PREVALENCE OF LOTKON (*Baccaurea ramiflora*) DISEASES IN NARSINGDI DISTRICT OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled, **" PREVALENCE OF LOTKON (*Baccaurea ramiflora*) DISEASES IN NARSINGDI DISTRICT OF BANGLADESH''** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M. S.) IN PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **AHAMMED ULLAH RAYHAN** bearing **Registration No.10-04077** 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 duly been acknowledged.

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PREVALENCE OF LOTKON (*Baccaurea ramiflora*) DISEASES IN NARSINGDI DISTRICT OF BANGLADESH

ABSTRACT

A field survey was conducted during 10th April to 20th July 2016 to study the disease incidence and severity of lotkon (*Baccaurea ramiflora*) in Narsingdi district of Bangladesh. In the survey, two diseases namely Anthracnose and Scab were identified and pathogen were isolated as *Colletotrichum gloeosporioides* and *Venturia inaequalis* respectively and their incidence and severity were recorded. The highest percentage of disease incidence was recorded in Belabo Upazila (16.83% for anthracnose and 14.33% for scab) followed by Raipura Upazila (15.17% for anthracnose and 13.31% for scab) the lowest incidence was observed in Shibpur Upazila (11.33% for anthracnose and 10.33% for scab). In the survey of leaf blight was observed but pathogen could not be isolated and confirmed. The maximum percentage of disease incidence was found in Raipura Upazila (20.5%) followed by Belabo Upazila (19.33%) and the lowest was observed in the Shibpur Upazila (17.67%). The maximum disease severity was observed in the Raipura Upazila (17.26% for anthracnose,19.69% for scab and 21.70% for blight) followed by Shibpur Upazila (13.50% for anthracnose,18.41% for scab and 17.71% for blight) and the lowest was observed in the Belabo Upazila (12.96% for anthracnose,15.86% for scab and 16.39% for blight) of all the diseases. Significant disease infestation was significantly recorded in all the three Upazilas in Narsingdi district of Bangladesh.

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CHAPTER 1

INTRODUCTION

Lotkon or Burmese grape (*Baccaurea ramiflora*), Family Phyllanthaceae is an important tropical and subtropical, evergreen, slow growing, dioecious fruit tree. The tree shows a good example for the fruits which grows directly from the main trunk (Hagens, 2000). It is one of the most popular and commercially important minor fruits of Bangladesh and locally called lotkon or bubi. lotkon is a native of Sumatra, Borneo and Java (Uji *et al*., 1992). It is widely cultivated in Southeast Asia including Myanmar, Nepal, Bangladesh, India, Srilanka, Thailand and regions of Indonesia, it also growing wild environment in those areas (Morton, 1987; Maniruzzaman, 1988; Subhadrabandhu, 2001). In Bangladesh it is mainly grown in the regions of Tangail, Mymensingh, Gazipur, Dhaka, Narshinghi, Sylhet, Chittagong hill tracts and more or less in other parts of the country. Considering it's importance, quality and availability, Bangladesh Agricultural Development Corporation (BADC) has expanded lotkan cultivation programme among the growers since last 1990 and it is exported about 1-1.5 tons by local exporters with vegetables in the local market as well as London and countries of Middle east and Europe during July to August (Kamaluddin, 2008). On an average, about 200 kg Lotkon are found from a 10 years aged tree (Kamaluddin, 2008).

Lotkon's nutritional value per 100g of edible portion is- water 35.6%, protein 5.8%, fat 0.73% (negligible), carbohydrate/sugar 51.9%, ash 3.85%. In case of minerals (mg)-75mg of calcium (7.5% RDI), 504mg of magnesium (144% RDI), 132mg of phosphorous (14.6% RDI), 730mg of potassium (16.2% RDI), 35mg of sodium (Kermasha *et al*, 1987).; Vitamins-

273 mg of vitamin C (455% RDI). It is observed that 93% of people of Bangladesh are suffering from deficiency of vitamin-C. The largest amount of iron, 5.34 mg/100g was observed in lotkon (Haque *et al.,* 2009). Fruit shows around 100 brix TSS, 4.42% total sugar and 2.1% acidity (Bhowmick, 2013). The fruit was reported to possess antiviral and antioxidant and the stem bark of the plant was reported to have diuretic activity (Hasan *et al.*, 2009). The health benefits of lotkon are significant. It contains twice as much potassium as bananas. Because high magnesium content, it helps to assimilate potassium, benefits blood pressure and heart diseases, keeps bones healthy and strong. It's high potassium content may contribute to a reduced risk of developing arthritis, cancer and digestive issues, aids in the performance of the heart, kidneys and other organs. The roots, bark and wood are harvested to treat skin diseases, to relieve eye inflammation and other medicinal uses (Hagens, 2000).

Burmese grape is conventionally propagated by seed worldwide. Also in Bangladesh, majority of the growers use seeds. Although it can be propagated by both sexual (seed) and asexual methods (i.e. layering, grafting and separation of root pieces). The asexual methods of propagation are desirable. Because, asexually propagated plants are true to- type and it has both male and female trees, vegetative propagation is recommended to make sure that the propagated trees will be male or female tree. Thus provides the owner early and high economic benefit (Kamaluddin, 2008).

However, there are some diseases generally that affect the grapes as well as lotkon such as- Anthracnose (*Colletotrichum* spp.), Leaf blight , Bacterial

leaf spot, Black rot (*Guignardia bidwellii*), Scab (*Venturia inaequalis*), Mould rot (*Botrytis cinerea*), Foot rot (*Cylindrocarpon* sp.) and so on.

In cultivation period scab disease is prevalent and causes the damage of fruit quality but in the post harvest period anthracnose disease is prevalent which causes rotting of fruit.

Keeping above facts in view, the present investigation was undertaken to analyze the prevalence of lotkon in Narsingdi of Bangladesh with the following objectives:

i. To identify the diseases of lotkon and identification of their casual agents.
ii. To measure the incidence and severity of lotkon disease in three Upazila of Narsingdi district.
iii. To assess the nature of fruit damage symptoms and its yield loss due to lotkon fruit diseases.

CHAPTER II
REVIEW OF LITERATURE

Bhowmick *et al.* (2016) studied the Burmese Grape (*Baccaurea ramiflora* Muell. Arg.), a dioecious under utilized fruit crop which is commonly grown in homestead condition under the sub-Himalayan *terai* region of West Bengal. The crop not only holds good nutritional properties but also has its ritual values in this part of the globe. Since, it is a cross pollinated crop; therefore, it is believed to have a high degree of variability. So, in this study an effort was made to group the available variability based on flowering and fruiting characteristics. Correlation and cluster analysis studies indicated strong relationships between some parameters and broad two sub-groups of Burmese grape.

Deb and Bhowmick (2015) described Burmese grape (*Baccaurea ramiflora*) is an underexploited fruit crop grown mainly backyard plantation and as forest plant. It is native to the South East Asian region. In West Bengal the fruits are grown mainly homestead condition in northern parts of states; i.e., Cooch Behar, Jalpaiguri, Darjeeling districts. Locally the fruit is known as 'latka'. It is a mild acidic fruit and mainly used as fresh fruit consumption. It flowers during the summer months and fruits are mature in rainy season. Bearing habit of Burmese grape is adventitious or cauliflory in nature. The fruit is oval to round in shape and turns yellow or yellowish brown in ripen condition. The type of fruit is berry and edible portion is aril which is covered by leathery rind. Burmese grape is propagated by seeds and as it is dioecious in nature so variation is present among the present plant population. In the present study, an attempt has been made to evaluate the

physical and biochemical properties of different Burmese grape genotypes in northern parts of West Bengal. The maturity for fruit harvesting is least in Accession No. 9 (78.33 days) followed by Accession No. 8 (80.66 days) and it is maximum (90.33 days) for the Accession No. 7. For most of the desirable attributes, the Accession No. 11 showed better response like maximum length (3.383 cm), diameter (3.513 cm), weight (19.93 gm), juice content (74.10 ml/10 fruit) and lowest acidity (1.996%).

Shams *et al.* (2015) conducted a research and addressed the socio-cultural dimensions of agroforestry systems in the Narsingdi District, Bangladesh within a participatory research and development project. Using participatory rural appraisal methods in two communities results reveal that a jackfruit (*Artocarpus heterophyllus*) and Burmese grape (*Baccaurea ramiflora*) agroforestry system has quickly emerged as the dominant land use. Within this context, certain socio-cultural dimensions associated with farmers' agroforestry practices are identified. These include: relationship with the agricultural environment, traditional practices and knowledge, linkages with friends and families, cultural traditions and specific gendered roles. Each of these dimensions involves a set of cultural norms, values and practices that help maintain agroforestry; for example a local sharing system of planting materials, reliance on local germplasm and dependence on available local resources. The study highlights the importance of understanding and adapting to such cultural characteristics within the design of agroforestry research and extension.

Rahman *et al.* (2014) studied on the fruit characteristics, yield contributing characters and yield of five promising lotkon genotypes was carried out at

the fruit Research Farm and Laboratory of Citrus Research Station, Bangladesh Agricultural Research Institute, Jaintiapur, Sylhet, during fruiting season of 2013. Five promising lines of lotkon *viz*., BS-Jai 001, BS-Jai 002, BS-Jai 003, BS-Jai 004 and BS-Jai 005 were included in the study. A wide variation was observed among the genotypes in respect of different characteristics under the present study. Percent edible portion and total soluble solids (TSS%) were the highest in BS-Jai 001 (47.2% and 13.6%, respectively) while the lowest edible portion in BS-Jai 002 (36.7%) and the lowest TSS in BS-Jai 003 (12.4%). Heaviest (15.2 g) fruits with length and diameter of 3.4 cm and 3.4 cm was recorded from the genotype BS-Jai 003 whereas the lightest (8.6 g) with length and diameter of 2.2 cm and 2.7 cm was noted in BS-Jai 004. The highest number of fruits per plant (6000) as well as highest yield per plant (55.4 kg/plant) and per hectare (56.8 t/ha) were produced from the genotype BS-jai 005. BS-Jai 001 found superior in respect of fruit attractiveness, color, juicyness, flesh texture and eating quality.

Bhowmick *et al*.(2013) reported the Burmese grape or *Baccaurea ramiflora* Muell. Arg. is an underutilized fruit crop and it is grown as homestead condition of northern districts of West Bengal. Locally the fruit is called as latka, or latkon. Very limited document is available regarding the fruit quality and its changing pattern during fruit growth and development pattern. The present study on developmental pattern of Burmese grape was carried out in respect to different physical and biochemical aspects of fruits on the plants of farmers house hold nearer to the Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal. All the plants aged between ten to fifteen years and were well grown, free from pests and

diseases. After fruit set, the required fruit bunches were tagged on all sides to obtain uniform result. The fruits were harvested and subjected for studies on physico-chemical changes from 30th days after fruit set and continued up to 86th days after fruit set at weekly interval. Fruit size (length and diameter), juice content increased while peel thickness and seed weight decreased with advancement of maturity. Notable increase in TSS, sugar content and sugar: acid ratio were observed with decrease in acidity. At the time of harvest the fruits have attained TSS around 12.13% OBrix, acidity less than 2.16%, 4.18% total sugar and pericarp colour should be yellowish green.

