

Published with Open Access at **Journal BiNET**

Vol. 07, Issue 01: 590-599

Journal of Bioscience and Agriculture ResearchHome page: www.journalbinet.com/jbar-journal.html

Investigation on foot and root rot of betel vine (*Piper betle* L.) in Kushtia district of Bangladesh

Afsana Jahan^a, Md. Rafiqul Islam^a, Md. Mahfuzar Rahman^b, Md. Harun-Or-Rashid^a and Md. Jannatul Adan^a

^aDept. of Plant pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

^bDept. of Agronomy, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

ABSTRACT

Betel vine crop is mainly attacked by foot and root rot disease in Kushtia. Young stems were found more prone to attack than the old ones. Pathogenicity test showed Sclerotium rolfsii produced characteristic symptoms on betel vine and proved to be the causal pathogen of the disease. An investigation on the diseases of betel vine was done in six upazilla of Kushtia district, viz. Bheramara, Daulatpur, Khoksha, Kumarkhali, Kushtia Sadar and 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.

Key words: *Piper betel* L., *Sclerotium rolfsii*, Paan Boroj, disease incidence and disease severity

Please cite this article as: Jahan, A., Islam, M. R., Rahman, M. M., Rashid, M. H. & Adan, M. J. (2016). Investigation on foot and root rot of betel vine (*Piper betel* L.) in Kushtia district of Bangladesh. *Journal of Bioscience and Agriculture Research*, 07(01), 590-599.

This article is distributed under terms of a Creative Common Attribution 4.0 International License.

I. Introduction

Betel vine (*Piper betel* L.) is a perennial dioecious creeper belonging to the family *Piperaceae*. 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. 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; Samanta, 1994). Desi Bangla, Bangla, Kali Bangla, Jhali, Sanchi, Bhabna, Mitha, Geso, Bonhoogly etc. betel vine cultivars are found in Bangladesh. 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 and the average yield is 2.27 tons per acre (Huq, 2011). Its cultivation is concentrated in the greater district of Barisal, Cox's Bazar, Rajshahi, Maulavi- Bazar, Satkhira, Jessore, Kushtia, Jhinidah, Pabna etc. 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 (Aycok, 1966). It has a wide host range and has been referred as an almost omni pathogenic 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 are often become 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 are 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 upazilla, viz. Kushtia sadar, Mirpur, Bheramara, Daulatpur, Khoksha, Kumarkhali etc. grows betel vine on a 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 seems to be alarming for inoculum potential. Betel vine growers are in troubles to grow betel vine due to this disease problem. Thus, this is pivotal to instigate detailed research program to address this acute disease problem in Kushtia district. On the basis of above facts the present investigation was undertaken 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 conduct a survey on the disease incidence and disease severity of foot and root rot of betel vine in major growing areas of Kushtia district.

II. Materials and Methods

Locations and survey methodology: 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. 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. The *In vitro* and *In vivo* experiments were conducted during July 2012 to October 2014. Survey was conducted at different upazilla of major betel vine growing locations in the Kushtia district. Six upazilla, 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. 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 betel vine growing upazilla, five survey plots in a "boroj" were randomly selected for data recording. Each spots covered an area of approx. 1200 square meter farmer plots. Cultivars of betel vine available in those areas were considered for investigation. Two visits were made to each spot in each month during the study period. Ten plants were selected randomly from each plot. Every selected plant

was observed carefully and symptoms of the diseases were recorded. Completely Randomized Design (CRD) was followed for the laboratory and in the nursery experiments. Randomized Completely Block Design (RCBD) was followed for field experiments. Data were analyzed for ANOVA using MSTAT-C program (MSTAT, 1991). Least Significance Difference (LSD) test were performed to determine the level of significant differences and to separate the means within the parameters (Gomez and Gomez, 1984).

Assessment of disease incidence and severity: Assessment of disease incidence and severity of the diseases were calculated by the following formula (Rai and Mamatha, 2005):

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

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

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

Collection and preservation of betel vine samples: Diseased stem samples of betel vine (*Piper betle* L.) were collected from different “boro” 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*.

Sterilization of materials and equipment: Liquid materials, such as media and distilled water were sterilized in an autoclave at 121°C and 15 pound per square inch (p.s.i) for 20 min following the method of (Hazra, 1988). For surface sterilization 0.1% sodium hypochlorite (NaOCl) was used for plant materials such as leaf, stem, seed etc. and rectified spirit was used for other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

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 soil dilution method.

