PREVALENCE OF FUNGI IN THE SEEDS AND SEEDLINGS OF BLACKBERRY

A THESIS
BY

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MASTER OF SCIENCE
IN
PLANT PATHOLOGY

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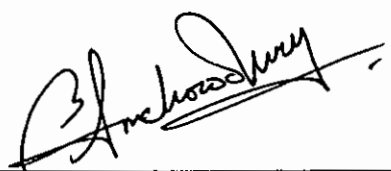

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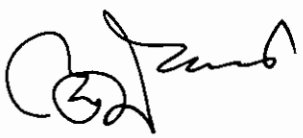

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This is to certify that the thesis entitled, **"PREVALENCE OF FUNGI IN THE SEEDS AND SEEDLINGS OF BLACKBERRY"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY**, embodies the result of a piece of bonafide research work carried out by **Nusrat Jahan, Registration No. 01520,** under my supervision and guidance. No part of the thesis has been submitted for any other degree in any other institutes.

I further certify that any help or sources of information, received during the course of this investigation have been duly acknowledged.

Dated:
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Dedicated to
My
Beloved Parents

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The author

PREVALENCE OF FUNGI IN THE SEEDS AND SEEDLINGS OF BLACKBERRY

ABSTRACT

Prevalence of fungi in seeds of blackberry plant collected from six different sources under Netrakona and Dhaka district were recorded. Seven different fungi, representing six genera, were detected in the seeds of blackberry. The seven seed borne fungi observed on blackberry seed , were *Pestalotia psidii, Curvularia lunata, Fusarium equiseti, Aspergillus flavus, Aspergillus niger, Rhizopus* sp. and *Penicillium* sp. All these seven fungi on the seeds of blackberry appear to be new records for Bangladesh. Prevalence of all these fungi varied significantly (P=1%) with respect to location. Pathogenicity test reveals that *Curvularia lunata, Pestalotia psidii* and *Fusarium equiseti* are pathogenic to blackberry tested in five selected nurseries in two different districts. In survey, four fungi species such as *Puccinia psidii, Pestalotia psidii, Fusarium equiseti* and *Curvularia lunata* were isolated from the leaves and stems of blackberry.

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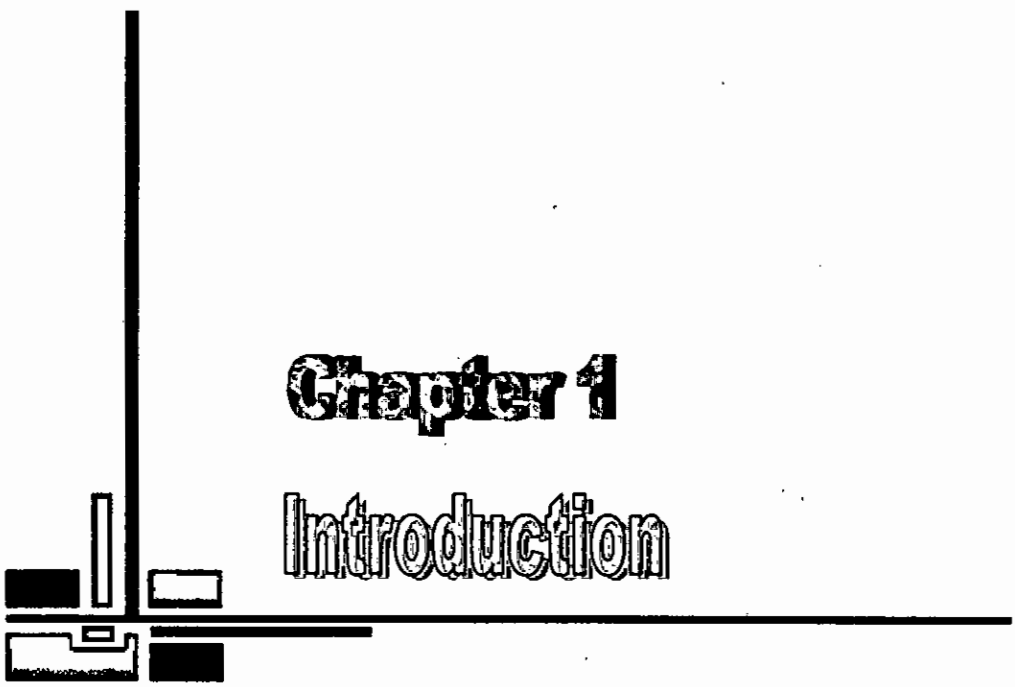


Chapter 1

Introduction

1. INTRODUCTION

Blackberry (*Syzygium cuminii*) belongs to the family Myrtaceae and is a native fruit of India, Burma, Srilanka, and Bangladesh. The tall tree with evergreen foliage is an excellent roadside tree and varies often used as wind break. It is widely grown both in north and south part of Bangladesh. Small dark purple colored fruits with sub-arid spicy flavor are eaten fresh.

There is a standard variety in blackberry. In north India, a type Known as "Ra Jamun" with big (2-5 cm long fruits) fruits are normally grown. A small (1.5-2.0 cm long and 1-1.5 cm. diameter) fruited type is also grown for late harvesting. A wide range of variability exists in blackberry and survey conducted (Keskar *et al.* 1989) in Pune and Ahmednagar districts in Maharashtra state revealed. The variation in fruit weight (3.5 to 16.5 g), pulp contents (54-85%), TSS (4.5 – 17%) and acidity (0.16 to 0.55%). Some promising lines have been selected. Blackberry has got various industrial uses too.

Bangladesh has the lowest per capita arable land due to its high population density. This limited land is inadequate for fulfillment of the requirements for the people. Therefore, much emphasis was not given on fruits production which is an important source of nutrition. The minimum dietary requirement of fruits per capita is 85 gm, whereas the availability is only 30-35 gm.

Fruits are important source of vitamins and minerals. The availability and consumption of fruits in Bangladesh are much less than it should be. Fruits are very costly and therefore, the majority of people cannot afford to buy them. As a result there has been a widespread malnutrition in the country. It may be over come by eating indigenous country fruits like blackberry, bel, amloki, guava, kamranga, jujube etc. It is less costly but rich of nutrition.

Bangladesh has been exporting fresh fruits since several decades. However, in the more recent years, export promotional activities for fresh fruits have received greater attention. The fresh fruits productions which were only 1631 metric tones in year 2002-2003 rose to 1772 metric tones during 2003-2004. According to Bangladesh Bourne of Statistics 2003-2004, per acre yield rate was 3306 kg.

It is said that a country needs at least 25% forest cover to maintain its ecological balance. But we have only 16% forest land. Among them actual free coverage of only 5.4% area (Amin, 1994). It is undoubtedly insufficient for ecological requirements. Blackberry may be introduced as agro forest tree. Being Bangladesh overwhelming with it booming population the forest area could only be increased through social afforestration, homestead gardening as well as intercropping.

Seed is the most important input for crop production. In modern agriculture seed health is a recognized factor for increased production. Pathogen free healthy seeds are considered as vital input for desired plant population and a good harvest. Many plant pathogens are seed borne which can cause enormous crop losses. Out of 16% annual crop losses due to plant disease at least 10% losses are incurred due to seed borne disease (Fakir, 1983).

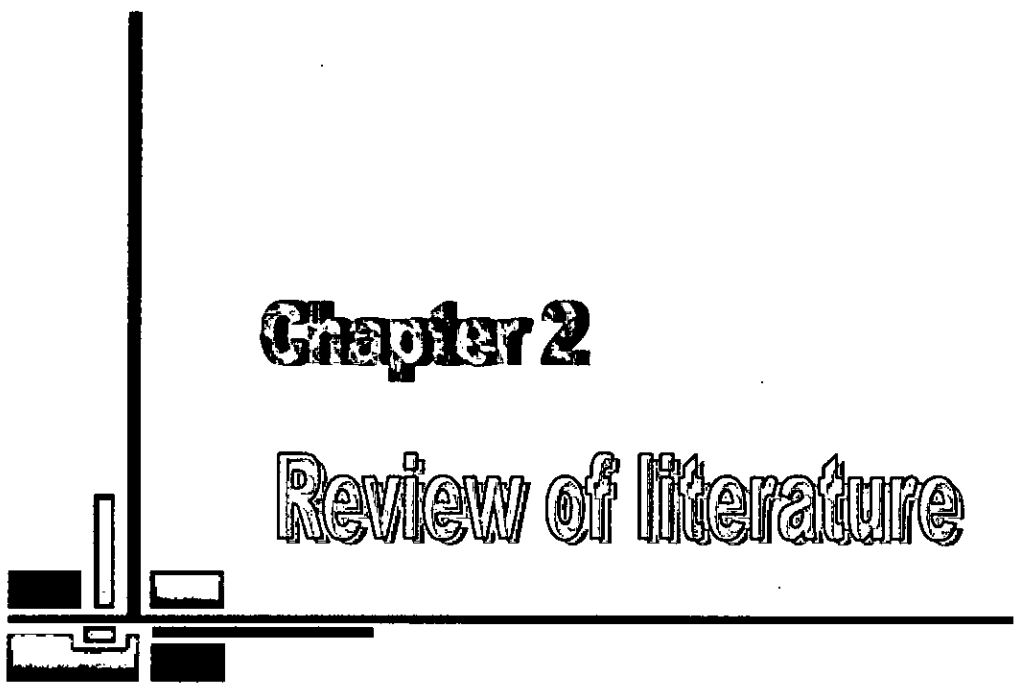
Along with blackberry, pineapple, turmeric, zinger etc, can be cultivated as a intercrop with blackberry without the addition of cultivated area, cultivation of blackberry at pond side and homestead areas might be important for increasing forest area minimizing the ecological imbalance as well as meeting the demand of food crisis. The woods are used to make furniture, spokes for wheels, arms for easy chairs, knees for all kinds of boats, beams for construction, frames for musical instruments (violins, guitars etc.) and packing cases. It is also popular for general turnery. It is not durable in the ground and is prone to attack by dry-wood termites. The tree grows rapidly after cutting to a stump and consequently yields a continuous supply of small wood for fuel. Blackberry and rose apple wood makes very good charcoal (Morton, J. 1987).

During the past two decades, research on diseases of fruits crops has made considerable progress. However, tremendous amounts of losses in yield and

quality of fruits caused every year dew to diseases incited by different plant pathogen viz. fungi, bacteria etc. In Bangladesh considerable amount of work has been conducted on determining the prevalence of seed born pathogen of cereals, vegetables and some fruit seeds but no work has been conducted on blackberry seeds and seedlings. The present study has been undertaken with the following objectives:

Objectives:

1. To study the prevalence of fungi in the seeds of black berry.
2. To determine the pathogenicity of the fungi associated with the seeds of blackberry.
3. To study the seedling disease (s) of blackberry.



Chapter 2

Review of literature

2. REVIEW OF LITERATURE

Blackberry is a popular fruit in our country. Millions of farmer plant blackberry in their homestead garden. The tropical weather is suitable for blackberry production. But the production qualities of blackberry are hampered due to different pathogens and diseases, especially, the seed borne ones. Pathogens attack seeds and leaves of blackberry. However, to the best of our knowledge, no work has been done in our country and least work has been conducted in abroad on the detection of seed borne fungi of blackberry. Therefore, reviews on similar work on blackberry and its relatives under same family pertinent to the present problem are presented below:

Diseases of blackberry

Gupta *et al.* (1955) stated that *B. theobromae* is one of the most serious pathogen of mulberry plant. It cause die-back and attack many plantation crops like pear, rose apple, mango, blackberry etc.

Edward *et al.* (1964) stated *Syzygium cuminii, Psidium cattleianum,* Chinese guava and wild Philippine guava were found to be resistant to *Fusarium* wilt of guava.

Morton (1987) reported that *Syzygium cuminii* is susceptible to several diseases like white spongy spot, leaf spot, leaf blight and lesions on leaves.

