INTEGRATED APPROACH FOR THE MANAGEMENT OF FOOT AND ROOT ROT DISEASE OF BETELVINE (*PIPER BETLE* L.)

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CERTIFICATE

This is to certify that the thesis entitled, "INTEGRATED APPROACH FOR THE MANAGEMENT OF FOOT AND ROOT ROT DISEASE OF BETELVINE (*PIPER BETLE* l..)'' submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY, embodies the result of a piece of bona fide research work carried out by Md. Hafizur Rahman, bearing Registration no.: 23913/00166 under my supervision and guidance. No part of the thesis has been submitted any where for any other degree or diploma.

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

Dated:
Place : Dhaka,Bangladesh

Prof. Dr. Md. Rafiqul Islam
Chairman
Advisory Committee

Dedicated

to

My Parents and Child

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ABSTRACT

Betelvine (*Piper betle* L.) is an important cash as well as exporting crop of Bangladesh. The crop is attacked by various diseases. Of those foot and root rot caused by *Sclerotium rolfsii* is the most devastating one, which causes considerable losses of the crop in the country. An investigation was undertaken to develop an integrated disease management (IPM) approach against the disease. Preliminary experiments were conducted to find out present severity status of the disease in major betelvine growing areas of Bangladesh, to select IPM components and finally to evaluate the efficacy of integrated application of the selected IPM components against the disease. It was found that incidence of the disease in five upazillas varied within range of 5.60 – 28.80, 4.00 – 10.40 and 4.00 – 7.20% in late summer (August), late winter (February) and midsummer, respectively. The maximum incidence of 15.46% was recorded from Gournadi where soil pH was 5.4, air temperature 32.61°C, RH 82.46 and light intensity 53x100 lux, and the lowest incidence of 4.53% from Sitakunda where soil pH 6.6, air temperature 28.78°C, RH 72.67% and light intensity 74x100lux. Other than foot and root rot disease leaf spot or anthracnose (*Colletototrichum piperis*), leaf rot (*Phytophthora parasitica*) and stem rot (*Phytophthora parasitica*) were recorded from different upzillas. An experiment was conducted to identify the isolates of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from different areas of Bangladesh. Altogether 19 isolates of *S. rolfsii* were found to be associated with foot and root rot infected betelvine plants in different locations. The mycelial growth, colony colour, colony consistency, formation of sclerotia, number, shape, size and colour of sclerotia varied remarkably among the isolates. All 19 isolates were pathogenic. The most pathogenic isolate was isolate-9 from Kaligonj upazilla of Jhenaidah. Thirteen betelvine cultivars designated as PB 001 to PB 013 were collected from different locations of Bangladesh and found remarkable variations in vegetative growth, morphological features and disease reaction. Based on incidence of foot and root rot (8.33-100.00%) on those cultivars, PB 001 was graded as resistant, PB 011 and PB 013 as moderately susceptible and rest of ten cultivars as susceptible. *In-vitro* and *in-vivo* experiments were conducted and based on promising results, four chemical fungicides (Provax 200, Tilt 250 EC, Score 250 EC, Pencozeb 80 WP), two plant extracts (garlic clove, Allamanda leaf), two soil amendments (poultry manure, Vermicompost) and a bioagent (*T. harzianum*) were selected as IPM components. Efficacy of integrated application of those IPM components, in 22 treatment combinations including a control, was evaluated under inoculated conditions to control foot and root rot of betelvine. Visible symptoms of the disease did not appear on the betelvine plants up to 120 days after inoculation (DAI) under the treatment with *T. hazianum* + Provax-200. The first visible symptoms of the disease appeared within 9 to 116 DAI under other treatments. At 120 DAI, the maximum reduction of disease incidence of 100% was found under treatments *T. harzianum* + Provax 200 and *T. harzianum* + Score 250 EC, which were followed by Soil amendment with Poultry manure +Score 250 EC, Poultry manure + Garlic clove extract, Soil amendment with vermicompost + Provax 200, Vermicompost + Score 250 EC, Vermicompost + Pencozeb 80 WP, Soil amendment with *Trichoderma harzianum* + Tilt 250 EC and *T. harzianum* + Garlic clove extract showing 91.67% disease reduction of disease incidence. The highest number of leaf (20.25/plant), weight of leaf (105.25 g/pant) and leaf yield (8.25 t/ha) were obtained with the treatment *T. harzianum* + Provax 200. The second highest leaf number, leaf weight and leaf yield of 18.75/plant, 97.68g/plant and 81.25 t/ha, respectively were obtained with the treatments with Vermicompost + Provax 200 and Vermicompost + Score 250 EC. All treatments with IPM components increased leaf yield by 55.63-82.78% over control. Based on findings of the experiment it was noted that the maximum reduction of disease incidence of 100% was recorded from treatments Soil amendment with *Trichoderma harzianum* + Provax 200 and Soil amendment with *Trichoderma harzianum* + Score 250 EC, which were followed by Soil amendment with Poultry manure + Score 250 EC, Soil amendment with Poultry manure + Garlic clove extract, Soil amendment with vermi-compost + Provax 200, Soil amendment with vermi-compost + Score 250 EC, Soil amendment with vermicompost + Pencozeb 80 WP, Soil amendment with *T. harzianum* + Tilt 250 EC and Soil amendment with *T. harzianum* + Garlic clove extract where reduction of disease incidence was 91.67%. As a conclusion it may be mentioned that as integrated approach application of either Provax 200 (0.2%) or Score 250 EC (0.1%) or Garlic clove extract (1:2 w/w) in combination with soil application of *Trichoderma harzianum* or Vermicompost was found as the suitable approach for the management of foot and root rot disease of betelvine that reduced up to 100% disease incidence increasing yield upto 82.78% over control.

CONTENTS

LIST OF SOME ABBREVIATE FORM AND THEIR ELABORATIONS

ABBREVIATE FORM	ELABORATION
AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
et al.	And others / Co-workers
N	Nitrogen
TSP	Triple Super Phosphate
MoP	Murate of Potash
RCBD	Randomized complete block design
DAI	Days after inoculation
ha^{-1}	Per hectare
g	gram (s)
Kg	Kilogram
SAU	Sher-e-Bangla Agricultural University
No.	Number
Wt.	Weight
cm	Centimeter
LSD	Least Significant Difference
^0C	Degree Centigrade
NS	Not significant
mm	Millimeter
Max	Maximum
Min	Minimum
%	Percentage
CV	Coefficient of variance
Hr	Hour
ha	Hectare
t	Ton
T	Treatment
viz.	Videlicet (namely)

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CHAPTER I
INTRODUCTION

Betelvine (*Piper betle* L.) is a kind of dioecious perennial creeper vine belonging to the family Piperaceae. It is cultivated largely for its leaves. It is an important cash crop of Bangladesh. Betelvine leaves have a strong pungent aromatic flovour and are widely used as masticatory. Mature leaves are used for chewing with smeared hydrated lime plus catechu, arecanut, cardamom, clove etc. Betelvine leaf chewing is considered as a good and cheap source of dietary calcium. Usually the people of South-Asia, South-east Asia, Gulf States and Pacific Islands chew betel leaves. All classes of people of Bangladesh chew not only as a habit but also as an item of rituals, etiquette and manners.

Morphologically betelvine leaves are shiny, broadly ovate and green heart-shaped with bleaching quality and softness. The stems are semi woody, climbing by many short adventitious roots. Fruits sparingly produced, quite immersed in the fleshy spike, which is about 5 cm long and pendulous.

The crops are grown 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 grown in the area where rainfall about 2250 – 4750 mm, relative humidity and temperature ranging from 40 – 80% and 15 – 90^{o}C, respectively (Guha and Jain, 1997).

There are about 100 varieties of betelvine across the world out of which 30 varieties are from West Bengal and Bangladesh (Guha, 1997). A good number of betelvine cultivars viz., Desi Bangla, Bangla, Kali Bangla, Jhali, Sanchi, Goyeshi, Bhabna, Mitha, Geso, Bonhoogly, etc. are found in Bangladesh. The origin of place of betelvine is thought to be Malayasia (Chattopadhyay and Maity, 1967). In ancient times, pan (betelvine) was cultivated in all parts of Bengal, preferably in some districts like Dinajpur, Rangpur and Chittagong. Bangladesh exports betel leaves to many countries of Asia and Europe including India, Pakistan, Saudi Arabia, United Arab Emirates, England, Italy and Germany. Export quality betel leaves are presently grown in the

districts of Natore, Kushtia, Rajshahi, Barisal, Khulna and Chuadanga. Bangladesh started exporting pan to Europe in 1974-75 and to Saudi Arabia in 1991. Basically pan is purchased and consumed by the people of Bangladesh, India and Pakistan. Pan contains some vitamins, enzymes, thiamine, riboflavin, tannin, iodine, iron, calcium, minerals, protein, essential oil and medicine for liver, brain and heart diseases (Chopra *et al*. 1956).

Total cultivated area under the crop in Bangladesh in 2015-16 was about 24,055 hectares and the total annual production was about 2,17,611 metric tons. The average yield per hectare is 9.04 metric tons (BBS, 2017). Pan leaf is usually plucked throughout the year but maximum production obtained in the months of July to October.

In Bangladesh, betelvine is cultivated mainly under an erected structure, known as Baroj, or Bheet, which is a kind of hut made of bamboo poles and splinter. Bamboo as well as jute sticks placed around the sides and roof is made of jute stick on a light frame work of bamboo. To cultivate the betelvine, low light intensity, mild temperature (10°C to 30°C), high humidity with moderate sunshine and 1450-1700 mm rainfall and frequent irrigation are needed throughout the year (Guha and Jain, 1997a).

This perennial crop is found to be infected by various diseases of which powdery mildew, foot rot, leaf rot caused by pathogens, *Phytophthora parasitica* and *Colletotrichum capsici*, *Sclerotium rolfsii* are the major constraints for cultivation of the crop (Goswami *et al.* 2002). Leaf rot can damage the crop within a week when it attacks the vine (Chaurasia 2001). Leaf rot and foot rot have been reported to be caused by *Phytophthpra palmivora* and leaf rot may cause 30-100% leaf yield loss (Maiti and Sen, 1982).

Different diseases are the limiting factors of betelvine production. Among the diseases foot and root rot caused by *Sclerotium rolfsii* is the most devastating disease that decreases the production of betel leaf to a great extent (Sayeeduzzaman, 1988: Islam, 2005). Humid and moist shaded conditions are favorable for growth and also favor a variety of root and foliage disease development (Goswami *et al*., 2002).

Sclerotium rolfsii Sacc is a serious soil borne fungus and harmful to many crops which are economically valuable in most of the tropical and subtropical regions of the world (Aycock, 1966). It has a wide host range and has been referred as an almost omnipathogenic organism (Talukdar, 1974). The fungus *S. rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of resting structure calledsclerotia (Ahmed, 1980). *Sclerotium rolfsii* is very difficult to control even by the use of chemical fungicides. Some fungicides such as Cupravit, Dithane M-45, Copper oxychloride, Difolatan and Bordeaux mixture were found to be effective to control foot rot disease of betlevine caused by *Sclerotium rolfsii* (Patil *et al.*, 1986).

Botanical extracts are biodegradable and their use in crop protection is a sustainable alternative. It reduces environmental contamination and health hazards (Grange and Ahmed, 1988). Research on the bio-active ingredients, application methods, rate of fungicides and environmental impact of botanical fungicides is a pre-requisite for sustainable agriculture. Few works have been done by using tobacco, neem, garlic, and some other plant extracts to control some fungi. Different natural biocides also used separately or in combination with plant extracts to control some fungi. Antifungul activities of garlic, neem, allamanda, have been reported by many researchers against plant pathogens (Islam, 2005).

Biological control could be successful alternative to control plant pathogens. Biological control of soil borne pathogens offer environmentally safe, durable and cost effective alternative to chemicals. Many species of fungi and bacteria are reported to be effective as bio-agents against soil borne plant pathogens (Mukhopadhyay, 1994). *Trichoderma* spp. is known antagonists of plant pathogenic fungi and have been shown to be very potential bio-agents against soil borne plant pathogenic fungi. *Trichoderma* spp. was reported found to be effective against different sclerotia forming fungi like *Rhizoctonia solani* and *Sclerotium rolfsii*.

At present, betelvine has a worldwide market. But in competition with India and other betelvine producing countries, Bangladesh has a very small share of the world betelvine market for lower production of quality betelvine due to various diseases and insect pests

(Goswami *et al.*, 2002). To increase export of betel leaves it production needs to be enhance through management of diseases, especially foot and root rot.

In Bangladesh, no resistant varieties are known to available against this disease. The betelvine growers are seriously discouraged to cultivate betelvine as they have no sustainable approach for controlling foot and root rot of betelvine.

Huge number of betelvine garden 'Baroj' ruined every year due to the severe attack of foot and root rot disease. If such a situation continued, the betelvine cultivation would face a great threat and the country will lose a huge foreign income. Thus, the problem needs to give an argent attention. To save the crop and it's growers, development of economic management tactics are required. Under the above facts the present piece of research was undertaken with following objectives:

i. To survey on incidence of foot and root rot of betelvine caused by *S. rolfsii* under prevailing environmental condition in major betelvine growing areas of Bangladesh

ii. To isolate and identify of *S. rolfsii* causing foot and root rot of betelvine collected from different growing regions of Bangladesh

iii. To test pathogenicity of *S. rolfsii* isolates causing foot and root rot disease of betelvine

iv. To screen out resistant cultivars of betelvine available in Bangladesh against *S. rolfsii* causing foot and root rot disease

v. To bioassay botanicals, fungicides and bioagents against *S. rolfsii* causing foot and root rot of betelvine under *in-vitro* condition

vi. To evaluate the selected botanicals, fungicides, bio-agent and soil amendments against foot and root rot (*S. rolfsii*) of betelvine in *in-vivo* condition

vii. To find out the integrated effect of selected IPM components for the management of foot and root rot (*S. rolfsii*) of betelvine

CHAPTER II

REVIEW OF LITERATURE

A good number of researches on foot and root rot disease caused by *Sclerotium rolfsii* Sacc have been caried out throughout the world. Most of the researchers are concentrated on etiology, epidemiology, incidence, method of inoculation of *S. roifsii* and management of the disease**.** The present review gives an overview of foot and root rot disease of betelvine caused by *S. rolfsii*.

2.1. Causal fungus and symptoms of foot and root rot

Sclerotium rolfsii Sacc.is a well known polyphagous, ubiquitous, omnivorous and most destructive soil borne fungus. The fungus is also a facultative saprophyte and can maintain contunity of generation under adverse situation by formation of sclerotia. The fungus was first reported by Rolfs (1892) as a cause of tomato blight from Florida in U.S.A. Later, Saccardo (1911) named the fungus as *Sclerotium* sp.

In India, Shaw and Ajrekar (1915) isolated the fungus from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. However, later studies showed that, the fungus involved is *S. rolfsii* (Ramakrishnan, 1930). However, its perfect stage was first studied by Curzi (1931) and proposed generic name as *Corticium*. Mundkar (1934) successfully isolated the perfect stage of *Sclerotium rolfsii*.

According to Chet *et al.* (1994) state that *S. rolfsii* causes the disease known as southern blight in wide variety of crops and *Sclerotium rolfsii* form brownish sclerotia that can survive in the soil for longer period of time.

Alexander and Stewart (1994) worked on *S. rolfsii* (Telemorph; *Athelia rolfsii*) and found that it causes serious root and stem rots of a range of economically important fruit and vegetable crops. Sclerotia are the important propagules for the survival of the pathogen. Under favourable conditions, sclerotia may germinate to cause infection usually occurs on soil surface or just below the soil surface and symptoms includes yellowing, browning and wilting of entire plants.

Aycock (1966) reported that stem rot disease also known as southern blight, *S.* wilt, and white mold attacted all part of the plant at any growth stage but stem infection is most common. Formation of deep brown lesions around the meristem below the soil surface are the first characteristics symptom. The lesions become covered with radiating mycelium which encircles the affected portion of the stem, resulting in the development of yellowing and wilting of the whole or part of the plant.

Aycock (1966) also stated that host range of *S. rolfsii* is very wide and includes not only many important horticultural and agronomic crops but also many of noneconomic important plants. It is not possible to estabilish precise totals for the species reported as host; nevertheless the soil borne plant pathogenic fungus *S. rolfsii* attacking more than 500 spp. of plants belonging to over 100 families.

Bisth (1982) described that the pathogen infected the potato plants at collar region causing wilting of plants. White or brown sclerotia were developed at maturity in the root and collar regions of the infected plants. The infection spread within few days either by irrigated water or by farm implements used for cultural practices. The pathogen damaged either stem or root.

Jahan *et al*. (2016) reported that the leaves and shoots of foot and root rot that caused by *S. rolfsii* infected betelvine plants turned yellow withered and finally dried out to a pale brown color. The fungus found to attack the roots and stem near the soil level. Black lesions are developed following necrosis of the plant cells. The mycelium invaded the stem and rotten the affected portions. As a result, the plant became wilted and gradually died. Abundant white mycelium and small light brown sclerotia formed on the rotten plants. The rotting spread through older roots and ultimately reached the foot or collar region of the plant. The soft tissues of old roots are found completely decomposed leaving only the fibrous portion.

2.2. Environmental factors on the disease development

According to Jana (1995), in the areas with lower rainfall (1500 - 1700 mm) the crop is cultivated with small and frequent irrigations, i.e. every day in summer and every 3 - 4 days in winter, whereas adequate drainage is required during the rainy season.

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

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

An epidemiological studies were reported that the maximum temperature, maximum relative humidity and rainfall played an important role in the development of both the diseases of (*Piper betel* L.) (Anonymous, 2006; Maiti and Sen, 1982).

Chattopadhyay and Maiti (1990) observed that the plants are cultivated in conservatories under shady and humid conditions that also favor the development of many diseases.

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

Farr *et al*. (1989) found that, fungus *S. roifsii* attacks all plant parts in the contact with the soil under favourable environmental conditions including stems, roots, and fruits. Gondo (1962) reported that the optimum soil temperature was 30^0C for mycelia and 25^0C for sclerotia development of *S. rolfsii*.

Hari *et al.* (1991) reported the radial growth of *Sclerotium rolfsii* causing collar rot of groundnut at pH range of 2 to 9 but the maximum growth was at pH 6.

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

Jahan *et al*. (2016) an investigate on the diseases of foot and root rot of betelvine in six upazilla of Kushtia district, viz. Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar and Mirpur. Disease incidence and severity of foot and root rot ranged from 24.00 to 58.00% and 17.65 to 34.75%, respectively, where the maximum disease was recorded in Mirpur and the minimum was in Khoksha in the month of July and October. Disease incidence and severity of foot and root rot ranged from 50.00 to 58.00% and 33.25 to 34.20%, respectively in Mirpur where the maximum disease was recorded in July and the minimum was in October. In Kushtia Sadar, disease incidence and severity of foot and root rot ranged from 27.00 to 37.00% and 18.45 to 20.39% respectively. In Bheramara, disease incidence and severity of foot and root rot ranged from 27.00 to 37.00% and 18.30 to 20.38%, respectively. In Kumarkhali, the disease incidence and severity were 45.00 to 50.00% and 27.95 to 30.60% respectively. In Khoksha, disease incidence and severity of foot and root rot ranged from 24.00 to 31.00% and 17.65 to 18.80% respectively. In Daulatpur, disease incidence and severity of foot and root rot ranged from 39.00 to 46.00% and 22.30 to 24.35% respectively, considering all the locations of Kushtia District, the maximum disease was recorded in the month of July and the minimum was in October.

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

Lingaraju (1977) reported that, the saprophytic activity of the fungus was more at 10% soil moisture and the fungus did not survive when the soil moisture was raised to 50 percent and above.

Mollah (2012) found that, in case of foot and root rot of in Satkhira district, highest disease incidence were found in August (12.50% to 32.50%) and lowest disease incidence were found in December (0% to 8.33%) in 2010. The highest disease incidence were found in August (18.75% to 50%) and the lowest disease incidence were found in December (0% to 2.08%) in 2011.

Palakshappa (1986) studied the effect of different soil moisture levels on foot rot of betelvine caused by *Sclerotium rolfsii* and reported that, the fungus survived better at low soil moisture than at higher levels. The survival ability was highest between 20 and 40 per cent soil moisture. However, higher saprophytic activity of the fungus was observed at 40 percent moisture level and the least was at 60 and 70 percent soil moisture where the saprophytic activity of the fungus was found to be very less. rot of soybean caused by *Sclerotium rolfsii.*

Mitchell *et al*. (1990) and Alexander and Stewart (1994) showed more rapid sclerotial degradation and reduced survival in soil with higher clay content and relatively low pH (~6), and lower survival in clay loam than in sandy loam.

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

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

2.3. Disease incidence and severity of leaf rot, stem rot and leaf spot of betelvine

Huq (2011) reported that the incidence of leaf rot was observed in the month of June, July and August. The peak infection was noted in the second week of August, 2002 when the average temperature, relative humidly and rainfall were 29.60ºC, 94.6% and 13.4 mm respectively. The incidence of leaf spot was observed during months of March, April and May. But gradually infection reached maximum when average temperature, relative humidly and rainfall parameters were 26.7ºC, 88.3% and 19.4 mm respectively. The meteorological factor which had greatly influenced initiation of leaf spot was less favourable for leaf rot disease.

Mollah (2012) investigated a survey on leaf rot of betelvine in different upazillas under the Satkhira district. The disease incidence and severity were significantly differing.

The highest disease incidence (46.94%) and severity (34.17%) were recorded in August at Tala and the lowest disease incidence (3.17%) and severity (2.05%) were recorded in December at Satkhira Sadar. In 29 ^{0}C and 85% R_H, the disease incidence and severity of leaf rot of was the highest and with the decrease of temperature and humidity the incidence and severity gradually decreased and was the lowest when the temperature laid around 18.7 ^{0}C and the RH laid around 75%.

Palakshappa (1986) surveyed the incidence of *S. rolfsii* on *Piper betle* L. in different areas of Karnataka state during 1984-85 and recorded 35 to 39 percent disease incidence.

Rahman and Sultana (2011) found that, in Jamalpur region, the incidence and severity of Sclerotial rot is more or less highest and lowest throughout the year.

2.4. Morphological variability of *Sclerotium rolfsii* isolates

Abida *et al.* (2008) reported that 12 isolates of *Sclerotium rolfsii* Sacc varied in colony morphology, mycelial growth rate, sclerotial formation, sclerotial size and color. Variability among the isolates of *Sclerotium rolfsii* was determined on the basis of their sensitivity to different fungicides.

Karthik Pandi *et al*. (2017) reported that eight isolates of *S. rolfsii* were grown in PDA medium. Isolate SFSR1 in petriplates showed significantly maximum mycelial growth per day (31.45 mm) followed by SFSR3 and SFSR6. The isolate SFSR4 showed minimum mycelial growth per day (21.62 mm). Isolate SFSR4 produced higher number of sclerotia (360 per plate) followed by SFSR1 (359.75), SFSR3 (324.25), SFSR6 (320.22), SFSR5 (290.68), SFSR2 (279.00) and SFSR8 (279.00). The isolate SFSR7 produced minimum number of sclerotia (274 /plate). Among the isolates the biggest sclerotia (1224 µm) was produced by SFSR1 isolate followed by SFSR3 (1080 µm) and SFSR6 (1076 µm). The smallest sclerotia (1002 µm) was observed in SFSR8.

Manu *et al*. (2018) reported that different pathogenic isolates of *S. rolfsii* were obtained from different regions and crops of southern Karnataka. The morphological studies on the pathogen showed variation among different isolates. The colony diameter of all the

isolates varied from 4.10 to 8.00 cm after 72 h of incubation, sclerotial number per plate ranged from 261.7 to 1048.7. However, the sclerotial colour ranged from light to dark brown, and their size varied from 1.10 to 2.10 mm with spherical to round shape. The test weight of sclerotial bodies ranged between 40.40 to 71.00 mg. Among the liquid media, ragi flour broth showedmaximum dry mycelial weight (352.00 mg) whereas, sclerotial production was more in malt extracts broth (247.70).

Mejda *et al*. reported that S. tuber rot incited by *S. rolfsii* is an emergent potato disease in Tunisia. The known effects of temperature on several post-harvest pathogens of potato, they focused on the assessment of pathogen development in vitro and in vivo under different thermal conditions. They showed significant differences in the mycelial growth rate of *S. rolfsii*, as measured by mean colony diameter recorded after 24, 48 and 72 hr at various temperatures (5-40°C) where the optimum was found to be 30-35°C on PDA. Significant differences in pathogen external and internal development were also noted on inoculated potato cv. 'Spunta' tubers. In fact, the maximum lesion diameter noted at tuber surface was observed at 30°C. However, the most severe soft (atypical) rot and the highest percentage of rotten tissue were recorded after 8 days of incubation at 35°C. Statistically significant positive correlations were noted between the tuber lesion diameter, pathogen penetration and the percentage of rotten tissue

2.5. Pathogenicity of *Sclerotium rolfsii* isolates

Datar and Bindu (1974) proved the pathogenicity of *S. rolfsii* on sunflower by soil inoculation method under glass house conditions. The inoculum was prepared by growing the fungus on sterilized maize bran medium and mixed with the sterilized soil one week. Typical symptoms were produced within a week of germination which was identical to those produced in the field.

Jahan *et al*. (2016) investgate a survey of crop on foot and root rot disease in Kushtia**.** Young stems were found more prone to attack than the old ones. Pathogenicity test showed *Sclerotium rolfsii* produced characteristic symptoms on and proved to be the causal pathogen of the disease.

Meah (2007) tested the pathogenicity of 10 isolates of *S. rolfsii* on eggplant (var. Dohazari) and he found that all the isolates of *S. rolfsii* significantly influenced the germination, pre-emergence death, damping off, foot rot and plant stand.

Mirsha and Bais (1987) used 15 days old fungal culture grown on sand corn meal medium for proving pathogenicity of root rot of barley caused by *Sclerotium rolfsii* mixing upper 4-5 layer of soil with inoculums at the rate of one flask per pot.

Palakshappa (1986) observed considerable foot rot infection when inoculated with two and three percent inoculum of *S. rolfsii*. They recorded percent infection at four percent and above inoculum levels.

Sengupta and Das (1970) studied the cross inoculation of isolates of *Sclerotium rolfsii* from groundnut, wheat, potato, guava, and benglagram. They concluded that, benglagram was the most susceptible host for *S. rolfsii*.

Siddaramaiah and Chandrapa (1988) proved the pathogenicity of *Sclerotium rolfsii* on cardamon in pot culture studies by inoculating 25 days old sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week inoculation.

Thammsak *et al*. (1982) made an investigation on the pathogenicity of *Sclerotium rolfsii*, and reported that the pathogen could infect its host cotton severly; disease severity in average was 84%. The pathogen caused pre and post emergence damping off symptoms of cotton seedlings. They also found the soil amendment decreased disease intensity e.g. crop refuses, nitrogen fertilizers and lime. Seed dressing with five fungitotoxicants showed that vitavax gave a complete protection when grown in infested soil.

2.6. Resistant cultivars

Reports on varietal resistance in betelvine are not available.

2.7. Physio-morphological characters of cultivars

Medda *et al*. (2011) conducted a field experiment to screen out the suitable cultivars of betelvine for Terai zone of West Bengal. The experiment was laid out in randomized

complete block design with twelve betelvine cultivars with three replications. Different cultivars exhibited significant variations with respect to their growth, yield and yield attributing characters. The sanchi cultivars showed significantly higher monthly linear growth having larger inter-nodal length with relatively larger size leaves. The cu/tivar Utkal sudam produced moderately higher leaf yield (66.03 lakh ha^{-1}) with significantly larger size leaves (173.33 cm2) having shortest inter-nodal length (3.98 cm) among all the bangla cultivars which led to give the highest benefit cost ratio (3.70). On economic point of view, the cv. Utkal sudam, Kotki can be considered as the suitable variety for cultivation in Terai zone of West Bengal.

Mohanta and Pariar (2015) studied the effect of various climatic factors on betelvine in an established baroj during December 2012 to November 2013. Temperature and RH were found to be the most important factors for variation in leaf characters in different cultivars. Simurali Jhal showed superior performance with respect to leaf length (16.45 cm^2), leaf breadth (13.76 cm^2) and leaf area (274.35 cm^2) in rainy season. But according to storability, CARI-2 (57.75 %) performed better in winter season. In rainy season the growth and chlorophyll content (Simurali Sanchi - 2.58 mg g-1 tissue) of betel leaves was maximum, but storability was minimum (Simurali Jhal - 31.75 %).

Shivashankara *et al*. (2000) studied effect of different light intensities on growth and yield of betelvine". Betelvine is mainly grown under closed conservatories where light intensity available for growth and development is very low. Total number of leaves produced per unit time will also be reduced because of low light intensity. Therefore, the present study was conducted to find unit of the optimum light requirement for better growth and yield. Three light intensities (10, 35 and 60% of full light) and two cultivators Desi Bangla Mahoba and Kapoori (Maharastra) were used for the study. Four to Six times increase in the number of leaves produced was seen in 35 and 60% light when compared to 10% light. Leaf size and internodes length were more in 35% light on the other hand, specific leaf weight was more in 60% light significantly higher photosynthesis, atomatal conductance and transpiration rate were seen in 35 and 60% light intensities when compared to 10%. However, the chlorophyll content was higher in 10% light intensity. Data clearly indicated that betelvine required 35% of sun light for production of maximum number of quality leaves. Optimization of light requirement was further proved by the saturation of photosynthesis at 35% light.

2.8. *In-vitro* evaluation of botanicals against *Sclerotium rolfsii* Sacc

Masuduzzaman *et al*. (2008) conducted an experiment to determine their inhibitory efficiency of Allamanda leaf extract against *Sclerotium rolfsii.* Higher concentration (1:1, 1:2) completely inhibited *Sclerotium rolfsii* whereas lower concentration (1:3, 1:4) arrested its growth to some extent.

Parvin *et al.* (2016) conducted an experiment to determine the effect of botanicals on radial mycelial growth of *S.rolfsii in-vitro.* Plant extracts showed profound and significant effect on reduction of radial mycelial growth of the fungus. The performance of Garlic in reduction of radial mycelial growth was the best followed by Onion, Ginger, Neem, Allamonda at 4 days after incubation. The highest radial mycelial growth (9.00 cm) was recorded in untreated control followed 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 showed strong effect to produce percent mycelial growth inhibition of *S.rolfsii* in culture media. The highest percent inhibition (25.56%) was recorded in case of Garlic followed by Onion (1.56%), Allamonda (1.67%) and Ginger (1.89%) at 4 days after inoculation.

Sahana *et al*. (2017) conducted an experimentto to evaluate the efficacy of botanicals viz. neem leaf extract, eucalyptus leaf extract, jathropa leaf extract, tulsi leaf extract, garlic bulb extract, onion bulb and marigold leaf extract at three different concentration (5, 10 and 15 %). Among the botanicals tested against *Sclerotium rolfsii* under *in vitro* conditions onion bulb extract showed 100% inhibition at all the three concentrations followed by garlic bulb extract (97.77%, 98.88% and 100% at 5, 10 and 15% concentration, respectively) while least inhibition of mycelium was observed (22.55, 24.44 and 44.07% inhibition at 5, 10 and 15% concentration, respectively) in jathropa leaf extract.

Yasmin (2016) conducted an experiment to investigate the effectiveness of three botanical extracts namely garlic, ginger and neem at different concentrations (5%, 10% and 15%) to reduce the mycelial growth of *Bipolaris sorokiniana, Fusarium oxysporum* and *Sclerotium rolfsii.* The different botanical extracts in different concentrations inhibited the mycelial growth of fungi significantly ($p<0.01$). At 15% concentration

garlic maximum inhibited *Sclerotium rolfsii* 72.20%, neem and ginger maximum inhibited in *F. oxysporum* 56.41% and 55.80% respectively.

2.9. *In-vitro* effect of chemical fungicide against *Sclerotium rolfsii*

Parvin *et al*. (2016) conducted an experiment to find out the effect of fungicides on radial mycelial growth of *S. rolfsii in-vitro.* All tested fungicides significantly reduced radial mycelial growth of the fungus. 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. The highest radial mycelium growth (9.00 cm) was recorded in untreated control followed by by Ridomil Gold (7.19 cm), Rovral (7.34 cm)and Dithane M- 45 (7.70 cm). Bavistin was found promising in reducing the growth of the fungus in the laboratory followed by Ridomil Gold. Furthermore, 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. Randon *et al*. (1995) reported that the carbendazim (Bavistin), Kasumin and Tecto used at five concentrations under *in-vitro* conditions were the most effective in inhibiting mycelial growth and sclerotia formation of *S. rolfsii* at low concentration.

Suryawanshi *et al*. (2015) reported that in *in-vitro* evaluation the highest average mycelial growth inhibition of *Sclerotium rolfsii* was obtained with fungicides, Vitavax (100.00%), Tebuconazole (99.25%) and Penconazole (99.03%). Aqueous extracts of all botanicals tested @ 10 and 20% exhibited antifungal potential and significantly highest average mycelial growth inhibition with *Azadirachta indica* (71.17%).