Banik *et al.* (2013) evaluated Northern parts of West Bengal possess ample scope for cultivating underutilized tropical and subtropical fruit species. Burmese grape (*Baccaurea ramiflora* Muell. Arg.) can be grown well in home-stead and intercropped with rambutan (*Nephelium lappaceum*) and mango (*Mangifera indica*). It is good source of vitamin C and minerals like calcium and iron. The average diet of rural communities in West Bengal, India is highly deficient in calories, nutrients (calcium, iron) and vitamins (A and C). The crops grown in home-stead are the only sources of protective food for people living in villages in meeting their requirements of vitamins and minerals.

Howlader *et al.* (2011) evaluated the purpose of the study was to find out the cytotoxic activity of the *n*-hexane, chloroform and carbon tetrachloride fractions of the ethanolic extracts of the leaves and stems of *Baccaurea ramiflora* (Lour.). Ethanolic extracts of the leaves and stems of *Baccaurea ramiflora* were subjected to solvent-solvent partitioning using *n*-hexane, chloroform and carbon tetrachloride to obtain *n*-hexane leaves

fraction, chloroform leaves fraction, *n*-hexane stems fraction, chloroform stems fraction and carbon tetrachloride stems fraction. Each fraction was assayed for their cytotoxic effect using brine shrimp lethality bioassay. Among the fractions, the *n*-hexane fractions of leaves and stems showed significant cytotoxic effects having LC50 values of 7.79 µg/ml (95% confidence interval 6.48-9.37) and 5.78 µg/ml (95% confidence interval 4.76-6.99) respectively as compared to vincristine sulfate (LC50=2.81 µg/ml (95% confidence interval 1.97-4.01) which was used as positive control. The results support the traditional uses of *B. ramiflora* for various medicinal purposes and thus demand the isolation and identification of active principles and thorough bioassay.

Review of literature on other grape diseases

Cassandra *et al.* (2016) performed a research to study the Brown spot, caused by *Cladosporium* spp., is becoming a problematic postharvest disease of late season table grape (*Vitis vinifera*) in the California central valley, and management is hindered by knowledge gaps in disease etiology and epidemiology. Brown spot is herein described as a pre- and postharvest dry rot typified by an external brown to black spot or black mycelium which encases the placenta. Isolates in the *Cladosporium herbarum* and *C. cladosporioides* species complexes were recovered from 85 and 5% of brown-spot affected berries, respectively. Five isolates in the *C. herbarum* species complex, representing three phylogenetically distinct species (*C. limoniforme*, *C. ramotenellum*, and *C. tenellum*), and one *C. cladosporioides* isolate all caused brown spot symptoms under cold-storage conditions, with and without mechanical wounding. Isolate virulence was similar ($P > 0.05$) based on disease incidence and severity on intact berries

but severity varied on wounded berries ($P < 0.001$). Surface disinfestation reduced severity of cluster rot development following 2 weeks in cold storage ($P = 0.027$) but incidence was not affected ($P = 0.17$). This work provides foundational information on brown spot patho system etiology and biology in late-harvest table grape, which can be used to improve management.

Mohamed *et al.* (2015) conducted an experiment to study the Grapevine disease caused by *Botryosphaeriaceous* fungi include leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood, and perennial cankers among the most dangerous Grapevine disease. However, there are no studies about the status (occurrence or absence) of these important diseases in KSA generally or in Taif region has been reported. Therefore, the resent study was aimed to use the modern molecular genetics tools to report the status of the *Botryosphaeria* spp. and its associated diseases in Taif. In this respect, the symptoms of four diseases caused by *Botryosphaeria* spp. (Black rot, Macrophoma rot, Black dead arm and Botryosphaeria canker) were monitored in the grapevine farms in Taif. Among the four diseases, only the symptoms of Black rot were observed in some farms. Subsequently, the symbiotic samples of the Black rot were collected and their associated fungus spp were isolated in the lab. Ten isolates were obtained, characterized and identified at the morphological level. However, the fungus of the black rot disease, *G. bidwellii*, was not identified. Alternatively, the phylogenetic analyses of ribosomal DNA internal transcribed spacer (ITS) regions performed by conventional PCR. Sequencing analysis of the amplified fragment was obtained and aligned to

the known sequences in the gene bank. A homologues percentage between the obtained sequence and the sequence of *G. bidwellii*was 99%.

Gautam *et al.* (2014) studied *Colletotrichum*is one of the most common and unusual plant pathogenic fungal genera causing variety of diseases. The genus is primerily responsible for significant disease; anthracnose inhabiting a wide range of host's worldwide. They reviewed various diseases caused by *Colletotrichum* in India with special reference to plant diseases reported in present century (i.e. 2000-2012). About 25 plant diseases caused by different species of *Colletotrichum* namely, *C. gloeosporioides*, *C. capsici*, *C. falcatum*, *C. truncatum*, *C. sansevieriae*, *C. acutatum* and *C. Coccodes*were reported in present century, in which *C. gloeosporioides* found more prevalent anthracnose pathogen. The study showed that even a single species of *Colletotrichum* can affect multiple hosts.

Uaciquete *et al.* (2013) evaluated anthracnose of cashew (*Anacardium occidentale*) was studies on various genotypes and locations in Mozambique. *Colletotrichum gloeosporioides* was identified as the anthracnose causal agent using polymerase chain reaction. The relationships between incidence and severity of anthracnose on cashew genotypes were statistically analyzed by regression. Anthracnose leafincidence, which is practically easy to evaluate, was consistently associated with leaf severity, and their relationships can be estimated using the restricted exponential function across locations, crop seasons, genotype and fungicide trials. Pooled data enabled estimation of initial incidence of 1.43% with percentage variance accounting for 83.2 and standard error of 8.3. By computing incidence data into the summary equation, 24 changes of 0, 1, 5, 10 and

40%, resulted in changes of severity estimates of 0.01, 0.05, 0.10, 0.50 and 1.00%, respectively. The maximum disease incidence was estimated as 80% when the severity reached only 5%. Increase in severity was observed afterward, approached a maximum of 25% when leaf detachment is observed. The use of incidence data for epidemic comparisons, genotype and fungicide evaluation in cashew orchards is recommended.

Khajuria *et al.* (2012) carried out an experiment to study the scab caused by fungal pathogen *Venturia inaequalis* (Cke.) Wint., which is a heterothallic, haploid ascomycete, reproducing sexually and asexually. Understanding the genetic variability of the pathogen is very important for devising the strategies to control it. DNA based molecular markers have been successfully used for studying the genetic variation in different fungal pathogens. For investigation, samples were collected from the different areas of Kashmir valley, during 2004 to 2008.

Kimberly (2011) studied *Venturia inaequalis*, the causal agent of apple scab, is controlled primarily by fungicides. Long-term, extensive fungicide use has led to the development of resistance to multiple fungicides. To assess fungicide resistance, isolates of *V. inaequalis* were collected from Indiana and Michigan orchards. Single-spore derived isolates were evaluated by mycelium growth assays with previously determined discriminatory doses on media containing dodine, kresoxim-methyl, myclobutanil, or thiophanate-methyl. Of 195 isolates tested, 5.2, 0.7, 57.0, and 92.6% of isolates were found to be resistant to dodine, kresoxim-methyl, mymyclobutanil, and thiophanate-methyl, respectively. This is the first report of kresoxim-methyl field resistance in these states. Isolates resistant or shifted to a single

fungicide were often found to have multiple fungicide resistance. Of all isolates tested, 38% were identified as resistant or shifted to two fungicides, and 12% were resistant or shifted to all four fungicides tested.

Patzak *et al.* (2011) described the presence of genes for resistance to scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) was studied using molecular markers in a sample of 279 apple cultivars from the Czech collection of apple genetic resources. The sample comprised 37 cultivars supposed to have the *Vf*gene for scab resistance, 97 reference world cultivars and 145 old and local cultivars. Six PCR molecular markers for the scab resistance genes *Vf*, *Vm*, *Vbj*, *Vr*and *Vh*and three PCR molecular markers for the powdery mildew resistance genes *Pl-w*, *Pl-1* and *Pl-d* were used. The marker for the major scab resistance gene *Vf*was detected in all cultivars supposed to have *Vf*, except in Romus 1, and in the three small-fruited cultivars *Malus* Evereste, Golden Gem and Hilleri. The markers of the *Vr*and *Vh*scab resistance genes were detected in 22 cultivars in combination with the marker for *Vf*, in 56 reference world cultivars and in 82 old and local apple cultivars. PCR molecular markers for one or two of the powdery mildew resistance genes were detected in the small-fruited cultivars *Malus* Evereste, Golden Gem, prof. Sprengeri and Hilleri; and in the larger fruited cultivars Hagloe Crab, Borovinka and TitaZetei. We did not find markers for the scab resistance genes *Vm* and *Vbj*in any of the studied cultivars.

Mmbag *et al.* (2011) isolated the isolates of *Alternaria* sp. from collected leaf blight samples of lilacs in the field. The internal transcribed spacer (ITS)

region and morphological characterization were used to identify lilac blight pathogen. Based on 100% ITS nucleotide sequence identities to the *Alternaria* genus in the Gen Bank and morphological features, these isolates were identified as *A. alternata*. Disease symptoms were reproduced in lilac plants inoculated with *A. alternate* mycelial plugs and sprayed with a fungus-free culture filtrate, indicating that pathogenesis in lilac involves secondary metabolites or toxins. Diagnostic primerswere developed to detect *Alternaria* sp. and *A. alternate* leaf blight based on ITS region and four known genes associated with pathogenesis in *A. alternata*: mixed-linked glucanase precursor, endopolygalacturonase, hsp70, and histone genes. The results from our study indicated *A. alternata* is a primary pathogen in lilac leaf blight, and these diagnostic primers can be used as a tool for the fast detection of *A. alternata*.

CHAPTER III
MATERIALS AND METHODS

The aim of this chapter is to deal with the materials and methods that were used to conduct the experiment. The location of the experiment, weather, materials used and methods followed in different operations during the experiment as well as in data collection are described here under the following sub-heading:

3.1 Survey locations and period

3.1.1 Study area

Detailed survey was carried out in three Upazillas of Narsingdi district namely Belabo, Shibpur and Raipura from April to July, 2016, to record the incidence and severity of lotkon fruit and leaf diseases (Figure 1).

3.1.2 Post harvest Survey

The incidence and severity of lotkon fruit in the local market of each upazila namely Morjal in Raipura , CNB bazar in Shibpur and Belabo sadar were surveyed to assess the nature of fruit damage and its yield loss due to the lotkon fruit diseases (Plate.1.). The survey was recorded accordingly with five days interval within fifteen days of fruit availability in market.