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.

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 liter 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 to prevent the coagulation. The pH of the media was adjusted to 6.5 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°C with 15 PSI pressure for about 20 minutes. All the procedures were done aseptically inside the laminar air flow cabinet.

Isolation in agar plate method: At first the diseased plant parts (stem) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into small pieces (5 mm) from advancing end of the lesions. The cut portion was surface sterilized with 1% chlorox (NaOCl) for 1 minute, 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.

Isolation of soil fungi (*Sclerotium rolfsii*) by soil dilution method: Dilution plate technique (Warcup, 1955) for isolation of soil microbes was followed.

Preparation of working area and sample: 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. For every dilution of soil samples, working sample was prepared from the composite sample which was made by collecting soil samples from the boroj (betel vine garden) of Kustia district.

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

Isolation procedures: 20 ml of warm (approx. 45°C) melted PDA medium was poured in each sterile Petri-plate. 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. The inoculated PDA plates were incubated for 7-10 days at room temperature (25±1°C). 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.

Identification, multiplication and preservation of the pathogen: Pure culture of the isolates were prepared following hyphal tip methods (Tuite, 1969) 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.

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

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 (Haider et al., 2013).

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 (Haider et al., 2013). In 23 March, 2014, betel vine cuttings were planted in the field. 120 cuttings were used for cultivation. Row-to-row spacing was 50-60 cm and plant-to-plant spacing was 15.

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.

III. Results

Isolation and identification of pathogen

Pathogen isolated from the infected diseased samples of betel vine was confirmed as *Sclerotium rolfsii* (Amin et al., 2013). 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 color. 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* was globose to oval, single celled and smooth to rough walled.

Symptomology study

The leaves and shoots of foot and root rot infected plants turned yellow withered and finally dried out to a pale brown color. The fungus found to attack the roots and stem near the soil level. Black lesions 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.

Results of artificial inoculation of *Sclerotium rolfsii*

Sclerotium rolfsii isolated from diseased betel vine sample was subjected to pathogenicity tests by Koch's postulates. On inoculation to healthy plant in the nursery, the inoculated plants exhibited typical symptoms. 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. The forming sclerotia were gradually turned blue to black and started to germinate producing white mycelia. Finally the artificially inoculated plants developed characteristics symptoms resulting foot and root rot disease.

Table 0. Result of pathogenicity test of *Sclerotium rolfsii*

| Isolates | Observation | | | | Mortality % |
|------------------|-------------|---------------------|---------------------|---------------------|-------------|
| | Inoculation | Mycelium formation | Wilting | Killing | |
| KMS ₁ | 18 Aug. | 2 nd day | 3 rd day | 4 th day | 100 |
| KKS ₂ | 24 Aug. | 2 nd day | 4 th day | 5 th day | 100 |

KMS₁= Sample-1: (K=Kushtia, M=Mirpur, S=Sample), KKS₂= Sample-2: (K=Kushtia, K=Kushtia Sadar, S=Sample)

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.

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 upazilla of Kushtia district were significantly varied from one upazilla to another upazilla and one boroj to another boroj (Table 02). The maximum disease incidence was recorded in Mirpur upazilla where disease incidence ranged

594

from 54.00% to 64.00% and the minimum disease incidence was recorded in Khoksha upazilla where disease incidence ranged from 28.00% to 34.00%. The highest disease incidence was found in boroj-1 in Mirpur (64.00%) and the lowest disease incidence was found in boroj-1 and boroj-3 in Khoksha (28.00%).

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 upazilla of Kushtia district were significantly varied from one upazilla to another upazilla and one boroj to another boroj (Table 02). The maximum disease incidence was recorded in Mirpur upazilla where disease incidence ranged from 46.00% to 52.00% and the minimum disease incidence was recorded in Khoksha upazilla where disease incidence ranged from 20.00% to 28.00%. The highest disease incidence was found in boroj-2 in Mirpur (52.00%) and the lowest disease incidence was found in boroj-1 in Khoksha (20.00%). Mean disease incidence for July and October, 2014 was shown in (Figure 01).

