EVALUATION OF BETEL VINE CULTIVARS AGAINST *SCLEROTIUM ROLFSII* CAUSING FOOT AND ROOT ROT DISEASE

SAMIA TASNIM

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

SHER-E-BANGLA NAGAR, DHAKA-1207

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EVALUATION OF BETEL VINE CULTIVARS AGAINST *SCLEROTIUM ROLFSII* CAUSING FOOT AND ROOT ROT DISEASE

BY

SAMIA TASNIM
Registration No: 15-06485

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Approved by:

…..…….	.....……..……..
(Dr. Md. Rafiqul Islam)	**(Dr. F. M. Aminuzzaman)**
Professor	Professor
Department of Plant Pathology	Department of Plant Pathology
Supervisor	Co-supervisor

……
(Abu Noman Faruq Ahmmed)
Professor & Chairman
Examination Committee
Department of Plant Pathology

Dr. Md. Rafiqul Islam
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Cell: +8801711937902
Email: rafiqsau64@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled**, "EVALUATION OF BETEL VINE CULTIVARS AGAINST *SCLEROTIUM ROLFSII* CAUSING FOOT AND ROOT ROT DISEASE"** 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 results of a piece of bona fide research work carried out by **SAMIA TASNIM** bearing Registration No**. 15-06485** 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: 30 July, 2022
Place: Dhaka, Bangladesh

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Prof. Dr. Md. Rafiqul Islam
Supervisor
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Dedicated
To
My Beloved Parents
&
Grandparents

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
Colony Forming Unit	CFU
Centimeter	cm
Commonwealth Mycological Institute	CMI
Complete Randomized Design	CRD
Coefficient of Variance	CV
Days After Inoculation	DAI
Degree Centigrade	°C
Duncan's Multiple Range Test	DMRT
Gram	g
Hectare	ha
Hour	hr
Journal	J.
Kilogram	kg
Least Significant Difference	LSD
Litre	L
Metric Ton	MT
Millimeter	mm
Milliliter	ml
Namely	viz.
Negative Logarithm of Hydrogen Ion Conc.	pH
Number	no.
Percentage	%
PDA	Potato Dextrose Agar
Pounds per square inch	psi
Randomized Complete Block Design	RCBD
Relative Humidity	RH
Sher-e-Bangla Agricultural University	SAU
Species	spp.

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The author

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ABSTRACT

A study was conducted at the Central Farm and at the Plant Pathology Laboratory of Sher-e-Bangla Agricultural University, Dhaka-1207 from July/2021 to June/2022 to isolate, identify and conduct pathogenicity test of the isolated organism and to screen out resistant cultivar(s) of betel vine against *Sclerotium rolfsii* causing foot and root rot disease. The causal organism of foot and root rot disease was isolated from infected root samples and identified as *Sclerotium rolfsii*. It was mass multiplied in barley grains and its pathogenicity was confirmed by Koch's Postulates. Nine different cultivars of betel vine viz. ChL-01 (Chuadanga Local), MoL-02 (Moheskhali Local), KiL-03 (Kishoreganj Local), SaL-04 (Satkhira Local), CuL-05 (Cumilla Local), RaL-06 (Rajshahi Local), BP-07 (BARI Pan-1), BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) were evaluated against *Sclerotium rolfsii* by screening experiment. The results were compiled based on physio-morphological features, days required for the visual expression of mycelial growth on soil surface, days required for the appearance of 1st disease symptom, disease reactions, yield and yield contributing characters. The vegetative growth parameters and morphological features of different cultivars of betel vine varied significantly to some extent. After inoculation, the days required for the appearance of mycelial growth on soil varied noticeably. The lowest 3 days were required for Satkhira Local, BARI Pan-2 and BARI Pan-3 and the highest 10 days were required for Rajshahi Local. No mycelial growth was seen on soil in Kishoreganj Local. The appearance of 1st disease symptoms among the betel vine cultivars differed significantly. The highest incubation period (18 days) was found in Rajshahi Local and the lowest (4 days) was found in BARI Pan-2 and BARI Pan-3. No disease symptom was noticed in Kishoreganj Local. The disease incidence ranged from 0%-100%. Among the betel vine cultivars, Kishoreganj Local showed resistant reactions while three cultivars viz. Chuadanga Local, Rajshahi Local and BARI Pan-1 showed moderately resistant reactions. Other three cultivars viz. Moheskhali Local, Satkhira Local and Cumilla Local showed moderately susceptible reactions and the rest two cultivars viz. BARI Pan-2 and BARI Pan-3 showed highly susceptible reactions against *Sclerotium rolfsii* causing foot and root rot disease of betel vine. CFU/gram soil was observed the highest ($3.04x10^6$) in BARI Pan-3 and the lowest ($0.06x10^6$) in Kishoreganj Local.

CHAPTER-1
INTRODUCTION

CHAPTER I

INTRODUCTION

Betel vine (*Piper betle* L.) is both an important horticultural and cash crop of Bangladesh which is under the order Piperales and is belonging to the family Piperaceae. Betel vine is cultivated largely for its leaves and the shiny heart-shaped leaves are commonly known as 'Pan' in Bangladesh. The leaves are widely consumed for masticator purposes along with areca nut. Generally, the people of South Asia, Southeast Asia, Gulf States and Pacific islands chew betel leaves in their social, cultural and religious events for hospitality, marriage, puja (religious festivals), sraddha ceremony (religious function done after cremation) and other occasions. It is also used as a special gift provided to guests to show respect and because of this long-standing tradition of betel leaf use in the society, the leaf still stands out today (Guha, 1997).

Morphologically, the betel vine is a semi-woody, dioecious (male and female plants are different), perennial plant having glossy, heart-shaped, deep green leaves with bleaching quality, softness, pungency and aroma. Betel vine is a shade-loving evergreen creeper and the type of stem is climbing by many short adventitious roots arising from the nodes (Hassan and Shahadat, 2005). Betel vine plants have two types of branches namely, orthotropic (vegetative) and plagiotropic (reproductive).

The origin place of the betel vine is thought to be in Malaysia, Sumatra and possibly Java (Chattopadhayay and Maiti, 1967). It is cultivated mostly in South and Southeast Asia, from Pakistan to Papua New Guinea. There are about 100 varieties of betel vine all over the world and among them, 40 varieties are found in India and the other 30 varieties are in west Bengal and Bangladesh (Guha, 1997; Maity, 1989 and Samanta, 1994). Eighteen species are found in Sri Lanka and among them, three are endemic (Dassanayake and Fosberg, 1981). In ancient times, betel vine was cultivated in all parts of

Bengal, preferably in some districts like Dinajpur, Rangpur and Chittagong. Now it is widely cultivated in Sylhet, Moulvibazar, Jessore, Khulna, Kushtia, Kishoreganj, Bagerhat, Satkhira, Barisal, Faridpur, Cumilla, Rajshahi, Rangpur, Gaibanda, Cox's Bazar and in greater Chittagong district of Bangladesh. A good number of betel vine cultivars namely Desi Bangla, Bangla, Kali Bangla, Jhali, Sanchi, Goyeshi, Bhabna, Mitha, Geso, Bonhoogly, etc. are grown in Bangladesh. All classes of people in Bangladesh chew betel leaves as a habit and an item of rituals, etiquette and manners.

Bangladesh trades betel leaves to many foreign countries like India, Pakistan, Saudi Arabia, United Arab Emirates, England, Italy and Germany. Bangladesh started exporting betel leaves to Europe in 1974-75 and to Saudi Arabia in 1991. Basically, betel leaf is purchased and consumed by the people of Bangladesh, India and Pakistan. Bangladesh has over 14000 hectares of betel vine cultivation, making it the world's second largest producer. The crop's total annual yield in Bangladesh is around 72,500 tons. The average yield per acre is 2.27 tons (Anonymous, 2006). The total cultivated area under the crop in Bangladesh in 2016-17 was about 23803.20 hectares, the total annual production was about 2,14,252 metric tons and the average yield per hectare was 9 metric tons (BBS, 2018).

Betel vine is grown under cool, shaded conditions in tropical and sub-tropical regions where humidity and temperature do not fluctuate abnormally and high humidity with moderate sunshine and good supply of soil moisture prevail throughout the year. 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 meters (Chaurasia, 2001). Betel vine is grown in areas where rainfall is about 2250 – 4750 mm, relative humidity and temperature are ranging from 40 – 80% and 15 – 90°C, respectively (Guha and Jain, 1997).

In Bangladesh, the betel vine is cultivated basically under an artificially erected structure, locally known as Baroj, Bareja or Bheet. The baroj is a kind of garden which is fenced by jute straw and the roof is covered by coconut leaf or

wheat/rice straw on a framework of bamboo. To cultivate the betel vine, 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, 1997). 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 the Ashar pan is the worst. Betel leaf is usually plucked throughout the year but maximum production is obtained in the months of July to October. Betel vine is grown in two different ways in the world: naturally and under regulated conditions.

Betel vine leaves are economically and medically important. The medicinal properties of betel leaf were recognized during 600 AD when the ayurvedic medicine came into practice. Betel leaf chewing is considered as a good and cheap source of dietary calcium. The leaves are found to contain the enzyme diastase and catalyze; vitamins A, B and C; thiamine, riboflavin, tannin, iodine, iron, calcium, minerals, protein, essential oils and it is supposed to be a tonic for the brain, liver and heart in human beings (Chopra *et al*., 1956). Antiseptic, anti-rheumatic, anti-rhodant, anti-cough, and anti-noctornel emission are all properties of betel leaf. Antiseptic properties have been discovered in a volatile oil extract from betel leaf (Chattopadhyay, 1967). When combined with lime, it boosts digestive capacity. It also functions as a blood purifier and neutralizes acidity. It is also good for the respiratory system and is used in treatment of bronchitis, cough and cold (Chopra *et al.,* 1956). Betel leaves are used as a mouth refresher and a mild energizer.

The acreage of betel vine is decreasing gradually because of some physical and socio-economic barriers like unavailability of credit facilities, and uncontrolled marketing system and an infestation of diseases and pests (Islam, 2005). Different diseases are the prominently known limiting factors in betel vine cultivation (Rahman, 2017). The susceptibility of the betel vine against diseases, pests and natural calamities is extremely high (Sayeeduzzaman, 1988). The most severe disease of the betel vine is *Sclerotium rolfsii* which

causes foot and root rot and significantly reduces betel vine yield. The extent of losses varies from 5 to 90 percent (Dasgupta and Sen, 1999; Dasgupta *et al*., 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). Environmental factors play an important role in the development of disease and in the expression of susceptibility or resistance of the host after infection. Because those environmental factors help the pathogen for growth, dissemination and infection (Walker, 1965).