The poisoned food technique was followed for *in vitro* studies on the effect of certain fungicides on *S. rolfsii*. The best inhibition was showed by Calixin, Vitavax, Duter, Ferbam, Ceresan wet, Sandoz seed dressing 6334 and Brassicol which allowed no growth of fungus (Chauhan, 1978).

Toorray *et al*. (2007) evaluated seven fungicides (each at 1000, 1500, 2000 ppm) against *S. rolfsii* under *in-vitro* condition. Complete inhibition of growth of *S. rolfsii* was recorded under Captan, Thiram, Mancozeb, Hinosan (edifenphos) and Antracol whereas Cholrothalonil showed partial inhibition at low concentration.

2.10. *In-vitro* effect of bio-agents against *Sclerotium rolfsii*

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

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.

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 *Sclerotium 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*. (2008) compared antagonistic properties of *Trichoderma harzianum* which is less effective than *G. virens* in suppressing *S. rolfsii* and *R. solani* for various mechanisms of antagonism *in vitro*, viz., antagonism in dual culture/hyphal parasitism, parasitism of sclerotia and antibiosis. *G. virens* and *T. harzianum* were equally effective in parasitizing the hyphae of *R. solani*. Only T. harzianum parasitized the hyphae of *S. rolfsii,* and the two antagonists were comparable with respect to antibiosis on the test pathogens. However, *G. virens* readily parasitized the sclerotia of the test pathogens and was found to be more effective than *T. harzianum* in destroying the sclerotia. Under SEM, *G. virens* was found to colonize, penetrate, and sporulate inside the sclerotia of the test pathogens. Parasitism of sclerotia is suggested as the principal mechanism of biological control of *S. rolfsii* and *R. solani* by *G. virens*.

Parvin *et al*. (2016a) conducted an experiment of bio-agents in inhibition of mycelial growth of *Sclerotium rolfsii* in dual culture method. Bio-agents have significant effect on reduction of radial mycelial growth of the fungus. The performance of *Trichoderma harzianum* in reduction of radial mycelial growth was the best followed by *Pseudomonus fluorescens* irrespective of days after inoculation. All the tested bio-

agents have strong effect to produce percent growth inhibition against *S.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.

Parvin *et al*. (2016b) were evaluated *in-vitro* of two bio-agents namely *Trichoderma harzianum* and *Pseudomonas florescens* for their efficacy against *Sclerotium rolfsii*. A remarkable inhibition of mycelium growth, number of sclerotia of *Sclerotium rolfsii* was achieved by treating *Trichoderma harzianum* and showed better performance *in vitro* compared to untreated control.

Rekha *et al.* (2012) conducted an experiment to reduce mycelial growth and formation of sclerotial bodies of *S. rolfsii* by *Ttrichoderma* spp. Forty four isolates of *Trichoderma* (Tri-1 to Tri-44) were screening against *S. rolfsii* through dual culture technique for know their efficacy. Among the 44 tested isolates 10 isolates *viz.,* Tri-8, Tri- 13, Tri-15, Tri- 16, Tri- 19, Tri-23, Tri-27, Tri- 29, Tri- 41 and Tri- 44 were found be efficient in reducing both mycelial growth and formation of sclerotial bodies by the pathogens. Further, the effective isolates were tested for production of volatile metabolites. Isolates Tri-13 (*T. viride*) and Tri-29 (*T. viride*) were found to reduce the growth of *S. rolfsii* through volatile metabolites compare to other tested isolates and control.

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 *Sclerotium 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.*

2.11. Effect of fungicides, botanicals and bioagents against *S. rolfsii* under *in-vivo* condition

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

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.

Mathur and Sarbhoy (1978) observed that the comparative effectiveness of *T. viride* and *T. harzianum* under glasshouse conditions against root rot of sugar beet caused by *Sclerotium rolfsii*. Both species of *Trichoderma* appeared to be strongly antagonistic against *S. rolfsii* caused only 13.3% and 20% infection in presence of *T. viride* and *T. harzianum*, respectively compared to 100% infection recorded in absence of any of the antagonists.

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 *Sclerotium rolfsii*) under field conditions.

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

Pal and Choudhary (1983) reported the efficacy of vitavax as soil drenching fungicide against *Sclerotium rolfsii* in reducing the sunhemp seedling mortality. However, they reported that, bayleton and sicorol were also found highly effective for soil drenching.

Patil *et al*. (1986) observed that, soil drenching of captan, copperoxychloride and difolaton were found effective in controlling wilt of betelvine caused by *Sclerotium rolfsii*.

Parvin *et al*. (2016a) conducted an experiment under field condition to evaluate some fungicide to control foot and root rot disease of betelvine. Betelvine stems were spraying with seven treatments comparising Bavistin, Topgan, Tilt 250EC, Garlic clove extracts, Neem leaf extracts, *Trichoderma harzianum* based bio-fungicides and control were explored in the experiment. The lowest severity was found in Bavistin (0.71%) 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. Stem treated with Bavistin by spraying at seven days interval after inoculation minimized disease incidence, severity and increased yield. Among the eco-friendly approach, Garlic clove extract and *T. harzianum* showed better performance in controlling foot and root rot disease of compared to control. Furthermore, *T. harzianum* based Bio-fungicide increased 30.15% yield of betel leaves over control.

Rjurkar *et al.* (1998) reported the antagonistic effect of *Trichoderma* spp. on the wilt causal organism *Sclerotium rolfsii.* Suryawanshi *et al*. (2015) reported that the *in-vivo* evaluation revealed significantly highest seed germination (80.00%) was recorded with the treatment Vitavax (ST@1.5g/kg) +Thiram (ST@1.5g/kg) + *P. fluorescens* (ST@10g/kg) + NSC (SA@50g/kg soil). Significantly highest reduction in pre-emergence (69.96%), post-emergence (55.43%) and average (62.37%) mortality were recorded with treatment of Vitavax +Thiram + *P. fluorescens* + NSC.Thus, it is concluded that brinjal collar rot can be managed effectively by seed treatment with fungicides (Vitavax,Thiram), bioagent (*P. fluorescens*) and soil amendment with neem seed cake.

Toorray *et al*. (2007) evaluated seven fungicides (each at 1000, 1500, 2000 ppm) against *Sclerotium rolfsii* under *in-vitro* condition. Complete inhibition of growth of *Sclerotium rolfsii* was recorded by Captan, Thiram, Mancozeb, Hinosan (edifenphos), Antracol where as Cholrothalonil showed partial inhibition at low concentration. Bavistin (carbendazim) did not show much inhibition at all concentrations. In *in-vivo* evaluation of chickpea with the 7 fungicides (captan at 3 g, thiram at 3 g, Bavistin at 3 g, mancozeb at 3 g, Hinosan at 1 g, Antracol at 3 g and Kavach at 3 g/kg) and two biological control agents (*Trichoderma harzianum* and *T. viride*, each at 4 g/kg) against *Sclerotium rolfsii*, captan, Kavach and thiram showed some reduction in preemergence

mortality, while both the biological control agents did not show protection against pre-emergence mortality due to *Sclerotium rolfsii*.

2.12. Use of soil amendments

Ahamed *et al.* (2012) conducted field trials in a farmer's field in Talagang (Chakwal) in 2009 to evaluate the effect of manuring on root rot disease caused by *Fusarium solani* and agronomic characters of groundnut. The experiment was comprised seven treatments viz. (i) Control (no amendment and no inoculation); (ii) *F. solani* (FS)-inoculated control; (iii) poultry manure + FS; (iv) farmyard manure + FS; (v) cattle manure + FS; (vi) *Brassica campestris* straw + FS; (vii) *Cicer ariantum* straw + FS. In the *Fusarium* inoculated control, disease incidence and plant mortality was 85 and 22.2%, respectively whereas, disease incidence and plant mortality were both 0% in non inoculated control. All the manuring treatments managed the disease to variable extent and influenced agronomic characters of groundnut. Poultry manure was the most effective in disease management followed by cattle manure.

Ersahin *et al*. (2009) reported that disease suppressiveness of vermicompost produced from agricultural wastes consisting of cattle manure, tree bark (Salix spp.), potato culls, and apples was assayed on damping-off of two days-old cucumber (*Cucumis sativus* cv. *Cevher*) seedlings infected by *Rhizoctonia solani* Kühn (AG-4). Suppression effect was assessed at the rates of 0, 10, 20 and 30% (v/v) vermicompost, either blended with *Trichoderma harzianum* Rifai (KRL-AG2), amended with potting mixtures consisting of sand and garden soil (1:1, v/v). Effect of water extracts of vermicompost on growth of R. solani mycelium in Petri dishes was also analyzed. Disease suppression effect increased in proportion to the pot amendment rate of vermicompost. Vermicomposts not blended with *T. harzianum* effectively controlled damping-off of cucumber by *R. solani* (AG-4) at the rate of 20% and 30%. Vermicompost not blended with T. *harzianum* improved plant growth as well as that blended with *T. harzianum*. Analysis of the effect of water extracts of vermicompost on growth of *R. solani* mycelium in Petri dishes revealed antagonistic activity of a putative bacterium. Heat sterilization eliminated the suppressive and antagonistic effect by vermicompost and its water extracts, respectively. Activity of an antagonistic bacterium, which expressed a strong inhibition of growth of the pathogen mycelium, indicated that the type of suppressiveness.

Punja *et al.* (2002) evaluated the disease suppression potential of three composts (greenhouse waste, windrow dairy solids, and vermicompost dairy solids) and commercially available biological control agents (BCA) to reduce disease incidences of Fusarium root and stem rot, caused by *F. oxysporum* f.sp. *radicis-cucumerinum*, and *Pythium* damping-off and crown rot, caused by *P. aphanidermatum*. They reported that all three composts reduced root and stem rots to some degree, and autoclaved compost lost its suppression effect suggesting the microbial antagonism

2.13. Integration of IPM components in *in vivo* condition

Appana *et al*. (2011) reported the efficacy of integratd application of biocontrol agent, chemical fungicide and organic amendments *S. rolfsii* and *Bradyrhizobium* sp. NC-92. The treatment combinations were (1) untreated control, (2) *Pseudomonas florescens* FPD-10, (3) *Pseudomonas florescens* FPD-15, (4) *Trichoderma harzianum*, (5) Nem cake, (6) Captan, (7) *Pseudomonas florescens* FPD-10 + *Trichoderma harzianum* and applied with Neem cake + Captan and (8) *Pseudomonas florescens* FPD-15+ *Trichoderma harzianum* and applied with Neem cake + Captan. At the time of sowing, seeds were inoculated with *Sclerotium rolfsii* and *Bradyrhizobium* sp. NC-92. All treatments tested significantly reduced pods infected with *S. rolfsii* and resulted in higher pod yield compare to untreated control. The highest pod yield was recorded in plant receiving *Pseudomonas florescens* FPD-10 followed by combination of different treatment with FPD-10 and combination of different treatment with *Pseudomonas florescens* FPD-15. Although individual application of either *Trichoderma harzianum* or neem cake or captan did not give similar results as single inoculation of either FPD-10 or FPD-15, it significant reduced the pod infection caused by *S. rolfsii* and improved pod yield.

Banyal *et al*. (2008) conducted an experiment to evaluate ten fungicides namely carbendazim 50 WP, carbendazim + mancozeb 75 WP, captan 50 WP, chlorothalonil 80 WP, thiabendazole 80 WP, mancozeb 75 WDG, carboxin 75 WP, propineb 70 WP, mancozeb 75 WP and tebuconazole 5 DS and five bioagents, *Trichoderma harzianum* (local strain), *Trichoderma viride* (local strain), *Gliocladium virens* (local strain), *Paecilomyces lilacinus* (Bhubaneswar strain) and *T. viride* (Ecoderma) to find out their efficacy against *Sclerotium rolfsii* causing collar rot of tomato.

Tebuconazole and carboxin @ 50 µg/ml gave complete inhibition of mycelial growth of the pathogen, whereas carbendazim (Bavistin) @ 1500 µg/ml failed to inhibit the growth of the pathogen. *Trichoderma viride* (local strain) was found to be highly effective against the pathogen. Seedling dip with tebuconazole and carboxin gave a total control of the disease in pots. Integrated effect of soil application of *T. viride* (local strain), seedling dip with tebuconazole (0.05%) and soil drenching with tebuconazole (0.05%) resulted in complete control of collar rot of tomato in pot culture.

Madhavi *et al*. (2011) conducted an experiment on dry root rot disease caused by *Sclerotium rolfsii* (Sacc.) of Chillies under rainfed conditions. *In-vitro* evaluation of nine fungicides following poison food technique showed that tebuconazole and combination of carbendazim + mancozeb were effective in inhibiting the mycelial growth (94.1%) followed by difenconazole (93.3%). *In-vivo* soil drenching with same fungicides proved to be effective in controlling the pathogen at 1000, 2000 and 3000 ppm. Integration of different treatments including seedling dip with carbendazim+mancozeb, addition of vermicompost, drenching with fungicide and application of *T. harzianum* (7%) were found to be effective in management of disease in comparison with individual treatments.

CHAPTER III

MATERIALS AND METHODS

A series of seven experiments were conducted during the investigation to achieve the objectives. The experiments were as follows:

1. Survey on the incidence of foot and root rot (*Sclerotium rolfsii*) of betelvine under prevailing environmental condition in major betelvine growing areas of Bangladesh

2. Isolation and identification of the isolates of *Sclerotium rolfsii* causing foot and root rot of betelvine collected from different regions of Bangladesh

3. Pathogenicity test of *Sclerotium rolfsii* isolates causing foot and root rot disease of betelvine

4. Screening of betelvine cultivars available in Bangladesh against *Sclerotium rolfsii* causing foot and root rot disease of betelvine

5. Bioassay of botanicals, fungicides and bioagents as IPM components against *Sclerotium rolfsii* causing foot and root rot of betelvine under *in-vitro* condition

6. Evaluation of botanicals, fungicides, bio-agent and soil amendments against foot and root rot (*Sclerotium rolfsii*) of betelvine *in-vivo* as components of IPM

7. Efficacy of integrated application of selected IPM components to control foot and root rot (*Sclerotium rolfsii*) of betelvine in Bangladesh

3.1. Experiment 1. Survey on the incidence of foot and root rot (Sclerotium rolfsii) of betelvine under prevailing environmental condition in major betelvine growing areas of Bangladesh

3.1.1. Location and duration of survey

The survey was conducted at major betelvine growing areas of five selected upazillas of Bangladesh. The upazillas were Gouronadi, Kaligonj, Mirpur, Mohanpur and Sitakunda under the districts of Barisal, Jhenaidah, Kushtia, Rajshahi and Chittagong, respectively. Five betelvine gardens (barojes) of each upazilla were visited thrice in a year for recording data.

The duration of survey was 2015, 2016 and 2017 in the moths of August, February and May. The scheduled was prepared based on variations in temperature, relative humidity and rainfall during the survey period. Hot and humid condition occurs in rainy season with high rainfall. Scanty rainfall and plenty of sunshine were prevailing during winter season. Data were collected during late summer (August), late winter (February) and mid summer (May) seasons of the years 2015-2017.

3.1.2. Data collection

In each upazilla, five barojes were selected for data recording. From each baroj, an area of approximately 1000 m^2 were selected. Cultivars available in those areas were considered for investigation. Nine visits were made to each garden during the survey period. Plants were selected randomly from the central part of the garden. Altogether 100 plants of each garden and 500 plants of each location were considered for the survey.

Incidence of foot and root rot of betelvine – The incidence of the disease (Plate 3.1.1) was computed based on the following formula:

$$\text{Percent disease incidence} = \frac{\text{Number of infected plant in the area covered}}{\text{Number of inspected plant}} \text{X } 100$$

The incidence and severity of leaf spot and leaf rot observed during survey in the visited betelvine baroj (garden) was recorded following the formula (Goswami *et al*., 2002).

The incidence of leaf spot and leaf rot was recorded following a '0 - 5' scale proposed by Goswami *et al*. (2002), where 0= Apparently healthy plants, 1= 1-5% leaf area infected, 2= 6-15% leaf area infected, 3= 16-30% leaf area infected, 4= 31-50% leaf area infected and >50% leaf area infected for leaf spot and 0= no or a few lesion on leaf, 1= <10% leaf area infected, 2= 11-25% leaf area infected, 3= 26-50% leaf area infected, 4= 51-75% leaf area infected and >75% leaf area infected for leaf rot.

The percent Disease Index (PDI) was computed using the formula of Islam (2005) as shown below:

$$\text{Disease severity (PDI)} = \frac{\text{Sum of total disease rating X 100}}{\text{Number of observation X Highest grade in the scale}}$$

Data on environmental factors were collected using digital hygro thermometer, soil pH and moisture meter and lux meter. Soil pH and light intensity in garden were recorded using a Survey Instrument (Model: 4 in 1: AMT-300). Air temperature and RH were determined by Digital HygroThermometer (Model: SH-110, G H Zeal ltd., London SW 19 3UU England) (Plate 3.1.2 A, B and C). Pooled data of five borojes per location collected during 2015, 2016, 2017 in late summer (August), late winter (February) and mid summer (May) are presented.

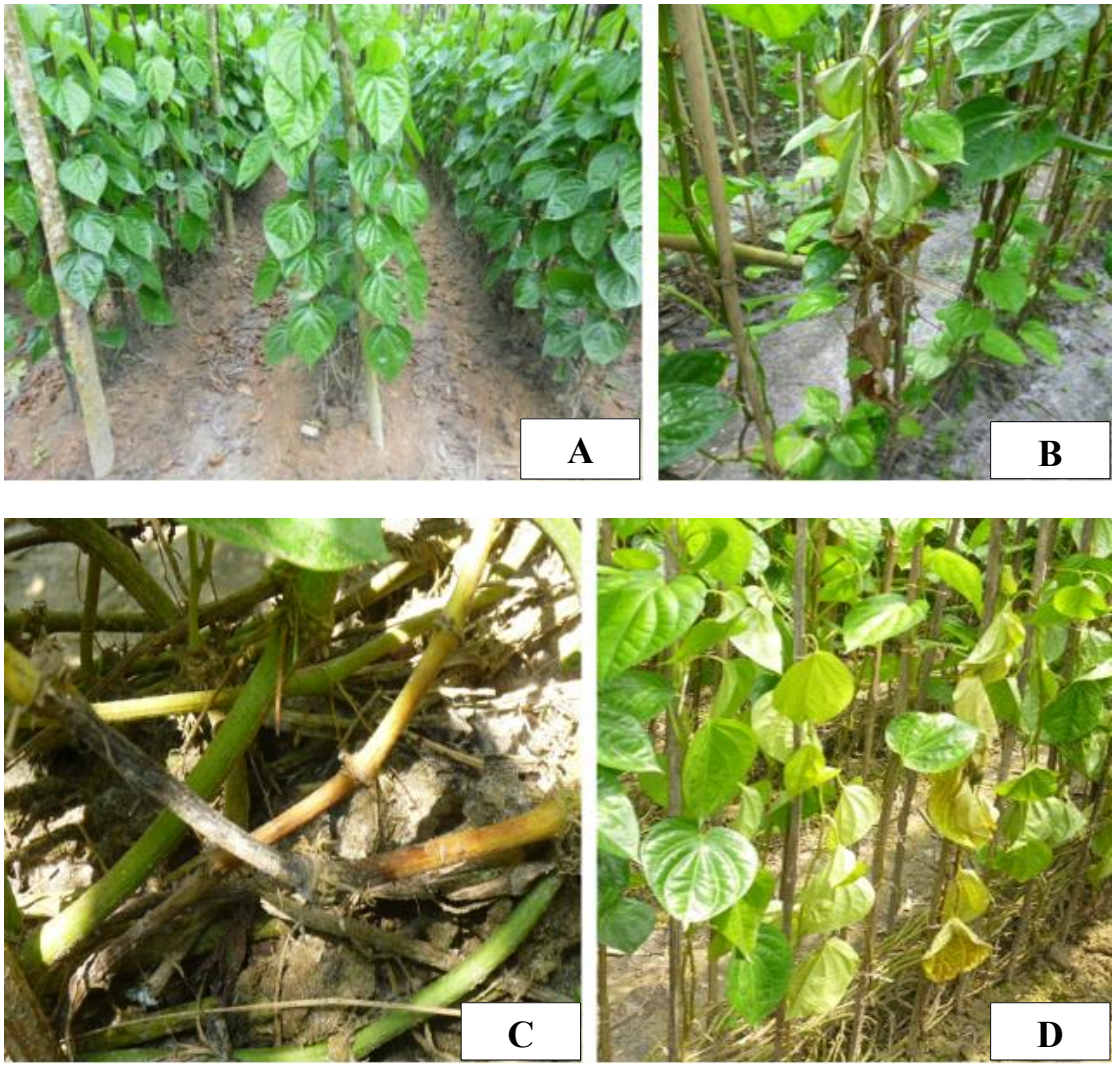


Plate 3.1.1. Orchared view of healthy betelvine plants (A) and *Sclerotium rolfsii* infected betelvine plants (B, C, D)

A

B

C

Plate 3.1.2. Epidemiological data collected by A. Digital hygro thermometer, B. Soil pH and moisture meter and C. Lux meter

3.2. Experiment 2. Isolation and identification of the isolates of *Sclerotium rolfsii* causing foot and root rot of betelvine collected from different regions of Bangladesh

3.2.1. Isolation of pathogen of foot and root rot from infected betelvine plants

The pathogens associated with the foot and root rot disease of betelvine were isolated from the collected diseased samples from major growing areas following tissue planting method (Tuite, 1969; Mian,1995). The diseased basal stems were thoroughly washed with tap water to remove soil and sand particles. After washing infected plant parts were cut into small pieces (5 cm) from advancing end of the lesions. The cut pieces were surface sterilized with 1.0% 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 (5±2^{o} C) for 3-5 days and examined daily for any fungal growth. Growing cottony mycelium was transfer to PDA plates to purify and multiply.

3.2.2. Preperation of pure culture

Pure culture of the isolates were prepared following hyphal tip method (Islam *et al.*, 2001; Tuite, 1969 and Mian, 1995) and subsequently transferred to fresh PDA plates and test tube slants and were characterize for physio-morphological features at room temperature (25±2^{o}C). Petridishes and test tube slants containing pure culture of *S. rolfsii* were stored at 4°C for future use.

3.2.3. Collection of morphological characteristics of isolates and identification

Pure cultures of the isolates were grown on fresh PDA in Petri dishes and data on mycelial growth, colony and sclerotial characters were recorded. The important identifying characteristics of isolates of *S. rolfsii* viz. consistency and colour of colony were recorded. Days to sclerotia formation, number of sclerotia per plate, size and shape of sclerotia, arrangement of sclerotia in Petri plate were also recorded. The characteristics were compared with appropriate key book (Ellis, 1971) and identified as isolate of *S. rolfsii*. The collected isolates were designated based on the location and source as suggested by Aminuzzman *et al.* (2010).

3.2.4. Isolation and identification of pathogen of other diseases of betelvine

The pathogens associated with leaf spot, leaf rot and stem rot diseases of betelvine were isolated following tissue planting method as described above (Tuite 1969, Mian1995) using stem or leaf sections as inocula. V8 medium was used to culture and multiply for *Phytophthora parasitica.* For other pathogens PDA was used. Identification of the pathogens were done by direct observation of disease specimens under sterio microscope and under compound microscope by making slides from the pathogenic culture. The pathogens were identified with the help of relevant literature (Ellis, 1971).

To prepare V8 agar culture medium, first V8-juice was prepared by mixing juices from 400 g carrots, 200 g celery, 400 g tomatoes, 250 g beet root, 200 g sweet peppers, 200 g spinach, 200 g lettuce and 100 g parsley. The juice was passed through two-ply chese cloth for several times. To obtain one liter of medium, 100 ml V8 juice, 15 g agar and 3 g calcium carbonate ($CaCO_3$) were added and then filled with distilled water up to 1 liter. After homogenization the media was autoclaved at 121ºC under 1.1 kg/cm_2 pressure for 15 minutes (Jeffers and Martin, 1986; Iacob *et al*., 2016).

3.3. Experiment 3. Pathogenicity test of *Sclerotium rolfsii* isolates causing foot and root rot disease of betelvine

3.3.1. Pathogenicity test of isolates of *Sclerotium rolfsii*

Pathogenicity test of 19 isolates of *S. rolfsii* isolated from foot and root rot infected stems of betelvine was conducted under *in-vivo* (pot culture) condition in a betelvine baroj. The boroj was constructed in the experimental field of the Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. Each isolate was cosidered as a treatment. For each treatment three plants were used where one plant was grown in earthen pot. The experiment was laid out in a randomized complete block design (RCBD) with 4 replications. Duncan's multiple range test (DMRT) was performed for comparison of means of various treatments (Gomez and Gomez, 1983).

3.3.2. Growing betelvine plants

Apparently healthy betelvine stem of variety Misti pan collected from Rajshahi were used to prepare cuttings. Forty centimeter long cutting having five nodes were prepared and grown in 16 inches diameter earthen pot containing potting medium at one plant/pot. The pots were placed inside a boraj of experimental farm of Sher-e-Bangla Agricultural University, Dhaka, and allowed to grow providing necessary care and management practices. One to two internodes below the bud point was dipped into the soil, kept touching with surface soil. Potting medium was prepared by mixing soil, sand and well decomposed cow-dung in the proportion of 2:1:1 and were sterilized by formaldehyde. Formalin solution (4%) @ 200 ml/cft soil were mixed with the soil heap and the soil was covered with a polythenc sheet for 48 hr for sterilization. After 7 days, surface sterilized earthen pots of 16 inches dia were filled with the sterilized soil (Dashgupta, 1988). Irrigation was applied as per requirement of the delicate betelvine plant with regular intervals. Weeding and mulching were also done as and when necessary to keep the betelvine plant free from weeds and for better soil aeration and conservation of soil moisture. Betelvine plants were fasten with bamboo sticks of baroj.

3.3.3. Inoculum preparation and inoculation with the causal pathogen

The isolates of *S. rolfsii* were multiplied on barley grains (Gupta and Kolte, 1982). Colonized barley grains were pre-soaked in 2% sucrose solution overnight, drained off excess solution and boiled in fresh water for 30 minutes and drained off again. These were transferred into 250 ml conocal flasks @ 80 g and autoclaved at 121.6^0C

temperature, under 1.1 kg/cm^2 pressure for 20 minutes. The conical flasks were allowed to cool at room temperature and were inoculated with 5 mm discs of 3 to 4 days old culture of *Sclerotium rolfsii* grown on PDA. Seven discs per flask were added and flasks were incubated for three weeks at 25±2^{0}C. Ninteen isolates were cultured for inoculation of the betelvine plants.

After six months of plantation each isolates of causal pathogen (*S. rolfsii*) were inoculated separately. The plants were prepared for inoculation by removing top soil within 5 cm of the stem to a depth of 2 cm. A table spoon of inoculum was placed in direct contact of entire circumference of the exposed stem. Finally, the inoculum was lightly covered with top soil for infection. The symptomalogy was studied to test the pathogenicity of the causal pathogen.

3.3.4. Data collection

The plant showing apperant wilting and rotting symptoms at collar region were consideder as infected plant and the plant without these symtoms were considered as healthy. The data on the following parameters were recorded untill 30 days after inoculation:

i. Days required for visible growth of mycelia on soil surface near base of the plant
ii. Days required for appearance of visible disease symptom
iii. Lesion length (cm)
iv. Incidence of disease of foot and root rot of betelvine in pots were recorded base on total number of plants checked.

3.4. Experiment 4. Screening of betelvine cultivars available in Bangladesh against *Sclerotium rolfsii* causing foot and root rot disease of betelvine

3.4.1. Betelvine cultivars screened against *Sclerotium rolfsii*

A total of 13 betelvine cultivars were collected from different betelvine growing areas of Bangladesh as described in Table 3.4.1. The cultivars were screened for their resistant against *S. rolfsii*, causal pathogen of foot and root rot of betelvine. The experiment was conducted *in-vivo* in a betelvine baroj. The baroj was constructed in the experimental field of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. The experiment was conducted during March, 2015 to March, 2016 and April, 2016 to April, 2017. Data on growth, yield, yield attributing parameters and disease incidence were collected.

Table 3.4.1. List of betelvine cultivars used in screening experiment against *Sclerotium rolfsii* causing foot and root rot disease of betelvine

Accession No.	Name of Cultivars	Location of collection (Upazilla and Zilla)
PB 001	Laldingi pan	Pakundia, Kishoregonj.
PB 002	BARI Line	Spices Research Centre, BARI, Bogura.
PB 003	Chalitaguti pan	Gouronadi, Barisal.
PB 004	Sanchi pan	Kaligonj, Jhenaidah
PB 005	BARI Line	Spices Research Centre, BARI, Bogura.
PB 006	Misti pan	Mohanpur, Rajshahi
PB 007	BARI Line	Spices Research Centre, BARI, Bogura.
PB 008	BARI Line	Spices Research Centre, BARI, Bogura.
PB 009	BARI Pan -1	RARS, Barisal.
PB 010	Bangla pan	Mirpur, Kushtia.
PB 011	Jhal pan	Sitakunda, Chittagong.
PB 012	Bhabna pan	Kaligonj, Jhenaidah
PB 013	Gayasur pan	Pakundia, Kishoregonj.

3.4.2. Procedures of cutting preparation, planting, intercultural operations and inoculation

Procedures of cutting preparation, planting of cuttings, preparation of inoculum and inoculation of betelvine plants were done following the same as described under experiment 3.3.

3.4.3. Data collection grading of cultivars

Data on morphological features of the cultivars, days required for appearance of visible symptom and incidence of foot and root rot in inoculated pots and control were recorded. The tested cultivars were graded as rasistant (R), moderately rasistant (MR), moderately susceptible (MS) and susceptible (S) based on disease incidence, which was determined on a 0-5 scale (Manandhar *et al*., 2016), where disease incidence 0-10% = rasistant (R), 11-30% = Moderately rasistant (MR), 31-50% = Moderately susceptable (MS) and More that 50% incidence = Susceptible (S).

3.5. Experiment 5. Bioassay of botanicals, fungicides and bioagents as IPM components against *Sclerotium rolfsii* causing foot and root rot of betelvine under *in- vitro* condition

3.5.1. *In-vitro* bioassay of botanicals, fungicide and bioagent

Three *in-vitro* experiments were conducted to determine the effect of botanical extract, chemical fungicide and bioagent on *in-vitro* myceliumgrowth of *S. rolfsii*.

3.5.1.1. Bioassay of botanicals against *Sclerotium rolfsii* following poison food technique

A total of 11 plant species were collected from different parts of Bangladesh (Table 3.5.1). Their inhibitory effect on *in-vitro* growth *Sclerotium rolfsii* was performed following Poison food technique using potato dextrose agar (PDA) as basic medium (Islam, 2005). Water extracts of suitable parts of botanicals (Table 3.5.1) were prepared according to Islam (2005). The plant parts were collected, washed with tap water, cut into small pieces, weighed on an electric balance and again washed with sterilized water. After soaking with blotting paper, weighed plant parts were blended in an electric blender adding equal amount of sterile water for preparing 1:1 botanical suspension (100 ml water for 100 g plant parts). The blend was filtered through sterile cheesecloth. For getting 1:2 suspension, another 100 ml sterilized water was added to the filtrate. PDA medium was prepared following the procedures as mentioned earlier and poured into conical flasks and autoclaved. Requisite quantity of botanical extracts was added to the medium before solidification and mixed thoroughly by stirring, and pour into the sterilized petriplate at 20 ml/plate.

The culture of the pathogen were grown on PDA medium. Five days old culture of the pathogen were cut into 5 mm small discs with a sterile cork borer and transfer aseptically in the centre of the Petri plate containing the medium. The petriplate of PDA without botanical extract was maintained for control. The inoculated Petri plates were incubated at 25±2°C temperature in an incubator. Each treatment was replicated 4 times. The linear growth of mycelium of the causal pathogens was recorded at 1 day's interval until the colony reached the rim of control plates (Islam *et al.*, 2001; Nene and Thaplial, 1979).

3.5.1.2. *In-vitro* screening of fungicides against *S. rolfsii*

Eleven fungicides reported as effective against *S. rolfsii* by different authors were evaluated *in-vitro* following poison food Technique in grove (Table 3.5.2). Fungicide suspensions were prepared in requisite quantity of water. The suspensions were poured into 250 ml Erlenmeyer. Flasks were labeled appropriately and shaken thoroughly before use.

Petri plates were prepared with 15 ml PDA media. After solidification, 5 mm discs of the medium were scooped from PDA plate in three places maintaining an equal distance from the centre using 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 fungicide into the medium around the hole. The next day, one 5 mm blocks of 5 days old PDA culture of *S. rolfsii* were cut with sterilized disc cutter and one block was placed at the centre of the plate. The plates were placed in an incubation chamber at 25±2°C. The radial mycelial growth of *S. rolfsii* was recorded at 24 hr interval until the the colony reached the rim of petridishes in control plates (Islam *et al*., 2001; Islam, 2005).

3.5.1.3. *In-vitro* screening of bioagents against *S. rolfsii*

Two bioagents, *Tricoderma harzianum* and *Pseudomonas florescens* were evaluated against *Sclerotium rolfsii* following growth inhibition techniques in Dual culture method (Islam, 2005). The bioagents were collected from Plant Pathology Division, Bangladesh Agriculture Research Institute (BARI). The fungal antagonists *Tricoderma harzianum* was cultured on PDA and the bacteria *Pseudomonas florescens* was cultured on Nutrient Agar (NA) medium.

