SCREENING OF RESISTANT CULTIVARS OF BETELVINE (*Piper betle* L.) AGAINST FOOT AND ROOT ROT DISEASE CAUSED BY *Sclerotium rolfsii*

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DECEMBER, 2020

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A Thesis
Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY
SEMESTER: July- December, 2020

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CERTIFICATE

This is to certify that the thesis entitled**,** "**SCREENING OF RESISTANT CULTIVARS OF BETELVINE (*Piper betle* L.) AGAINST FOOT AND ROOT ROT DISEASE CAUSED BY *Sclerotium rolfsii***" submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in PLANT PATHOLOGY** embodies the result of a piece of bona fide research work carried out by Registration No**. 18-09236** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma elsewhere in the country or abroad.

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

Dated: 04 April, 2021
Place: SAU, Dhaka, Bangladesh

…….....
Prof. Dr. Md. Rafiqul Islam
Supervisor

Dedicated
To
My Beloved Parents,
Wife & Daughters

List of Abbreviations of Technical Symbols and Terms

Full Words	**Abbreviation/ Symbol**
Agro-Ecological Zone	AEZ
And	&
And others	*et al.*,
Bangladesh Bureau of Statistics	BBS
Centimeter	cm
Commonwealth Mycological Institute	CMI
Complete Randomized Design	CRD
Coefficient of Variance	CV
Days After Inoculation	DAI
Degree Centigrade	^{o}C
Duncan's Multiple Range Test	DMRT
Gram	g
Hectare	ha
Journal	*J.*
Kilogram	kg
Least Significant Difference	LSD
Muriate of Potash	MoP
Namely	viz.
Negative Logarithm of Hydrogen Ion Conc.	p^{H}
Percentage	%
Per Square Inch	PSI
Potato Dextrose Agar	PDA
Randomized Complete Block Design	RCBD
Sher-e- Bangla Agricultural University	SAU
Sodium Hypo chloride	NaOCl
That is	i.e
Triple Super Phosphate	TSP

ACKNOWLEDGEMENT

*All the praises and gratitude to the omniscient Almighty '**Allah Rabbul Al-Amin**,' the supreme ruler of the universe who enabled the author to pursue his higher study and to complete the research work.*

*The author sincerely desires to express his deepest sense of gratitude, respect, profound appreciation and indebtedness to his research Supervisor **Dr. Md. Rafiqul Islam,** Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his kind and scholastic guidance, untiring effort, valuable suggestions, inspiration, co-operation and constructive criticisms throughout the entire period of research work and the preparation of the manuscript.*

*The author expresses heartfelt gratitude and indebtedness to the Co-Supervisor and Chairman **Professor Dr. Fatema Begum**, Department of plant Pathology, SAU, Dhaka for her precision advice, instructions, inspirations and cordial co-operation to complete the courses and the research work successful.*

*The author also expresses heartfelt gratitude and indebtedness to the Member of Advisory Committee, **Prof. Dr. Nazneen Sultana,** and **Prof. Dr. Md. Belal Hossain,** Department of plant Pathology, SAU, Dhaka for their precision advice, instructions, inspirations and cordial co-operation to complete the courses and the research work successful.*

Special thanks and indebtedness to the Chairman and all the respective teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their valuable teaching, sympathetic co-operation and inspiration throughout the period of the study.

The author thankfully remembers the students of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their cooperation in the entire period of study. The author also expends his thanks to all the staff of the

Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their help and cooperation during the research work.

The author also likes to give thanks to all of his friends for their support and inspiration throughout his study period in Sher-e-Bangla Agricultural University, Dhaka.

The author like to express his heartful gratitude to the authority of ***Sher-e-Bangla Agricultural University (SAU)*** *for giving the opportunity to pursue higher education by granting deputation for the degree.*

The author also likes to express his heartful gratitude to the ***Dean, Faculty of Agribusiness Management*** *Sher-e-Bangla Agricultural University (SAU) for giving the opportunity to use the office his study period and specially thanks to my colleague and all staff for his cordial cooperation in the study period.*

The author also likes to expresses thanks to classmates ***Rakibul Hasan Nitol, Md Sajeduzzaman*** *and* ***Md Al Sabbir Mahmud****, for their cordial support, cooperation and inspiration in preparing this thesis.*

The author is specially thankful to ***Tanvir Ahmed*** *for his helpful co- operation in preparing research field and collecting data and for giving technical support to prepare the thesis paper.*

The author grateful to his parents for their support and inspiration throughout his study period.

Finally, the author found no words to thank his wife ***Sharmin Akter*** *and daughters* ***Nur-E-Jannat (Nuha)*** *and* ***Nasiba Alima (Hiba)*** *for their unquantifiable love and continuous support, their sacrifice, never ending affection, immense strength and untiring efforts for bringing his dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of his studies.*

The author

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ABSTRACT

The causal organism of foot and root rot disease was isolated from diseased specimen with typical symptoms collected from affected betelvine. The isolate was pure cultured and identified as *Sclerotium rolfsii* The *Sclerotium rolfsii* was mass multiplied and the pathogenicity was confirmed by Koch's Postulates. Eight betelvine cultivars viz. PB 001 (Chalitaguti) PB 002 (Chuadanga Pan), PB 003 PB (Moheskhali Pan), 004 (Laldingi Pan), PB 005 (Satkhira Pan), PB 006 (BARI Pan-1), PB 007 (BARI Pan-2) and PB 008 (BARI Pan-3), were explored in the screening experiment. Results were compiled based on physio-morphological features, days required for appearance of 1st disease symptom, disease reactions, yield and yield contributing characters Betelvine cultivars were showed differential reactions against *S. rolfsii* causing foot and root rot disease. The vegetative growth parameters and morphological features of different cultivars of betelvine varied remarkably. The maximum vine increment per month (90.97 cm) was recorded in PB 003 and the lowest increment (46.87 cm) was in PB 006. The maximum length of internode (8.27 cm) was recorded in PB 004 and the minimum length (4.50 cm) was in PB 006. The maximum vine girth (1.63 cm) was recorded in PB 007 and the minimum girth (0.93 cm) was in PB 002. Significantly the highest length of leaf (22.07 cm) was recorded in PB 005 and the lowest (13.17 cm) was in PB 006. The leaf breadth (13.03 cm) was recorded the highest in PB 003 and the lowest (6.93 cm) in PB 006. The highest petiole length (8.63 cm) was recorded in PB 008 and the lowest length (4.03 cm) was in PB 002. The petiole breadth (1.13cm) was recorded the highest in PB 004 and the lowest (0.43 cm) in PB 008. The weight (g) of 100 petiole was recorded the highest in PB 004 (83.33 g) and the lowest weight (38.33 g) was in PB 006.The fresh weight of 100 leaves with petiole was recorded the highest in PB 004 (553.33 g) and the lowest weight (206.67 g) was in PB 006. The PB 006 produced significantly the highest number (23.00) of leaves per meter vine and the lowest number (13.67) of leaf was recorded in PB 003. The leaf number per plant per year was recorded the highest in PB 006 (414 leaves) and the lowest number was found in PB 003 (282 leaves). After inoculation, the time interval (days) required for appearance of 1st disease symptoms among the betelvine cultivar differed significantly. The lowest incubation period (8 days) required for the cultivars PB 007 (BARI Pan-2) and PB 008 (BARI Pan-3). The highest incubation period (14 days) was required for PB 006 (BARI Pan-1). No symptom was appeared in the cultivars PB 004 (Laldingi pan). The disease incidence ranged from 0.00% - 100%. Among the betelvine cultivars, PB 004, (Laldingi pan) showed resistant (R) reaction while three cultivars viz. PB 006 (BARI Pan-1), PB-003 Moheskhali Pan) and PB 002 (Chuadanga) showed moderately resistant (MR) reaction. Two cultivars viz. PB 001 (Chalitaguti) and PB 005 (Satkhira) showed moderately susceptible (MS) reaction and the rest cultivars PB 007 (BARI Pan-2) and PB 002 (BARI Pan-3) showed highly susceptible reaction against *S. rolfsii* causing foot and root rot of betelvine.

CHAPTER-1
INTRODUCTION

CHAPTER I

INTRODUCTION

Betelvine (*Piper betle* L.) leaf familiarly known as 'pan' is an important horticultural crop in Bangladesh. It is a kind of dioecious perennial creeper vine belonging to the family Piperaceae and grown in tropical and sub-tropical regions in the world. The betel leaf is cultivated largely for its leaves, which is an important cash crop of Bangladesh and used as a masticatory. Betelvine leaves are shiny, broadly ovate and green heart-shaped with bleaching quality, softness, pungency and aroma. 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.

Leaves of betel vine are chewed along with areca nut as a masticator. Usually the people of South-Asia, Southeast Asia, Gulf States and Pacific islands chew betel leaves. All classes of people of Bangladesh chew betel vine not only as a habit but also as an item of rituals, etiquette and manners.