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 to as an omnipathogenic organism (Talukdar, 1974). The fungus *S. rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situations by the formation of a resting structure called sclerotia (Ahmed, 1980). Farmers growing *Piper betle* in three Upazilas of Rajshahi incurred a huge loss as foot rot disease damaged about 60% of the cultivation in the year 2004 (Islam, 2005).

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 caused by *Sclerotium rolfsii* (Patil *et al*., 1986). The continuous and indiscriminate use of chemicals to manage crop disease results in the accumulation of harmful chemical residues in the soil, water and plants. In a third-world country like Bangladesh, farmers are illiterate and they seldom follow the appropriate methods in handling chemicals which creates health hazards and also breaks the natural ecological balance by killing the beneficial and/or antagonists microorganisms. The continuous and spontaneous chemical application also induced the development of resistant isolates of the pathogens which sometimes become more virulent. Hence, efforts have to be made to retain pathogen activity below the economic threshold level by choosing

methods alternative to chemicals only. Biological control could be a successful alternative for controlling this pathogen. Due to foot and root rot disease, the basal part of the stem becomes rotten and caused a huge loss for the betel vine-growers reducing the quality of betel leaf and hence, the farmers deprived of the usual market price. For continuous cultivation, the betel stem rot infestation seems to be alarming for inoculum potential. The betel vine growers are in trouble growing betel vine due to this disease problem.

At present, betel vine has a worldwide market. But in competition with India and other betel vine producing countries, Bangladesh has a very small share of the world betel vine market for lower production of quality betel vine due to various diseases and insect pests (Goswami *et al*., 2002). To increase the export of betel leaves production needs to be enhanced through the management of diseases, especially foot and root rot.

In Bangladesh, among the diseases of betel vine, foot and root rot caused by *Sclerotium rolfsii* is the most devastating disease which decreases the production of betel leaf to a great extent. The betel vine growers are presently discouraged to cultivate it as they have no suitable and sustainable approach for controlling this disease and also no resistant varieties are known to be available against it in the country. A huge number of betel vine gardens known as "*Baroj*" are ruined every year due to severe attacks of foot and root rot disease. If such a situation let continues to happen, the betel vine cultivation would face a great threat and the country will lose a huge foreign income. Therefore, considering the above facts, the present study has been carried out with the following objectives:

1. To isolate, identify and conduct pathogenicity test of the isolated organism.
2. To screen out resistant or tolerant cultivar(s) against *Sclerotium rolfsii* causing foot and root rot disease of betel vine.

CHAPTER-2
REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The betel vine (*Piper betel* L.*)* is highly susceptible to different kinds of diseases, pests and some natural calamities. Foot and root rot is one of the most devastating diseases of betel vine caused by a facultative soil-borne fungus, *Sclerotium rolfsii* which causes severe loss. As a management strategy, searching of resistant cultivar (s) can play a major role in reduction of losses caused by this pathogen. Hence, in this chapter an attempt was made to compile the relevant reviews for the present investigation.

2.1. History

The foot and root rot disease of betel vine has been reported in almost all growing regions in the world including Bangladesh (Turner, 1969; Roy, 1948) and Indonesia, Myanmar, and Srilanka (Paul, 1939). In India, the disease has been reported in all the betel vine gardens of the country. According to Dasgupta and Sen (1997), the highest intensity of this disease has been recorded in Midnapore and Nadia district of West Bengal. The extent of losses may vary from 30-100% in the case of foot and root rot leading to almost total crop failure (Dasgupta *et al*., 2000; Maiti and Sen, 1982).

2.2. Environmental factors on the development of disease

Al-Askar *et al.* (2013) reported that the disease conditions caused by *Sclerotium rolfsii* occur primarily in warm climates, especially at high temperatures.

According to Mollah (2012), in Satkhira area of Bangladesh, the disease incidence and severity was the highest at 29ºC temperature and 85% RH and was the lowest around 18.7ºC and around 75% RH.

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

Khan (1996) investigated the incidence and severity of collar rot of sunflower in fifteen varieties which were grown at 3 agro-ecological zones (AEZ) of Bangladesh during Rabi (1993-94) and Kharif-I (1994) and he noticed that at flowering and harvesting stages, incidence of the disease was the minimum in Rabi season and a higher percentage of plants were killed in Kharif-I season.

Alexander and Stewart (1994) and Mitchell *et al*. (1990) found more rapid sclerotial degradation and reduced survival in clay soil and at relatively low pH (~6).

Alexander and Stewart (1994) also showed that lower survival occured in clay loam with higher water holding capacity which affected drying and wetting of soil resulting in greater microbial activity than in sandy loam.

According to Tu *et al*. (1991), the percent 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 also observed that the highest sclerotial germination occurred at 20-28ºC on plates of soil.

Hari *et al.* (1991) studied the radial growth of the fungus and found that the maximum growth of *S. rolfsii* causing collar rot of groundnut at pH 6.

Chattopadhyay and Maiti (1990) reported that the betel vine plants which are cultivated under shady and humid conditions also favor the development of several diseases including foot and root rot.

Farr *et al*. (1989) noticed that under favorable environmental conditions, *S. roifsii* attacks all plant parts including stems, roots, and fruits that are in contact with the soil.

Punja *et al.* (1988) mentioned temperature to be one of the major limiting factors in the geographic distribution of *S. rolfsii*. Maximum disease occurs at 25 - 35ºC which is also the optimum range for mycelia growth and sclerotia germination. The disease rarely occurs where the average daily minimum temperature is below 0ºC or freezing point.

Harlapur (1988) found that the optimum soil temperature for growth and activity of *Sclerotium rolfsii* was 25-30ºC and the growth was completely ceased at 45ºC.

Hari *et al*. (1988) observed that 26ºC was the optimum temperature for mycelial growth of *S. rolfsii* and the maximum growth was induced at 30ºC.

According to a study of Palakshappa (1986), *S. rolfsii* causing foot and root rot of betel vine survived better at low soil moisture than at higher. The survival ability was highest between 20-40 % soil moisture and higher saprophytic activity was observed at 40% and the least was at 60 and 70% moisture level.

An epidemiological study found that maximum temperature, maximum relative humidity and maximum rainfall played an important role in the development of foot ant root rot disease of betel vine. (Maiti and Sen, 1982; Anonymous, 2006).

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.

Gondo (1962) found that the optimum soil temperature was 30ºC for the growth of mycelia and 25ºC for the development sclerotia.

2.3. The causal pathogen and its morphological characteristics

The pathogen, *Sclerotium rolfsii* is a well-known soil-borne fungus that is polyphagous, ubiquitous, and omnivorous in nature. Also, this destructive fungus is a facultative saprophyte that has the capacity to continue its generation under adverse conditions by the formation of sclerotia (Ahmed, 1980).

The pathogen, *Sclerotium rolfsii* had been reported by Rolfs in 1892 as a cause of tomato blight from Florida in the U.S.A. and in 1911, this fungus was named *Sclerotium rolfsii* by Saccordo. However, its perfect stage was first studied by Curz in 1931 and he proposed a generic name as *Corticium*. It is Mundkar who successfully isolated the perfect stage of *S. rolfsii* in 1934. It has an extensive host range, at least 500 species in 100 families are susceptible and the most common hosts are legumes, crucifers, and cucurbits (Chupp and Sherf, 1960). It is an economically important pathogen on numerous crops worldwide (Aycock, 1966).

Manu *et al*. (2018) investigated on morphological studies of different isolates of the pathogen. Pathogenic isolates of *S. rolfsii* were obtained from various crops of southern Karnataka. The colony diameter varied from 4.10 to 8.00 cm after 72 hours of incubation, the sclerotial number ranged from 261.7 to 1048.7 per plate; color ranged from light to dark brown and size varied from 1.10 to 2.10 mm with spherical to round. The test weight of sclerotial bodies ranged between 40.40 to

71.00 mg. Among the media, sclerotial production was more in malt extracts broth (247.70) but ragi flour broth showed maximum dry mycelial weight (352.00 mg).

Pandi (2017) collected eight isolates of *S. rolfsii* from the sunflower plants during the disease survey from different locations of India and used them for morphological variability studies. Among the eight isolates studied, three isolates produced fluffy, dull-white colonies, while other isolates produced compact and dull white colonies. The mycelial growth was recorded at 31.45 mm/day (maximum) and 21.62 mm/day (minimum). All the eight isolates produced sclerotial bodies and their color was grouped under three categories viz., light brown, dark brown, and reddish-brown; and the maximum number of sclerotia was found 360 per plate and the minimum number of sclerotia was 274 /plate. Among the produced sclerotia, the biggest was 1224 mm and the smallest one was 1002 mm in diameter.

Khalequzzaman and Hossain (2012) investigated the growth habit, conidial and sclerotial characters of *Sclerotium rolfsii* causing foot and root rot diseases in pulses. They found that radial mycelial growth ranged from 75.9 to 88.8 mm at 7 DAI and colony color was off-white to creamy white. The shape of the colony was regular and compactness was fluffy to medium fluffy. The colour of sclerotia was found brownish and brown, and the shape was round.

Rekha *et al*. (2012) reported that pathogen produced sclerotia resembling mustard seed, which were resistant to degradation in soil and served as inocula for the next season helping in spreading of the disease to other plants.

Sharma *et al.* (2002) investigated the variability in growth and basidial stage production of 26 isolates of *Sclerotium rolfsii*. The isolates of *Sclerotium rolfsii*

varied in all of the characteristics such as colony morphology, mycelia growth rate, sclerotial production, basidiocarp induction, sclerotial size and color.

Surendranath (1999) observed that fully grown sclerotia of *S. rolfsii*, the causal agents of white rot of onion were spherical to ellipsoidal and size was measured from 0.5 mm to 1.75 mm in diameter.

Siddique (1997) studied histopathology of foot rot and showed that *Sclerotium rolfsii* penetrated through the cuticle and spread both intra and intercellularly and destroyed the cortex most rapidly and then advanced towards the vascular bundle. The pathogen is not systematic.

Punja and Rahe (1992) stated that the sclerotia producing fungi are characterized by small tan to dark brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla which placed in the genus *Sclerotium.*

Butler (1966) reported that being very resistant to biological degradation, some septate fungi are capable of forming sclerotia consisting of firm aggregates of vegetative hyphae and serve as a means of survival for these fungi.

In 1954, Townsend and Willetts investigated the development and the structure of several types of sclerotia by the light microscope and found out that a mature sclerotium of *Sclerotium rolfsii* Sacc. is composed of four distinct cell layers: (a) a fairly thick skin, (b) a rind, two to four thick cells made of broad and tangentially flattened cells, (c) a cortex having densely staining cytoplasm with thin-walled cells, and (d) a medulla consisting of loosely arranged, ordinary filamentous hyphae filling with dense contents.