PDA media was prepared and sterilized in an autoclave at 121°C under 1.1 kg/cm^2 for15 minutes and poured into sterilized Petri dishes (90 mm) at 20 ml/dish. Five millimiter diameter blocks of 5 days old PDA culture of both bioagents and pathogen were cut separately with the help of sterilized cork bores (5 mm). The culture discs of pathogen and bioagent was transferred aseptically and placed at periphery of Petri dish at oposite to each other. The inoculated Petri plates was transferred into an incubator and incubated at 25°C. The inoculated plate with pathogen culture without antagonists was maintained for control. The growth of the pathogen was observed periodically and

measured the colony diameter in each Petri plate. The percent inhibition of the mycelium covered petriplates control plates.

3.5.2. Measurement of radial growth and computation percent inhibition

After 24 hours of incubation, radial colony growth of *Sclerotium rolfsii* in petridishes was recorded. The radial growth of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony. The linear growth of mycelium of the causal pathogens were recorded at 24 hrs interval until the colony reached the rim of control plates (Islam, et al. 2001; Nene and Thaplial, 1979).

Inhibition percentage 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 *Sclerotium rolfsii* in control petridishes.
Y= Average radial growth (cm) of *Sclerotium rolfsii* in treated petridishes.

Table 3.5.1. List of plant species tested in bioassayed against *Sclerotium rolfsii*

Local name	English name	Scientific name	Plant parts
Neem	Margosa	*Azadiracta indica*	Leaf
Biscatali	Knotweed	*Polygonum hydropiper*	Leaf
Allamanda	Allamanda	*Allamanda cathartica*	Leaf
Lemon grass	Lemon grass	*Cymbopogon citratus*	Leaf
Korobi	Yellow oleander	*Cascabela thevetia*	Leaf
Tamak	Tobacco	*Nicotiana tabacum*	Leaf
Durba	Burmuda grass	*Cynodon dactylon*	Leaf
Roshun	Garlic	*Allium sativum*	Clove
Ada	Ginger	*Gingiber officinales*	Rhizome
Peaz	Onion	*Allium cepa*	Bulb
Mahogany	Mahogany	*Swietenia mahagoni*	Seed

Table 3.5.2. List of fungicides tested in the bioassay *in vitro* against *Sclerotium rolfsii*

Tread name	Chemical name	Active ingredient	Conc. used
Tilt 250 EC	1-[2- (2,4-Dicholorophenyl4-propyle- 1 ,3-diooxalane-2 EI-Methyl]-IH, 1,2,4-Triyazole	25% Propiconazole	0.1 %
Score 250 EC	Difenconazol	25% Difenconazole	0.05 %
Rovral 50 WP	3-(3,5 dichlorophenyl)-N-(1methyl ethyl)-2,4 dioxuimidazolidene Carboxamide $(C_{13}H_{13})_3N_3C_{12}$	50 % Iprodione	0.2 %
Bavistin 50 WP	Mythyl-2-Benzimidazole Carbamate	50 % Carbendazim	0.2 %
Provax 200	$C_{12}H_{13}NO_2S + C_6H_{12}N_2S_4$	Carboxin 17.5%+ Thiram 17.5%	0.25%
Topgan	Copper-oxychloride	50% Copper- oxychloride	0.2 %
Ridomil Gold MZ-68 WP	N- (2,6 dimethyl phenyl)-N-(methoxyacetyl)-alanine methyl ester (C14H2N04)	Metalaxil- 4% Mancozeb-64%	0.5 %
Pencozeb 80 WP	Mancozeb	80 % Mancozeb	0.45 %
Cuprofix 30 D	Copper + Mancozeb	30% Mancozeb+ 12% Copper	0.45%
Bordeaux mixture	Copper sulphate + Calcium Hydro-oxide + Water	$CuSO_4$ - 5 lbs $Ca(OH)_2$ - 5 lbs H_2O - 50 gallons	100.00%

3.6. Experiment 6. Evaluation of botanicals, fungicides, bio-agent and soil amendments against foot and root rot (*Sclerotium rolfsii*) of betelvine *in-vivo* as components of IPM

3.6.1. Treatments

An *in-vivo* experiment was conducted to evaluate the efficacy of four fungicides (Provax 200, Tilt 250 EC, Score 250 EC and Pencozeb 80 WP), two botanicals (Garlic clove extract, Allamanda leaf extract), two soil amendments (Poultry waste, Vermi-compost) and one bio-agent (*T. harzianum*) to control foot and root rot disease (*S. rolfsii*) of betelvine under inoculated pot conditions. Altogether 10 treatments comprising botanicals, fungicides, bioagent and soil amendments were tested in the experiment. The treatments were: T_1 = Provax 200, T_2 = Tilt-250 EC, T_3 = Score 250 EC, T_4 = Pencozeb 80 WP, T_5 = Garlic clove extract, T_6 = Allamunda leaf extract, T_7 = Vermi-compost, T_8 = Poultry manure, T_9 = *Tricoderma harzianum* and T_{10} = Control.

3.6.2. Experimental site, duration and design

The experiment was conducted in a betelvine orchared (baroj) in the experimental farm of Sher-e-Bangla Agricultural University, Dhaka during June, 2017 to April, 2018. The experiment was laid out in RCBD with 4 replications maintaining plot size (0.30 x 0.60) m^2, block to block distance 30 cm, row to row distance 90 cm and plant to plant spacing 20 cm.

3.6.3. Land preparation, collection of betelvine cultivar, fertilization, cuttings preparation, treatment and planting of cuttings

A piece of medium high land with well drainage system was selected and deep ploughing was done during early summer at the end of April month. After ploughing, upper soil is left exposed in the sun for two months. During the first week of June, two to three ploughing was done for well pulverized tilth condition. Weeds and stubbles were removed. Drainage trenches of 90 cm width by 15 cm depth were dugged in between two adjacent beds. A suseptible betelvine cultivar Misti pan cutting was collected from a betelvine orchared in Rajshahi. Forty centimeter long cuttings with three to five nodes were cut from the cultivar. For treatment betelvine cuttings were deeped into suspension of of fungicides, plant extracts and bioagent for 20 minutes. The cutting were then drained off, air dried and planted in the field. For control treatment the cutting were treated with plain water.

For fertilization Urea - 130, TSP - 220, MoP - 36, Zypsum - 50 and Zinc Sulphate - 15 kg/ha were used. Cow-dung and Mustard oil cake were applied @ 20t and 6t, respectively as suggested by Masudul Haque *et al*. (2013). All the fertilizers except urea were applied during final land preparation. Urea was applied in five splits at 60, 90, 120, 150 and 180 days after plantation. Mustard oil cake was applied in twelve splits @ 500kg/split after two months of plantation at 30 days interval.

Treated vine cuttings were planted in the experimental field under partially shaded and humid environment in the betelvine baroj. Planting was done with the help of khurpi (a hand operated implement). For planting, a hole was made with khurpi, so that the internodes below the bud point is dipped in soil, but rest part of cutting must be touching with surface soil. The cuttings of 3-5 years old vines were planted in the furrows (8-10 cm deep). The hole was completely packed with the help of thumb finger. The planted cuttings were watered twice a day untill the vines were established. Sticking of vines, irrigation and fertilization/manuring were done as per requirement of the orchared. Weeding was done as and when necessary.

3.6.4. Inoculum preparation and inoculation of pathogen

The procedures of inoculum preparation and inoculation of young betelvine plants were the same as described under experiment 3.

4.6.5. Spraying of fungicides and botanicals

Three times were spraying of fungicides and botanicals. The selected plant extracts were prepared using the method of Islam (2005) as described in earlier experiment. The selected plant extracts were sprayed at 3 days after inoculation at the base of the plant and base soil. Then plant extract sprayed at 7 days intervals by hand sprayer. Precautions were taken to avoid drifting of spray materials to neighboring plants.

Selected fungicide solutions were prepared separately by taking requisite amount of fungicides for each dose. The fungicides were sprayed after 3 days of inoculation at the base of the plant and base soil at 7 days interval by hand sprayer. Precautions were taken to avoid drifting of spray materials to neighboring plants.

3.6.6. Application of bio-agent, poultry manure and vermi-compost

Inoculum of the selected bioagent, *T. harzianum* was prepared using the method as described in experiment 3. Cultured *T. harzianum* on barley grains were applied at the base and root zone of the plants before 10 days of *S. rolfsii* inoculation. Poultry manure and vermi-compost were applied idividually at the base and root zone of the plants before 10 days of *S. rolfsii* inoculation. Precautions were taken to avoid drifting of application materials to neighboring plants.

3.6.7. Data collection

The data on days required for appearance of disease symptom, disease incidence and severity and yield of betel leaf were recorded following the procedures as mentioned earlier.

3.7. Experiment 7. Efficacy of integrated application of selected IPM components to control foot and root rot (*Sclerotium rolfsii*) of betelvine in Bangladesh

3.7.1. Components of IPM tested

As promising components of integrated disasee management, four chemical fungicides (Provax 200, Tilt 250 EC, Score 250 EC, Pencozeb 80 WP), two plant extracts (Garlic clove, Allamanda leaf), two soil amendments (Poultry manure, Vermicompost) and a bioagent (*T. harzianum*) were selected based on resuls of *in-vitro* and *in-vivo* experiments. Efficacy of the selected IPM components were further evaluated in 22 treatment combinations for the management of foot and root rot disease of betelvine, each of which represented a treatment. The treatments were T_1 (Soil amendment with Poultry manure), T_2 (T_1+ Provax-200), T_3 (T_1+ Tilt 250 EC), T_4 (T_1+Score 250 EC), T_5 (T_1+ Pencozeb 80 WP), T_6 (T_1+ Garlic clove extract), T_7 (T_1+Allamanda leaf extract), T_8 (Soil amendment with Vermicompost), T_9 (T_8+ Provax 200), T_{10} (T_8 + Tilt 250 EC), T_{11} (T_8+ Score 250 EC), T_{12} (T_8+ Pencozeb 80 WP), T_{13} (T_8 + Garlic clove extract), T_{14} (T_8 + Allamanda leaf extract), T_{15} (Soil amendment with *T. harzianum*), T_{16} (T_{15}+ Provax 200), T_{17} (T_{15}+ Tilt 250 EC), T_{18} (T_{15}+ Score 250 EC), T_{19} (T_{15}+ Pencozeb 80 WP), T_{20} (T_{15}+ Garlic clove extract), T_{21} (T_{15}+ Garlic clove extract) and T_{22} (No IPM component).

3.7.2. Applications of treatments

The methods of preparation of fungicidal suspensions and treatment of cuttings were the the same as used in *in-vivo* experiment 3.6. *Trichoderma harzianum*, Poultry manure and Vermicompost were applied idividually at the base and root zone of the plants before 10 days of *Sclerotium rolfsii* inoculation. Precautions were taken to avoid drifting of application materials from plant to neighboring plants.

3.7.3. Procedures of collection of betelvine, preparation of cutting, treatment of cuttings

Procedures of collection of betelvine cultivar, preparation of cutting and treatment of cuttings were the same as followed in the *in-vivo* experiment 3.6.

3.7.4. Experimental site, duration, design and planting of cuttings

The experiment was conducted in the experimental farm of Sher-e-Bangla Agricultural University, Dhaka. The experiment was laid out in a RCBD with 4 replications maintaining plot size 0.30 m x 0.60 m, block to block distance 30 cm, row to row distance 90 cm and plant to plant

spacing 20 cm. Methods of land preparation, application of fertilizers and manures, collection and prepation of vitelvine cuttings, treatment and plantation of cuttings and intercultural operations were the same as described and followed in the experiment 3.6. The selected bio-agent *Trichoderma harzianum* was prepared following the methods as described in laboratory experiment 3.3.

3.7.5. Data collection

The data on days to appear symptoms of foot and root rot, disease incidence and betel leaf yield were collected following the procedures as done under previous experiments.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Experiment 1. Survey on the incidence of foot and root rot (Sclerotium rolfsii) of betelvine under prevailing environmental condition in major betelvine growing areas of Bangladesh

4.1.1. Data on environmental factors in different locations during survey

4.1.1.1. Soil pH

During survey, the soil pH of different upzillas ranged 5.40 – 6.60. The highest pH level was found Sitakunda which was statistically similar to Mohanpur and Kaligong. The lowest sol pH was found in Gouranadi, which was statistically similar to Mirpur (Table 4.1.1).

4.1.1.2. Air temperature

The prevailing air temperature in different locations were 28.78-32.61^{o}C. The maximum temperature was found in the borjes at Gouranadi, which was statistically similar to Mirpur. The lowest air temperature was recorded from Sitakundu, which was statistically similaaaar to Mahonpur and Kaligonj (Table 4.1.2).

4.1.1.3. Relative humidity

Relative humidity (RH) in borojes of various locations varied from 72.67-82.46%. The highest RH was found at Gouranadi, which was statistically similar to Mirpur. The lowest RH was recorded from borejes of Sitakundu, which was not significantly different from Mahonpur and Kaligonj upzila (Table 4.1.1).

4.1.1.4. Light intensity

Significantly the highest light insity was recorded from Sitakundu. The lowest light intensity was recorded from Gournadi followed by Mirpur, Kaligonj and Mahonpur. The parameter under those four Upzilla was significantly different (Table 4.1.1).

4.1.2. Incidence of foot and root rot of betelvine in different locations and seasons

Incidence of foot and root rot of betelvine in different seasons and five upazillas of Bangladesh varied remarkably. In case of late summer (August), the plant infection ranged from 5.60 – 28.80% where maximum plant infection was recorded at Gouranadi,

Barisal followed by Mirpur, Kustia and the minimum plant infection was recorded at Sitakunda, Chittagong followed by Mohanpur, Rajshahi. In late winter (February), the plant infection ranged from 4.00 – 10.40%. The highest plant infection was recorded at Gouranadi and Mirpur followed by Kaligonj, Jhenaidah. The lowest plant infection recorded at Sitakunda followed by Mohanpur. At mid summer (May), the plant infection ranged from 4.00 – 7.20%. The highest plant infection was recorded at Kaligonj followed by Mohanpur. The lowest plant infection recorded at Sitakunda and Gouranadi followed by Mirpur (Table 4.1.2).

Table 4.1.1. Data on environmental factors in different locations of Bangladesh recorded during survey (pooled data of five boroj per location and three years (2015, 2016 and 2017)

Upzilla (Location)	Soil pH	Air temperature (°C)	Relative humidity (%)	Light intensity (x10 Lux)
Gouranadi, Barisal	5.40 c[α]	32.61 a	82.46 a	530 e
Kaligonj, Jhenaidah	6.03 b	29.64 bc	76.46 bc	641 c
Mirpur, Kustia	5.8 bc	31.13 ab	79.93 ab	619 d
Mahonpur, Rajshahi	6.40 ab	29.24 c	73.86 c	726b
Sitakuda, Chittagong	6.60 a	28.78 c	72.67 c	738 a

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

Table 4.1.2. Incidence of foot and root rot of betelvine in different locations and seasons (pooled of five boroj per location and the years 2015, 2016 and 2017)

Name of the location	**Disease incidence (%)**		
	Late summer (August)	**Late winter (February)**	**Mid summer (May)**
Gouranadi, Barisal	28.80 a[α]	10.40 a	7.20 a
Kaligonj, Jhenaidah	13.60 c	8.00 b	4.00 d
Mirpur, Kustia	21.60 b	10.40 a	4.80 c
Mohanpur, Rajshahi	7.20 d	5.66 c	5.60 b
Sitakunda, Chittagong	5.60 e	4.00 d	4.00 d

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

4.1.3. Incidence of foot and root rot under different environmental facors

The environmental factors viz. soil pH, air temperature, relative humidity and light intensity showed remarkable impact on the incidence of foot and root rot of betelvine (Fig. 4.1.1).

4.1.3.1. Disease incidence at different level of soil pH

The disease incidence decreased gradually with the increase of soil pH level. The highest disease incidence of 15.46% was recorded at soil pH of 5.4 at Gouranadi upazilla. The lowest disease incidence was recorded from Sitakunda, where the soil pH was maximum of 6.6. The results reveal that low pH favoured the development of the disease (Table 4.1.1).

Relationship between incidence of foot and root rot of betelvine and soil pH was linear and negative. Their relationship could be explained by the regression equation $y = -9.2856x + 65.527$, where x = soil pH and y = disease incidence. The R^2 value was 0.9819 which indicates that the contribution of soil pH to the incidence of foot and root rot disease of betelvine is 98.19% (Fig. 4.1.1. and Fig. 4.1.2).

4.1.3.2. Air temperature

In case of air temperature, the highest disease incidence of 15.46% was recorded at 32.61^0C in Gouranadi followed by 12.26% at 31.13^0C in Mirpur, Rajshahi, respectively. The lowest disease incidence of 4.53% was recorded at 28.78^0C in Sitakunda. The higher disease incidence was observed while the temperature was above 30^0C (Fig. 4.1.1).

A significant and positive correlation between air temperature and foot and root rot incidence was observed. The linear relationship between air temperature and disease incidence could be expressed by the equation $y = 2.8094x - 75.684$, where x = air temperature and y = disease incidence. The R^2 value of 0.9768 indicates that the contribution of air temperature to the incidence of foot and root rot of betelvine is 97.68% (Fig. 4.1.1. and Fig. 4.1.3).

4.1.3.3. Relative humidity

In case of relative humidity, it was revealed that the higher humidity favoured the disease. The disease gradually decreased with the decrease of relative humidity (Fig. 4.1.1). A significant positive correlation between relative humidity and foot and root rot disease of betelvine were observed for the year (Fig. 4.1.1)

The linear relationship between relative humidity and disease incidence could be expressed by the equation y= 1.0887x – 74.527, where x = relative humidity and y = disease incidence. The R^2 value 0.9974 indicates that the contribution of relative humidity to the incidence of foot and root rot disease of betelvine contributes by 99.74% (Fig. 4.1.1. and Fig. 4.1.4).

4.1.3.4. Light intensity

Comparatively low light intensity favoured development of foot and root rot of betelvine. The highest disease incidence of 15.46% was recorded while the light intensity was 53 X 100 lux at Gouranadi upazilla followed by 12.26% disease incidence was found in Mirpur upazilla at 62 X 100 lux light intensity. The lowest disease incidence of 4.53% was recorded from Sitakunda at 74 X 100 lux light intensity preceded by 6.15% disease incidence at 73 X 100 lux in case of Mohanpur upazilla (Fig. 4.1.1).

The relationship between disease incidence and light intensity was negative and could be explained by the regression equation y = -0.5028x +42.171, where x = light intensity and y = disease incidence. The co-efficient of determination, R^2 = 0.9451 and the regression equation equation, The R^2 value indicates that the contribution of light intensity to the incidence of foot and root rot disease of betelvine is 94.51% (Fig. 4.1.1. and Fig. 4.1.5).

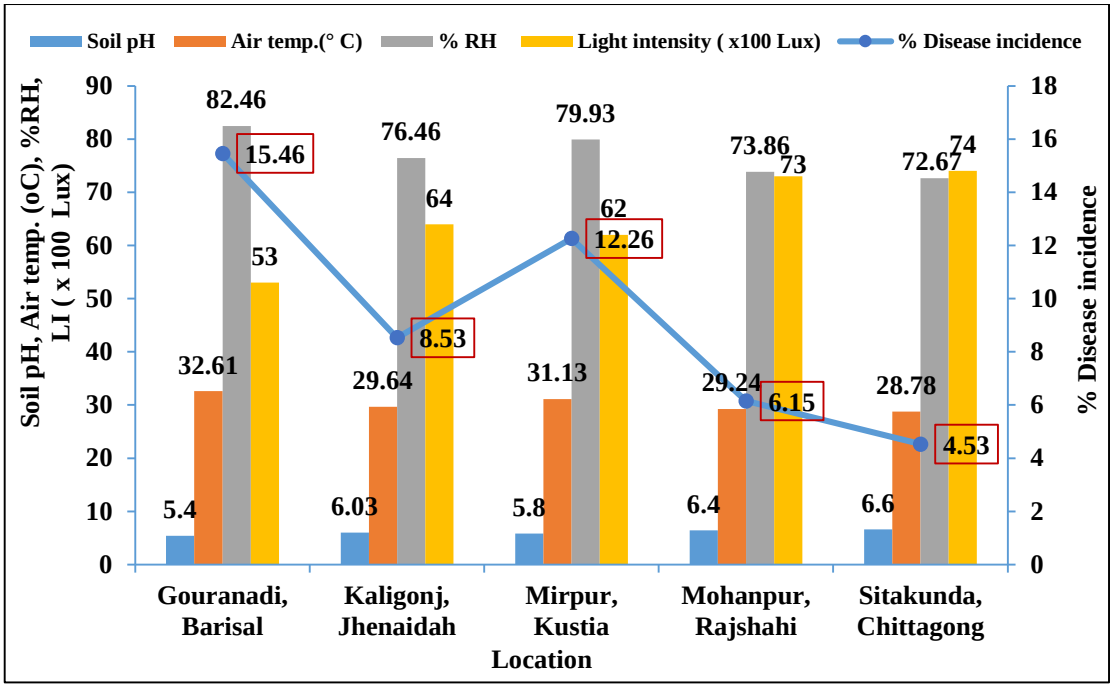


Figure 4.1.1. Effect of different weather factors on disease incidence of foot and root rot of betelvine

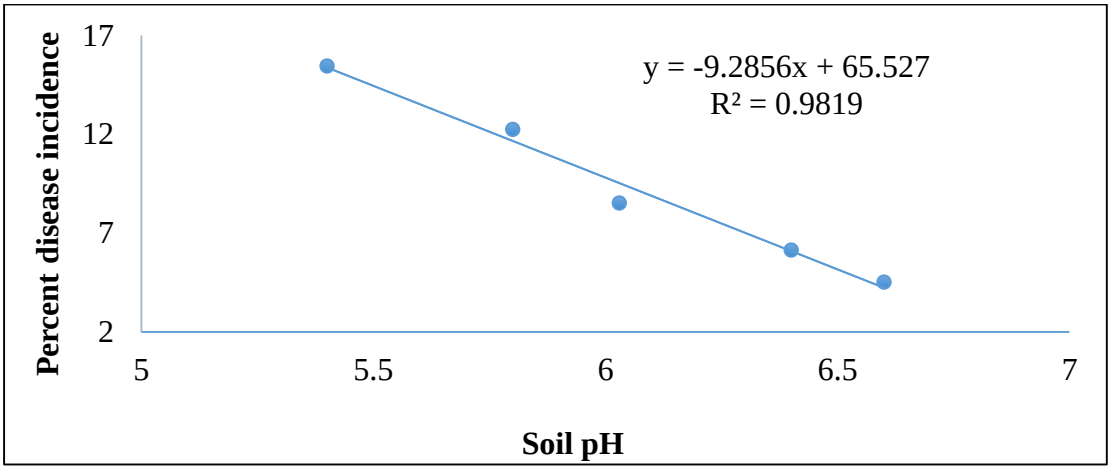


Figure 4.1.2: Relationship between incidence of foot and root rot of betelvine and soil pH

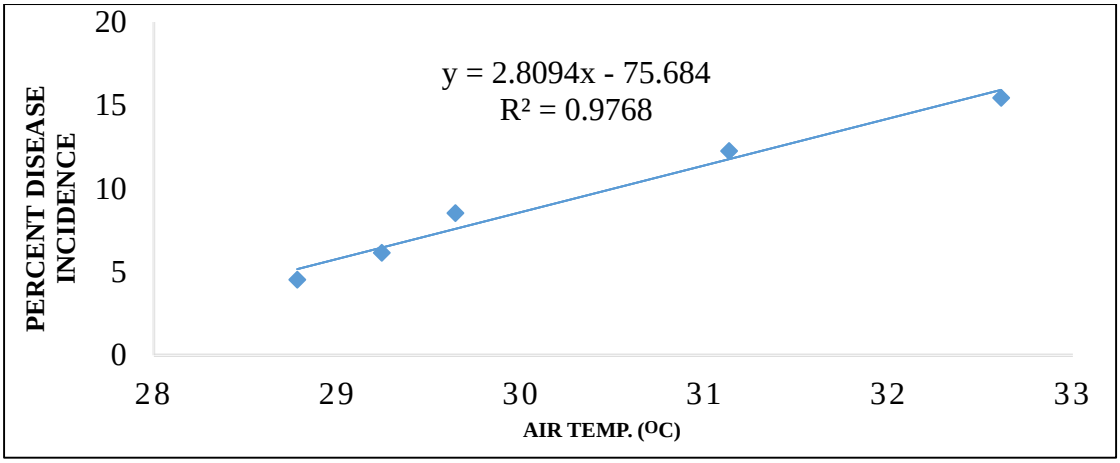


Figure 4.1.3: Relationship between air temperature and and incidence of foot and root rot disease of betelvine

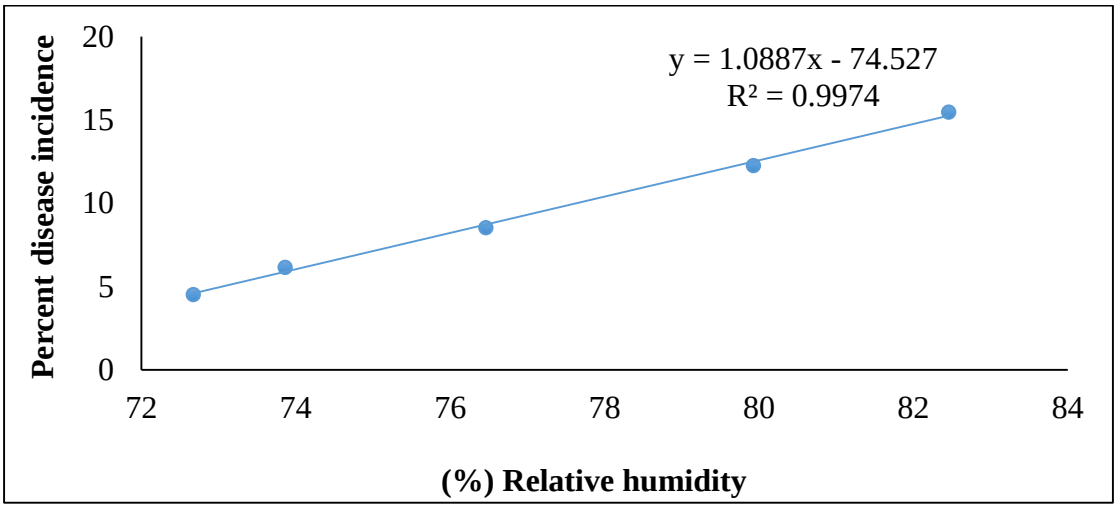


Figure 4.1.4: Relationship between foot and root rot disease of betelvine and relative humidity

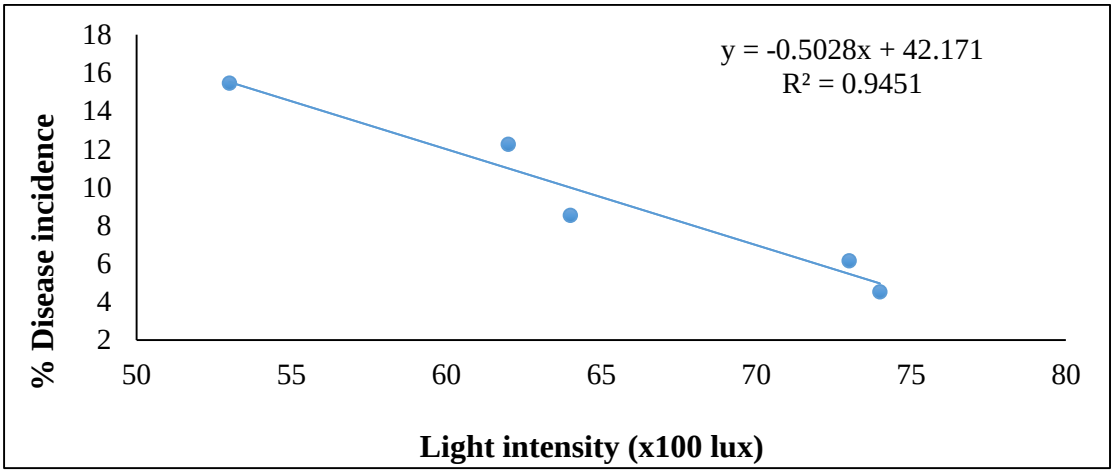


Figure 4.1.5: Relationship between incidence of foot and root rot disease of betelvine and light intensity

4.1.4. Other diseases observed in betelvine baroj in the surveyed areas

Other than foot and root rot of betelvine, leaf spot (*Colletototrichum piperis*), leaf rot (*Phytophthora parasitica*) and stem rot (*Phytophthora parasitica*) of betelvine diseases were found to occur in the surveyed areas (Plate 4.1.1 and 4.1.2). Their incidence and severity in different locations were recorded and presented in Tables 4.1.3, 4.1.4 and 4.1.5.

4.1.4.1. Leaf spot

Incidence of leaf spot of betelvine varied from one upazilla to another upazilla and one month to another month. The maximum disease incidence of was recorded in Mohanpur upazilla in the month of August where disease incidence ranged from 13.70-56.94%. The minimum disease incidence was found in Sitakunda in the month of May where disease incidence ranged from 8.34-11.42%. Disease incidence was comparatively higher in the month of August. Disease incidence was gradually decreased from August to May in every locations (Table 4.1.3).

The severity of leaf spot of betelvine in different upazillas found to vary appreciably. The highest disease severity was recorded in Mohanpur upazilla in the month of August where disease severity ranged from 4.75-28.30%. The lowest disease severity was found in Gouranadi in the month of May where disease severity ranged from 2.70-4.64%. Comparatively higher disease severity was recorded in the month of August. Disease severity was gradually increased from August to May in every locations (Table 4.1.3).

Table 4.1.3. Incidence and severity of leaf spot of betelvine in different upazillas (pooled data of five borojes per location for the year 2015, 2016 and 2017)

Name of the location	% Disease incidence			% Disease severity		
	August	February	May	August	February	May
Gouranadi, Barisal	14.30 d[α]	12.46 c	8.78 c	4.75 e	5.08 c	2.70 c
Kaligonj, Jhenaidah	40.40 c	20.54 a	10.22 b	23.36 c	10.25 ab	4.51 a
Mirpur, Kustia	50.33 b	20.08 ab	10.60 b	25.37 b	9.06 b	3.79 b
Mohanpur, Rajshahi	56.94 a	18.59 b	11.42 a	28.30 a	12.34 a	4.64 a
Sitakunda, Chittagong	13.70 d	19.16 ab	8.34 c	6.22 d	11.59 a	2.81 c

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

4.1.4.2. Leaf rot

Incidence of leaf rot of betelvine in different upazillas found to vary from location to location and season to season. Maximum disease incidence was recorded from Mohanpur upazilla in the month of August where disease incidence ranged from 6.42-42.0%. The lowest disease incidence was found in Kaligonj in the month of May where disease incidence ranged from 4.45-17.53%. Disease incidence was comparatively higher in the month of August and decreased gradually from August to May in every locations (Table 4.1.4).

Severity of leaf rot of betelvine in different locations also vary from location to location and season to season. Maximum disease severity was recorded in Mohanpur upazilla in the month of August where disease severity ranged from 5.35-21.69%. The lowest disease severity was found in Kaligonj in the month of May where disease severity ranged from 1.84-8.42%. Disease severity was comparatively higher in the month of September and decreased gradually from August to May in every locations (Table 4.1.4).

Table 4.1.4. Disease incidence and Severity of leaf rot of in different upazillas (pooled of five boroj per location for the year 2015, 2016 and 2017)

Name of the location	% Disease incidenc			% Disease severity		
	August	February	May	August	February	May
Gouranadi, Barisal	9.42 c[α]	14.27 d	7.76 c	5.35 d	7.447 c	2.84 c
Kaligonj, Jhenaidah	34.60 b	20.98 b	4.45 e	18.24 b	11.08 b	1.84 d
Mirpur, Kustia	36.11 b	17.30 c	17.53 a	19.57 b	9.83 b	8.42 a
Mohanpur, Rajshahi	42.00 a	27.30 a	9.72 b	21.69 a	14.40 a	5.41 b
Sitakunda, Chittagong	6.42 d	14.53 d	7.08 d	9.68 c	8.04 c	2.97 c

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

4.1.4.3. Stem rot

Incidence of stem rot of betelvine varied remarkably from upazilla to upazilla and season to season. The highest disease incidence was recorded from Gouranadi upazilla in the month of August where disease incidence ranged from 6.66-29.53%. The lowest disease incidence was found in Sitakunda in the month of May where disease incidence ranged 1.73-7.13%. Disease incidence was comparatively higher in the month of August and decreased gradually from August to May in every locations (Table 4.1.5).

