INVESTIGATION ON FOOT AND ROOT ROT OF BETEL VINE (*Piper betle* L.) IN KUSHTIA DISTRICT OF BANGLADESH

BY

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INVESTIGATION ON FOOT AND ROOT ROT OF BETEL VINE (*Piper betle* L.) IN KUSHTIA DISTRICT OF BANGLADESH

BY

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ABSTRACT

Betel vine is a perennial dioecious creeper and important cash crop of Bangladesh. This crop suffers from several diseases. Betel vine crop is mainly attacked by foot and root rot disease in Kushtia**.** The young stems were found more prone to attack than the old ones. Pathogenicity test showed *Sclerotium rolfsii* produced characteristic symptoms on betel vine and proved to be the causal pathogen of the disease. An investigation on foot and root rot of betel vine was done in six upazillas of Kushtia district viz. Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar, Mirpur. Disease incidence and severity of foot and root rot of betel vine ranged from 24.00 to 58.00% and 17.65 to 34.75%, respectively where the maximum disease was recorded in Mirpur and the minimum was in Khoksha in the month of July and October. Disease incidence and severity of foot and root rot of betel vine ranged from 54.00 to 64.00% and 34.00 to 35.60%, respectively in Mirpur where the maximum disease was recorded in July and the minimum was in October. In Kushtia Sadar, disease incidence and severity of foot and root rot of betel vine ranged from 27.00 to 37.00% and 18.45 to 20.39%, respectively. In Bheramara, disease incidence and severity of foot and root rot of betel vine ranged from 27.00 to 37.00% and 18.30 to 20.38%, respectively. In Kumarkhali, the disease incidence and severity were 45.00 to 50.00% and 27.95 to 30.60%, respectively. In Khoksha, disease incidence and severity of foot and root rot of betel vine ranged from 24.00 to 31.00% and 17.65 to 18.80%, respectively. In Daulatpur, disease incidence and severity of foot and root rot of betel vine ranged from 39.00 to 46.00% and 22.30 to 24.35%, respectively. Considering all the locations of Kushtia District, the maximum disease was recorded in the month of July and the minimum was in October.

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The author

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CERTIFICATE

This is to certify that the thesis entitled, "INVESTIGATION ON FOOT AND ROOT ROT OF BETEL VINE (Piper betle L.) IN KUSHTIA DISTRICT OF BANGLADESH" 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* ***Afsana Jahan,*** *bearing Registration No****. 13-05751*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

.....
Dated: 20 November, 2014
Place: Dhaka, Bangladesh

Dr. Md. Rafiqul Islam
Professor
Supervisor

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CHAPTER I

INTRODUCTION

Betel vine, (*Piper betel* L.) is a perennial dioecious creeper belonging to the family Piperaceae. It is thought to have originated in Malaysia, Sumatra and possibly Java (Chattopadhayay and Maiti, 1967). It is a climbing plant with shiny, green, heart-shaped leaves and white catkin. The stem is climbing by many short adventitious roots (Hassan and Shahadat, 2005). Leaves of betel vine are chewed along with areca nut as a masticator. Usually the people of South Asia, Southeast Asia, Gulf States and Pacific islands chew betel leaves. All classes of people in Bangladesh chew betel vine not only as a habit but also as an item of rituals, etiquette and manners.

The deep green heart shaped leaves of betel vine are popularly known as *Paan* in Bangladesh. It is an important cash crop in Bangladesh. It is also known as Nagaballi, Nagurvel, Saptaseera, Sompatra, Tamalapaku, Tambul, Tambuli, Vaksha Patra, Vettilai, Voojangalata etc in different parts of India (CSIR, 1969; Guha and Jain, 1997).

Betel vine (*Piper betle* L.) is an important horticultural crop of aesthetic and commercial values. The perennial climber is grown throughout the country. There are about 100 varieties of betel leaf (paan) across the world of which 40 are encountered in India and 30 in west Bengal and Bangladesh (Guha 1997; Maity, 1989; Samanta, 1994). In Sri Lanka, 18 species are found and three are endemic (Dassanayake and Fosberg 1981). Desi Bangla, Bangla, Kali Bangla, Jhali, Sanchi, Bhabna, Mitha, Geso, Bonhoogly etc. betel vine cultivars are found in Bangladesh.

Paan is grown in moist tropical region in the world. It has been grown under two conditions i.e., natural conditions and controlled conditions. In natural condition in the tropical forest region on the tree it can grow as tall as the tree (Western regions & north eastern regions). Cultivation under controlled conditions (bareja) is in practice in the sub-tropics. The betel vine cultivation is practiced, the south region where humidity and temperature do not fluctuate abnormally and high humidity with moderate sunshine prevails throughout the year. The cultivation under controlled condition is practiced where there is relative humidity is often low and temperature remains high (above 40°C) in summer and low (below 10°C) in winter, respectively (Guha and Jain, 1997). In Bngladesh, Betel vine is cultivated mainly under an artificially erected structure, known as Boroj, Bareja or Bheet, which is kind of hut which sides and roof are made of jute slaths or 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.

Bangladesh is the second largest grower of betel vine on about 14000 hectares. Total annual production of the crop in Bangladesh is about 72,500 tons. The average yield is 2.27 tons per acre (Anonymous, 2006). Its cultivation is concentrated in the greater district of Barisal, Cox's Bazar, Rajshahi, Maulavi- Bazar, Satkhira, Jessore, Kushtia, Jhinidah, Pabna etc.

Betel vine is an evergreen, perennial climber with a semi arid stem. It is trained on poles or trellis. The leaves of this plant are economically and medically important. The medicinal properties of paan was recognized during 600 AD. when Ayurvedic system of medicine came into practice.

Betel leaves are beneficial to the throat and remove viscidity in human beings. It is also good for the respiratory system and is used in treatment of bronchitis, cough and cold (Chopra *et al*, 1965). It increases digestive capacity when used with lime. Besides, it neutralizes the acidity and acts as blood purifier. Main constituents of betel leaves are vitamin B and C, carotene, and other elements. Paan 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; Khanra 1997).

Betel leaves regarded as an excellent mouth freshener and mild vitalizer, routinely served on the social, cultural and religious occasions like marriage, Puja (religious festivals), Sraddha ceremony (religious function performed after cremation) etc. It is also used as a special item offered to the guests in order to show respect and for such traditional use of betel leaf in the Indian society, the leaf really stands alone without any parallel even today (Guha, 1997; Mehrotra, 1981).

In fact, this edible leaf has achieved an esteemed position in the human society right from the dawn of civilization, particularly in the countries like Bangladesh, Myanmar, China, India, Indonesia, Malaysia, Nepal, Pakistan, Philippines, South Africa, Sri Lanka, Thailand etc. (Jana, 1996; Khoshoo, 1981; Samanta, 1994). Where betel leaves are traditionally used with sliced areca nut, lime, coriander, clove, cardamon, sweetener, coconut scrapings, ashes of diamond, pearl, gold and silver (Ayurvedic preparations), jelly, pepper mint, flavouring agent, fruit pulp etc. (CSIR, 1969).

The acreage of betel vine is decreasing gradually because of some physical and socioeconomic barriers like unavailability of credit facilities, uncontrolled marketing system and infestation of diseases and pest (Islam, 2005). Disease is one of several known limiting factors. The betel vine is highly susceptible to diseases, pests and some natural climates (Sayeeduzzaman, 1988). Among the diseases of betel vine, foot and root rot caused by *Sclerotium rolfsii* Sacc. are the most devastating diseases which decrease the production of betel vine to a great extent. In 2004, Sixty percent betel vine damaged due to foot rot disease in 3 upazilla of Rajshahi (Islam, 2005).

Sclerotium rolfsii Sacc. is a soil borne pathogenic fungus and harmful to many crops which are economically valuable in most of the tropical and subtropical region of the world (Aycock, 1966). It has a wide host range and has been referred as an almost omnipathogenic organism (Talukder, 1974). The fungus is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of sclerotia (Ahmed, 1980). It is very difficult to control even by the use of chemical fungicide.

As a creeper crop the basal part of the betel vine stem to be kept in soil by folding and it's a continuous process as the part of the cultivation practice. The stem kept in the soil often affected by the soil borne fungus *Sclerotium rolfsii.* The basal part of the stem become rotten and caused a huge loss of the betel vine growers, reduces the quality of betel leaf and hence the farmers deprived from the usual market price. Kushtia is a well known and the major betel vine growing district in Bangladesh. Most of the marginal farmers are involved in betel vine cultivation, as it is a continuous source of income. The farmers of different upazillas viz. Kushtia sadar, Mirpur, Bheramara, Daulatpur, Khoksha, Kumarkhali etc grows betel vine regular basis and contribute a lot to meet up the

national demand as well as exporting betel vine leaf abroad. For continuous cultivation, the betel stem rot infestation seem to be alarming for inoculum potential. The betel vine growers are in troubles to grow betel vine due to this disease problem. Thus, investigation on this acute disease problem in Kushtia district need to accomplish.