2.4. Morphological variabilities of *Sclerotium rolfsii* isolates

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

Rasu *et al.* (2013) studied morphological and genomic variability among *S. rolfsii* populations and reported that out of 17 isolates tested for their cultural morphology, most of them were observed with compact colonies and few were fluffy colonies. Based on growth rate, they were categorized into three groups such as slow growing, fast growing and intermediate.

According to Abida *et al*. (2008), 12 isolates of *Sclerotium rolfsii* Sacc varied in terms of mycelial growth rate, sclerotial formation, size and color of colony and sclerotia.

Akram *et al*. (2007) reported mycelial incompatibility and morphological diversity between 12 isolates of *S. rolfsii* collected from different locations of Pakistan (Panjab) and reported that mycelial incompatibility and compatibility between different isolates of *S. rolfsii.*

Kokub *et al.* (2007) evaluated comparative growth, morphological and molecular characterization of indigenous *S. rolfsii* strains isolated from different locations of Pakistan and reported that out of 8 strains, growth rate of 8 fungal strains of *Sclerotium rolfsii* on potato dextrose agar plates at 28ºC ranged from 0.86-1.35 mm/hr. All strains produced round shaped sclerotia with an average diameter of 0.5-2.0 mm.

2.5. Symptoms and effects of the disease

Mahadevakumar (2018) observed the outbreak of southern blight disease of China aster during the post rainy season in Mysore and Mandya districts of Karnataka, India. Symptoms were noticed as distinctive water-soaked lesions on leaves, stems, and the lower stem followed by rapid wilting of the entire plant with an ample number of sclerotia at the soil level.

In 2016 Jahan *et al*. reported that betel vine plants caused by *Sclerotium rolfsii*, turned yellow, withered and finally dried out to a pale brown color. The fungus was 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 were formed on the rotten plants. The rotting spread through older roots and finally reached the foot or collar region of the plant. The soft tissues of old roots were completely decomposed, leaving only the fibrous portion.

Kwon (2010) reported that stem rot disease was found in garlic (*Allium sativum* L.) cultivated in vegetable gardens in Korea. Initially typical water-soaked spots were found, which gradually progressed to rotting, wilting, blighting, and eventually death. White mycelial strands had spread over the lesions near the soil line, and sclerotia had formed over the mycelial strands on the stem as well as adjacent soil surface. The sclerotia were globoid in shape, size was 1~3mm, and color was tan to brown.

Lievens *et al.* (2004) observed that a severe collar rot was found on two months old wilted tomato (*Lycopersicum esculentum)* plants in a commercial green house setting in Belgium. Symptom development was restricted to lower plant parts with severe rotting of the whole root system with dark lesions girdling the stem base.

Anahosur (2001) reported that dark brown lesions were found on the stem slightly below the surface soil followed by drooping and wilting of affected leaves and ultimately wilting of the whole plant. Such wilted plants showed whitish mycelial growth having sclerotial bodies resembling mustard seeds on collar region and also on roots.

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.

Kulkarni *et al.* (1994) stated that the pathogen affected either stem or root or tubers. The infected stem produced dark brown lesions at collar region causing wilting and ultimately plants dried up. Brownish minute sclerotia look like mustard seed developed at later stages on the root and collar regions of the infected plants. Finally the tubers get infected and rotten.

Khanna and Jyotsama (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 eventually 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 as well as on soil.

Okoli *et al.* (1991) stated that *Sclerotium rolfsii* caused severe infestation on sunflower plants. Initially, acropetal wilting of the entire plant occurred and then gradually dried out but remained green and attached to the stem. Within 24 hours

of wilting, a white mycelial mat formed around the discolored site on the stem base. Within 1-2 days, the mycelia had rounded off into miniature white balls and characteristics of brown mature sclerotia within 24 hours.

Mridha and Alamgir (1989) noticed that sclerotial wilt of betel vine in thirty selected gardens in Chittagong district. Plants showed decay at the collar region and below the soil level. It has been reported that infected plants lost luster, leaves turned yellow and the entire plant wilted and eventually died. The infected portion of stem was covered with white cottony mycelial mats having small, light to deep brown sclerotia on the base of stem.

Singh (1970) observed that damping-off symptoms are brown lesion on the base of hypocotyl, early and falling-over on the ground of seedling with wilting whereas those of stem rot are brown lesion on the stem base, yellowing, wilting, dying of stems remaining upright, with white mycelium in the lesion. The fungus infects the base of stems producing strands of silky white mycelium and tiny round sclerotia which were initially white and gradually darkened.

Choudhury (1967) reported that foot rot disease of brinjal is caused by *Sclerotium* sp. Tiny mustard like structure, adheres to the stem at gourd level was found and mycelia enter the stem and block the vessels.

Dastur (1935) studied the symptoms of foot and root rot of betel vine. A well-accepted description came out from his study. He found that the leaves and shoots turn yellow, wither, and eventually dry out to a pale brown color. *Sclerotium rolfsii* attacks the roots and stem near the soil level and black-colored lesions develop following necrosis of the cells. Invading the stem, the mycelium rots the affected portions, and therefore the plant wilts and gradually dies. Abundant white mycelium and little brown sclerotia form on the rotted portions. In due succession, the rotting spreads through older roots and ultimately reaches the foot or collar

region of the plant. The disease in internodes is often easily detected by the blackening of the tissues inside. The infection to aerial parts does not usually extend beyond one or two internodes because the plant is killed before the disease progresses further.

2.6. Host range of *Sclerotium rolfsii*

Garibaldi *et al. (*2006) observed severe basal rot symptoms of potato (*Solanum tuberosum* L.) in a commercial field near Alessandria (northern Italy).

During a pot experiment, Yaqub and Shahzad (2005) observed that *Sclerotium rolfsii* is highly pathogenic on sunflower, and mildly pathogenic on tomato, lentil, sweet pumpkin and cabbage and non-pathogenic on cauliflower plant.

A study of Sharma *et al*. (2002) found that *Sclerotium rolfsii* Sacc. has an extensive host range and it cannot be controlled easily by chemical means.

Sugha *et al*. (1991) reported that *Sclerotium rolfsii* caused collar rot of chickpea. A total of 210 lines and cultivars of chickpea tested by placing on wheat grain fully covered with mycelium of *S. rolfsii* at the collar rot of 7 days old seedling in pot of sterilized garden soil. All were find to be susceptible to collar rot.

In 1985, Chowdhury and Ahmed tested the reaction of twenty-two different crop plants against *Sclerotium rolfsii* in the pot-house. They observed that maize, wheat, gram, khesari, lentil, mashkalai, mungbean, soybean, sunflower, sesame, brinjal, bitter gourd, bottle gourd, cowpea, cucumber, okra, radish, tomato, radish, chilli, coriander, garlic and onion are susceptible to this pathogen under experimental conditions. Before sowing the seeds, sterilized soil in pots was inoculated with a culture of *S. rolfsii* grown in oats. Up to 45 days after germination, data were collected on pre and post emergence mortality. The rate of pre-emergence mortality was found 5.6% in garlic and 85% in gram.

Debnath (1979) evaluated the reaction of twelve cultivars of soybean to collar rot and root rot disease caused by *Sclerotium roifsii* and found that all the varieties were susceptible and infected by the pathogen.

Hsiehh (1979) reported from Taiwan that *Sclerotium rolfsii* was found to be the causal organism of rot of three ornamentals viz. *Saintpaulia, Jonantha gloxinia and Streptocarpus hybridus.*

In 1966, Aycock reported 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 establish 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.

2.7. Physio-morphological characters of betel vine cultivars

Mohanta and Pariari (2015) studied the effect of various climatic factors on the growth of betel vine. Temperature and RH were found to be the most important factors for variation in leaf characters in different cultivars. Simurali Jhal showed superior performance in respect to leaf length (16.45 cm), leaf breadth (13.76 cm) and leaf area (274.35 cm^2) in rainy season.

Medda *et al*. (2011) conducted a field experiment to screen out the suitable cultivars of betel vine for Terai zone of West Bengal. Different cultivars exhibited significant variations in respect to their growth, yield and yield attributing characters. Among the cultivars, sanchi recorded higher monthly growth having larger inter-node length with larger sized leaves. The cultivar, Utkal sudam produced moderately higher leaf yield (66.03 lakh/ha) with significantly larger sized leaves (173.33 cm^2) having shortest inter-node length (3.98 cm).

Shivashankara *et al*. (2000) reported that under 35% and 60% light intensity the number of leaves increased 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. Betel vine requires 35% of sun light for production of maximum number of quality leaves.

According to CSIR (1969); Guha and Jain (1997) , betel vines grows best under the shaded, tropical forest ecological conditions with a rainfall of about 2250-4750 mm, relative humidity and temperature ranging from 40-80% and 15- 40°C, respectively. A well-drained fertile sandy or sandy loam or sandy clay soil with pH range of 5.6 to 8.2 is considered suitable for its cultivation.

2.8. Maintenance and Mass multiplication of the pathogen

For maintaining *S. rolfsii*, Potato Dextrose Agar (PDA) was found to be the most effective supporting medium (Gaur *et al*., 2005; Raoof *et al*., 2006; Gupta and Ashu, 2004 and Harinath, 2000).

Rahman (2017) used pre-soaked barley grains (2% sucrose) for mass multiplication of the pathogen and grains were inoculated with 5 mm discs of 3 to 4 days old culture of *Sclerotium rolfsii* grown on PDA. 7discs/250 ml flask containing 80gm barley were added and incubated for 3 weeks at 25±20°C.

Ramarao and Usharaja (1980) also noticed that *S. rolfsii* can be maintained on potato sucrose agar medium.

2.9. Method of inoculation of *Sclerotium rolfsii*

According to Rahman (2017), after six months of plantation 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 inocula (multiplied in barley grains) was placed in direct

contact of the exposed stem and then it was lightly covered with the top soil for infection.

Islam (2008) inoculated the eggplants by following the soil inoculation technique and using the barley culture of the pathogen (*Sclerotium rolfsii*). All the varieties were infected ranging from 66.66 to 100%. Varieties varied in percent mortality.

Kashem (2005) used the soil infestation method for the inoculation of *S. rolfsii*. He found that soil infestation with grain culture at the rate of 0.1% weight basis of dry soil before sowing seeds caused the heavy infestation.

Babar (1999) used 10 gms of colonized dried oat grains or poured fungal suspension with soil near plant base for inoculation and covered with moist cotton. Older plants (60-90 days age) developed infections quicker (8-10 days) and larger-sized lesions than those younger ones (10-45 days age).

Waraitch *et al*. (1986) used the soil mixing method of inoculation of *S. rolfsii* (multiplied on sterilized sorghum seeds which were pre-soaked in 2% sucrose solution) was mixed in the soil near the plants @ three 500 ml flasks per 100 m^2.

Chakravarty and Bhowmik (1983) used both soil infestation and toothpick method for inoculation of *S. rolfsii*. Both techniques effectively induced the disease where soil: inoculums ratio was 50:1(weight basis) causing heavy infection.