3.1.3 Field Survey

A detailed field survey was carried out in three Upazila of Narsingdi district such as- Belabo, Shibpur and Raipura from April to July, 2016, to record the disease incidence and disease severity of lotkon (Figure 1). Two lotkon

orchards were selected for each mentioned upazilas. In three upazilas total six fields were selected. During survey period total six times survey were conducted at a regular 20 days intervals.

Figure. 1. Survey areas of Narshindi district (Belabo, Shibpur and Raipura) in Bangladesh.

The information about the disease incidence and severity of lotkon fruit and tree was recorded in the cultivated fields during survey. The first survey was made on 10th April 2016. The times of data collection were determined on the basis of variation in temperature, relative humidity and rainfall during the growing seasons

3.2 Observation of the symptoms

Symptoms of the disease were studied by visual observation. Sometimes hand lens were used for critical observation of the disease and a disease was identified based on matching the observed symptoms from the infected plants. The symptoms of the disease were recorded according to following description of Amador (2002); Ferguson (2002) and Reddy and Marti (1990). Identification of all the fungal disease was finally confirmed by identification of the associated fungal organism through isolation.

3.3 Collection of disease specimen

During the survey infected fruits and leaves were collected from different regions of Narsingdi in 2016 (April to July), and put in a polyethylene bags (Malik, 1996). Fifty numbers of fruits and fifty numbers of leaves among the 20 lotkon trees were collected from Lotkon yard of 6 fields randomly during 6 periods of investigation.

In case of market survey, five fruits sellers were selected from each of three market randomly. The infected fruits were selected from the 1 kg lotkon of each fruit sellers and 50 fruits were selected for random collection for the determination of disease incidence and severity.

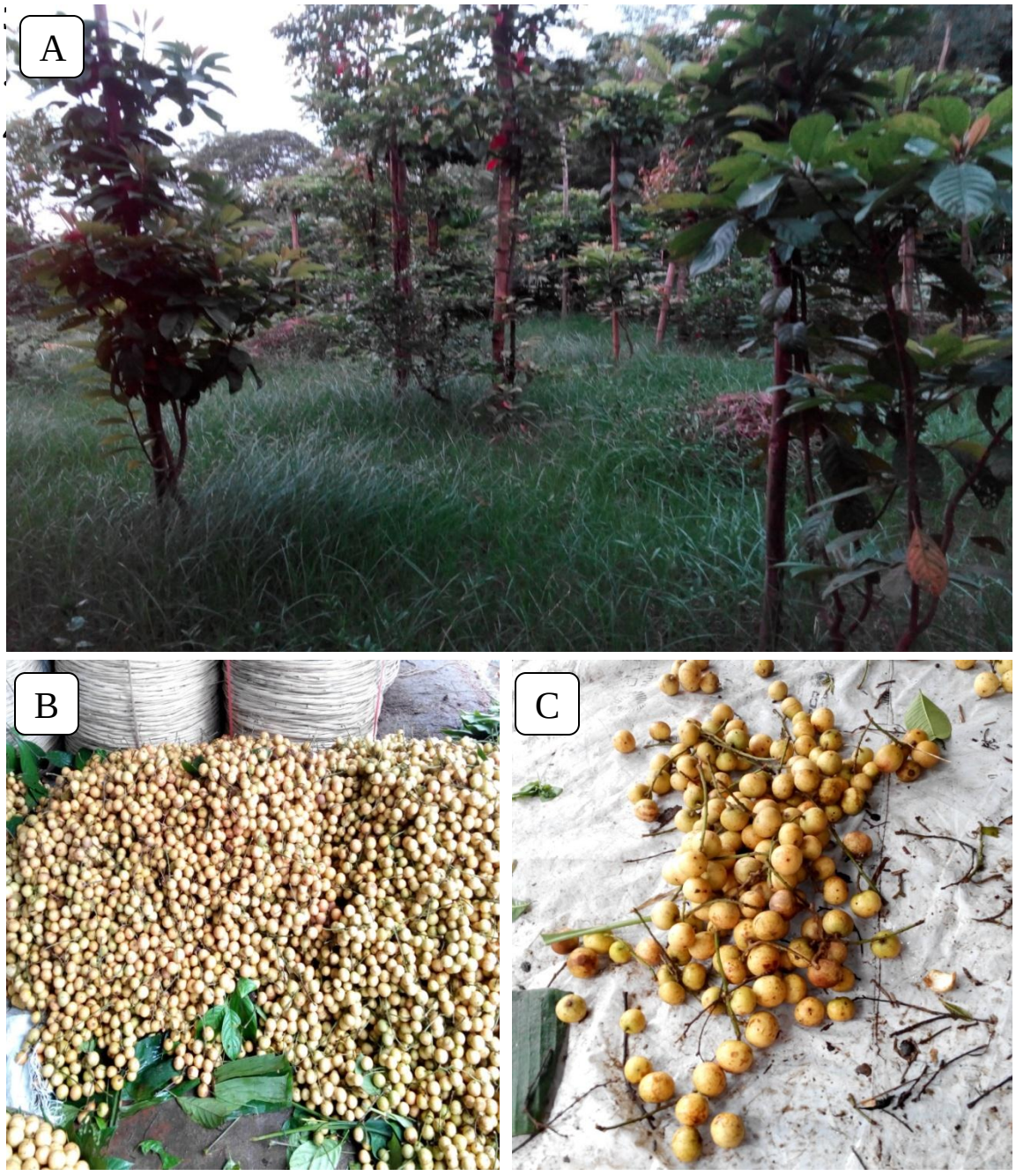

Plate.1. Field view (A); Fruits in the market (B,C).

3.4 Determination of disease incidence and severity

For the calculation of disease incidence and severity of lotkon fruits and leaves were counted from the infected leaves and fruits and then expressed in percentage. Assessment of the disease incidence and severity of lotkon fruits was determined by the following formula (Rai and Mamatha, 2005):

$$\text{Fruit Incidence (\%)} = \frac{\text{No. of diseased fruits affected by particular disease}}{\text{Total number of inspected fruits}} \times 100$$

$$\text{Fruit Severity (\%)} = \frac{\text{Total infected fruit tissue area}}{\text{Total fruit tissue area}} \times 100$$

$$\text{Leaf Incidence (\%)} = \frac{\text{No. of diseased leaves affected by particular disease}}{\text{Total number of inspectedleaves}} \times 100$$

$$\text{Leaf Severity (\%)} = \frac{\text{Total infected leaves tissue area}}{\text{Total leaves tissue area}} \times 100$$

3.5 Isolation of causal organism was made by following method

3.5.1 Tissue planting method

The pathogen associated with the diseased plant parts (fruits and leaves) were cutting in to several pieces by scissors and placed on the moist filter paper (Whatman no. 1). Three pieces of filter papers were moistened by dipping in distilled water. The pertidishes with the diseased specimens were incubated at 22 ± 2^0C under 12/12 alternating cycles of NUV and darkness in the incubation room of the Pathology laboratory for three to five days. After

incubation the plates were examined under stereo microscope for primary identification of the organisms (fungi) (Ko *et al.*, 2001). The fungi were transferred to PDA plates for proper sporulation and purification.

3.5.2 Preparation of Potato Dextrose Agar Media (PDA)

First, 200g peeled potato slices and 500 ml water were boiled together for 15 minutes so that the potato tissues were soften. Then the prepared pulp was sieved through a muslin cloth; 20g of Agar and 500 ml water were heated together and a solution was made. The 20 g of dextrose were added to it and volume upto 100 ml. Then 1000 mL of potato dextrose was prepared.

3.5.3 Agar plate method

At first the diseased plants (fruits and leaves) thoroughly washed to remove soil and some particles. Then infected plant parts were cut into 5mm pieces from advancing end of the lesions.The cut portions were surface sterilized by dipping them in 0.001% $HgCl_2$ solution for 1.5 minutes and washed three times with sterile water and there after placed on PDA plates aseptically. The plates were incubated at 28 ± 1^0C for several days and examined daily for any fungal growth and continued for 10 days .After incubation period the inoculated PDA plates were observed to identify the causal organisms.

3.6 Isolation of Pathogens

The fruits samples were surface sterilized for 3 minutes with 1% NaOCl and rinsed in four successive changes of sterile distilled water (Djeugap *et al.*, 2009). The plates were incubated in an inverted position at 20±1°C for 2-3 days and observed for fungal growth and later sub cultured into fresh PDA medium.

3.6 Identification of Pathogens

Pure isolates of pathogens obtained were identified on the basis of macro and micro morphological characteristics. Morphological characteristics of the fungi (mycelium coloration or pigmentation, presence or absence of septate, spore morphology) were recorded. In some cases, the infected tissues were stained by cotton blue and Lactophenol (McClenny, 2005) and observed under microscope. Morphological identification of fungi was based on the morphology of the fungal culture colony or hyphae, the characteristics of the spores and reproductive structures (Barnett and Hunter, 1972; Alexopoulos and Mims, 1996; Agrios, 2005).

3.7 Inoculum preparation and inoculation

The plates were incubated at the optimal temperature for the growing of the selected pathogen. After the desired level of growth was achieved, agar plates were stored upside down in a refrigerator for an extended period of time to keep pathogens for future experiments.The inoculum and inoculation procedures were followed by the description and put in microhumidity chamber (Pelczar, 1993; Cheesbrough, 2000).

i) Added 100 ml of distilled water in a beaker and 10 drops of Tween 20 using pasteur pipet stir using magnetic stir plate.

ii) Put 10 ml of water solution on fungal plate and scraped the spores using spatula.

iii) Placed the spore mixture in a small beaker put stired bar and stir for 10 minutes.

iv) Filtered the mixture using cheese cloth and funnel and quantify the spores using Hemacytometer and light microscope.

v) Adjusted the spore suspension to ideal concentration of $1.2X10^6$ spores/ml

vi) Inoculated into the lotkon fruits using sterile scapel

vii) Placed the plants in the mist chamber for 48 hours and 100% Relative Humidity.

viii) Evaluated after 7 days regular intervals from the inoculation.

The pathogenic infection thereafter can be detected by the symptomatic expression.

3.8 Meteorological data collection

The generalized environment variables were selected for their effect on the incidence and severity of disease of lotkon. Day to day meteorological data in temperature, humidity and rainfall were collected from Meteorological Department, Agargaon, Dhaka-1207. The taken data were analyzed and calculated for the monthly mean of minimum and maximum temperature, mean of minimum and maximum relative humidity, and mean of minimum and maximum rainfall throughout the study period from April, 2016 to July, 2016. (Appendix I).

CHAPTER IV

RESULTS AND DISCUSSION

A survey was carried out in the Narsingdi district to study the Diseases incidence and Severity of lotkon (burmese grape) fruit and leaves. The survey was conducted at regular interval to assess the diseases of lotkon. The results are interpreted and discussed below:

4.1 Description of collected lotkon (*Baccaurea ramiflora*) fruits.

The fruit was green colored while young turned yellow, red or even deep purple as they matured. Juicy white to pinkish white, translucent pulp was enclosed by leathery rind and in the middle of the pulp there were several small, hard seeds, flat-elliptic or rotund, 1–1.3 cm with purplish red testa which was edible. The fruits have a succulent, sour and sweet taste. Its texture resembles a lychee fruit, but is less juicy and more fibrous.