Ramaswamy *et al.* (1988) reported that *Pestalotia psidii* is the causal agent of guava canker. The pathogen is also isolated from infected coconut palm, mango, Jackfruit, blackberry and litchi in inoculation tests.

Ramaswamy *et al.* (1998) isolated *Pestalotia psidii* from infected guava, coconut, palm, mango, eucalyptus, achrous sapota, litchi. Inoculation tests confirmed *P. psidii* the pathogen.

Lyman *et al.* (2004) reported that fungus *Cercosporella rubi* is an important blackberry pathogen in the southeastern United States. This pathogen severely reduces fruit production and its management has been erratic due to a limited understanding of the host-pathogen relationship.

Diseases plants under same family of blackberry

Chattopadhyay *et al.* (1955) isolated both *Rhizoctonia solani* and *Fusarium solani* from diseased guava plants. The disease was considered a serious problem in commercial plantings. Other guava diseases reported from India were *Fusarium* wilt caused by *Fusarium oxysporium* f.sp. *psidii* and a condition characterized by intraveinal chlorosis and die-back of leaders associated with zinc deficiency.

Mathur (1956) reported one deficiency disease and a number of fungal diseases on common guava grown as a fruit crop in India. Wilting was first observed in Allahabad in 1935 and subsequently caused extensive damage in Uttar pradesh. Both *Cephalosporium* sp. and *Fusarium* were initially isolated from diseased plants, the latter was identified as *Fusarium oxysporiem* f.sp. *psidii*. A serious fruit canker caused by *Pestalotia psidii* was reported very common in several state of India. Canker or scab caused by *Glomerella psidii* was also reported.

Glasscock and Rosser (1958) observed that the seedling of *Eucalyptus Perrineana* and *E. gunnij* became severely affected by a species of oidium in July. Various kinds of Eucalyptus leaf spots due to *Cercospora epicocooides, Mycosphaerella molleriona* and *Readeriella onirabilis;* tar spots due to *Phytisma eucalypti* and rust caused by *Puccinia psidii* were reported.

Edward (1960) reported that *F. oxysporum* sp. *psidii* exists in a variety of clonal forms that differ in pathogenicity and in morphological and cultural characteristics. No correlation was observed between the cultural characters and pathogenicity.

Edward (1961) reported that wilt disease of guava caused by *F. oxysporium* f.sp. *psidii* was the most serious of the diseases to which guava is

susceptible. However, guava relatives, including *Psidium cattleinaum* var. *lucidum* and *Syzygium cuminii* reportedly had never been observed to be attacked by the disease. Inoculation experiments subsequently demonstrated that seedlings of *Syzygium cuminii* were immune.

Kapoor and Tandon (1970) isolated the fungus *Curvularia tuberculata* was reportedly newly from guava fruits stored in local markets in India. Previously *Curvularia tuberculata* had been reported as causing die-back disease of citrus in India. The disease on guava caused by the fungus appeared as a circular yellow spot, which later changed to brown and followed by decay.

Rahman and Zethner (1971) found considerable fungal damage of seeds of forest tress occurs in Bangladesh. They obtained 36 isolates of fungi from the seeds of eleven different forest tree species. Genera of fungi identified by them were *Acremonium* sp., *Penicillium* sp., *Fusarium* sp. and *Candida* sp. on *Syzygium grandies*.

Galli *et al.* (1980a) reported disease of *Psidium guajava* caused by *Puccinia guajava, Puccinia psidii, Colletotrichum gloeosporidies, Sphaceloma psidii, Pseudomonas* sp., *Phyllorsticta guajava.*

Galli (1980b) described diseases of *Psidium guajava* with emphasis on guava rust *(Puccinia psidii)* in Brazil. He also mentioned fruit diseases

caused by the fungi *Phyllosticta* sp., *Colletotrichum gloeosporidies, Sphaceloma psidii, Pseudomonas* sp., *Phyllorsticta guajavae*

Pandey *et al.* (1984) studied the fungi colonizing the phylloplane (leaf surface) and internal tissue of guava leaves from bud stage to senescence in summer, rainy and winter seasons in India. The total numbers of fungi were highest in the rainy season and lowest during the summer. Fungi were categorized into 3 groups, those exclusively seasonal *Pestalotia psidii, Fusarium oxysporum* f.sp. *Psidii* and *Colletotrichum gloeosporioides* were isolated from the foliage in different seasons in different levels of dominance. The number of phylloplane micro flora increased with leaf age.

Adisa (1985) conducted a survey on guava fruit rot diseases in 16 locations of Nigeria. Two types of rots, soft rot and dry rot were recorded. *Aspergillus niger, Rhizoctonia solani, Colletotrichum gloeosporioides, Botryodiplodia theobromae, Erwinia* sp., *Rhizopus stolonifer, Rhizopus oryzae* were established as soft rot organisms. While *Penicillium* sp., *Fusarium equiseti* and *F. oxysporium* were established as dry rot organisms.

Kuthubutheen *et al.* (1988) encountered species of *Collectotrichum, Pestalotiopsis, Fusarium, Botryodiplodia* on healthy leaves of several fruit trees and these fungi were all capable of lesion formation and subsequent leaf damage.

Pandey (1990) studied the mycoflora of common guava. He found the leaves were colonized by a limited range of fungi such as, *Alternaria alternata, Aspergillus niger,* pink yeasts and white yeasts. Four consistently pathogenic species: *Colletotrichun gloeosporioides, Fusarium oxysporium* f. sp. *psidii, Pestalotia psidii,* and *phoma psidii* were consistently present during all seasons.

Jan *et al.* (1991) isolated the *Macrophonina phaseolina* (Tassi) Goid. On guava fruists from orchards. Pathogenicity was confirmed by inoculations of guava fruits.

Hossain *et al.* (1992) surveyed the prevalence of guava fruit anthracnose in 3 major guava producing areas of Bangladesh during 1987-1988. All plants and 90-100% of fruits surveyed were severely diseased. *Pestalotiopsis psidii, Glomerella cingulata,* and *Botryodiplodia theobromae* were isolated from infected fruits and pathogenicity was confirmed.

Hossain and Meals (1992) monitored the prevalence of guava anthracnose in Chittagong, Barisal and Mymensingh during 1987-1988 and found 100% plant infection and 90-100% fruit infection.

Dwivedi *et al.* (1994) reported that disease incidence in guava orchards ranged from 3.9 to 30%. Symptoms included defoliation, die-back and bark cracking. Among the associated pathogens *F. solani, F. oxysporum* and *F.*

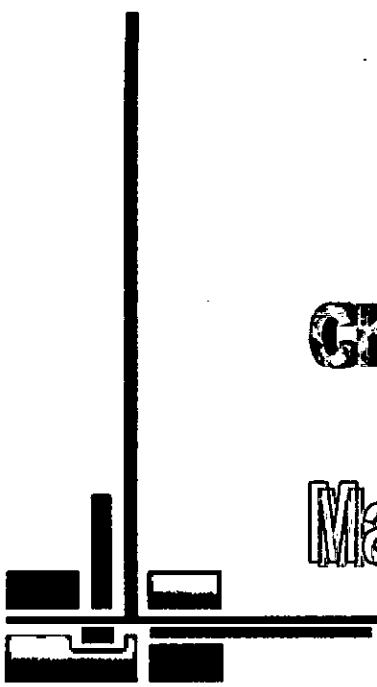
oxysporum f.sp. *psidiis* predominated in all seasons. Accompanying saprophytes included *Alternaria alternata, A. flavus, A. niger, Penicillium citrinum, Trichoderma harzianum* in summer, while *A. luchuemis, Humicola* sp. and *F. solani* were dominant in rainy and winter seasons. Wilted twigs yielded isolates of *Cunninghamella, Fusarium* sp and *Macrophomina phaseolina,* while roots yielded *F. solani, F. longipus, F. monilifarmae, F. oxysporum* f. sp. *psidii* and *M. phaseolina.* The maximum soil temperature (43°C) was recorded in the summer while the organic matter content of the soil was highest (2.1%) in the rainy season.

Alahakoon and Brown (1994) isolated *Colletotrichum gloeosporioides* from 23 fruit crops in Srilanka. This was the first record of *G. eingulata* on durian, mangostein, pini jambu (*Syzygium jambos*), trees in Srilanka. Symptoms of infection by *G. cingulata* on fruit tree seedlings especially mango and rambutan which can cause up to 40% loss of planting stock were previously believed to be a physiological disorder.

Smith *et al.* (1998) observed die-back of eucalyptus species, clones and hybrids during survey of forest plantations in the Mpumalanga and kwazulu-Natal provinces, South Africa. This symptom was often associated with environmental stress (drought, frost and hot winds). *Botryodiplodia dothidea* was frequently isolated from twigs showing die-back symptoms. In some

cases, *C. gloeosporioides* was also isolated. Artificial inoculations of 3 years old clones of *E. grandis* with both fungi resulted in lesion development.

Coutinho *et al.* (1998) describe the symptoms of the disease. He stated the disease first begin as tiny bright yellow powdery eruptions in a circular pattern on the leaf or stem surface. These infection loci or spots expand and become necrotic, and spread over the entire leaf, stem or shoot. Leaves and stems can be deformed by the disease, and growing tips can die back if the infection is severe.



Chapter 3

Materials and methods

3. MATERIALS AND METHODS

3.1. Experimental site and period

The laboratory experiments on the detection of fungi on blackberry seeds were conducted in the Plant Pathology laboratory of Sher-e-Bangla Agricultural University, Dhaka, and Plant Pathology Laboratory, Bangladesh Agricultural University, Mymensingh during the period May to August, 2006.

Two places were selected for survey to determine the disease of blackberry. The places were Mymensingh sadar in the district of Mymensingh and Netrakona sadar in the district of Netrakona. Five nurseries were selected from the two places for survey. The nurseries are-

Nursery and total number of plants served

District	Nursery	Total no. of plants surveyed
Netrakona	1. Bono Bevagh nursery	2910
	2. Sotabdhy nursery	2574
	3. Mohammed nursery	1500
Mymensingh	1. Mamun nursery	1500
	2. Tulip nursery	1000
Total		9984

3.2. Collection of seed sample

Seeds of blackberry species were collected from Netrakona and Dhaka districts. Three sources were included from each district for seed collection. In Dhaka, DAE Horticulture center, Asadgate, karwan bazer and Tongi bazer were selected for seeds collection. And in Netrakona seeds were collected from the Government nursery, Choto bazer and Modonpur. Totally 800 seed samples from the two selected seed sources. Seeds were collected during May- June, 2005 and 2006. After collection of seeds the samples were plated in the laboratory for the study.

The seed sources are presented bellow

District	Source
Netrakona	1. Government nursery 2. Choto bazer 3. Modonpur
Dhaka	1. Karwan bazer 2. Tongi bazer 3. Asadgate nursery

3.3. Inspection of dry seed

Dry Inspection of seeds were done, 4 hundred seeds of each district were taken randomly and grouped into two categories viz. I. healthy seeds II. Discolored and calculated the percentages of each group (Photo.-1).

3.4. Incubation test

3.4.1. Blotter method

The seed samples were tested by blotter method for the presence of the seed-borne infection of fungi following the International Rules for seed health testing (ISTA, 1999). In this method, three pieces of whatman no. 1 filter paper were soaked in sterile water and placed at 9 cm petridishes. In another method, the seeds were treated with Mercuric chloride and Alcohol for 30 seconds then wash three times under running fresh water and then plated on the wet filter paper in the petridishes. Seeds were plated at the rate of 9 seeds per petridish. In this way 36 seeds in 4 replicates were plated per sample. The Petridishes, with the seed plated were incubated at 22 ± 2°c under 12/12 hours alternating cycles of NUV and darkness in the incubation room of the seed pathology laboratory for 20 days. After incubation, the plates were examined under stereomicroscope (Photo.-2).