2.10. Pathogenicity

Tanjila (2018) conducted an experiment where twenty-two isolates of *S. rolfsii* were collected from foot and root rot-affected portions of the betel vine gardens of

different regions of the Northern parts of Bangladesh. Those isolates were tested for pathogenicity and the most virulence isolate was found to be Baghmara-1 which caused 23.42% disease severity.

Jahan *et al*. (2016) investigated a survey on the foot and root rot disease of betel vine in Kushtia and the pathogenicity test showed that *S. rolfsii* produced characteristic symptoms and proved to be the causal pathogen of the disease.

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

According to a study by Yaqub and Shahzad (2005), *S. rolfsii* was found to be highly pathogenic on sunflowers and on the contrary, mildly pathogenic on tomato, lentil, sweet-pumpkin and cabbages; and nonpathogenic on cauliflower.

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

Mirsha and Bais (1987) used 15-day old fungal culture grown on sand corn meal medium for proving the pathogenicity of root rot of barley caused by S. rolfsii mixing the upper 4-5 layers 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 4% and above inoculum levels**.**

2.11. Resistance against *Sclerotium rolfsii*

Shirsole *et al*. (2018) studied 185 chickpea entries under field conditions in 2016-2017 to identify sources of genetic resistance against collar rot disease caused by *Sclerotium rolfsii* and found that GNG 2331, JG 2016-9605, IPC 2012-98, RVSSG-38 and GL 12003, these 5 entries showed moderate resistance while the remaining were susceptible to highly susceptible.

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

In 2011, Iqbal and Ahmad evaluated 11sugar beet genotypes during the year 2009 in Pakistan, for their resistance against *Sclerotium rolfsii*. Inoculation of eleven genotypes with *S. rolfsii* exhibited resistant response only in SDPAK-09/07 and moderately resistant in SD-PAK-07/071. The remaining nine genotypes performed susceptible to highly susceptible responses to the pathogen.

2.12. Disease incidence and severity of foot and root rot of betel vine

According to Hanif (2020) no disease incidence was recorded in case of Laldingi pan after 30 days of inoculation.

Rahman (2017) reported that the lowest disease incidence was 8.33% in Laldingi pan after 30 DAI.

Jahan (2016) made an investigation on foot and root rot of betel vine in six upazillas of Kushtia district. Disease incidence and severity of foot and root rot of betel vine ranged from 24 to 58% 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 in 2014.

Mollah (2012) foot and root rot of betel vine in Satkhira district, the highest disease incidence were found in August (12.50% to 32.50%) and the 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.

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

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.

CHAPTER-3
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

For conducting the present piece of research, different materials were used and methodologies were followed. Those are purposely described in this chapter.

3.1. Experimental location and duration

The *in vivo* experiments were performed in the temporary built betel vine garden (*pan baroj*) at the Central Farm and the *in vitro* experiments were conducted at the Plant Pathology Laboratory of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. The experiments were conducted from July, 2021 to June, 2022.

3.2. Experimental design

The *in vitro* experiments were conducted following CRD and the *in vivo* experiments were conducted following RCBD to achieve the objectives of the study where 5 replications were exercised.

3.3. Soil type

The soil of the experimental site belonged to the Agro-ecological zone of "Madhupur Tract" (AEZ No. 28). It was Deep Red Brown Terrace soil and belonged to "Nodda" cultivated series. The top soil was 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:

Table 1. 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

3.4. Materials

3.4.1. Equipments and instruments

The common laboratory equipments and instruments used in the experiments were autoclave, hot air oven, incubator, laminar air flow cabinet, refrigerator, weighing balance, compound microscope, physical balance, etc.

3.4.2. Glassware

Different types of Corning and Borosil glassware were used in the experiments. The common glassware includes petri dishes, test tubes, conical flasks, measuring cylinder, glass rod, cover slips, 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

A sets of experiments were conducted during the study period to screen out resistant cultivar(s) against foot and root rot disease of betel vine**.** The experiments were as follows:

1. Isolation, purification, identification and pathogenicity test of causal pathogen of foot and root rot disease collected from major betel vine growing areas in the country and

2. Screening out resistant / tolerant cultivar (s) against *Sclerotium rolfsii* causing foot and root rot disease of betel vine

3.5.1. Isolation, purification, identification and pathogenicity test of causal of pathogen

At first, the diseased samples were collected from major betel vine growing regions of Bangladesh viz. Rajshahi, Cumilla, Kishoregonj, Moheshkhali, Satkhira etc. and after that, tissue planting method was followed to isolate the pathogens responsible for foot and root rot disease of betel vine (Tuite, 1969; Mian,1995). At the beginning of the experiment, the affected basal stems were washed fully with running tap water for removing soil and other dust particles. Then, those were cut into convenient pieces of 5 cm from advancing end of the lesions and surface sterilized with 1.0% chlorox (NaOCl) for 5 minutes followed by 3 times washing with sterile distilled water. Those sterilized stems were transferred on potato dextrose agar (PDA) media in 90 mm petri dishes and were placed for incubation at room temperature (25±2°C) for 3-5 days and regularly observed to figure out the growth of whitish cottony mycelia (Plate 1).

After growing of cottony mycelia, pure cultures were prepared by following hyphal tip culture method (Islam *et al.,* 2001; Tuite, 1969; Mian, 1995). They were subsequently transferred to fresh PDA media and incubated at 25 ± 2°C temperature for getting pure culture (Plate 2). When maximum growth was monitored, pure cultures were stored at 4°C for future use. Sub-cultures were

also prepared once in a month for retaining the viability of the pathogen. The pathogen was identified with its key characteristics with the help of identifying key book (Ellis, 1971) and it was identified as an isolate of *Sclerotium rolfsii.*

Pathogenicity test of isolated *Sclerotium rolfsii* was conducted under *in vivo* (pot culture) conditions by following Koch's Postulate method (Mitchell *et al*., 1990) in a betel vine garden (*pan baroj*). The symptomatology was studied to test the pathogenicity of the inoculated organism.

All the needed glass wares, equipments and distilled water were autoclaved under 121°C for 20 min. The total sterilization and transfer procedures were carried out in the laminar flow which was wiped by 70% ethanol and was sterilized by ultra violet (UV) ray for 20 minutes.

Infected stem | **Planting of diseased tissue on PDA** | **Cottony mycelia of isolated pathogen**

Plate 1. Isolation of *Sclerotium rolfsii* growing on PDA (Potato Dextrose Agar) media

Scooping of hypha | **Mycelial growth on PDA** | **Pure culture**

Plate 2. Purification of *Sclerotium rolfsii* growing on PDA (Potato Dextrose Agar) media

3.5.1.1. Preparation of Potato Dextrose Agar (PDA) media

Potato Dextrose Agar (PDA) media were used to isolate and get pure culture of the isolated organism. Firstly, 200 grams of peeled potato were taken and then the potato was cut into small pieces and boiled for 30 minutes in one-liter distilled water. When potato became soft, it was sieved using a muslin cloth and the liquid extract was poured into a 1000 ml conical flask. After that, 20 grams dextrose and 20 grams agar were mixed slowly with it and stirred continuously to prevent any kind of coagulation. The final volume was made 1000 ml by adding distilled water and then the media was sterilized in an autoclave under 121°C temperature and 15 psi pressure for around 30 minutes. After autoclaving, the media was poured into petri dishes and kept for some 20 minutes for cooling in laminar air flow chamber.

3.5.2. Screening of resistance of betel vine cultivar(s) available in the country against *Sclerotium rolfsii* causing foot and root rot disease of betel vine

3.5.2.1. Collection of cultivars

A total of nine betel vine cultivars were collected from major betel vine growing regions of Bangladesh. The cultivars were used as treatments to screen out the resistant or tolerant cultivars against the pathogen, *Sclerotium rolfsii* causing foot and root rot disease (Table 2). The study was conducted in field condition in a betel vine garden (*pan 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 betel vine from each treatment. The experiments were conducted during the year July, 2021 to June, 2022.

Table 2. List of betel vine cultivars used for screening experiment against *Sclerotium rolfsii* causing foot and root rot disease

Sl. No.	Accession No.	Name of Cultivars	Collection sources/area
1	ChL-01	Chuadanga Local	Chuadanga Sadar, Chuadanga
2	MoL-02	Moheskhali Local	Moheskhali, Chattagram
3	KiL-03	Kishoreganj Local	Pakundia, Kishoregonj.
4	SaL-04	Satkhira Local	Kaligong Upazilla, Satkhira
5	CuL-05	Cumilla Local	Debidwer, Comilla
6	RaL-06	Rajshahi Local	Mohanpur, Rajshahi
7	BP-07	BARI Pan-1	Spices Research Centre, BARI, Bogura.
8	BP-08	BARI Pan-2	Spices Research Centre, BARI, Bogura.
9	BP-09	BARI Pan-3	Spices Research Centre, BARI, Bogura.

3.5.2.2. Construction of betel vine garden (*pan baroj*)

Pan baroj is a closed hut like structure with doors for entry and exit. The baroj was constructed on a bamboo structure where the bamboo sticks with a height of 2-2.5 meters were covered with straw and coconut leaves for providing shade (Figure 1).

Figure 1. Construction of betel vine garden (*pan baroj*)

3.5.2.3. Collection of disease specimens

Affected root samples of betel vine were collected from different *baroj* in Bangladesh. Collected samples were put in polyethylene bags immediately after collection to protect them from drying. Then the samples were preserved in refrigerator at 4°C temperature.

3.5.2.4. Preparation of pot and potting media

Sterilized earthen pots having 16 inches diameter were taken and potting media 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 with a polythene sheet for 48 hours for sterilization. After 7 days, earthen pots were filled up with the sterilized soil up to 14 inches diameter (Dashgupta, 1988) (Plate 3).

A **B** **C**

Plate 3. Sterilization of soil with formaldehyde (**A**), soil covered with polythene sheet for 48 hours (**B**), earthen pots with sterilized soil for planting betel vine cuttings (**C**)

3.5.2.5. Growing of betel vine cuttings

Forty centimeters long, healthy betel vine cuttings with five nodes were taken and grown in earthen pots under the betel vine garden. 1-2 internodes below the

bud point are dipped in soil and kept touching with surface soil. Irrigation was applied as per requirement of the plants with regular intervals. Weeding and mulching were also done when necessary to keep the betel vine plant free from weeds and for better soil aeration and conservation of soil moisture. Betel vine plants were fasten with bamboo sticks of betel vine garden (*baroj*).