During the investigation, there were some common diseases were observed from the selected Upazilas and three diseases were identified viz.– Anthracnose, Scab and blight of lotkon fruits. But the disease infection were varied from Upazila to Upazila.

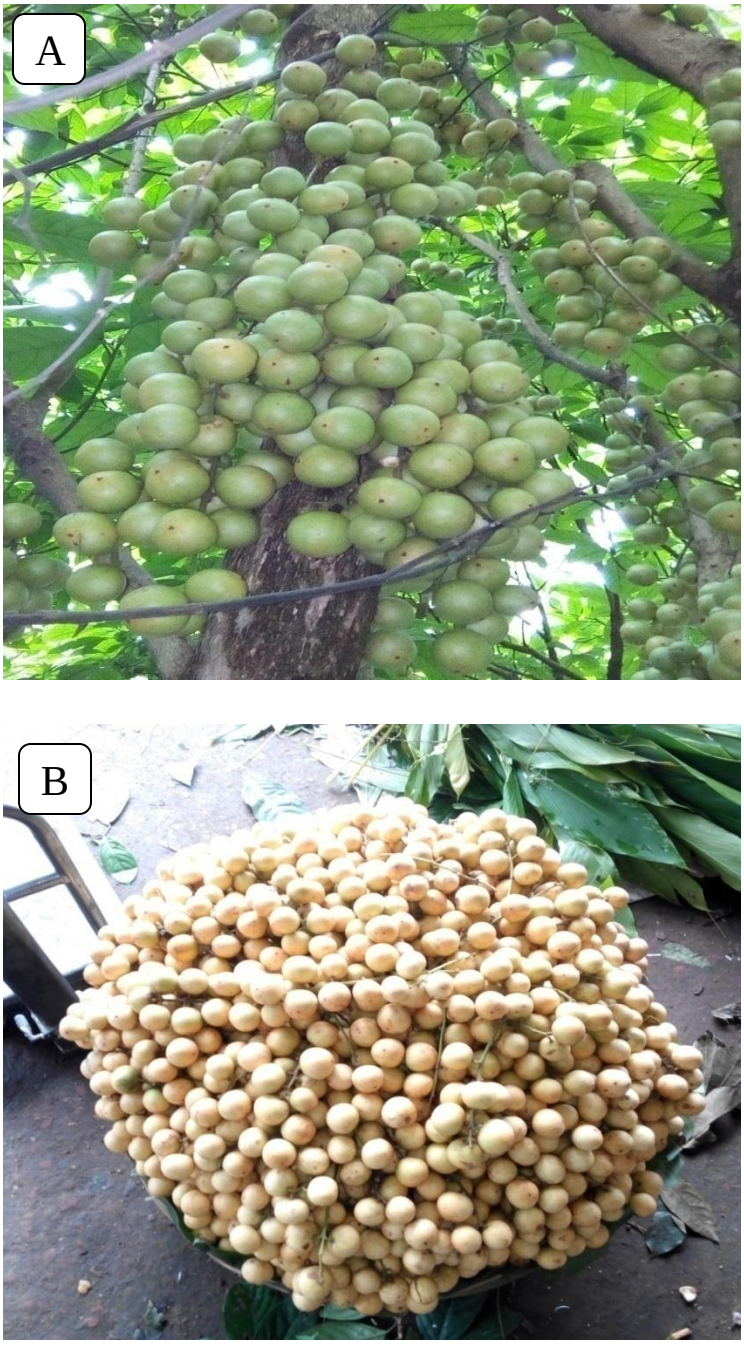

Plate.2. Mature stage (A), Harvested lotkon fruits (B).

4.2 Symptoms of the disease and identification of pathogen

4.2.1 Anthracnose of lotkon

Anthracnose disease symptoms as dark red mostly irregular shaped lesions were observed on fruits. Disease appeared as round to oval, water-soaked dark green areas turned circular spots with tan to light brown. The symptoms on the fruits initially appeared as a small brown-red, circular spots gradually enlarge with the central part being light brown and dark brown to black margin. Centre of the lesion became reddish brown to brown color as the spots expand and loss of mucilaginous gel and death of infected leaves was also observed (Plate.1)

The pathogen isolated from the disease symptom was identified as *Colletotrichum* sp. (Plate 3 to 7). There were great variations in size and shape of conidia among different *Colletotrichum* spp. Conidia may be oblong with obtuse ends, and were generally broader, elliptic to fusiform, falcate, and gradually tapered to each end, and straight to slightly curved with abruptly tapered and obtuse ends.

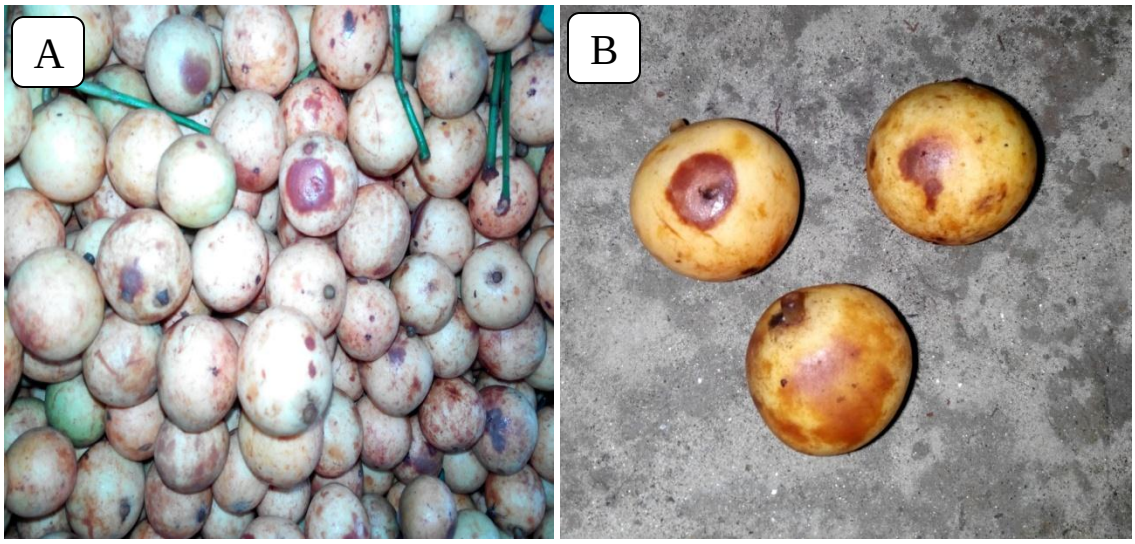

Plate. 3: Infected lotkon fruits with spots, (A,B).

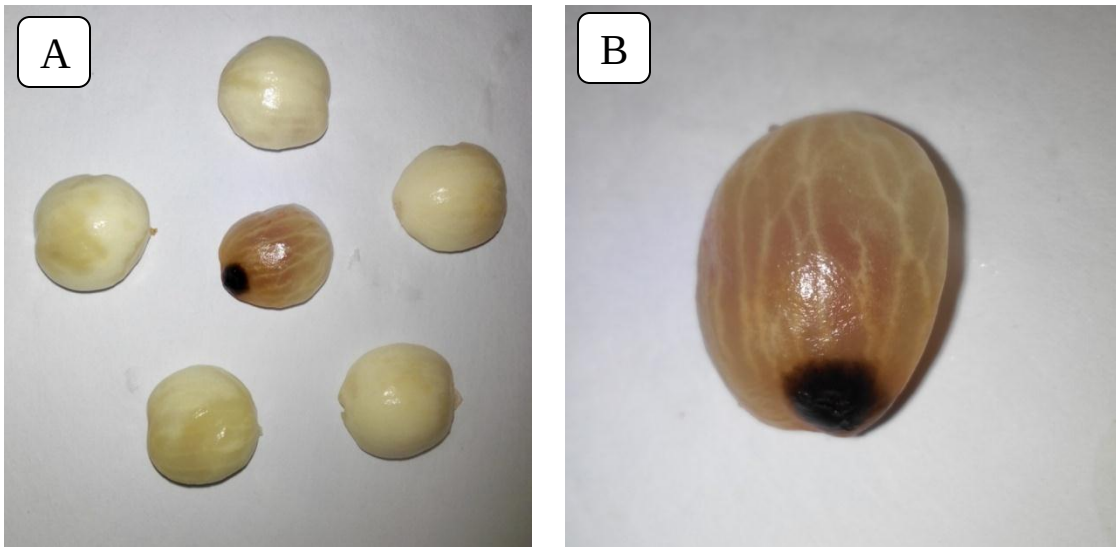

Figure 4. Infected pulp caused by Anthracnose disease (A,B).

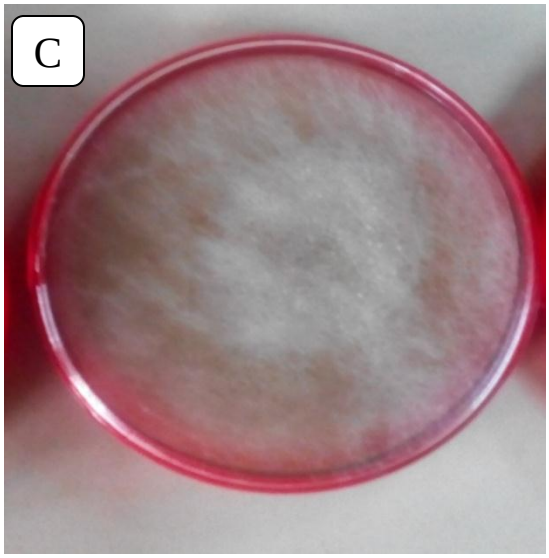

Figure 5 : Pure culture (A) of *Colletotrichum* sp.

Plate 4. Anthracnose of lotkon.

Figure. 2: Symptoms after inoculation of *Colletotrichum* sp.

4.2.2 Scab of lotkon

The earliest noticeable symptom on fruit was raised areas which developed into velvety, reddish to olive-brown lesions. Infections of young fruit caused fruit distortion and found cracked symptoms. The disease manifested as dull black or grey-brown lesions on the fruits surface. Lesions appeared less frequently on the fruit tissues of the lotkon (Fig. 3).

The pathogen isolated from the disease symptom was identified as *Venturia inaequalis* (Fig. 3). The ascospores were brown, two-celled, and have a characteristic "footprint" shape. Culturing of *V. inaequalis*was a difficult step because of cross contamination of other fungi. *Venturia* was very slow growing fungus, and takes 10–15 days for growth.(Fig .4.)

Figure. 3. Scab disease of lotkon caused by *Venturia inaequalis*.