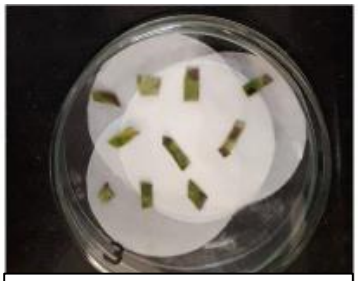
Severity of stem rot of betelvine varied from upazilla to upazilla and season to season. Maximum disease severity was recorded in Gouranadi upazilla in the month of August where disease severity ranged 1.71-20.49%. The lowest disease severity was found in Sitakunda in the month of May where disease severity ranged 1.14-3.81%. Disease severity was comparatively higher in the month of August and decreased gradually from August to May in every locations (Table 4.1.5).

Table 4.1.5. Incidence and severity of stem rot of betelvine in different upazillas (pooled data of five borojes per location for the year 2015, 2016 and 2017)

Name of the location	**% Disease incidence**			**% Disease severity**		
	August	February	May	August	February	May
Gouranadi, Barisal	29.53 a[α]	10.46 a	3.20 c	20.49 a	3.40 c	2.41 c
Kaligonj, Jhenaidah	13.46 c	8.06 b	7.13 a	10.60 b	12.74 a	3.81 a
Mirpur, Kustia	21.51 b	10.33 a	6.27 b	19.55 a	4.66 b	3.21 b
Mohanpur, Rajshahi	7.20 d	4.80 c	2.43 d	2.90 c	3.16 c	1.94 d
Sitakunda, Chittagong	5.66 e	4.100c	1.73 e	1.71 c	2.46 d	1.14 e

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

Leaf spot of betel leaf

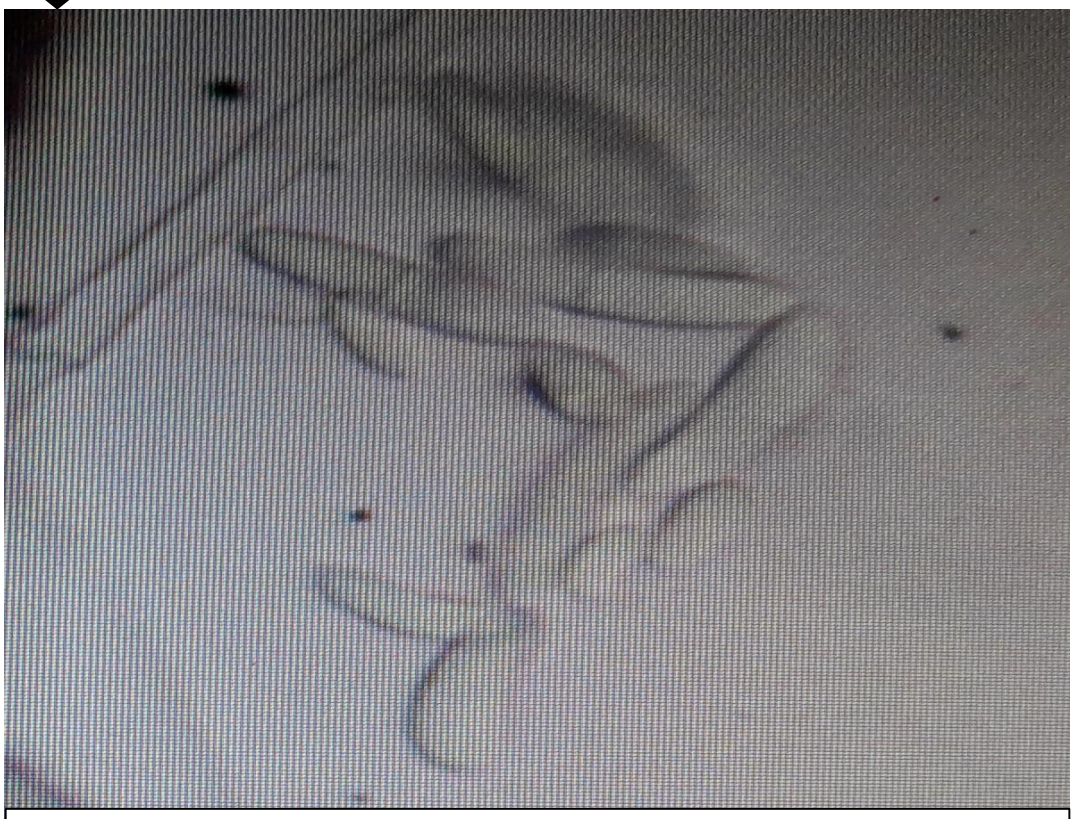


Isolation of pathogen by tissue culture method

Pure culture of pathogen on PDA media

Conidia of *Colletotrichum piperis* seen under compound microscope

Setae *of Colletotrichum* sp.



Conidia of *Colletotrichum piperis*

Plate 4.1.1. Symptoms of leaf spot and characteristics of its causal fungus *Collitotrichum piperis*

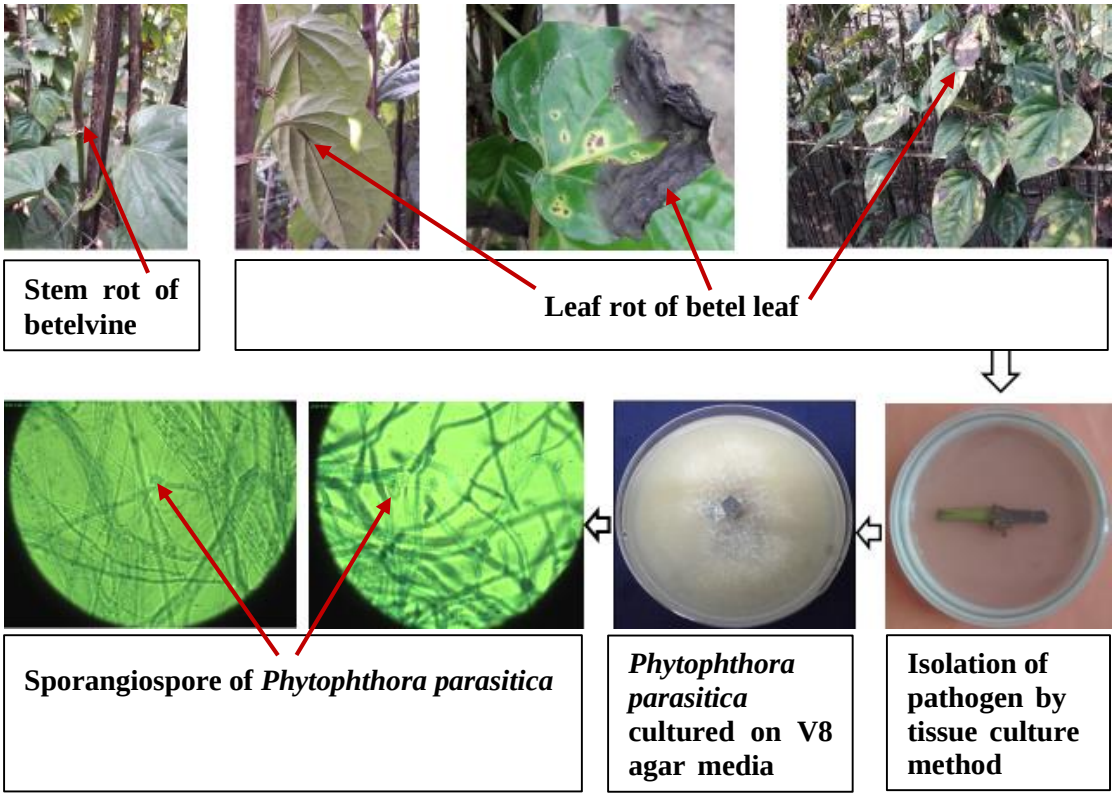


Plate 4.1.2. Symptoms of stem rot of betelvine and characteristics of its causal fungus *Phytophthora parasitica* isolated from infected stem

4.2. Experiment 2. Isolation and identification of the isolates of *Sclerotium rolfsii* causing foot and root rot of betelvine collected from different regions of Bangladesh

4.2.1. Characteristic features and identification of isolates of *S.rolfsii*

The fungus isolated from diseased betelvine samples was identified as *Sclerotium rolfsii* based on mycological characteristics.

4.2.1.1. Mycelial growth of the isolates of *Sclerotium rolfsii*

The isolates of *S.rolfsii* grown on PDA plates and revealed different mycelial growth speed of different isolates varied up to 72 hours. The maximum (1.47 mm/hr) mycelial growth recorded in Isolate -16 followed by Isolate – 15 (1.41 mm/hr). The minimum mycelial growth (0.96 mm/hr) was yielded in Isolate - 2 (Table 4.2.1).

4.2.1.2. Colony characters

Microscopic examination of the fungal culture showed hyaline, thin walled, septate, profusely branched mycelium with clamp connections. Among the isolates, colony of isolates 1, 2, 7, 11, 13, 14, 18 and 19 showed embeded growth on the surface of the culture medium and thin to profusely thick consistency. Fluffy colony consistency was found in isolates 3, 4, 6, 8, 12, and 17. In other isolates the colony consistency was wooly. The colony colour was offwhite in isolates 11, 12, 17 and 18, white in 1, 13, 14, 15, 16 and 19, and brightly white in rest of the isolates (Table 4.2.2 and Plates 4.2.1 and 4.2.2). Based on the physio – morphological variations, the isolates of *S. rolfsii* are grouped into 3 groups viz, a. Embeded growth, b. Fluffy and c. Wooly colony consistency representing all the isolates collected from major belelvine growing areas of Bangladesh

4.2.1.3. Sclerotium characters

When the colony attained maturity (within 9 - 15 days of culture), small mycelial knots were found to form, which were turned to mustard seed like sclerotia. The isolate - 9 produced sclerotia within 9 days; isolate-2, 4, 5, 12, 17, 18 and 19 developed sclerotia within 10 days; isolate 10 within 12 days; isolate 11 within 13 days; isolate 1, 3, 6, 13, 15 and 16 within 14 days and isolate 7, 8 and 14 within 15 days of age. The colour of fully matured sclerotia varied in different isolates as light brown, brown and dark brown. Colour of sclerotia was light btown in isolates 5, 6 and 7, brown in islates 2, 3,

4, 3, 9, 11, 14 and 19 and rest of the isolates produced dark brown sclerotia in culture (Table 4.2.2 and 4.2.3. and Plates 4.2.1- 4.2.3).

Number of sclerotia produced by different isolates on culture plates varied 14-288 per plate. The highest number of sclerotia was produced by isolate-16 followed by Isolate - 1 (270/plate). The lowest number of sclerotia was produced by isolate -10.

Sclerotial shape was round or irrigular or spherical to irrigular in different isolates. Sclerotia of isolates 4, 5 and 6 were spherical to irregular and Isolate 18 produced round to irregular sclerotia. The average size of the sclerotia in the isolates varied 1.0-2.2 mm in diameter. The biggest sclerotium was produced by isolate -10 followed by isolate-7 (2.2 mm). The smallest sized S. (1.0 mm) was observed in isolate -3 and 9. The weight of 100 sclerotia ranged 72-553 mg. The highest 100 sclerotial weight was found in isolate-7 and the lowest in isolate-15. Altogether 19 isolates of *S.rolfsii* were identified considering characteristics of colony and sclerotia (Tables 4.2.2 and 4.2.3).

Findings of the present studies reveal that most of the morphological characteristics of the cultures of *S. rolfsii* isolated from foot and root rot infected betelvine plants were vary clearly. Based on those variations in morphological characteristics, the 19 isolates demonstrate that these were independent 19 isolates.

Table 4.2.1. Growth of the 19 isolates of *Sclerotium rolfsii* on PDA plates at room temperature (25^{o}C)

Isolates	Radial mycelial growth (mm) at the incubation period (hr.)								Average Growth mm h^{-1}
	12	24	36	48	60	72	84	96	
Isolate - 1 (BGPBSr - 1)	12 f	22 de	42 de	56 de	80 c	87	90	NM	1.33 c
Isolate - 2 (BGPBSr - 2)	13 ef	17 f	32i	41 k	58 j	66	83	90	0.96 j
Isolate - 3 (BGPBSr - 3)	14 def	22 de	40 ef	50 hi	68 h	75	90	NM	1.13 h
Isolate - 4 (BGPBSr - 4)	17 abcd	23 cde	38 fg	51 ghi	67 h	75	87	90	1.11 h
Isolate - 5 (BGPBSr - 5)	18 abc	25 bcd	45 bcd	58 cd	78 cd	85	90	NM	1.30 cd
Isolate - 6 (BGPBSr - 6)	17 abcd	26 bc	47 b	60 bc	80 c	87	90	NM	1.33 c
Isolate - 7 (BGPBSr - 7)	13 ef	23 cde	42 de	57 de	77 de	84	90	NM	1.28 de
Isolate - 8 (JKPBSr - 1)	13 ef	20 ef	39 fg	52 gh	74 fg	84	90	NM	1.23 fg
Isolate - 9 (JKPBSr - 2)	14 def	21 e	37 gh	49 i	67 h	76	88	90	1.11 h
Isolate - 10 (JKPBSr - 3)	15 cdef	25 bcd	43 cd	55 ef	75 ef	85	90	NM	1.25 ef
Isolate - 11 (KMPBSr - 1)	20 a	26 bc	45 bc	61 b	80 c	88	90	NM	1.33 c
Isolate - 12 (KMPBSr - 2)	13 ef	22 de	43 cd	56 de	78 cd	88	90	NM	1.30 cd
Isolate - 13 (KMPBSr - 3)	14 def	21 e	44 cd	57 de	80 c	90	NM	NM	1.33 c
Isolate - 14 (RMPBSr - 1)	16 bcde	25 bcd	45 bc	62 b	80 c	87	90	NM	1.33 c
Isolate - 15 (RMPBSr - 2)	20 a	28 ab	51 a	66 a	85 b	90	NM	NM	1.41 b
Isolate - 16 (RMPBSr - 3)	19 ab	30 a	52 a	68 a	88 a	90	NM	NM	1.47 a
Isolate - 17 (CSPBSr - 1)	18 abc	25 bcd	42 de	56 de	75 ef	82	90	NM	1.25 ef
Isolate - 18 (CSPBSr - 2)	15 cdef	21 e	35 h	45 j	63 i	71	85	90	1.05 i
Isolate - 19 (CSPBSr - 3)	16 bcde	28 ab	40 ef	53 fg	72 g	78	90	NM	1.20 g

Note:

NM= No measurement after petriplate had been covered

BG = Barisal-Gouronadi
PB = *Piper betle*
Sr = *Sclerotium rolfsii*
CS = Chittagong-Sitakunda
JK = Jhenaidah-Kaligonj
KM = Kushtia-Mirpur
RM = Rajshahi-Mohanpur

Table 4.2.2. Four morphological characteristics (Colony consistency, colony colour, Days to sclerotia formation, colour of sclerotia) of the 19 isolates of *Sclerotium rolfsii* on PDA at room temperature (25^{o}C)

Accession of Isolates	Colony consistency	Colony colour	Days to sclerotia formation	Colour of sclerotia
Isolate - 1 (BGPBSr - 1)	Embeded and thick	White	14	Dark brown
Isolate - 2 (BGPBSr - 2)	Embeded and thick	More white	10	Brown
Isolate - 3 (BGPBSr - 3)	Fluffy and thick	More white	14	Brown
Isolate - 4 (BGPBSr - 4)	Fluffy and thick	More white	10	Brown
Isolate - 5 (BGPBSr - 5)	Wooly	More white	10	Light brown
Isolate - 6 (BGPBSr - 6)	Fluffy	More white	14	Light brown
Isolate - 7 (BGPBSr - 7)	Embeded and thick	More white	15	Light brown
Isolate - 8 (JKPBSr - 1)	Fluffy at the pheripheral area	More white	15	Dark brown
Isolate - 9 (JKPBSr - 2)	Wooly	More white	09	Brown
Isolate - 10 (JKPBSr - 3)	More wooly	More white	12	Dark brown
Isolate - 11 (KMPBSr - 1)	Embeded and thin	Off white	13	Brown
Isolate - 12 (KMPBSr - 2)	Fluffy	Off white	10	Dark brown
Isolate - 13 (KMPBSr - 3)	Embeded and thick	White	14	Dark brown
Isolate - 14 (RMPBSr - 1)	Embeded profuse and thick	White	15	Brown
Isolate - 15 (RMPBSr - 2)	Wooly at the pheripheral area	White	14	Dark brown
Isolate - 16 (RMPBSr - 3)	Wooly at the pheripheral area	White	14	Dark brown
Isolate - 17 (CSPBSr - 1)	Fluffy	Off white	10	Dark brown
Isolate - 18 (CSPBSr - 2)	Embeded and thick	Off white	10	Dark brown
Isolate - 19 (CSPBSr - 3)	Embeded and thick	White	10	Brown

Table 4.2.3. Characteristics of sclerotia of the 19 isolates of *Sclerotium rolfsii* isolated from foot and root rot infected betelvine plants on PDA at room temperature (25^{o}C)

Accession of isolates	Characteristics of Sclerotia				
	Sclerotial arrangement on culture plate	**Sclerotia per plate**	**Shape**	**Size (mm)**	**Wt. of 100 sclerotia (mg)**
Isolate - 1 (BGPBSr - 1)	Scattered	270 b$^{\alpha}$	Round	1.06 gh	85.5 l
Isolate - 2 (BGPBSr - 2)	Scattered	60 k	Round	1.03 gh	105 i
Isolate - 3 (BGPBSr - 3)	Cluster and scattered	50 l	Round	1.00 h	100 j
Isolate - 4 (BGPBSr - 4)	Scattered	83 j	Spherical to irrigular	1.05 gh	106 i
Isolate - 5 (BGPBSr - 5)	Scattered	63 k	Spherical to irrigular	1.72 c	282.5 d
Isolate - 6 (BGPBSr - 6)	Scattered	25 m	Spherical to irrigular	1.67 c	304.11 c
Isolate - 7 (BGPBSr - 7)	Cluster at the site of petriplates	48 l	Irrigular	2.00 b	553 a
Isolate - 8 (JKPBSr - 1)	Scattered	249 c	Round	1.42 d	154 g
Isolate - 9 (JKPBSr - 2)	Scattered and cluster at the peripheri	230 d	Round	1.00 h	102 j
Isolate - 10 (JKPBSr - 3)	Scattered	14 n	Irrigular	2.20 a	473 b
Isolate - 11 (KMPBSr - 1)	Scattered	89 i	Round	1.13 g	96 k
Isolate - 12 (KMPBSr - 2)	Scattered but cluster at peripheral ring	147 f	Round	1.70 c	245 e
Isolate - 13 (KMPBSr - 3)	Scattered	118 h	Round	1.09 gh	73.5 m
Isolate - 14 (RMPBSr - 1)	Scattered one side	132 g	Round	1.1 gh	74 m
Isolate - 15 (RMPBSr - 2)	Cluster at peripheral ring	209 e	Round	1.25 f	72 m
Isolate - 16 (RMPBSr - 3)	Scattered	288 a	Round	1.40 de	130 h
Isolate - 17 (CSPBSr - 1)	Scattered	79 j	Round	1.62 c	280.5 d
Isolate - 18 (CSPBSr - 2)	Scattered	51 l	Round to irrigular	1.50 d	212 f
Isolate - 19 (CSPBSr - 3)	Scattered	213 e	Round	1.30 ef	131 h

$^{\alpha}$Values within the same column with a common letter(s) do not differe significantly (P=0.01).

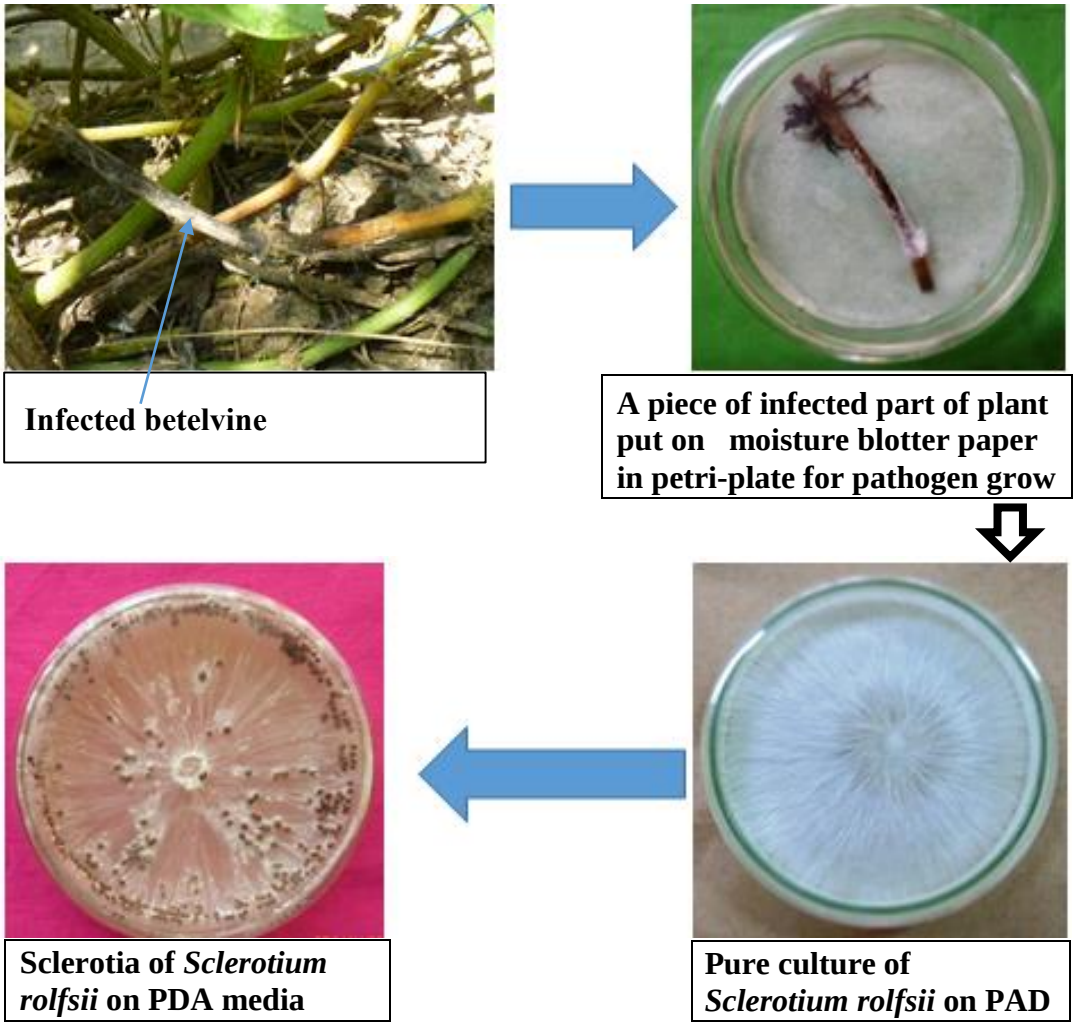


Plate 4.2.1. Infected betelvine vines, vine pieces on PDA and pure culture and sclerotia of *Sclerotium rolfsii*

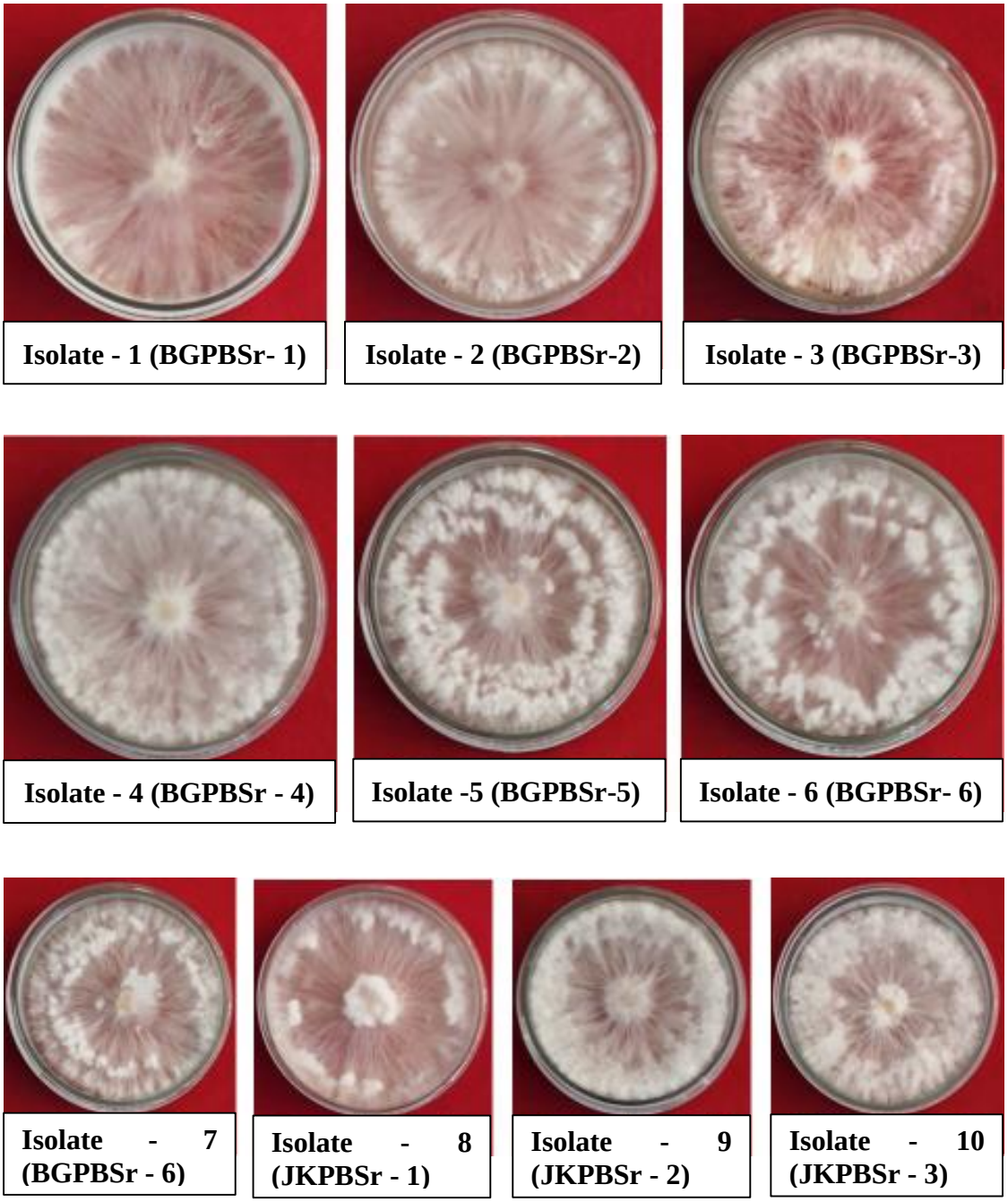
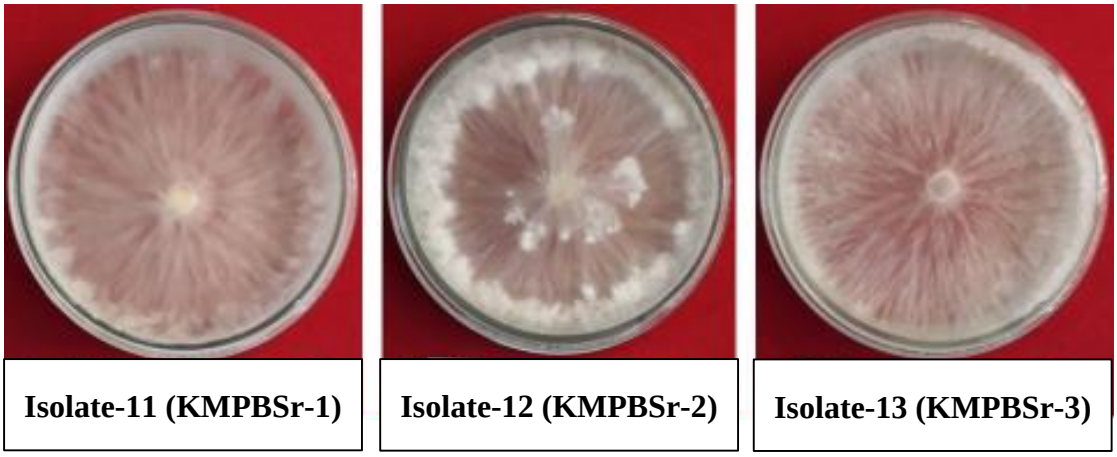
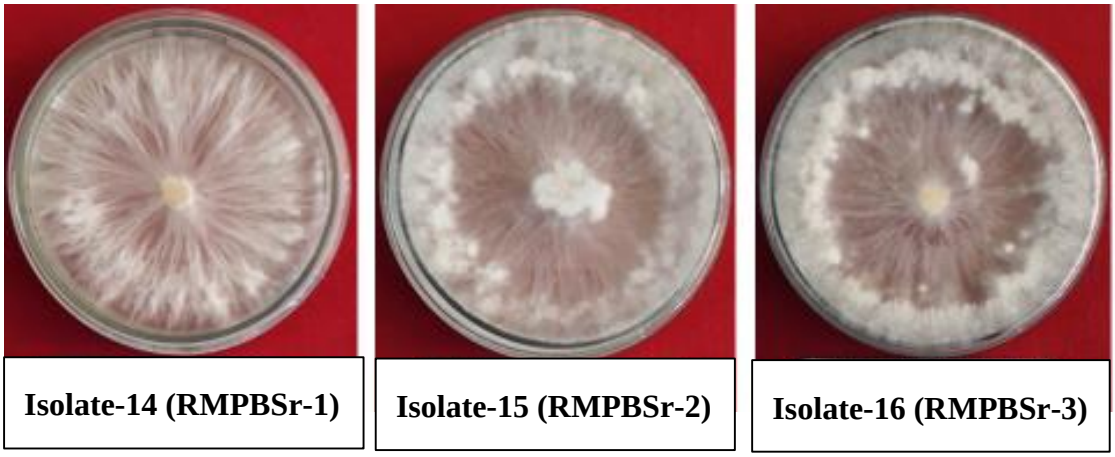


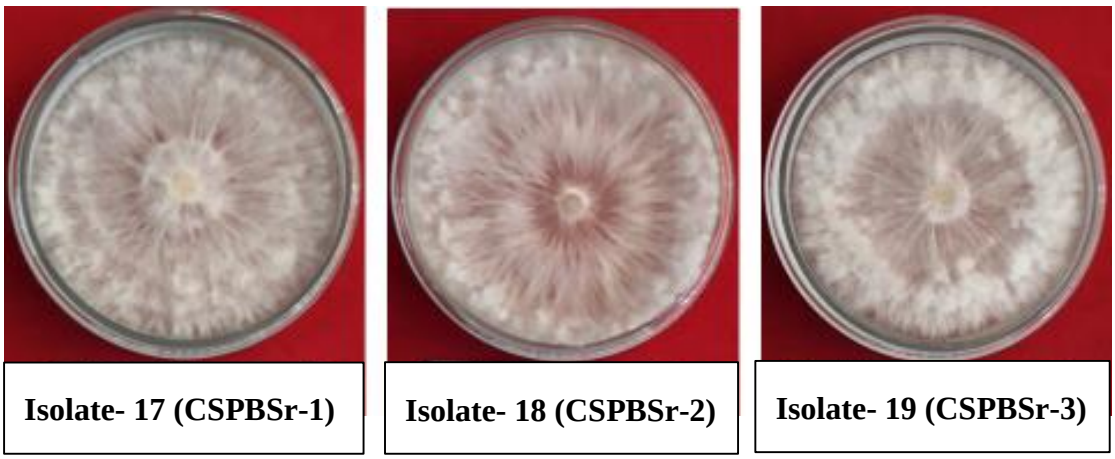
Plate 4.2.2. Cultural plate showing mycelial growth of isolates of *Sclerotium rolfsii* on PDA petri-plate



Isolate-11 (KMPBSr-1) | **Isolate-12 (KMPBSr-2)** | **Isolate-13 (KMPBSr-3)**



Isolate-14 (RMPBSr-1) | **Isolate-15 (RMPBSr-2)** | **Isolate-16 (RMPBSr-3)**



Isolate- 17 (CSPBSr-1) | **Isolate- 18 (CSPBSr-2)** | **Isolate- 19 (CSPBSr-3)**

Plate 4.2.2. Cont'd

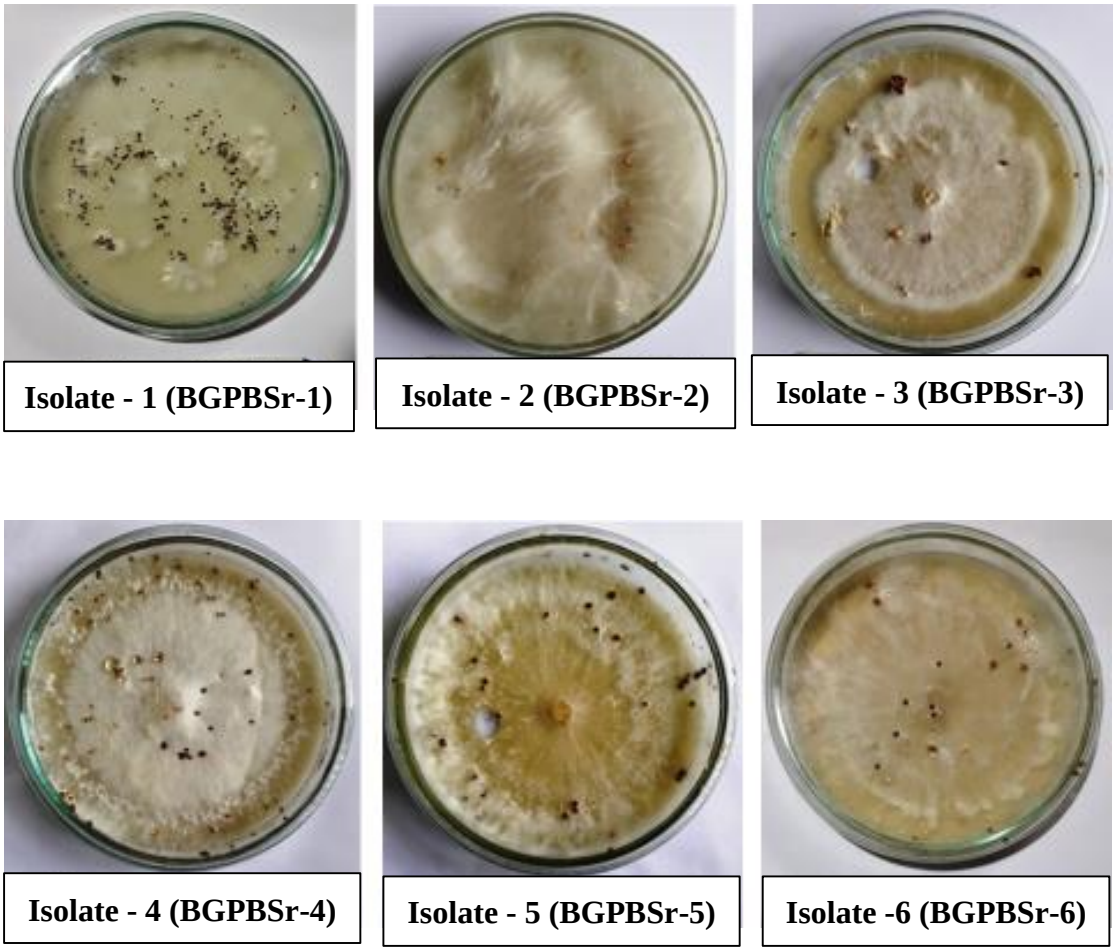


Plate 4.2.3. Mycelia and sclerotia of the different isolates of *Sclerotium rolfsii* on PDA medium

Isolate- 11 (KMPBSr-1)

Isolate-12 (KMPBSr-2)

Isolate- 13 (KMPBSr-3)

Isolate-14 (RMPBSr-1)

Isolate-15 (RMPBSr-2)

Isolate-16 (RMPBSr-3)

Isolate-17 (CSPBSr-1)

Isolate-18 (CSPBSr-2)

Isolate- 19 (CSPBSr-3)

Plate 4.2.3. (Cont'd)

4.3. Experiment 3. Pathogenicity test of *Sclerotium rolfsii* isolates causing foot and root rot disease of betelvine

4.3.1. Infection of betelvine plant after inoculation with *Sclerotium rolfsii*

Pathogenicity of the isolates of *S. rolfsii* causing foot and root rot disease of betelvine was tested in earthen pots, which were placed in betelvine barojes (orchard). The pathogenecity was determinied based on mycelial growth on soil surface, early appearance of disease symptomps, lesion length and disease incidence.