The betelvine thrives cultivate under tropical conditions having a cool, shade, considerable humidity and a good supply of soil moisture. Geographically it belongs to the region bounded by 68° E to 118° W longitudes and 30° N to 12° S latitudes. It is grown from sea level to an altitude of about 900 m (Chaurasia, 2001). It is 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).

Betelvine leaves have various medicinal values and also used as an antiseptic. Betel leaves are also known for its medicinal attributes containing some vitamins, enzymes, thiamine, tannin, iodine, iron, calcium, riboflavin, minerals, protein, essential oil and medicine for liver, brain and heart diseases (Chopra *et al.*, 1956). The leaves are found to contain enzyme diastase and catalyzes and vitamins A and C. It is supposed to be tonic to the brain, liver and heart in human beings. Betel leaf is also anti-rheumatic, antirhodant, anti-cough and

antinoctornel emission. Volatile oil extract from betel leaf has been found to have antiseptic (Chattopadhyay, 1967).

There are about 100 varieties of betel leaf (pan) across the world of which 40 are encountered in India and 30 in 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 most probable place of origin of pan is Malayasia (Chattopadhyay and Maity, 1967). In ancient times, pan 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 of 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 2016-17 was about 23,813 ha and the total annual production was about 2,14,252 metric tons. The average yield per hectare is 9.00 metric tons (BBS, 2017). Betel leaf is usually plucked in the month of *Kartik, Phalgun* and *Ashar*. The Kartik pan is considered by consumers to be the best and Ashar pan the worst. When plucking, it is a rule to leave at least sixteen leaves left on the vine.

In Bangladesh, Betel vine is cultivated mainly under an artificially erected structure, known as Baroj, Bareja or Bheet, which is a kind of hut which sides and roof are made of jute straw on a light frame work of bamboo. To cultivate the betel vine, low light intensity, mild temperature (10°C to 30°C), high humidity with moderate sunshine & 1450-1700 mm rainfall and frequent irrigation are needed throughout the year.

Disease damage to the crop is one of the prominently known limiting factors for betel vine production. The betel vine is highly susceptible to diseases, pests and natural calamities (Sayeeduzzaman, 1988). Humid and moist shaded conditions are favorable for betel vine growth and also favor a variety of root and foliage disease development (Goswami *et al*., 2002). Among the diseases of betelvine, foot and root rot known to cause by *Sclerotium rolfsii* is the most devastating disease which decreases the production of betel leaf to a great extent (Islam, 2005). *Sclerotium rolfsii* Sacc. is a serious soil borne fungus and has wide host range of economically valuable substantial crops and has been referred as an almost omni-pathogenic organism in the tropical and subtropical region of the world (Aycock, 1966; Talukdar, 1974). *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). The continuous and indiscriminate uses of chemicals result in accumulation of harmful chemicals residues in the soil, water and plants. In a third world country like Bangladesh, farmers are illiterate and they could not properly handle the use of chemicals, which created health hazards. The nonjudicious use of chemicals not only hazardous to living being but also hamper the natural ecological balance by killing the beneficial and/or antagonists microorganisms. The continuous and spontaneous chemical application also induced the development of resistant isolates of the pathogens, which sometimes become more virulent.

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 the various insect pests and pathogenic attack of the export-oriented crop (Goswami *et al.,* 2002).

In Bangladesh, foot and root rot of betelvine was initially a minor disease but now has become a major disease and the incidence of the disease is increasing day by day. At present no resistant varieties are known to available against this disease in the country. The betelvine growers are presently discouraged to cultivate betelvine as they have no suitable approach for controlling foot and root rot disease. Huge number of betelvine garden 'Baroj' become 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 loss a huge income of foreign currency. Thus, the problem needs to give urgent attention to ensure the smooth production of such an important and economically potential crop. Search of resistant cultivars might be a good option.

Based on the above facts the present piece of research has been conducted to achieve the following objectives.

i. To isolate and identify the pathogen causing foot and root rot disease of betelvine
ii. To evaluate the disease incidence against foot and root rot disease of betelvine
iii. To screen out the resistant/tolerant cultivars of betelvine against foot and root rot disease

CHAPTER-2
REVIEW OF LITERATURE

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. The present review is an overview of foot and root rot disease of betelvine caused by *S. rolfsii*. Most of the researcher were concentrated on etiology, epidemiology, incidence, method of inoculation of *Sclerotium roifsii* and management of the disease**.** The relevant literatures are cited in this chapter.

2.1. The pathogen

Sclerotium rolfsii is a well-known polyphagous, ubiquitous, omnivorous and most destructive soil borne fungus. The fungus *Sclerotium rolfsii* Sacc. 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 *S. rolfsii* sp. But, in India, Shaw and Ajrekar (1950) isolated the fungus from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. However, later studies showed that, the fungus was *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 *S. rolfsii*.

2.2. Symptoms of foot and root rot disease

According to Chet *et al.,* (1994), *Sclerotium rolfsii* Sacc. causes the disease known as southern blight in wide variety of crops. *Sclerotium rolfsii* from brownish sclerotia that can survive in the soil for longer period of time in adverse condition.

Alexander and Stewart (1994) worked on *Sclerotium rolfsii* (Telemorph; *Athelia rolfsii*) and found to cause serious root and stem rots of a good range of economically important fruit and vegetable crops. Sclerotia are the important propagules for the survival of this pathogen. Under favorable conditions,

sclerotia may germinate to cause infection usually occurs at 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, *Sclerotium* wilt, *Sclerotium* blight and white mold which is affected all part of the plant at any stage of crop growth 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) stated that host range of *Sclerotium rolfsii* is very wide and includes many important horticultural and agronomic crops. It is not possible to estabilish precise totals for the species reported as host; nevertheless, the soil borne plant pathogenic fungus *Sclerotium 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 stem and root.

Choudhury (1967) stated that foot rot disease of brinjal is caused by *Sclerotium* sp. Minute mustard like structure, adhere to the stem at gourd level. These put out mycelia which enter the stem and choke the vessels. This is spread from one plant to the other by irrigation water. It is difficult to control the disease as the fungus persists in the soil. Crop rotations with non-host crops and drenching of copper fungicides in the soil before planting helps to control the disease.

Das *et al.*, (2000) found that the disease symptoms of foot and tuber rot of tuberose caused by *Sclerotium rolfsii* is preceded by the appearance of

prominent coarse mycelia masses on leaf surfaces at or near the soil surface. The infected leaves detached from the plant. More or less round sclerotia, brown in color, are formed on and around the infected leaves. As a result, the infected plants become weak and send out few or none of the flowering shoots in case of severe damage.

Khanna and Jyotsama Sharma (1993) described the symptoms of *Sclerotium* rot of potato as dark brown lesions appearing on the stem just below the soil surface followed by wilting of lower leaves and gradually drying of the entire plant. Such wilted plants showed white cover of fungal threads, girdling the basal part of stem, which moved above and below to the stem and roots. Sclerotia resembling mustard seeds, developed on infected plant parts and also on soil.

Jahan *et al*., (2016) reported betelvine plants caused by *Sclerotium rolfsii*, 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 were 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 were found completely decomposed, leaving only the fibrous portion.

2.3. Environmental factors on the disease development

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

Alexander and Stewart (1994) attributed lower survival in clay loam to 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 study was reported that the maximum temperature, maximum relative humidity and rainfall played an important role in the development of foot and root rot diseases of betel vine (*Piper betel* L.) (Anonymous, 2000-2006; Maiti and Sen, 1982).

Chattopadhyay and Maiti (1990) observed that the betelvine plants are cultivated under shady and humid conditions that also favor the development of many diseases including foot and root rot.

Flados (1958) found that the reduction in growth of the fungus *Sclerotium rolfsii* increase in moist soil.

Giganate (1950) stated that potatoes grown in northern and central Italy were occasionally attacked by *Sclerotium rolfsii*. The fungus affected collar region to cause collar rot, the plants turning yellow and rapidly wilting. Spherical, elliptical or irregular sclerotia of 1-3 mm in diameter were found in the affected part of tuber. The disease occurred mostly in sandy or compact clay soils and was favoured by hot moist weather.

Gondo (1962) reported the optimum soil temperature of 30^0C for mycelia and 25^0C for sclerotia.

Hari *et al*. (1991) reported that the maximum radial growth of *Sclerotium rolfsii* was observed at 30ºC at 72 hours of incubation followed by 25ºC at 96 hours of incubation and they found that the 25-30ºC temperature was the best for the growth of *Sclerotium rolfsii* collar rot of groundnut.

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

Hari *et al*., (1988) reported that, 26^0C was optimum temperature for growth of *S. rolfsii* and the maximum growth of *S. rolfsii* was at 30^0C.

Harlapur (1988) reported that the optimum soil temperature for growth and activity of *Sclerotium rolfsii* was found to be 25-30^0C and the growth was completely ceased at 45^0C.