On the basis of above facts the present investigation was undertaken with the following objectives

1. To isolate and identify the causal pathogen of foot and root rot of betel vine.
2. To study the pathogenicity of the isolated organism.
3. To survey on disease incidence and disease severity of foot and root rot of betel vine in major growing areas of Kushtia district.

CHAPTER II

REVIEW OF LITERATURE

Betel vine (*Piper betle* L.) is prone to attack of many diseases at all stages of growth. The diseases of betel vine have been studied in Bangladesh to a limited extent. Humid and moist shaded conditions are favorable for its growth. But these humid and moist shaded conditions are very prone to root and foliage disease development of betel vine. Foot and root rot of betel vine caused by *Sclerotium rolfsii* Sacc. is also a devastating soil borne fungus with a wide host range of agricultural and horticultural crops and very much difficult to control. The fungus *Sclerotium rolfsii* Sacc. is also a devasting soil brone pathogenic fungus with a wide host range of agricultural and horticultural crops and very much difficult to control. The fungus *Sclerotium rolfsii* Sacc. is also a facultative saprophyte and can maintain contunity of generation under adverse situation by formation of sclerotia.

Sclerotium rolfsii, the causal agent of foot rot or collar rot of many crops (Aycock, 1966) having wider host range (Talukdar, 1974; Bhattacharrya *et al*.,1977) attracted the attention of plant pathologist and professional researcher throught the world. The pathogen is known to cause diseases of cereals, pulses, oil crops, betel vine, potatoes, vegetables, ornamentals and nursery seedlings of fruits and forest trees.

In Bangladesh, disease caused by *Sclerotium rolfsii* in different crops have been reported among many others by Talukder (1974) and Meah and Khan (2003).

In this chapter attempt has been made to review the available literature about foot and root rot disease. Some important literatures supporting the symptom, effect of the diseases, method of inoculation of *Sclerotium roifsii*, host range of *Sclerotium roifsii*, histopathology are reviewed here.

2.1. History

The foot and root rot of betel vine have been reported from almost all betel vine growing countries in the world including Indonesia, Myanmer, Srilanka (Paul, 1939) and Bagladesh (Roy, 1948; Tuner, 1969) etc. in west Bengal, the highest intensity of foot and root rot have been recorded in Midnapore and Nadia district (Dasgupta an *Sclerotium roifsii* d Sen, 1997 & 1999). The extent of losses may vary from 30-100% in case of foot and root rot (Maiti and Sen, 1982; Dasgupta *et al*., 2000).

Islam (2005) observed that farmers growing *Piper betel* in three upazilas of Rajshahi incurred a huge loss as foot rot disease damaged about 60% of the cultivation in the year of 2004.

2.2. Environmental factor

An epidemiological studies were reported that the maximum temperature, maximum relative humidity and rainfall played an important role in the development of both the diseases of betel vine (*Piper betel* L.) (Anonymous, 2000-2006; Maiti and Sen, 1982).

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 25-35^0C which is also

optimum range for mycelia growth and sclerotia germination of the fungus.

Farr *et al*. (1989) found that, fungus *Sclerotium roifsii* attacks all plant parts in the contact with the soil under favourable environmental conditions including stems, roots, and fruits.

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

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

CSIR (1969); Guha and Jain (1997) observed that 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.

Mollah (2012) found that 29^0C and 85% R_H, the disease incidence and severity of foot and root rot of betel vine was the highest and was the lowest when the temperature laid around 18.7^0C and the R_H laid around 75% in Satkhira district.

Al-Askar *et al.* (2013) found that sclerotial diseases caused by *Sclerotium rolfsii* occur primarily in warm climates, especially at high temperatures.

2.3. Symptom and effect of the disease

Bertus (1929) stated that *Sclerotium rolfsii* possessed the ability to cause damping off of the seedlings of certain plants when the pathogen was brought in contact with stem of these plants. Conditions that appeared to be necessary for the fungus were its presence in the upper four inches of the soil, high temperature and humidity. On a number of other hosts like chilli, tomato, groundnut etc it caused collar rot of the plants.

Ramkrishan *et al.* (1930) and Smith (1932) found that *Sclerotium rolfsii* causing wilt, it was common on Irish potatoes, sweet potatoes and almost all kinds of vegetables and flowers in Jamaica.

Dastur (1935) observed that the foot and root rot of betel vine, the leaves and shoots turn yellow, wither and finally dry out to a pale brown colour. The fungus attack the roots and stem near the soil level. Black lesion develops following necrosis of the plant cells. The mycelium invades the stem and rots the affected portions. As a result, the plant wilts and gradually dies. Abundant white mycelium and small light brown Sclerotia form on the rotted plants.

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 fall on the soil surface. More or less round sclerotia, brown in colour, 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.

Aycock (1966) stated that host range of *Sclerotium rolfsii* is very wide and includes not only many important horticultural and agronomic crops but many of noneconomic importance. 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.

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.

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.

Choudhury (1967) stated that, foot rot disease of brinjal is caused by *Sclerotium* sp. Minute mustard like structure, adheres 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. It is difficult to control the disease as the fungus persists in the soil. Crop rotations with crops which are not affected by this and mixing some copper fungicides in the soil before planting the soil is helped to control the disease.

Mostofa (1973) stated that the presence of any amount of *Sclerotium rolfsii* in would produce collar rot and ultimately wilting of betel vines.

Dutta *et al.* (2002) observed that *Sclerotium rolfsii* initially attacks roots of tuberose plant and later advances to the tubers and petioles to cause 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.

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

Singh (1970) observed that, damping-off symptoms are brown lesion on the hypocotyl base, 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 causal fungus infects the base of stems producing a fan of silky white mycelium and round sclerotia which are initially white and gradually darken. When pathogen attack the seedlings in that's time invades quickly and plant die rapidly.

Aycock (1966) and Punja *et al.* (1988) reported that root-rot disease caused by *Sclerotium rolfsii* being one of the most important diseases of crops.

Ahmed and Hossain (1985) reported that collar rot, foot and root rot disease caused by *sclerotium rolfsii* caused considerable damage both in seedling and adult stages of Indian spinach, and there existed variations in the incidence of the disease in different parts of Bangladesh.

Chakravarty and Bhowmik (1983) studied on symptoms and techniques of inducing collar-rot of sunflower caused by *Sclerotium rolfsii* Sacc. The fungus caused pre- and post-emergence damping off of sunflower seedling and collar rot of adult plants. Affected plants developed round-elliptical basal lesions, brown-tan colored whitish entre and producing chaff seeds. Collar rot was serious in the wet seasons of 1977 at the University Farm, Kalyani and occurred at IARI, Delhi. Disease development was highest in 60 day old plants, with maximum rotting in internal tissues and highest incidence in the field at the stage.

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

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

Gurkins and Jenkins (1985) reported that carrot became diseased by *Sclerotium rolfsii* about 90-100 days of planting when the plant canopies shade the soil surface and create a micro environment suitable for southern blight development in the North Carolina.

Yasmin-Ahmed *et al*. (1988) reported that *Sclerotium rolfsii* caused collar rot of maize. The pathogen was isolated from infected maize and pure culture was subsequently inoculated into Maize cv. Shaheen sown in soil infested with the pathogen. Within 15 days of seedling emergence,

sclerotia were seen on the soil surface and around the seedling. Seedlings were killed within 10-15 days.

Wangihr *et al.* (1988) reported that an outbreak of collar and root rot was observed on Capsicum in Maharastra, India during the first week of October, 1985.The disease was most severe on cultivars Jurala and CA960. The causal agent was identified as *Sclerotium (Coeticium) rolfsii*.

Montealegre and Esterio (1989) reported that outbreak of *Sclerotium rolfsii* affected more than 150 hectares of *Phaseolus vulgaris* occurred in Chile in January 1987.The disease was unequally distributed with large area of dead plants and patches of small plants showed wilt and chlorosis, Adult plants which were infected but did not die produced fewer fruits and smaller seeds than uninfected plants.

Mridha and Alamgir (1989) observed sclerotial wilt of betel vine in thirty selected gardens in Chittagong. 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 whole plant wilted and died. The infected portion of stem was covered with white cottony mycelia strands with small, light to deep brown sclerotia on the stem as well as adjacent soil surface.