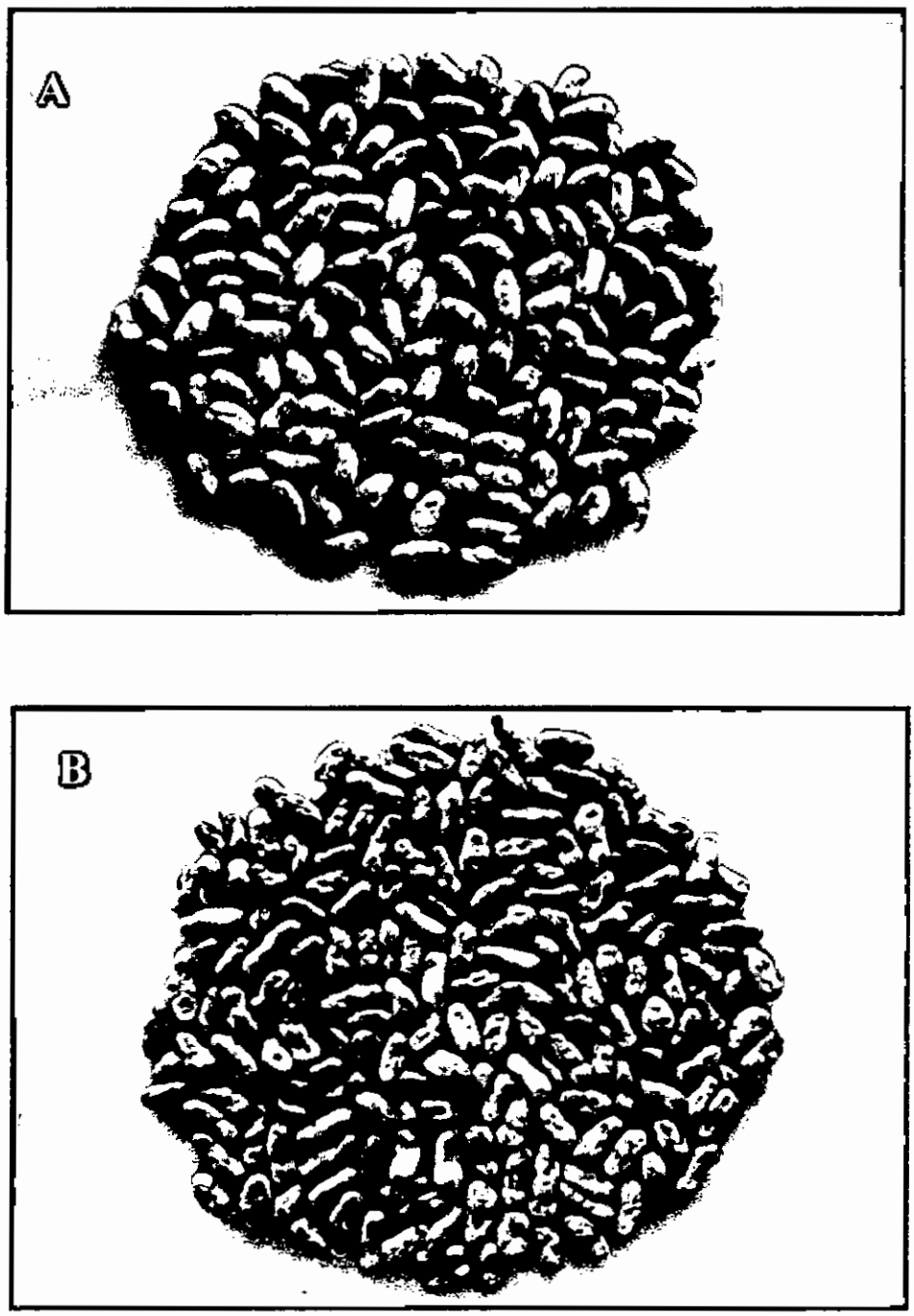
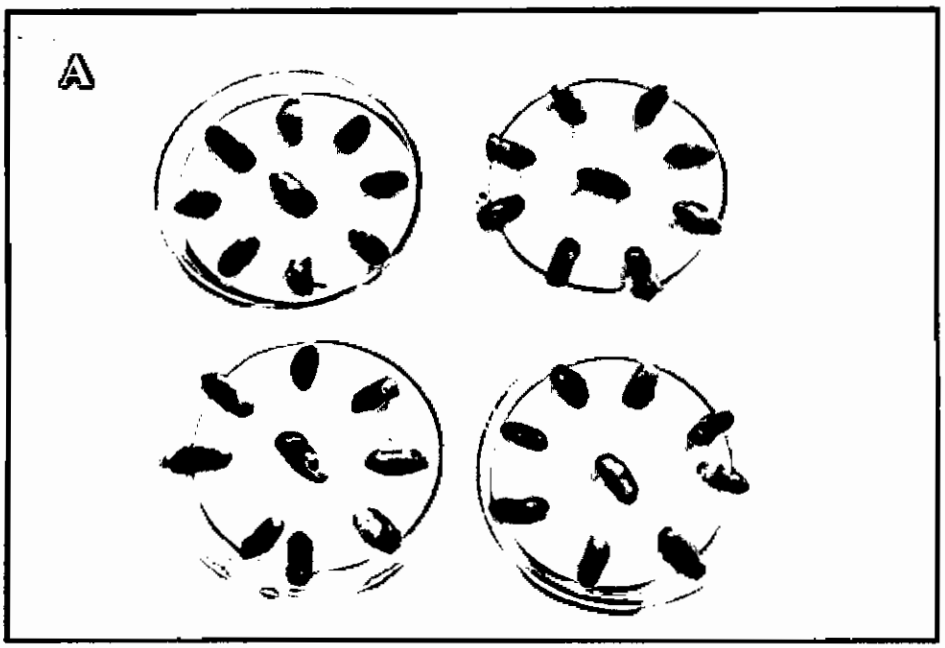


Photo. 1. A. Healthy seeds of blackberry
B. Infected, shriveled and dead seeds of blackberry



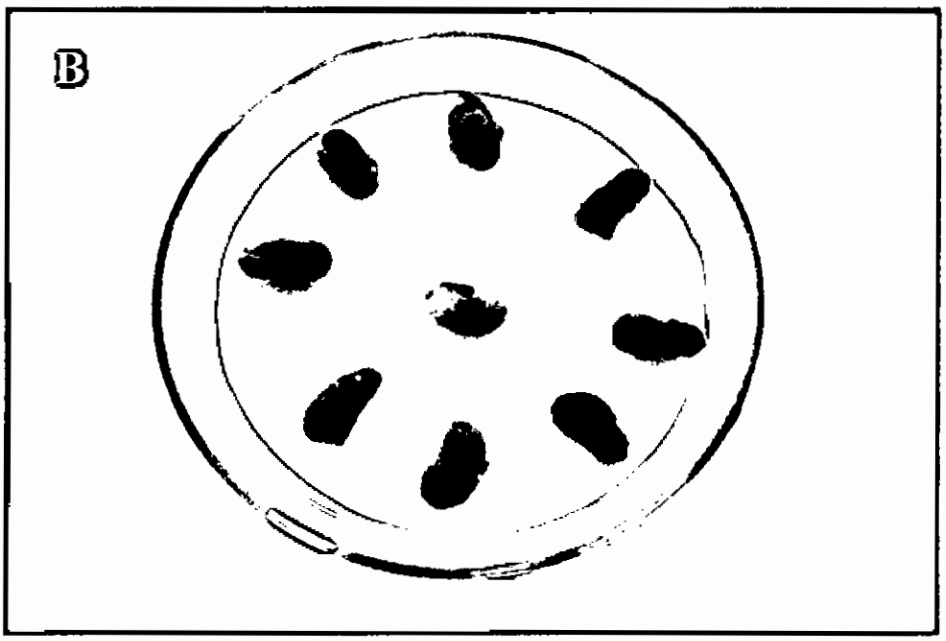


Photo. 2. A, B. Dishes ready to examine under a stereo-microscope

3.4.2. Sterilization of incubating papers and dishes

Often contaminants interfere with the detection of seed borne pathogen in blotter technique. Filter paper and petridishes were sterilized by autoclave. Whatman no. 1 filter paper and glass petridish were packed in sterile condition for future use. Germination percentage and presence of major pathogens were recorded following the procedures described in 3.4.1.

3.4.3. Pre treatment of seeds

Seeds were pretreated by soaking in 0.05% Hg_2cl_2 for 30 second. And in another experiment the seeds were pretreated by 70% ethyl alcohol. Seed borne infection by the major seed borne pathogen and germination were noted following the procedures described in section 3.4.1.

3.4.4. Agar plate technique

In agar plate technique, Potato Dextrose Agar (Potato 200gms, Agar- 15 gm and H_2O 1000 ml) having pH 6.5 was used. About 15 ml of the media were poured in each sterilized glass petridish. Controlled seeds were plated. Then, Hg_2cl_2 0.05% and alcohol 70% were used to pretreated the seeds. Seeds were dipped in each concentration of chemicals for 30 seconds. The pretreated seeds were plated in the petridishes. All the petridishes were kept in the glass chamber for avoiding contamination.

3.5. Isolation and identification of fungi

The fungi associated with seeds, seed coat and cotyledons were isolated in pure form and grown on the acidified PDA. Seed borne infections of fungi observed under the stereo microscope and identified by measuring their growth characters on the incubated seeds. The fungi were identified to species level, wherever possible, following the keys of Malone and Muskette (1964), RamNath *et al.* (1970), Booth (1971), Ellis (1971), Barnette and Barry (1972), Mathur and Kangsdal (2003). Identification of pathogen was done by preparing slides and examined them under the compound microscope.

3.6. Germination test

The germination test was carried out by blotter method, plastic tray and agar plate method. In blotter method, two layer of whatmen paper 1 was used. 9 seeds were plated in each petridish. In case of agar plate method, PDA media was prepared and then media and glass petridishes were sterilized in the autoclave. 15 ml PDA media poured in each the petridishes. Then 9 seeds were plated in each dish. Germination test was also determined in soil on plastic tray measuring 1 × 2 ft. The soil was collected from the field laboratory of the department of plant pathology. SAU, Dhaka. 50 seeds were sown in each tray. 3 plastic trays were used for this purpose. Data on

germination test were recorded after 7, 15 and 21 days of sowing. After 21 days of sowing, data of germination test was taken for final count.

3.7. Pathogenicity test

3.7.1. Pathogenicity of the causal organism

Pathogenicity of causal organism was conducted in seed pathology laboratory of department of plant pathology, Bangladesh Agricultural University, Mymensingh. Young leaves of the blackberry seedlings included for pathogenicity test of the fungus.

3.7.2. Fungi included

Pathogenicity test was conducted with the three fungi isolated from seeds of blackberry, collected from two locations. The fungi used for inoculation were *Curvularia lunata, Pestalotia psidii* and *Fusarium equiseti.* The fungi *Aspergillus niger, Aspergillus flavus, Penicillium* sp. and *Rhizopus* sp. were not included in the pathogenicity test because they are predominantly saprophytic and storage fungi. The three fungi were included for the pathogenicity test because they were frequently associated with incubated seeds in the blotter test.

3.7.3. Inoculation of leaves

3.7.3.1. Raising of seedling

Leaves of two months old seedlings of blackberry were inoculated by the test fungi in order to determine the pathogenic potentiality of these fungi. Seedlings were grown in earthen pot filled with soil. Four seeds were sown per pot. There were eight replications. At two months of rising seedling one were uprooted and the rest were kept for experiment. Watering and necessary aftercares were taken throughout the growing period of the seedlings during the experiments.