Khan (1996) reported the incidence and severity of collar rot of sunflower in fifteen (15) varieties which were grown at 3 agro-ecological zones (AEZ) of Bangladesh. Survey was conducted during Rabi (1993-94) and Kharif-I (1994) at flowering and harvesting stages of the crop. He observed that young plants were more susceptible to collar rot. Incidence of the disease were minimum in Rabi season and a higher percentage of plants were killed in Kharif-I season.

Khati *et al*., (1983) reported that the survival of *S. rolfsii* was highest at soil moisture levels between 30-50% of water holding capacity.

Kulkarni and Kulkarni (1998) found the maximum saprophytic activity of *Sclerotium rolfsii* was 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% and above.

Manjappa (1979) reported that the sunflower isolate of *S. rolfsii*, made maximum growth at 30^0C which was significantly superior to the growth at all the temperature levels followed by 25^0C. They concluded that the optimum temperature for the growth of *S. rolfsii* was between 25-30^0C. However, there was no significant difference between the temperatures of 20^0C and 35^0C and 15^0C. Least growth was noticed at 40^0C.

Mathur and Sinha (1978) observed that the infection of *S. rolfsii* in guar was maximum (54.2%) at pH 6.6 and in gram (89.6%) at pH 5.7. Alkaline condition reduced the disease in both the crops.

Meah (1994) reported the incidence and severity of collar rot of sunflower in fifteen (15) varieties which were grown at 2 (two) agro-ecological zones (AEZ) of Bangladesh. At Bangladesh Agricultural University (BAU), Mymensingh, collar rot was prevalent throughout the crop season. All varieties at BAU were heavily affected with collar rot and 3.0-5.0% plants were killed at flowering stage.

Mollah (2012) found that 29^0C and 85% R^H had the highest influenced on the disease incidence and severity of foot and root rot of betelvine and the lowest when the temperature laid around 18.7^0C and the R^H laid around 75%.

Palakshappa (1986) studied the effect of different soil moisture levels on foot rot of betelvine caused by *S. rolfsii* and reported that, the fungus survived better at low soil moisture than at higher levels. The survival ability was the highest between 20-40% soil moisture. However, the highest saprophytic activity of the fungus was observed at 40% moisture level and the least was at 60-70% soil moisture.

Prabhu (2003) studied the effect of inoculum density on foot rot of soybean and reported that 100% pre-emergence disease incidence was noticed in 4% inoculum levels and the maximum post emergence disease incidence was noticed in 3% inoculum level. The disease intensity ranged from 36.70 to 90.00% in seed and seedling rot of soybean caused by *S. rolfsii.*

Prasad *et al.*, (1986) found the best mycelial growth of *S. rolfsii* at pH 5.0 while the best sclerotial formation was at pH 7.0. He also reported that the best mycelial growth was observed at 30 ± 0.5ºC whereas 25 ± 0.5ºC for the *sclerotial* formation.

Singh and Gandhi (1991) reported that maximum mortality of guar seedling was observed at pH 6.1 and pH 8.4 significantly reduced disease incidence of *Sclerotium rolfsii*. They also reported that the incidence of disease caused by *Sclerotium rolfsii* was 100% at 30ºC in treated seedlings of guar, while at 25ºC and 35ºC temperature seedling mortality was 95 and 83%, respectively.

Singh *et al.,* (1987) proposed that, the disease incidence varied with soil moisture and soil temperature, while a maximum of 72% disease incidence was recorded at high relative humidity and moderate temperature in case of collar rot of pigeon pea infected by *S. rolfsii*. Soil texture and pH may also be affected the survival of sclerotia. For *S. sclerotiorum*, 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). Lower survival was noticed in clay loam than in sandy loam.

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

Tu *et al*., (1991) reported that the percent germination of sclerotia of *S. rolfsii* 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.

Singh and Thapliyal (1998) reported inoculum density levels of 2.5 to 10 g kg^{-1} soil significantly increased the pre-emergence rot which ranged from 36.7 to 90% seed and seedling rot of soybean caused by *S. rolfsii*, respectively.

Weerapat and Schroeder (1966) observed that the optimum soil temperature for the growth of rice seedlings and the two strains of *S. rolfsii* were 30-35^0C. Infection occurred at all temperatures and severe disease development occurred between 25 to 35^0C. However, the severity of the disease was highest at 30^0C.

2.4. Disease incidence and severity of foot and root rot of betelvine

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

Rahman M.M. and Sultana N. (2011) found that, in Jamalpur region, the incidence and severity of Sclerotial rot of betel vine is higher comprised to rest of the surrounded areas.

2.5. 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 *S. rolfsii* was determined on the basis of their sensitivity to different fungicides.

Karthik Pandi *et al*., (2017) reported that eight isolates of *Sclerotium rolfsii* were grown in PDA medium. Isolate SFSR1 in petri plates recorded significantly maximum mycelial growth per day (31.45 mm) followed by SFSR3 and SFSR6. The isolate SFSR4 has recorded minimum mycelial growth per day (21.62 mm). In the production of sclerotia the isolate SFSR4 has produced significantly higher number of sclerotia (360 per plate).

Manu, T.G. *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.

2.6. Resistance against *Sclerotium rolfsii*

Fakir *et al*., (1991) reported that sowing of lentil during third week of November was found to reduce the incidence of collar rot and root rot caused by *Sclerotium rolfsii and Fusarium oxysporum* compared to early sowing. Artificial inoculation often selected genotypes of lentil to collar rot pathogen, *Sclerotium rolfsii* showed that all the lines were susceptible to the test pathogen.

Rakholiya and Jadeja was screened groundnut cultivars against stem and pod rot during *Kharif* 2006 and 2007 in field conditions. Fourteen groundnut cultivars *viz.*, J-11, GG-2, GG-4, GG-5, GG-6, GG-7, JL-24, TAG-24, TG-26, GG-20, GG-13, GG-11, BAU-13 and ICGV-86564 were screened for their resistance against *S. rolfsii*. Spreading type groundnut GG-11 and GG-13 were moderately resistant, while eight varieties *viz.*, J-11, GG-4, GG-6, JL-24, TG-26, TAG-24, BAU-13 and ICGV-86564 were susceptible. Four varieties *viz.*, GG-2, GG-5, GG-7 and GG-20 were highly susceptible to *S. rolfsii*.

Ramesh Amule *et al*., (2014) conducted an experiment during 2009 and 2010 to find out the most effective screening techniques for identifying host plant resistance against chickpea collar rot caused by *Sclerotim rolfsii* in pot house. Out of four techniques employed, chickpea 'grain inoculation techniques' was found the best. The minimum post emergence mortality (6.7%) occurred at 4.0% concentration of Pyraclostrobin which is significantly less comprised to control (26.8%) during the two-consecutive year of testing. Among 88 chickpeas desi genotype GNG 1958 was found resistant to disease.

Iqbal and Ahmad (2011) evaluated eleven sugar beet genotypes at National Agricultural Research Centre, Islamabad, Pakistan, during the year 2009 for their resistance against root rot caused by *Sclerotium rolfsii*. Mass culturing of pathogen was prepared through sorghum seed inoculum technique. Inoculation of eleven genotypes with *S. rolfsii* exhibited resistant response only in SD-PAK-09/07 and moderately resistance in SD-PAK-07/071. The remaining nine genotypes showed susceptible to highly susceptible response to the pathogen.

Shirsole *et al*., (2018) evaluated one hundred eighty-five chickpea entries received from AICRP (All India Coordinated Research Project) on Chickpea at Raipur under field condition during the year 2016-17 to identify sources of genetic resistant against collar rot disease incited by the fungus *Sclerotium rolfsii*. Out of 185 chickpea entries only 5 entries viz., GNG 2331, JG 2016-9605, IPC 2012-98, RVSSG-38 and GL 12003 exhibited moderately resistant

response while, the remaining were susceptible to highly susceptible for collar rot of chickpea.

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. Different cultivars exhibited significant variations in respect to their growth, yield and yield attributing characters. The sanchi cultivars recorded significantly higher monthly linear growth having larger inter-node length with larger sized leaves. The cultivar Utkal sudam produced moderately higher leaf yield (66.03 lakh ha^{-1}) with significantly larger sized leaves (173.33 cm2) having shortest inter-node length (3.98 cm).

Mohanta and Pariari (2015) studied the effect of various climatic factors on betelvine. Temperature and R^H were found to be the most important factors for variation in leaf characters in different cultivars. SimuraliJhal showed superior performance in respect to leaf length (16.45 cm^2), leaf breadth (13.76 cm^2) and leaf area (274.35 cm^2) in rainy season. The growth and chlorophyll content (SimuraliSanchi - 2.58 mg g-1 tissue) of betel leaves was maximum in SimuraliJhal.

Shivashankara *et al*., (2000) made a study on "Effect of Different Light Intensities on Growth and Yield of Betel Vine". And reported that under 35% and 60% light intensity increased the number of leaves compared to 10% light. Leaf size and internodes length were more in 35% and specific leaf weight was more in 60% light intensity. The chlorophyll content was higher in 10 percent light intensity. Betelvine required 35% of sun light for production of maximum number of quality leaves.