Okoli *et al. (*1991) reported that *Sclerotium rolfsii* caused heavy infestation on sunflower, plants wilted and dried out with basal stem dry rot. Symptoms included an initial acropetel wilting of the entire plants. Affected plants gradually dry-out but remained green and attached to stem. They found that within 24 hrs of wilt onset, a white mat of mycelia formed around the discolored site on the stem base. Within 1-2 days, the mat had rounded off into small white balls and characteristics brown mature sclerotia within 24 hrs.

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 one 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.

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.

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

Kulkarni *et al.* (1994) reported 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 sclerotia resembling mustard seed developed at later stages on the root and collar regions of the infected plants. After that, tubers get infected and rotten in the field.

The pathogens of sclerotial diseases cause damping-off of seedlings, stem canker, crown blight, root-rot, crown rot, bulb, tuber and fruit rots.

Sclerotial diseases frequently affect a wide variety of plants, including most vegetables, flowers, legumes, cereals, forage plants and weeds (Agrios, 1997 and Farr, *et al*., 1989).

Mullen (2001) reported Southern blight, Southern stem blight, White mold caused by *Sclerotium rolfsii.*

Anahosur (2001) observed the dark brown lesion on the stem just below the surface followed by drooping and wilting of infected leaves and gradually wilting of the entire plant. Such wilted plants showed whitish mycelia growth with sclerotial bodies resembling mustard seeds on collar region and also roots.

Lievens *et al.* (2004) studied that a severe rot and collar/foot rot was observed on two month old wilted tomato (*Lycopersicum esculentum)* plants in a large scale (2.5 ha) commercial green house setting in Belgium. Symptom development was restricted to lower plant parts with severe rotting of the entire root system and dark lesions girdling the stem base.

Yaqub and Shahzad (2005) proved *Sclerotium rolfsii* highly pathogenic on sunflower, and mildly pathogenic on tomato, lentil, sweet pumpkin and cabbage and non-pathogenic on cauliflower plant in a pot experiments. Increase in inoculum density of *Sclerotium rolfsii* caused gradual relation in growth parameters of sunflower and mungbean plants where as a positive correlation was observed between root colonization and population of *Sclerotium rolfsii* in soil.

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

According to Daami-Remadi *et al*. (2007), potato tubers showing a fan-like mycelia growth at their surface and severe soft rot symptoms were observed in traditional potato storage at Essaida (North of Tunisia).

2.4. Method of inoculation of *Sclerotium rolfsii*

Islam (2008) inoculated the eggplants following soil inoculation technique 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 soil infestation method for 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 heavy infestation.

Babar (1999) used 10 gms of colonized dried oat grains or pouring 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 that in younger ones (10-45 days age).

Hiremath *et al*. (1998) compared 5 methods of inoculation of *S. rolfsii*. Soil infestation technique was the most efficient for inducing infection of seedlings by *S. rolfsii*. Incorporation of 2% inoculum with soil was sufficient to produce high disease levels. Disease incidence on plants inoculated at 30-60 days by toothpick method increased with plant age.

Waraitch *et al* (1986) used soil mixing method of inoculation of *S. rolfsii* (multiplied on sterilized sorghum seeds pre-soaked in 2% sucrose solution) was mixed in soil near the plants @ three 500 ml flask 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, soil : inoculums ratio of 50:1(w:w) caused heavy infection.

2.5. Host range of *Sclerotium rolfsii*

Dutta and Das (2002) observed that *Sclerotium rolfsii* Sacc.had a host range of more than 500 species of cultivated and wild plants in tropical region. The pathogen causes pre- and post-emergence root/collar rot and wilt of the seedlings. The disease results from infection by germinating sclerotia produced by the pathogen which can be controlled through host resistance or fungicides is difficult. As an alternative, in recent times.

Sharma *et al*. (2002) observed that *Sclerotium rolfsii* Sacc. has very wide host range and not easily controlled by chemical means.

Chowdhury and Ahmed (1985) tested the reaction of twenty-two different crop plants 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 to *Sclerotium rolfsii* in the pot-house. Before sowing the seeds, sterilized soil in pots was inoculated with a culture of *S. rolfsii* grown in oats. Records of pre- and post-emergence mortality, up to 45 days after germination, was taken. All the crops were found to be susceptible under experimental conditions. The rate of pre-emergence mortality varied from 5.6% in garlic to 85% in gram.

2.6. Pathogen

Sclerotium rolfsii fungus had been described by Rolfs (1892) and named *Sclerotium rolfsii* by Saccordo in 1911. It is an economically important pathogen on numerous crops worldwide (Aycock, 1966). It is an omnivorous and destructive parasite of many plants. It has an extensive host range, at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers, and cucurbits (Chupp and Sherf, 1960).

Ahmed (1980) reported that the fungus *S. rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of sclerotia.

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

Surendranath (1999) reported that, fully matured Sclerotia of *S. rolfsii* the causal agents of white rot of onion were spherical to ellipsoidal and measured 0.5 mm to 1.75 mm in diameter.

Sarma *et al.* (2002) investigated the variability in growth and basidial stage production of 26 isolates *Sclerotium rolfsii*. The isolates of *Sclerotium roifsii* varied in all of the charaters such as colony morphology, mycelia growth rate, sclerotial production, basidiocarp induction, sclerotial size and color.

Rekha *et al*. (2012) reported that pathogen will produce mustard seed like sclerotia, which are very resistant to degradation in soil and serve as inoculums for the next season and also, help in spreading of the disease to other plant.

2.7. Histopathology

Babar (1999) studied histopathology of sunflower and showed that the pathogen produced mycelia over the epidermis of an infected stem and caused deformed and disorganized epidermis over a large tangential area along corresponding disintegrated cortical tissue resulting big cavities.

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

Chan and Sackston (1973) observed that *S. bataticola* formed appressoria on the epidermis of inoculated sunflower seedlings. These may aid both mechanical and chemical penetration of adult stems by mass action hyphae which are intra and intercellular. Longitudinally spread in the cortex is in the intracellular spaces. Hyphae are closely associated with cell walls and the walls of xylem vessels are penetrated through the pits. A virulent isolate formed chlamydospore like resting structures first and sclerotia later. Sclerotial formation appeared to reflect the level of nutrition rather than relationship of isolates.

2.8. Maintenance of the pathogen

Narain and Mishra (1979) found that malt extract agar supported the large number and size of sclerotia of ragi isolate of *S. rolfsii*.

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

Potato Dextrose Agar (PDA) was found to be the best supporting medium for *S. rolfsii* (Harinath Naidu, 2000, Amarsingh and Dhanbir Singh, 1994, Gupta and Ashu Sharma, 2004., Gaur *et al*., 2005 and Raoof *et al.,* 2006).

2.9. Mass multiplication of the pathogen

Gupta and Kolte (1982) reported that the pathogen was multiplied on sorghum grains. Sorghum grains were pre-soaked in 2 percent sucrose solution overnight, drained and boiled in fresh water for 30 minutes and drained again. This was transferred into 1000 ml flasks @ 400 g and autoclaved at 15 lb psi (121.6 ^{0}C) for 20 minutes. The flasks were allowed to cool at room temperature and 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 28 ± 2^{0}C.

2.10. Pathogenicity

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

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

Thammsak-Sommat *et al*. (1982) made an investigation on the pathogenicity of *Sclerotium rolfsii*, and reported that the pathogen could

infect its host cotton severly; disease severity in average was 84%. The pathogen caused pre and post emergence damping off symptoms of cotton seedlings. They also found the soil amendment decreased disease intensity e.g. crop refuses, nitrogen fertilizers and lime. Seed dressing with five fungitotoxicants showed that vitavax gave a complete protection when grown in infested soil.

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

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

Siddaramaiah (1988) confirmed the pathogenicity of *Sclerotium rolfsii* on *Desmodium uncimatum* Desv. and *Cotonoris ainesii* Eckl and Zeyh, two important forage legumes of hill zone by similar producer.

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

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.

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

2.11. Incidence and severity of *Sclerotium rolfsii.*

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

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. Survey was conducted during (Kharif-I) July, 1994. He observed that almost all the varieties were affected by collar rot. At Bangladesh Agricultural University (BAU), Mymensingh collar rot was prevalent throughout the crop season. All varieties at BAU were heavily affected with collar rot. Some 3.0-5.0% plants were killed at flowering stage.

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.

Further report of Meah (1997) includes the incidence and severity of collar rot of sunflower in thirty (30) varieties which were grown at 2 (two) agro ecological zones (AEZ) of Bangladesh. Survey was conducted during March, 1997. He observed that collar rot affected almost all the sunflower varieties except MSFH-17 and MSFH-592.

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

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

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental site

3.1.1. *In vitro* and *In vivo* experiments

The experiment was conducted at the M.S. Laboratory and in the nursery house of Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka- 1207.