Table 02. Disease incidence of foot and root rot of betel vine in different upazilla of kushtia

| Location | % Disease incidence (July) | | | % Disease incidence (October) | | |
|-----------------------------|----------------------------|----------|----------|-------------------------------|----------|----------|
| | Boroj 1 | Boroj 2 | Boroj 3 | Boroj 1 | Boroj 2 | Boroj 3 |
| Mirpur | 64.00 a | 62.00 a | 54.00 a | 50.00 a | 52.00 a | 46.00 a |
| Kushtia Sadar | 32.00 c | 38.00 c | 42.00 b | 22.00 c | 32.00 c | 30.00 bc |
| Bheramara | 32.00 c | 34.00 c | 42.00 b | 22.00 c | 26.00 c | 32.00 bc |
| Kumarkhali | 50.00 b | 54.00 ab | 50.00 ab | 40.00 ab | 46.00 ab | 44.00 a |
| Khoksha | 28.00 c | 34.00 c | 28.00 c | 20.00 c | 28.00 c | 22.00 c |
| Daulatpur | 44.00 b | 50.00 b | 50.00 ab | 34.00 b | 42.00 b | 40.00 a |
| <i>LSD_(0.05)</i> | 7.69 | 10.25 | 11.32 | 10.50 | 7.84 | 11.85 |
| <i>CV (%)</i> | 13.99 | 17.13 | 19.36 | 25.40 | 15.78 | 25.18 |

In a column, having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly as per 0.05 level of probability.

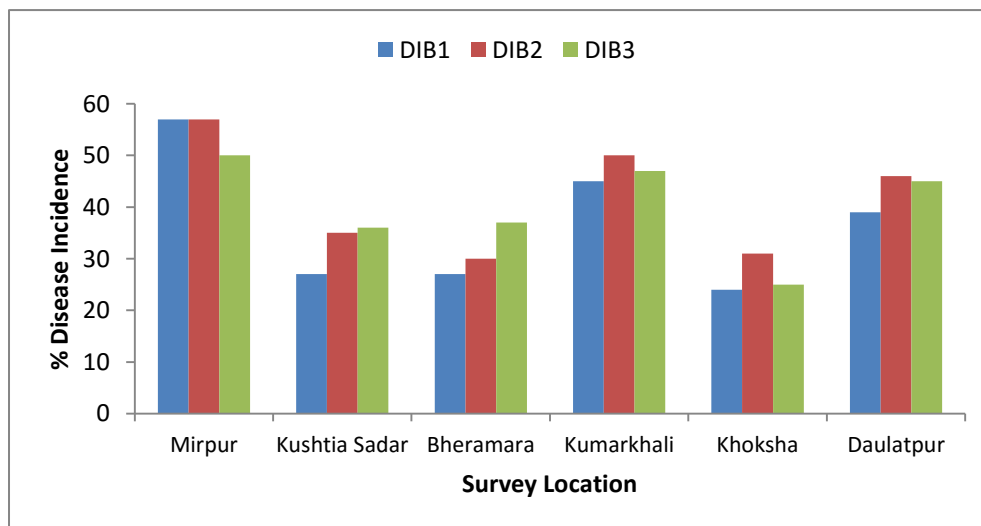


Figure 01. Mean disease incidence of foot and root rot of betel vine in different upazilla of Kushtia district in July and October, 2014. Where, DIB1= Disease incidence, Boroj-1, DIB2= Disease incidence, Boroj-2, DIB3= Disease incidence, Boroj-3.

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 upazilla of Kushtia district were found to vary from one upazilla to another upazilla and one boroj to another boroj (Table 03). The maximum disease severity was recorded in Mirpur upazilla, where disease severity ranged from 34.00% to 35.60% and the minimum disease severity was recorded in Khoksha upazilla where

disease severity ranged from 18.60% to 19.50%. The highest disease severity was found in boroj-1 in Mirpur (35.60%) and the lowest disease severity was found in boroj-2 in Khoksha (18.60%).

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 upazilla of Kushtia district were found to vary from one upazilla to another upazilla and one boroj to another boroj (Table 03). The maximum disease severity was recorded in Mirpur upazilla, where disease severity ranged from 32.50% to 33.90% and the minimum disease severity was recorded in Khoksha upazilla where disease severity ranged from 16.70% to 18.10%. The highest disease severity was found in boroj-1 in Mirpur (33.90%) and the lowest disease severity was found in boroj-1 in Khoksha (16.70%). Mean disease severity for July and October, 2014 was shown in (Figure 02).