4.3.2. Days required for appearance of mycelium growth on soil surface

Soil inoculated with *S. rolfsii* exhibited mycelial growth on the soil surface and around the base of the plant within 2 - 4 DAI. Only 2 DAI were required to manifest cottony colony on soil surface near root zone of inoculated betelvine plants in isolate-3, 5, 7, 9 and 12. Isolate-1, 2, 4, 6, 10, 11, 13, 15, 17 and 18 required 3 days to produce sclerotia. Other isolates needed 4 days to develop sclerotia (Table 4.3.1).

4.3.3. Days required for appearance of 1st symptom of plants

The data were recorded on the appearance of first symptom of foot and root rot disease on betelvine. The first disease symptoms were observed within 6 to 16 DAI among different isolates of *S. rolfsii*, where the minimum days were required by Isolate - 9 and the maximum by isolate-2 and 14 (Table 4.3.1).

4.3.4. Lesion length

Lesion length ranged 1.25 - 6.50 cm due to inoculation with different isolates of *S. rolfsii* on the base of betelvine plants. The highest length (6.50 cm) was observed while inoculated with isolate-9 and Isolate - 13. The lowest length (1.25 cm) was recorded while the plants were inoculated with isolate-2 followed by isolate - 14, 19 and 5 (Table 4.3.1).

4.3.5. Severity of foot and root rot in inoculated plants

In severely infected plants, soft watery rotting symptoms and brown lesions advanced above the soil level appeared at the collar region. On the lesions, white mycelial growth having white and brown sclerotia depending on the maturity was observed (Plate 4.3.1 and 4.3.2).

4.3.6. Disease incidence

The disease did not appear in uninoculated control plants at all stages of data collection. disease incidence was 100.0% recorded from plants inoculated with isolate-8 and 9 at 15 DAI; with isolate-8, 9 and 14 at 20 DAI; with 3, 4, 7, 8, 9, 12, 13, 15, and 16 at 25 DAI; and Isolate-3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16 and 18 at 30 DAI (Table 4.3.2).

The highest disease incidence of 91.7% was observed when the plants were inoculated with the isolate - 9 followed by isolate-15 (83.3%) and Isolate - 13 (83.3%) at 10 days after inoculation. No disease incidence was observed when plants were inoculated with isolate-1, 2, Isolate - 4, Isolate - 14, Isolate - 17 and Isolate - 19 and untreated control pots after 10 days of inoculation (Table 4.3.2).

At 15 days after inoculation with isolates of *S. rolfsii*, the highest disease incidence of 100% was observed when the plants were inoculated with the Isolate-8, 9 and 15 and no disease incidence was observed when plants were inoculated with the Isolate-2 and Isolate-14 and Control (No isolates) pots (Table 4.3.2).

At 20 days after inoculation, the highest disease incidence of 100% was observed when plants were inoculated with isolate-8, 9, 11, 12 and 15 and the lowest disease incidence of 33.33% was observed when plants were inoculated with the isolate-2 and 14. No disease incidence was noticed in uninoculated control (Table 4.3.2).

At 25 days after inoculation, 100% disease incidence was recorded when plants were inoculated with isolate-3, 4, Isolate - 7, 8, 9, 11, 12, 13, 15 and 16, while the disease incidence was 58.33% in case of isolate-10 and 14. Disease incidence was absent under control (Table 4.3.2).

After 30 days of inoculation with *S. rolfsii*, the disease incidence in isolate-1, 2, 14, 17 and 19 was 83.3, 83.3, 75.00, 91.67 and 66.66%, respectively. The maximum 100.0% disease incidence was recorded from plants inoculated with isolate - 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16 and 18 (Table 4.3.2).

Results of the pathogenicity test revealed that among 19 isolates, 14 caused 100.0% disease incidence in inoculated plants. All the isolates are found to be pathogenic but in some cases, disease delayed due to their degree of pathogenicity.

Table 4.3.1. Pathogenicity test of various isolates of *Sclerotium rolfsii* on betelvine plant in pot culture

Isolates	Days to appear mycelium on soil	Days to appear disease symptom	Lesion length at 20 DAI*
Isolate - 1 (BGPBSr - 1)	3	12	2.00 fgh[α]
Isolate - 2 (BGPBSr - 2)	3	16	1.25 h
Isolate - 3 (BGPBSr - 3)	2	10	3.25 de
Isolate - 4 (BGPBSr - 4)	3	12	2.25 efgh
Isolate - 5 (BGPBSr - 5)	2	10	1.75 gh
Isolate - 6 (BGPBSr - 6)	3	6	5.25 bc
Isolate - 7 (BGPBSr - 7)	2	8	4.50 c
Isolate - 8 (JKPBSr - 1)	3	7	5.25 bc
Isolate - 9 (JKPBSr - 2)	2	6	6.50 a
Isolate - 10 (JKPBSr - 3)	3	8	3.00 ef
Isolate - 11 (KMPBSr - 1)	3	8	4.25 cd
Isolate - 12 (KMPBSr - 2)	2	7	6.00 ab
Isolate - 13 (KMPBSr - 3)	3	7	6.50 a
Isolate - 14 (RMPBSr - 1)	4	16	1.50 gh
Isolate - 15 (RMPBSr - 2)	3	7	4.50 c
Isolate - 16 (RMPBSr - 3)	4	7	5.00 bc
Isolate - 17 (CSPBSr - 1)	3	12	2.50 efg
Isolate - 18 (CSPBSr - 2)	3	8	3.25 de
Isolate - 19 (CSPBSr - 3)	4	14	1.50 gh
Control (No isolates)	-	-	-

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

*DAI = Days after inoculation

Table 4.3.2. Incidence of foot and root rot due to inoculation with various isolates of *Sclerotium rolfsii* at 10-30 days after inoculation in pathogenicity test

Isolates	Disease incidence at different days after inoculation (DAI)				
	10	15	20	25	30
Isolate - 1 (BGPBSr - 1)	0.00 g[α] (0.083)	41.66 fg (38.71)	41.7 cd (38.71)	66.66 de (52.79)	83.33 bc (69.81)
Isolate - 2 (BGPBSr - 2)	0.00 g (0.083)	0.00 h (0.083)	33.3 d (34.02)	66.66 de (52.79)	83.33 bc (69.81)
Isolate - 3 (BGPBSr - 3)	41.70 ef (38.71)	83.33 bc (69.81)	91.7 a (78.32)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 4 (BGPBSr - 4)	0.00 g (1.59)	58.33 ef (48.10)	75.0 b (61.31)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 5 (BGPBSr - 5)	33.3 f (34.02)	33.33 g (34.02)	41.7 cd (38.71)	83.33 bc (69.81)	100.0 a (86.82)
Isolate - 6 (BGPBSr - 6)	50.00 def (43.41)	66.66 de (52.79)	66.7 bc (52.79)	75.00 cd (61.31)	100.0 a (86.82)
Isolate - 7 (BGPBSr - 7)	66.70 cde (52.79)	75.0 cd (61.31)	91.7 a (78.32)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 8 (JKPBSr - 1)	75.0 abc (65.12)	100.0 a (86.82)	100.0 a (86.8)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 9 (JKPBSr - 2)	91.7 ab (78.32)	100.0 a (86.82)	100..0 a (86.82)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 10 (JKPBSr - 3)	33.30 f (34.02)	66.66 de (52.79)	75.0 b (61.31)	58.33 ab (78.32)	100.0 a (86.82)
Isolate - 11 (KMPBSr - 1)	66.7 cde (52.79)	91.67 ab (78.32)	100.0 a (86.82)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 12 (KMPBSr - 2)	66.7 bcd (56.61)	83.33 bc (69.81)	100.0 a (86.82)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 13 (KMPBSr - 3)	83.3 ab (69.81)	91.67 ab (78.32)	91.7 a (78.32)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 14 (RMPBSr - 1)	0.00 g (0.083)	0.00 h (0.083)	33.3 d (34.02)	58.3 e (48.10)	75.0 cd (61.31)
Isolate - 15 (RMPBSr - 2)	83.30 a (69.81)	100.00 a (86.82)	100.0 a (86.82)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 16 (RMPBSr - 3)	66.7 cde (52.79)	66.66 de (52.79)	91.7a (78.32)	100.0 a (86.82)	100.0 a (86.82)
Isolate - 17 (CSPBSr - 1)	0.00 g (0.083)	33.33 g (34.02)	66.7 bc (52.79)	83.33 bc (69.81)	91.67 ab (78.32)
Isolate - 18 (CSPBSr - 2)	33.3 f (34.02)	33.33 g (34.02)	50.0 cd (43.41)	66.66 de (52.79)	100.0 a (86.82)
Isolate - 19 (CSPBSr - 3)	0.00 g (0.083))	33.33 g (34.02)	50.0 cd (43.41)	66.66 de (52.79)	66.66 d (52.79)
Control (No isolates)	0.00 g (0.083)	0.00 h (0.083)	0.0 e (0.083)	0.00 f (0.083)	0.00 e (0.083)

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

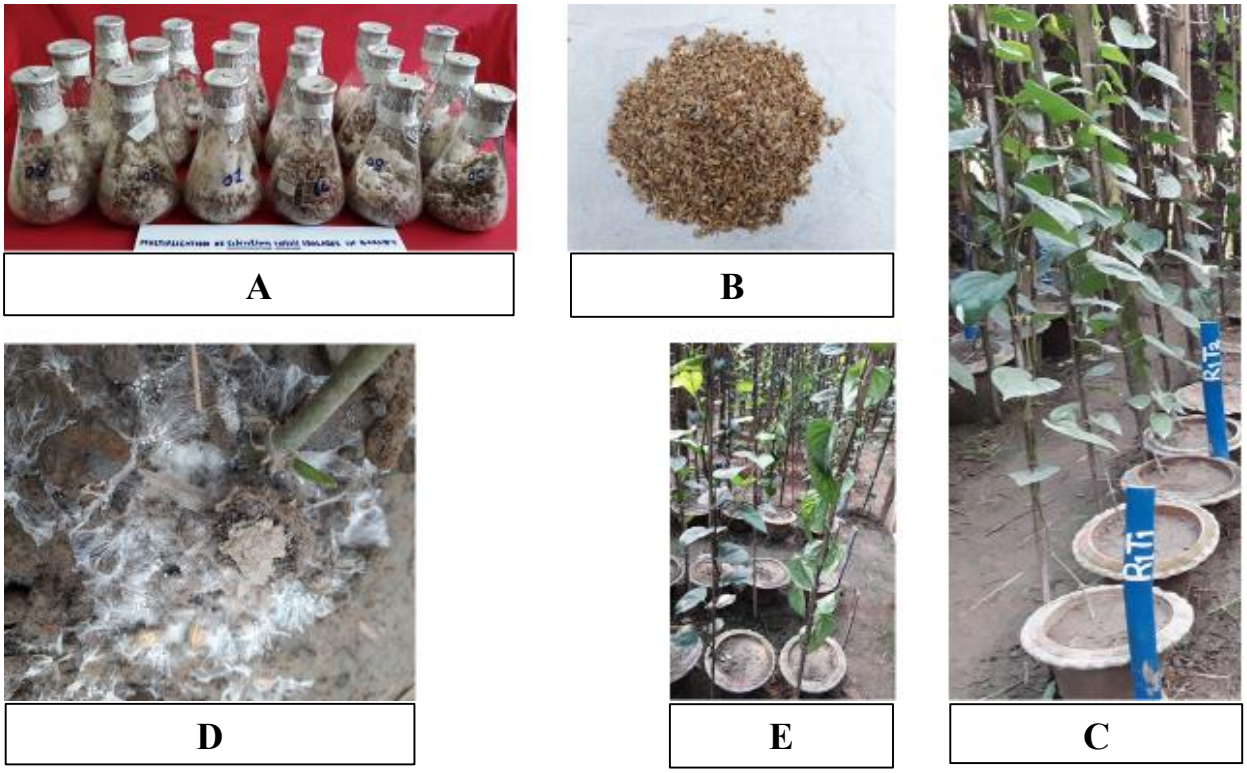


Plate 4.3.1. Barley grains colonized barley with *Sclerotium rolfsii* in conical flasks (A), air dried colonized barly grains used as inoculum (B) for inoculation of pot soil (C) and mycelium of *Sclerotium rolfsii* appeared on the soil surface (D)

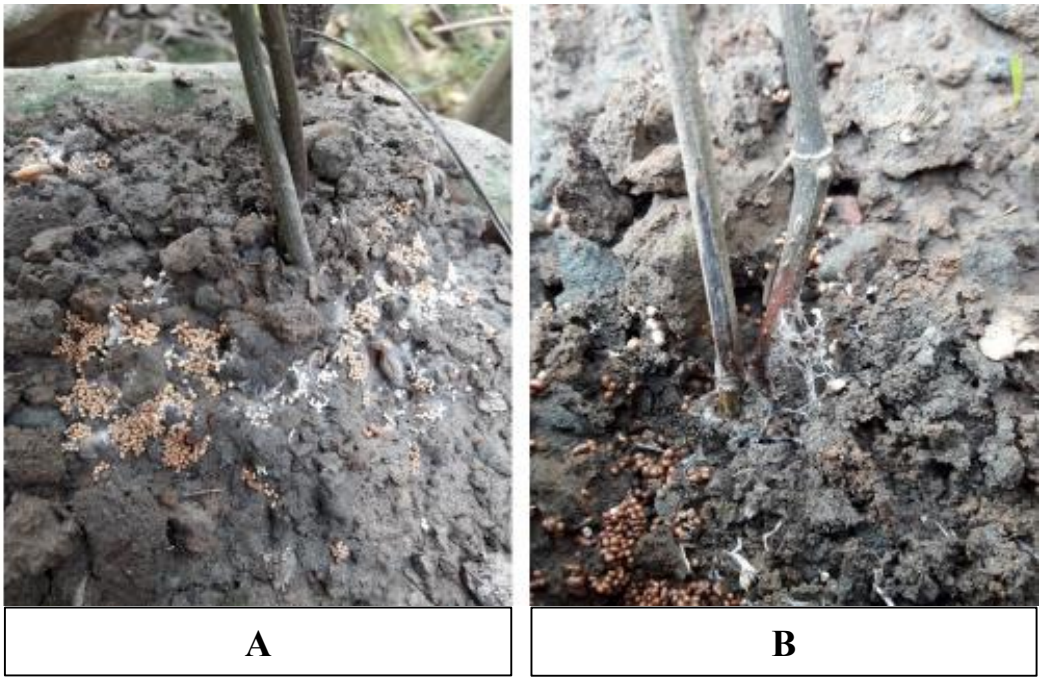


Plate 4.3.2. Sclerotia *S. rolfsii* developed on the soil surface around the stem base of betelvine plant (A and B)

4.4. Experiment 4. Screening of betelvine cultivars available in Bangladesh against *Sclerotium rolfsii* causing foot and root rot disease of betelvine

4.4.1. Vegetative growth parameters of betelvine cultivars

The vegetative growth parameters and morphological features of different germplasm of betelvine varied remarkably. The vine elongation in different cultivars of betelvine ranged 37.46 -50.34 cm/month. Significantly the maximum vine increment per month was recorded in PB 005 followed by PB 006, PB 003 cm and PB 012. The lowest vine increment was recorded in PB 008. Increase in internode per month ranged 6.75-10.08 cm in different cultivars. The highest length of internode was recorded from PB 004 that was statistically similar to PB 006 and the minimum length was found in PB 007 and PB 013. The vine girth of different betelvine cultivars varied 0.445-0.7475 cm. Significantly the maximum vine girth was recorded in PB 005 and the minimum vine girth was found in PB 012 followed by PB 002 and PB 013. Leaf length of various cultivars ranged 17.13-27.35 cm. The highest length of leaf was recorded from PB 012, which was statistically similar to PB 003, 004, 009 and 010. The lowest leaf length was recorded in PB 013. The leaf breadth in different cultural was 8.33 to 16.20 cm. The highest leaf breadth was recorded from PB 004 and the lowest from PB 007. The length of petiole was the highest in PB 010 which was statistically similar to PB 007 and PB 009. The lowest length of petiole was recorded in PB 013. The maximum petiole breadth was found in PB 004 and the lowest in PB 007 (Table 4.4.1).

4.4.2. Yield and yield contributing characters of different betelvine cultivars

The 100 petiole weight was the highest in PB 010 followed by PB 012 and the lowest weight in PB 013. The maximum fresh weight of 100 leaf with petiole was recorded from PB 009 followed by PB 008 and the lowest from PB 007. The highest dry matter content was recorded from cultivar PB 007 followed by PB 002, PB 011, and PB 001 and the lowest from PB 010 followed by PB 012. The PB 013 produced significantly the highest number of leaves per meter vine followed by PB 007 and the lowest number of leaves per meter vine was recorded from PB 004. Leaf number per plant per year was found the highest in PB 005 and the lowest from PB 004. The highest yield per plant annually was from in PB 009 followed by PB 006 and the lowest yield per plant per year was from in PB 002 followed by PB 007. The highest annual yield per hectare was from in PB 009 followed by PB 006 and the lowest annual yield was from in PB 002 followed by PB 007 (Table 4.4.2).

4.4.3. Morphological features of the betelvine cultivars

Morphological characteristics of 13 betelvine cutlivars screened in the present experiment have described below and presented in Table 4.4.3 and Plate 4.4.1 (A-M).

PB 001 (Laldingi pan)

The colour of PB 001 (Laldingi pan) vine was found green. The leaf was green coloured, less soft after maturation, cordate in shape, acute in tip and medium pungent (Plate 4.4.1 A).

PB 002 (BARI Line)

The leaf was found soft after maturation, dark green coloured, cordate in shape, acute in tip and less pungent. The vine colour of PB 002 was recorded violet (Plate 4.4.1 B).

PB 003 (Chalitaguti pan)

The vine of PB 003 (Chalitaguti pan) was recorded greenish with light pinkish line in colour. The leaf was recorded soft after maturation, green coloured, cordate in shape, acute in tip and less pungent (Plate 4.4.1 C).

PB 004 (Sanchi pan)

The vine of PB 004 (Sanchi pan) was violet in colour. The leaf was soft after maturation, dark green coloured, cordate to ovate in shape, acute in tip and pungent aroma (Plate 4.4.1 D).

PB 005 (BARI Line)

The vine colour of PB 005 was greenish with light pinkish line. The leaf was soft after maturation, dark green coloured, cordate in shape, acute in tip and highly pungent (Plate 4.4.1 E).

PB 006 (Misti pan)

The leaf was found soft after maturation, green coloured, cordate in shape, acute in tip and no pungent. Vine colour was recorded dark green in PB 006 (Misti pan) (Plate 4.4.1 F).

PB 007 (BARI Line)

Vine colour was violet in PB 007. The leaf was found soft after maturation, dark green coloured, cordate in shape, acute in tip and medium pungent (Plate 4.4.1 G).

PB 008 (BARI Line)

The leaf was found soft after maturation, dark green coloured, cordate in shape, acute in tip and medium pungent and vine colour was violet in PB 008 (Plate 4.4.1 H).

PB 009 (BARI Pan-1)

Green vine colour was found in PB 009 (BARI Pan-1). The leaf was soft after maturation, light green coloured, cordate to ovate in shape, acuminate in tip and medium pungent (Plate 4.4.1 I).

PB 010 (Bangla pan)

The vine colour was found green in cultivar PB 010 (Bangla pan). The leaf was soft, dark green coloured, cordate in shape and medium pungent (Plate 4.4.1 J).

PB 011 (Jhal pan)

The vine colour of PB 011 (Jhal pan) was found dark green. The leaf of the cultivar was soft after maturation, light green coloured, cordate in shape, acute in tip and highly pungent (Plate 4.4.1 K).

PB 012 (Bhabna pan)

The leaf of PB 012 (Bhabna pan) was soft after maturation, dark green coloured, cordate in shape, acute in tip and less pungent. The vine was greenish with pinkish line colour (Plate 4.4.1 L).

PB 013 (Gayasur pan)

The vine of PB 013 (Gayasur pan) was found green. The leaf was soft after maturation, light green coloured, cordate in shape, acute in tip and less pungent (Plate 4.4.1 M).

Table 4.4.1. Variations in **v**egetative growth parameters of different betelvine cultivars screened in the present experiment

Accession no. of betelvine cultivars	Vine elongation /month(cm)	Length of internode (cm)	Girth of vine (cm)	Leaf size with petiole		Petiole size (cm)	
				Length	Breadth	Length	Breadth
PB 001 (Laldingi pan)	38.77 de[α]	8.43 bc	0.5775 d	22.81 ef	12.95 b-d	7.45 c-e	0.35 b-e
PB 002 (BARI Line)	37.86 e	7.15 de	0.4538 fg	19.60 g	9.33 ef	7.95 c-e	0.30 ef
PB 003 (Chalitaguti pan)	43.17 bc	7.50 de	0.5325 de	25.65 a-d	14.35 b	8.70 b-d	0.37 b-d
PB 004 (Sanchi pan)	41.91 c	10.08 a	0.6500 bc	26.23 a-c	16.20 a	6.98 de	0.47 a
PB 005 (BARI Line)	50.34 a	7.73 cd	0.7475 a	22.60 ef	12.93 b-d	8.75 bc	0.39 b
PB 006 (Misti pan)	44.86 b	9.38 a	0.5925 cd	24.80 cd	13.50 b-d	9.98 ab	0.33 c-f
PB 007 (BARI Line)	41.01 cd	6.75 e	0.5150 ef	21.35 fg	8.33 f	10.95 a	0.28 f
PB 008 (BARI Line)	37.46 e	8.33 bc	0.6575 b	24.00 de	12.15 d	10.38 ab	0.38 bc
PB 009 (BARI Pan-1)	37.53 e	8.55 b	0.5400 de	25.63 a-d	12.25 cd	11.23 a	0.33 b-f
PB 010 (Bangla pan)	41.37 c	8.45 bc	0.6775 b	27.03 ab	12.85 b-d	11.45 a	0.37 b-d
PB 011 (Jhal pan)	38.36 e	7.05 de	0.5600 de	25.58 b-d	14.35 b	6.65 e	0.32 d-f
PB 012 (Bhabna pan)	42.42 c	7.33 de	0.4450 g	27.35 a	13.85 bc	10.43 ab	0.39 b
PB 013 (Gayasur pan)	37.47 e	6.75 e	0.4700 fg	17.55 h	10.28 e	4.83 f	0.38 b-d

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

Table 4.4.2. Yield and yield contributing characters of different betelvine cultivars screened in the present experiment

Accession no. of betelvine cultivars	100 Petiole wt. (gm)	Fresh wt. of 100 leaf (g)	Dry matter (%)	Leaf no. /meter vine	Leaf no. /plant/ year	Yield/ plant /year (g)	Yield (t/ha/year)
PB 001(Laldingi pan)	5.12i[α]	515.50b	14.14ab	11.07cde	55.19def	284.54abc	22.76abc
PB 002 (BARI Line)	57.07j	289.38g	13.83abc	12.81b	64.92 c	187.89f	15.03f
PB 003 (Chalitaguti)	103.55f	317.05f	14.26ab	12.41bc	69.39 bc	220.09e	17.61e
PB 004 (Sanchi pan)	122.95d	445.00c	13.06abc	9.46f	50.16 f	223.07e	17.85e
PB 005 (BARI Line)	128.77c	346.20e	13.83abc	12.74b	78.44 a	272.11c	21.77c
PB 006 (Misti pan)	130.82c	515.25b	13.31abc	10.05ef	57.71 de	297.12a	23.77a
PB 007 (BARI Line)	86.54g	264.38h	14.49a	15.38a	73.05 ab	193.19f	15.46f
PB 008 (BARI Line)	130.15c	544.13a	12.80bc	12.05bcd	54.16 def	293.21ab	23.46ab
PB 010 (Bangla pan)	165.74a	416.25d	12.28c	11.22cde	58.98 d	245.51d	19.64d
PB 011 (Jhal pan)	77.02h	408.03d	14.35ab	13.35b	65.89 c	268.51c	21.48c
PB 012 (Bhabna pan)	148.38b	313.25f	12.57c	13.20b	70.70 bc	221.66e	17.73e
PB 013 (Gayasur pan)	46.38k	411.00d	13.27abc	16.35a	66.59 c	273.69bc	21.89bc
PB 009 (BARI Pan -1)	114.90e	565.03a	12.79bc	10.72def	52.70 ef	297.78a	23.82a

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

Table 4.4.3. Physio-morphological characters of betelvine cultivars grown in betelvine orchard (Pan baroj) at SAU campus

Accession no. of cultivars	Vine colour	Leaf colour	Leaf shape	Leaf tip	Leaf softness	Pungency of leaf
PB 001 (Laldingi pan)	Green	green	Cordate	Acute	less soft	Medium pungent
PB 002 (BARI Line)	Violet	Dark green	Cordate	Acute	Leaf soft	Less pungent
PB 003 (Chalitaguti pan)	Greenish with light pinkish line	Green	Cordate	Acute	Leaf soft	Less pungent
PB 004 (Sanchi pan)	Violet	Dark green	Cordate to ovate	Acute	Leaf soft	Pungent aroma
PB 005 (BARI Line)	Greenish with light pinkish line	Dark green	Cordate	Acute	Leaf soft	Highly pungent
PB 006 (Misti pan)	Dark green	Green	Cordate	Acute	Leaf soft	No pungent
PB 007 (BARI Line)	Violet	Dark green	Cordate	Acute	Leaf soft	Medium pungent
PB 008 (BARI Line)	Violet	Dark green	Cordate	Acute	Leaf soft	Medium pungent
PB 010 (Bangla pan)	Green	Dark green	Cordate	Acute	Leaf soft	Medium pungent
PB 011 (Jhal pan)	Dark green	Light green	Cordate	Acute	Leaf soft	Highly pungent
PB 012 (Bhabna pan)	Greenish with pinkish line	Dark green	Cordate	Acute	Leaf soft	Less pungent
PB 013 (Gayasur pan)	Green	Light green	Cordate	Acute	Leaf soft	Less pungent
PB 009 (BARI Pan -1)	Green	Light green	Cordate to ovate	Acuminate	Leaf soft	Medium pungent

PB 001 (Laldingi Pan)

PB 002 (BARI Line)

PB 003 (Chalitaguti Pan)

PB 004 (Sanchi Pan)

PB 005 (BARI Line)

PB 006 (Misti Pan)

PB 007 (BARI Line)

PB 008 (BARI Line)

PB 009 (BARI Pan - 1)

Plate 4.4.1. Morphological variations of leaves of different betelvine cultivars screened in present experiment

Continued Plate 4.4.1.

PB 010 (Bangla Pan)

PB 011 (Jhal Pan)

PB 012 (Bhabna Pan)

PB 013 (Gayasur Pan)

4.4.4. Days required for appearance of 1st disease symptom

The time interval required for appearance of 1st disease symptoms after inoculation among the betelvine cultivars differed considerably compared to control. The lowest incubation period (8 days) required for the cultivars PB 005 (BARI Line), PB 006 (Misti pan), PB 009 (BARI Pan - 1) and PB 010 (Bangla pan). The highest incubation period (22 days) was required for the cultivars PB 001 (Laldingi pan), PB 011 (Jhal pan) and PB 013 (Gayasur pan) (Table 4.4.4).

4.4.5. Disease incidence

Among the 13 betelvine cultivars, no disease incidence was observed in case of cultivars PB 001, PB 002, PB 003, PB 007, PB 008, PB 011 and PB 012 whereas PB 009 showed maximum disease incidence against the foot and root rot disease at 10 days after inoculation of pathogen (Table 4.4.4).

At 15 days after inoculation of pathogen, the 100% incidence was observed in cultivars PB 009 (BARI Pan-1) and no disease incidence (0%) was observed in PB 001, PB 011 and PB 013 (Table 4.4.4).

At 20 days after inoculation, the highest disease incidence was observed in PB 005, PB 006, PB 009 and PB 010, followed by PB 007 and the lowest disease incidence was recorded in PB 001 and PB 013 followed by PB 011 (Table 4.4.4).

At 25 days after inoculation, the betelvine cultivars showed more or less similar reactions as were in 20 days after inoculation among themselves against the disease. The minimum disease incidence was recorded in case of PB 001 (Laldingi) (8.33%) followed by PB 008, PB 011 and PB 013. The maximum disease incidence (100%) was recorded from PB 005, PB 006, PB 007, PB 009 and PB 010 (Table 4.4.4).

At 30 DAI, the disease incidence of 13 different betelvine cultivars showed remarkable difference among them. The lowest disease incidence was recorded in case of PB 001 (Laldingi). The highest disease incidence was recorded from PB 002, PB 003, PB 004, PB 005, PB 006, PB 007, PB 008, PB 009, PB 010 and PB 012 followed by PB 011 and PB 013 (Table 4.4.4).

4.4.6. Grading of cultivars

Based on responses (disease incidence) of the betelvine cultivars, they are categorized as Resistant (R), Moderately susceptible (MS) and Susceptible (S). Among the cultivars, only one cultivar PB 001 (Laldingi pan) showed resistant reaction (R), while cultivars PB 011 (Jhal pan) and PB 013 (Goyasur pan) showed moderately susceptible reactions (MS) and rest of the cultivars showed susceptible reactions (S) (Table 4.4.4).