3.1.2. Survey experiments

Survey on the incidence and severity of foot and root rot of betel vine were conducted in major betel vine growing location in the district of Kushtia during 2013-2014.

3.2. Experimental period

The *In vitro* and *In vivo* experiments were conducted during July 2012 to October 2014.

3.3. Location of survey

Survey was conducted at different upazillas of major betel vine growing locations in the Kushtia district. Six upazillas viz. Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar, Mirpur were the survey area. Three borojes (betel vine garden) in an upazilla were considered for recording diseases of betel vine.

3.4. Topography and soil

The experimental site was situated in the sub-tropical zone. The soil of the experimental site lies in Agro-Ecological Zone (AEZ No.11)-High Ganges River Floodplain (AEZ-BARC/FAO/UNDP, 1988). Most areas have a complex relief of broad and narrow ridges and inter-ridge depression, separated by areas with smooth broad ridges and basins. The upper parts of ridges stand above normal flood level. Lower parts of ridges and basin margins are seasonally shallowly flooded. General soil types predominantly include calcareous dark grey floodplain soils and calcareous brown floodplain soils. Soils are slightly alkine in reaction. The land type of the different upazillas in Kushtia are high to medium high, the soil is clay and silty loam in texture having a pH 6.1 to 7.9. Soil colour is dark grey, olive brown and mottled brown. Generally fertility level is low, organic matter content in the brown ridge soils is low but higher in the dark grey soils.

3.5. Weather and climate

The experimental location is situated at $23^{0}44^{I}\ 0^{II}$ N latitude and $89^{0}29\ ^{I}0^{II}$ E longitude in Kushtia district and it is approximately 52.49 feet high above the sea level (Appendix-II). The macro-climate of the experimental areas are sub-tropical in nature characterized by two distinct seasons, the monsoon or rainy season extending from April to September and Rabi season extending from mid October to early March. Hot and humid condition occurs in rainy season with high rainfall. Scanty rainfall and plenty of sunshine prevailing during Rabi season.Details of the meteorological data regarding temperature, rainfall, relative and sunshine during the period (2014) of experiment were collected from the Weather Yard. http://www.accuweather.com and Kushtia Sugar Mills Ltd. presented in Appendix-I.

3.6. Procedures of survey

During the survey, the cultivation area of betel vine, name of the cultivars and different diseases observed in the three “borojes” were recorded in each upazilla. In each growing upazilla, five survey plots in a “boroj” randomly selected for data recording. Each spots covered an area of approx. 1200 sq-m. farmers plots. Cultivars of betel vine available in those area were considered for investigation. Two visit were made to each spot in each month during the study period. Ten plants were selected randomly from each plot. Every selected plants was observed carefully and symptoms of the diseases were recorded.

Table 1: Survey area of Kushtia district and prevalence of betel vine disease

Upazilla	**Name of varieties**	**Name of the disease**
Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar, Mirpur.	Mainly Jhalpaan, Sanchipaan, Banglapaan	Foot and root rot of betel vine

Data were recorded on the following parameters:

a) Total betel vine plants/selected spot

b) Diseased plants/selected spot

c) % Stem area diseased

d) Total Stem area

e) % Plant infection

f) % Disease severity

3.7. Assessment of disease incidence and severity

Assessment of disease incidence and severity of the diseases were calculated by the following formula:

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

Disease severity was calculated using the formula of Johnston (2000) as:

$$\% \text{ Disease severity} = \frac{\text{Area of stem tissue infected}}{\text{Total stem area inspected}} \times 100$$

3.8. Collection and preservation of betel vine samples

Diseased stem samples of betel vine (*Piper betle* L.) were collected from different “boroj” in selected upazilla of Kushtia district**.** Collected

samples were put in polyethylene bags immediately after collection to protect them from drying. The collected betel vine samples was brought to the laboratory and subject to the preliminary cleaning and then store in paper packet at 4°C in refrigerator for isolation of *Sclerotium rolfsii*.

Plate 1: Betel vine sample affecting by foot and root rot of betel vine

Table 2: Source or location of fungal isolates of *Sclerotium rolfsii*

Isolates	**Source/ location**
KMS_1	Mirpur, Kushtia (Farmer field)
KKS_2	Kushtia Sadar, Kushtia (Farmer field)

KMS_1= Sample-1: (K=Kushtia, M=Mirpur, S=Sample)

KKS_2= Sample-2: (K=Kushtia, K=Kushtia Sadar, S=Sample)

3.9. Sterilization of materials and equipment's

Liquid materials, such as media and distilled water were sterilized in an autoclave following the method (Hazra, 1988) at 121°C and 15 pound per square inch (p.s.i.) for 20 min for surface sterilization 0.1% sodium

hypochlorite (NaOCl) was used for plant materials such as leaf, stem, seed etc. and rectified spirit used for other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

3.10. Isolation and identification of *Sclerotium rolfsii*

The pathogens associated with the foot and root rot disease of betel vine were isolated following tissue planting method (Tuite 1969 and Mian 1995) and soil dilution method.

3.10.1. Tissue Planting Method

3.10.1.1. Moist blotter method

The pathogen associated with the diseased plant parts vine/stem of betel vine were cut into several pieces by scissors and placed on the moist filter (Whatman no.1). Three pieces of filter paper were moistened by dipping in sterile water. The petridishes with the diseased specimens were incubated at 22 ±2°C in the incubation room for 3 to 5 days. After incubation the plates were examined under compound microscope for primary identification of the organisms (fungi). The fungi were transferred to PDA plates by tip culture method and purified.

Plate 2: Betel vine sample were placed in blotter paper

3.10.1.2. Preparation of Potato Dextrose Agar (PDA) media

Potato Dextrose Agar (PDA) medium was prepared following the standard procedure. At first, 200g potato was taken followed by washing with tap water. Then the potato was peeled and cut in a slice and boiled in one litre water. When potato was soft fully, it was sieved. After that 20g dextrose and with a few minutes interval, 15g Agar were mixed slowly with it and stirred properly so that it cannot be coagulated. The pH was adjusted to 6.5 of the media by using pH meter with the help of HCL (1N) or NaOH (1N) and kept the media in the conical flask and then sterilized the media in an autoclave at temperature of 121^0C with 15 PSI pressure for about 20 minutes. All the procedures were done aseptically inside the laminar air flow cabinet.

3.10.1.3. Isolation in agar plate method

At first the diseased plant parts (stem) were thoroughly washed to remove soil and sand particals. Then infected plant parts were cut into small pieces (5 mm) from advancing end of the lesions. The cut portion were surface sterilized with 1% chlorox (NaOCl) for 1 minutes, and rinsed with sterilized water for 3 times. Surface sterilized plant pieces were placed on PDA media in 90 mm petridishes and incubated at room temperature of 22± 20°C for 7-10 days and examined daily for any fungal growth. After incubation period the inoculated plates were observed to identify the causal organism.

Plate 3: Betel vine sample were placed in agar media

3.10.2. Isolation of soil fungi (*Sclerotium rolfsii*) by soil dilution method

Dilution plate technique (Warcup, 1955) for isolation of soil microbes was followed:

3.10.2.1. Preparation of working area

Since the bacteria and fungi are always present as contaminants in the soil, it is important to exclude them as much as possible from the surface of the working area and the equipment to be used. The surface of the working area was disinfected with cotton soaked in methylated spirit (70%). The hands were disinfected by the same. The glass wares (Test tubes, Petri dishes, Pipettes, Beakers etc.) were sterilized in dry oven. Then these were placed in laminar flow cabinet.

3.10.2.2. Preparation of working samples

For every dilution of soil samples, working sample was prepared from the composite sample that was made after soil sample collection from the boroj (betel vine garden) from Kushtia district.

3.10.2.3. Making suspension (soil dilution)

a. 1gm of the soil was placed in test tube containing 9 ml of sterile water and stirred thoroughly for few minutes in order to obtain an uniform 1:10 dilute soil suspension. This was used as stock suspension.

b. 1ml of that 1:10 stock suspension was transferred with the help of sterile pipette into the 2nd test tube containing 9 ml sterile water and shaken thoroughly thus resulting 10^{-1} dilution.

c. 1ml of the dilution was transferred to 3rd test tube containing 9 ml sterile water by sterile pipette thus making 10^{-2} dilution. In this way dilution was made up to 10^{-4} (Fig. 1).

