungal growth. The next day, one 5-mm block of 7 days old fungal culture (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, 1997).

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

Two bio-agents comprising one fungi and one bacteria were evaluated against *S. rolfsii* following cup method and dual culture method.

3.1.7.7. Isolation / collection of biocontrol agents

Biocontrol agents *Trichoderma harzianum* were collected from Bangladesh Agricultural University and *Pseudomonas fluorescens* were collected from Laboratory of Sher-e-Bangla Agricultural University department of Plant Pathplogy (Plate. 3).The fungal antagonists were cultured in Potato Dextrose Agar (PDA) medium and the bacteria in Nutrient Agar (N.A) medium.



A **B**

Plate 3. Bio-agents used to test antifungal activity against *Sclerotium rolfsii* A. Pure culture of *Trichoderma harzianum* and B. Pure culture of *Pseudomonas fluorescens*

3.1.7.8. Dual culture method for screening bio-agent against *Sclerotium rolfsii*

PDA media was prepared and sterilized in an autoclave at 121^{o}C for 15minutes 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^{o}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.

3.1.7.9. 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:

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

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.

3.1.7.10. Counting of sclerotia

After 30 days the sclerotia of each petridish were separated by using camel hair brush and number of sclerotia of each petridish was counted manually. Here a petridish was maintained as control to compare it with others.

3.2. Field experiment

3.2.1. Experimental site

The field experiments were conducted in the field of Malonchi upazila in Pabna district under natural condition.

3.2.2. Experimental period

The field experiment was carried out during the period from January, 2013 to July, 2013.

3.2.3. Soil type

The experimental site was situated in the sub-tropical zone. The soil of the experimental site lies in Agro-Ecological Zone (AEZ No.11) -High Ganges River Floodplain. This tract represents the riverine lands of the Gangetic plains. The soils are rich and are characterized by high lime content and are well supplied with phosphate. The texture varies from clay loam to light sandy loam according to its formation from the silt of the various tributaries of the Ganges. The PH varies from 7 to 8.4.

3.2.4. Design and layout of the experiment

The experiment was carried out in Randomized Complete Block Design (RCBD) with three replications. The field was divided into seven blocks with three unit plots in each. Each block contains one hill of betel vine and each hill contains three plants.

3.2.5. Land preparation

A piece of medium high land with well drainage system was selected. The soil was well pulverized for tilth condition. Weeds and stubbles were removed. Provide drainage trenches of 0.5 m width by 0.5 m depth in between two adjoining beds.

3.2.6. Plantation of betel vine

Selected healthy and disease free 63 betel vine sets were planted in the experimental field. Planting is done with the help of khurpi (a hand operated implement). For planting, a hole is made with khurpi, so that the internodes below the bud point is dipped in soil, but must be touching with surface soil. The hole is completely packed with the help of thumb finger. After that, planted material is covered with straw. This planted betel vine plant was watered twice a day with the help of watering cane or sprinkler.

3.2.7. Inoculum preparation

6 petri plates (90 mm) of pure culture of *S. rolfsii* were cultured and pure mycelia were collected from pure culture with the help of camel hair brush and blended with the help of electric blender to make mycelial suspensions @ 50 mg/ml of distilled water. Then pure mycelial suspensions were mixed with 4 kg 200 g sterilized soil.

3.2.8. Inoculation of pathogen

Mycelial suspensions of *S. rolfsii* mixed soil were incorporate with the base of each 21 hills @ 200 g soil / hill.

3.2.9. Treatments

T_1 =Vine treatment with Bavistin

T_2 =Vine treatment with Topgan

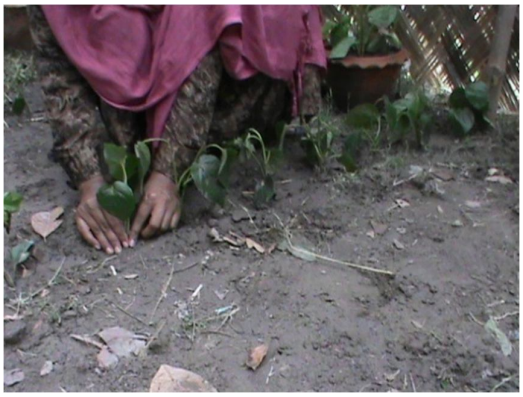
T_3 =Vine treatment with Tilt 250 EC

T_4 =Vine treatment with Garlic clove extract

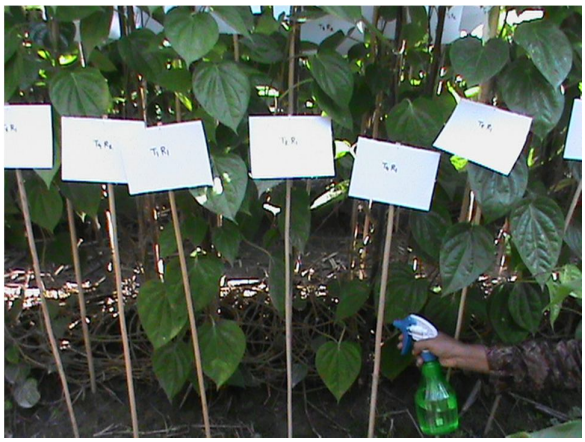
T_5 =Vine treatment with Neem leaf extract

T_6 =Vine treatment with *Trichoderma* spp.

T_7 = Untreated vine (Untreated control)



A **B**



C

Plate 4. A. Plantation of betel vine B. Field view of experimental plot and C. Spraying of fungicides in the field of betel vine

3.2.10. Irrigation

Irrigate the field immediately after planting and afterwards once in a week.

3.2.11. Weeding

Weeding was done fourth time in the whole experimental period at 20 days after planting, 40 days after planting, 55 days after planting and 7o days after planting.

3.2.12. Evaluation of suitable management strategies for controlling foot and root rot disease of betel vine in field condition caused by *Sclerotium rolfsii*

3.2.12.1. Screening of fungicides, plant extracts and bio-agents against *Sclerotium rolfsii* in field condition

3.2.12.2. Application of fertilizer and manures

The following dose of fertilizers and manures were applied to the plot for betel vine cultivation. First application at 15 days after lifting the vines and second and third dose at 40 - 45 days intervals.

Time of application	N	P	K
	(kg/ha)		
Basal dressing	37	100	50
Top dressing @ 3 split doses	112	0	0

3.2.12.3. Spraying of fungicides

Fungicide solutions were prepared separately by taking requisite amount of fungicides for each dose. The fungicides were sprayed at 7 days interval for two times by hand sprayer. Precautions were taken to avoid drifting of spray materials from plant to neighboring plants.