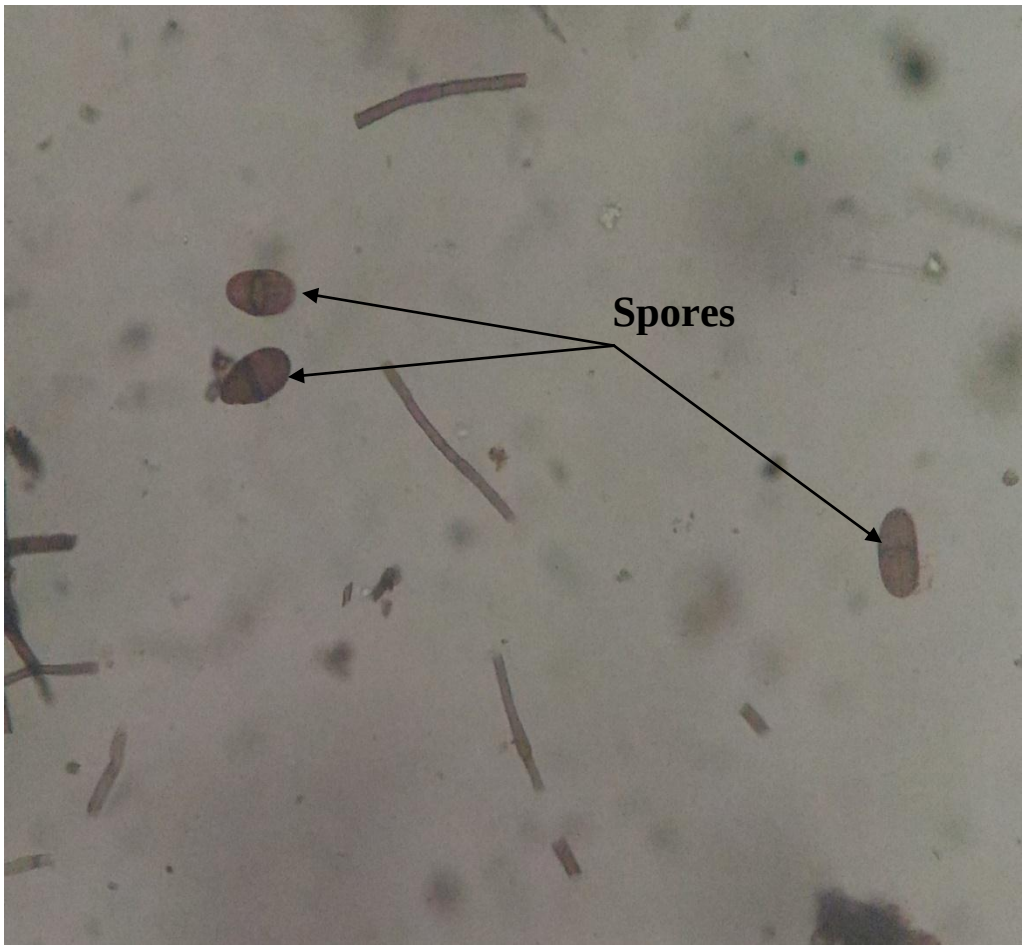

Figure. 4. Two-celled ascospores of *Venturia inaequalis* responsible for scab disease of lotkon.

4.2.3 Leaf blight disease of lotkon

The disease attacked on the leaves. Small yellowish spots first appeared along the leaf margins, which gradually enlarged and turne into brow or blight with concentric rings. Mature leaves near the crown of the plant are often infected first. Severely infected leaves turn browned, curl upwarded, wither and died.(Figure.5.).The causal organism of leaf blight could not be identified and isolated.

Figure .5. Observed leaf blight disease of lotkon.

4.3 Incidence and severity of anthracnose disease of lotkon

4.3.1 Disease incidence and severity of Anthracnose of lotkon (*Colletotrichum*sp.) at different upazillas of Narshindi districts.

The average disease incidence of anthracnose of lotkon was observed 15.17% in Raipura at 120 days (20th July,2016) after fruits come out during observation. But the average disease incidence was found 11.33% in Shibpur, whereas the average disease incidence of anthracnose was observed 16.83% in Belaboupazilla (Table 1). In case of severity, the disease severity of anthracnose was observed 3.2% in Raipuraupazilla, whereas 1.3 %and 1.8% were found in Shibpur and Belaboupazillas, respectively (Table 2)

4.3.2 Highest and lowest disease incidence and severity of Anthracnose of lotkon (*Colletotrichum* sp.) in growing period of Narshindi districts.

The highest infection of anthracnose was found 25, 22 and 26% in Raipura, Shibpur and Belabo Upazilas, respectively during survey period 20th July, 2016. The lowest disease incidence was observed 4% in Raipura Upazila, whereas 2% and 8% were found in Shibpur and Belabo Upazilas, respectively in 10th April, 2016 (Table 1).

The highest severity of anthracnose was observed 30.23, 28.56 and 24.67% in Raipura, Shibpur and Belabo upazillas, accordingly in 20th July, 2016. And the lowest severity was found 3.2, 1.3 and 1.8%, respectively in 10th April, 2016 (Table 2).

Table 1. Disease incidence of anthracnose of lotkon fruits (Market).

Date of Observation	Disease Incidence (%)			Average Disease Incidence (%)
	Raipura	**Shibpur**	**Belabo**	
10th April	4	2	8	4.60
30th April	6	4	11	7.00
20th May	16	8	16	13.30
10th June	18	12	19	16.30
30th June	22	20	21	20.30
20th July	25	22	26	24.30
Average	15.17	11.33	16.83	

Table 2. Disease severity of Anthracnose of lotkon fruits (Market).

Date of observation	Disease Severity (%)			Average Disease Severity (%)
	Raipura	**Shibpur**	**Belabo**	
10th April	3.2	1.3	1.8	2.1
30th April	11.2	7.8	6.5	8.5
20th May	16.45	9.8	11.32	12.52
10th June	19.89	14.78	15.56	16.74
30th June	22.56	18.78	17.89	19.74
20th July	30.23	28.56	24.67	27.82
Average	17.26	13.50	12.96	

4.3.3 Mean disease incidence and severity of Scab disease of lotkon (*Venturia inaequaliqs*) at different Upazilas of Narshindi districts.

The average disease incidence of Scab disease of lotkon was observed 13.33% in Raipuraon 20th July, 2016. But the infestation of average disease incidence was found 10.33% in Shibpur, whereas the average disease incidence of scab was observed 14.33% in Belaboupazilla(Table 4). In case of severity, the average disease severity of scab disease of lotkon was observed(20thJuly,2016) at 19.69%, 18.41% and 15.86%, respectively (Table 5).

4.3.4 Highest and lowest disease incidence and severity of scab of lotkon (*Venturia inaequalis*.) during growing period of Narshindi districts.

The highest disease incidence of scab was observed 26%, 23% and 29% in Raipura, Shibpur and Belabo Upazillas, accordingly in 20th July, 2016.The lowest scab disease of lotkon was found 2% and 1% in 10th April, 2016 (Table 4).

The highest infestation of was found 17.20%, 16.78% and 15.45% in Raipura, Shibpur and Belabo upazillas, respectively in 10th April, 2016. And the lowest disease severity observed 4.56%, 5.65% and 3.89%, respectively of the upazillas in 10th April, 2016 (Table 5).

Table 3. Disease Incidnce of Scab of lotkon fruits (Field).

Date of observation	Disease Incidnce(%)			Average Disease Incidence (%)
	Raipura	**Shibpur**	**Belabo**	
10th April	2	0	1	1.0
30th April	6	3	2	3.60
20th May	11	8	12	10.30
10thjune	16	13	19	16.0
30thjune	19	15	23	19.0
20thjuly	26	23	29	26.0
Average	13.33	10.33	14.33	

Table 4.Disease severity of Scab of lotkon fruits (Field).

Date of observation	Disease Severity (%)			Average Disease Severity (%)
	Raipura	**Shibpur**	**Belabo**	
10th April	4.56	5.65	3.89	4.7
30th April	21.6	18.92	15.76	18.76
20th May	28.32	25.67	22.98	25.65
10thJune	25.23	24.67	19.89	23.26
30thJune	21.23	18.78	17.21	17.82
20thJuly	17.2	16.78	15.45	16.48
Average	19.69	18.41	15.86	

4.3.5 Mean disease incidence and severity of observed leaf blight disease of lotkon at different upazilas of Narshindi districts.

The average disease incidence of leaf disease of lotkon was observed 20.5% in Raipura at 20th July, 2016. But the infestation of average disease incidence was found 17.67% in Shibpur, whereas the average disease incidence of anthracnose was observed 19.33% in Belabo Upazila.The average disease severity of leaf blight disease of lotkon was observed 21.70%, 17.71% and 16.39%, respectively.

4.3.6 Highest and lowest disease incidence and severity of leaf blight disease of lotkon in growing upazilas of Narshindi districts.

The highest incidence of leaf disease was observed 28, 26 and 31% in Raipura, Shibpur and Belabo upazilas, accordingly during 20th July, 2016. When the observation was carried out 30th June, 2016 the incidence of leaf blight was observed 25, 23 and 26%, respectively. The lowest leaf blight incidence 11%, 8% and 7% were found in 10th April, 2016.

The severity of leaf blight disease was observed 26.12%, 21.89% and 19.87%, respectively in 30thJune, 2016. The infestation of leaf blight disease was rapidly increased at 120 days after observation (20th July, 2016) and found 31.89, 26.54 and 23.78%, accordingly.Then the lowest disease severity of leaf blight disease was observed 14.20, 11.56 and 8.92%, respectively.

Table .5. Disease Incidnce of observed leaf blight disease of lotkon (Field).

Date of Observation	Disease Incidnce (%)			Average Disease Incidence (%)
	Raipura	**Shibpur**	**Belabo**	
10th April	11	8	7	8.67
30th April	16	13	11	13.33
20th May	21	17	18	18.67
10th June	22	19	23	19.33
30th June	25	23	26	24.67
20th July	28	26	31	28.33
Average	20.5	17. 67	19.33	

Table 6. Disease Severity of leaf blight disease of lotkon (Field).

Date of Observation	Disease Severity (%)			Average Disease Incidence (%)
	Raipura	**Shibpur**	**Belabo**	
10th April	14.2	11.56	8.92	11.56
30th April	16.59	13.46	12.56	14.20
20th May	18.59	15.59	14.67	16.28
10th June	22.78	17.23	18.54	19.52
30th June	26.12	21.89	19.87	22.63
20th July	31.89	26.54	23.78	27.40
Average	21.70	17.71	16.39	

4.4 Effect of weather components on the disease incidence and severity

4.4.1.a Relationship between anthracnose disease incidence and temperature

Apositive correlation between anthracnose disease incidence and temperature was observed. The relationship between disease incidence and temperature could be expressed by the equation Y=6.891x - 197.0 (R^2=0.960), where x= temperature and y=disease incidence.