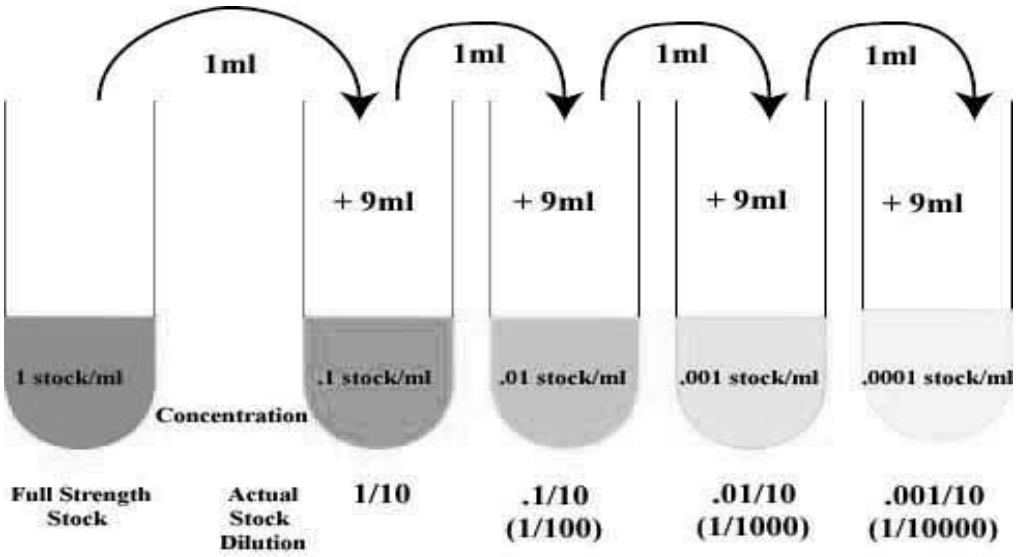


Fig.1. Preparation of dilution series (Soil dilution)

3.10.2.4. Isolation procedures

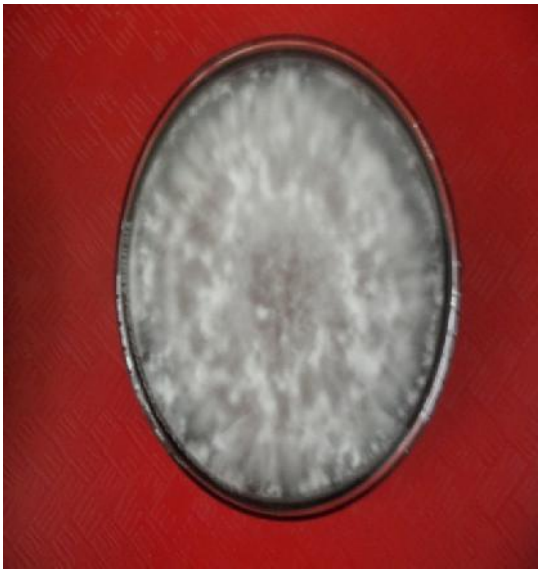
a. 20 ml of warm melted PDA medium was (approx. 45°C) poured in each sterile Petri-plate.

b. 1 ml of diluted soil sample (10^{-4}) was placed at the center of PDA and spreaded. Four Petri-dishes each were inoculated with 1 ml of diluted sample. This was repeated with every soil sample.

c. The inoculated PDA plates were incubated for 7-10 days at room temperature (25±1^{o}C).

d. The colonies were grown out on PDA were recorded after 3-5 days. 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.

a. Isolation from soil dilution technique



b. Pure culture of *Sclerotium rolfsii*

Plate 4: Isolation of *Sclerotium rolfsii*

3.11. Identification, multiplication and preservation of the pathogen

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

3.12. Pathogenicity Test :

3.12.1. Betel vine variety used and source

Bangla paan variety was used in the experiment. Stem cuttings of betel vine were collected from Kushtia district.

3.12.2. Land preparation

The land of the experimental plot was prepared well by 4–5 ploughings and land should be raised by 5–10 cm from the adjacent areas, providing proper gradient on both sides for quick drainage. Afterwards, field beds of suitable size (15cm high and 30cm broad) were prepared. Before planting the cuttings, stubbles were removed (Ciju, 2013).

3.12.3. Construction of boroj

A (5×7) sq-m. sized boroj was prepared in the nursery of the Department of Plant Pathology Sher-e-Bangla Agricultural University (SAU) for plantation of betel vine for the pathogenicity test of *Sclerotium rolfsii.*

Plate 5: Experimental plot (Boroj)

3.12.4. Planting of betel vine cuttings

Stem cuttings having 3–5 nodes were used for propagation and these were planted in such a manner that 2–3 nodes were buried in the soil. A single node cutting with a mother leaf was also planted. Cuttings of the apical and middle portions of the vine were used for planting (Ciju, 2013). In **23 March, 14** betel vine cuttings were planted in the field. 120 cuttings were used of cultivation. Row-to-row spacing is 50-60 cm and Plant-to-plant spacing is 15.

Plate 6: Planting of betel vine cuttings

3.12.5. Fertilizer application

Betel leaves are picked once in every 3-4 weeks and with that substantial quantity of nutrient is removed from the field. Therefore application of fertilizer is essential for higher yield and better growth (Ciju, 2013).

Table 3: Fertilizers application in the field

Fertilizer	**Amount/ Bed**
Urea	195g
Triple Super Phosphate	65g
Muriate of Potash	100g

Organic fertilizer

Mustard oilcakes were applied to the rows after two months and it was mixed well with soil without damaging to the newly planted cuttings for better leaves color. Again mustard oilcakes were applied at two months intervals.

Plate 7: Field view of experiment in the boroj of nursery in SAU

3.12.6. Sticking and adding Soil

After the four months of planting, adding soil (Gasti) and bamboo stick (washi) into the soil. It was done again at two months of intervals.

Plate 8: Adding bamboo stick and soil in the plants

3.12.7. Intercultural operation

Since betelvine requires high soil moisture, frequent light irrigation is necessary depending upon the season. Irrigation should be need-based and proper drainage is essential during rainy season. Weeding was done as and when necessary.

3.12.8. Inoculation of betel vine with the *Sclerotium rolfsii*

Three plants were individually inoculated in each row by adding 2-3 discs of mycelium of ***Sclerotium rolfsii*** from the pure culture plate near the plant base and covered with moist cotton. Inoculation was done in the afternoon, cotton was kept moist by adding water as required.

Plate 9: Inoculation of betel vine with *Sclerotium rolfsii* at the base of plant

Plate 10: Wrapping with moist cotton to create favourable environment for disease development

3.12.9. Observation and recording of symptoms

The inoculated plant were keenly observed and recorded the typical symptoms produced by the pathogen.

3.12.10. Re-isolation and identification of pathogen

The re-isolation and identification of pathogen were done as described in 3.10 at page 30.

3.13. Design of experiment

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

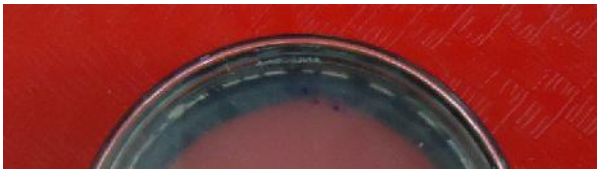
CHAPTER IV

RESULTS

The experiments were conducted on various aspects of foot and root rot disease of betel vine including investigation of isolation, identification, pathogenicity test and survey. The results of investigation on foot and root rot disease of betel vine are presented in this chapter.

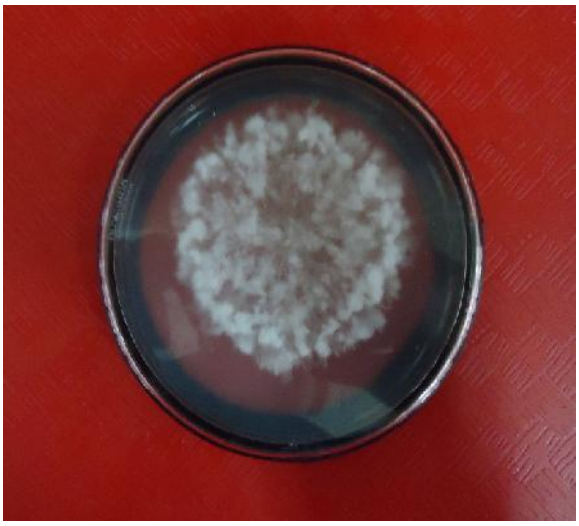
4.1. Isolation and identification of pathogen

Pathogen isolated from the infected diseased samples of betel vine was confirm as *Sclerotium rolfsii***.** On PDA media *Sclerotium rolfsii* grew rapidly covering the petriplate within 9 days. The fungus produced fluffy mycelium. The mycelium was sparse to dense, white to dull white in colour. The microscopic observation revealed the production of branched mycelium. The mycelium was hyaline, superficial, septate and branched. Numerous sclerotia were produced from the mycelium after 15 days of incubation in cultures. *Sclerotium* were globose to oval, thick celled and smooth to rough walled.