3.2.12.4. Collection and preparation of plant extracts

The plant extracts were prepared using the method of Ashrafuzzaman and Hossain (1992) as described in laboratory experiment (3.1.7.4).

3.2.12.5. Spraying of plant extracts

The different plant extracts were sprayed at 7 days intervals for 60 days after transplanting.

3.2.12.6. Spraying of BAU-biofungicides / bio-agent

A formulated product of *Trichoderma harzianum*, developed by Prof. Dr. Ismail Hossain, Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh (Hossain, 2003) was sprayed at 2% solution at 7 days interval for two times by hand sprayer.

3.2.13. Data collection

The data were recorded on the following parameters at an interval of 30 days as shown below:

1. Number of leaf / plant
2. Number of sclerotia / plant
3. Percent disease incidence
4. Percent disease severity or percent foot area diseased
5. Yield (ton / ha)

3.2.14. Assessment of disease incidence were calculated by the following formula:

Percent disease incidence was calculated using the formula as:

$$\text{Percent disease incidence} = \frac{\text{Number of diseased plant}}{\text{Number of total plants observed}} \times 100$$

Percent disease severity was calculated using the formula as:

$$\text{Percent disease severity} = \frac{\text{Area of stem tissue infected by disease}}{\text{Total stem area inspected}} \times 100$$

3.2.15. Statistical analysis of data:

Completely Randomized Design (CRD) was followed for the laboratory experiments. Randomized Completely Block Design (RCBD) was followed for field experiments. The data were statistically analyzed by using computer package program (MSTAT-C). The significant difference of the treatment means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS

This chapter includes the experimental results. Effect of the treatments in controlling foot and root rot disease of betel vine caused by *Sclorotium rolfsii* was assessed *in vitro* and *in vivo* method. The results were compiled based on the inhibition of radial mycelium growth, number of leaf / plant, number of sclerotia / plant, percent disease incidence, percent disease severity and yield (ton / ha).

4.1. Laboratory experiment

4.1.1. *In vitro* efficacy of fungicides in inhibition of mycelial growth of *Sclerotium rolfsii* in poisoned food technique (cup method)

Efficacy of fungicides on radial mycelial growth of *Sclerotium rolfsii* is shown in Table 3. and Plate 5. Fungicides have profound effect on reduction of radial mycelial growth of the fungus. All the tested fungicides significantly reduced radial mycelial growth of the fungus. Radial mycelial growth for all the tested fungicides ranged from 2.70 cm to 9.00 cm recorded after inoculation of 4 days. The lowest radial mycelial growth (1.39 cm, 2.29 cm, 2. 48 cm, 2.70 cm) of *Sclerotium rolfsii* was recorded in case of Bavistin at 1 day, 2 days, 3 days, 4 days after inoculation respectively. The performance of Bavistin in reduction of radial mycelial growth was the best followed by Topgan, Tilt 250 EC, Ridomil Gold, Rovral and Dithane M-45 irrespective of days after

incubation. The highest radial mycelium growth (9.00 cm) was recorded in untreated control preceded by Ridomil Gold (7.19 cm), Rovral (7.34 cm) and Dithane M-45 (7.70 cm) at 4 days after inoculation. Bavistin was found promising in reducing the growth of the fungus in the laboratory followed by Ridomil Gold.

All the tested fungicides have strong effect to produce percent inhibition against *Sclerotium rolfsii* in culture media. The highest percent inhibition (70%) was recorded in case of Bavistin preceded by Rovral (18.44%) and Dithane M-45 (14.44%) at 4 days after inoculation. No growth inhibition was found in case of untreated control treatment.

Table 3. *In vitro* efficacy of fungicides in inhibition of mycelial growth of *Sclerotium rolfsii* in poisoned food technique (cup method)

Treatments	Radial mycelial growth (cm)				% Inhibition of mycelial growth (4 DAI)
	1 DAI	2 DAI	3 DAI	4 DAI	
Bavistin 50 WP	1.39 g	2.29 g	2.48 g	2.70 g	70
Topgan	1.50 f	2.49 f	2.77 f	3.10 f	65.55
Tilt 250 EC	1.55 e	2.56 e	2.81 e	3.14 e	65.11
Ridomil Gold	1.60 d	4.20 d	5.20 d	7.19 d	20.11
Rovral 50 WP	1.65 c	4.26 c	5.30 c	7.34 c	18.44
Dithane M-45	1.70 b	4.34 b	5.43 b	7.70 b	14.44
Control	2.13 a	6.03 a	8.41 a	9.00 a	-
LSD (0.05)	0.00055	0.05538	0.00055	0.00055	-

In a column, DAI = Days after inoculation

A **B** **C**

D **E** **F**

Plate 5. Radial mycelial growth of *S. rolfsii* against A. Bavistin 50 WP, B. Topgan, C. Tilt 250 EC, D. Rovral 50 WP, E. Ridomil Gold, F. Control after 4 days of inoculation

4.1.1.2. *In vitro* efficacy of plant extracts in inhibition of mycelial growth of *Sclerotium rolfsii* in poisoned food technique (cup method)

Efficacy of plant extracts on radial mycelial growth of *Sclerotium rolfsii* is shown in Table 4. and Plate 6. Plant extracts have profound and significant effect on reduction of radial mycelial growth of the fungus. Radial mycelial growth for all the tested plant extracts ranged from 6.70 cm to 9.00 cm recorded after incubation of 4 days. The lowest radial mycelial growth (1.70 cm, 3.26 cm, 5.03 cm, 6.70 cm) of *Sclerotium rolfsii* was recorded in case of Garlic at 1 day, 2 days, 3 days, 4 days after inoculation respectively. The performance of Garlic in reduction of radial mycelial growth was the best followed by Onion, Ginger, Neem, Allamonda irrespective of days after inoculation. The highest radial mycelial growth (9.00 cm) was recorded in untreated control preceded by Onion (8.86 cm), Allamonda (8.85 cm) and Ginger (8.83 cm) at 4 days after incubation. Garlic was found promising in reducing the growth of the fungus in the laboratory followed by Onion and Allamonda.