3.5.2.6. Inoculum preparation and inoculation with the causal pathogen

The isolated pathogen, *Sclerotium rolfsii* was multiplied on barley grains (Gupta and Kolte, 1982). At first barley grains were pre-soaked in 2% sucrose solution overnight and drained. Then it was transferred into 1L conical flasks @ 500g and autoclaved at 121°C temperature, 15 psi for 30 minutes. The flasks were allowed to cool in laminar airflow and were inoculated with five mm discs of 3 to 4 days old culture of *S. rolfsii* grown on PDA. Fifteen discs per flask were added and flasks were incubated for 28 days at 25±2°C (Plate 4).

After six months of plantation each variety was inoculated by *S. rolfsii* separately. The top soil from the upper 5 cm was removed and 2 cm depth was made around the exposed stem.15 grams of *Sclerotium rolfsii* inoculated barley seeds were directly placed on and around the exposed betel vine stem (Figure 2). Finally, the inoculum was lightly covered with top soil for infection. (Fery *et al*., 2002). Screening of betel vine cultivars was achieved on the basis of disease incidence.

A **B** **C**

Plate 4. Mass multiplication of *Sclerotium rolfsii* in barley grains; growth of *S. rolfsii* after 1 day (**A**), after 15 days (**B**) and after 28 days (**C**) of inoculation

Figure 2. Soil inoculation with *Sclerotium rolfsii* multiplied in barley seeds

3.5.3. Soil dilution plate method

The purpose of soil dilution plate method was to count colony forming unit (CFU) of the fungi. Soil was taken from experimental pots inoculated with *Sclerotium rolfsii* and serial dilution was set up carefully. One gram of soil from the working sample was taken in a test tube containing 10 ml of sterile water and stirred thoroughly and this suspension was used as stock solution. 1ml of that stock solution was taken into the 2nd test tube containing 9 ml sterile water with the help of sterile pipette and shaken vigorously to make 1:10 dilution. The 3rd test tube was inoculated with 1 ml from 2nd test tube to give 1:100 dilutions. By following the same technique, dilution was made up to 1:1000.

15 ml of warm melted PDA medium was poured in each sterile petri dish. 0.1 ml of diluted soil sample was placed at the center of PDA and spreaded thoroughly by an “L” shaped glass rod. Then these plates were incubated at room temperature for 3 days and then the colonies were counted (Adesemoye *et al*., 2006). Sub cultures were made by transferring a small colony to a new petri dish on the basis of color and morphology of the colony. Further transfers were made for purification. The contaminated plates were discarded. The cultures were identified on the basis of macroscopic (colonial morphology, color, texture, shape and appearance of morphology) (Plate 5) and microscopic

characteristics (septation in mycelium, presence of specific reproductive structures) (Zafar *et al*., 2006).

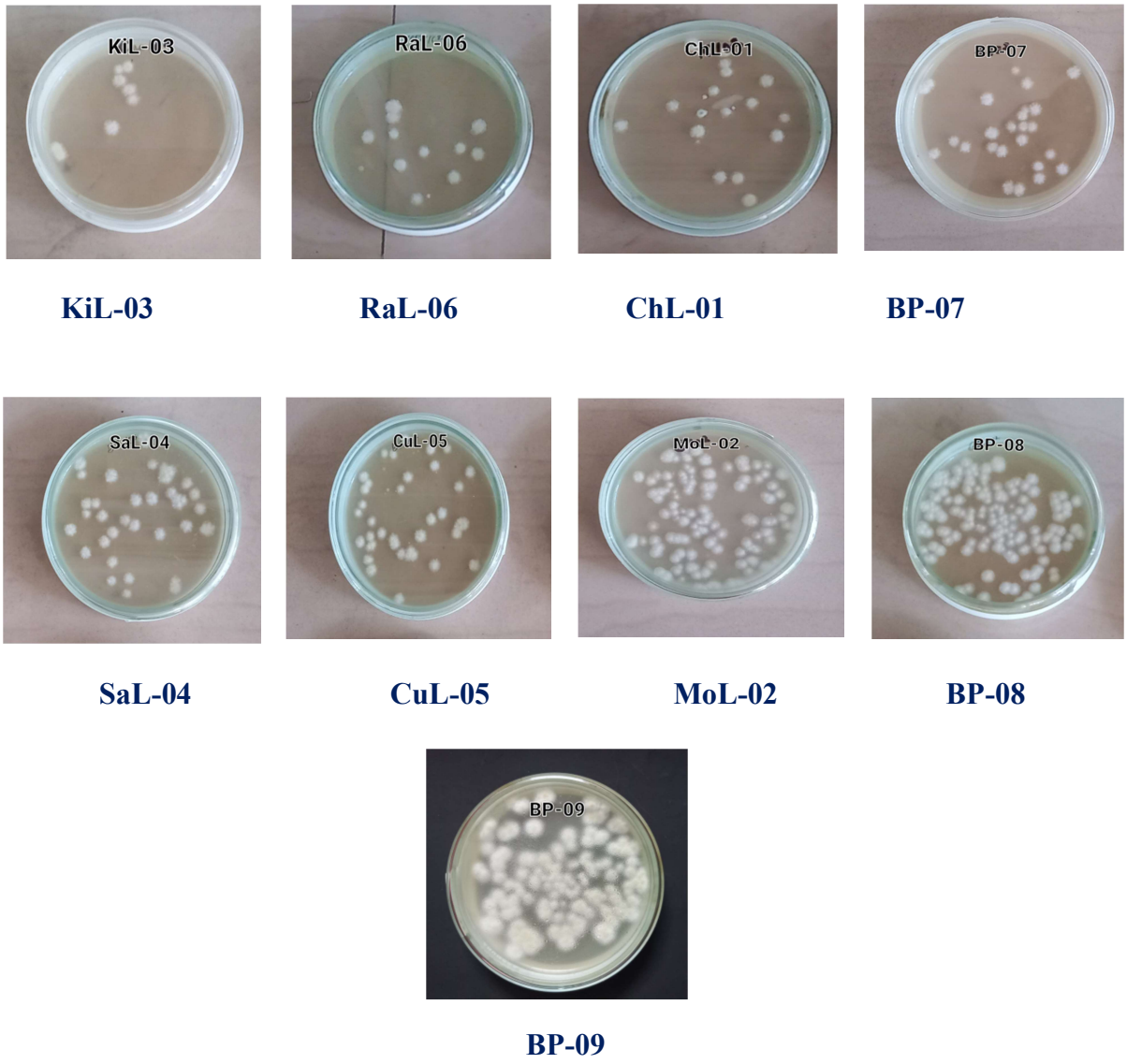

Plate 5. CFU (Colony Forming Unit) counting of *Sclerotium rolfsii* on PDA

3.5.4. Data collection

The data were recorded on following parameters:

i. Morphological features of the cultivars
ii. Days required for visible growth of mycelia on inoculated soil surface near the plant base
iii. Days required for appearance of 1st disease symptom
iv. Disease incidence/severity

3.5.4.1. Disease incidence

Assessment of disease incidence/severity of the foot and root rot disease was calculated by the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Number of total plants inspected}} \times 100$$

The tested cultivars were graded as Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS) and susceptible (S) on the basis of percent of disease incidence (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

Completely Randomized design (CRD) for *in vitro* experiments and Randomized Complete Block Design (RCBD) for *in vivo* experiments with 5 replications were used in this study. 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

Experiment 1.1. Isolation and identification of causal pathogen of foot and root rot disease of betel vine

Pathogen isolated from the infected root samples of betel vine was confirmed as *Sclerotium rolfsii* based on its key characteristics (CMI description no. 410). On PDA media, *Sclerotium rolfsii* grew rapidly covering the whole petri dish within 7 days. The fungus produced fluffy mycelium which was sparse to dense and white to dull white in color (Plate 6). The observation under compound microscope proved the production of branched mycelium which was hyaline, septate and superficial.

Numerous sclerotia were produced from the grown mycelium after 20-25 days of incubation in pure cultures. The sclerotia were mustard seed-like, globose to oval, thick walled and smooth to rough. Initially the sclerotia were found white and with maturation those became dark brown in color (Plate 6). The present findings are kept in with the findings of Hanif (2020), Tanjila (2018), Rahman (2017) and Jahan (2016).

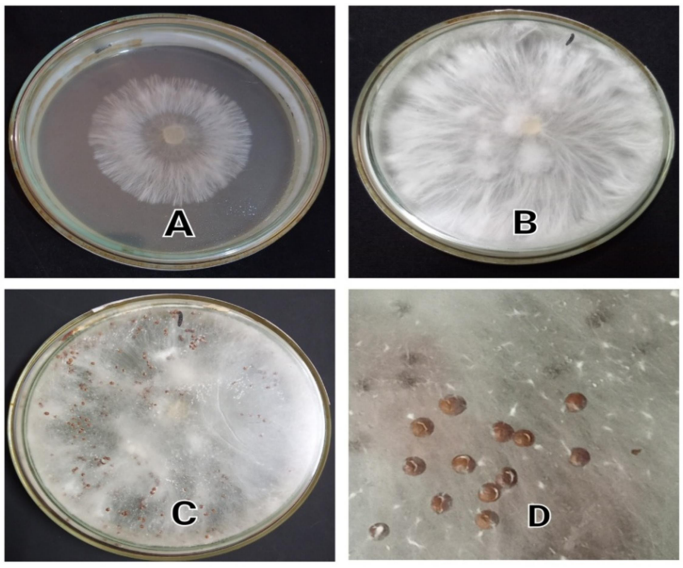

Plate 6. Colony texture (A & B) and sclerotia formation (C & D) of *Sclerotium rolfsii* on PDA media

Experiment 1.2. Pathogenicity test of *Sclerotium rolfsii* causing foot and root rot disease of betel vine

4.1.2.1. Symptomatological results of artificial inoculation

Sclerotium rolfsii isolated from diseased betel vine sample was subjected to pathogenicity tests by Koch's postulates. When pathogen was inoculated in pot soil of healthy betel vine plants, the inoculated plants exhibited typical symptoms. After five days of inoculation, white mycelial growth was noticed on soil surface near the plant base. On seventh days after inoculation, white mycelial mat was formed which spread rapidly towards the plant base. The leaves and shoots of foot and root rot infected plants turned yellow, withered and finally dried out to a pale brown color (Plate 7).

Immature white rounded sclerotia were also observed on soil surface near the plant base within fifteen days. The forming sclerotia were gradually turned light brown to dark and started to germinate producing white mycelia. The fungus was found to attack the roots and stem near the soil level. Black lesions were developed following necrosis of the plant tissues. The mycelium invaded the stem and rotten the affected portions. As a result, the plants became wilted and gradually died. Abundant white mycelium and small light brown sclerotia were formed on the rotten plants. The rotting spread through older roots and ultimately reached to the foot or collar region of the plants. In the diseased plants, 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 stem portion. (Plate 7).

The findings of pathogenicity test are in agreement with the findings of other researchers. 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.

Siddaramaiah (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 after a week inoculation.