a. 1 day old culture of *S. rolfsii* on PDA

b. 3 day old culture of *S. rolfsii* on PDA



c. 5 day old culture of *S. rolfsii* on PDA

d. 7 day old culture of *S. rolfsii* on PDA

e. 9 day old culture of *S. rolfsii* on PDA

f. 15 day old culture of *S. rolfsii* on PDA

Plate 11: Pure culture of *Sclerotium rolfsii*

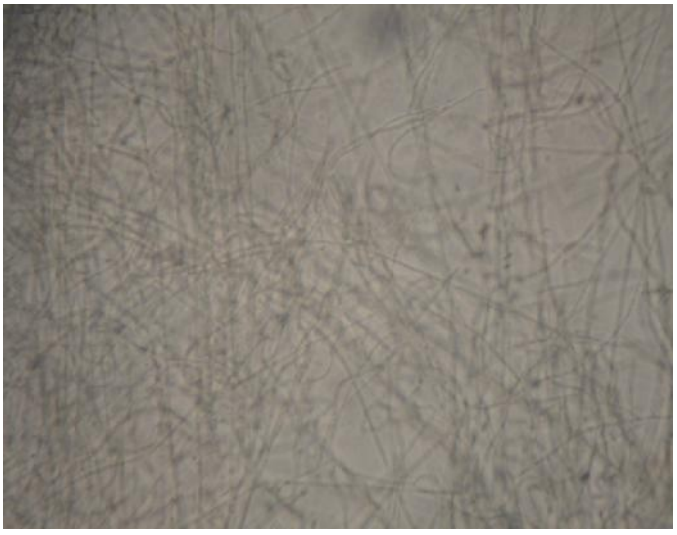


Plate 12: Observed mycelium of *Sclerotium rolfsii* under compound microscope (100x)

4.2. Pathogenicity Test

4.2.1. 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-13).

a. Healthy plant b. Infected plant

c. Infected stem

Plate 13: Field view of betel vine

4.2.2. Results of artificial inoculation of *Sclerotium rolfsii*

Sclerotium rolfsii isolated from diseased betel vine sample was subjected to pathogenecity 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 (Plate-14). Finally the artificially inoculated plants developed characteristics symptoms resulting foot and root rot disease (Plate-14).

a. Healthy plant

b. Inoculated plant

c. Infected plant showing wilting symptom

d. Infected betel vine plant totally collapsed killed

c. Typical foot and root rot symptom, sclerotia were formed within 15 days

Plate 15: Development of foot and root rot in betel vine

Table 4: Result of pathogenicity test of *Sclerotium rolfsii*

Isolates	Observation					Mortality %
	Inoculati-on	Age of host	Mycelium formation	Wilting	Killing	

KMS_1	18 Aug.	5 months old	5^{th} day	7^{th} day	10^{th} day	100
KKS_2	24 Aug.		5^{th} day	8^{th} day	11^{th} day	100

KMS_1= Sample-1: (K=Kushtia, M=Mirpur, S=Sample)

KKS_2= Sample-2: (K=Kushtia, K=Kushtia Sadar, S=Sample)

4.2.3. 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 re-isolated pathogen was *Sclerotium rolfsii* that was responsible for causing foot and root rot of betel vine.

4.3. Survey on disease incidence and severity

In Kushtia district, six upazillas were surveyed to investigate on foot and root rot disease of betel vine. The upazillas were Kushtia Sadar, Mirpur, Bheramara, Daulatpur, Kumarkhali and Khoksha. Altogether six upazillas were found to cultivate betelvine commercially in approximate 630 ha of land and production 1210 MT (Table-5). Mainly local cultivars Banglapaan, Jhalpaan and Sanchipaan were found to cultivate in that area. Banglapaan occupied the 95% area of the cultivated land. The observation of disease in six upazillas were as follows:

Table 5: Survey areas of betel vine in Kushtia District

Upazilla	Cultivars name	Growing area (ha)	Production(MT)
Kushtia Sadar	Jhalpaan Sanchipaan	200	425

	Banglapaan		
Mirpur	Jhalpaan Sanchipaan Banglapaan	80	130
Bheramara	Jhalpaan Sanchipaan Banglapaan	90	160
Daulatpur	Jhalpaan Sanchipaan Banglapaan	130	200
Kumarkhali	Jhalpaan Sanchipaan Banglapaan	70	130
Khoksha	Jhalpaan Sanchipaan Banglapaan	60	165
Total		630	1210

Source: (Agricultural extension office, Kushtia)

4.4. Disease incidence (%) of foot and root rot of betel vine in July, 2014

Disease incidence of foot and root rot of betel vine in different upazillas of Kushtia district were significantly varied from one upazilla to another upazilla and one boroj to another boroj (Table-6). The maximum disease incidence were recorded in Mirpur upazilla where disease incidence ranged from 54.00% to 64.00% and the minimum disease incidence were recorded in Khoksha upazilla where disease incidence ranged from 28.00% to 34.00%. The highest disease incidence were found in boroj-1 in Mirpur (64.00%) and the lowest disease incidence were found in boroj-1 and boroj-3 in Khoksha (28.00%).

4.5. Disease incidence (%) of foot and root rot of betel vine in October, 2014

Disease incidence of foot and root rot of betel vine in different upazillas of Kushtia district were significantly varied from one upazilla to another upazilla and one boroj to another boroj (Table-7). The maximum disease incidence were recorded in Mirpur upazilla where disease incidence ranged from 46.00% to 52.00% and the minimum disease incidence were recorded in Khoksha upazilla where disease incidence ranged from 20.00% to 28.00%. The highest disease incidence were found in boroj-2 in Mirpur (52.00%) and the lowest disease incidence were found in boroj-1 in Khoksha (20.00%).

Mean disease incidence for July and October, 2014 was shown in (Figure-2).

Table 6: Disease incidence of foot and root rot of betel vine in different upazillas of kushtia district in July

LOCATION	% DISEASE INCIDENCE		
	Boroj 1	**Boroj 2**	**Boroj 3**
MIRPUR	64.00 a	62.00 a	54.00 a
KUSHTIA SADAR	32.00 c	38.00 c	42.00 b
BHERAMARA	32.00 c	34.00 c	42.00 b
KUMARKHALI	50.00 b	54.00 ab	50.00 ab
KHOKSHA	28.00 c	34.00 c	28.00 c
DAULATPUR	44.00 b	50.00 b	50.00 ab
LSD(0.05)	7.69	10.25	11.32
CV (%)	13.99	17.13	19.36

Table 7: Disease incidence of foot and root rot of betel vine in different upazillas of kushtia district in October

LOCATION	% DISEASE INCIDENCE		
	Boroj 1	**Boroj 2**	**Boroj 3**
MIRPUR	50.00 a	52.00 a	46.00 a
KUSHTIA SADAR	22.00 c	32.00 c	30.00 bc
BHERAMARA	22.00 c	26.00 c	32.00 bc
KUMARKHALI	40.00 ab	46.00 ab	44.00 a
KHOKSHA	20.00 c	28.00 c	22.00 c
DAULATPUR	34.00 b	42.00 b	40.00 a
$LSD_{(0.05)}$	10.50	7.84	11.85
CV (%)	25.40	15.78	25.18

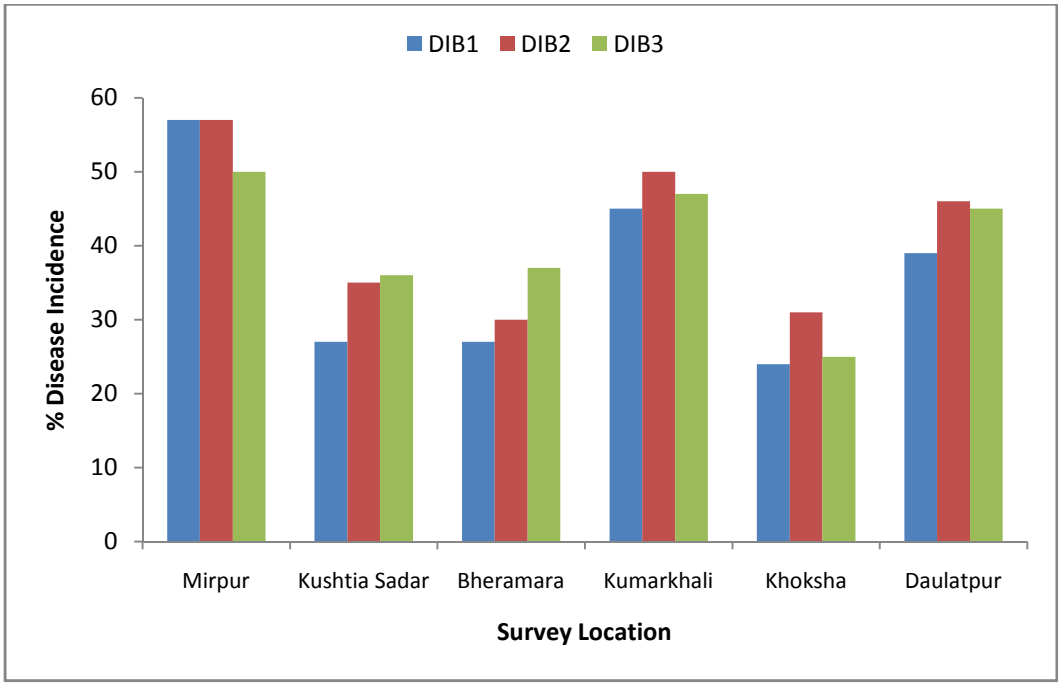


Figure 2: Mean disease incidence of foot and root rot of betel vine in different upazillas of Kushtia district in July and October, 2014