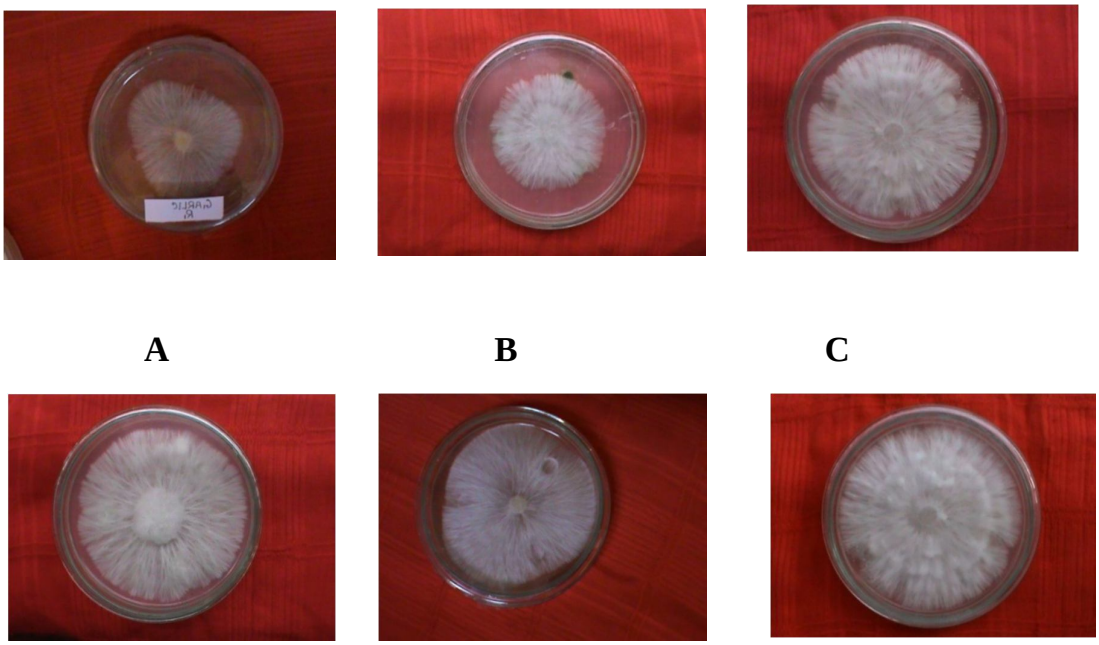
All the tested plant extracts have strong effect to produce percent mycelial growth inhibition of *Sclerotium rolfsii* in culture media. The highest percent inhibition (25.56%) was recorded in case of Garlic preceded by Onion (1.56%), Allamonda (1.67%) and Ginger (1.89%) at 4

days after inoculation. No percent inhibition was found in case of untreated control treatment.

Table 4. *In vitro* efficacy of plant extracts in inhibition of mycelial growth of *Sclerotium rolfsii* in poisoned food technique (cup method)

Treatments	Radial mycelial growth (cm)				% Inhibition of mycelial growth (4 DAI)
	1DAI	2DAI	3DAI	4DAI	
Garlic	1.70 c	3.26 d	5.03 c	6.70 c	25.56
Onion	2.00 ab	5.85 a	8.35 a	8.86 a	1.56
Ginger	1.98 ab	5.41 b	8.38 a	8.83 a	1.89
Neem	1.74 c	3.43 d	5.41 c	7.00 b	22.22
Allamonda	1.91 b	4.91 c	7.40 b	8.85 a	1.67
Control	2.13 a	6.03 a	8.43 a	9.00 a	-
LSD (0.01)	0.1932	0.4247	0.3943	0.1932	-

In a column, DAI = Days after inoculation



A **B** **C**

D E F

Plate 6. Radial mycelial growth of *S. rolfsii* against A. Garlic extracts, B. Neem leaves extracts, C. Allamonda extracts, D. Onion extracts, E. Ginger extracts, F. Control after 4 days of inoculation

4.1.1.3. *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 5. and (Plate. 7) Bio-agents have significant effect on reduction of radial mycelial growth of the fungus. Radial mycelium growth of S. 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, 5.15 cm) of Sclerotium rolfsii was recorded in case of Trichoderma harzianum at 1 day, 2 days, 3 days, 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 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 5. *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 (4 DAI)
	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	2.13 a	6.03 a	8.41 a	9.00 a	-
LSD (0.05**)**	0.00063	0.08935	0.00063	0.00063	-

In a column, DAI = Days after inoculation

A

B

C

Plate 7. Radial mycelial growth of *S. rolfsii* against A. *Trichoderma harzianum*, B. *Pseudomonas fluorescens* and C. Control after 4 days of inoculation

4.1.1.4. *In vitro* efficacy of fungicides, plant extracts and bio-agents on sclerotia formation of *Sclerotium rolfsii*

Efficacy of fungicides, plant extracts and bio-agents on sclerotia formation of *Sclerotium rolfsii* is shown in Table 6. and Plate 8. All the tested fungicides, plant extracts and bio-agents have profound effect on decreased sclerotia formation of the fungus. All the tested fungicides, plant extracts and bio-agents significantly reduced the number of sclerotia formation of the fungus. Number of sclerotia for all the treatments ranged from 114.7 to 529.0 recorded after inoculation of 30 days. The lowest number of sclerotia (114.7) of *Sclerotium rolfsii* was recorded in case of Bavistin at 30 days after incubation. In case of Topgan the number of sclerotia (122.7) that was statistically similar with the number of sclerotia (124.3) of Tilt 250 EC. Among 5 plant extracts garlic clove extract (209.0) and neem leaf extracts (214.0) showed better performance and among 2 bio-agents *Trichoderma harzianum* (132.3) showed better performance. Numbers of sclerotia produced at others fungicides, plant extracts and bia-agents were significantly different among each other. The highest number of sclerotia (529.0) of *Sclerotium rolfsii* was recorded in case of untreated control treatment preceded by *Trichoderma harzianum*, Garlic, Topgan at 30 days after inoculation. Bavistin was found promising in reducing the number of sclerotia formation of the

fungus in the laboratory followed by Topgan, Ridomil Gold, Garlic, Neem, *Trichoderma harzianum*.

All the tested fungicides, plant extracts and bio-agents have strong effect to produce percent reduction of number of sclerotia against *Sclerotium rolfsii* in culture media. The highest percent reduction of number of sclerotia (78.32%) was recorded in case of Bavistin preceded by Rovral (41.39%), Garlic (60.49%), Neem (59.55%) and *Trichoderma harzianum* (74.99%) at 4 days after inoculation.