CHAPTER-3
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental site

3.1.1. *In vivo* Experiments

The *in vivo* experiments were conducted in the temporary built betelvine garden (Baroj) at the Central Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh.

3.1.2. *In vitro* Experiments

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

3.1.3. Soil type

The soil of the experimental site belongs to the Agro-ecological zone of "Madhupur Tract" (AEZ No. 28). It was Deep Red Brown Terrace soil and belongs to "Nodda" cultivated series. The top soil is silty clay loam in texture. Organic matter content was very low (0.82%) and soil pH varied from 5.47 - 5.63. The information about AEZ 28 (Anonymous, 1994) is stated bellow:

Characteristics of AEZ-28

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

3.2. Experimental period

The experiments were conducted during June, 2019 and July, 2020. *In vivo* experiments were done starting from June, 2019 to December, 2020.

3.3. Experimental design

Randomized Complete Block Design (RCBD) with 3 replications was used for *in vivo* experiments.

3.4. Materials

3.4.1. Equipment and instruments

The common laboratory equipments and instruments used in the experiments such as, autoclave, hot air oven, incubator, laminar air flow chamber, refrigerator, electronic weighing balance, research microscope, physical balance, etc.

3.4.2. Glassware

Different types of Corning and Borosil glassware were used in experimental work. The common glasswares were petri-plates, test tubes, conical flasks, measuring cylinder, glass rods, coverslips, beakers, pipette, etc.

3.4.3. Other materials

Miscellaneous materials viz., marking pencils, rubber bands, sticky labels, muslin cloth, blotting paper, inoculating needle, forceps, spirit lamp, filter paper, disc cutter, funnel, glass jar, thin wrapping tape, polypropylene bags, mercuric chloride and sodium hypochlorite stock solutions etc. were used.

3.5. Experiments

Experiments were conducted during the study period in order to screening of resistant variety against foot and root rot disease of betelvine**.** The experiments were as follows:

1. Isolation, purification, identification and pathogenicity test of causal organism from diseased samples collected from different betelvine growing regions of Bangladesh.
2. Screening of resistant betelvine cultivars available in the country against foot and root rot disease of betelvine.

3.5.1.1. Isolation and identification of pathogen

The diseased samples (betelvine) were collected from different “baroj” from major betelvine growing areas. The pathogens associated with the foot rot disease of betel vine were isolated following tissue planting method (Tuite 1969, Mian,1995). At first the diseased plant parts (basal stem) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into small pieces (5 cm) from advancing end of the lesions. The cut portion were surface sterilized with 1% chlorox (NaOCl) for 5 minutes, and rinsed with sterilized water for 3 times. Surface sterilized plant pieces were plated on PDA media in 90 mm petridishes and incubated at room temperature of 25 ± 2^{o} C for 3-5 days and examined daily for any fungal growth. After growing embeded cottony mycelium, re-isolation was done by mycelial tip culture method. The pathogen was identified with its key characteristics (Ellis, 1971). Pathogenicity test of the isolate of *S. rolfsii* was done by Koch’s Postulate method (Mitchell *et al*., 1997).

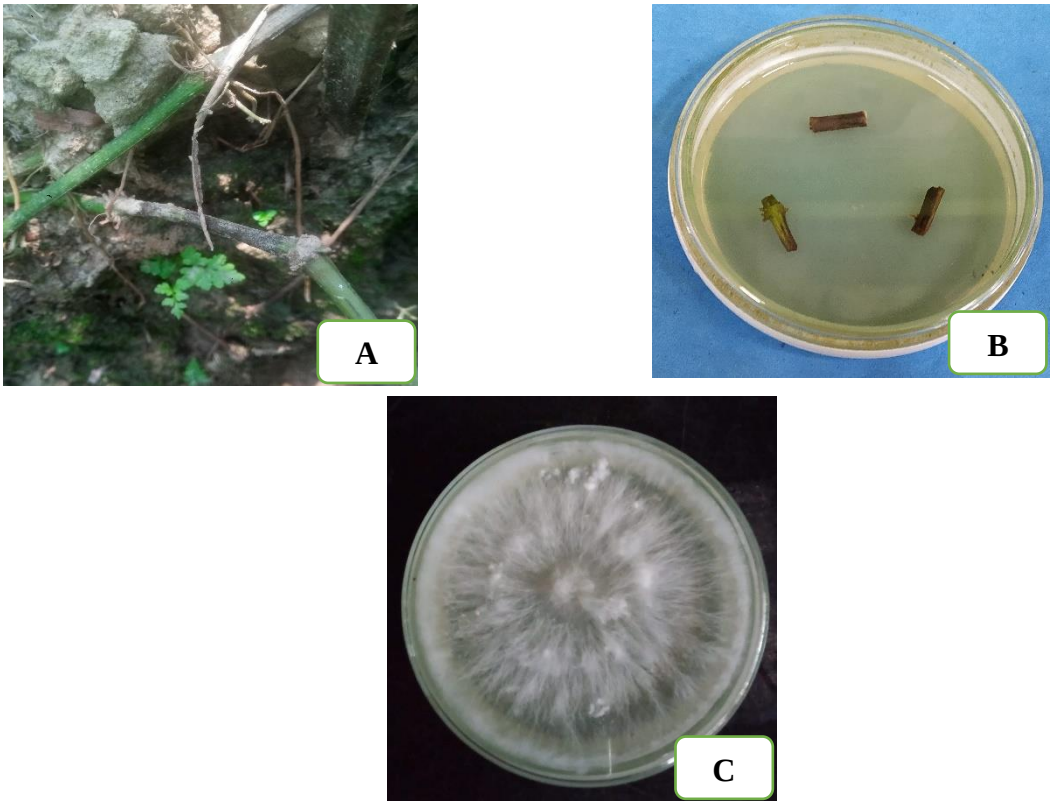

Plate 1. Isolation and identification procedure of *Sclerotium rolfsii*, A. infected plant parts, B. Piece of infected part of plant put on medium in petri-plate for pathogen grow & C. Pure culture of *Sclerotium rolfsii* on PDA plate.

3.5.1.2. Preparation of Potato Dextrose Agar (PDA) media

PDA media were used to isolate and culture the isolated causal organism. Composition of PDA media preparation:

Peeled potato (decoction)	: 200g
Dextrose	: 20g
Agar	: 20g
Distilled water	: 1000 ml

At first, 200g potato was taken and cleaned followed by washing with tap water. Then the potato was peeled and cut in a small slice and boiled about 30-40 minutes in one-liter water. When potato become soft, it was sieved. After that, 20g dextrose and with a few minutes' interval, 20g Agar were mixed slowly with it and stirred properly so that it cannot be coagulated. Then the media was sterilized in an autoclave at temperature of 121^0C temperature under 15 PSI pressure for about 30 minutes. After autoclaving, the media was kept 20-30 minutes for cooling in laminar air flow cabinet.

3.5.2. Screening of betelvine resistant cultivars available in the country against *Sclerotium rolfsii* causing foot and root rot disease of betelvine

Eight betelvine cultivars were collected from different betelvine growing areas of the country. The cultivars used in the experiment to screen out the resistant or tolerant cultivars against the pathogen *S. rolfsii* causing foot and root rot disease of betelvine (Table 1). The study was conducted in *in vivo* condition in a betelvine garden (baroj). The baroj was constructed in the experimental field at the Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh.

The observation was made on disease incidence/severity, yield and yield attributing parameters of the betelvine from each treatment. The experiments were conducted during the year June, 2019 to December, 2020.

Table 1. List of betelvine cultivars used in screening experiment against *Sclerotium rolfsii* causing foot and root rot disease of betelvine

Sl. No.	Accession No.	Name of Cultivars	Collection sources/area
01	PB 001	Chalitaguti Pan	Debidwer, Comilla
02	PB 002	Chuadanga Pan	Chuadanga Sadar, Chuadanga
03	PB 003	Moheskhali Pan	Moheskhali, Chattagram
04	PB 004	Laldingi Pan	Pakundia, Kishoregonj.
05	PB 005	Satkhira Pan	Kaligong Upazilla, Satkhira
06	PB 006	BARI Pan-1	Spices Research Centre, BARI, Bogura.
07	PB 007	BARI Pan-2	Spices Research Centre, BARI, Bogura.
08	PB 008	BARI Pan-3	Spices Research Centre, BARI, Bogura.

3.5.2.1. Land preparation

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 sun for two months. During the first week of June, 2019 two or three ploughing was done for well pulverized tilth condition. Weeds and stubbles were removed. Provide drainage trenches of 90 cm width by 15 cm depth in between two adjacent beds.