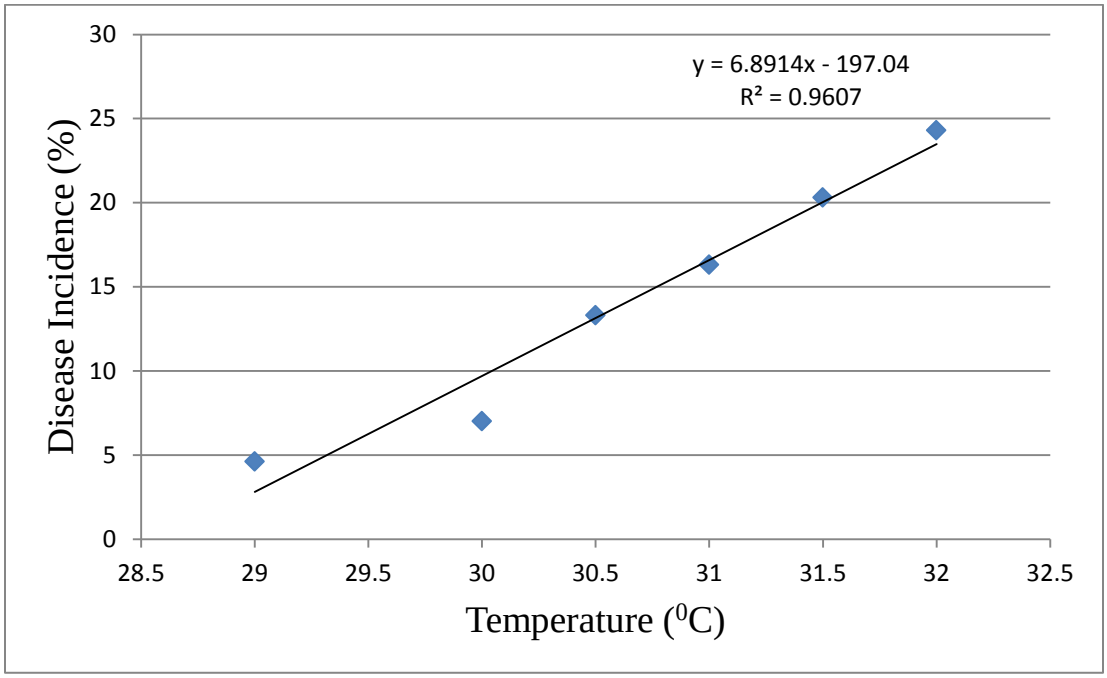

Figure. 8. Linear regression analysis of the effect of temperature on the incidence of anthracnose of lotkon.

4.4.1.b Relationship between anthracnose disease incidence and relative humidity

A positive correlation between anthracnose disease incidence and relative humidity was observed. The relationship between disease incidence and temperature could be expressed by the equation Y=1.374x - 89.02(R^2=0.818), where x= relative humidity and y=disease incidence.

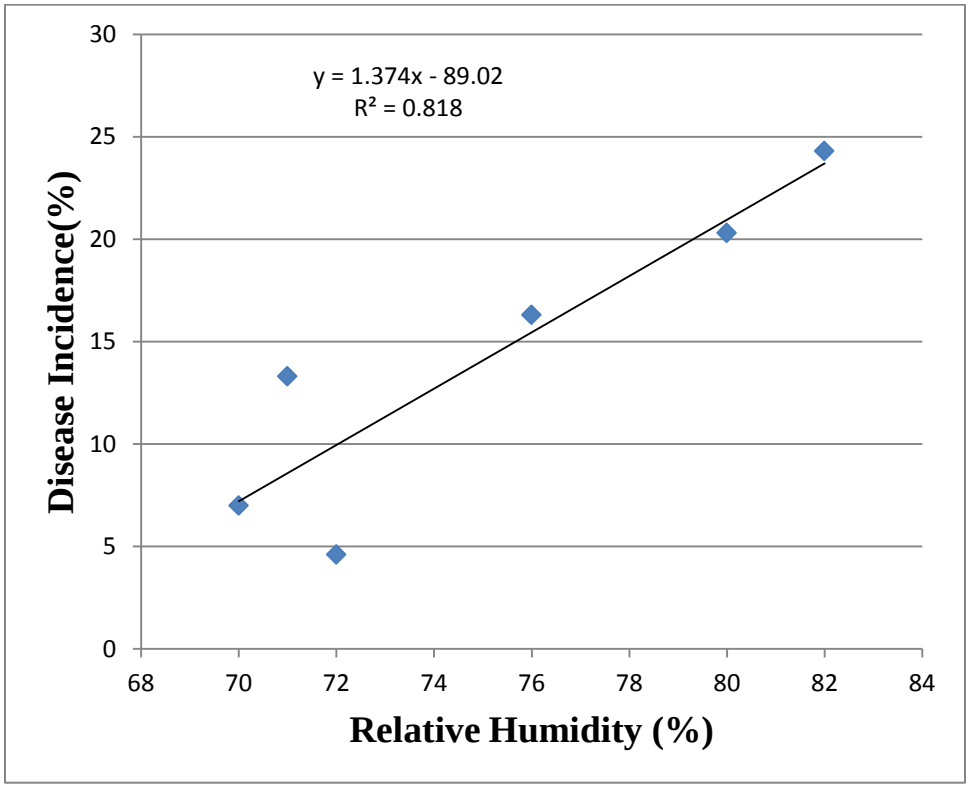

Figure 9 Linear regression analysis of the effect of relative humidity on the incidence of anthracnose of lotkon.

4.4.1.c Relationship between anthracnose disease incidence and rainfall

A positive correlation between anthracnose disease incidence and rainfall was observed. The relationship between disease incidence and rainfall could be expressed by the equation Y=2.620x - 3.931(R^2=0.912), where x= rainfall and y=disease incidence. Here, the R^2 value indicates that the contribution of rainfall was on (91.20%) the incidence of anthracnose of lotkon.

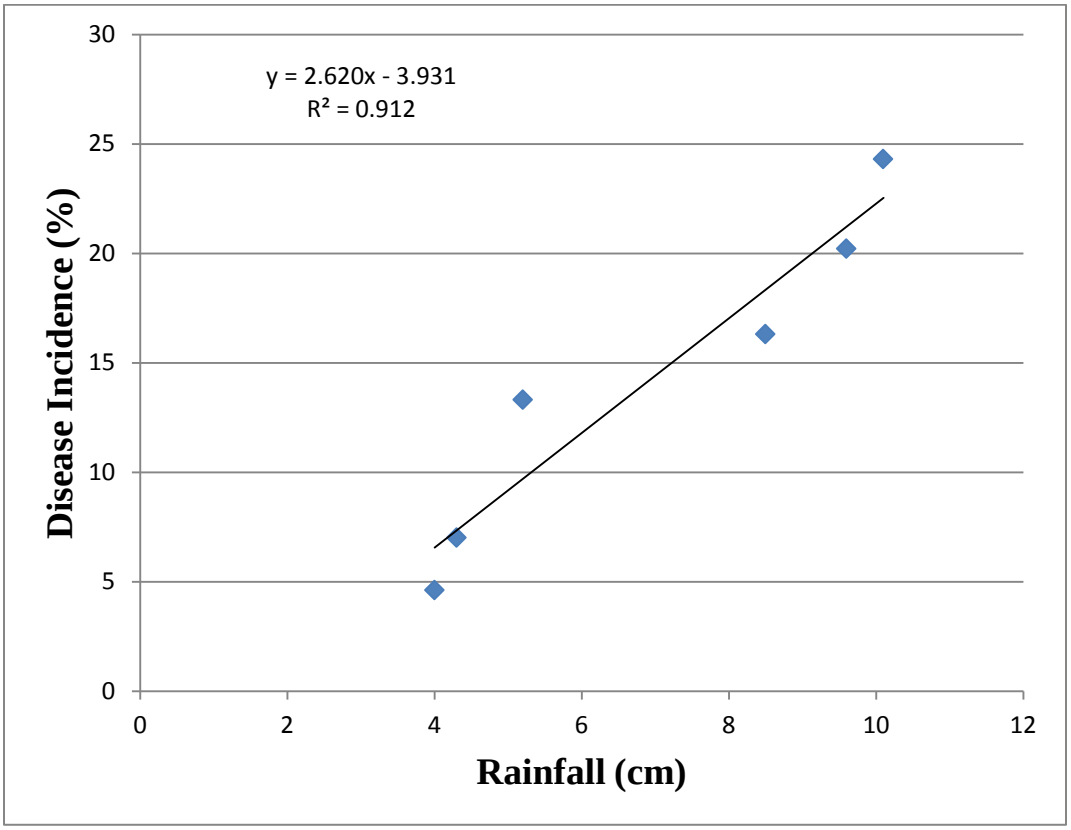

Figure 10. Linear regression analysis of the effect of rainfall on the incidence of anthracnose of lotkon.

4.4.2.a Relationship between scab disease incidence and temperature

A positive correlation between scab disease incidence and temperature was observed. The relationship between disease incidence and temperature could be expressed by the equation Y=8.58x - 250.4 (R^2=0.948), where x= temperature and y=disease incidence.

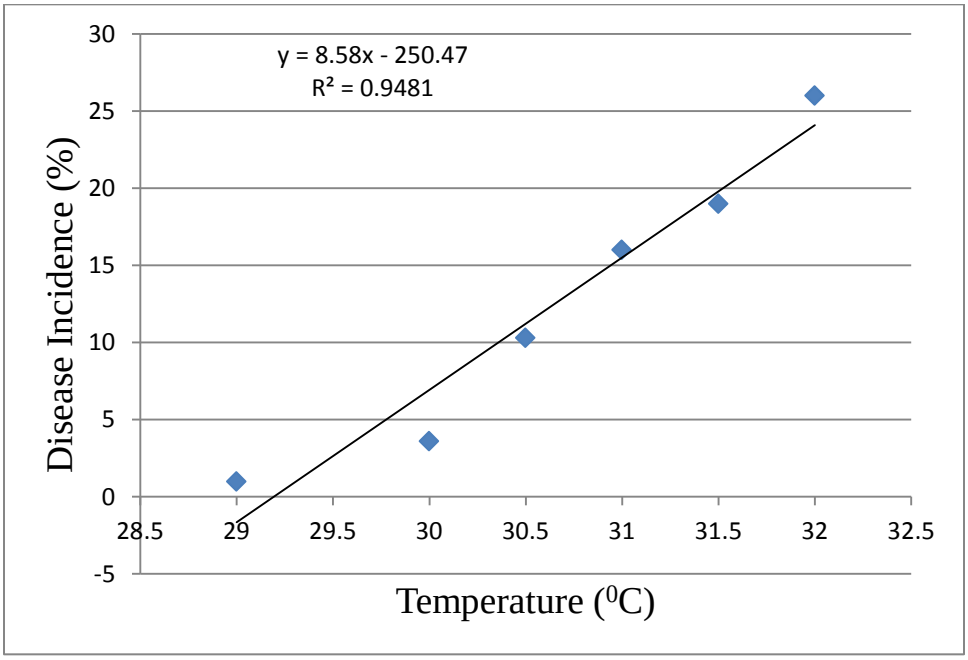

Figure 11 Linear regression analysis of the effect of temperature on the incidence of scab of lotkon.