Table 6. ***In vitro*** **efficacy of fungicides, plant extracts and bio-agents on sclerotia formation of** ***Sclerotium rolfsii***

Treatments	Number of sclerotia	% Reduction of number of sclerotia over control
Bavistin 50 WP	114.7 m	78.32
Topgan	122.7 l	76.81
Tilt 250 EC	124.3 l	76.51
Ridomil Gold	305.0 g	42.34
Rovral 50WP	310.3 f	41.39
Dithane M-45	314.3 e	40.59
Garlic	209.0 j	60.49
Onion	349.7 c	33.89
Ginger	353.3 b	33.21
Neem	214.0 i	59.55
Allamonda	318.3 d	39.88
Trichoderma harzianum	132.3 k	74.99
Pseudomonas fluorescens	232.3 h	56.09
Control	529.0 a	-
LSD (P = 0.01)	2.913	-

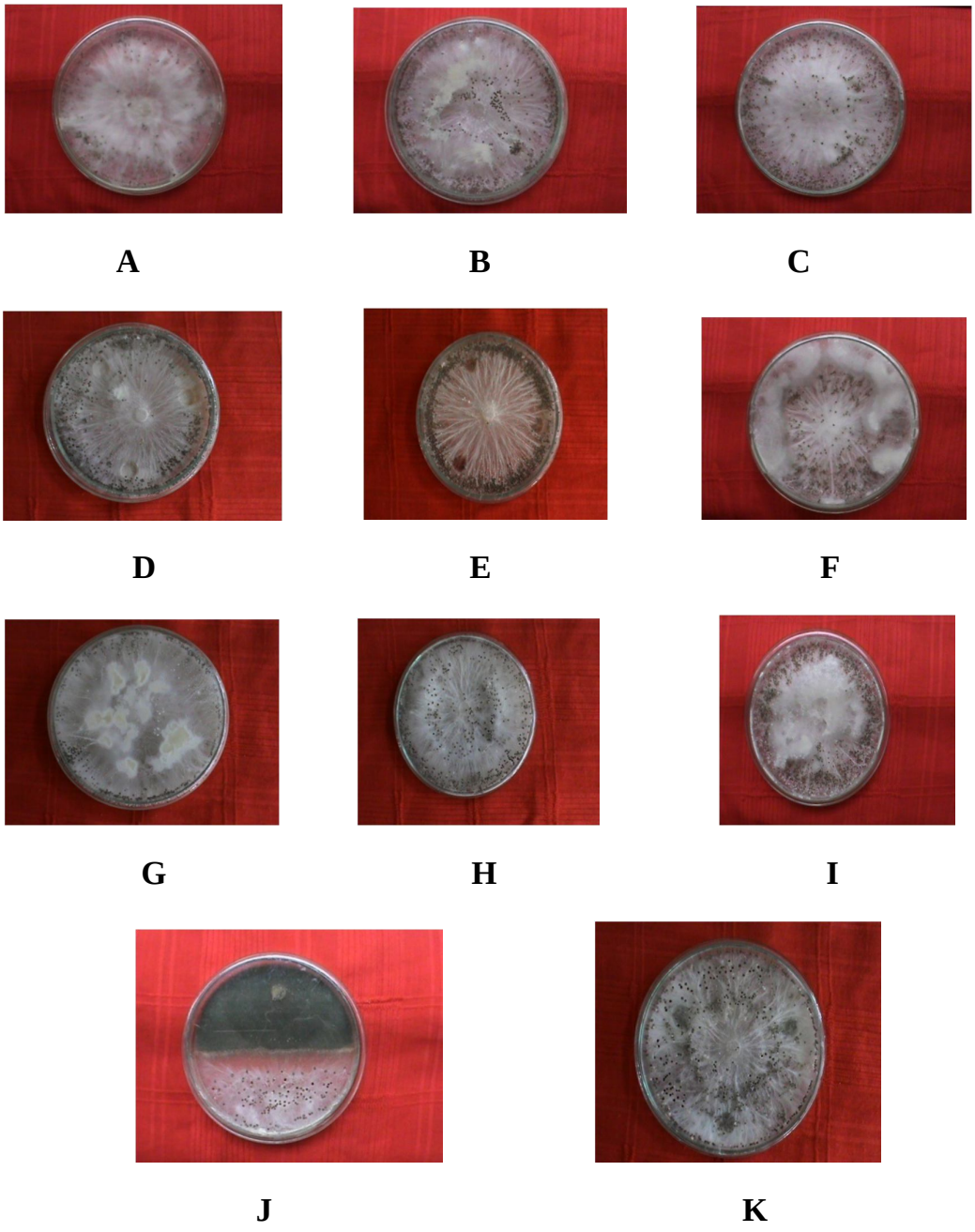


Plate 8. Sclerotia formation under different treatments A. Bavistin 50 WP, B. Tilt 250 EC, C. Topgan, D. Ridomil Gold, E. Rovral, F. Garlic extracts, G. Neem extracts, H. Allamonda Extracts, I. Onion extracts, J. *Trichoderma harzianum* and K. Control

4.2. Field experiment

4.2.1. Efficacy of fungicides, plant extracts and bio-agents on number of leaf / plant in field condition

The effect of the treatments on number of leaf / plant was determined and presented in Table 7. All the treatments have strong effect to increasing the number of leaf / plant with the increase of the age of the plant. The highest number of leaf / plant (19) was found in Bavistin treated plot followed by Topgan (17.33) and the lowest number of leaf / plant (12.67) was found in untreated control condition at 120 DAT. Number of leaves / plant while garlic clove extract and *Trichoderma harzianum* treated plots were 15.00 and 15.33 respectively. Among all the treatments, Bavistin was the best for percent increasing number of leaf / plant (49.96%) followed by Topgan (36.77%).

Table 7. Efficacy of fungicides, plant extracts and bio-agents on number of leaf / plant in field condition

Treatments	Number of leaf / plant			% Increase of number of leaf over control
	60 DAT	90 DAT	120 DAT	
Bavistin 50 WP	10.00 hi	15.67 cd	19.00 a	49.96
Topgan	9.66 i	14.33 de	17.33 b	36.77
Tilt 250 EC	9.33 ij	13.33 ef	16.67 bc	31.57
Garlic	8.66 ijk	12.33 fg	15.00 d	18.39
Neem	8.00 jk	11.33 gh	14.33 de	13.10
Trichoderma harzianum	8.66 ijk	12.33 fg	15.33 cd	20.29
Control	7.33 k	9.66 i	12.67 fg	-
LSD (0.05)	1.38	1.38	1.38	-