3.7.3.2. Techniques used

Leaves of two months old plant (at the stage of five to six leaves) was chosen for inoculation in pathogenicity test. The leaves were washed with sterile water and then taken in glass house. Inocula of the test pathogen were prepared for inoculation. During inoculation selected parts of leaves were first 5 pricked with fine sterile needle. And then 5 mm block of 7-8 days young culture of the test pathogen was attached in the pricked zone. Then the spot was wrapped with wet cotton pad. The inoculation was made with injury. For development of leaf spot, some leaves were inoculated at a point 1 cm away from the tip. 2 plants per pot were inoculated and one plant kept

for control. The experiment was conducted in the glass house of the seed pathology laboratory in BAU, Mymensingh.

After inoculation, both the inoculated and control pots were kept at chamber. After 48 hours, the wet cotton pads were removed. During incubation, the leaves were examined for disease development from time to time.

3.7.3.3. Isolation and purification of fungi

The three fungi namely *Curvularia lunata, Fusarium equiseti* and *Pestalotia psidii* were included for the test. These fungi were obtained from the infected leaves after inoculation. The fungi growing on the leaves were transferred to PDA plates. Pure culture of each of the test fungi was prepared by single hyphal tip transfer method as followed by Riker and Riker (1921). The transferred single hyphal tip of the each test fungi was allowed to grow on PDA at 22±2°C for seven days.

3.8. Method of survey

Each of the selected nurseries was inspected at seven days interval for three months. This was done in the months of June, July and August in the year of 2006. In each nursery 1000 seedlings of blackberry were randomly selected for observation. The total number of affected plants in each nursery

by each disease was recorded. Since leaf diseases were found in a few numbers through the seedlings.

In the field different diseases were recognized by visual observation of the symptoms. Then the samples of diseased specimens were brought to the laboratory for isolation of the causal organism and confirmation of the diseases (Photo.-3).

3.8.1. Symptom observation

In the five selected nursery more or less similar kinds of diseases were observed viz. leaf spot, leaf blight and leaf rust.

The characteristics symptoms of leaf spot of blackberry were examined in details on infected leaf with naked eyes. The symptoms were also studied by hand lens or under a low power stereo-binocular microscope to observe the presence of signs of the pathogen.

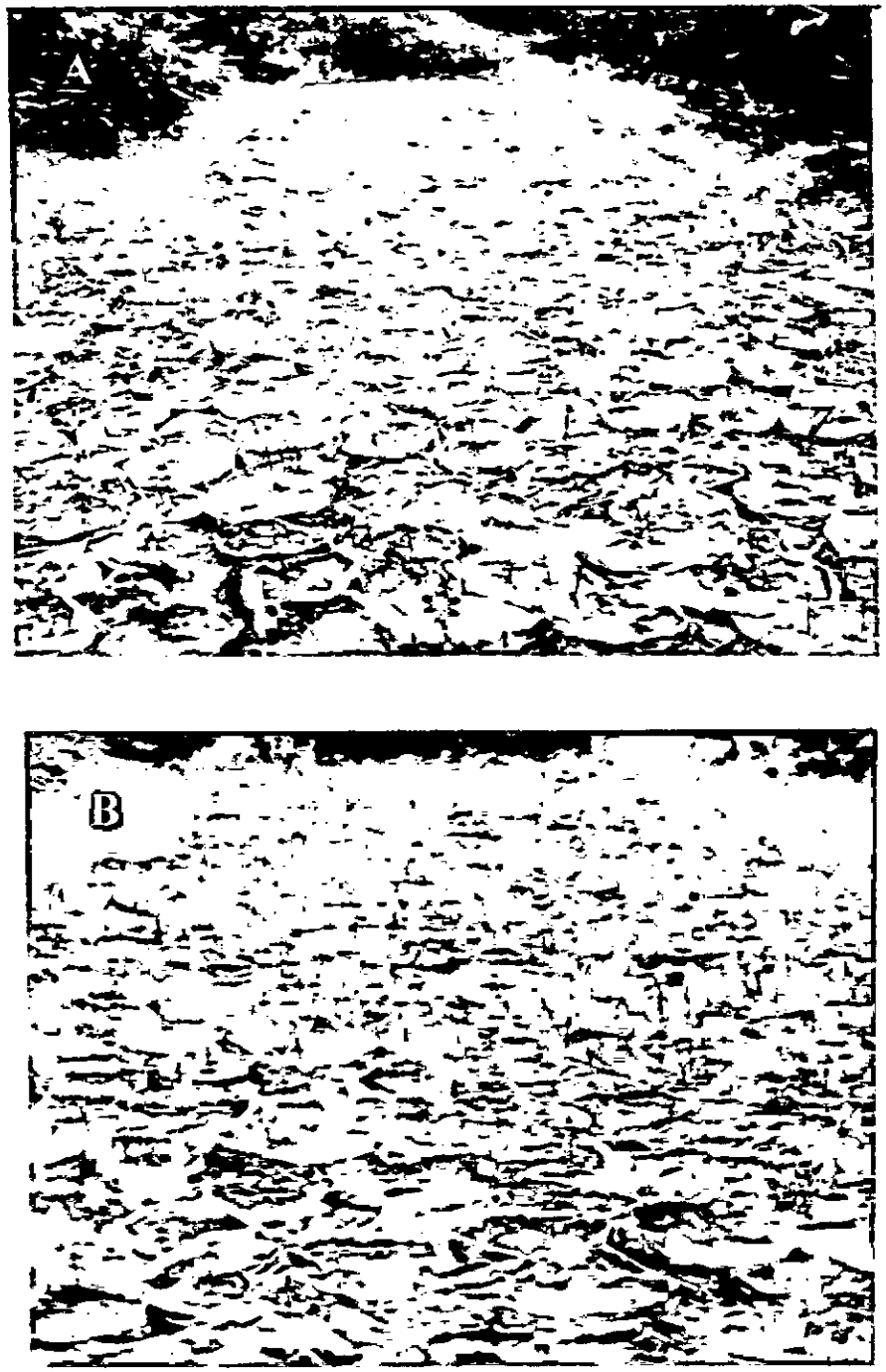


Photo. 3. A. A nursery survey in Netrakona (Sotabdy nursery)

B. A nursery survey in Mymensingh (Tulip nursery)

3.8.2. Collection, isolation, purification and identification of causal organisms

3.8.2.1. Collection of diseased specimen

Diseased leaves were collected from the infected plants representing the different area of survey. The specimens were preserved in the laboratory following standard procedure until isolation was made.

3.8.2.2. Isolation of causal organisms

Isolation of causal organism was done by tissue planting method. Diseased samples were collected for isolation of the causal fungus. The tissue approximately 1.5 cm length and 1cm in width were cut out of the infected leaf area. Then the inocula were washed thrice in sterile water. The inocula were then placed on acidified potato dextrose agar media. The Petri-dishes containing the inocula were incubated at room temperature (24±2°C) for several days and examined daily for any fungal growth.

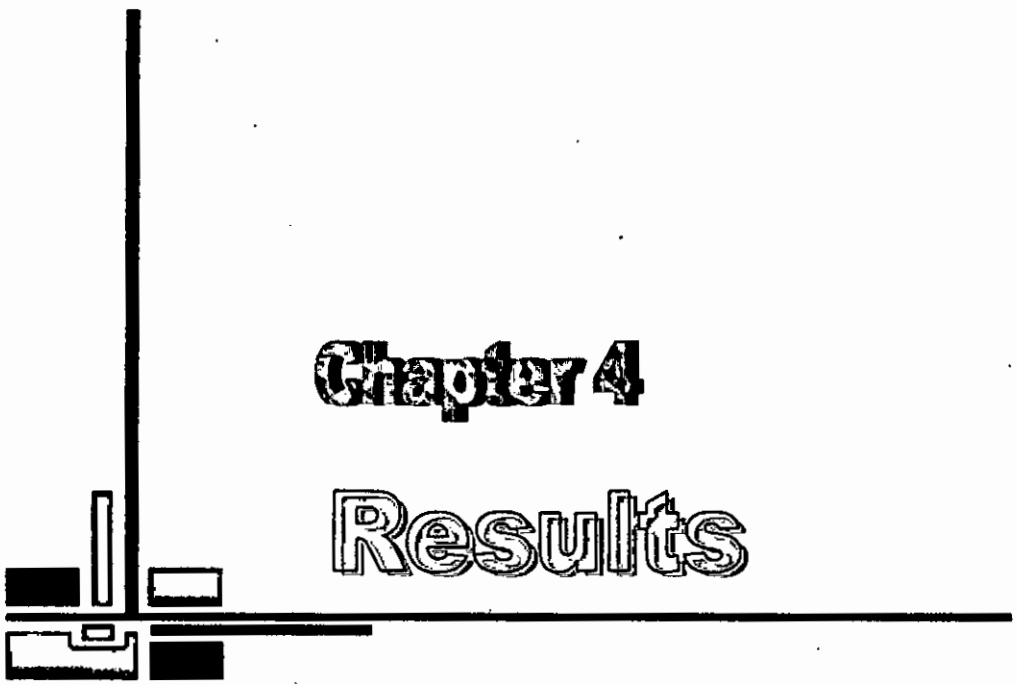
3.8.2.3. Purification and identification of causal organisms

The fungi which grew out from the inocula were transferred to fresh culture plates. The sub-cultures were made into PDA and purified the pathogen by transferring hyphal tip. Morphological characters of each colony were studied on PDA. The fungus was identified by observing

colony characters, linear growth, color and speculation (Arx, 1970; Kulshrerthe *et al.* 1976; Sutton, 1980).

3.9. Statistical analysis

The data were analyzed following the Completely Randomized Design (CRD). The mean differences for efficiency of the treatments were judged by least significant difference (LSD) test.



Chapter 4

Results

4. RESULTS

4.1. Inspection of dry seeds

The results of dry inspection of seeds, that were collected from two different sources viz. Dhaka and Netrakona districts. The results of dry inspection of seeds are presented in Table-1.

It was observed that the two categories of seeds viz. i. apparently healthy seeds ii. Discolored and shriveled seeds ranged from 3% and 8.5% in Netrakona and Dhaka district, respectively. The percentage of apparently healthy seeds was the highest in Netrakona (87%) and lowest in Dhaka (71%) districts.

4.2. Germination test

The germination test was carried out by blotter incubation test, agar test and pot soil test. The germination of blackberry seeds was the highest in pot soil which was 75% and 70% respectively in Netrakona and Dhaka districts. The lowest germination was recorded in agar media 25% and 22%, in Netrakona and Dhaka, respectively (Table-2).

Table -1. Dry inspection of the selected seed of blackberry

Source of Seed	Dry infection (%)	
	Healthy seeds	Shriveled seeds
Netrakona	87.00	3.0
Dhaka	71	8.5

Table-2. Germination percentage of the selected seeds observed in different tests.

Source	Germination %		
	Pot soil	Agar	Blotter
Netrakona	75	25	68
Dhaka	72	22	65

4.3. Prevalence of fungi

4.3.1. Total seed borne fungal infection

In blotter method, out of 800 seeds, a total 183 seeds were found infected by fungal pathogens. The prevalence of total seed borne fungal infections varied significantly in respect of sources of collection of six samples from different location.

In general, more seed borne fungal infections 50% were observed in seeds collected from Dhaka district compared to Netrakona 30%.

In Netrakona, three sources were selected for seed collection. Among them the highest numbers of *Aspergillus flavus* were observed in seeds collected from forest nursery (7%) and the lowest was *Curvularia lunata* (0.0%). The highest *Rhizopus* sp. (8.1%) was recorded in the seeds collected from Choto bazer and *Penicillium* sp. was the lowest (0.0%) in number. The presence of *Pestalotia psidii* was also lowest (0.0%) in the seeds collected from Choto bazer. The infections of fungi in the seeds of Modonpur were *Aspergillus niger* (6.9%), *Aspergillus flavus* (6.4%), *Rhizopus* sp. (6.1%), *Curvularia lunata* (3%), *Pestalotia psidii* (2.2%) and *Penicillium* sp. (3%). In Dhaka, accept *Aspergillus niger*, the highest number seed borne fungal infections were recorded in the seeds collected from karwan bazer. The highest *Aspergillus niger* (8%) found in the blackberry seeds collected from

Tongi bazer. *Aspergillus flavus* (6.9%) *Aspergillus niger* (6.5%), *Fusarium equiseti* (1%), *Rhizopus* sp. (2%), *Pestalotia psidii* (3.2%), *Penicillum* sp. (0.0%) and *Curvularia lunata* (0%), respectively observed in the seeds collected from Asadgate (Table-3).