4.4.2.b Relationship between scab disease incidence and relative humidity

A positive correlation between scab disease incidence and relative humidity was observed. The relationship between disease incidence and temperature could be expressed by the equation Y=1.747x - 118.7(R^2=0.841), where x= relative humidity and y=disease incidence.

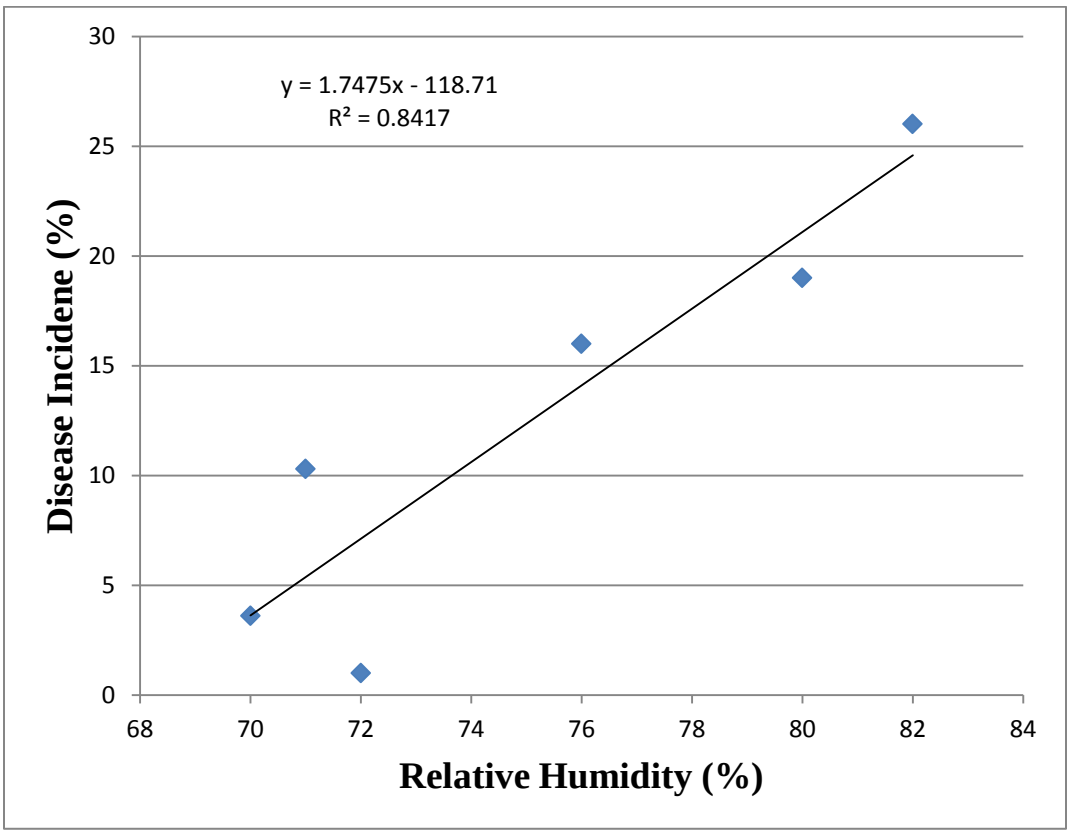

Figure 12. Linear regression analysis of the effect of relative humidity on the incidence of scab of lotkon.

4.4.2.c Relationship between scab disease incidence and rainfall

A positive correlation between scab disease incidence and rainfall was observed. The relationship between disease incidence and rainfall could be expressed by the equation Y=3.318x - 10.41(R^2=0.927), where x= rainfall and y=disease incidence.

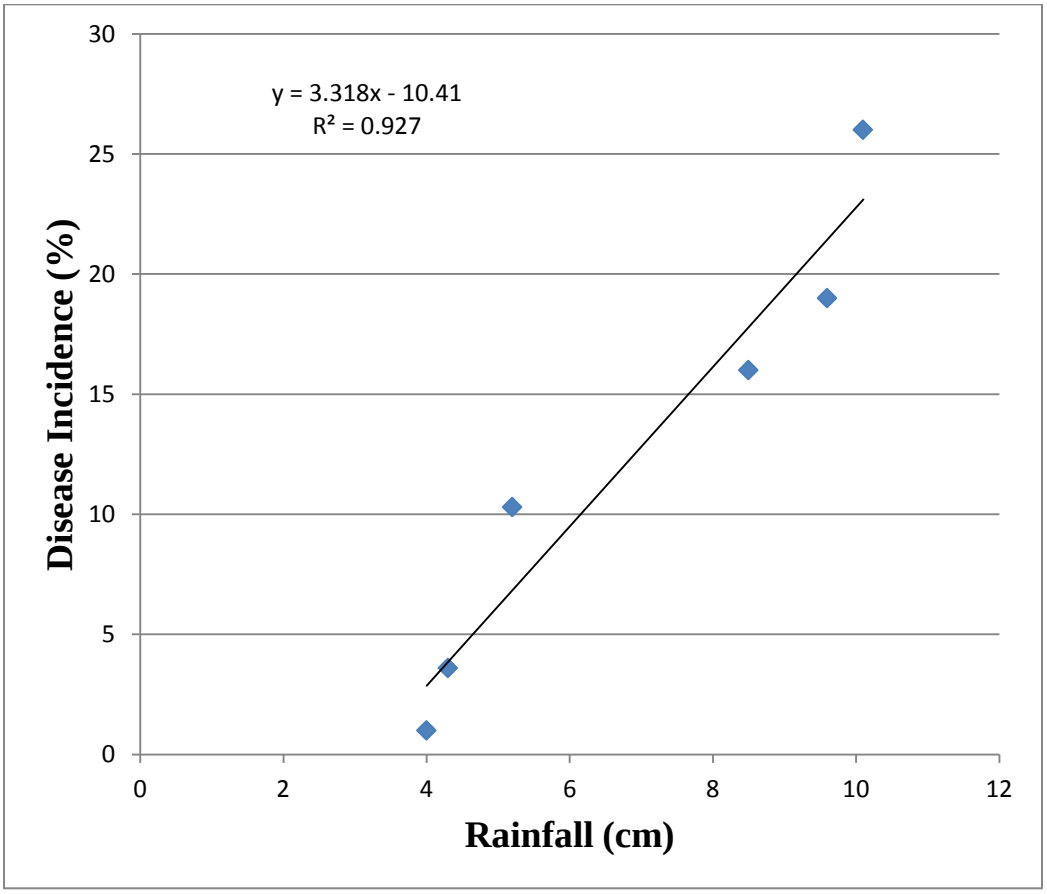

Figure 13. Linear regression analysis of the effect of rainfall on the incidence of scab of lotkon.

4.4.3.a Relationship between leaf blight disease incidence and temperature

A positive correlation between observed leaf disease incidence and temperature was observed. The relationship between disease incidence and temperature could be expressed by the equation Y=6.570x - 182.6 (R^2=0.975), where x= temperature and y=disease incidence.

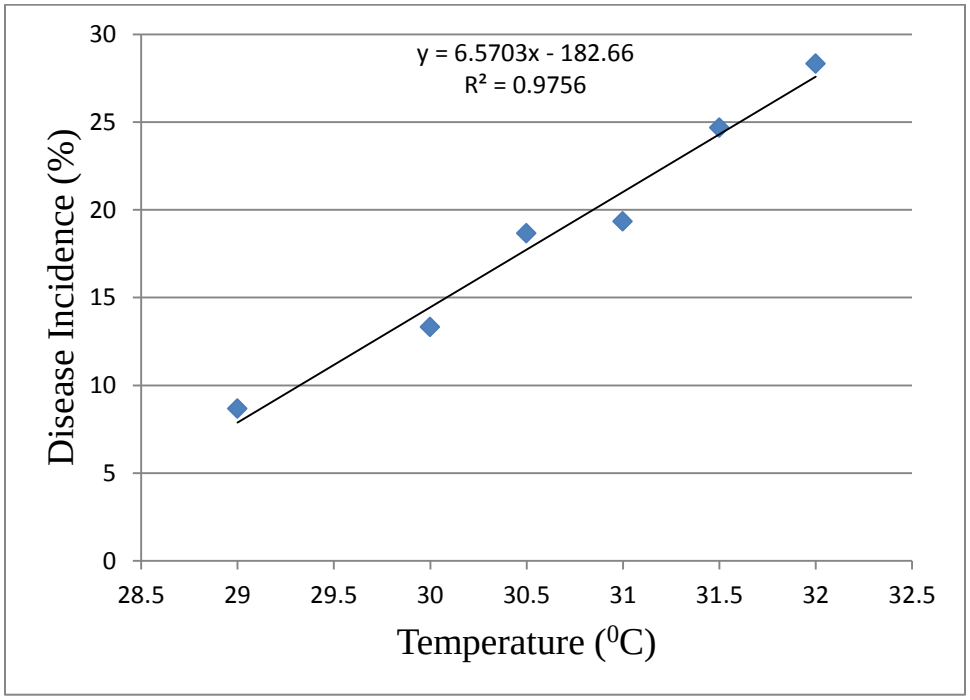

Figure 14. Linear regression analysis of the effect of temperature on the incidence of leaf blight disease of lotkon.

4.4.3.b Relationship between leaf blight disease incidence and relative humidity

A positive correlation between observed leaf disease incidence and relative humidity was observed. The relationship between disease incidence and temperature could be expressed by the equation Y=1.253x - 75.05 (R^2=0.744), where x= relative humidity and y=disease incidence.