In a column, DAT = Days After Transplanting

4.2.2. Efficacy of fungicides, plant extracts and bio-agents on number of sclerotia / plant in field condition

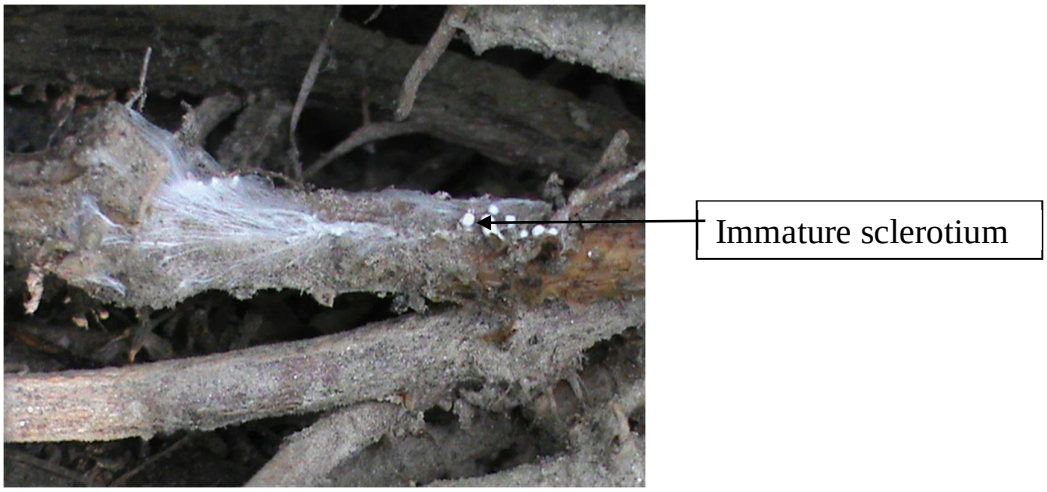
The effect of the treatments on number of sclerotia / plant was determined and presented in Table 8. All the treatments have strong effect in reducing the number of sclerotia / plant. The lowest number of sclerotia / plant (0.71) was found in Bavistin treated plot followed by garlic clove extract (1.57) and the highest number of sclerotia / plant (3.69) was recorded in untreated control condition at 120 DAT. The maximum percent reduction of number of sclerotia was recorded in Bavistin (80.77%) treated plot followed by garlic clove extract (57.29%).

Table 8. Efficacy of fungicides, plant extracts and bio-agents on number of sclerotia / plant in field condition

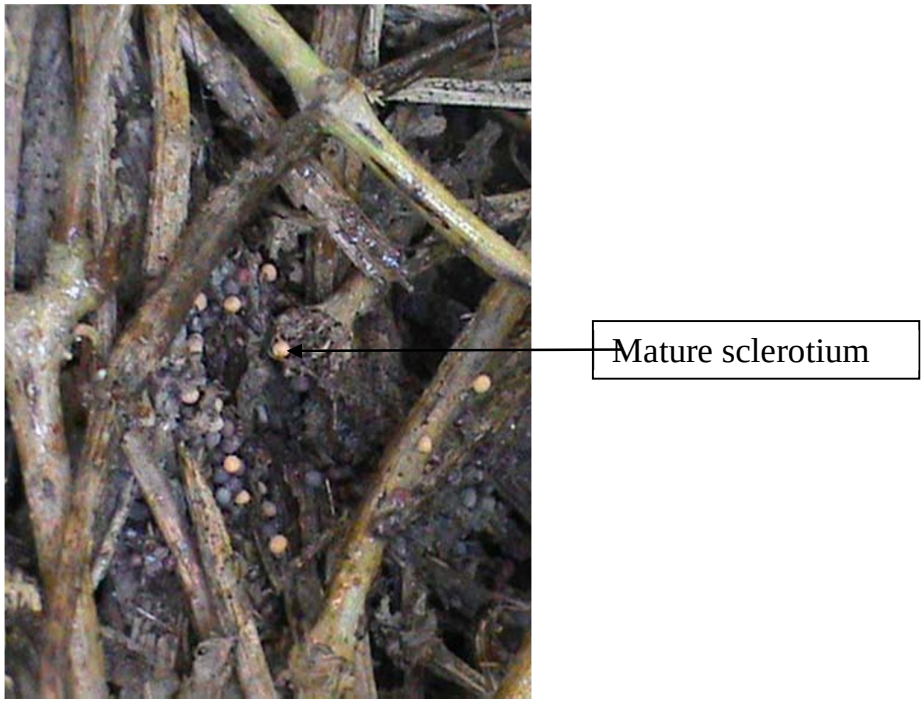
Treatments	Number of sclerotia / plant		% Reduction of number of sclerotia over control
	90 DAT	120 DAT	
Bavistin 50 WP	0 (0.71) c	0 (0.71) c	80.77
Topgan	0 (0.71) c	0 (0.71) c	80.85
Tilt 250 EC	0 (0.71) c	0 (0.71) c	80.85
Garlic	2.33 (1.38) bc	3.66 (1.57) bc	57.29
Neem	3.0 (1.49) bc	7.67 (2.49) b	32.41
Trichoderma harzianum	0 (0.71) c	7 (2.39) b	35.15
Control	5.67 (2.23) b	13.67 (3.69) a	-
LSD (0.05)	1.14	1.15	-

Data in parenthesis denotes the transformed values

In a column, DAT = Days After Transplanting



A



B

Plate 9. Mycelium and sclerotia formation of *Sclerotium rolfsii* in the infected vine A. Mycelium with initiation of sclerotia and B. Mature sclerotium

4.2.3. Efficacy of fungicides, plant extracts and bio-agents on percent disease incidence of foot rot disease of betel vine in field condition

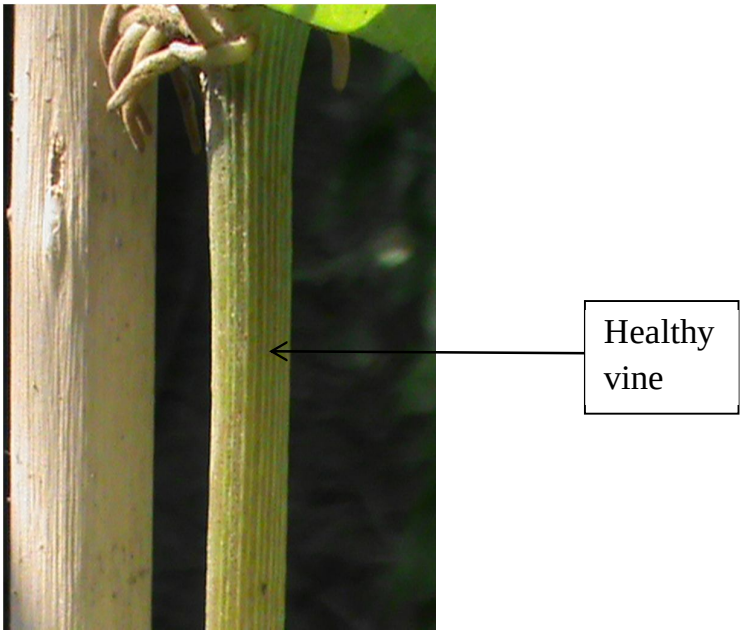
Data recorded on percent disease incidence of foot rot disease of betel vine as affected by the application of different fungicides, plant extracts and bio-agent were summarized and presented in Table 9. The effects of different treatments recorded at different days after transplanting (DAT) differed significantly as compared to control. The results showed that the spraying of Bavistin gave the lowest disease incidence that were (0.71%) at 120 DAT followed by Topgan (2.39%). The highest disease incidence was recorded in control treatment which were (4.08%), (7.36%) and (8.16%) respectively at 60 DAT, 90 DAT and 120 DAT while the garlic clove extract and *Trichoderma harzianum* treated plot showed (4.88%) and (5.67%) disease incidence respectively at 120 DAT. It was noted that the percent disease incidence was gradually increased with the increase of age of the plant and increasing rate was must slower in Bavistin and Topgan treated polt compared to control. Among all the treatments, Bavistin was the best for reducing percent disease incidence (91.30%) of betel vine.