4.3.2. Fungi identified and their frequency of occurrence in Netrakona District

Out of 74 seed borne fungal infections, 6 species of fungi representing, 5 genera were identified. The identified fungi were *Pestalotia psidii* (Photo.-5B, 5C, 7) *Fusarium equiseti* (Photo.-5A, 8A), *Aspergillus flavus*(Photo-4C), *Aspergillus niger* (Photo.-4B,8B), *Curvularia lunata* (Photo.-4A,6), *Rhizopus* sp. (Photo.-8C). *Penicillium* sp. (Photo. - 9B). Of all these fungi, most predominant fungi were *Aspergillus niger* (29.72%), followed by *Aspergillus flavus* (25.6%), *Rhizopus* sp. (21.62%) and *Fusarium equiseti* (10.81%).

The *Curvularia lunata* had the lowest (4.05%) occurrence and the *Aspergillus niger* was the highest (29.72%) once. *Penicillium* sp. was not found in the seeds of blackberry in Netrakona (Table-4).

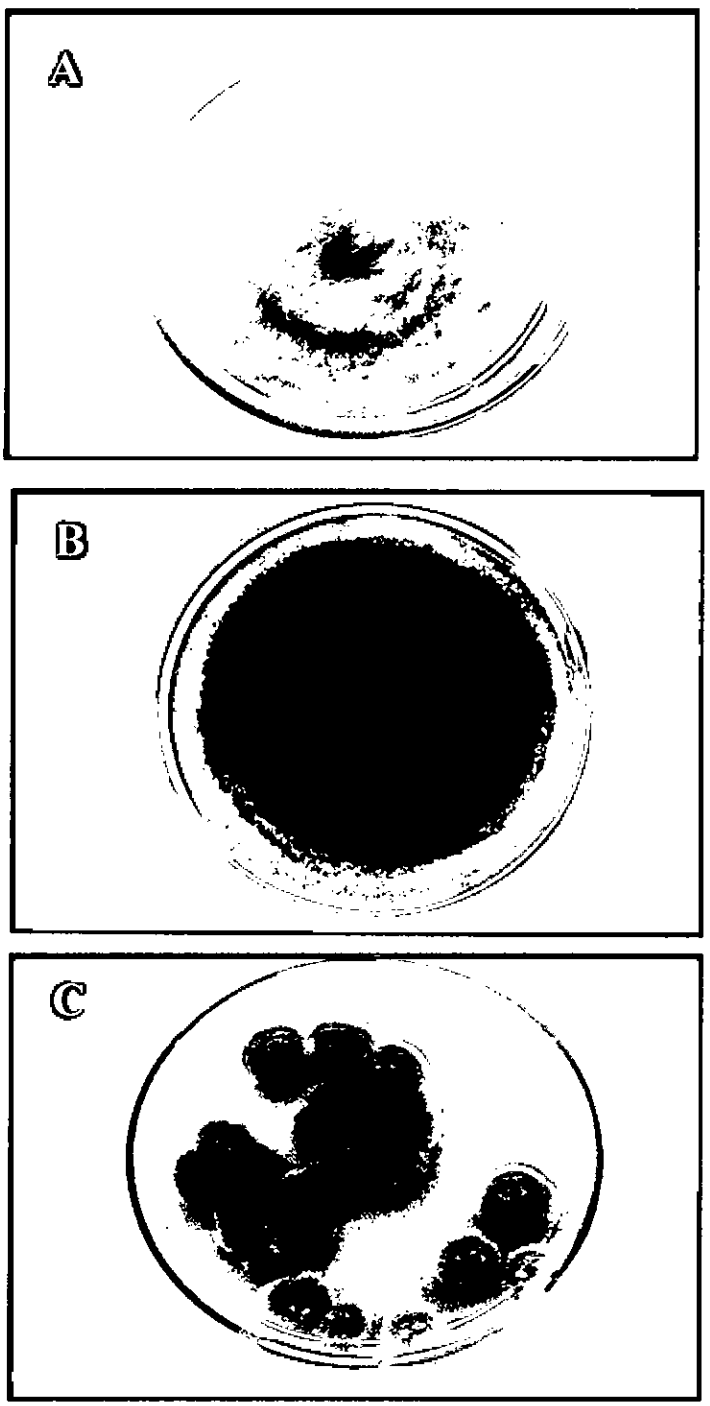


Photo 4. A. Pure culture of *Curvularia lunata* of blackberry seeds on PDA (40x)
B. Pure culture of *Aspergillus niger* of blackberry seeds on PDA (40x)
C. Pure culture of *Aspergillus flavus* of blackberry seeds on PDA (40x)

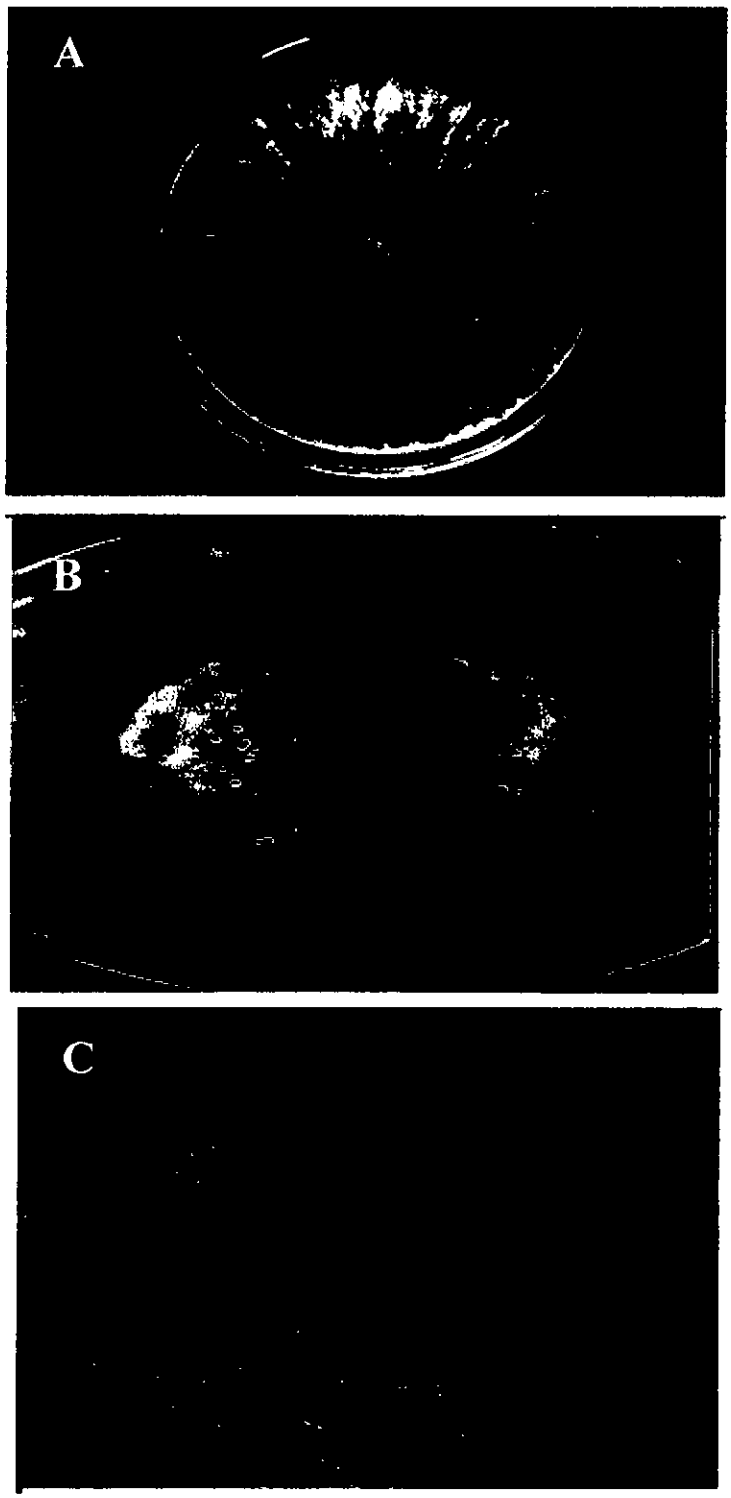


Photo. 5. A. Pure culture of *Fusarium equiseti* of blackberry seeds of PDA (40x)
B. Pure culture of *Pestalotia psidii* of blackberry seeds of PDA (40x)
C. Mature fruiting bodies of *Pestalotia psidii* of blackberry seeds of PDA (40x)

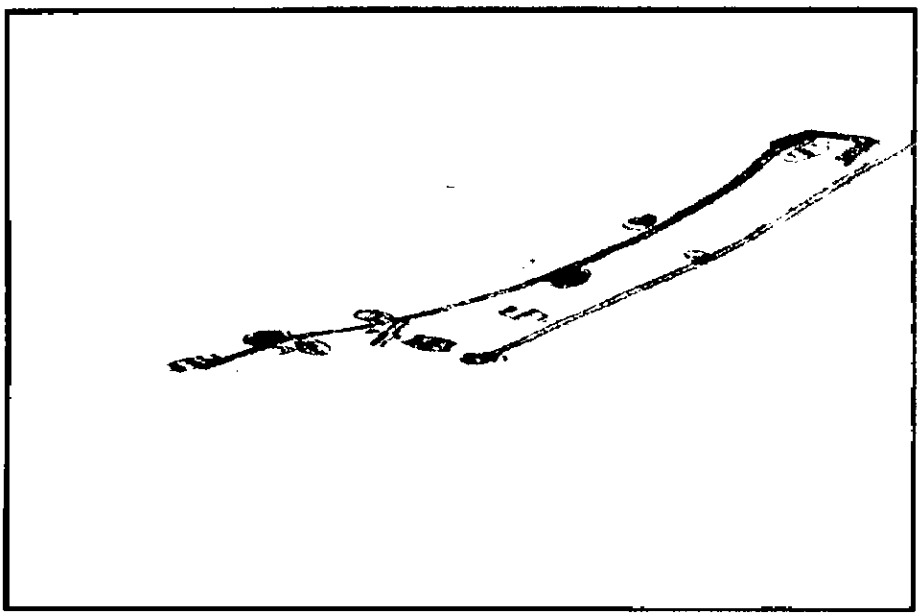


Photo. 6. Conidia of *Curvularia lunata* seen under microscope (400x)

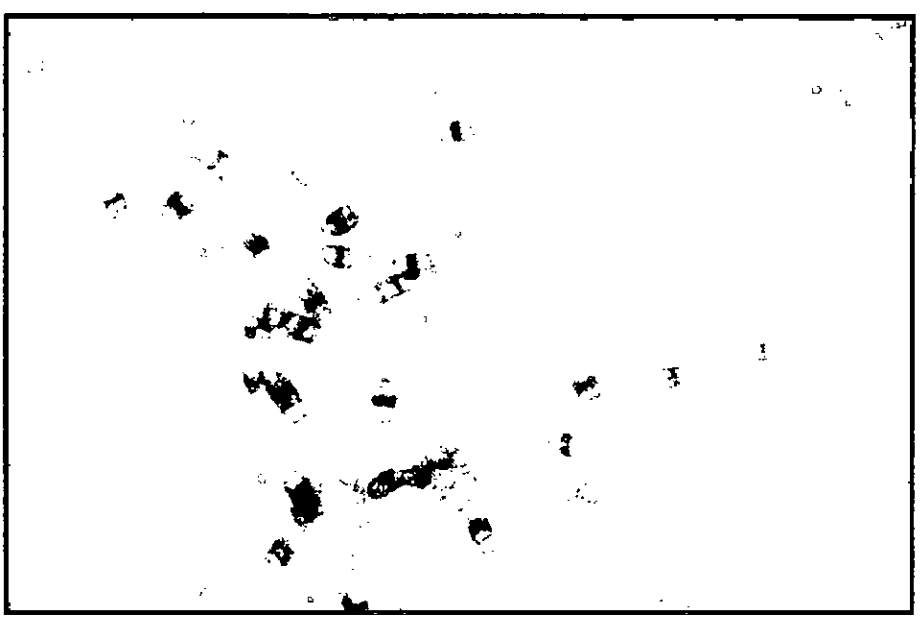


Photo. 7. Conidia of *Pestalotia psidii* seen under microscope (400x)