Table 4.4.4. Screening of different betelvine cultivars for resistance against foot and root rot disease caused by *Sclerotium rolfsii*

Betelvine cultivars	Days to appear symptom	Percent disease incidence at different days after inoculation (DAI)					Disease reaction
		10	15	20	25	30	
PB 001 (Laldingi pan)	22	0.00c[α] (0.083)	0.00f (0.083)	0.00d (0.083)	8.33d (8.56)	8.33d (8.56)	R
PB 002 (BARI Line)	12	0.00c (0.083)	33.33e (34.017)	33.33c (34.017)	66.66b (52.79)	83.33ab (69.81)	S
PB 003 (Chalitaguti pan)	11	0.00c (0.083)	66.66cd (52.799)	66.66b (52.799)	66.66b (52.80)	83.33ab (69.81)	S
PB 004 (Sanchi pan)	9	41.66b (38.71)	58.33de (48.104)	66.66b (52.799)	66.66b (52.80)	91.66a (78.32)	S
PB 005 (BARI Line)	8	41.66b (38.71)	75.00bc (65.115)	100.00a (86.822)	100.00a (86.82)	100.00a (86.82)	S
PB 006 (Misti pan)	8	58.33a (48.10)	91.67ab (78.316)	100.00a (86.822)	100.00a (86.82)	100.00a (86.82)	S
PB 007 (BARI Line)	11	0.00c (0.083)	66.66cd (52.799)	91.67a (78.316)	100.00a (86.82)	100.00a (86.82)	S
PB 008 (BARI Line)	11	0.00c (0.083)	33.33e (34.017)	33.33c (34.017)	33.33c (34.02)	66.66bc (52.80)	S
PB 009 (BARI Pan -1) (Control)	8	66.66a (52.79)	100.00a (86.822)	100.00a (86.822)	100.00a (86.82)	100.00a (86.82)	S
PB 010 (Bangla pan)	8	33.33b (34.02)	83.33b (69.811)	100.00a (86.822)	100.00a (86.82)	100.00a (86.82)	S
PB 011 (Jhal pan)	22	0.00c (0.083)	0.00f (0.083)	8.33d (8.56)	33.33c (34.02)	58.33c (48.10)	MS
PB 012 (Bhabna pan)	9	0.00c (0.083)	49.99de (43.408)	66.66b (52.799)	75.00b (61.31)	91.66a (78.32)	S
PB 013 (Gayasur pan)	22	0.00c (0.083)	0.00f (0.083)	0.00d (0.083)	25.00c (25.91)	49.99c (43.41)	MS

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

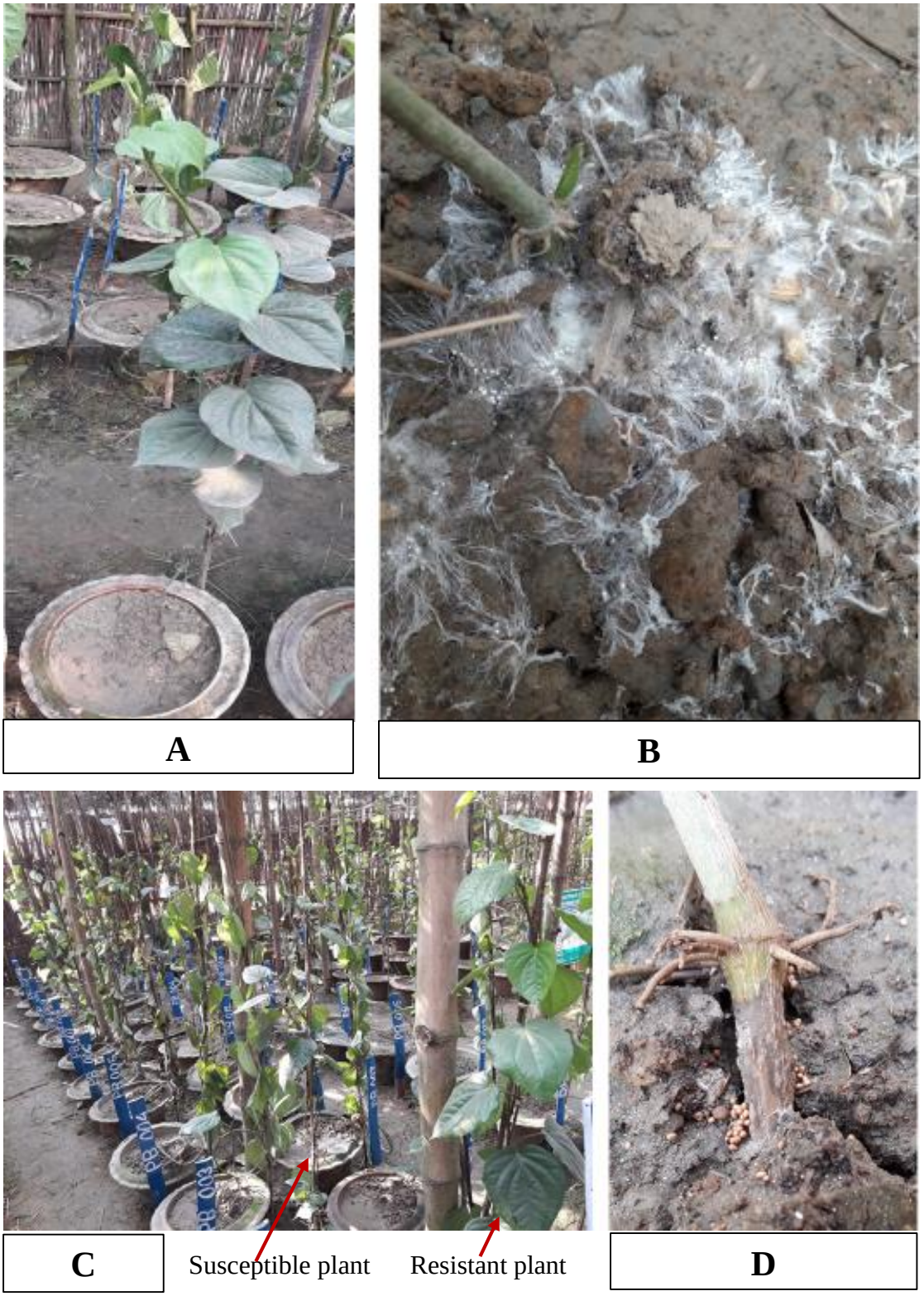


Plate 4.4.2. *Sclerotium rolfsii* inoculated plant (A), mycelia formation on inoculated earthen pot (B), susceptible and resistant plant against *Sclerotium rolfsii* (C) and lesion on foot and root of betelvine plant (D)

4.5. Experiment 5. Bioassay of botanicals, fungicides and bioagents as IPM components against *Sclerotium rolfsii* causing foot and root rot of betelvine under *in-vitro* condition

4.5.1. Effeffect of botanicals on *in-vitro* mycelial growth of *Sclerotium rolfsii*

The effect of plant extracts on *in-vitro* mycelial growth of *S. rolfsii* varied considerably with plant extract and days after inoculation (DAI). The inhibition of colony growth over control was observed in Petri dishes containing PDA amended with botanical.

At 1, 2 and 3 DAI, *in-vitro* radial myucelium growth of *S. rolfsii* was 22.75, 49.75 and 74.25 mm under control, which were reduced significantly due to amendment of PDA with all botanicals. The pathogen failed to grow on PDA amended with garlic extract and the lowest colony diameter was recorded from Allamanda leaf extract amendment (Table 4.5.1).

At 1 DAI, the *inv-itro* colony growth of *S. rolfsii* ranged 0.00-21.50 mm. The colony diameter under the leaf extracts of Neem, Bishkatali and Korobi, zinger rhizome, onion bulb ranged 16.25-16.50 mm, which were statistically similar and significantly lower compared to othe treatments except garlic and Allamanda. The second highest diameter was observed under Lemon grass, which was statistically similar to Tobacco and Bermutha grass (Table 4.5.1).

At 2 DAI, the *in-vitro* radial colony diameter of *S. rolfsii* under different botanicals ranged 0.00-47.50 mm. Radial colony diameter under Bishkatali, Allamanda and Zinger was statistically similar and significantly lower compared to all other treatments except Garlic clove under which the fungal coloy failed to grow. Significantly the highest colony diameter was recorded from PDA amended with Bermuda grass followed by Tobacco leaf (Table 4.5.1).

At 3 DAI, the colony diameter was 74.25 mm under control, which was significantly inhibited by different botanical extracts. The colony diameter was 0.00 mm under Garlic clove and the maximum of 66.75 mm colony diameter was recorded from Tobacco. The lowest colony diameter of 31.75 was found under Allamanda followed by Bishkatali, Zinger and Korobi leaf. Their differences were significant. The second

highest radial colony diameter was recroded from PDA ameded with Tobacco followed by Bermutha grass and Mehagony seed (Table 4.5.1).

At 4 DAI, the radial diameter of intro colony growth under different botanicals and control ranged 3.00-89.50 mm. The maximum colony growth was found under control, which was statistically similar to Mehagony seed, Bermutha grass and Tobacco but other botanicals significantly reduced the colony growth over control. Significantly the highest growth inhibition was obtained with Garlic cloves followed by Allamanda, Bishkatali, Bermuda grass and Zinger (Table 4.5.1).

Among 11 botanicals tested Allamanda and Garlic were recorded as highly effective materials to inhibit mycelium growth of *Sclerotium rolfsii*. Colony growth increased gradually with the progress of incubation under every botanical (Table 4.5.1).

4.5.2. Effect of fungicides on *in-vitro* colony growth of *S. rolfsii*

Among 10 fungicides some showed suppressive effect on reduction of radial mycelial growth of *S. rolfsii*. At 1, 2 and 3 DAI, the colony diameter under control were 24.25, 54.75 and 81.25 mm, respectively. All treatments with the fungicides reduced *in-vitro* radial growth to 0.00 - 21.25, 0.00 - 49.25 and 0.00 - 78.75 mm at 1, 2 and 3 DAI, respectively and the reduction was significant compared to control. The *in-vitro* redial mycelium growth was 0.00, at all stages of data collection due to use of Provax-200. After Provax-200, fungicides Score 250 EC, Tilt 250 EC, Rovral 50 WP and Pencozeb 80 WP reduced *in-vitro* mycelium colony diameter 10.25 - 11.50, 12.75 - 22.50, 17.50 - 49.50 mm at 1, 2, 3 and DAI. At 4 DAI, (final stage of data collection) maximum reduction in *in-vitro* mycelial colony growth was achieved with Provax-200, Score 250 EC, Tilt 250 EC, Rovral 50 WP and Pencozeb 80 WP giving respectively 100.00, 78.15, 75.64, 59.10 and 44.53% inhibition colony growth. These five fungicides were noted as highly effective components of IPM.

4.5.3. *In vitro* evaluation of bio-agents against *S. rolfsii* in dual culture Technique

The *in-vitro* radial colony diameter of *Sclerotium rolfsii* in dual plate technique was 23.75, 62.00, 81.00 and 90.00 mm at 1, 2, 3 and 4 DAI, respectively. Bio-agent, *Trichoderma harzianum* reduced the colony diameter to 12.50, 20.75, 31.00 and 39.25 mm and *Pseudomonus fluorescens* to 19.00, 32.25, 45.25 and 90.00 mm at 1, 2, 3 and

4 DAI. The reduction was significant compared to control. The effectiveness of *T. harzianum* to reduce *in-vitro* colony growth was higher compared to *P. fluorescens* (Table 4.5.3).

Table 4.5.1. Efficacy of plant extracts in inhibition of in-vitro mycelial growth of *Sclerotium rolfsii*

Botanical treatments components	**Radial colony diameter (mm) at different days after inoculation (DAI)**				**% Inhibition of mycelial growth over control**
	1	**2**	**3**	**4**	
Neem leaves extract	16.50 d[α] (4.12)*	36.25 c (6.06)	53.00 de (7.31)	66.50 d (8.19)	25.69 c[£] (29.37)
Biscatali leaves extract	16.25 d (4.09)	28.25 e (5.36)	36.50 g (6.08)	50.00 e (7.11)	44.14 b (40.16)
Allamanda leaves extract	14.75 e (3.90)	26.00 e (5.15)	31.75 h (5.68)	43.75 e (6.65)	51.12 b (44.03)
Garlic clove extract	0.00 f (0.71)	0.00 f (0.71)	0.00 i (0.71)	3.00 f (1.41)	96.67 a (81.66)
Zinger rhizome extract	16.75 d (4.15)	27.25 e (5.27)	39.50 f (6.32)	50.75 e (7.16)	43.30 b (39.69)
Lemon grass leaves extract	21.50 b (4.69)	37.25 c (6.14)	52.00 de (7.25)	74.25 bcd (8.65)	17.04 d (23.51)
Onion bulb extract	16.25 d (4.09)	35.50 c (6.00)	54.50 d (7.42)	70.50 cd (8.43)	21.22 cd (26.46)
Korobi leaves extract	16.25 d (4.09)	31.00 d (5.60)	51.00 e (7.18)	72.25 bcd (8.53)	19.27 cd (25.11)
Tobacco leaves extract	20.50 b (4.58)	43.50 b (6.63)	66.75 b (8.20)	81.25 ab (9.04)	9.22 e (17.02)
Bermuda grass leaves extract	21.25 b (4.66)	47.50 a (6.93)	62.75 c (7.95)	82.50 ab (9.11)	7.82 e (15.65)
Mehagony seed extract	18.75 c (4.38)	31.25 d (5.63)	54.50 d (7.41)	79.50 abc (8.94)	11.17 e (18.83)
(Control)	22.75 a (4.82)	49.75 a (7.09)	74.25 a (8.64)	89.50 a (9.49)	00.00 f (0.062)

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

*Data given in parenthesis are square root or arc-sine transformed values

[£]% growth inhibition of pathogen over control, $I = \frac{C-T}{C}$ x100 (Vincent, 1947)

Where C = Colony diameter in control (T_{10}) and T= Colony diameter in treatment ($T_{n,\ n=1-9}$)

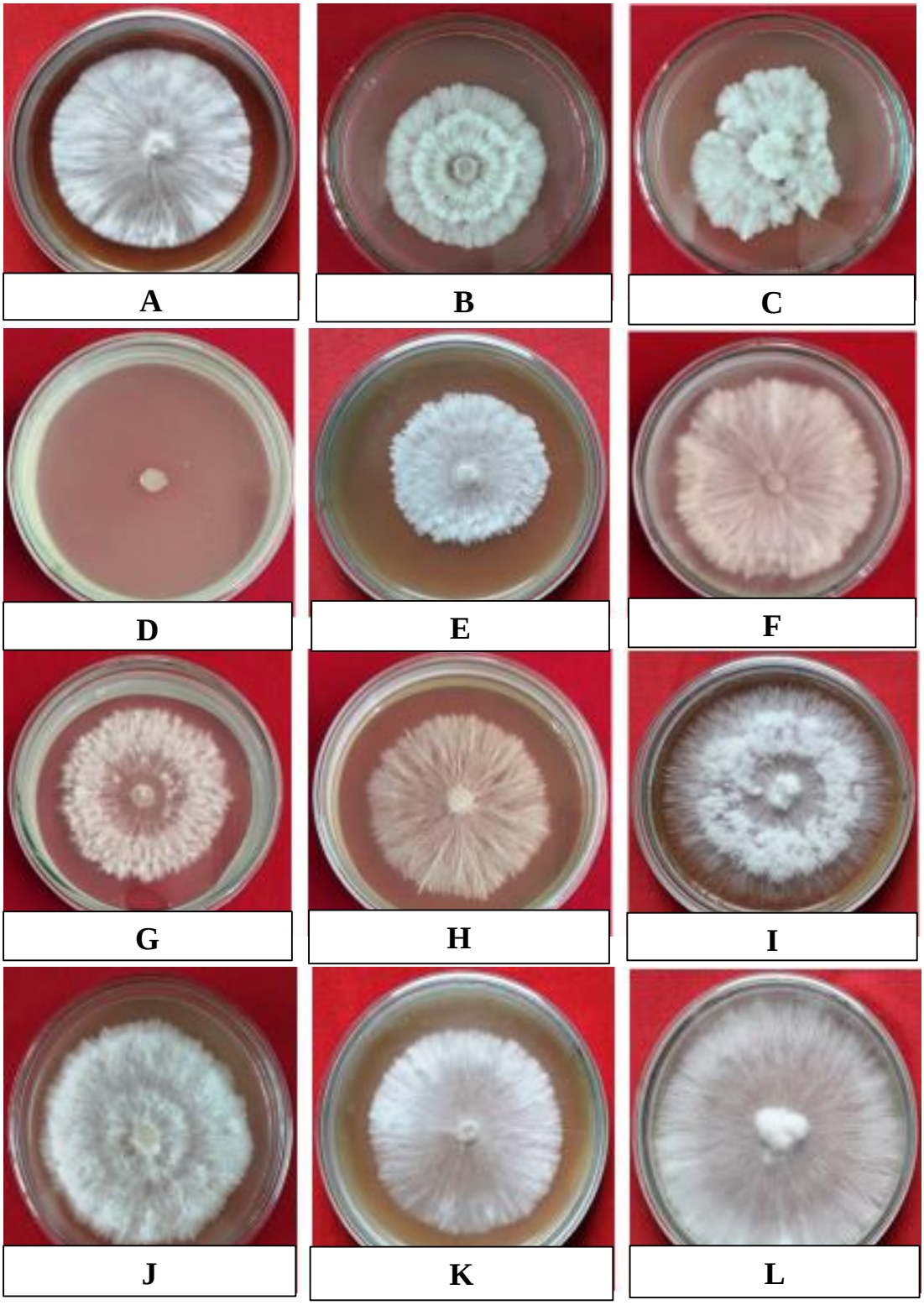


Plate 4.5.1 Radial mycelial growth of *Sclerotium rolfsii* in Petri dishes containing PDA amended with botanicals [(A) Neem leaves extract, (B) Biscatali leaves extract, (C) Allamanda leaves extract, (D) Garlic clove extract, (E) Zinger rhizome extract, (F) Lemon grass leaves extract, (G) Onion bulb extract, (H) Korobi leaves extract, (I) Tobacco leaves extract, (J) Bermuda grass leaves extract, (K) Mehagony seed extract and (L) Control at 4 DAI of pathogen]

Table 4.5.2. Efficacy of fungicides to inhibit *in-vitro* mycelial growth of *Sclerotium rolfsii* on PDA Growth Inhibition Technique (Cup method)

Fungicidal components of IPM	**Radial colony growth (mm) of *S. rolfsii* at different days after inoculation (DAI)**				**% Inhibition of mycelial growth over control**
	1	**2**	**3**	**4**	
Tilt-250 EC	10.50 ef[α] (3.31)*	14.75 g (3.90)	18.25 h (4.32)	21.75 f (4.72)	75.64 b (58.29)
Score 250 EC	10.25 f (3.28)	12.75 h (3.64)	17.50 h (4.24)	19.50 g (4.47)	78.15 b (59.95)
Rovral 50 WP	11.50 e (3.46)	16.75 f (4.15)	26.25 g (5.17)	36.50 e (6.08)	59.10 c (48.47)
Bavistin 50 WP	19.50 c (4.47)	45.50 c (6.78)	57.00 e (7.58)	65.00 c (8.09)	27.16 e (30.29)
Provax 200	0.000 g (0.71)	0.00 i (0.71)	0.000 i (0.71)	0.00 h 0(.71)	100.00 a (86.82)
Topgan	19.00 c (4.42)	40.00 d (6.36)	63.00 d (7.97)	79.00 b (8.92)	11.48 f (19.08)
Ridomil Gold MZ-68 WP	21.25 b (4.66)	48.25 b (6.9)	72.25 c (8.53)	87.75 a (9.40)	1.67 g (6.48)
Pencozeb 80 WP	11.00 ef (3.39)	22.50 e (4.79)	36.00 f (6.04)	49.50 d (7.07)	44.53 d (40.38)
Cuprafix 30 D	16.25 d (4.09)	40.75 d (6.42)	63.00 d (7.97)	79.25 b (8.93)	11.19 f (18.80)
Bordeaux mixture	17.50 d (4.24)	49.25 b (7.05)	78.75 b (8.90)	87.75 a (9.39)	1.69 g (5.84)
(Control)	24.75 a (5.02)	54.75 a (7.43)	81.25 a (9.04)	89.25 a (9.47)	0.00 h (0.062)

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).

*Data given in parenthesis are retransformed (square root and arc-sine) values

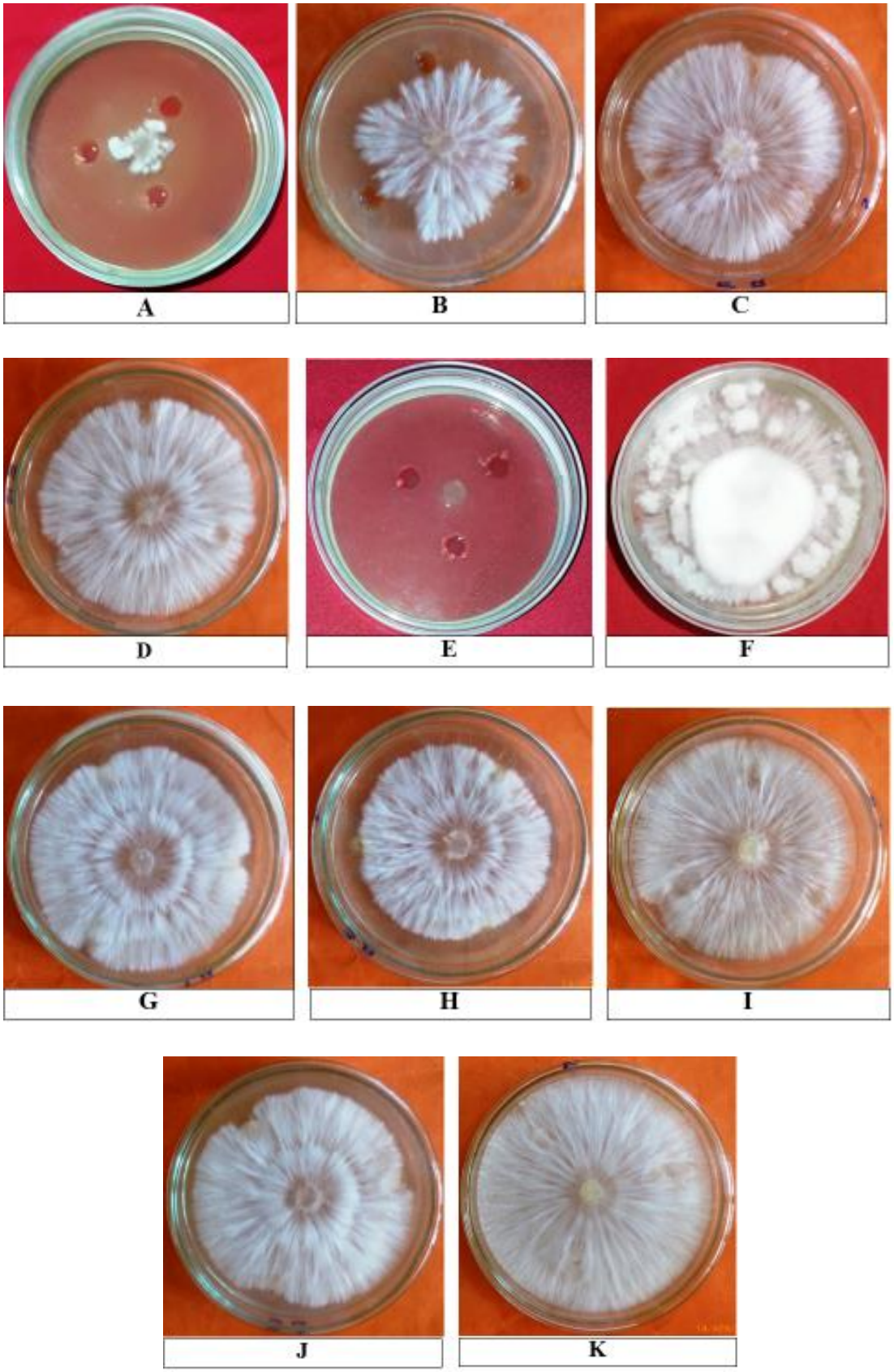


Plate 4.5.2. Radial mycelial growth of *Sclerotium rolfsii* against (A) Tilt-250 EC, (B) Score 250 EC, (C) Rovral 50 WP, (D) Bavistin 50 WP, (E) Provax 200, (F) Topgan, (G) Ridomil Gold MZ- 68 WP, (H) Pencozeb 80 WP, (I) Cuprafix 30 D, (J) Bordeaux mixture and (K) Control after 4 days of inoculation

Table 4.5.3. Efficacy of bio-agents to inhibit *in-vitro* radial mycelial growth of *Sclerotium rolfsii* in dual culture method

Treatments	Radial mycelial growth (mm) at different days after inoculation (DAI)				% Inhibition of mycelial growth over control
	1	2	3	4	
Trichoderma harzianum	12.50 c[α]	20.75 c	31.00 c	39.25 c	56.39 a (46.96)*
Pseudomonas fluorescens	19.00 b	32.25 b	45.25 b	60.50 b	32.78 b (33.69)
Control	23.75 a	62.00 a	81.00 a	90.00 a	00.00 c (0.062)

[α]Values within the same column with a common letter(s) do not differe significantly (P=0.01).
*Data given in parenthesis are retransformed values

Plate 4.5.3. Radial mycelial growth of *S. rolfsii* against (A) *Trichoderma harzianum*, (B) *Pseudomonas fluorescens* and (C) Control at 4 days after inoculation of pathogen

4.6. Experiment 6. Evaluation of botanicals, fungicides, bio-agents and soil amendments against foot and root rot (*Sclerotium rolfsii*) of betelvine *in-vivo* as components of IPM

4.6.1. Days required for appearance of disease symptom

Under inoculated conditions, duration required for first appearance of visible symptoms of foot and root rot of betelvine were 95, 91, 87, 86, 76, 70, 47, 44, 29 and 10 days under treatments with Provax 200 (T_1), Garlic Clove Extract (T_5), *T. harzianum* (T_9), Score 250 EC (T_3), Tilt-250 EC (T_2), Allamunda leaf extract (T_6), Pencozeb 80 WP (T_4), Vermi-compost (T_7), Poultry manure (T_8) and control, respectively. At 30 DAI, visible symptoms of foot and root rot of betelvine did not apperar due to application of fungicides, botanicals, bio-agent and soil amendments under treatments T_1 to T_7 and T_9. At 45 DAI, visible symptoms of the disease did not appear under T_1 to T_6 and T_9.

4.6.2. Disease incidence

The highest disease incidence of 58.33, 66.66, 74.99, 74.99, 83.33, 91.67 and 91.67 was observed under control at 30, 45, 60, 75, 90, 105, 120 DAI, respectively. The disease incidence increased gradually with the progress of duration after inoculation showing the maximum of 91.67% at 105 and 120 DAI. Application of treatments significantly reduced the disease incidence over control at every stage of data collection (Table 4.6.1).

At 30 DAI, visible symptoms of foot and root rot of betelvine did not apperar due to application of fungicides, botanicals, bio-agents and soil amendments under treatments T_1 to T_7 and T_9. Under T_8 the disease incidence was 16.67% (Table 4.6.1).

At 45 DAI, the minimum of 16.67% disease incidence was recorded in case of treatment T_7 followed by T_8. At 60 DAI, the disease incidence was absent under T_1, Tilt-250 EC T_2, T_3, T_5, T_6 and T_9. At 75, no disease incidence was observed in T_1, Tilt-250 EC T_2, T_3, T_5 and T_9. At that stage of data collection, the lowest incidence of 16.16.67% was recorded from T_6 which was statistically similar to T_4 but significantly higher compared to T_7 and T_8 (Table 4.6.1).

At 90 DAI, only T_1 aand T_5 completely suppressed the disease. At that stage of data collection, disease incidence under T_2, T_4, T_7 and T_8 were statistically similar but significantly higher compared to T_6 and T_9 (Table 4.6.1).

At 105 DAI, the disease incidence was absent only in T_1. Minimum of 16.66% foot and root rot incidence of betevine was found under T_3 and T_9, which were statistically similar to T_2 and T_5. The disease incidence was 58.33, 41.66, 58.33 and 66.66% under T_4, T_6, T_7 and T_8, respectively. Their differences were not significant (Table 4.6.1).

At 120 DAI, the lowest disease incidence of 8.33% was recorded from T_1, which was statistically similar to only T_3 (16.67%) but significantly higher compared to other treatments. The disease incidence was reduced to 33.33% under treatments T_2, T_5 and T_9, 49.99% under T_6, 58.32% under T_7 and 66.66% under T_4, and T_8 (Table 4.6.1).

At this stage of data collection, the maximum of 90.91% reduction in disease incidence over control was found under T_1, which was statistically similar to only T_3 but significantly higher compared to all other treatments. The lowest reduction was found under T_4 followed by T_8, T_6 and T_7. The disease incidence was 63.64 under treatments T_2, T_5 and T_9 which were significantly higher compared to T_4, T_6, T_7 and T_7. Among the treatments, Provax 200 was noted as the most effective fungicide followed by Score 250 EC (Fig. 4.6.2).

4.6.3. Yield

The yield of betel leaf per hectare per 120 days was 7.57, 7.24, 7.16, 7.14 and 6.59 tons under the treatments with Provax 200 (T_1), Score 250 EC (T_3), *T. harzianum* (T_9), Garlic clove extract (T_5) and Tilt-250 EC (T_2), respectively. The lowest yield of 1.76 t/ha was found under control (T_{10}) followed by Poultry manure (T_8), Allamunda leaf extract (T_6).

Application of fungicides, Provax 200, Tilt-250 EC, Score 250 EC, botanical Garlic clove extract and *Tricoderma harzianum,* the yield increase over control ranged 274.4 -330.1%. These five were noted as very effective treatments to control the disease.

Table 4.6. 1. Effect of fungicides, plant extracts, bio-agent and soil amendments selected as IPM components on incidence of foot and root of betelvine *in-vivo* under pot condition

Components of IPM tested	Day[α]	Percent disease incidence at different days after inoculation (DAI)						
		30 DAI	45 DAI	60 DAI	75 DAI	90 DA	105 DAI	120 DAI
T_{1} = Provax 200	95	0.00 c[β] (0.083)[δ]	0.00 d (0.083)	0.00 c (0.083)	0.00 d (0.083)	0.00 e (0.083)	0.00 e (0.083)	8.33 e (8.57)
T_{2} = Tilt- 250 EC	76	0.00 c (0.083)	0.00 d (0.083)	0.00 c (0.083)	0.00 d (1.59)	33.33 bc (34.02)	33.33bcd (34.02)	33.33 cd (34.17)
T_{3} = Score 250 EC	86	0.00 c (0.083)	0.00 d (0.083)	0.00 c (0.083)	0.00 d (0.083)	0.00 e (0.083)	16.67 de (17.05)	16.67 de (17.05)
T_{4} = Pencozeb 80 WP	47	0.00 c (0.083)	0.00 d (0.083)	8.33 c (9.70)	24.99 bc (25.53)	33.33 bc (34.02)	58.33 b (48.10)	66.66 b (52.80)
T_{5} = Garlic clove extract	91	0.00 c (0.083)	0.00 d (0.083)	0.00 c (0.083)	0.00 d (0.083)	0.00 e (0.083)	24.99 cd (25.53)	33.33 cd (34.01)
T_{6} = Allamunda extract	70	0.00 c (0.083)	0.00 d (0.083)	0.00 c (0.083)	16.67 cd (17.05)	24.99 cd (25.53)	41.66 bc (38.71)	49.99 bc (43.41)
T_{7} = Vermi-compost	44	0.00 c (0.083)	16.67 c (17.05)	33.33 b (34.02)	41.66 b (38.71)	49.99 b (43.41)	58.33 b (48.10)	58.32 bc (48.10)
T_{8} = Poultry manure	29	16.67 b (17.05)	33.33 b (34.02)	41.66 b (38.71)	49.99 b (43.41)	49.99 b (43.41)	66.66 b (52.80)	66.66 b (52.80)
T_{9} = *T. harzianum*	87	0.00 c (0.083)	0.00 d (0.083)	0.00 c (0.083)	0.00 d (0.083)	16.66 d (17.05)	16.67 de (17.05)	33.33 cd (30.22)
T_{10} = Control	10	58.33 a (51.91)	66.66 a (56.60)	74.99 a (65.11)	74.99 a (65.11)	83.33 a (69.81)	91.67 a (78.32)	91.67 a (78.31)
LSD	-	14.48	13.67	15.69	18.45	16.57	18.95	18.04

[α] Days required to apprear symptms of foot and root rot of betelvine

[β] Figures within same column having a common letter(s) donot differ significantly at 1.00% level of significance.

[δ] Data within parenthesis are arc-sine transformed values.

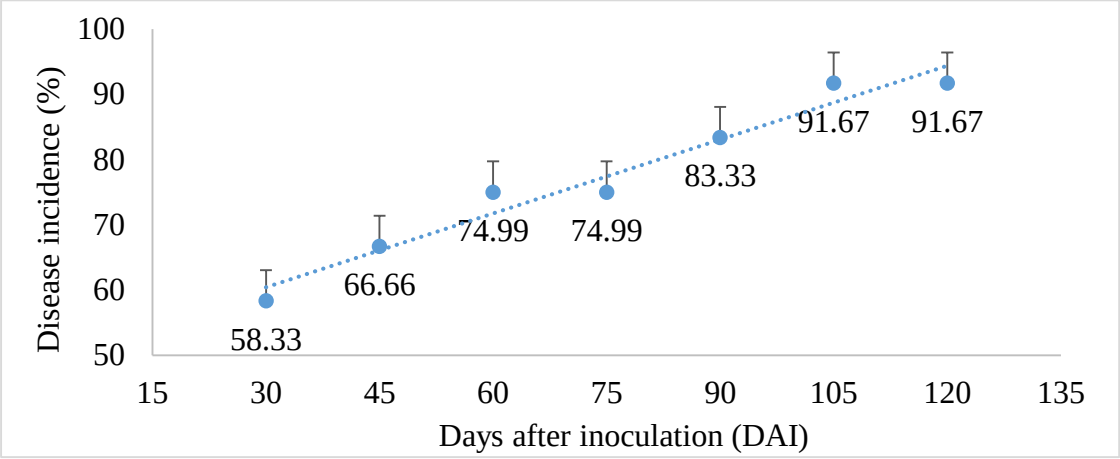


Figure 4.6.1. Progress of incidence of foot ant and root rot of betelvine with the progress of duration after inoculation under control where no treatment was used (T_{1} = Provax 200, T_{2} = Tilt- 250 EC, T_{3} = Score 250 EC, T_{4} = Pencozeb 80 WP, T_{5} = Garlic clove extract, T_{6} = Allamunda leaf extract, T_{7} = Vermi-compost, T_{8} = Poultry manure, T_{9} = *Tricoderma harzianum* and T_{10} = Control (No chemicals, plant extract, bioagents and soil amendments).