Table 03. Disease severity of foot and root rot of betel vine in different upazilla of Kushtia district in July and October

| Location | % Disease severity (July) | | | % Disease severity (October) | | |
|-------------------|---------------------------|---------|---------|------------------------------|---------|---------|
| | Boroj 1 | Boroj 2 | Boroj 3 | Boroj 1 | Boroj 2 | Boroj 3 |
| Mirpur | 35.60 a | 34.00 a | 34.30 a | 33.90 a | 32.50 a | 32.80 a |
| Kushtia Sadar | 18.80 d | 20.10 d | 21.04 c | 18.50 de | 18.10 d | 19.74 d |
| Bheramara | 21.18 d | 18.90 d | 20.12 c | 19.58 d | 17.70 d | 18.52 d |
| Kumarkhali | 30.10 b | 29.00 b | 31.70 a | 28.40 b | 26.90 b | 29.50 b |
| Khoksha | 19.50 d | 18.60 d | 18.80 c | 16.70 e | 17.10 d | 18.10 d |
| Daulatpur | 24.90 c | 23.10 c | 25.10 b | 23.60 c | 21.10 c | 23.10 c |
| <i>LSD</i> (0.05) | 2.55 | 2.98 | 2.83 | 2.46 | 2.65 | 2.75 |
| <i>CV</i> (%) | 7.71 | 9.49 | 8.53 | 7.96 | 9.02 | 8.82 |

In a column, having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

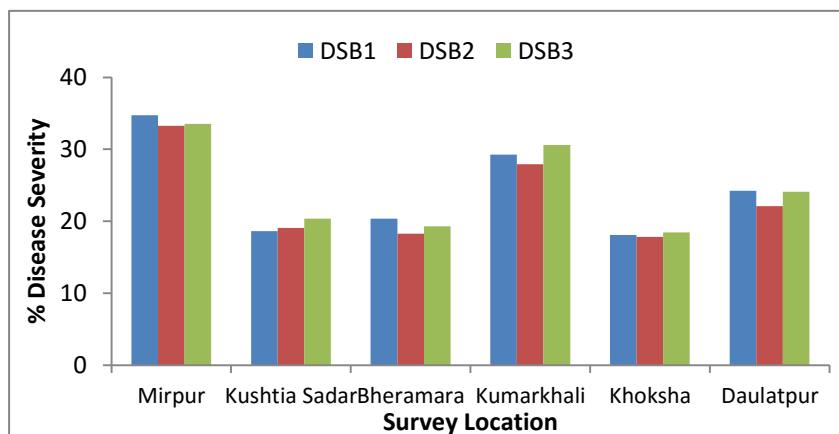


Figure 02. Mean disease severity of foot and root rot of betel vine in different upazillas of Kushtia district in July and October, 2014. Where, DSB1= Disease Severity, Boroj-1, DSB2= Disease Severity, Boroj-2, DSB3= Disease Severity, Boroj-3

IV. Discussion

Betel vine (*Piper betel* 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 favor 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. 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 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 confirmed as *Sclerotium rolfsii*. The fungus produced fluffy mycelium. The mycelium was sparse to dense, white to dull white in color. 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* was 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 color. The fungus found to attack the roots and stem near the soil level. Black lesions were developed following necrosis of the plant cells. The mycelium invaded the stem and rotten the affected portions. 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. 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. The forming sclerotia were gradually turned blue to black and started to germinate producing white mycelia. Finally the artificially inoculated plants developed characteristics symptoms resulting foot and root rot disease. Symptoms of foot and root rot observed on betel vine was quite similar to those described by the previous workers (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 damaging the crop causing complete death of the plant. Siddique (1997) had also the similar findings while working with tomato varieties. 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 was 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 locations 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.

V. Conclusion

There is a wider possibility of betel vine cultivation. Foot and root rot of betel vine was the most prevalent disease in different upazilla of Kushtia district and there prevalence was higher in July and lower in October irrespective of the different betel vine growing areas of Kushtia district. In pathogenicity test, *S. rolfisii* isolates were found to be highly virulent against the of betel vine. In the surveyed areas, there is an urgent need for improvement of management practices using pesticides, fertilizer, irrigation, etc. The growers should be trained keeping in view the management of diseases. 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 is needed. However further investigations are need to be carried out to ascertain the present findings for consecutive years.