3.5.2.2. Raising of betelvine cuttings

Collected forty centimeter long, healthy with five nodes betelvine cuttings were grown in 16 inches dia earthen pots under the boraj with necessary care and management. One to two internodes below the bud point are dipped in soil, kept touching with surface soil. Pots were 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 by a polythenc sheet for 48 hours for sterilization. After 7 days, surface sterilized earthen pots of 14 inches dia were filled up with the sterilized soil (Dashgupta, 1988).

3.5.2.3. Fertilizers and manures

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

3.5.2.4. Intercultural operations

Irrigation was given as per requirement of the delicate betelvine plant with regular intervals. Weeding and mulching were done as and when required to keep the betelvine plant free from weeds and for better soil aeration and conservation of soil moisture. Betelvine plant were tide with stick when nessasary.

3.5.2.5. Inocula preparation and inoculation with the causal pathogen

The isolate pathogen *Sclerotium rolfsii* were multiplied on barley grains (Gupta and Kolte, 1982). Barley grains were pre-soaked in 2% sucrose solution overnight and drained. This was transferred into 250 ml flasks @ 80 g and autoclaved at 121^0C temperature, 15 lb psi for 20 minutes. The flasks were allowed to cool at room temperature and were inoculated with five mm discs of 3 to 4 days old culture of *S. rolfsii* grown on PDA. Seven discs per flask were added and flasks were incubated for three weeks at 25 ± 2^0C.

After six months of plantation each variety was inoculated by causal pathogen (*S. rolfsii*) separately. The plants were prepared for inoculation and were done by removing top soil within 5 cm of the stem to a depth of 2 cm. A table spoon of inoculam was placed in direct contact of entire circumference of the exposed stem. Finally, the inoculum was lightly covered with top soil for infection. (Fery *et al*., 2002). The symptomalogy was studied to test the pathogenicity of the inoculated organism.

Plate 2. Multiplication of *S. rolfsii*

3.5.2.6. Data collection

The data were recorded on following parameters:

i. Morphological features of the cultivars

ii. Days required for appearance of 1st disease symptom

iii. Disease incidence/severity

iv. Disease reaction

Disease reaction was recorted following host responsed (Manandhar *et al*., 2016):

Percent of disease incidence	Host response
0-10	Resistant (R)
11-30	Moderately resistant (MR)
31-60	Moderately susceptible (MS)
61-100	Susceptible (S)

3.6. Statistical analysis of data

Randomized Complete Block Design (RCBD) with 3 replications was used for *in vivo* experiments. Duncan's Multiple Range Test (DMRT) was explored for comparison of means (Gomez and Gomez, 1983). Statistical package program 'Statistic 10' was used for analysis of the experimental data.

CHAPTER-4
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Isolation and identification of Pathogen

The causal fungus isolated from typical diseased samples collected from different betelvine growing regions of Bangladesh was identified as *Sclerotium rolfsii* based on its key characteristics (CMI Description No. 410).

On PDA medium, the fungus grew rapidly covering the Petrid ishes (9 cm) within 7 days. The fungus produced fluffy whitish mycelium. The microscopic observation of fungal culture revealed that the mycelium was hyaline, thin walled, superficial, septate, profusely branched with clamp connection. A huge number of Sclerotia were produced in the Patri plate after 15-20 days of inoculation. Sclerotia were globose to oval, thick walled like mustard seed. The present findings are kept in with the findings of Jahan (2016) and Rahman (2017).

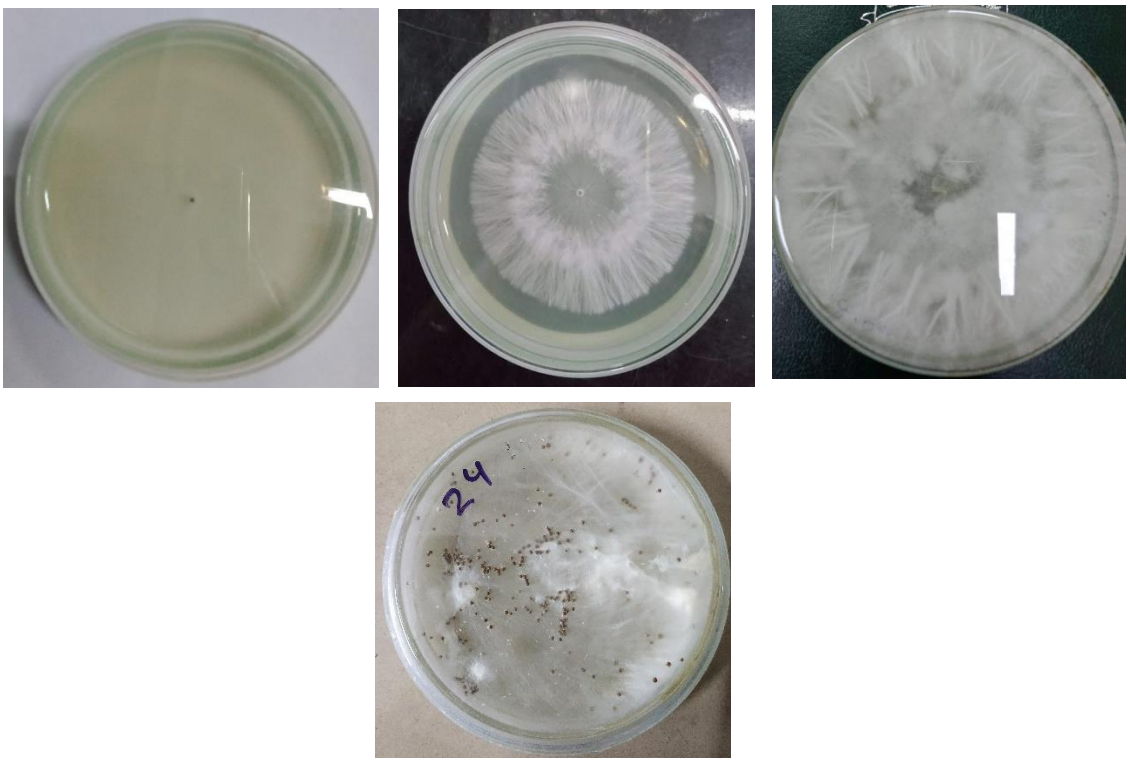

Plate 3. Colony texture and formation of Sclerotia on PDA media

4.1.1. Results of artificial inoculation of *Sclerotium rolfsii*

Sclerotium rolfsii isolated from diseased betel vine sample was subjected to pathogenicity tests by Koch's postulates. On inoculation to healthy plant in the nursery, the inoculated plants exhibited typical symptoms. Five days after inoculation by mycelium of the *Sclerotium rolfsii*, the betel vine plants exhibited white mycelial growth on soil surface near the plant base. On seventh day of inoculation, white mycelial mat was formed which spread rapidly towards the plant base. Immature white rounded sclerotia were also observed on soil surface near the plant base within fifteen days. The forming sclerotia were gradually turned blue to black and started to germinate producing white mycelia. Finally, the artificially inoculated plants developed characteristics symptoms resulting foot and root rot disease.

4.1.2. Result of re-isolation

On re-isolation from the artificially inoculated diseased betel vine plant, it was found that the pathogen exhibited same characteristics in respect of mycelia and sclerotia on PDA culture as found earlier on isolation from naturally infected betel vine plant caused by *Sclerotium rolfsii*. Thus, the reisolated pathogen was *Sclerotium rolfsii* that was responsible for causing foot and root rot of betel vine.

4.1.3. Pathogenicity Test

Symptomology study

The leaves and shoots of foot and root rot infected plants turned yellow, withered and finally dried out to a pale brown colour. The fungus found to attack the roots and stem near the soil level. Black lesions were 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. In a diseased plant, the whole underground portion got more or less rotten. The soft

tissues of old roots and the inter-nodal portion of the cuttings were found completely decomposed, leaving only the fibrous portion.

Plate 4. A. Pathogen inoculated on healthy plant, B. Mycelia formation on inoculated earthen pot, C. Susceptible and resistant plant of experiment and D. Lesion on foot of betelvine plant

4.2. Evaluation of betelvine cultivars available in the country against *Sclerotium rolfsii* causing foot and root rot disease of betelvine by different growth and yield contributing characters

Eight different betelvine cultivars viz. PB 001 (Chalitaguti Pan), PB 002 (Chuadanga Pan), PB 003 (Moheskhali Pan), PB 004 (Laldingi Pan), PB 005 (Satkhira Pan), PB 006 (BARI Pan-1), PB 007 (BARI Pan-2), PB 008 (BARI Pan-3), were assayed against the *Sclerotium rolfsii* causing foot and root rot disease in betelvine orchard (Pan baroj) in earthen pot. Results were compiled based on physio-morphological features, days required for visual expression of mycelia on above ground, days required for appearance of 1st disease symptom, disease reaction and yield & yield contributing characters (Table 1, 2, 3, 4 & 5).