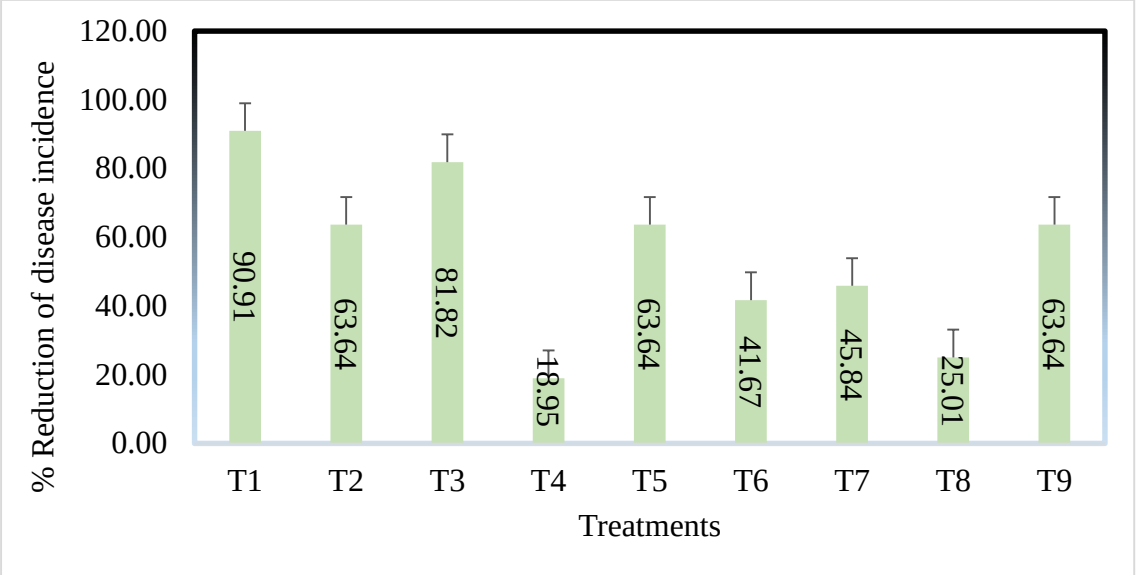


Figure 4.6.2. Percent reduction of incidence of foot and root rot of betelvine due to treatment with IPM components over control at 120 days after inoculation
(T_1 = Provax 200, T_2 = Tilt- 250 EC, T_3 = Score 250 EC, T_4 = Pencozeb 80 WP, T_5 = Garlic clove extract, T_6 = Allamunda leaf extract, T_7 = Vermi-compost, T_8 = Poultry manure, T_9 = *Tricoderma harzianum* and T_{10} = Control (No chemicals, plant extract, bioagents and soil amendments).

Table 4.6.2. Effect of fungicides, plant extracts, bio-agents and soil amendments on yield of beteleaf under field condition

Treatments	**Yield (t/ha) at 120 days**	**% Yield increase over control**[£]
T_1 = Provax 200	7.57 a	330.1
T_2 = Tilt- 250 EC	6.59 abc	274.4
T_3 = Score 250 EC	7.24 a	311.4
T_4 = Pencozeb 80 WP	5.94 bcd	237.6
T_5 = Garlic clove extract	7.14 ab	300.5
T_6 = Allamunda extract	5.59 cde	218.0
T_7 = Vermi-compost	5.29 de	201.0
T_8 = Poultry manure	4.48 e	154.5
T_9 = *T. harzianum*	7.16 a	306.8
T_{10} = Control	1.76 f	-

[£]$\%\ yield\ increase\ over\ control\ = \frac{x-y}{y}\ x100$

Where x = Mean under treatments ($T_{n,\ n=1-9}$) and y= Mean under control (T_{10})

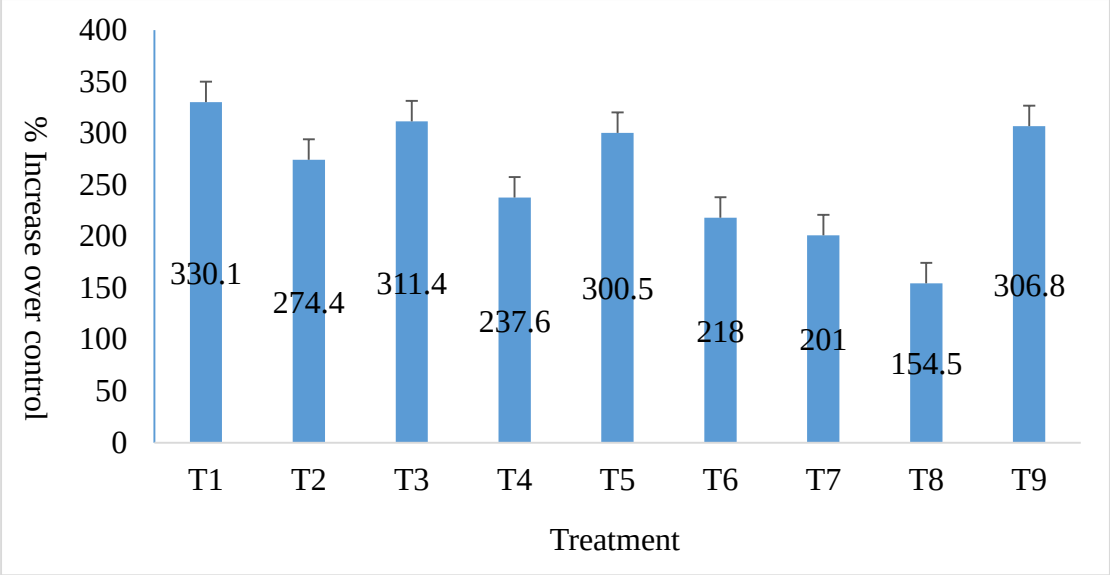


Figure 4.6.3. Percent yield increase of betelvine due to treatment with IPM components over control at 120 days after inoculation (T_1 = Provax 200, T_2 = Tilt- 250 EC, T_3 = Score 250 EC, T_4 = Pencozeb 80 WP, T_5 = Garlic clove extract, T_6 = Allamunda leaf extract, T_7 = Vermi-compost, T_8 = Poultry manure, T_9 = *Tricoderma harzianum* and T_{10} = Control (No chemicals, plant extract, bioagents and soil amendments).

4.7. Experiment 7. Efficacy of integrated application of selected IPM components to control foot and root rot (*Sclerotium rolfsii*) of betelvine in Bangladesh

4.7.1. Days required for appearance of disease symptom

Visible symptoms of foot and root rot disease did not appear on the betelvine plants up to 120 DAI under the treatments, Soil amendment with *T. hazianum* + Provax-200 and Soil amendment with *T. hazianum* + Score 250 EC. The visible symptoms first apeeared within 9 DAI under control. Under all other treatments, first appearance of visible symptoms of the disease were observed within 9 to 116 DAI. The minimum days after inoculation for appearing first disease symptom under control followed by treatments T_1 and T_7. The days after inoculation required for symptms appearance were 116 under T_4, T_9 and T_{17}, 115 under T_{11}, 114 under T_6, T_{13} and T_{20}, and 110 under T_3. The results indicate that the most effective treatment is T_{16} followed by T_4, T_9 and T_{17}; T_{11}; T_6, T_{13} and T_{20}; T_{18}; and T_3 to delay appearance of foot and root rot disease (Table 4.7.1).

4.7.2. Disease incidence

At 30, 60, 90 and 120 DAI, the highest disease incidence of 41.66, 83.33, 100.00 and 100.00%, respectively was found under control (T_{22}). Application of all IPM components in different treatment combinations, except T_1, significantly reduced disease incidence over control. At all stages of data collection, the second highest disease incidence was found under T_1, which was significantly higher compared all other treatments. Only at 120 DAI, the disease incidence under control and T_1 were statistically similar. Plants were free from visible symptms of foot and root rot under treatments T_2-T_{21} at 30 and 60 DAI, under T_4, T_6, T_9, T_{10}, T_{13} and T_{16}-T_{20} at 90 DAI, and under T_{16} and T_{18} at 120 DAI (Table 4.7.2). At 90 DAI, the disease incidence under T_5, T_7, T_8, T_{12}, T_{14} and T_{21} were statistically similar but significantly higher compared to the treatments T_2, T_{10}, and T_{15}, where the disease incidence was 8.33%. At 120 DAI (last day of harvest), the lowest disease incidence of 0.00% was recorded from treatments T_{16} and T_{18} and the second lowest incidence of 8.33% from T_4, T_6, T_9, T_{11}, T_{17} and T_{20} . Under treatments T_3, T_5, T_7, T_8, T_{10}, T_{12}, T_{13}, T_{14}, T_{15}, T_{19} and T_{21}, the disese incidence ranged 24.99 to 49.99% which were statistically similar but significantly lower compared to T_1 and T_{22} but significantly higher compared to other treatments. At that stage of data collection, the reduction in disease incidence over

control due to varius treatments were 100% under T_{16} and T_{18}; 91.67% under T_4, T_6, T_{19}, T_9, T_{11}, T_{17} and T_{20}; and 50.01 – 83.34% under other treatments (Table 4.7.2).

Data collected at 120 DAI showed that the maximum reduction of disease incidence of 100% was found under treatments *T. harzianum* + Provax 200 and *T. harzianum* + Score 250 EC. The second highest reduction in disease incidence of 91.67% was recorded from treatment combinations Soil amendment with Poultry manure +Score 250 EC, Poultry manure + Garlic clove extract, Soil amendment with vermicompost + Provax 200, Vermicompost + Score 250 EC, Vermicompost + Pencozeb 80 WP, Soil amendment with *Trichoderma harzianum* + Tilt 250 EC and *T. harzianum* + Garlic clove extract. Under other treatment combinations the reduction in disease incidence ranged 8.3-83.34% (Table 4.7.2).

4.7.3. Number and weight of leaves per plant at 120 DAI

At 120 DAI, the number and weight of leaves per plant of betelvine were 3.51 and 18.32 g under control (T_{22}). Both the parameters increased significantly over control due to T_1 to T_{21}. The increase in leaf number was achieved with different treatments within the range of 8.20-20.00/plant. The highest leaf number was obtained with T_{16}, which was statistically similar to T_9, T_{11}, T_{17} and T_{18}. The lowest leaf number per plant was recorded from T_1 followed by T_8, T_7 and T_{12}, T_{21} and T_5. Under other treatments the leaf number per plant ranged 16.02-20.25. The leaf weight per plant ranged 42.74-105.50 g under different IPM components (T_1-T_{21}). The highest leaf weight was obtained with T_{16}, which was statistically similar to T_9, T_{11}, T_{17}, and T_{18}. The IPM combinations T_2, T_4, T_6, T_{13}, T_{15}, T_{19} and T_{20} produced 91.58-95.65 g leaves per plant. Their differences were not significant. Significantly the lowest leaf weight was recorded from T_1 followed by T_8, T_7 and T_{12}, and T_{21} (Table 4.7.2).

4.7.4. Leaf yield (t/ha)

At 120 DAI, the lowest leaf yield of 1.47 t/ha was recorded in the control (T_{22}). All treatments with IPM components (T_1-T_{21}) increased leaf yield significantly over control. The highest leaf yield of 8.44 t/ha was recorded from T_{16}. The second highest yield of 7.81 t/ha was obtained with T_{11}, T_{17} and T_{18}. The efficacy of four treatments were statistically similar. Leaf yield under T_2, T_3, T_4, T_5, T_{13}, T_{15} T_{19}, and T_{20} was 7.00-7.65 t/ha, which were not significantly different. The second lowest yield was recorded

from T_1 followed by T_8. The yield increase over control ranged 132.65-474.16%. The lowest percentage of increase of yield was recorded in treatment T_1. The treatments T_{16} was the best performe in increasing of percent yield of betelvine leaf over control (Table 4.7.2).

Table 4.7.1. Efficacy of integrated application of selected IPM components to control foot and root rot (*Sclerotium rolfsii*) of betelvine

Treatments	Days to appear symptom	% Disease incidence at different days after inoculation (DAI)				% Didease reduction at 120 DAI over control
		30 DAI	60 DAI	90 DAI	120 DAI	
T_1 = Soil amendment with poultry manure	28.0	16.67b[α] (17.05)[β]	58.32b (48.10)	74.99b (64.57)	91.67a (88.18)	8.34e (8.56)
T_2 = T_1 + Provax 200	87.0	0.00c (0.083)	0.00d (0.083)	8.33 d (8.57)	16.67def (17.05)	83.34 bc (76.36)
T_3 = T_1 + Tilt 250 EC	110.0	0.00c (0.083)	0.00d (0.083)	0.00 d (0.08)	41.66bc (38.71)	58.34d (48.11)
T_4 = T_1 + Score 250 EC	116.0	0.00c (0.083)	0.00d (0.083)	0.00 d (0.08)	8.33ef (8.56)	91.67ab (88.13)
T_5 = T_1 + Pencozeb 80WP	84.0	0.00c (0.083)	0.00d (0.083)	24.99c (25.53)	49.99b (43.41)	50.0 d (43.41)
T_6 = T_1 + Garlic clove extract	114.0	0.00c (0.083)	0.00d (0.083)	0.00 d (0.08)	8.33ef (8.56)	91.67ab (88.13)
T_7 = T_1 + Allamunda leaf extract	89.0	0.00c (0.083)	0.00d (0.083)	41.66c (38.71)	49.99b (43.41)	50.01d (43.41)
T_8 = Soil amendment with Vermicompos	46.0	0.00c (0.083)	33.33c (30.23)	41.66c (38.71)	41.66bc (38.71)	58.34d (48.11)
T_9 = T_8 + Provax 200	116.0	0.00c (0.083)	0.00d (0.083)	0.00 d (0.08)	8.33ef (8.56)	91.67ab (88.13)
T_{10} = T_8 + Tilt 250 EC	88.0	0.00c (0.083)	0.00d (0.083)	8.33 d (8.57)	33.33bcd (34.02)	66.67d (52.81)
T_{11} = T_8 + Score 250 EC	115.0	0.00c (0.08)	0.00d (0.083)	0.00 d (0.08)	8.33ef (8.56)	91.67ab (88.13)
T_{12} = T_8 + Pencozeb 80 WP	86.0	0.00c (0.08)	0.00d (0.083)	41.66c (38.71)	49.99b (43.41)	50.01d (43.41)
T_{13} = T_8 + Garlic clove extract	114.0	0.00c (0.08)	0.00d (0.083)	0.00 d (0.08)	33.33bcd (34.02)	66.67d (52.81)
T_{14} = T_8 + Allamunda leaf extract	82.0	0.00c (0.08)	0.00d (0.083)	24.99c (25.53)	33.33bcd (34.02)	66.67d (52.81)
T_{15} = Soil amendment with *T. harzianum*	88.0	0.00c (0.08)	0.00d (0.083)	8.33 d (8.57)	24.99cde (25.53)	75.00cd (64.58)
T_{16} = T_{15} + Provax 200	-	0.00c (0.08)	0.00d (0.083)	0.00 d (0.08)	0.00 f (0.083)	100.0 a (99.91)
T_{17} = T_{15} + Tilt 250 EC	112.0	0.00c (0.08)	0.00d (0.083)	0.00 d (0.08)	8.33ef (8.56)	91.67ab (88.13)
T_{18} = T_{15} + Score 250 EC	-	0.00c (0.08)	0.00d (0.083)	0.00 d (0.08)	0.00f (0.083)	100.0 a (99.91)
T_{19} = T_{15} + Pencozeb 80 WP	96.0	0.00c (0.083)	0.00d (0.083)	0.00 d (0.08)	33.33bcd (34.02)	66.67d (52.81)
T_{20} = T_{15} + Garlic clove extract	114.0	0.00c (0.083)	0.00d (0.083)	0.00 d (0.08)	8.33ef (8.56)	91.67ab (88.19)
T_{21} = T_{15} + Allamunda leaf extract	70.0	0.00c (0.083)	0.00d (0.083)	33.33c (34.02)	33.33bcd (34.02)	66.67d (52.81)
T_{22} = Control	9.0	41.66a (38.71)	83.33a (76.35)	100.0a (99.91)	100.00a (99.91)	-

-= Disease did not appear at all times.

[α]Values with parentheses are arcsign transformed values.

[β]Figures within the same column with a common letter(s) do not differ significantly (P=0.01).

Table 4.7.2. Efficacy of integrated application of selected IPM components against foot and root rot (*Sclerotium rolfsii*) of betelvine and to improve plant growth parameters and betel leaf yield

Treatments	At 120 days after inoculation (DAI)			
	Number of leaf/plant	Leaf weight (g/plant)	Yield (t/ha)	Yield increase over control (%)
T_1 = Soil amendment with poultry manure	8.20 h[α]	42.74 h	3.42 h	132.65
$T_2 = T_1$ + Provax 200	17.58 b-d	91.58 b-d	7.33 b-d	398.63
$T_3 = T_1$ + Tilt 250 EC	16.86c-e	87.51 c-e	7.00 c-e	376.19
$T_4 = T_1$ + Score 250 EC	18.36 bc	95.65 bc	7.65 bc	420.40
$T_5 = T_1$ + Pencozeb 80 WP	15.23 ef	79.37 ef	6.35 ef	331.97
$T_6 = T_1$ + Garlic clove extract	18.36 bc	95.65 bc	7.65 bc	420.40
$T_7 = T_1$ + Allamunda leaf extract	14.45 fg	75.30 fg	6.02 fg	309.52
T_8 = Soil amendment with Vermicompos	13.28 g	69.19 g	5.54 g	276.87
$T_9 = T_8$ + Provax 200	18.75 ab	97.68 ab	7.81 ab	431.29
$T_{10} = T_8$ + Tilt 250 EC	16.02 d-f	83.44 d-f	6.68 d-f	354.42
$T_{11} = T_8$ + Score 250 EC	18.75 ab	97.68 ab	7.81 ab	431.29
$T_{12} = T_8$ + Pencozeb 80 WP	14.45 fg	75.30 fg	6.02 fg	309.52
$T_{13} = T_8$ + Garlic clove extract	17.19 b-d	89.54 b-d	7.16 b-d	387.07
$T_{14} = T_8$ + Allamunda leaf extract	16.02 d-f	83.44 d-f	6.68 d-f	354.42
T_{15} = Soil amendment with *T. harzianum*	17.50 b-d	91.17 b-d	7.29 b-d	395.91
$T_{16} = T_{15}$ + Provax 200	20.25 a	105.50 a	8.44 a	474.14
$T_{17} = T_{15}$ + Tilt 250 EC	18.75 ab	97.68 ab	7.81 ab	431.29
$T_{18} = T_{15}$ + Score 250 EC	18.75 ab	97.68 ab	7.81 ab	431.29
$T_{19} = T_{15}$ + Pencozeb 80 WP	17.19 b-d	89.54 b-d	7.16 b-d	387.07
$T_{20} = T_{15}$ + Garlic clove extract	17.58 b-d	91.58 b-d	7.32 b-d	397.95
$T_{21} = T_{15}$ + Allamunda leaf extract	14.84 fg	77.33 fg	6.19 fg	321.08
T_{22} = Control	3.52 i	18.32 i	1.47 i	-

[α] Figures within the same column with a common letter(s) do not differ significantly (P=0.01).

A field survey was conducted to find out incidence of foot and root rot of betelvine caused by *Sclerotium rolfsii* under prevailing environmental factors in major betelvine growing upazillas of Bangladesh during late summer, late winter and mid-summer. The incidence of the disease in different upzillas ranged 5.60 – 28.80, 4.00 – 10.40 and 4.00 – 7.20% in late summer (August), late winter (February) and mid summer (May), respectively. The maximum incidence was recorded from Gournadi of Barisal in late summer and late winter and from Kaligonj of Jhenaidah in mid-summer. The lowest incidence was found in Sitakuda of Chittagong in every season. The incidence of the disease decreased gradually with the increase of soil pH and light intensity, on the contrary, increased with the increase of air temperature and relative humidity. The findings of the survey reveal that low soil pH and light intensity, and high air temperature and relative humidity are favourable for the development of foot and root rot of betelvine and vice versa. The variations in disease incidence may be attributed to the variations of environmental factors like soil pH, air temperature and relative humidity in the surveyed upzillas.

Similar findings are also reported by other researchers. Anonymous (2006) and Maiti and Sen (1982) reported that temperature, relative humidity and rainfall played an important role in the development of foot and root rot of betelvine. Mollah (2012) reported that at 29^0C and 85% RH, the disease incidence and severity of foot and root rot was the highest, and at around 18.7^0C temperature and 75% RH was the incidence was lowest in Satkhira district. He found that the highest disease incidence was found in August (12.50- to 32.50%) and the lowest disease in December (0.00-8.33%) in 2010. According to Punja *et al.* (1988), temperature is the principal limiting factor in the geographic distribution of *S. roifsii*. The disease rarely occurs where average daily minimum winter temperatures are below freezing (0^oC). Maximum disease occurs at $25\text{-}35^0$C which is also optimum range for mycelia growth and sclerotia germination of the fungus.

Other diseases of betelvine recorded from surveyed areas were leaf spot or anthracnose (*Colletototrichum piperis*), leaf rot (*Phytophthora parasitica*) and stem rot (*Phytophthora parasitica*). The incidence vary considerably from one upazilla to another and one season to another. The incidence and severiety were comparatively higher in the month of August, which gradually decreased from August to May in every

locations. Similar results were recorded by Mollah (2012) in different upazillas under the Satkhira district. The highest disease incidence (46.94%) and severity (34.17%) were recorded in August at Tala upzilla and the lowest disease incidence (3.17) and severity (2.05%) were recorded in December at Satkhira Sadar. At 29 ^{0}C and 85% RH, the disease incidence and severity of leaf rot was the highest and the lowest when the temperature was around 18.7^{0}C and the RH around 75%.

An experiment was conducted to identify the isolates of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from different five upzillas of Bangladesh. Altogether 19 isolates were isolated and identified. These were found to occur in different locations. The mycelial growth, colony colour, colony consistency, formation of sclerotia, number, shape, size and colour of sclerotia varied remarkably among the isolates. Mycelia of eight isolates namely Isolate - 1, 2, 7, 11, 13, 14, 18 and 19 showed embedded growth on the surface of PDA medium with thin to profusely thick colony consistency. Mycelium of Isolate -3, 4, 6, 8, 12 and 17 was fluffy and thin with offwhite to very white mycelial colour. Isolate-5, 9, 10, 15 and 16 was wooly to very wooly myceliaum growth with white to very white colour. Sclerotia were found to form on PDA plates within 9 - 15 days of culture. Isolate-2, 3, 4, 9, 11 and 19 produced brown sclerotia; isolate-1, 13, 18, 8, 10, 12, 15, 16 and 17 produced dark brown; and rest of the isolates produced light brown sclerotia. Isolates-3, 7, 9, 12 and 15 produced and other isolates were developed in scattered on Petri plates. Isolate-16 produced the highest number (288/plate) and isolate-10 produced lowest number (14/plate) of sclerotia. The 100-sclerotial weight ranged from 72 to 553 mg.

Findings on characterisistics of isolates of *S. rolfsii* recorded from the preset investigation are in agreement with the findings of the previous investigations (Karthik Pandi *et al*., 2017; Manu *et al*., 2018). Karthik Pandi *et al*. (2017) reported maximum mycelial growth (31.45 mm/day) in isolate SFSR1. The minimum mycelium growth found in isolate SFSR4 (21.62 mm/day). Isolate SFSR4 produced higher number of sclerotia (360 per plate) while the isolate SFSR7 produced minimum number of sclerotia (274 /plate). The biggest size sclerotia (1224 µm) was produced by SFSR1 and the smallest sclerotia (1002 µm) was observed in SFSR8. Manu *et al*. (2018) found variation among different pathogenic isolates of *Sclerotium rolfsii*. He also reported the variation of colony diameter, sclerotial number, the sclerotial colour and sclerotial

weight among the isolates of *Sclerotium rolfsii* where the sclerotial number ranged from 261.7 to 1048.7 and the sclerotial size ranged from 1.10 – 2.10 mm with light to dark brown colour.

Pathogenicity of the isolates of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from different areas was tested in earthen pots, which were placed in betelvine baroj. The mycelial growth, appearance of disease symptoms of foot and root rot, lesion length and disease incidence were recorded. Soil inoculated with *S. rolfsii* exhibited mycelial growth on the surface and around the base of the plant within 2-4 DAI. The disease symptoms were observed within 6 to 16 DAI among different isolates of *S. rolfsii*, where the least days after inoculation was required by isolate-9 and the maximum DAI was required by isolate-2 and 14. Lesion length ranged 1.25 - 6.50 cm due to inoculation of betelvine plants with different isolates. The highest lesion length was observed when inoculated with isolate-9 and isolate–13 and the lowest length was recorded when inoculation was done with isolate-2. Out of 19, forteen isolates caused 100.0% disease incidence in inoculated plants. Most of the isolates tested were pathogenic but some of them delayed disease development. The isolate-9 (JKPBSr - 2) collected from Kaligonj upazilla was noted as the most virulent.

The findings of pathogenicy test are in agreement with the findings of other researchers. Datar and Bindu (1974) proved the pathogenicity of *Sclerotium rolfsii* on sunflower by soil inoculation method under glass house conditions. Meah (2007) tested the pathogenicity of 10 isolates of *S. rolfsii* on eggplant (var. Dohazari) and found that all the isolates of *S. rolfsii* tested influenced the germination, pre-emergence death, damping off, foot rot and finally plant stand. Sommat *et al*. (1982) made an investigation on the pathogenicity of *S. rolfsii* and found that the pathogen could infect its host (cotton) severly. Siddaramaiah and Chandrapa (1988) proved the pathogenicity of *Sclerotium rolfsii* on cardamon in pot culture studies by inoculating 25 days old sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week inoculation.

Thirteen betelvine cultivars were collected from betelvine growing upazillas of Bangladesh. These were designated as PB 001, PB 002, PB 003, PB 004, PB 005, PB

006, PB 007, PB 008, PB 009, PB 010, PB 011, PB 012 and PB 013. These were screened for their growth, yield, yield attributes and susceptbility against foot and root rot disease under inoculated conditions. The vegetative growth parameters and morphological features of different cultivars varied remarkably. The vine elongation, increase in internode length, vine girth, leaf length, leaf breadth and petiole length of 13 cultivars ranged 37.46 -50.34 cm/month, 6.75-10.08 cm/month, 0.445-0.7475 cm, 17.13-27.35 cm, 8.33 to 16.20 cm and 4.83 cm-11.45, respectively. Fresh weight of 100-petiole, 100-leaves, dry matter content, leaf number per meter vine, leaf number per plant/year, yield per plant/year and per hectare yield t/ha of the crop were 50.07-165.74g, 289.38-565.25g, 12.57-14.49%, 9.46-15.38, 50.16-78.44, 187.89-297.12g and 15.03-23.82t, respectively. Variations were also found in leaf colour of cultivars. Among the betelvine cultivars, PB 001 showed resistant reaction, PB 011 and PB 013 showed moderately susceptible reaction and rest of the cultivars showed susceptible reactions. Reports on such screening test against foot and root rot of betelvine cultivars are not available in Bangladesh or elsewhere. Therefore, it may be concluded that this is the first report from Bangladesh about susceptibility of betelvine cultivars against foot and root rot disease.

An *in-vitro* experiment was conducted to determine the effect of 11 botanical extracts on mycelium colony growth of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from majore betelvine growing upzillas. Potato dextrose agar (PDA) was amended with the individual botanical extracts and the fungus was grown on amended PDA and data on radial colony diameter was recorded at 1, 2, 3 and 4 DAI. At 1, 2 and 3 DAI, it was found that *in-vitro* radial mycelium diameter was 22.75, 49.75 and 74.25 mm under control. The growth was reduced to 0.00-21.50, 0.00-47.50 and 0.00-66.75 mm, respectively due to amendment of PDA with different botanicals. During first three days, the fungus failed to grow on PDA amendment with Garlic and the lowest colony diameter was recorded from Allamanda leaf extract. At 4 DAI, the *in-vitro* colony diameter under different treatments including control ranged 3.00-89.50 mm. The maximum growth was observed under control and the minimum under Garlic extract. The highest growth inhibition was obtained with Garlic followed by Allamanda and Bishkatali. Garlic and Allamanda were noted as most effective botanicals to inhibit colony growth of *S. rolfsii*. Colony growth increased gradually with the progress of

incubation under every botanical and control. Garlic extracts and Allamanda are noted as the components of IPM.

Efficacy of botanicals to inhibit colony of *Sclerotium rolfsii* has also been reported by many other investigators (Masuduzzaman *et al*., 2008; Parvin et al., 2016; Yasmin *et al*., 2016; Shaarma *et al*., 2017;). Masuduzzaman *et al*. (2008) found that at higher concentrations of 1:1 and 1:2 completely inhibited *in-vitro* growth of *S. rolfsii* whereas at lower concentrations of 1:3 and 1:4 its growth was suspended to some extent. Parvin *et al.* (2016) conducted an *in-vitro* experiment to find out efficacy of botanicals to inhibit radial mycelial growth of *S. rolfsii.*

All the tested plant extracts showed strong inhibitory effect on myceliual growth of the pathogen. The highest growth inhibition was obtained with Garlic extract (25.56%) followed by Ginger (1.89%), Allamonda (1.67%) and Onion (1.56%) compared to control at 4 DAI. Sahana *et al*. (2017) conducted an experiment to evaluate the efficacy of leaf extracts Neem, Eucalyoptus, Jathropa, Tulsi and Marigold, extracts of Garlic clove and Onion bulb at 0, 5, 10 and 15% concentrations to inhibit *in-vitro* coliny growth of *S. rolfsii*. Among the botanicals tested in the present investigation Onion bulb extract showed 100% inhibition at all the three concentrations followed by Garlic clove showing 97.77, 98.88 and 100% inhibition at 5, 10 and 15% concentrations, respectively. The least inhibition of mycelium diameter was 22.55, 24.44 and 44.07% at 5, 10 and 15% concentrations, respectively in Jathropa leaf extract. Yasmin (2016) conducted an experiment to investigate the effectiveness of garlic, ginger and neem extracts at 0, 5, 10 and 15% concentrations to inhibit mycelial colony growth of *Bipolaris sorokiniana, Fusarium oxysporum* and *S. rolfsii.* Radial diameter of colony was inhibited remarkably by three botanical extracts at different concentrations. The maximum growth inhibition of 72.20% was obtained with Garlic extract, with Neem wxtract (56.41%) and with Ginger in *F. oxysporum* (55.80%) at their highest concentration.

In the present investigation, effect of 10 fungicides on *in-vitro* radial mycelial growth of *S. rolfsii* was evaluated following Poison food technique in Cup method. At 1, 2, 3 and 4 DAI, the colony diameter under control was 24.25, 54.75, 81.25 and 19.50-87.75 mm, respectively. The fungus failed to grow when PDA was amended with Provax-

200. Other nine fungicides reduced the colony growth of the pathogen within the ranges of 10.25-21.25, 12.75-49.25, 17.50-78.75 and 9.50-87.75 mm, respectively. At the final day of data collection, reduction in colony growth was 100.00, 78.15, 75.64 and 44.53% under Provax-200, Score 250 EC, Tilt 250 EC and Pencozeb 80 WP, which were noted as highly effective fungicides to inhibit mycelial growth of *S. rolfsii*.

Similar reports about the efficacy fungicides to inhibit *in-vitro* mycelial growth of *S. rolfsii* are available (Chauhan, 1978; Randon *et al*., 1995; Toorray *et al*. (2007; Suryawanshi *et al*., 2015; Parvin *et al*., 2016). Chauhan (1978) followed poisoned food technique to study the effect of certain fungicides on *in-vitro* growth of *S. rolfsii* and found the highest growth inhibition in Calixin, Vitavax, Duter, Ferbam, Ceresan wet, Sandoz seed dressing 6334 and Brassicol.