VI. References

- [1]. Ahmed, F. (1980). Control of foot and root rot disease of wheat. M.S. thesis, Dept. of Plant Pathology Department, Bangladesh Agricultural University (BAU), Mymensingh.
- [2]. Amin, R., Sarker, B. C., Adhikary, S. K., Sultana, S. & Zubair, T. (2013). Effect of some botanical extracts and cow's urine on *Sclerotium rolfisii* causal agent of foot and root rot of betel vine. *The International Journal of Engineering and Science*, 2(9), 77-82.
- [3]. Aycock, R. (1966). Stem rot and other diseases caused by *S. rolfisii*. Tech. Bull. No. 174. Agric. Expt. Station, North Carolina State University, Raleigh. p. 202.
- [4]. Chattopadhyay, S. B. & Maiti, S. (1990). Diseases of betel vine and spices. Indian Council of Agricultural Research, New Delhi. p. 160.
- [5]. Fakir, G. A. (2001). List of seed borne diseases of important crops occurring in Bangladesh. Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh.
- [6]. Gomez, K. A. & Gomez, A. A. (1984). Statistical procedure for agricultural research. Second Edn. Intl. Rice Res. Inst. John Wiley and Sons. New York. pp. 1-340.
- [7]. Guha, P. (1997). "Paan Theke Kutir Silpa Sambhabana" (In Bengali). "Exploring betel leaves for cottage industry". In: Krishi, Khadya-O- GraminBikash Mela – A Booklet published by the Agricultural and Food Engineering Department, IIT, Kharagpur, India. pp. 15- 19.
- [8]. Haider, M. R., Khair, A., Rahman, M. M. and Alam, M. K. (2013). Indigenous management practices of betel-leaf (*Piper betel* L.) cultivation by the Khasia community in Bangladesh. *Indian Journal of Traditional Knowledge*, 12(2), 231-239.
- [9]. Hassan, S. A. and Shahadat, S. (2005). Disease affecting betel vine. *Journal of Plant Development Science*, 3(2), 4-5.
- [10]. Hazra, S. (1988). Pathogenic variability in sorghum anthracnose incited by *Colletotrichum graminicola* (Ces.) Wilson, MS Thesis, Department of Plant Pathology College of Agriculture, Rajendranagar Acharya N.G. Ranga Agricultural University Rajendranagar, Hyderabad-500030.
- [11]. Huq, M. I. (2011). Studies on the epidemiology of leaf rot and leaf spot diseases of betel vine (*Piper betel* L.). Bangladesh J. Sci. Ind. Res. 46(4), 519-522.
- [12]. Islam, M. (2005). Country news, Holiday Publication Limited. 8: 3-4.
- [13]. Mollah, M. I. (2012). Investigation on The leaf rot and foot and root rot of betel vine (*Piper betel* L.) in Satkhira district of Bangladesh. MS Thesis, Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207.
- [14]. MSTAT. (1991). User's manual for MSTAT-C. Michigan State University, East Lansing, Michigan. p. 450.
- [15]. Rai, V.R. and Mamatha, T. (2005). Seedling diseases of some important forest tree species and their management. A working paper of the finish forest research institute. India. p. 11.
- [16]. Samanta, C. (1994). A report on the problems and solutions of betel vine cultivation. A booklet published by Mr. H. R. Adhikari, C-2/16, Karunamoyee, Salt Lake City, Kolkata-64 (WB), India.
- [17]. Sayeeduzzaman, M. (1988). An economic geographical study of betel leaf cultivation in Bangladesh. A M.Sc. thesis submitted to Geography, University of Dhaka. pp. 45-47

- [18]. Siddique, M. A. B. (1997). Study on varietal reactions of brinjal to foot rot and its control through chemicals and organic soil amendments. MS thesis, Dept. of Plant Pathology. Bangladesh Agricultural University, Mymensingh. Bangladesh. p.119.
- [19]. Talukder, M. (1974). Plant diseases of Bangladesh. *Bangladesh J. of Agril. Res.*, 1(1), 64-68.
- [20]. Tuite, J. (1969). Plant Pathological Methods. Fungi and Bacteria Burgess Pub. Co. Minneapolis, Minn. USA. p. 293.
- [21]. Warcup, J. H. (1955). On the origin of fungi developing on soil dilution plates. *Trans. Br. Mycol. Soc.*, 38(1955), pp. 298-301. [http://dx.doi.org/10.1016/S0007-1536\(55\)80075-7](http://dx.doi.org/10.1016/S0007-1536(55)80075-7)