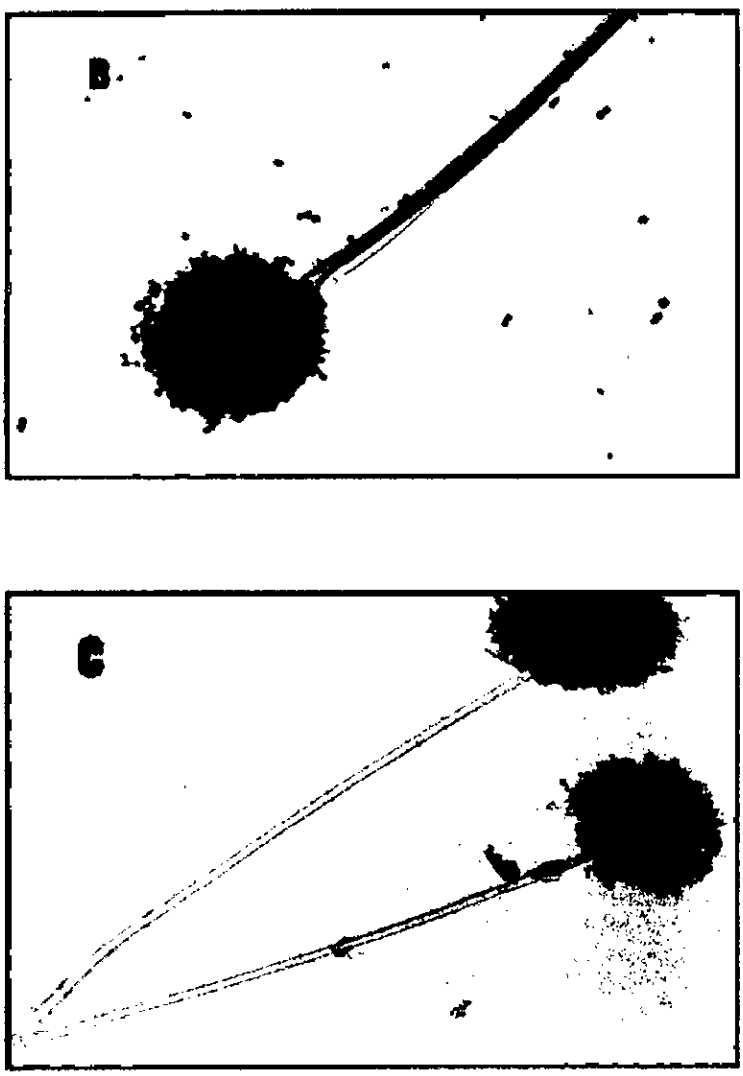


Photo. 8. A. Conidiophore, vesicle and conidia of *Aspergillus niger* (400x)
B. Sporangiophores, columella and sporangiosphores of *Rhizopus* sp. (400x)

Table--3. Prevalence of Seed borne infections of individually fungi detected in blackberry seeds collected from six different sources.

District	Source	% of Seed borne infection						
		A. flavus	*A. niger*	*F. equiseti*	*Rhizopus* sp.	*Penicillium sp.*	*C. lunata*	*P. psidii*
Netrakona	Government nursery	7.000a	7.700a	5.000a	2.00d	0.100a	0.000d	1.500b
	Choto Bazer	6.100c	6.800b	0.400d	8.100a	0.000c	2.400c	0.000d
	Modonpur	6.400bc	6.900b	2.900b	6.104b	0.300b	3.090a	2.200a
Dhaka	Karwan bazer	7.200a	7.100b	5.200a	8.300a	6.000a	2.200c	2.270a
	Tongi bazer	6.800ab	8.000a	5.100a	4.500c	4.800a	1.700b	1.400c
	Asadgate nursery	6.900a	6.5b	1.000c	2.000d	0.000c	0.00d	3.210a
LSD		0.445	0.3929	0.305	0.4050	0.1196	0.235	0.135
cv(%)		7.38%	6.05%	10.62%	8.73%	7.39%	14.22%	6.79%

Means bearing the same letter (s) in a column did not differ significantly at 1% level by DMRT.

Table- 4. Frequency of occurrence of fungi observed in the seeds of Netrakona.

Fungi	No. of infection	% of total infections[a]	No. of Sample infections[b]
Aspergillus flavus	19	25.6	3
A. niger	22	29.72	3
Rhizopus sp.	16	21.62	3
Fusarium equiseti	8	10.81	2
Pestalotia psidii	6	8.10	2
Curvularia lunata	3	4.05	2
Total	74		

[a]Total number of seed borne fungal infections recorded of the seeds of Netrakona.

[b]Total no. of seed samples were 3

4.3.3. Fungi identified and their frequency of occurrence in the seed of Dhaka district

Out of 109 seed borne fungal infections recorded on 400 blackberry seeds that represent 3 locations of Dhaka districts, seven different fungi were identified. The identified fungi, in order of prevalence were *Aspergillus niger, Aspergillus flavus, Rhizopus* sp., *Fusarium equisiti, Penecillium* sp., *Pestalotia psidii* and *curvularia lunata.* Of these, the most predominant seed borne fungus was *Aspergillus niger;* constituting 29.35% of the total fungal infections. Out of the 3 seed samples tested, 3 samples were found to be infected by 5 fungi viz. *Aspergillus flavus, Aspergillus niger, Rhizopus* sp, *Fusarium equiseti* and *Pestalotia psidii* (Table-5).

4.3.4. Prevalence of fungi in blotter paper

Among the three treatments, the prevalence of fungi was the highest in control seeds (8%), followed by seeds treated with alcohol and mercuric chloride. In control seeds, the occurrence of *Rhizopus* sp. (8%) was the highest followed by, *Fusarim equiseti* (5.62%), *Aspergillus flavus* (5.27%), *Aspergillus niger* (3.87%), *Penicillium* sp. (3%), *Pestalotia psidii* (2.62%) and *Curvularia lunata* (2%). In case of alcohol treatment, the incidence of *Rhizopus* sp. (5.7%) was also the highest, followed by *Fusarium equiseti* (4%), *Aspergillus flavus* (4.8%), *Aspergillus niger*

(3.2%), *Penicillium* sp. (2.1%) *Pestalotia psidii* (2%) and *Curvularia lunata* (1.5%). In case of Hg_2cl_2 treated seeds, the incidence of *Aspergillus flavus* was the highest (3.8%), followed by *Fusarium equiseti* (3.2%), *Rhizopus* sp. (3%), *Aspergillus niger* (2.25%), *Pestalotia psidii* (1.25%), *Penicillium* sp. (0.9%) and *Curvularia lunata* (0.8%) (Table-6).

4.3.5. Prevalence of fungi in PDA media

The incidence of fungi was relatively lower in PDA media. The occurrence of *Aspergillus flavus* was the highest (6.62%) in PDA media in control seeds followed by *Fusarium equiseti* (6%), *Rhizopus* sp. (6.1%), *Aspergillus niger* (4.8%) and *Penicillium* sp. (1.9%). In case of Hg_2cl_2 treated seed, the incidence of *Aspergillus flavus* was the highest (3.1%) while *Fusarium equiseti* was the lowest (1.1%). The alcohol treated seeds showed medium incidence. Where, *Rhizopus* sp. caused the highest incidence (5%) followed by *Aspergillus flavus* (4.02%), *Fusarium equiseti* (3.8%) and *Aspergillus niger* (3.7%). No incidence of *Curvularia lunata* and *Pestalotia psidii* was observed (Table-7).

Table- 5. Frequency of occurrence of fungi observed in the seeds of Dhaka.

Fungi	No. of infection	% of total infections [a]	No. of Sample infections [b]
Aspergillus flavus	20	18.34	3
Aspergillus niger	32	29.35	3
Rhizopus sp.	24	22.01	3
Fusarium equiseti	11	10.09	3
Penicillium sp.	11	10.09	2
Pestalotia psidii	7	6.42	3
Curvularia lunata	4	3.66	2
	109		

[a]Total number of seed borne fungal infections recorded of the seeds of Netrakona.

[b]Total no. of seed samples were 3

Table–6. Prevalence of fungi in blotter method with different treatments

Treatment	*Fusarium equiseti*	*Aspergillus flavus*	*A. niger*	*Curvularla lunata*	*Pastalotia psidii*	*Penicicillium sp.*	*Rhizopus sp.*
Control	5.625a	5.275a	3.875a	2.000a	2.625a	3.000a	8.000a
Hg_2d_2 (%)	3.250b	3.814b	2.250b	0.875b	1.250c	0.900c	3.020c
Alcohol (%)	4.000b	4.875ab	3.250a	1.500a	2.000b	2.110b	5.727b
LSD 0.5	1.049	1.214	0.959	0.594	0.417	0.592	0.357

Table –7. Prevalence of fungi in PDA media with different treatments

Treatment	*Fusarium equiseti*	*Aspergillus flavus*	*A. niger*	*Curvularla lunata*	*Pastalotia psidii*	*Penicicillium sp.*	*Rhizopus sp.*
Control	6.000a	6.625a	4.875a	--	--	1.980a	6.147a
Hg_2d_2 (%)	1.125b	3.100b	2.750c	--	--	0.000b	2.992c
Alcohol (%)	3.875b	4.025b	3.750b	--	--	0.000b	5.000b
LSD 0.5	2.026	1.968	0.935	--	--	0.998	0.208

4.4. Pathogenicity

4.4.1. Leaves inoculation

The three fungi *Fusarium equiseti, Pestalotia psidii* and *Curvularia lunata* tested for pathogenicity on blackberry. In case of infection caused by *Fusarium equiseti* raddish lesions were developed in infected leaves later the leaves turn black and died out (Table- 8). On the leaves of two months old blackberry seedlings, *Pestalotia psidii* caused leaf spots. The centers of the spots were whitish with brown border. The fungus caused 10 spots on 5 leaves in 4 seedlings out of 8 inoculated plants. A few larger spots were found to coalesce to form bigger irregular lesions resulted in blight symptom. Infected cotyledon leaves with blight symptom ultimately dropped off (Table-9). *Curvularia lunata* caused characteristic dark brown to black colored lesions on the tip of the inoculated leaves of blackberry (Table-10). The fungus caused shriveling of the leaves and irregular dark brown lesions. The fungus caused altogether 3 lesions on 3 leaves in 2 plants out of the 8 inoculated plants (Photo.-9A, B, and C).