Table 9. Efficacy of fungicides, plant extracts and bio-agents on percent disease incidence of foot rot disease of betel vine in field condition

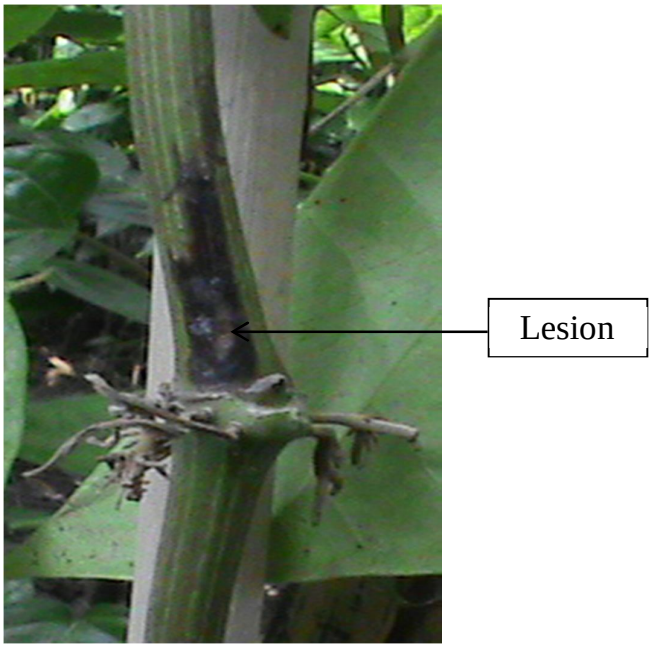
Treatments	Disease incidence			% Reduction of disease incidence over control
	60 DAT	90 DAT	120 DAT	
Bavistin 50 WP	0 (0.71) c	0 (0.71) c	0 (0.71) c	91.30
Topgan	0 (0.71) c	11.11 (2.39) bc	11.11 (2.39) bc	70.65
Tilt 250 EC	0 (0.71) c	11.11 (2.39) bc	11.11 (2.39) bc	70.65
Garlic	11.11 (2.39) bc	33.33 (4.88) abc	33.33 (4.88) abc	40.21
Neem	11.11 (2.39) bc	33.33 (4.88) abc	44.44 (5.67) ab	30.44
Trichoderma harzianum	0 (0.71) c	22.22 (4.08) abc	44.44 (5.67) ab	30.44
Control	22.22 (4.08) abc	55.55 (7.36) a	66.66 (8.16) a	-
LSD (0.05)	4.45	4.45	4.46	-

Data in parenthesis denotes the transformed values

In a column, DAT = Days After Transplanting



A



B

Plate 10. A. Healthy vine and B. Foot and root rot infected vine with lesion caused by *Sclerotium rolfsii*

4.2.4. Efficacy of fungicides, plant extracts and bio-agents on percent foot area diseased (% FAD) of betel vine in field condition

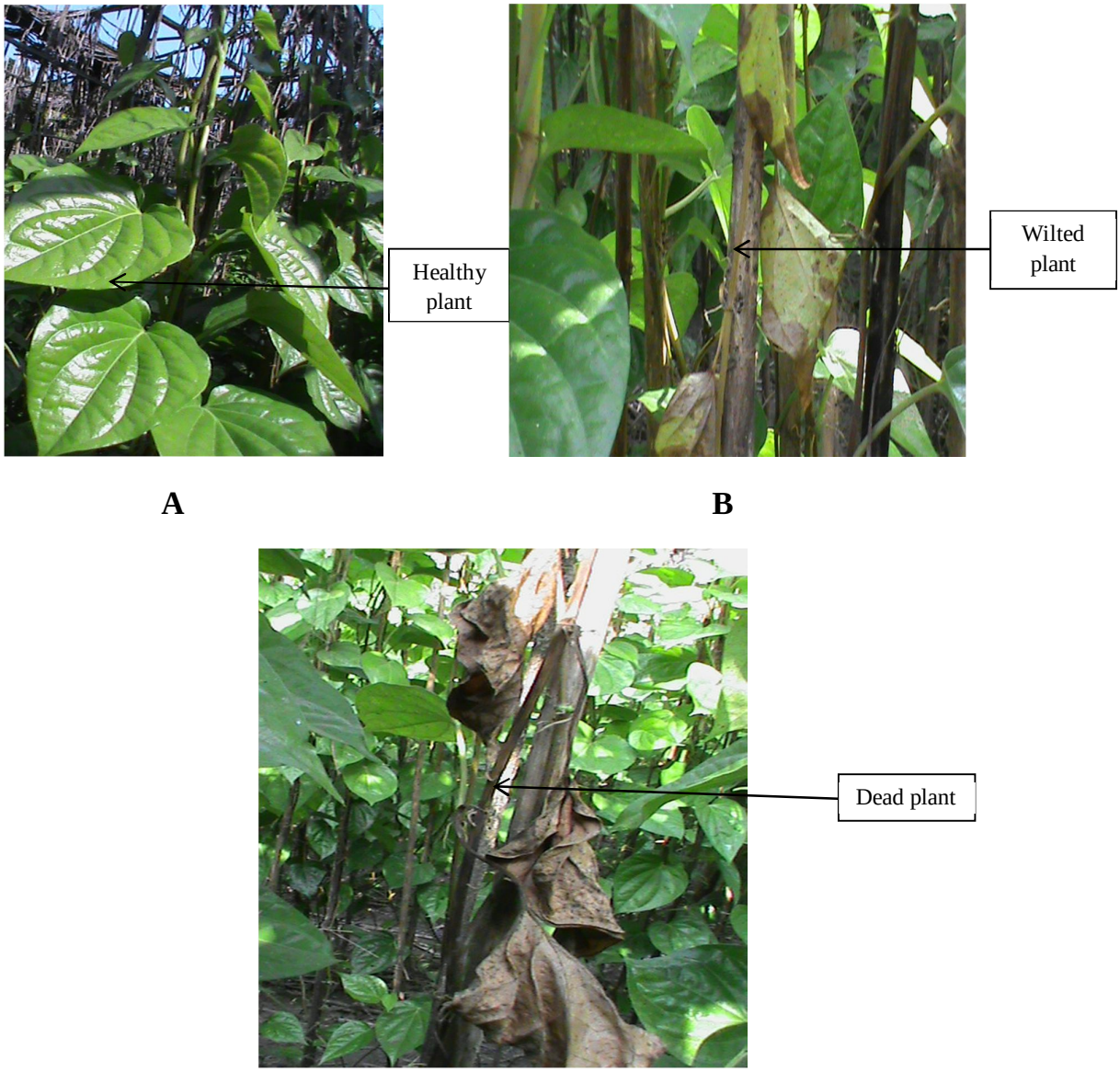
The effect of all the treatments on disease severity of percent foot area diseased of betel vine stem was determined and presented in Table 10. The percent foot area diseased under different treatments was found to differ significantly from one to another. The lowest percent foot area diseased of betel vine was found with the Bavistin (0.71%) followed by Topgan (0.94%) and the highest in the untreated control (2.87%) condition at 120 DAT. Among plant extracts and bio-agent garlic clove extract (1.69%) and *T. harzianum* (1.60%) showed better performance compared to control. Among all the treatments, Bavistin was the best for reducing percent foot area diseased (75.26%) of betel vine.