4.2.1. Vegetative growth parameters

4.2.1.1. Vine elongation/ month

The vegetative growth parameters and morphological features of different cultivars of betelvine varied remarkably. The maximum vine elongation per month (90.97 cm) was recorded in PB 003 followed by PB 008 (79.17 cm), PB 007 (77.17 cm), PB 001 (76.30 cm) and PB 004 (75.87 cm). The lowest vine increment (46.87 cm) was recorded in PB 006 (Table 1).

4.2.1.2. Length of internode (cm)

The maximum length of internode (8.27 cm) was recorded in PB 004 that was significantly similar with PB 001 (7.53 cm) and the minimum length (4.50 cm) and (5.50 cm) was in PB 006 and PB 002 (Table 1).

4.2.1.3 Girth of vine (cm)

The maximum vine girth (1.63 cm) was recorded in PB 007 and the minimum vine girth (0.93 cm) was in PB 002 (Table 1).

Table 1. Vegetative growth parameters (vine elongation and girth) of different betel vine cultivars grown in orchard (Pan baroj) at SAU Campus

Accession no. of Betelvine cultivars	Vine elongation /month (cm)	Length of internode (cm)	Girth of vine (cm)
PB 001 (Chalitaguti pan)	76.30 ab	7.53 ab	1.50 ab
PB 002 (Chuadanga pan)	63.33 bc	5.50 bc	0.93 b
PB 003 (Moheskhali pan)	90.97 a	6.90 ab	1.53 ab
PB 004 (Laldingi pan)	75.87 ab	8.27 a	1.53 ab
PB 005 (Satkhira pan)	54.17 bc	7.03 ab	1.53 ab
PB 006 (BARI pan-1)	46.87 c	4.50 c	1.17 ab
PB 007 (BARI pan-2)	771.7 ab	6.37 a-c	1.63 a
PB 008 (BARI pan-3)	79.17 ab	6.50 a-c	1.33 ab
CV (%)	**22.12**	**19.74**	**27.85**
LSD	**27.31**	**2.27**	**0.68**

4.2.1.4. Leaf size (cm)

Significantly the highest length of leaf (22.07 cm) was recorded in PB 005 and the lowest (13.17 cm) was recorded in PB 006. The leaf breadth (13.03 cm) was recorded the highest in PB 003 and the lowest (6.93 cm) in PB 006 (Table 2).

4.2.1.5. Petiole size (cm)

The length of petiole was the highest (8.63 cm) in PB 008 which was significantly similar with PB 003 (7.90 cm) and PB 004 (7.43 cm). The lowest length of petiole (4.03 cm) was recorded in PB 002. The petiole breadth (1.13cm) was recorded the highest in PB 004 and the lowest (0.43 cm) in PB 008 (Table 2).

Table 2. Vegetative growth parameters (leaf and petiole) of different betel vine cultivars grown in orchard (Pan baroj) at SAU Campus

Accession no. of betelvine cultivars	Leaf size with petiole (cm)		Petiole size (cm)	
	Length	Breadth	Length	Breadth
PB 001 (Chalitaguti pan)	21.20 a	10.97 a-c	6.27 bc	0.83 b
PB 002 (Chuadanga pan)	17.50 bc	10.37 bc	4.03 d	0.60 c
PB 003 (Moheskhali pan)	21.53 a	13.03 a	7.90 ab	1.10 a
PB 004 (Laldingi pan)	21.33 a	11.97 ab	7.43 ab	1.13 a
PB 005 (Satkhira pan)	22.07 a	12.67 ab	7.23 ab	0.83 b
PB 006 (BARI pan-1)	13.17 d	6.93 d	4.63 cd	0.43 c
PB 007 (BARI pan-2)	17.23 c	9.20 cd	6.40 bc	0.53 c
PB 008 (BARI pan-3)	20.80 ab	9.10 cd	8.63 a	0.43 c
CV (%)	**10.20**	**13.67**	**19.30**	**16.90**
LSD	**3.46**	**2.52**	**2.22**	**0.23**

4.2.2. Yield and yield contributing characters

4.2.2.1. 100-Petiole weight, fresh weight of 100 leaf

The 100-petiole weight (83.33 g) was recorded the highest in PB 004 followed by PB 003 (80.00 g) and the lowest 100 petiole weight (38.33 g) was recorded in PB 006. The fresh weight of 100 leaf. with petiole was recorded the highest in PB 004 (553.33 g) followed by PB 003 (476.67 g) and the lowest fresh leaf weight (206.67 g) was recorded in PB 006. The PB 006 produced significantly the highest number (23.00) of leaves per metre vine followed by PB 002 (18.00) and the lowest number (13.67) of leaves per metre vine was recorded in PB 003(Table 3).

4.2.2.2. Yield of betelvine leaf

The leaf numbers of betelvine cultivars essayed in the experiment was ranged from 282.00 to 414.00 per plant per year. The highest leaf number (414) per plant per year was recorded in PB 006 followed by PB 002 (324.00) and PB 004 (300) while the lowest number (282) was counted in PB 003(Table 3).

Table 3. Yield and yield contributing characters of different betel vine cultivars grown in orchard (Pan baroj) at SAU campus

Accession no. of betelvine cultivars	100 Petiole wt. (g)	Fresh wt. of 100 leaf (g)	No. of Leaf/ Meter Vine	Yield (No. of Leaf)/ Plant/Year
PB 001 (Chalitaguti pan)	59.00 cd	398.33 c	16.00 bc	288.00 bc
PB 002 (Chuadanga pan)	63.33 bc	290.00 de	18.00 b	324.00 b
PB 003 (Moheskhali pan)	80.00 ab	476.67 b	13.67 c	282.00 c
PB 004 (Laldingi pan)	83.33 a	553.33 a	16.67 bc	300.00 bc
PB 005 (Satkhira pan)	61.67 c	473.33 b	16.00 bc	288.00 bc
PB 006 (BARI pan-1)	38.33 e	206.67 f	23.00 a	414.00 a
PB 007 (BARI pan-2)	41.67 de	243.33 ef	16.00 bc	288.00 bc
PB 008 (BARI pan-3)	73.33 abc	310.00 d	16.67 bc	300.00 bc
CV (%)	**16.45**	**8.16**	**7.71**	**7.71**
LSD	**18.03**	**52.71**	**2.33**	**41.91**

4.3. Physio-morphological features of the betelvine cultivars

PB 001 (Chalitaguti pan)

The vine of PB 001 (Chalitaguti pan) was found greenish with light pinkish line in colour. The leaf was observed soft after maturation, green coloured, cordate in shape, acute in tip and less pungent (Table 4).

PB 002 (Sanchi pan)

The vine of PB 002 (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 (Table 4).

PB 003 (Moheskhali Pan)

The vine colour was found green in cultivar PB 003 (Moheskhali Pan). The leaf seemed soft, dark green coloured, cordate in shape and medium pungent (Table 4).

PB 004 (Laldingi pan)

The colour of PB 004 (Laldingi pan) vine was found green. The leaf was green coloured, less soft after maturation, cordate in shape, acute in tip and medium pungent (Table 4).

PB 005 (Satkhira Pan)

The vine colour of PB 005 (Satkhira 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 (Table 4).

PB 006 (BARI Pan-1)

Vine colour was found green in PB 006 (BARI Pan-1). The leaf was soft after maturation, light green coloured, cordate to ovate in shape, acuminate in tip and medium pungent (Table 4).

PB 007(BARI Pan-2)

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 (Table 4).

PB 008 (BARI Pan-3)

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 008(BARI Pan-3) (Table 4).

Table 4. 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 (Chalitaguti pan)	greenish with light pinkish line	Green	Cordate	Acute	Leaf soft	Less pungent
PB 002 (Chuadanga pan)	Violet	Dark green	Cordate to ovate	Acute	Leaf soft	Pungent aroma
PB 003 (Moheskhali pan)	Green	Dark green	Cordate	Acute	Leaf soft	Medium pungent
PB 004 (Laldingi pan)	Green	Green	Cordate	Acute	less soft	Medium pungent
PB 005 (Satkhira pan)	Dark green	Light green	Cordate	Acute	Leaf soft	Highly pungent
PB 006 (BARI pan-1)	Green	Light green	Cordate to ovate	Acuminate	Leaf soft	Medium pungent
PB 007 (BARI pan-2)	Violet	Dark green	Cordate	Acute	Leaf soft	Medium pungent
PB 008 (BARI pan-3)	Dark green	Green	Cordate	Acute	Leaf soft	No pungent

Plate 5. Pictorial view of different betelvine cultivars

4.4. Evaluation of different betelvine cultivars against foot and root rot disease by disease incidence

4.4.1. Numbers of days for mycelia formation

Analyzed pooled data of two consecutive experiment showed significant variations of the screening parameters. Soil infested with *S. rolfsii* exhibited mycelia growth on soil surface and around the foot zone of the plants after 2 days of inoculation (plate 4.B). In severely infected plants, soft watery rot symptoms and brown lesions advanced above the soil level appeared at the collar region (plate 4.D). On the lesions, white mycelial growth having white and brown sclerotia depending on the fungal maturity was observed.