Randon *et al*. (1995) reported that the carbendazim (Bavistin), Kasumin and Tecto used at five concentrations under *in-vitro* conditions were the most effective in inhibiting mycelial growth and sclerotia formation. Toorray *et al*. (2007) evaluated seven fungicides at 0, 1000, 1500, 2000 ppm against *S. rolfsii* under *in-vitro* condition. Complete inhibition of growth of *Sclerotium rolfsii* was obtained with Captan, Thiram, Mancozeb, Hinosan (edifenphos) and Antracol, whereas Cholrothalonil showed partial inhibition. Bavistin (carbendazim) did not show much inhibition at any concentration. Suryawanshi *et al*. (2015) conducted an *in-vitro* experiment and found that Vitavax, Tebuconazole and Penconazole caused 100.00, 99.25 and 99.03% mycelial growth inhibition of *S. rolfsii*. Parvin *et al*. (2016) conducted another experiment with fungicides to find out their efficacy to inhibit radial mycelial growth of *S. rolfsii in-vitro*. All the tested fungicides significantly reduced radial mycelial growth of the fungus over control. 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 at 4 days after inoculation. The highest growth inhibition of 70% was recorded in case of Bavistin pfollowed by Dithane M-45 (14.44%) and Rovral (18.44%).

Effectiveness of two bio-agents, *Trichoderma harzianum* and *Pseudomonus fluorescens* were tested against *in-vitro* mycelial growth of *S. rolfsii* following dual plate technique. The radial colony diameter was 23.75, 62.00, 81.00 and 90.00 mm at 1, 2, 3 and 4 DAI, respectively. *Trichoderma harzianum* reduced the colony diameter

to 12.50, 20.75, 31.00 and 39.25 mm and *P. fluorescens* to 19.00, 32.25, 45.25 and 90.00 mm at 1, 2, 3 and 4 DAI. The effectiveness of *T. harzianum* to reduce *in-vitro* colony growth was higher compared to *P. fluorescens.*

The findings of the present investigation are in agreement with many other investigators (Almeida and Landim, 1981; Bagwat, 1997; Ikotun and Adekunle, 1990; Iqbal *et al*., 1995; Mukherjee *et al*., 1995; Parvin *et al*., 2016). Almeida and Landim (1981) reported that an isolate of *Trichoderma* sp. was hyper parasite of *S. rolfsii* on PDA culture and found to be most effective in contrillings *S. rolfsii* on cowpea in green house. The findings are in agreement with other researchers. Bagwat (1997) tested the antagonistic organisms against *S. rolfsii*. Among them, *T. harzianum* was found to be superior and it produced the inhibition zone of 3.27 mm. Maximum reduction of sclerotial bodies was observed in *T. harzianum* followed by *T. viride*. 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 organism *S. rolfsii* and prevented their continued growth. Iqbal *et al*. (1995) tested the micro-organisms, *Trichoderma harzianum, Trichoderma koningii, Trichoderma viride, Gliocladium virens* Miller, *Aspergillus canditus* Link, *Paecilomyces lilacinus* (Thom) Samson and *Bacillus* spp. for their antagonism against *S. rolfsii.* All the organisms significantly inhibited the mycelial growth of *S. rolfsii*. *Trichoderma harzianum, T. koningii* and *T. 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 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.

Parvin *et al*. (2016) conducted an experiment of bio-agents in inhibition of mycelial growth of *S. rolfsii* in dual culture method. Bioagents have significant effect on reduction of radial mycelial growth of the fungus. The performance of *T. harzianum* in reduction of radial mycelial growth was the best followed by *P. fluorescens* irrespective of days after inoculation. All the tested bioagents have strong effect to produce percent growth inhibition against *S. rolfsii* in culture media. The highest percent inhibition (42.77%) was recorded in case of *T. harzianum* preceded by *P. fluorescens* (27.66%)

at 4 days after inoculation. Rekha *et al.* (2012) conducted an experiment to reduce mycelial growth and formation of sclerotial bodies of *S. rolfsii* by *Ttrichoderma* spp. Fourty four isolates of *Trichoderma* (Tri-1 to Tri-44) were screened against *S. rolfsii* through Dual culture technique. Among 44 tested isolates 10 isolates *viz.*, Tri-8, Tri-13, Tri- 15, Tri- 16, Tri- 19, Tri-23, Tri-27, Tri- 29, Tri- 41 and Tri- 44 were found to be efficient in reducing both mycelial growth and formation of sclerotial bodies by the pathogens. Further, the effective isolates were tested for production of volatile metabolites. Isolates Tri-13 (*T. viride*) and Tri-29 (*T. viride*) were found to reduce the growth of *S. rolfsii* through volatile metabolites compare to other tested isolates and control.

In the present investigation, a pot experiment was conducted to find out the effect of fungicides, botanicals, soil amendments and biocontrol agent on development of foot and root rot disease of betelvine under inoculated condition. The duration required for appearance of visible symptoms of the disease were 95, 76, 86, 47, 91, 70, 44, 29, 87 and 10 days under treatments with Provax 200, Tilt-250 EC, Score 250 EC, Pencozeb 80 WP, Garlic Clove Extract, Allamunda leaf extract, Vermi-compost, Poultry manure and *Tricoderma harzianum*, respectively. The highest disease severity of 58.33, 66.66, 74.99, 74.99, 83.33 and 91.67 was observed under control at 30, 45, 60, 75, 90, 105 and 120 DAI, repectively. The disease incidence increased gradually with the progress of duration after inoculation showing the maximum of 91.67% at 105 and 120 DAI. The yield of betel leaf per hectare per 120 days was 7.57, 7.24, 7.16, 7.14 and 6.59 tons under the treatments with Provax 200, Score 250 EC, *T. harzianum*, Garlic clove extract and Tilt-250 EC, respectively. The yield increase under these treatments ranged 274.4 -330.1% over control. The lowest yield of 1.76 t/ha was found under control (T_{10}).

The results of the present investigation are in agreement with findings of many other mresearchers. Previously reported that Carboxin (Provax 200/ Vitavax 200) are effective in controlling collar rot of Sun-flower and Sunhemp caused by *S. rolfsii* (Pal and Choudhary, 1983). Others reported that Garlic clove extract found effective both *in-vitro* and *in-vivo* in controlling foot and root rot of betelvine. As a bioagent *T. harzianum* and *T. viride* have been proved to be highly promissing against soil borne pathogen, *S. rolfsii* (Mathur and Sarbhoy, 1978; Ellil *et al*. 1998; Rekha *et al*., 2012; Parvin *et al.*, 2016).

Efficacy of integrated application of selected integrated disease management (IPM) components was tested under inoculated field conditions to control foot and root rot (*S. rolfsii*) of betelvine. The components of IPM were four chemical fungicides (Provax 200, Tilt 250 EC, Score 250 EC, Pencozeb 80 WP), two plant extracts (Garlic clove, Allamanda leaf), two soil amendments (Poultry manure, Vermicompost) and a bioagent (*T. harzianum*). Efficacy of the components were evaluated in 22 treatment combinations including a control. Visible symptoms of foot and root rot disease did not appear on the betelvine plants in the experiment up to 120 days after inoculation (DAI) under Soil amendment with *T. hazianum* + Provax-200. The first visible symptoms of the disease appeared within 9 to 116 DAI under other different treatments. The lowest disease incidence of 8.33% was recorded from treatments, Soil amendment with poultry manure +Score 250 EC, Poultry manure + Garlic clove extract, Vermicompost + Provax 200, Vermicompost + Score 250 EC, *T. harzianum* + Tilt 250 EC and *T. harzianum* + Garlic clove extract. Data collected at 120 DAI showed that the maximum reduction of disease incidence of 100% was found under treatments *T. harzianum* + Provax 200 and *T. harzianum* + Score 250 EC.

The second highet reduction of 91.67% was recorded from Soil amendment with Poultry manure +Score 250 EC, Poultry manure + Garlic clove extract, Soil amendment with vermicompost + Provax 200, Vermicompost + Score 250 EC, Vermicompost + Pencozeb 80 WP, Soil amendment with *Trichoderma harzianum* + Tilt 250 EC and *T. harzianum* + Garlic clove extract. The lowest leaf yield of 1.47 t/ha was recorded under control. Under other treatments the reduction in disease incidence ranged 8.34-83.34%. All treatments with IPM components increased leaf yield to 3.42-8.44 t/ha i.e. 132.65-474.16% over control.

The lowest percentage of increase in yield was recorded under treatment Soil amendment with poultry manure alone and the highest under Soil amendment with poultry manure+ Provax 20 0. The highest number of leaf (20.25/plant), weight of leaf (105.25 g/pant) and leaf yield (8.25 t/ha) were obtained with the treatment *T. harzianum* + Provax 200. The second highest leaf number, leaf weight and leaf yield of 18.75/plant, 97.68g/plant and 81.25 t/ha were obtained with the treatments with Vermicompost + Provax 200 and Vermicompost + Score 250 EC. Based on findings of the experiment it was noted that the maximum reduction of disease incidence of 100% was recorded from treatments Soil amendment with *T. harzianum* + Provax 200 and Soil amendment

with *T. harzianum* + Score 250 EC, which were followed by Soil amendment with Poultry manure +Score 250 EC, Soil amendment with Poultry manure + Garlic clove extract, Soil amendment with Vermicompost+Provax 200, Soil amendment with vermicompost + Score 250 EC, Soil amendment with vermicompost + Pencozeb 80 WP, Soil amendment with *T. harzianum* + Tilt 250 EC, and Soil amendment with *T. harzianum* + Garlic clove extract, where reduction of disease incidence was 91.67%.

Results of the present integration with different IPM components including soil application of *T. harzianum* and soil drenching with Provax 200 (0.2%) or Score 250 EC (0.1) or Garlic clove extract (1:2 w/v) clearly showed outstanding performances for the management of foot and root rot of betelvine and to enhane yield and yield contributing characters.

Similar reports on integrated management of foot and root rot of betelvine are not available. So, it may be mentioned this is the first report about integrated application of soil amendments, fungicides, botanicals and bioagent for the management of foot and root rot disease of betelvine. However, similar studies with other crops and IPM coponents are available. In support of the present findings the following reaserch results are presented below:

Appana *et al*. (2011) reported an experimental result to effect of management on Collar rot of ground nut caused by *Sclerotium rolfsii*. In the experiment, different biocontrol agent, chemical treatment and organic amendments were tested. The treatment combinations were (1) untreated control, (2) *Pseudomonas florescens* FPD-10, (3) *Pseudomonas florescens* FPD-15, (4) *Trichoderma harzianum*, (5) Nem cake, (6) Captan, (7) *Pseudomonas florescens* FPD-10 + *Trichoderma harzianum* and applied with Neem cake + Captan and (8) *Pseudomonas florescens* FPD-15+ *Trichoderma harzianum* and applied with Neem cake + Captan. All treatment were inoculated with *Sclerotium rolfsii* and *Bradyrhizobium* sp. NC-92 at the time of sowing. All treatment showed significantly lower percentage of pods infected with *Sclerotium rolfsii* and resulted in higher pod yield compare to untreated control. The highest pod yield was obtained with *Pseudomonas florescens* FPD-10 followed by combination of different treatments with FPD-10 and combination of different treatments with *Pseudomonas florescens* FPD-15. Although individual application of either *T. harzianum* or Neem

cake or Captan did not give similar results as single inoculation of either FPD-10 or FPD-15, it did significantly reduction in the pod infection caused by *S. rolfsii* and improved pod yield.

Banyal *et al*. (2008) conducted an experiment to test 10 fungicides against *S. rolfsii* causing collar rot of tomato. Ten fungicides were Carbendazim 50 WP, Carbendazim + mancozeb 75 WP, Captan 50 WP, Chlorothalonil 80 WP, Thiabendazole 80 WP, Mancozeb 75 WDG, Carboxin 75 WP, Propineb 70 WP, Mancozeb 75 WP and Tebuconazole 5 DS and five bioagents, *T. harzianum* (local strain), *T. viride* (local strain), *Gliocladium virens* (local strain), *Paecilomyces lilacinus* (Bhubaneswar strain) and *T. viride* (Ecoderma) were evaluated against *Sclerotium rolfsii*. Tebuconazole and Carboxin @ 50 µg/ml gave complete inhibition of mycelial growth of the pathogen, whereas Carbendazim (Bavistin) @ 1500 µg/ml failed to inhibit the growth of the pathogen. *Trichderma viride* (local strain) was found to be highly effective against the pathogen. Seedling dip with tebuconazole and carboxin gave a total control of the disease in pots. Integrated effect of soil application of *T. viride* (local strain), seedling dip with Tebuconazole (0.05%) and soil drenching with Tebuconazole (0.05%) resulted in complete control of Collar rot of tomato in pot culture.

Madhavi *et al*. (2011) conducted an experiment on dry root rot disease caused by *S. rolfsii* (Sacc.) of Chillies under rainfed conditions. *In vitro* evaluation of nine fungicides by poison food technique showed that Tebuconazole and combination of Carbendazim+Mancozeb were effective in inhibiting the mycelial growth (94.1%) followed by Difenconazole (93.3%). *In vivo* soil drenching with same fungicides proved effective in controlling the pathogen at 1000, 2000 and 3000 ppm. Integration of different treatments including seedling dip with Carbendazim+Mancozeb, addition of Vermicompost, drenching with fungicide and application of *T. harzianum* (7%) were found to be effective in management of disease in comparison with individual treatment.

CHAPTER V
SUMMARY AND CONCLUSION

Betelvine (*Piper betle* L.) is a kind of dioecious perennial creeper vine belonging to the family Piperaceae. It is an important cash and exporting crop of Bangladesh. The crop is grown under cool, shade, considerable humidity and a good supply of soil moisture. In Bangladesh, betelvine is cultivated mainly under an artificial shade structure, known as Baroj. Disease damage to betelvine crop is one of the prominently known limiting factors of betelvine cultivation.

Among the diseases attacking betelvine, foot rot caused by *Phytophthora parasitica*, and foot and root rot caused by *Sclerotium rolfsii* are common in Bangladesh. The foot and root rot decreases the production of betel leaf to a great extent. An investigation was undertaken to develop an integrated disease management (IPM) approach against the disease. A series of preliminary experiments were conducted to find out present severity status of the disease under various environmental conditions in major betelvine growing areas of Bangladesh and to select IPM components and finally to evaluate the efficacy of integrated application of the selected IPM components against the disease. The experiments were conducted in the Central farm and Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka and Laboratory of Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh during the period from January 2015 to June 2018.

A field survey was conducted to find out incidence of foot and root rot of betelvine caused by *S. rolfsii* under prevailing environmental factors in major betelvine growing upzillas namely Gouronadi, Kaligonj, Mirpur, Mohanpur and Sitakunda under the district of Barisal, Jhenaidah, Kushtia, Rajshahi and Chittagong, respectively. The survey was conducted during late summer (August), late winter (February) and midsummer (May). Five barojes were selected from each upazilla and data on disease incidence and impact of prevailing environmental factors on the disease incidence were recorded. The incidence of foot and root rot varied remarkably from upazilla to upazilla and season to season within the range 4.00 – 28.80% in different upzillas. The incidence ranged 5.60 – 28.80, 4.00 – 10.40 and 4.00 – 7.20% in late summer, late winter and midsummer, respectively in various locations. The maximum disease incidence was recorded from

Gournadi (Barisal) in late summer and late winter and from Kaligonj (Jhenaidah) in midsummer. The lowest incidence was found in Sitakuda (Chittagong) in every season. The incidence of foot and root rot decreased gradually with the increase of soil pH and light intensity. On the contrary, the disease incidence increased with the increase of air temperature and relative humidity. Other diseases of betelvine recorded during the survey were leaf spot or anthracnose (*Colletototrichum piperis*), stem rot and leaf spot or anthracnose (*Phytophthora parasitica*).

The findings of the survey reveal that the areas where pH and light intensity are low, and temperature and relative humidity are high, incidence of foot and root rot of betelvine is high and vice versa. The disease is favoured by high temperature (32.61^0C), low soil pH (pH 5.4), Low light intencity (530 X 10 Lux) and high relative humidity (82.46%). The results show that higher disease incidence is found in warmer environment.

A laboratory experiment was conducted to identify the isolates of *S. rolfsii* obtained from foot and root rot infected betelvine plants collected from different upzillas. Tissue planting method and PDA medium were used for isolation. Altogether 19 isolates of *S. rolfsii* were isolated. These were identified based on morphological features of colony and sclerocia. The mycelial growth, colony colour, colony consistency, formation of sclerotia, number, shape, size and colour of sclerotia varied remarkably among the isolates. Mycelia of eight isolates namely isolate-1, 2, 7, 11, 13, 14, 18 and 19 showed embedded growth in the surface of the culture medium with thin to profusely thick colony consistency. Mycelium of isolate-3, 4, 6, 8, 12 and 17 was fluffy and thin with offwhite to very white mycelium. Isolate-5, 9, 10, 15 and 16 were wooly to very wooly mycelia with white to brightly white colour. Sclerotia were found to form on PDA plates within 9-15 days of culture. Isolate-2, 3, 4, 9, 11 and 19 produced brown; isolate - 1, 13, 18, 8, 10, 12, 15, 16 and 17 produced dark brown; and rest of three isolates produced light brown sclerotia.

The number of sclerotia varied from 14 to 288 per plate. Isolate-16 produced the highest number and isolate-10 produced the lowest number of sclerotia. The fresh weight of 100 sclerotia ranged 72-553 mg.

The morphological characteristics were compared with standard key book and the isolates were identified as isolates of S. rolfsii. The findings revel that at least 19 are common in mBangladesh.

Pathogenicity test of 19 isolates of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from different upazillas was performed in earthen pots. The pots were placed on the floor of betelvine barojes. The pathogenicity was determined based on parameter related to foot and root rot disease development after inoculation. The parameter were number of days for mycelia formation, number of days for appearance of visible disease symptom, lesion length and disease incidence (percent plant infection). Pot soil was inoculated with barley grains colonized with *S. rolfsii*. Soil inoculated with *S. rolfsii* exhibited mycelial growth on the surface and around the base of the betelvine plant within 2-4 days after inoculation (DAI). The first disease symptoms were observed within 6 to 16 DAI among different isolates. The minimum DAI was required by isolate-9 and the maximum by isolate-2 and 14. Lesion length ranged 1.25 - 6.50 cm due to inoculation of betelvine plants with different isolates. The highest length was observed when inoculated with isolate-9 and isolate-13 and the lowest length was recorded when inoculation was done with isolate-2. Among the isolates, isolate-14 caused 100.0% disease incidence in inoculated plants.

The findings clearly indicate that most of the isolates are pathogenic but some isolates may delay the development of the disease. The disease incidence may become destructive to the crop before harvesting stage. The isolate-9 (JKPBSr-2) collected from Kaligonj upazilla of Zhenaidah district was noted as the most virulent.

Thirteen betelvine cultivars were collected from majore betelvine growing upazillas of Bangladesh. These were designated as PB 001, PB 002, PB 003, PB 004, PB 005, PB 006, PB 007, PB 008, PB 009, PB 010, PB 011, PB 012 and PB 013. These were screened for their growth, yield, yield attributes and susceptbility against foot and root rot disease under inoculated conditions. Data recorded during the test, the vegetative growth parameters and morphological features of different cultivars varied remarkably. The vine elongation, increase in internode length, vine girth, leaf length, leaf breadth and petiole length of 13 cultivars ranged 37.46 -50.34 cm/month, 6.75-10.08 cm/month, 0.445-0.7475 cm, 17.13-27.35 cm, 8.33 to 16.20 cm and 4.83 cm-11.45, respectively.

Fresh weight of 100-petiole, 100-leaves, dry matter content, leaf number per meter vine, leaf number per plant/year, yield per plant/year and per hectare yield t/ha of the crop were 50.07-165.74g, 289.38-565.25g, 12.57-14.49%, 9.46-15.38, 50.16-78.44, 187.89-297.12g and 15.03-23.82t, respectively.

Variations were also found in leaf colour of cultivars. Among the betelvine cultivars, PB 001 showed resistant reaction, PB 011 and PB 013 showed moderately susceptible reaction and rest of the cultivars showed susceptible reactions. Reports on such screening test against foot and root rot of betelvine cultivars are not available in Bangladesh or elsewhere. Therefore, it may be concluded that this is the first report from Bangladesh about susceptibility of betelvine cultivars against foot and root rot disease.

Three *in-vitro* experiments were conducted to determine the effect of botanical extract, chemical fungicide and bioagent on *in-vitro* mycelium growth of *S. rolfsii*. The first *in-vitro* experiment was conducted to determine the effect of 11 botanical extracts on mycelium colony growth of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from majore betelvine growing upzillas. Potato dextrose agar (PDA) was amended with the individual botanical extracts and the fungus was grown on amended PDA and data on radial colony diameter was recorded at 1, 2, 3 and 4 DAI. At 1, 2 and 3 DAI, it was found that *in-vitro* radial mycelium diameter was 22.75, 49.75 and 74.25 mm under control. The growth was reduced to 0.00-21.50, 0.00-47.50 and 0.00-66.75 mm, respectively due to amendment of PDA with different botanicals. During first three days, the fungus failed to grow on PDA amendment with Garlic and the lowest colony diameter was recorded from Allamanda leaf extract. At 4 DAI, the *in-vitro* colony diameter under different treatments including control ranged 3.00-89.50 mm. The maximum growth was observed under control and the minimum under Garlic extract. The highest growth inhibition was obtained with Garlic followed by Allamanda and Bishkatali. Garlic and Allamanda were noted as most effective botanicals to inhibit colony growth of *S. rolfsii*. Colony growth increased gradually with the progress of incubation under every botanical and control. Garlic extracts and Allamanda are noted as the components of IPM.

Three *in-vitro* experiments were conducted to determine the effect of botanical extract, chemical fungicide and bioagent on *in-vitro* mycelium growth of *S. rolfsii.* In first experiment, potato dextrose agar (PDA) was amended with water extracts of Margosa, Knotweed, Allamanda, Lemon grass, Yellow oleander, Tobacco, Burmuda grass, Garlic, Ginger, Onion and Mahogany. Amended PDA in Petri dishes (90 mm) were inoculated with mycelium blocks of *S. rolfsii* (1 block/dish) and data on colony diameter was recorded. At 4 DAI, the radial diameter of intro colony growth under different botanicals and control ranged 3.00-89.50 mm. The maximum of 89.50 mm colony diameter was found under control, which was statistically similar to Mehagony seed, Bermutha grass and tobacco.

Other six botanicals significantly reduced the colony growth over control. Significantly the highest growth inhibition was obtained with Garlic cloves followed by Allamanda, Bishkatali, Bermuda grass and Zinger. The inhibition of colony diameter due to amendment of PDA with 11 botanicals ranged 7.82-96.67% over control. The maximum of inhibition was obtained with Garlic extract followed by Allamanda, which were selected as components of IPM. The second *in-vitro* experiment was conducted to determine the effect of Tilt-250 EC, Score 250 EC, Rovral 50 WP, Bavistin 50 WP, Provax 200, Topgan, Ridomil Gold MZ-68 WP, Pencozeb 80 WP, Cuprafix 30 D and Bordeaux mixture on radial mycelial growth of *S. rolfsii* following Poison food technique in Cup method.

Some of the fungicides showed profound effect on reduction of radial mycelial growth of the pathogen. At 4 DAI, maximum reduction in *in-vitro* mycelial colony growth was achieved with Provax-200 followed by Score 250 EC, Tilt 250 EC, Rovral 50 WP and Pencozeb 80 WP giving 100.00, 78.15, 75.64, 59.10 and 44.53% reduction. These five fungicides were noted as highly effective components of IPM.

The third experiment was conducted following Dual plate technique to find out the effect of two bio-agents, *Trichoderma harzianum* and *Pseudomonus fluorescens* on *in-vitro* colony growth of the pathogen. *Trichoderma harzianum* reduced the colony diameter by 56.39% and *P. fluorescens* inhibited colony growth by 32.78% over control. Only T. harzianum was selected a components of IPM. Based on finds of three *in-vitro* tests, two botanicals (Garlic extract and Allamanda leaf extract), four

fungicides (Provax-200, Tilt 250 EC, Score 250 EC, Pencozeb 80 WP) and a bioagent (*T. harzianum*) were recorded as most effective materials and selected for further test against *S. rolfsii in-vivo* under pot conditions.

To find out the effect of fungicides, boanical, soil amendment and bioagent selected based on three *in-vitro* tests on the development of foot and root rot (*S. rolfsii*) of betelvine, an *in-vivo* experiment was conducted under pot culture conditions in a betelvine baroj of the experimental farm of Sher-e-Bangla Agricultural University. Treatments of the test were Provax 200, Tilt 250 EC, Score 250 EC and Pencozeb 80 WP, Garlic clove extract, Allamanda leaf extract, Poultry waste, Vermi-compost and *T. harzianum*. Pot soil was inoculated with barley grains colonized with *S. rolfsii*. The soil was amended with Poultry manure, Vermicompost and *T. harzianum* at 10 days before inoculation. The selected plant extracts and fungicides were sprayed at 3 days after inoculation at the base of the plant and soil for 7 days interval. Data on days to symptom appearance, disease incidence and leaf yield were recorded. The durations required for appearance of visible symptoms of the disease in inoculated as well as treated pot soils were 95, 76, 86, 47, 91, 70, 44, 29, 87 and 10 DAI under treatments with Provax 200, Tilt-250 EC, Score 250 EC, Pencozeb 80 WP, Garlic clove extract, Allamunda leaf extract, Vermi-compost, Poultry manure and *T. harzianum*, respectively.

The highest disease incidence of 58.33, 66.66, 74.99, 74.99, 83.33 and 91.67 was observed under control at 30, 45, 60, 75, 90, 105 and 120 DAI, respectively. The disease incidence increased gradually with the progress of duration after inoculation showing the maximum of 91.67% at 105 and 120 DAI. The yield of betel leaf per hectare at 120 DAI was 7.57, 7.24, 7.16, 7.14 and 6.59 tons under treatments with Provax 200, Score 250 EC, *T. harzianum*, Garlic clove extract and Tilt-250 EC, respectively. The yield increase under different treatments ranged 154.5 -330.1% over control. Results of *in-vivo* experiment reveal that the treatments may be selected as IPM components to control foot and root rot disease of betelvine.

Efficacy of integrated application of selected IPM components was tested under inoculated field conditions to control foot and root rot of betelvine. As promising components of IPM, four chemical fungicides (Provax 200, Tilt 250 EC, Score 250 EC, Pencozeb 80 WP), two plant extracts (garlic clove, Allamanda leaf), two soil amendments (poultry manure, Vermicompost)

and a bioagent (*T. harzianum*) were selected based on findings of *in-vitro* and *in-vivo* tests. Efficacy of the components were evaluated in 21 treatment combinations. A control was also maintained. *Trichoderma harzianum*, poultry manure and Vermicompost were applied to soil and based of betelvine plants at 10 days before inoculation with barley grains colonized with *S. rolfsii*. Botanicals and fungicides were applied as sprays on the base and root zone of the plant at 3 days after inoculation. The visible symptoms of foot and root rot disease did not appear on the betelvine plants up to 120 days after inoculation (DAI) under the treatment combinations, Soil amendment with *T. hazianum* + Provax-200 and Soil amendment with *T. hazianum* + Score 250 EC. The visible symptoms appeared within 9 DAI under control. Under other treatment combinations, the visible symptoms of the disease appeared within 28-116 DAI.

The maximum of 116 DAI was required to appear visible symptoms under Poultry manure + Score 250 EC, Vermicompost + Provax-200 and harzianum + Tilt 250 ECT. Application of all treatment combinations significantly reduced incidence of foot and root rot over control except the treatment with poultry manure alone at 30, 60 and 90 DAI. At 120 DAI, the maximum of 100.00% reduction in disease incidence was obtained with soil amendment with *T. harzianum* + Provax-200 and *T. harzianum* + Score 259 EC. The second highest reduction of 91.67% was found under the treatment combinations, Poultry manure +Score 250 EC, Poultry manure + Garlic clove extract, Vermicompost + Provax 200, Vermicompost + Score 250 EC, Vermicompost + Pencozeb 80 WP, *T. harzianum* + Tilt 250 EC and *T. harzianum* + Garlic clove. Under other treatment combinations the reduction ranged 8.3-83.34%.

At 120 DAI, the lowest number 3.51 of leaves/plant was recorded from control. The parameters increased significantly over control due to different treatment combinations within the range of 8.20-20.00/plant. The highest leaf number was obtained with *T. harzianum*+Provax-200, which was statistically similar to Vermicompost + Score 250 EC, Vermicompost + Provax-200, Vermicompost + Score 250EC, *T. harzianum*+Provax-200, and *T. harzianum*+Score 250 EC Provax-200. The lowest leaf number per plant was recorded from Poultry manure alone followed by Vermicompost alone, Poultry + Allamanda and Vermicompost + Pencozeb 80 WP, T. harzianum + Allamanda and Poultry manure + Provax-200. Under other treatments the leaf number per plant ranged 16.02-20.25.

Leaf weight per plant was 18.32 g under control, which was increased significantly over control ranging 42.74-105.50 g due to different treatment combinations. The highest leaf weight per plant was obtained with *T. harzianum* + Provax-200, which was statistically similar to Vermicompost + Provax-200, Vermicompost +Score 250 EC, *T. harzianum* + Tilt 250 EC, and *T. harzianum* + Score 250 EC. The treatment combinations Poultry manure + Provax-200, Poultry manure + Score 250 EC, Poultry manure + Garlic extract, Vermicompost + Garlic extract, *T. harzianum* alone, *T. harzianum* + Score 250 EC and *T. harzianum* + Pencozeb 80 WP produced 91.58-95.65 g leaves per plant. Their differences were not significant. Significantly the lowest leaf weight was recorded from Poultry manure alone followed by Vermicompost alone, Poultry manure + Allamanda and Vermicompost + Pencozeb 80 WP, and *T. hasrzianum* + Allamanda leaf extract.

The lowest leaf yield of 1.47 t/ha was recorded from the control. All treatment combinations significantly increase leaf yield per hectare. The highest leaf yield of 8.44 t/ha was obtained with *T. harzianum* + Provax-200. The second highest yield of 7.81 t/ha was obtained with Poultry manure + Score 250 EC, *T. harzianum* + Tilt 250 EC and *T. harzianum* + Score 250 EC. The efficacy of four treatments were statistically similar. Leaf yield under Poultry manure + Provax-200, Poultry manure + Tilt 250 EC, Poultry manure + Score 250 EC, Poultry manure + Pencozeb 80 WP, Vermicompost + Garlic extract, *T. harzianum* alone, *T. harzianum* + Pencozeb 80 WP, and *T. harzianum* + Garlic extract was 7.00-7.65 t/ha, which were not significantly different. At 120 DAI, the yield increase over control ranged 132.65- 474.16%. The maximum leaf yield increase over control was obtained with *T. harzianum* + Provax-200 (474.16%), which was statistically similar to Poultry manure + Provax-200 (398.63%), Poultry manure+Tilt 250 EC (376.19%), Poultry manure +Score 250 EC (420.40%), Poultry manure + Garlic extract (420.40%), Vermicompost + Provax-200 (431.29%), Vermicompost + Tilt 250 EC (354.42%), Vermicompost + Score 250 EC (431.29%), *T. harzianum* alone (395.91%), *T. harzianum* + Provax 200 (474.14%), *T. harzianum* + Tilt 250 EC (431.29%), *T. harzianum* + Score 250 EC (431.29%), *T. harzianum* + Pencozeb 80 WP (387.07%) and *T. harzianum* + Garlic extract (397.95%).

Considering findings of the present investigation, the following conclusions may made:

1. Foot and root rot of betelvine caused by *S. rolfsii* is widely distributed in the growing regions of Bangladesh and the disease is remarkably influenced by environmental factors like soil pH, temperature, humidity and light intensity. High temperature & high RH (%) and low light intensity & low soil pH (acidic soil) favours development of the disease.

2. Variabilities exist among the isolates of *S. rolfsii* associated with foot and root rot of betelvine in Bangladesh. The existence of physiological races of the pathogen in Bangladesh might be the reasons of diversified severity of the disease in different growing regions in the country

3. Results of a pathogenicity test of 19 isolates of *S. rolfsii* found to be pathogenic causing foot and root rot disease of betelvine and the isolates were sharply varied in terms of degree of pathogenicity.

4. Variations in terms of growth, yield, yield attributes and susceptibility against foot and root rot disease among 13 cultivars of betelvine collected from different areas of Bangladesh are existed. Among the cultivars of betelvine, most of the cultivars were found to be susceptible. Out of the cultivars only Laldingi (PB 001) showed resistant reaction while cultivars PB 011 & PB 013 showed moderately susceptible reaction.

5. Based on finds of three *in-vitro* evaluation, Garlic clove extract, Provax-200 & Score 250 EC fungicides and *T. harzianum* are found to be most effective against *S. rolfsii*.

6. Based on results of an *in-vivo* evaluation, Garlic clove extract, Provax-200 & Score 250 EC fungicides, *T. harzianum and* two soil amendments with Poultry waste & Vermi-compost are found to be most effective against *S. rolfsii* as IPM components.

7. Based on the integrated effect of IPM components, soil drenching with either garlic clove extract (1:2 w/w) or Provax 200 (0.2%) / Score 250 EC (0.1%) in combination of soil amendment with *T. harzianum* or Vermicompost showed promising performances for the eco-friendly management of foot and root rot disease of betelvine.

CHAPTER VI

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