DIB1= Disease incidence, Boroj-1

DIB2= Disease incidence, Boroj-2

DIB3= Disease incidence, Boroj-3

4.6. Disease severity (%) of foot and root rot of betel vine in July, 2014

Disease severity of foot and root rot of betel vine in different upazillas of Kushtia district were found to vary from one upazilla to another upazilla and one boroj to another boroj (Table-8). The maximum disease severity were recorded in Mirpur upazilla, where disease severity ranged from 34.00% to 35.60% and the minimum disease severity were recorded in Khoksha upazilla where disease severity ranged from 18.60% to 19.50%. The highest disease severity were found in boroj-1 in Mirpur (35.60%) and the lowest disease severity were found in boroj-2 in Khoksha (18.60%).

4.7. Disease severity (%) of foot and root rot of betel vine in October, 2014

Disease severity of foot and root rot of betel vine in different upazillas of Kushtia district were found to vary from one upazilla to another upazilla and one boroj to another boroj (Table-9). The maximum disease severity were recorded in Mirpur upazilla, where disease severity ranged from 32.50% to 33.90% and the minimum disease severity were recorded in Khoksha upazilla where disease severity ranged from 16.70% to 18.10%. The highest disease severity were found in boroj-1 in Mirpur (33.90%) and the lowest disease severity were found in boroj-1 in Khoksha (16.70%).

Mean disease severity for July and October, 2014 was shown in (Figure-3).

Table 8: Disease severity of foot and root rot of betel vine in different upazillas of Kushtia district in July

LOCATION	% DISEASE SEVERITY		
	Boroj 1	**Boroj 2**	**Boroj 3**
MIRPUR	35.60 a	34.00 a	34.30 a
KUSHTIA SADAR	18.80 d	20.10 d	21.04 c
BHERAMARA	21.18 d	18.90 d	20.12 c
KUMARKHALI	30.10 b	29.00 b	31.70 a
KHOKSHA	19.50 d	18.60 d	18.80 c
DAULATPUR	24.90 c	23.10 c	25.10 b
$LSD_{(0.05)}$	2.55	2.98	2.83
CV (%)	7.71	9.49	8.53

Table 9: Disease severity of foot and root rot of betel vine in different upazillas of Kushtia district in October

LOCATION	% DISEASE SEVERITY		
	Boroj 1	**Boroj 2**	**Boroj 3**
MIRPUR	33.90 a	32.50 a	32.80 a
KUSHTIA SADARz	18.50 de	18.10 d	19.74 d
BHERAMARA	19.58 d	17.70 d	18.52 d
KUMARKHALI	28.40 b	26.90 b	29.50 b
KHOKSHA	16.70 e	17.10 d	18.10 d
DAULATPUR	23.60 c	21.10 c	23.10 c
$LSD_{(0.05)}$	2.46	2.65	2.75
CV (%)	7.96	9.02	8.82

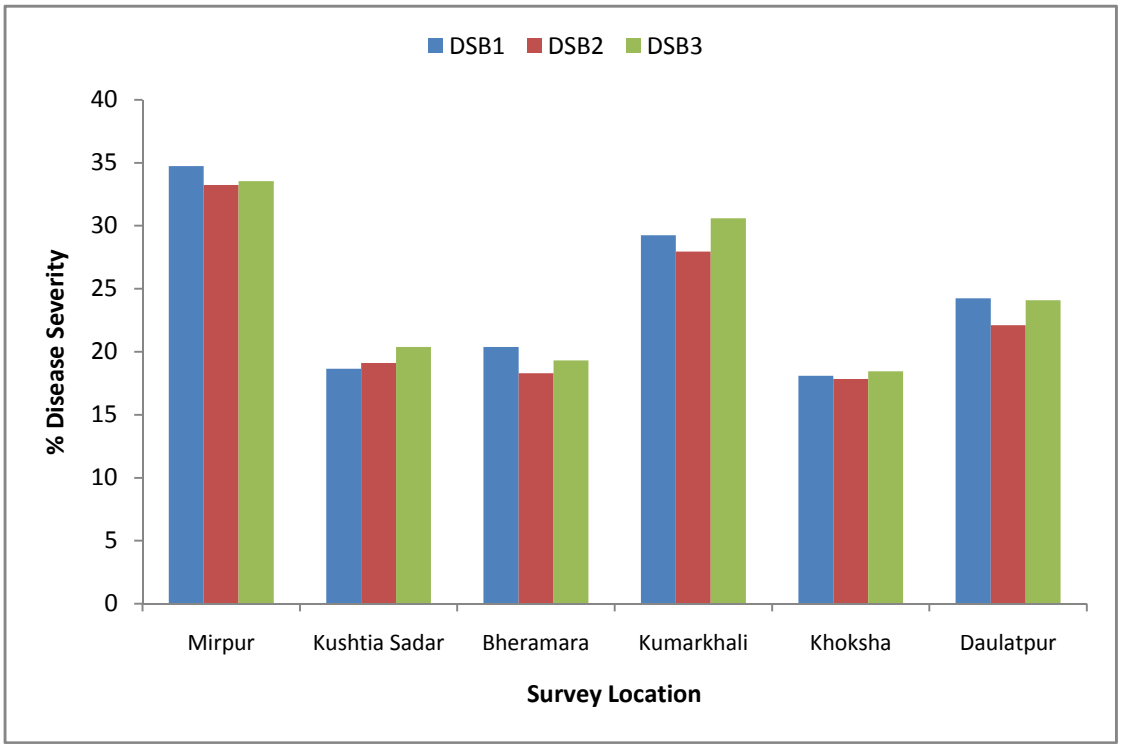


Figure 3: Mean disease severity of foot and root rot of betel vine in different upazillas of Kushtia district in July and October, 2014

DSB1= Disease Severity, Boroj-1

DSB2= Disease Severity, Boroj-2

DSB3= Disease Severity, Boroj-3

CHAPTER V

DISCUSSION

Betel vine (*Piper betle* L.) is a perennial climber, cultivated for its leaf. It is an important cash crop grown on a commercial scale in Kushtia district. The major constraint of cultivation of betel vine is foot and root rot disease that severely damage foot, stem, root and foliage. The climate of Bangladesh harbors plant pathogens and provide luxuriant environment for the growth and reproduction of pathogens (Fakir, 2001). Betel vine plants are cultivated in conservatories under shady and humid conditions that also favours the development of many diseases (Chattopadhyay and Maiti, 1990). Bangladesh is the second largest grower of betel vine on about 14000 hectares. Total annual production of the crop in Bangladesh is about 72,500 tons. The average yield is 2.27 tons per acre (Anonymous, 2006). Its cultivation is concentrated in the greater district of Barisal, Cox's Bazar, Rajshahi, Maulavi- Bazar, Satkhira, Jessore, Kushtia, Jhinidah, Pabna etc.

The present experiments were conducted on collection, isolation, identification, pathogenicity test of the causal pathogen of foot and root rot disease of betel vine and the survey on incidence and severity of the disease during July' 2012 to October' 2014.

Pathogen isolated from the infected diseased samples of betel vine was confirm as *Sclerotium rolfsii***.** The fungus produced fluffy mycelium. The mycelium was sparse to dense, white to dull white in colour. The microscopic observation revealed the production of branched mycelium. The mycelium was hyaline, superficial, aseptate and branched. Numerous sclerotia were produced from the mycelium after 15 days of incubation in

cultures. *Sclerotium* were globose to oval, single celled and smooth to rough walled.