Table 10. Efficacy of fungicides, plant extracts and bio-agents on percent foot area diseased (% FAD) of betel vine in field condition

Treatments	Percent foot area diseased (% FAD)			% Reduction of foot area diseased over control
	60 DAT	90 DAT	120 DAT	
Bavistin 50 WP	0 (0.71) d	0 (0.71) d	0 (0.71) d	75.26
Topgan	0 (0.71) d	0.86 (0.96) cd	0.49 (0.94) cd	67.22
Tilt 250 EC	0 (0.71) d	0.64 (0.98) cd	0.59 (0.97) cd	66.14
Garlic	1.04 (1.10) cd	1.87 (1.44) bcd	2.87 (1.69) bc	41.03
Neem	0.96 (1.08) cd	1.9 (1.47) bcd	3.12 (1.75) bc	39.04
Trichoderma harzianum	0 (0.71) d	1.0 (1.29) cd	2.49 (1.60) bc	44.02
Control	2.08 (1.50) bcd	4.6 (2.26) ab	7.74 (2.87) a	-
LSD (0.05)	0.85	0.85	0.852	-

Data in parenthesis denotes the transformed values

In a column, DAT = Days After Transplanting



A **B**

C

Plate 11. A. Healthy plant, B. Foot and root rot diseased wilted plant and C. Foot and root rot diseased with dead plant

4.2.5. Efficacy of fungicides, plant extracts and bio-agents on yield (**ton / ha**) **of betel vine in field condition**

Data recorded on yield of betel vine by the application of different fungicides, plant extracts and bio-agent were summarized and presented in Table 11. Yield of betel vine differed statistically due to the effect of different fungicides, plant extracts and bio-agent. Mean yield of betel vine ranged from 2.93 (ton / ha) to 1.74 (ton/ ha). The highest yield (2.93 ton / ha) was recorded in the plot treated with Bavistin followed by Topgan (2.59 ton / ha), neem leaf extract (2.12 ton/ ha). The lowest yield (1.74 ton / ha) was recorded in control plot. Application of garlic clove extract (2.25 ton / ha) and *Trichoderma harzianum* (2.26 ton / ha) was more effective compared to control.

Table 11. Efficacy of fungicides, plant extracts and bio-agents on yield (ton / ha) of betel vine in field condition

Treatments	Yield (ton / ha)	% Yield increase over control
Bavistin 50 WP	2.93 a	68.46
Topgan	2.59 b	49.05
Tilt 250 EC	2.58 b	48.30
Garlic	2.25 c	29.29
Neem	2.12 d	21.82
Trichoderma harzianum	2.26 c	30.15
Control	1.74 e	-
LSD (0.05)	0.07380	-

DISCUSSION

Effect of the treatments in controlling foot and root rot disease of betel vine caused by *Sclerotium rolfsii* was assessed based on the result of laboratory experiment and field experiment. Discussions on laboratory experiment and field experiment are presented in this chapter.

Laboratory experiment

The fungicides, plant extracts and bio-agents assay in the laboratory showed significant effect in reducing radial mycelial growth of *Sclerotium rolfsii*. It has been also found that Bavistin 50 WP and Topgan 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 stydy among five plant extracts it has been also found that Garlic clove extracts and Neem leaf extracts have strong effect to inhibit mycelial growth of *Sclerotium rolfsii* in culture media. 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 (1990) reported that, the viability of sclerotia was reduced when treated with neem oil.

In the present study among two bio-agents it has been also found that *Trichoderma harzianum* have strong effect to inhibit mycelial growth of *Sclerotium rolfsii* in culture media. 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 T8, T10 and T12 were effective against *S. rolfsii* and they over grew the pathogen up to 92%, 85% and 79% respectively *in vitro*.

Field experiment

The effect of fungicides, plant extracts and bio-agents in term of number of leaf/plant were found remarkable in the field experiment. Data on number of leaf/plant recorded at different DAT showed that spraying of Bavistin proved to be the most effective in percent increase of number of leaf / plant (49.96%) followed by Topgan (36.77%) and Tilt 250EC (31.57%). Among the plant extracts and bio-agents *Trichoderma harzianum* showed better performance in percent increase of number of leaf / plant (20.99%) followed by garlic clove (18.39%) and neem leaf extracts (13.10%).

In case of number of sclerotia / plant, the result showed that Bavistin proved to be the best potential among the fungicides, plant extracts and bio-agents used in the experiment followed by garlic clove, neem

leaf extracts and *Trichoderma harzianum*. Bavistin is the most effective in percent reduction of number of sclerotia (80.77%) followed by garlic clove (57.29%), neem leaf extracts (32.41%) and *Trichoderma harzianum* (35.15%) while Topgan and Tilt 250EC is simillar to the Bavistin. The present research work was supported by Singh and Dwivedi (1987) they observed that, hyphal dry weight and sclerotial production of *Sclerotium rolfsii* were significantly reduced by bark extracts of *Acacia arabica.* They also reported that bulb and leaf extracts of garlic and onion, leaf

extracts of *Rauvolfia serpentine, Lawsoni alba, Datura stramonium, Solanum xanthicarpum, Calotropis procera, Eucalyptus globus* and *Azadirachta indica* fruit and leaf extracts, *Emblica officinalis* and rhizome extracts of turmeric, ginger extracts against *S. rolfsii* and found that those extracts were more or less effective in inhibiting the fungus. In another study Singh *et al*. (1989) reported that, out of six plant oils tested against *S. rolfsii*, leaf oil of *Azadirachta indica* was found most effective followed by *Eucalyptus globules* and *Ocimum canum*.