Sommat *et al*. (1982) made an investigation on the pathogenicity of *S. rolfsii* and found that the pathogen could infect its host (cotton) severly.

Datar and Bindu (1974) proved the pathogenicity of *Sclerotium rolfsii* on sunflower by soil inoculation method under glass house conditions.

4.1.2.2. Results of re-isolation

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

Amin *et al.* (2013) and Dastur (1935) reported that *Sclerotium rolfsii* found to be associated as the causal pathogen of foot and root rot observed on betel vine.

Siddique (1997) found the similar findings while working with tomato varieties.

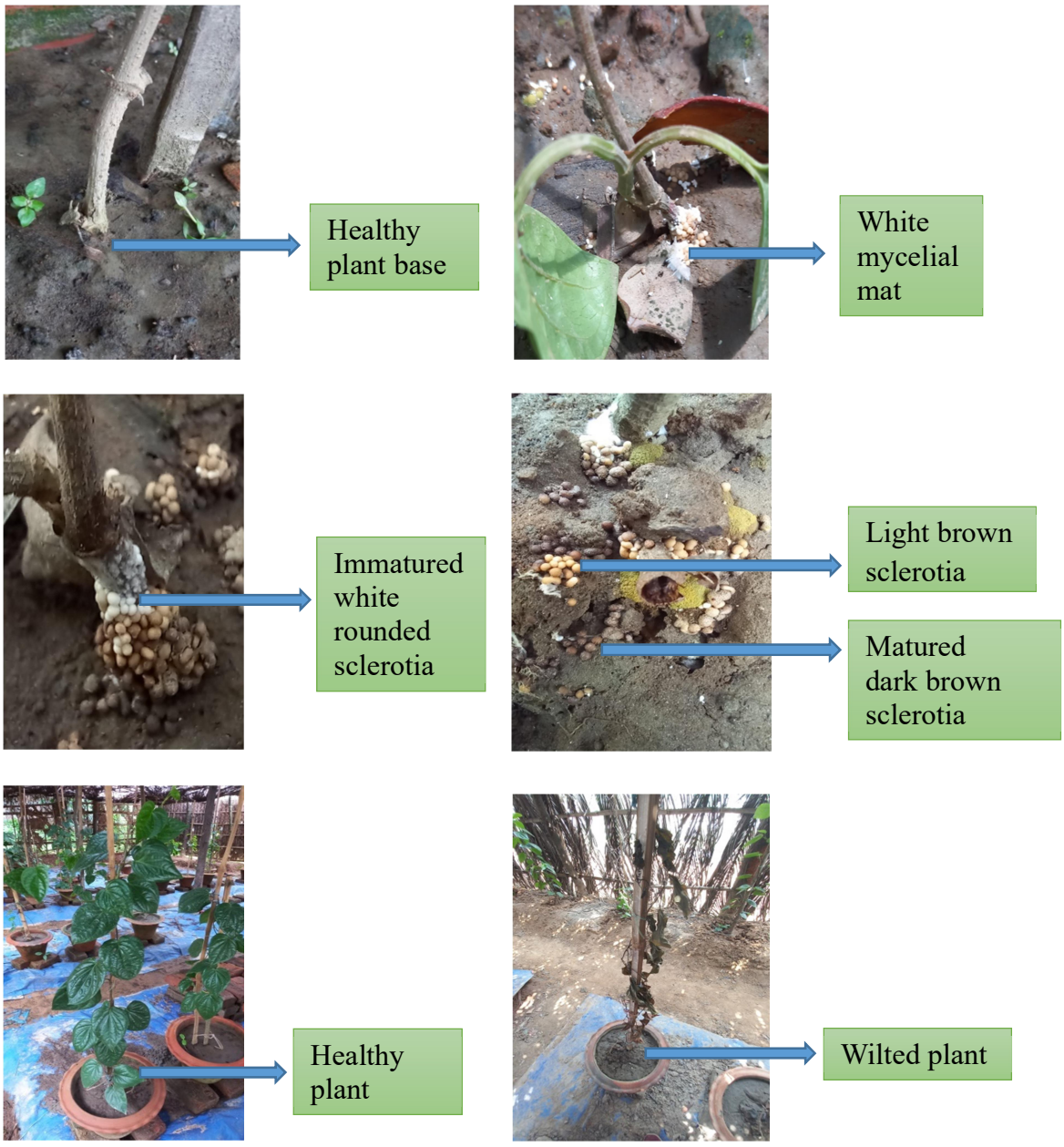

Plate 7. Symptoms of artificially inoculated betel vine plant by *Sclerotium rolfsii* causing foot and root rot disease

Experiment 2. Screening of betel vine cultivars available in Bangladesh against *Sclerotium rolfsii* causing foot and root rot disease

Nine (9) different cultivars of betel vine viz. ChL-01(Chuadanga Local), MoL-02 (Moheskhali Local), KiL-03 (Kishoreganj Local), SaL-04 (Satkhira Local), CuL-05 (Cumilla Local), RaL-06 (Rajshahi Local), BP-07 (BARI Pan-1), BP-08 (BARI Pan-2), BP-09 (BARI Pan-3) were evaluated against the pathogen, *Sclerotium rolfsii* causing foot and root rot disease of betel vine. The experiment was conducted in field condition in a betel vine garden (*pan baroj*).

The results were compiled based on physio-morphological features, days required for the visual expression of mycelia on soil, days required for the appearance of 1st disease symptom, disease reactions and yield and yield contributing characters (Table 3- 9).

4.2.1. Vegetative growth parameters

The vegetative growth parameters and morphological features of different cultivars of betel vine varied remarkably.

a) Vine elongation/ month (cm)

The maximum vine elongation per month (77.60 cm) was recorded in RaL-06 followed by KiL-03(67.61 cm), ChL-01 (57.71 cm), MoL-02 (53.32 cm) and CuL-05 (53.31 cm). The lowest vine increment (40.04 cm) was recorded in BP-07 (Table 3).

b) Length of internode (cm)

The maximum length of internode (10.33 cm) was recorded in RaL-06 followed by KiL-03 (7.93 cm) and the minimum length of internode (4.28 cm) was found in BP-07 (Table 3).

c) Vine girth (cm)

The maximum vine girth (2.24 cm) was recorded in RaL-06 and the minimum vine girth (1.03 cm) was in ChL-01 (Table 3).

Table 3. Vegetative growth parameters (vine elongation per month, length of internode and vine girth) of different betel vine cultivars used in the experiment

Accession no. of betel vine cultivars	Vine elongation /month (cm)	Length of internode (cm)	Girth of vine (cm)
ChL-01 (Chuadanga Local)	57.71 c	5.73 de	1.03 d
MoL-02 (Moheskhali Local)	53.32 cd	6.49 c	1.49 b
KiL-03 (Kishoreganj Local)	67.61 b	7.93 b	1.59 b
SaL-04 (Satkhira Local)	46.15 de	5.63 e	1.49 b
CuL-05 (Cumilla Local)	53.31 cd	7.02 c	1.53 b
RaL-06 (Rajshahi Local)	77.60 a	10.33 a	2.24 a
BP-07 (BARI Pan-1)	40.04 e	4.28 f	1.08 cd
BP-08 (BARI Pan-2)	46.51 de	6.44 cd	1.44 b
BP-09 (BARI Pan-3)	49.76 d	5.44 e	1.38 bc
CV (%)	10.78	8.59	17.68
LSD (0.05)	7.59	0.73	0.34

d) Leaf size (cm)

The highest length of leaf (21.00 cm) was recorded in RaL-06 followed by KiL-03 (19.10 cm) and the lowest leaf length (12.71 cm) was recorded in BP-07. The leaf breadth (15.71 cm) was recorded the highest in RaL-06 followed by KiL-03 (11.85 cm) and SaL-04(11.40 cm). Conversely, the lowest leaf breath (7.13 cm) was found in BP-07 (Table 4).

Table 4. Vegetative growth parameters (leaf size without petiole**)** of different betel vine cultivars used in the experiment

Accession no. of betel vine cultivars	Leaf size without petiole (cm)	
	Length	**Breadth**
ChL-01 (Chuadanga Local)	15.57 e	9.63 cd
MoL-02 (Moheshkhali Local)	18.62 bc	11.30 bc
KiL-03 (Kishoreganj Local)	19.10 b	11.85 b
SaL-04 (Satkhira Local)	17.10 d	11.40 b
CuL-05 (Cumilla Local)	17.93 cd	10.23 bd
RaL-06 (Rajshahi Local)	21.00 a	15.71 a
BP-07 (BARI Pan-1)	12.71 g	7.13 e
BP-08 (BARI Pan-2)	15.32 ef	8.53 de
BP-09 (BARI Pan-3)	14.35 d	8.54 de
CV (%)	5.11	12.98
LSD (0.05)	1.11	1.75

Hanif (2020) worked with eight different betel vine cultivars and recorded the maximum vine elongation (90.97 cm/month) in Moheshkhali pan, length of internode (8.27 cm) in Laldingi pan and vine girth (1.63 cm) in BARI Pan-2. He also recorded the highest leaf length (22.07 cm) and breadth (13.03 cm) in Satkhira pan and Moheshkhali pan, respectively.

Rahman (2017) found that the vegetative growth parameters and morphological features of 13 cultivars used in the experiment varied remarkably. The vine elongation per month, elongation of internode per month, vine girth, leaf length and leaf breadth ranged 37.46 -50.34 cm, 6.75-10.08 cm, 0.445-0.7475 cm, 17.13-27.35 cm and 8.33-16.20 cm, respectively.

4.2.2. Yield and yield contributing characters

a) Fresh weight of 100 leaf (gram)

The fresh weight of 100 leaf with petiole was recorded the highest in RaL-06 (675.80 g) followed by KiL-03 (556.00 g) and the lowest fresh leaf weight (180.00 g) was recorded in BP-07 (Table 5).

b) Number of Leaf per meter of vine

The highest number of leaves per meter vine was found in ChL-01 (19.00) followed by KiL-03 (18.40) and the lowest number (14.20) of leaves per meter vine was recorded in RaL-06 (Table 5).

c) Yield of betel vine leaf

The number of leaf of betel vine cultivars assayed in the experiment was ranged from 196.60 to 392.20 per plant per year. The highest leaf number (392.20) per plant per year was recorded in BP-07 followed by ChL-01 (308.80) and KiL-03 (299.20) while the lowest number (196.60) was counted in RaL-06 (Table 5).