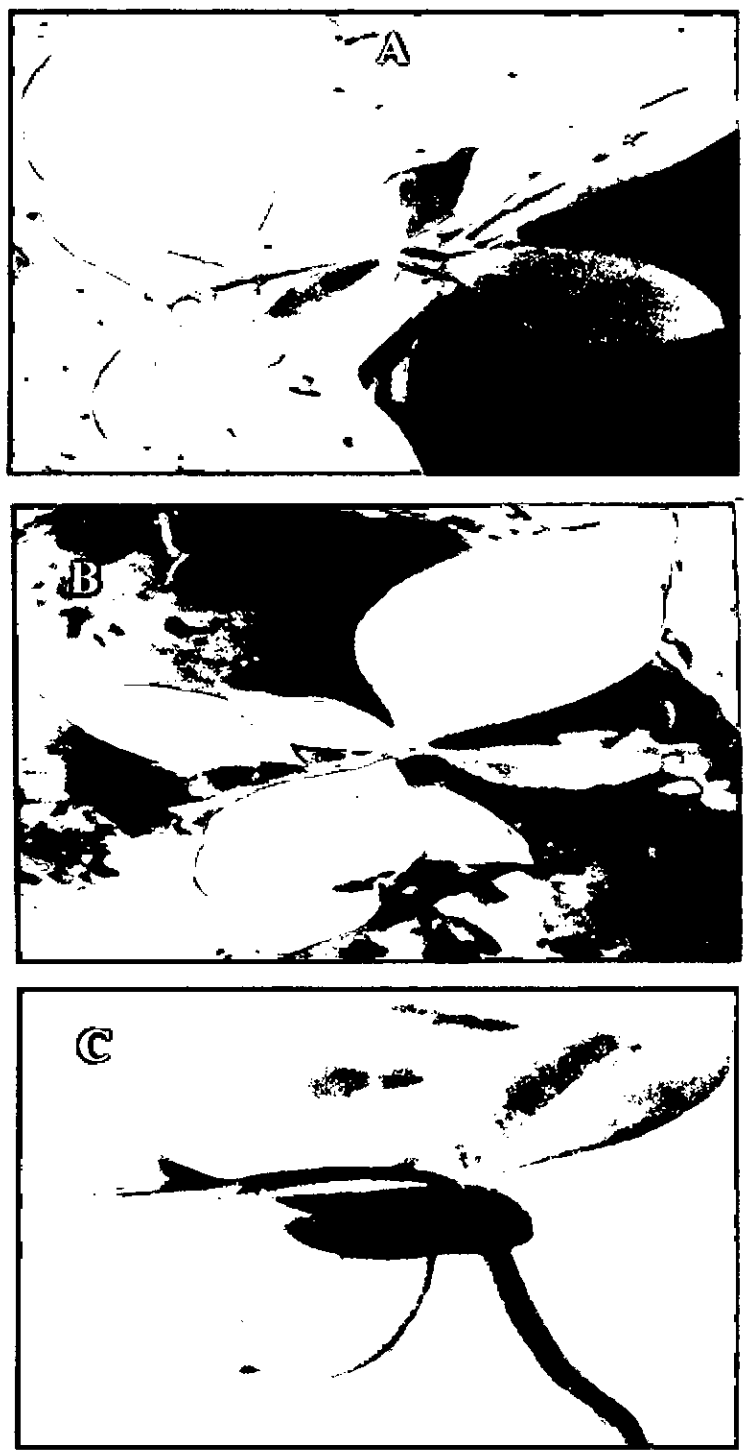


Photo. 9. A. Leaf blight symptoms developed after two months old leaves of blackberry by *Curvularia lunata.*
B and C. Leaf spot symptom developed after two months old leaves of Blackberry by *Pestalotia psidii* and *Fusarium equiseti.*

Table-8. Pathogenicity of *Fusarium equiseti* on two months old seedlings of blackberry as determined by leaves inoculation.

Fruit Plants	Total no. of plants inoculated	No. of infected plants	No. of infected leaves	No. of spots/ lesions	Remarks
Blackberry	8	2	3	3	In severe cases reddish lesions developed. Infected leaves become dried. It turned black and died out.

***Table- 9. Pathogenicity of Pestalotia psidii* on two months old seedlings of blackberry as determined by leaf inoculation.**

Fruit Plants	Total no. of plants inoculated	No. of infected plants	No. of infected leaves	No. of spots/lesions	Remarks
Blackberry	8	4	5	10	In severe cases blight symptom developed. Infected cotyledon ary leaves got fully blighted and dropped off.

Table-10. Pathogenicity of *Curvularia lunata* on two months old seedlings of blackberry as determined by leaf inoculation.

Fruit Plants	Total no. of plants inoculated	No. of infected plants	No. of infected leaves	No. of spots/ lesions	Remarks
Blackberry	8	2	3	3	In severe cases blight symptom developed. Infected cotyledon leaves got fully blighted and dropped off.

4.5. Occurrence of foliage diseases in nurseries

Totally five nurseries were surved in Netrakona and Mymensingh districts. Three nurseries in Netrakona, such as 1. Bonobevagh nursery 2. Sotabdy nursery 3. Mohammed nursery. In Mymensingh, 1. Mamun nursery and 2.Tulip nursery were surveyed. During the two months period of observation on foliage infection in three months old seedlings, the following diseases have been recorded:

1. Disease- Leaf blight of blackberry
   Pathogen- *Pestalotia psidii*
   Symptoms- Irregular light brown spots with white centre surrounded by brownish border; the white area covered with black spore (Photo-10A)

2. Disease - Red rust of blackberry
   Pathogen –*Puccinia psidii*
   Symptoms – leaf blade covered with brownish superficial fungal growth on the dorsal surface; lesions of various sizes scattered on the surface (Photo.-10C).

3. Disease – Leaf blight of blackberry
   Pathogen – *Curvularia lunata*
   Symptoms – Withering of leaves started from the tip; the blighted area turned dark brown to black color; the border with healthy pinkish part.

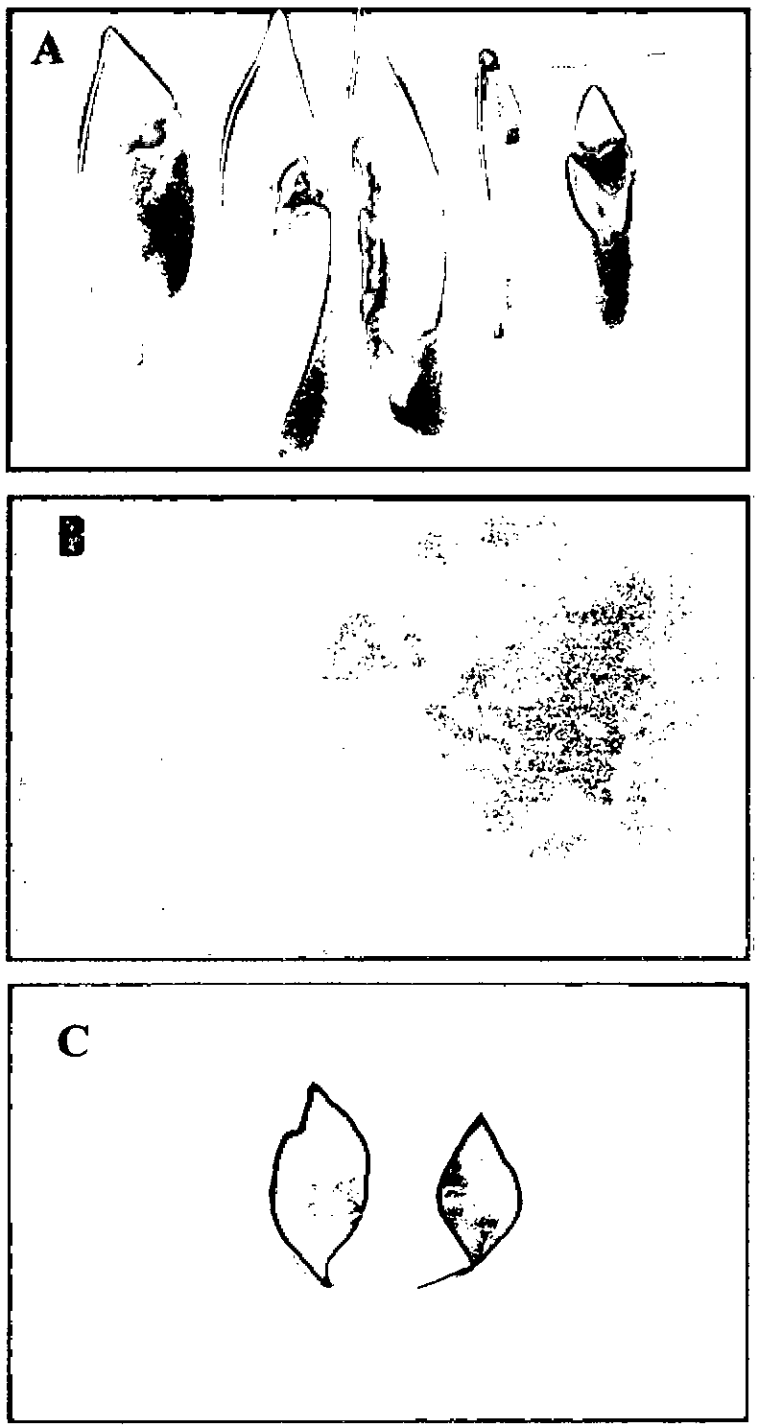


Photo. 10. A. Symptom of leaf blight diseases
B. Growth of *Penicillium sp.* on blackberry seed incubated on blotter (40x)
C. Symptom of leaf rust disease

4. Leaf spot of blackberry

Pathogen – *Fusarium* sp.

Symptoms - Irregular reddish brown spots of different sizes were shown on the leaf surface.

Intensity of disease incidence was determined by counting the number of plants showing leaf infection in the selected nurseries in two locations. Red rust of blackberry caused by *Puccinia psidii* was the least frequent.

Totally 9484 seedlings were survey in five nurseries. The germination was 75%. The disease severities were different in the nurseries. It depends on microclimate and locations.

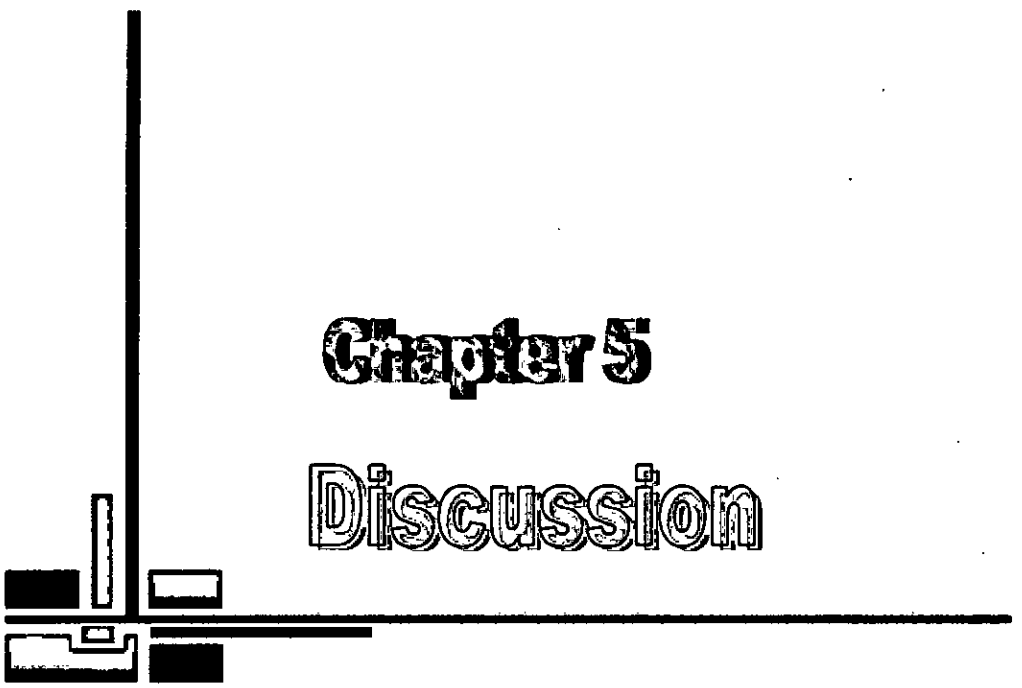
Only three diseases were noted during the period of survey. The disease were leaf spot, leaf blight and leaf rust which occurred in all the seasons and in all the age of the trees. In Netrakona, the highest incidence (40%) of leaf blight disease was found in Mohammad nursery caused by *Pestalotia psidii.* The incidence of leaf rust diseases caused by *Puccinia psidii* was the lowest (10%) (Table- 11). The highest infection was 80% due to *Pestalotia psidii* recorded in the nursery of Mymensingh (Table-12). The fungi isolated were *Fusarium equiseti, Curvularia lunata, Pestalotia psidii* and *Puccinia psidii.*

Table-11. Occurrence and intensity of foliage diseases of seedling raised in three nurseries of Netrakona.