4.4.2. Days required for appearance of 1st disease symptom

The time interval (days) required for appearance of 1st disease symptoms after inoculation among the betelvine cultivars differed significantly compared to control. The lowest incubation period (8 days) required for the cultivars PB 007 (BARI Pan-2) and PB 008 (BARI Pan-3). The highest incubation period (14 days) was required for PB 006 (BARI Pan-1). No symptoms were seen in the cultivars PB 004 (Laldingi pan) (Table 5).

4.4.3. Disease incidence

Among the 08 betelvine cultivars, no disease incidence (0%) was observed in case of cultivars PB 002 (Chuadanga pan), PB 004 (Laldingi pan) and PB 006 (BARI pan-1) whereas PB 008 (BARI pan-3) shown maximum (100 %) disease incidence against the foot and root rot disease at 10 days after inoculation of pathogen (Table 5).

At 15 days after inoculation of pathogen, the cent percent disease incidence (100 %) was observed in cultivars PB 002 (BARI Pan-2), PB 002 (BARI Pan-3) and no disease incidence (0%) was observed in PB 004.

At 20 days after inoculation, the highest disease incidence was observed in PB 001(60%) followed by PB 005 (40%) and the lowest (0%) disease incidence was

recorded in PB 004.

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 lowest (0%) disease incidence was recorded in PB 004 (Laldingi).

At 30 DAI, the disease incidence of 08 different betelvine cultivars showed remarkable difference among them. No disease incidence (0%) was noticed in case of PB 004 (Laldingi). The highest disease incidence (100%) was recorded in case of PB 007 and PB 008 followed by PB 001 (60%), and PB 005 (40%). The minimum (20%) disease incidence was recorded in PB 002, PB 003, and PB 006 (Table 5).

4.4.4. Disease reactions

Based on the disease responses (disease incidence) of the evaluated betelvine cultivars, they are categorized as Resistant (R), Moderately resistant (MR) Moderately susceptible (MS) and Susceptible (S). Among the cultivars, only one cultivar PB 004 (Laldingi pan) showed resistant reaction (R), while 3 cultivars viz. PB 002 (Chuadanga pan), PB 003 (Moheskhali pan) and PB 006 (BARI pan-1) showed moderately resistant reactions (MR). Two cultivars viz. PB 001 (Chalitaguti pan) and PB 005 (Satkhira pan) showed moderately susceptible reactions (MS) and rest of the cultivars showed susceptible reactions (Table 5).

Table 5. Disease reactions of different betelvine cultivars against foot and root rot of betelvine caused by *Sclerotium rolfsii*

Accession no.	Days required for appearance of 1st disease symptom	Percent disease incidence					Disease reactions
		10 DAI	15 DAI	20 DAI	25 DAI	30 DAI	
PB 001 (Chalitaguti pan)	10	20.00	60.00	60.00	60.00	60.00	MS
PB 002 (Chuadanga pan)	11	0.00	20.00	20.00	20.00	20.00	MR
PB 003 (Moheskhali pan)	10	20.00	20.00	20.00	20.00	20.00	MR
PB 004 (Laldingi pan)	Not seen	0.00	0.00	0.00	0.00	0.00	R
PB 005 (Satkhira pan)	10	20.00	40.00	40.00	40.00	40.00	MS
PB 006 (BARI pan-1)	14	0.00	20.00	20.00	20.00	20.00	MR
PB 007 (BARI pan-2)	08	40.00	100.00	100.00	100.00	100.00	S
PB 008 (BARI pan-3)	08	100.00	100.00	100.00	100.00	100.00	S

DISCUSSIONS

Betelvine (*Piper betle*L.) having the heart shaped deep green leaves are an important perennial horticultural crop of aesthetic and commercial values. In our social culture of the country, the betel leaves are generally offered to the guests as the symbol of hospitality. Thus, a group of marginal farmers cultivate betelvine year-round and enjoy a continuous source of earning from once built betelvine garden /Pan boroj. Such an important economic crop found to be affected by various diseases and disorders. Among them foot and root rot disease caused by *Sclerotium rolfsii* is treated as devastating disease.

In the present investigation eight different betelvine cultivars viz. PB 001 (Moheskhali Pan), PB 002 (BARI Pan-1), PB 003 (Chuadanga Pan), PB 004 (Satkhira Pan), PB 005 (Chalitaguti), PB 006 (BARI Pan-2), PB 007 (BARI Pan-3), PB 008 (Laldingi Pan), were assayed against the *Sclerotium rolfsii* causing foot and root rot disease in betelvine orchard (Pan baroj) in earthen pot. Results were compiled based on physio-morphological features, days required for visual expression of mycelia on above ground, days required for appearance of 1st disease symptom, disease reactions, yield & yield contributing characters. The vegetative growth parameters and morphological features of different cultivars of betelvine varied remarkably. The maximum vine increment per month (90.97 cm) was recorded in PB 003 and the lowest vine increment (46.87 cm) was in PB 006. The maximum length of internode (8.27 cm) was recorded in PB 004 and the minimum length (4.50 cm) was in PB 006. The maximum vine girth (1.63 cm) was recorded in PB 007 and theminimum vine girth (0.93 cm) was in PB 002. Significantly the highest length of leaf (22.07 cm) was recorded in PB 005 and the lowest (13.17 cm) was recorded in PB 006. The leaf breadth (13.03 cm) was recorded the highest in PB 003 and the lowest (6.93 cm) in PB 006. The length of petiole was the highest (8.63 cm) in PB 008 and the lowest length of petiole (4.03 cm) was in PB 002. The petiole breadth (1.13cm) was recorded the highest in PB 004 and the

lowest (0.43 cm) in PB 008. The 100-petiole weight (83.33 g) was recorded the highest in PB 004and the lowest weight (38.33 g) recorded in PB 006. The fresh weight of 100 leaf. with petiole was recorded the highest in PB 004 (553.33 g) and the lowest weight (206.67 g) was in PB 006. The PB 006 produced significantly the highest number (23.00) of leaves per metre vine and the lowest number (13.67) of leaves per metre vine was recorded in PB 003. Leaf number per plant per year was recorded the highest in PB 006 (414.00 no.) and the lowest in PB 003 (282.00 no.). Experiment of six months of plantation each cultivar was inoculated by causal pathogen (*S. rolfsii*) separately. The time interval (days) required for appearance of 1st disease symptoms after inoculation among the betelvine cultivars differed significantly compared to control. The lowest incubation period (8 days) required for the cultivars PB 007 (BARI Pan-2) and PB 008 (BARI Pan-3). The highest incubation period (14 days) was required for PB 006 (BARI Pan-1). No symptoms were seen in the cultivars PB 004 (Laldingi pan). In the experiment of screening of betelvine cultivars, the disease incidence ranged from 0.00% - 100%. Among the 08 betelvine cultivars PB 004 (Laldingi pan) was found resistant (R), while three others cultivars were found moderately resistant (MR), two others found moderately susceptible (MS) and two were found susceptible against *S. rolfsii* causing foot and root rot of betelvine.

Hafiz *et. al*., (2019) while working with 13 accession of betelvine reported that soil infestation with *S. rolfsii* exhibited mycelial growth on soil surface and around the root zone after 2 days of inoculation. He noticed that 1st disease appeared after 8 days of inoculation in the accessions PB 006, PB 005, PB 009 and PB 006, which indicated that those accessions possess susceptibility against foot and root rot disease. On the other hand, in case of PB 001 (Laldingi), PB 011 (Jhalpan) and PB 013 (Gayasur) exhibited first disease symptom after 22 days of inoculation which indicated that those accessions possess resistance against the foot rot disease caused by *S. rolfsii*.

Hafiz *et al*., (2019) also reported that among 13 different germplasms, the lowest disease incidence and severity was shown by PB 001(Laldingi pan) and was designated as resistant (R) cultivars based on disease reactions against *S. rolfsii* causing foot and root of betelvine.

CHAPTER-5
SUMMARY AND CONCLUSION

CHAPTER VI
SUMMARY AND CONCLUSION

The investigation was carried out to search out resistant cultivars of betelvine available in the country for the management of foot and root rot disease of betelvine. The experiments were conducted in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the years from 2019 to 2020. The pathogens *Sclerotium rolfsii* were found to be involved as causal organisms of the disease. This pathogen attacked and usually ruined the betelvine garden.