In pathogenecity tests by Koch's postulates, the typical symptoms of the disease noted as infected plants with yellowish leaves and wilted shoots 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. 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 and the inter-nodal portion of the cuttings were found completely decomposed, leaving only the fibrous portion (Plate-13). On inoculation to healthy plant in the nursery, the inoculated plants exhibited typical symptoms. Two 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 third 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. Then the sclerotia were gradually turned blue to black and started to germinate producing white mycelia (Plate-14). Finally the artificially inoculated plants developed characteristics symptoms resulting foot and root rot disease (Plate-14). Symptoms of foot and root rot observed on betel vine was quite similar to those described by the previous workers (Aycock, 1966; Ahmed 1980 and Amin *et al.* 2013). From the study it appeared that betel vine plant get infection by the pathogen *Sclerotium rolfsii* and there is significant role in damazing the crop causing complete death of the plant. Siddique (1997) had also the similar findings while working with tomato varieties. Dastur

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

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 disease severity (35.60%) in Boroj-1 of foot and root rot diseases of betel vine were found in July at Mirpur upazilla. On the contrary lower amount of disease incidence (28.00%) in Boroj-1 and Boroj-3 and disease severity (18.60%) in Boroj-2 of foot and root rot diseases of betel vine was found in July at Khoksha upazilla in Kushtia district. The maximum disease incidence (52.00%) in Boroj-2 and disease severity (33.90%) in Boroj-1 of foot and root rot diseases of betel vine were recorded in October at Mirpur upazilla. On the contrary minimum amount of disease incidence (20.00%) and disease severity (16.70%) in Boroj-1 of foot and root rot diseases of betel vine was found in October at Khoksha upazilla in Kushtia district. From the present survey investigation it revealed that there is a very little information regarding the presence, prevalence, epidemiology and management of diseases of betel vine in Bangladesh. As the disease poses a potential threat to betel vine causing enormous loss in leaf quality and disruption of production schedules, foot and root rot of betel vine disease was found in all the locatios under survey areas viz. Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar, Mirpur in Kushtia district. The incidence and severity of the diseases were found to vary from month to month, boroj to boroj as well as location to location. The present findings corroborate with the findings of the previous report (Mollah, 2012). He 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.

CHAPTER VI

SUMMARY AND CONCLUSION

Betel vine (*Piper betle* L.) having the heart shaped deep green leaves is an important horticultural crop of aesthetic and commercial values. The leaves of betel vine (*Piper betle* L.) have been traditionally used for chewing in our country. On account of its immense medicinal, social, religious and export value betel vine is a cash crop of economic importance and is extensively grown on large scale in different parts of Bangladesh. Kushtia is a major district in betel vine production. The present study has been designed to isolate and identify the causal pathogen of foot and root rot of betel vine,
to study the pathogenicity of the isolated organism through Koch's postulates and to survey on disease incidence and disease severity of foot and root rot of betel vine in major growing areas of Kushtia district.

Pathogenicity test showed *Sclerotium rolfsii* produced characteristic symptoms on betel vine and proved to be the causal pathogen of the disease. An investigation on the diseases of betel vine was done in six upazillas of Kushtia district viz. Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar, Mirpur. Disease incidence and severity of foot and root rot of betel vine ranged from 24.00 to 58.00% and 17.65 to 34.75%, respectively where the maximum disease was recorded in Mirpur and the minimum was in Khoksha in the month of July and October. Disease incidence and severity of foot and root rot of betel vine ranged from 50.00 to 58.00% and 33.25 to 34.20%, respectively in Mirpur where the maximum disease was recorded in July and the minimum was in October. In Kushtia Sadar, disease incidence and severity of foot and root rot of betel vine ranged from 27.00 to 37.00%

and 18.45 to 20.39%, respectively. In Bheramara, disease incidence and severity of foot and root rot of betel vine ranged from 27.00 to 37.00% and 18.30 to 20.38%, respectively. In Kumarkhali, the disease incidence and severity were 45.00 to 50.00% and 27.95 to 30.60%, respectively. In Khoksha, disease incidence and severity of foot and root rot of betel vine ranged from 24.00 to 31.00% and 17.65 to 18.80%, respectively. In Daulatpur, disease incidence and severity of foot and root rot of betel vine ranged from 39.00 to 46.00% and 22.30 to 24.35%, respectively considering all the locations of Kushtia District, the maximum disease was recorded in the month of July and the minimum was in October.

On the basis of field investigation in the surveyed areas of Kushtia district, it was observed that there is a huge possibilities of betel vine cultivation. Some of the important efforts required in this direction are presented here under:

1. Foot and root rot of betel vine was the most prevalent disease in different upazillas of Kushtia district and the prevalence was higher in July and lower in October irrespective of the different betel vine growing areas of Kushtia district.
2. In pathogenicity test, *S. rolfsii* isolates were found to be highly virulent against the of betel vine.
3. In the surveyed areas, there is an urgent need for improvement management practices using pesticides, fertilizer, irrigation, etc.
4. In the surveyed area generally boroj construction constitutes a major portion of cost during the production. Therefore, in the study area it needs to develop low cost techniques of boroj construction. As per availability of locally available raw materials change in

traditional techniques is to be effected to the benefit of growers by reducing the cost of boroj construction.

5. The growers should be trained keeping in view the management practices of diseases.
6. It was also observed that there were no linkages between the research institutes and growers. This affects the transfer of technology. Therefore, for smooth transfer of production technology from the institutions to the farm level need linkage between research, DAE people and farmers.
7. However further investigations are need to be carried out to ascertain the present findings for consecutive years.

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http://www.accuweather.com

Appendix 1: Month wise air temperature (^{0}C), relative humidity (%) and rainfall (mm) from July to October 2014, in Kushtia district.

Month	Average air temperature (^{0}C)			Average relative humidity (%)	Total rainfall (mm)	Average Sunshine (hr)
	Maximum	Minimum	Average			
January	21.6	11.1	16.5	77	0	4.84
February	24.7	15.7	22.2	74	0	7.78
March	31.6	22.5	27.5	72	1	8.58
April	35.6	27.0	31.3	73	69.60	7.46
May	34.6	27.4	31	79	120.90	4.61
June	33.2	24.9	29.5	85	107.70	7.32
July	31.8	26.3	29.5	87	194.56	6.60
August	32.1	24.8	28.5	86	320.04	5.71
September	32.5	26.4	29.1	87	68.83	6.00
October	32.3	24.3	28.3	84	0	4.15

Source: http://www.accuweather.com and Kushtia Sugar Mills Ltd.

Appendix 2: Map showing the location of experimental site

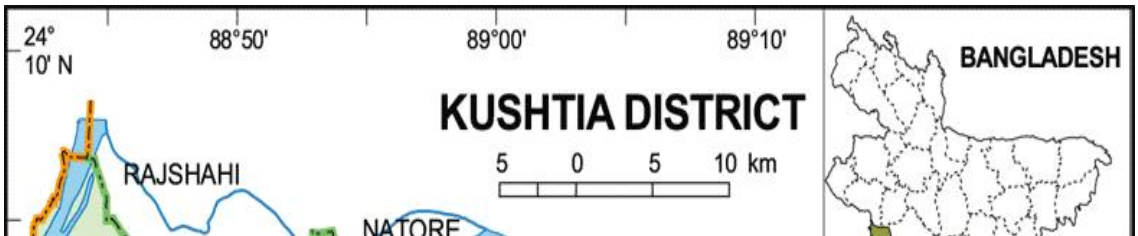

Figure: Kushtia district

Appendix 3: List of some abbreviation from and their elaboration

Abbreviation	**Elaboration**
AD	After death
AEZ	Agro- Ecological Zone
et al.	And others

@	At the rate
BARC	Bangladesh Agricultural Research Council
BAU	Bangladesh Agricultural University
Cm	Centimeter
CV	Coefficient of Variance
CRD	Completely Randomized Design
Conc.	Concentration
^{0}C	Degree Celsius
DI	Disease incidence
DS	Disease severity
DMRT	Duncan's Multiple Range Test
E	East
FAO	Food and Agricultural Organization
Ft	Foot, feet
e.g.	For example
G	Gram
Ha	Hectare
Hr	Hour
HCl	Hydrochloric Acid
pH	Hydrogen ion conc.
i.e.	In other words
IARI	Indian Agricultural Research Institute
LSD	Least significance Difference
MT	Metric ton
Ml	Mililitre
Mg	Milligram
Mm	Millimeter
Min	Minute
M	Miter
N	Normal
N	North
Psi	Per square inch
% RH	Percent Relative Humidity

Abbreviation	Elaboration
%	Percentage
PDA	Potato dextrose agar
Ib	Pound
RCBD	Randomized Completely Block Design
R_H	Relative Humidity
S. rolfsii	*Sclerotium rolfsii*
SAU	Sher-e-Bangla Agricultural University
NaOH	Sodium Hydroxide
NaOCl	Sodium Hypochlorite
m^2	Square meter
sq-m	Square- meter
T	Ton
UNDP	United Nations Development Programme
Viz.	Videlicet