Nurseries	Diseases	Causal organism	% seedling infected
Bono-bevagh nursery	leaf spot	*Fusarium equiseti*	27.00
	leaf rust	*Puccinia psidii*	20.00
	leaf blight	*Petalotia psidii*	33.50
Sotabdy nursery	leaf spot	*Fusarium equiseti*	14.50
	leaf rust	*Puccinia psidii*	10.00
	leaf blight	*Curvularia lunata*	28.00
	leaf bight	*Pestalotia psidii*	30.50
Mohammed nursery	leaf spot	*Fusarium equiseti*	17.00
	leaf rust	*Puccinia psidii*	11.70
	leaf blight	*Pestalotia psidii*	40.00

Table- 12. Occurrence and intensity of foliage diseases of seedling raised in three nurseries of Mymensingh.

Nurseries	Diseases	Causal organism	% seedling infected
Mamun nursery	leaf spot	*Curvularia lunata*	21.80
	leaf rust	*Puccinia psidi*	8.00
	leaf blight	*Fusarium equiseti*	15.00
Tulip nursery	leaf blight	*Pestalotia psidii*	80.00



Chapter 5

Discussion

5. DISCUSSION

Eight hundred seeds of blackberry were collected from two districts, Dhaka and Netrakona, and the seeds were tested for the prevalence of seed borne fungi. A total 183 seed borne fungal infections were recorded from 800 seeds of blackberry. The present study reveals that around 23% of the seeds were infected by fungi. Or in other words, at least one seed out of every four had fungal infection. This indicates that the seeds of the blackberry plants under study had considerable amount of infection by pathogens.

Seven species of fungi were detected from the seeds of blackberry. No fungi have been reported from the seeds of this fruit species earlier in the country. Thus, all these seven fungi recorded on the seeds of blackberry appears to be new records for Bangladesh.

In present study *Aspergillus flavus, Aspergillus niger, Fusarium equiseti, Rhizopus* sp., *Curvularia lunata, Pestalotia psidii, Penicillium* sp. has been recorded from the blackberry seeds. Rahman and Zethner (1971) isolated *Acremonium* sp., *Candida* sp., *Penicillium* sp. And *Fusarium* sp. from the seeds of *Syzygium grandies* which is close relative of blackberry. The study of prevalence of seed borne fungal pathogens on the blackberry seeds are very limited in abroad also. So, there is no confirm evidence of occurrence

of pathogen *Aspergillus flavus, Aspergillus niger, Curvularia lunata, Pestalotia psidii, Rhizopus* sp., *Penicillium* sp and *Fusarium equiseti* in blackberry seed available that can present in the thesis paper. The prevalence of the fungal infection varied with respect of fruit species and sources of seed collection. However, there is no report of variation in the prevalence of fungi in seeds of blackberry and no study has been made on this subject. Such variation of occurrence of seed borne fungi has been demonstrated in a number of crops like rice, kaon, mustard, blackgram, wheat, jute and chilli by different research workers (Hossain *et al.* 1977; Barma and Fakir, 1981; Dey and Fakir, 1988; kabir and Fakir, 1988; Rahman *et al.* 1988; Fakir and Islam, 1990; Basak *et al.* 1991 and Fakir and Halder, 1993). Variation in the occurrence of seed borne fungal infections on the seeds of blackberry could be due to the storage practice and climatic condition of the seed collected areas.

Three fungi, *Fusarium equisiti, Pestalotia psidii* and *Curvularia lunata* were tested for pathogenicity on two months old seedlings of blackberry. From pathogenicity test, it appears that *Fusarium equiseti, Pestalotia psidii Curvularia lunata* are pathogenic and caused spot and blighted symptoms on the leaves of blackberry. Morton (1987) reported several diseases like white spongy spot, leaf spot and leaf blight lesions on leaves of *Syzygium cuminii.* Kuthubutheen *et al.* (1988) encountered species of *Colletotrichum, Pestaloliopsis, Fusarium, Botryodiplodia* on leaves of several fruits trees

and stated that these fungi were all capable of lesion formation and subsequent leaf damage. Pandey (1984 and 1990) found leaves of guava, which is a close relative of blackberry under same family Myrtaceae, were colonized by *Colletotrichun gloeosporioides, Fusarium oxysporium* f. sp. *psidii, Pestalotia psidii,* and *phoma psidii* and these four were consistently pathogenic. Dwivedi *et al.* (1994) also reported that wilted twigs of guava yielded isolates of *Fusarium* sp. However, Edward (1961) reported that guava relative, *Syzygium cuminii* had never been observed to be attacked by wilt disease. As it is a very new finding in our country so further investigation in future by researchers are required to confirm the pathogenicity of the *Fusarium equiseti* fungi.

Pestalotia psidii caused irregular small spots (2-4 mm) of the leaves of seedlings of blackberry. Ramaswamy *et al.* (1988 and 1998) isolated *Pestalotia psidii* from blackberry and he confirmed the pathogenicity of the pathogen to blackberry by inoculation test. They also reported that *Pestalotia psidii* is the causal agent of guava canker and this report is in accordance with the findings of Pandey (1990).

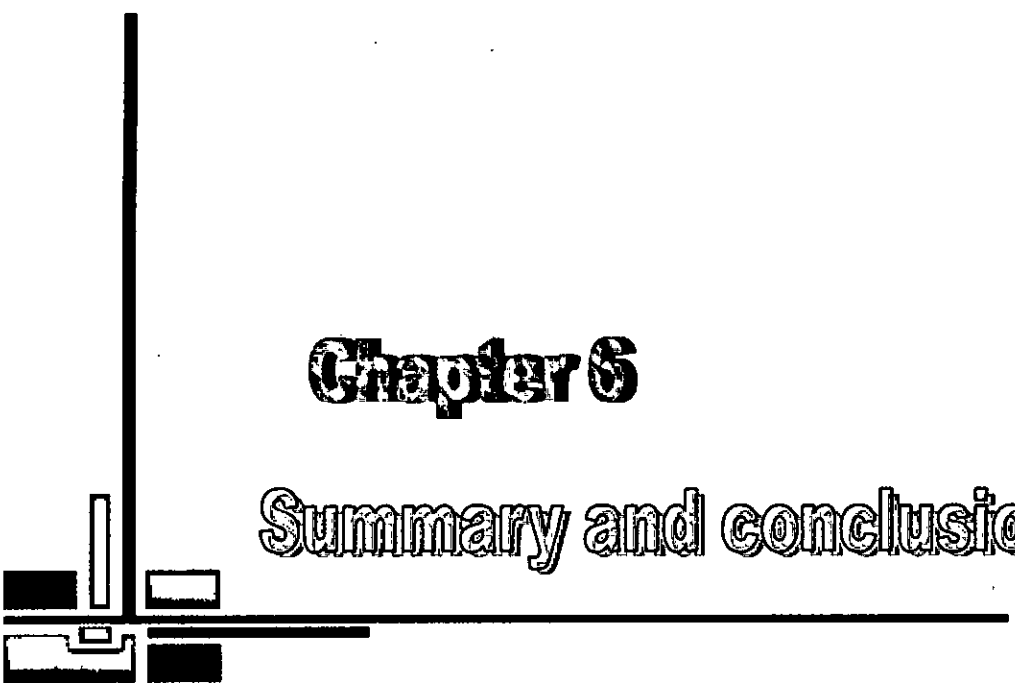
Curvularia lunata caused large lesions (3-7 mm) in leaves of seedling of blackberry. In severe case, blight symptom developed and often the infected cotyledonary leaves dropped off. No evidence of such pathogenicity could be presented due to lack of literature on blackberry but Kapoor *et al.* (1970)

isolated the fungus *Curvularia tuberculata* from guava which belongs to same family of blackberry. Previously *Curvularia tuberculata* had been reported as causing die-back disease of citrus in India.

In survey, 9984 seedlings were observed in five selected nurseries in Mymensing and Netrakona districts. At the time of survey, in addition to the three fungus viz., *Pestalotia psidii, Curvularia lunata and Fusarium equiseti, Puccinia psidii* has been encountered in the leaves of blackberry seedlings. This fungus is also recorded from the leaves of *Syzygium jambos* (White, 1990 and Galli, *et al.*1990), guava and eucalyptus (Galli, *et al,* 1990). It may be mentioned here that these are close relatives to blackberry and all are belongs to the same family of Myrtaceae.

Rust symptoms was first appeared as tiny, bright yellow, powdery eruptions that developed into circular, pustules on the stem and foliage. These pustules later expanded, coalesced, and became necrotic, spreading over the entire leaf and stem surfaces, and then leaves and stems were deformed and tip dieback ensued. Coutinho *et al.* (1998) describe the symptoms of the disease as first begin of tiny bright yellow powdery eruptions in a circular pattern on the leaf or stem surface. These infection loci or spots expand and become necrotic, and spread over the entire leaf, stem or shoot. Leaves and stems can be deformed by the disease, and growing tips can die back if the infection is severe.

As the study was limited to two districts only, further studies with more representative seed samples from different blackberry species, obtained from different parts of the country should be undertaken in order to depict the exact picture regarding the prevalence of fungi and the pathogenicity of the fungi under study in this experiment.



Chapter 6

Summary and conclusion

6. SUMMARY AND CONCLUSION

Prevalence and pathogenicity of fungi associated with the seeds of blackberry, collected form 6 sources under two districts of Dhaka and Netrakona, were studied in plant pathology lab, Sher-e- Bangla Agricultural University, Dhaka.

183 seed-borne fungal infections were recorded from 800 seeds of blackberry. In general, more seed-borne fungal infections were detected in seeds obtained from Dhaka district compared to Netrakona.

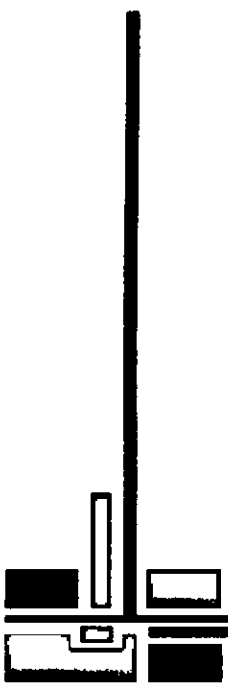
Of the total seed borne fungal infections recorded from the seeds of blackberry, seven fungi representing 6 genera were identified. The fungi identified were *Aspergillus flavus, Aspergillus niger, Fusarium equiseti, Pestalotia psidii, Curvularia lunata, Rhizopus* sp., *Peniecillium* sp.

The seven fungi recorded on the seeds of the one fruit species (blackberry) appear to be new records of seed-borne fungi of blackberry in Bangladesh.

Pathogenicity test conducted with *Curvularia lunata, Fusarium equiseti* and *Pestalotia psidii* and the fungi were found to be pathogenic to blackberry. As it is a very new finding, further investigations in future by researchers are required to confirm the pathogenicity of the above mentioned fungi. The

fungi produced dark brown to black spots and blight symptom on leaves of blackberry.

In survey, study four fungi *Curvularia lunata, Fusarium equiseti, Pestalotia psidii* and *Puccinia psidii* were isolated from the leaves and stems of seedling of blackberry. These fungi caused spot and blight symptom on the leaves of blackberry. Emphasis on under taking further research with more representative seed samples of different species of blackberry plants collected from various fruit growing areas is stressed in order to unveil the exact picture of the seed borne fungi, specially the pathogenic ones prevalent in fruit seeds in Bangladesh.



Chapter 7

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