Table 5. Yield and yield contributing characters of different betel vine cultivars used in the experiment

Accession no. of betel vine cultivars	Fresh wt. of 100 leaf with petiole(g)	No. of Leaf/ Meter Vine	Yield (No. of Leaf/ Plant/Year)
ChL-01 (Chuadanga Local)	223.20 f	19.00 a	308.80 b
MoL-02 (Moheshkhali Local)	323.00 de	16.00 c	258.40 b
KiL-03 (Kishoreganj Local)	556.00 b	18.40 a	299.20 bc
SaL-04 (Satkhira Local)	384.00 c	16.60 bc	259.20 d
CuL-05 (Cumilla Local)	366.00 cd	17.80 ab	276.80 cd
RaL-06 (Rajshahi Local)	675.80 a	14.20 d	196.60 e
BP-07 (BARI Pan-1)	180.00 f	16.00 c	392.20 a
BP-08 (BARI Pan-2)	213.00 f	15.20 cd	268.80 cd
BP-09 (BARI Pan-3)	280.60 e	16.00 c	281.40 bd
CV (%)	12.04	7.90	8.41
LSD (0.05)	55.19	1.69	30.58

The present findings have similarity with the findings of Hanif (2020) where he recorded the highest fresh weight of 100 leaf with petiole in Laldingi pan (553.33 g) and the lowest (206.67 g) in BARI Pan-1. He reported the highest number of leaves per meter vine (23.00) in BARI Pan-1 followed by Chuadanga pan (18.00). He also recorded the maximum yield in BARI Pan-1 (414 leaf/plant/year) followed by Chuadanga pan (324 leaf/plant/year) and Laldingi pan (300 leaf/plant/year).

4.2.3. Physio-morphological characteristics of betel vine cultivars

ChL-01 (Chuadanga Local)

The vine color of this cultivar was recorded as violet. The leaf was observed as soft, dark green, cordate to ovate and had acute tip with highly pungent (Table 6 and Plate 8).

MoL-02 (Moheshkhali Local)

The vine was green. The leaf was soft, dark green, cordate and had acute tip with medium pungent (Table 6 and Plate 8).

KiL-03 (Kishoreganj Local)

Vine color was green. The leaf was green, less soft, cordate in shape, acute in tip and had acute tip with medium pungent (Table 6 and Plate 8).

SaL-04 (Satkhira Local)

The vine colour was found dark green. The leaf was soft, light green, cordate, acute in tip and highly pungent (Table 6 and Plate 8).

CuL-05 (Cumilla Local)

The color of vine was greenish with light pinkish line. The leaf was soft after maturation, green coloured, cordate in shape with acute tip and less pungent (Table 6 and Plate 8).

RaL-06 (Rajshahi Local)

The vine color of was found dark green. The leaf of the cultivar was rough after maturation, dark green, cordate in shape with acute tip and highly pungent(Table 6 and Plate 9).

BP-07 (BARI Pan-1)

Vine was found green in color. The leaf was soft, light green colored, cordate to ovate in shape, acuminate in tip and pungency was medium (Table 6 and Plate 8).

BP-08 (BARI Pan-2)

The color of vine was violet with soft, dark green colored, cordate leaves having acute tip and medium pungency (Table 6 and Plate 8).

BP-09 (BARI Pan-3)

Vine colour was observed as dark green. After maturation, the leaf was found soft with green color, cordate shape, acute tip and no pungency (Table 6 and Plate 8).

Table 6. Physio-morphological characters of betel vine cultivars grown in the betel vine garden

Accession no. of betel vine cultivars	Color of vine	Color of leaf	Shape of leaf	Tip of leaf	Soft-ness of leaf	Pungency of leaf
ChL-01 (Chuadanga Local)	Violet	Dark green	Cordate to ovate	Acute	Soft	Highly pungent
MoL-02 (Moheskhali Local)	Green	Dark green	Cordate	Acute	Soft	Medium pungent
KiL-03 (Kishoreganj Local)	Green	Green	Cordate	Acute	Soft	Medium pungent
SaL-04 (Satkhira Local)	Dark green	Light green	Cordate	Acute	Soft	Highly pungent
CuL-05 (Cumilla Local)	Greenish with light pinkish line	Green	Cordate	Acute	Soft	Less pungent
RaL-06 (Rajshahi Local)	Dark green	Dark green	Cordate	Acute	Slightly rough	Highly pungent
BP-07 (BARI Pan-1)	Green	Light green	Cordate to ovate	Acuminate	Soft	Medium pungent
BP-08 (BARI Pan-2)	Violet	Dark green	Cordate	Acute	Soft	Medium pungent
BP-09 (BARI Pan-3)	Dark green	Green	Cordate	Acute	Soft	No Pungent

4.2.4. Evaluation of resistant betel vine cultivars against *Sclerotium rolfsii*

4.2.4.1. Days required for the appearance of mycelia on soil

Soil infested with *Sclerotium rolfsii* showed white mycelial growth on soil surface and around the foot and root regions of betel vine plants after inoculation. In case of SaL-04 (Satkhira Local), BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) mycelium formation was found after 3 days of inoculation whereas, in MoL-02 (Moheshkhali Local) and CuL-05 (Cumilla Local) white mycelial mat was noticed after 4 days of inoculation (Table 7).

Moreover, 7, 9 and 10 days were required for appearance of mycelia on soil after inoculation for ChL-01(Chuadanga Local), BP-07 (BARI Pan-1) and RaL-06 (Rajshahi Local), respectively. No conspicuous mycelial growth was noticed in case of KiL-03 (Kishoreganj Local) (Table 7).

Hanif (2020) and Rahman (2017) reported that soil infested with *S. rolfsii* exhibited mycelial growth on soil surface and around the root zone after 2 days of inoculation.

4.2.4.2. Days required for the appearance of 1st disease symptom

For appearance of 1st disease symptom after inoculation, the lowest incubation period (5days) was required for BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) and the highest incubation period (18 days) was required for RaL-06 (Rajshahi Local). In addition, 7, 8 and 11 days were required in case of CuL-05 (Cumilla Local), ChL-01 (Chuadanga Local) and BP-07 (BARI Pan-1) for appearance of 1st disease symptom, respectively. No symptoms were noticed in KiL-03 (Kishoreganj Local). On the lesions of infected plants, white mycelial growth having white and brown sclerotia depending on the fungal maturity was observed (Table 7).

The present findings have similarity with the previous report of Hanif (2020). He reported that the lowest incubation period (8 days) required for BARI Pan-2 and BARI Pan-3 and the highest incubation period (14 days) was required for

BARI Pan-1 for the appearance of 1st disease symptom. He also reported that no symptoms were seen in the cultivar, Laldingi pan (Kishoreganj Local).

Rahman (2017) while working with 13 accession of betel vine reported that the lowest incubation period (8 days) required for the cultivars, Misti pan, BARI Pan-1 and Bangla pan. The highest incubation period (22 days) was required for the cultivars, Laldingi pan, Jhal pan and Gayasur pan.

Table 7. Days required for appearance of mycelia on soil and 1st disease symptom

Accession no. of betel vine cultivars	Days required for appearance of mycelia on soil	Days required for appearance of 1st disease symptom
ChL-01 (Chuadanga Local)	7	8
MoL-02 (Moheshkhali Local)	4	5
KiL-03 (Kishoreganj Local)	Not seen	Not seen
SaL-04 (Satkhira Local)	3	5
CuL-05 (Cumilla Local)	4	7
RaL-06 (Rajshahi Local)	10	18
BP-07 (BARI Pan-1)	9	11
BP-08 (BARI Pan-2)	3	4
BP-09 (BARI Pan-3)	3	4

4.2.4.3. Disease incidence

Evaluation of resistance in cultivars was measured on the basis of disease incidence in plants of each cultivar after inoculation with the pathogen, *Sclerotium rolfsii*. A total of nine cultivars of betel vine were screened out for making the evaluation process (Plate 8).

After 3 days of inoculation, no disease incidence was found in any cultivars of betel vine while 80% disease incidence was noticed in BP-09 (BARI Pan-3) and 20% in MoL-02 (Moheshkhali Local), SaL-04 (Satkhira Local) and BP-08 (BARI Pan-2) after 6 days of inoculation (Table 8).

After 9 days of inoculation, disease incidence was found 40% in MoL-02 (Moheshkhali Local) and SaL-04 (Satkhira Local) and 60% in BP-08 (BARI Pan-2) while 20% was observed in ChL-01 (Chuadanga Local) and CuL-05 (Cumilla Local) (Table 8).

After 12 days of inoculation, BP-08 (BARI Pan-2) showed 80% whereas, BP-07 (BARI Pan-1) showed 20% disease incidence against *Sclerotium rolfsii* (Table 7). On the other hand, BP-08 (BARI Pan-2) showed 100%, SaL-04 (Satkhira Local) showed 60% and CuL-05 (Cumilla Local) showed 40% disease incidence after 15 days of inoculation (Table 8).

Even after 18 days of inoculation, no disease incidence (0%) was recorded in KiL-03 (Kishoreganj Local). The lowest disease incidence (20%) was recorded in RaL-06 (Rajshahi Local), ChL-01 (Chuadanga Local) and BP-07 (BARI Pan-1) followed by moderate disease incidence (60%) in MoL-02 (Moheshkhali Local), SaL-04 (Satkhira Local) and CuL-05 (Cumilla Local). After 18 days of inoculation, the highest disease incidence (100%) was recorded in BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) (Table 8).

The present findings support the data of previous researchers. Hanif (2020) reported that after 30 DAI, the disease incidence of eight different betel vine cultivars showed remarkable difference. No disease incidence (0%) was noticed in case of Laldingi pan. The highest disease incidence (100%) was recorded in case of BARI Pan-2 and BARI Pan-3 followed by Chalitaguti pan (60%) and Satkhira pan (40%). The minimum (20%) disease incidence was recorded in Chuadanga pan, Moheshkhali pan and BARI Pan-1.

Rahman (2017) reported that at 30 DAI, the lowest disease incidence was recorded in case of Laldingi pan. 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 among 13 different betel vine cultivars.

Jahan (2016) reported that foot and root rot of betel vine was recorded as a common disease in all the surveyed areas of the country. Regarding locations of survey, the highest disease incidence, 64.00% and 52.00% were found in July and October, respectively at Mirpur upazilla while the lowest disease incidence, 28.00% and 20.00% were observed in July and October, respectively at Khoksha upazilla in Kushtia district. She mentioned that disease incidence was found to vary from month to month, baroj to baroj as well as location to location.

Mollah (2012) made a survey on foot and root rot of betel vine in Satkhira district of Bangladesh and reported that disease incidence was ranged from 0.0 –50.00 %. He also reported that the disease incidence found to be varied in respect of growing areas and weather factors.

4.3.4.4. Disease reactions

Based on the disease responses (disease incidence) of the evaluated betel vine cultivars, they are categorized as Resistant (R), Moderately resistant (MR), Moderately susceptible (MS) and Susceptible (S). Among the cultivars, only one cultivar KiL-03 (Kishoreganj Local) showed resistant reaction (R), while three cultivars viz. ChL-01 (Chuadanga Local), RaL-06 (Rajshahi Local) and BP-07 (BARI Pan-1) showed moderately resistant reaction (MR). Three cultivars viz. MoL-02 (Moheshkhali Local), SaL-04 (Satkhira Local) and CuL-05 (Cumilla Local) showed moderately susceptible reaction (MS) and rest two cultivars viz. BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) showed susceptible reaction (Table 8).