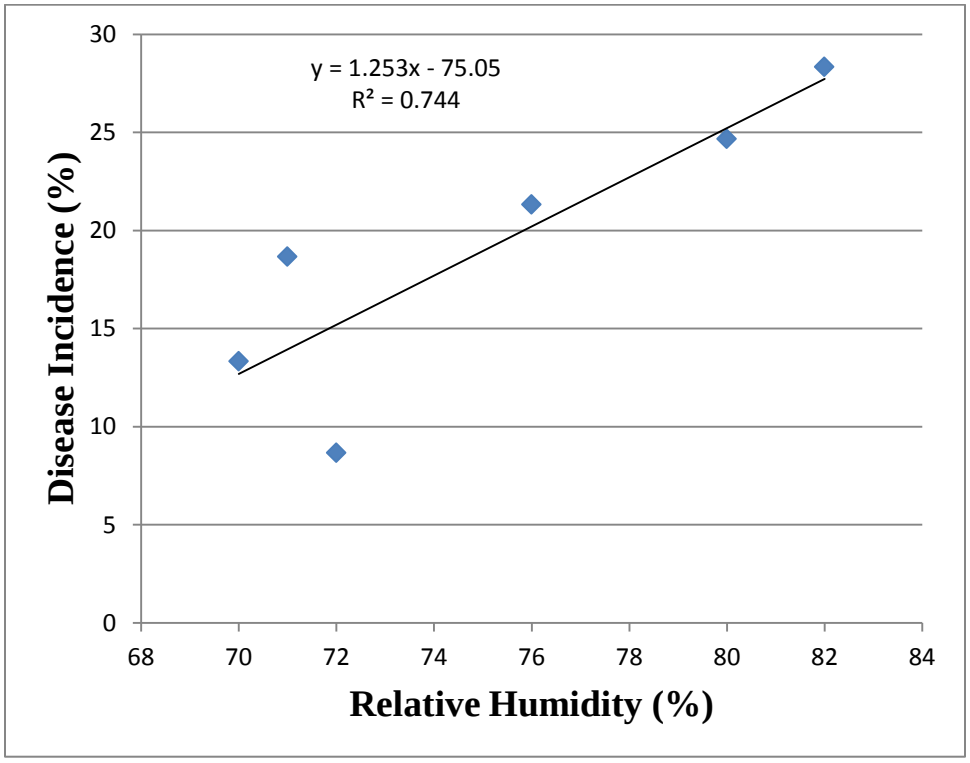

Figure 15. Linear regression analysis of the effect of relative humidity on the incidence of leaf blight disease of lotkon.

4.4.3.c Relationship between leaf blight disease incidence and rainfall

A positive correlation between observed leaf disease incidence and rainfall was observed. The relationship between disease incidence and rainfall could be expressed by the equation Y=2.467x + 2.016 (R^2=0.881), where x= rainfall and y=disease incidence.

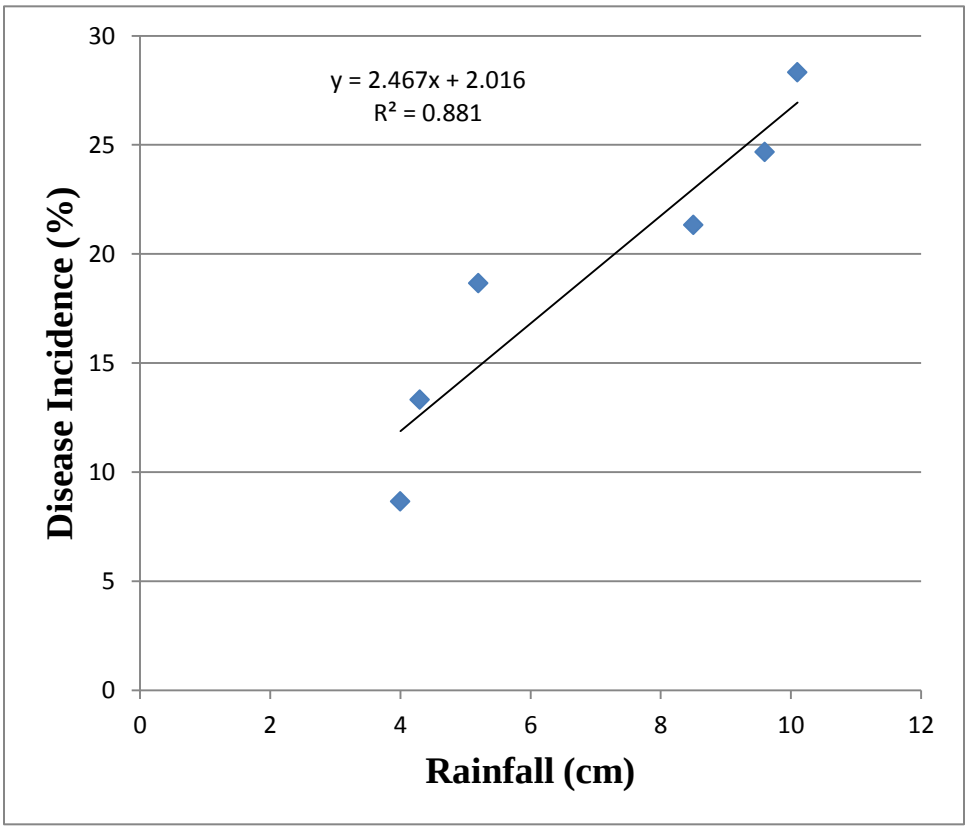

Figure 16. Linear regression analysis of the effect of rainfall on the incidence of leaf blight disease of lotkon.

DISCUSSION

In Bangladesh, Narsingdi district is considered as the main area for lotkon cultivation. The cultivation of the lotkon fruit (*Baccaurea ramiflora*) has been gaining popularity among farmers in Narsingdi. Lotkon is an under exploited fruit crop grown mainly in the background plantation (Deb and Bhowmik, 2013). Field and market survey conducted in the three upzilas of Narsingdi district and significant diseases namely, anthracnose, scab and leaf disease of lotkon were recorded. Research on diseases of lotkon is very meager. In the present study Anthracnose disease was recorded and *Colletotrichum gloeosporioides* were isolated from lotkon fruit. Association of C*olletotrichum gloeosporioides* in Anthracnose was reported by many researchers around the world (Chowdhury, 2009). Onyeani *et al.*, 2012 reported that over 30% of harvestable fruits are lost annually because of fruits abortion and abscission caused by Anthracnose disease and the fungus associated was *Colletotrichum gloeosporioides*. The study was carried out on 10th April, 2016 at 20 days after fruits set. The incidence and severity were observed six times throughout the fruit maturity process till 20thJuly, 2016. The highest disease incidence (24.30%) and severity (27.82%) of anthracnose on lotkon was observed at 120 days after fruits set during study. In a similar study on mango, Onyeani and Amusa (2015) showed that 60% of mango trees surveyed were found to be infected with anthracnose and over 34% of fruits produced on those trees were severely infected with the disease incidence (45.90%) and severity (38.10%) of anthracnose.In the study, of scab disease was recorded and *Venturea inaequalis* were isolated from lotkon fruit. Association of *Venturea inaequalis* causing scab in different fruits was reported by many researchers in around the

world (Khajuria *et al.*, 2012; Kimberly, 2011and Patzak *et al.*, 2011). The apple scab pathogen *Venturia inaequalis* cause extensive crop losses in all apple production areas (Kerik, 2012; Brun *et al.*, 2008). Apple scab, caused by the pathogen *Venturia inequalis*, is the most important disease involved in apple production across the world (MacHardy, 1996). In Indiana, the disease was responsible for yield reduction of nearly 50% in situations in which control measures were ineffective or absent (Suheri *et al.*, 1991). The highest disease incidence (26.0%) and severity (25.65%) of scab on lotkon was observed on 20th May after 60 days of fruit infection. In s similar study, the apple scab disease was observed with the highest disease incidence (71.90%) and severity (76.19%) in Uganda (Byarugaba *et al.*, 2013).

Determining the effects of temperature, rainfall and relative humidity on the incidence and severity of diseases in different pathosystems has been focused by many researchers worldwide (Rowe and Beute, 1975; Sutton, 19981; Rawal, 2005; Chowdhury, 2009 and Hossain, 2011). In the present study, a positive correlation was observed between prevalence of Anthracnose with temperature, rainfall and relative humidity. With the increase of temperature, rainfall and relative humidity both the disease incidence and severity increased significantly. Similar increase in incidence and severity were also observed with the increase of temperature, rainfall and relative humidity supported by previous workers (Tangonan, 2011; Tandon and Baligrami, 1957 and Chowdhury, 2009). Chowdhury (2009)reported that humidity and rainfall played more important role in the development of disease than by temperature. A positive correlation was observed between prevalence with temperature, rainfall and relative

humidity with the increase of temperature, rainfall and relative humidity both the disease incidence and severity increased significantly. The prevalence of scab of lotkon was observed in the month of April to July. The lowest prevalence (incidence and severity) observed in the month of April and the highest prevalence was observed in the month of July. In case of leaf blight disease, a positive correlation was observed between prevalence of leaf blight disease with temperature, rainfall and relative humidity. A positive correlation was observed between prevalence with temperature, rainfall and relative humidity with the increase of temperature, rainfall and relative humidity both the disease incidence and severity increased significantly. The lowest prevalence (incidence and severity) observed in the month of April and the highest prevalence was observed in the month of July.

CHAPTER V

SUMMARY AND CONCLUSION

Lotkon is very popular under exploited and background plantation fruit in Bangladesh. Although the total production of this fruit is very low but it is gaining popularity among the urban people of Bangladesh due to it's exceptional taste. Lotkon is one of the wonderful source of essential nutrients, minerals, vitamins. Consuming 100 gram of Burmese grapes offers 55 mg of vitamin C, 3.3 mg of iron, 10.5 g of carbohydrate, 0.7 g of Protein, 0.3 g of total fat and 2 mg of Calcium.

A field and post harvest survey was conducted from April to July, 2016 in Narsingdi district included three Upazilas namely Raipura, Shibpur and Belabo to identify the disease and isolation of casual agents of lotkon fruits. To measure the incidence and severity of lotkon diseases and observation of the nature of fruit damage with its yield loss. In the present study field survey was done by selecting six fields from three Upazilas (two in each Upazila) and was observed 20 to 25 lotkon trees in each field. In market survey, three markets namely Morjal, CNB and Belabo were selected and collected 1kg lotkon fruits each of five fruits sellers. The collected fruits from the sellers were used for the observation of disease symptoms (Amador, 2002 and Ferguson, 2002) and data collection (Rai and Mamatha, 2005). In the survey period disease incidence and severity data were collected. The observed diseases of lotkon were anthracnose, scab . In case of anthracnose of lotkon the disease incidence and severity were highest in the month of 20th July and lowest on 10th April. Similarly scab of lotkon disease, the highest incidence and severity were found on 20th May and the

lowest was on 10th April. For unidentified leaf disease, the highest incidence and severity were observed on 20th J

uly and lowest was on 10th April. The collected disease specimens were brought to the central laboratory of the Department of Plant Pathology in Sher-e-Bangla Agricultural University for the pathological test. In laboratory condition, tissue planting method and pure culture were done for the identification of causal organisms. The identified causal organisms were C*olletotrichum g sp.* for anthracnose, *Venturea inaequalis* for scab disease of lotkon. A positive correlation and regression analysis were observed in between the disease incidence and severity with the weather parameters viz. temperature, rainfall and relative humidity during the period of April to July, 2016 of the lotkon diseases.

From the finding of the present study, it may be concluded that the lotkon growers will get the valuable information about the diseases and it will be helpful to reduce the yield loss for the diseases of lotkon.

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APPENDIX

Table 1. Average temperature, relative humidity and rainfall of Narshindi district from 10th April to 20th July, 2016

Days of observation	Temperature (^{0}C)	Relative Humidity (%)	Rainfall (cm)
10th April, 2016	29	72	4.0
30th April, 2016	30	70	4.3
20th May, 2016	30.5	71	5.2
10th June, 2016	31.5	76	8.5
30th June, 2016	31.5	80	9.6
20th July, 2016	32	82	10.1