In case of disease incidence, Bavistin (91.30%) also proved to be the superior among the fungicides, plant extracts and bio-agents used in the experiment against percent reduction of disease incidence followed by Topgan (70.65%) and garlic clove extracts (40.21%) and *Trichoderma harzianum* (30.44%).

In respect of disease severity or percent foot area diseased (% FAD), Bavistin (75.26%) also to be the promising effect against percent reduction of disease severity followed by Topgan (67.22%), Garlic clove extracts (41.03%) and *Trichoderma harzianum* (44.02%). The present research work was supported by Patil *et al.* (1986). They found effective control of foot rot disease of *Piper betle* by soil drenches with Copper oxychloride, Cupravit, Dithane M-45, Difolatan and Bordeaux mixture. In another study Dutta (1975) reported that, soil application of Bavistin

(0.5-0.7%), Brassicol (0.1%), three times at 20 days interval has been effective in controlling foot and tuber rot disease of tuberose. In another study Mehrotra and Tiwari (1976) showed that dipping of cutting in a *Trichoderma viride* cell suspension effectively reduced the foot rot disease of betel vine.

In another study Muthamilan and Jeyarajan (1996) they 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.

Ellil *et al.* (1998) stated that, *T. harzianum reduced* root rot infection 6.7-45.0% in bean. *Trichoderma* spp. obviously antagonized the effects caused by the pathogen, *S. rolfsii* and *Fusarium solani*.

The effect of fungicides, plant extracts and bio-agents in term of yield (ton/ha) were found remarkable in the field experiment. Bavistin contributed the highest effect against foot and root rot of betel vine followed by Topgan, garlic clove extracts and *Trichoderma harzianum*. Bavistin treated plot showed the maximum yield (2.93 ton/ha) while yield of control was 1.74 ton/ha.

In this experiment data recorded on disease incidence and disease severity at different days after transplanting, it was noticed that the first onset of

infection and preliminary diseased development was more or less similar for all the treatments but the disease incidence and severity appeared to be distinct among the treatments in comparison to control with the progress of time due to the consecutive spraying with the fungicides, plant extracts and use of bio-agent. The findings of the study have ventilated an opportunity to the inhibitory effect of the treatments applied on the field for controlling the foot and root rot disease of betel vine with the increasing of yield. However, more investigation need to persue including more fungicides, plant extracts and bio-agents for consecutive year to confirm the result.

SUMMARY AND CONCLUSION

Betel vine (*Piper betle* L.) is an important cash crop in Bangladesh. Foot and root rot disease caused by *Sclerotium rolfsii* is a limiting factor of betel vine production in Bangladesh. The fungus reduces the yield and quality of leaves. The research program were undertaken to study the efficacy of some selected fungicides, plant extracts and bio-agents against foot and root rot disease (*Sclerotium rolfsii)* of betel vine in the field.

The experiment was carried out in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka 1207 and at the field of Malonchi upazila in Pabna district under natural condition during June 2012 to July 2013. The field experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications of each treatment. The *in vitro* effect of the fungicides, plant extracts and bio-agents were compiled based on inhibition of mycelial growth and number of sclerotia of *Sclerotium rolfsii* and *in vivo* effect of the treatments were compiled based on number of leaf / plant, number of sclerotia / plant, disease incidence, disease severity (stem lesion size) and yield (ton / ha).

In *in vitro* assay, Bavistin 50 WP performed the best result in inhibition of mycelial growth of *Sclerotium rolfsii* followed by Topgan. Among six

plant extracts, Garlic clove extracts showed better performance than other plant extracts in inhibition of mycelial growth of *Sclerotium rolfsii*. Between the two bio-agents *Trichoderma harzianum* showed better performance than *Pseudomonus fluorescens* in inhibition of mycelial growth of *Sclerotium rolfsii.* The highest number of sclerotia (529.0) was produced in untreated control condition and the lowest number of sclerotia (114.7) was produced in Bavistin in laboratory. The maximum percent reduction of number of sclerotia was recorded in case of Bavistin (78.32%).

In case of field experiment, the disease incidence were observed at different DAT. The minimum plant infectoin was observed in case of Bavistin treated plot. The lowest percent disease incidence (0.71%) was recorded in case of Bavistin at 120 DAT. The highest percent disease incidence (8.16%) was recorded in case of untreated control condition while garlic clove extract and *Trichoderma harzianum* treated plot showed (4.88%) and (5.67%) disease incidence respectively at 120 DAT. The plant infection gradually increased with the increase of the age of the plant and increasing rate was sharp in control plot but very slower in treated plot. The maximum percent reduction of disease incidence were recorded in Bavistin treated plot (91.30%).

From this study it is evident that the highest number of leaf / plant (19) was found in Bavistin treated plot and the lowest number of leaf / plant (12.67) was found in untreated control condition at 120 DAT. The lowest number of sclerotia / plant (0.70) was also found in Bavistin treated plot and the highest number of sclerotia / plant (3.69) was recorded in untreated control condition at 120 DAT.

Regarding disease severity, the lowest percent foot area diseased of stem were recorded in case of Bavistin (0.71%) treated plot at 120 DAT followed by Topgan (0.94%) and the highest percent foot area diseased of stem (2.87%) were recorded in case of untreated control condition while garlic clove extract and *Trichoderma harzianum* treated plot showed (1.69%) and (1.60%) percent foot area diseased of stem respectively at 120 DAT. The maximum percent reduction of FAD were Bavistin treated plot (75.26%).

In terms of yield, the highest yield (2.93 ton / ha) were achieved by applying Bavistin and the second highest performances were achieved by Topgan (2.59) and the lowest yield (1.74 ton / ha) were achieved by untreated control condition while (2.26 ton / ha) were achieved by *Trichoderma harzianum.*

From the findings of the present investigation it may be concluded that Bavistin had a promising effect in reducing the disease incidence and severity of foot and root rot disease of betel vine and also increasing the yield. Topgan also showed the second highest performance in suppressing the disease and increasing yield. Garlic clove extract and *Trichoderma harzianum* showed significantly better performances. Thus, the farmers may be suggested to use Bavistin wiyh Garlic clove extracts and *Trichoderma harzianum* for the control of foot and root rot disease of betel vine which is ecofriendly control the disease.

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