Hanif (2020) reported only Laldingi pan (Kishoreganj Local) showed resistant reactions (R), while Chuadanga pan, Moheskhali pan and BARI Pan-1 showed moderately resistant reactions and other two cultivars viz. Chalitaguti pan, Satkhira pan showed moderately susceptible reactions and BARI Pan-2 and BARI Pan-3 showed susceptible reactions against foot and root rot disease caused by *S. rolfsii*.

On the other hand, Mia (2017) reported that Bari Pan-2 and Bari Pan-3 were tolerant against root rot disease.

Rahman (2017) showed that among different germplasms of betel vine, the lowest disease incidence was found in Laldingi pan (Kishoreganj Local) and it was designated as resistant (R) cultivar while Jhal pan and Gayasur pan showed moderately susceptible reactions against *S. rolfsii* causing foot and root of disease of betel vine.

ChL-01 (Chuadanga Local)

MoL-02 (Moheshkhali Local)

KiL-03 (Kishoreganj Local)

SaL-04 (Satkhira Local)

CuL-05 (Cumilla Local)

RaL-06 (Rajshahi Local)

BP-07 (BARI Pan-1)

BP-08 (BARI Pan-2)

BP-09 (BARI Pan-3)

Plate 8. Pictorial view of different betel vine cultivars before (left) and after (right) inoculation of *Sclerotium rolfsii* causing foot and root rot disease

Table 8. Disease reactions of different betel vine cultivars against foot and root rot of betel vine caused by *Sclerotium rolfsii*

Accession no. of betel vine cultivars	Percent disease incidence						Disease reactions
	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI	18 DAI	
ChL-01 (Chuadanga Local)	0.00	0.00	20.00	20.00	20.00	20.00	MR
MoL-02 (Moheshkhali Local)	0.00	20.00	40.00	40.00	40.00	60.00	MS
KiL-03 (Kishoreganj Local)	0.00	0.00	0.00	0.00	0.00	0.00	R
SaL-04 (Satkhira Local)	0.00	20.00	40.00	40.00	60.00	60.00	MS
CuL-05 (Cumilla Local)	0.00	0.00	20.00	20.00	40.00	60.00	MS
RaL-06 (Rajshahi Local)	0.00	0.00	0.00	0.00	0.00	20.00	MR
BP-07 (BARI Pan-1)	0.00	0.00	0.00	20.00	20.00	20.00	MR
BP-08 (BARI Pan-2)	0.00	20.00	60.00	80.00	100.00	100.00	S
BP-09 (BARI Pan-3)	0.00	80.00	100.00	100.00	100.00	100.00	S

4.2.5. Population dynamics of *Sclerotium rolfsii* in root zone area of infested soil

For determination of colony forming unit (CFU) of *Sclerotium rolfsii* in inoculated and infested soil, soil dilution plate method was followed. During soil dilution plate method, colony counting was achieved at 1:1000 dilutions. The lowest population of *Sclerotium rolfsii* was found in case of KiL-03 ($0.06x10^6$ CFU/g **s**oil). The second and third lowest population density were observed in RaL-06 ($0.20x10^6$ CFU/g **s**oil) and ChL-01 ($0.46x10^6$ CFU/g **s**oil), respectively. On the other hand, the highest population density was found in BP-09 ($3.04x10^6$ CFU/g **s**oil) followed by BP-08 ($2.46x10^6$ CFU/g **s**oil) (Table 9).

Table 9. Colony forming unit (CFU) of *Sclerotium rolfsii* per gram soil extracted from rhizosphere soil of different betel vine cultivars by soil dilution plate method

Accession no. of betel vine cultivars	CFU/g soil ($x10^6$)
ChL-01 (Chuadanga Local)	0.46 g
MoL-02 (Moheshkhali Local)	1.38 c
KiL-03 (Kishoreganj Local)	0.06 i
SaL-04 (Satkhira Local)	0.90 e
CuL-05 (Cumilla Local)	0.98 d
RaL-06 (Rajshahi Local)	0.20 h
BP-07 (BARI Pan-1)	0.60 f
BP-08 (BARI Pan-2)	2.46 b
BP-09 (BARI Pan-3)	3.04 a
CV (%)	3.76
LSD (0.05)	54083

From the present findings it can be said that CFU/g soil for *Sclerotium rolfsii* was the lowest for KiL-03 (Kishoreganj Local) which showed resistant reactions against foot and root disease of betel vine. Possibly, there could have effects of root exudates or some other bio-chemicals secreted from Kishoreganj cultivar which might be the reason of the least inoculum formation of *S. rolfsii* comparing to the other cultivars.

CHAPTER-5
SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Betel vine (*Piper betle* L.) is an important cash crop having immense medicinal, social, religious and aesthetic values in many Southeast Asian countries and thus, it is regarded as "green gold". The present study was carried out to isolate and identify the causal organism of foot and root rot of betel vine, to study the pathogenicity of the isolated organism and to screen out resistant cultivar(s) of betel vine available in Bangladesh for the management of foot and root rot disease.

Pathogenicity test showed that *Sclerotium rolfsii* produced characteristic symptoms on betel vine and the cultural characteristics of the isolated organism matched with the CMI description No. 410 that proved to be the causal organism of foot and root rot disease of betel vine. Nine betel vine cultivars viz. ChL-01 (Chuadanga Local), MoL-02 (Moheskhali Local), KiL-03 (Kishoreganj Local), SaL-04 (Satkhira Local), CuL-05 (Cumilla Local), RaL-06 (Rajshahi Local), BP-07 (BARI Pan-1), BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) were assayed against *Sclerotium rolfsii* causing foot and root rot disease in betel vine garden (Pan baroj). Results were compiled based on physio-morphological features, days required for visual expression of mycelial growth on soil, days required for appearance of 1st disease symptom, disease reactions, yield and yield contributing characters.

From this study, it is evident that the vine increment per month was the highest (77.60 cm) in RaL-06 (Rajshahi Local) and the lowest (40.04 cm) in BP-07 (BARI Pan-1). The maximum length of internode (10.33 cm) was recorded in RaL-06 (Rajshahi Local) and the minimum (4.28 cm) was recorded in BP-07 (BARI Pan-1). The maximum vine girth (2.24 cm) was found in RaL-06 (Rajshahi Local) and the minimum (1.03 cm) was in ChL-01(Chuadanga Local).

The largest leaf length (21.00 cm) and leaf breadth (15.71 cm) were recorded in RaL-06 (Rajshahi Local) whereas the smallest leaf length (12.71 cm) and leaf breadth (7.13) were recorded in BP-07 (BARI Pan-1). The fresh weight of 100 leaves without petiole was recorded the highest (675.80 g) in RaL-06 (Rajshahi Local) and the lowest weight (180.00 g) in BP-07 (BARI Pan-1). ChL-01(Chuadanga Local) produced significantly the highest number (19.00) of leaf per meter vine and the lowest number (14.20) of leaf was produced in RaL-06 (Rajshahi Local).

In terms of yield, the highest leaf number per plant per year (392 leaves) was noticed in BP-07 (BARI Pan-1) and the lowest number (196 leaves) was in RaL-06 (Rajshahi Local).

After inoculation with *Sclerotium rolfsii*, days required for the appearance of mycelial growth on soil varied remarkably. The lowest 3 days were required for SaL-04 (Satkhira Local), BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) and the highest 10 days were required for RaL-06 (Rajshahi Local). No mycelial growth was seen on soil surface in case of KiL-03 (Kishoreganj Local). The appearance of 1st disease symptoms among the betel vine cultivars differed significantly. The highest incubation period (18 days) was found in RaL-06 (Rajshahi Local) and the lowest (4 days) was found in BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3).

Regarding disease incidence and severity, it ranged from 0.00%-100%. Among the betel vine cultivars, KiL-03 (Kishoreganj Local) showed resistant (R) reactions while three cultivars viz. ChL-01(Chuadanga Local), RaL-06 (Rajshahi Local) and BP-07 (BARI Pan-1) showed moderately resistant (MR) reactions. Three cultivars viz. MoL-02 (Moheskhali Local), SaL-04 (Satkhira Local) and CuL-05 (Cumilla Local) showed moderately susceptible (MS) reactions and the rest two cultivars BP-08 (BARI Pan-2) and BP-09 (BARI Pan-3) showed highly susceptible reactions against *Sclerotium rolfsii* causing foot and root rot disease of betel vine.

Population density for *Sclerotium rolfsii* was observed the highest ($3.04x10^6$CFU/g **s**oil) in BP-09 (BARI Pan-3) and the lowest ($0.06x10^6$CFU/g **s**oil) in KiL-03 (Kishoreganj Local).

From the findings of the present investigation it can be concluded that KiL-03 (Kishoreganj Local) showed a promising performance in terms of resistance against foot and root rot disease. The morphological characteristics and yield of KiL-03 (Kishoreganj Local) were desirable. Thus, the betel vine growers are suggested to use KiL-03 (Kishoreganj Local) cultivar against *Sclerotium rolfsii* causing foot and root rot of betel vine.

Further extensive study is recommended taking more cultivars available in the country to find out other resistant cultivar(s) if any, against *Sclerotium rolfsii* causing foot and root rot disease of betel vine. As *Sclerotium rolfsii* is a soil-borne pathogen and its survival in soil depends on various factors like micro climate, soil condition and soil type; therefore, zonal trials for consecutive years are needed to be carried out to confirm the present findings.

CHAPTER-6
REFERENCES

CHAPTER VI
REFERENCES

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CHAPTER-7
APPENDICES

CHAPTER VII

APPENDICES

Appendix I: Nutritional Composition of fresh betel leaf

Constituents	Approximate composition
Water	85-90%
Protein	3-3.5%
Fat	0.4-1.0%
Minerals	2.3-3.3%
Fibre	2.30%
Chlorophyll	0.01-0.25%
Carbohydrate	0.5-6.10%
Nicotinic acid	0.63-0.89 mg/100g
Vitamin C	0.005-0.01%
Vitamin A	1.2-2.9 mg/100g
Thiamin	10-70 µg/100g
Riboflavin	1.9-30 µg/100g
Tannin	0.1-1.3%
Nitrogen	2.0-7.0%
Phosphorus	0.05-0.6%
Potassium	1.1-4.6%
Calcium	0.2-0.5%
Iodine	3.4 µg/100g
Essential oil	0.08-0.2%
Energy	85-90%

Source: Guha, 2006.

Appendix II: Some pictorial view during field work and lab work