Eight different betelvine cultivars viz. PB 001 (Moheskhali Pan), PB 002 (BARI Pan-1), PB 003 (Chuadanga Pan), PB 004 (Satkhira Pan), PB 005 (Chalitaguti), PB 006 (BARI Pan-2), PB 007 (BARI Pan-3), PB 008 (Laldingi Pan), were assayed against the *Sclerotium rolfsii* causing foot and root rot disease in betelvine orchard (Pan baroj) in earthen pot. Results were compiled based on physio-morphological features, days required for visual expression of mycelia on above ground, days required for appearance of 1st disease symptom, disease reactions, yield & yield contributing characters. The vegetative growth parameters and morphological features of different cultivars of betelvine varied remarkably. The maximum vine increment per month (90.97 cm) was recorded in PB 003 and the lowest vine increment (46.87 cm) was in PB 006. The maximum length of internode (8.27 cm) was recorded in PB 004 and the minimum length (4.50 cm) was in PB 006. The maximum vine girth (1.63 cm) was recorded in PB 007 and the minimum vine girth (0.93 cm) was in PB 002. Significantly the highest length of leaf (22.07 cm) was recorded in PB 005 and the lowest (13.17 cm) was recorded in PB 006. The leaf breadth (13.03 cm) was recorded the highest in PB 003 and the lowest (6.93 cm) in PB 006. The length of petiole was the highest (8.63 cm) in PB 008 and the lowest length of petiole (4.03 cm) was in PB 002. The petiole breadth (1.13cm) was recorded the highest in PB 004 and the lowest (0.43 cm) in PB 008. The 100 petiole weight (83.33 g) was recorded the highest in PB 004and the lowest weight (38.33 g) recorded in PB 006. The fresh weight of 100 leaf. with petiole was recorded the

highest in PB 004 (553.33 g) and the lowest weight (206.67 g) was in PB 006. The PB 006 produced significantly the highest number (23.00) of leaves per metre vine and the lowest number (13.67) of leaves per metre vine was recorded in PB 003. Leaf number per plant per year was recorded the highest in PB 006 (414.00 no.) and the lowest in PB 003 (282.00 no.). Experiment of six months of plantation each cultivar was inoculated by causal pathogen (*S. rolfsii*) separately. The time interval (days) required for appearance of 1st disease symptoms after inoculation among the betelvine cultivars differed significantly compared to control. The lowest incubation period (8 days) required for the cultivars PB 007 (BARI Pan-2) and PB 008 (BARI Pan-3). The highest incubation period (14 days) was required for PB 006 (BARI Pan-1). No symptom was noticed in the cultivars PB 004 (Laldingi pan). In the screening experiment of betelvine cultivars against foot and root rot disease, the disease incidence ranged from 0.00% - 100%. Among the 08 betelvine cultivars PB 004 (Laldingi pan) showed resistant (R) reactions, while three others cultivars viz. PB 002 (Chuadanga pan), PB 003 (Moheskhali pan) and PB 006 (BARI pan-1) were found moderately resistant (MR). Two cultivars viz. PB 001 (Chalitaguti pan) and PB 005 (Satkhira pan) showed moderately susceptible (MS) reactions and the rest cultivars showed susceptible (S) reaction against *S. rolfsii* causing foot and root rot of betelvine. From the findings of the present investigation, the betelvine growers are suggested to use the betelvine cultivars PB 004 (Laldingi pan) against foot and root rot disease of betelvine.

Works on searching of betelvine cultivars against foot and root rot of betelvine are lacking in the country. The present findings regarding the resistance of betelvine cultivars might be a potential technology to the betelvine growers of the country.

- *S. rolfsii* was confirmed as the causal organism of foot and root rot of betelvine by Koch's Postulates.
- As per growth parameters PB 004 (Laldingi pan) showed the promising performance in respect of length of internode (8.27 cm), petiole breadth (1.13cm), 100-petiole weight (83.33 g), fresh weight of 100 leaf with petiole (553.33 g).
- In screening experiment, the local cultivars of Kishoregonj PB 004 (Laldingi pan) graded as resistant wild. PB 002 (Chuadanga pan), PB 003 (Moheskhali pan) and PB 006 (BARI Pan-1) were found moderate resistant.
- As the *S. rolfsii* is a soil borne pathogen and its survival in soil, depends on various soil condition/ soil type. Therefore, zonal trials for consecutive years need to be carried out to confirm the present findings.

CHAPTER-6
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CHAPTER VI

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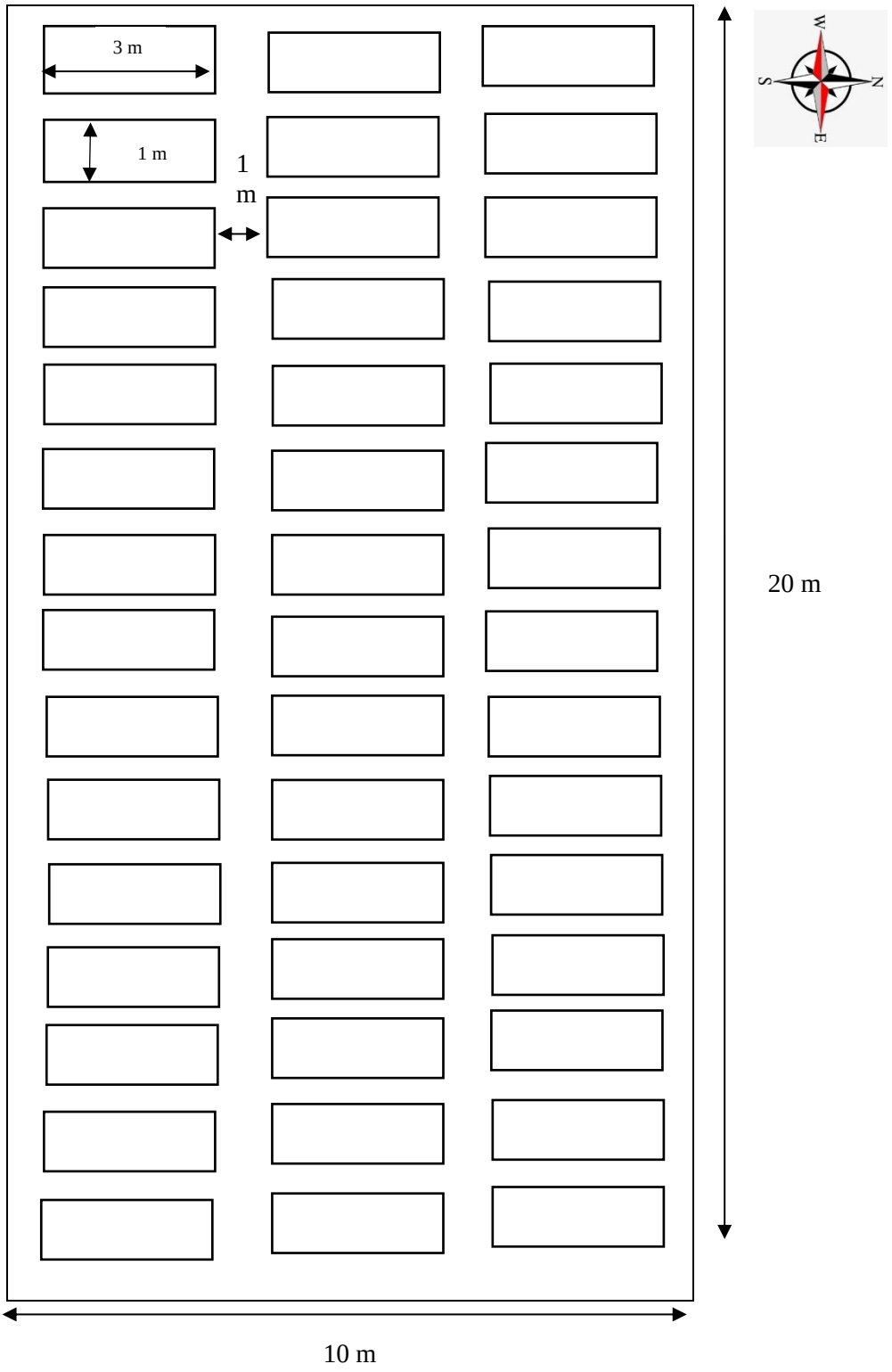
CHAPTER-7
APPENDICES

CHAPTER VII

APPENDICES

Appendix I: Experimental Layout for betelvine garden



Appendix II: Nutritional Composition of fresh betel leaf

Sl. No.	Constituents	Approximate composition
01	Water	85-90%
02	Protein	3-3.5%
03	Fat	0.4-1.0%
04	Minerals	2.3-3.3%
05	Fibre	2.30%
06	Chlorophyll	0.01-0.25%
07	Carbohydrate	0.5-6.10%
08	Nicotinic acid	0.63-0.89 mg/100g
09	Vitamin C	0.005-0.01%
10	Vitamin A	1.2-2.9 mg/100g
11	Thiamin	10-70 µg/100g
12	Riboflavin	1.9-30 µg/100g
13	Tannin	0.1-1.3%
14	Nitrogen	2.0-7.0%
15	Phosphorus	0.05-0.6%
16	Potassium	1.1-4.6%
17	Calcium	0.2-0.5%
18	Iron	0.005-0.007%
19	Iodine	3.4 µg/100g
20	Essential oil	0.08-0.2%
21	Energy	85-90%

Source: Guha, 2006.

Appendix III: Some pictural view during field work and Lab work