STUDY ON THE POST HARVEST DISEASES OF POTATO IN THE WHOLE SALE MARKETS OF DHAKA

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BY

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This is to certify that the thesis entitled **"STUDY ON THE POST HARVEST DISEASES OF POTATO IN THE WHOLE SALE MARKETS OF DHAKA"** submitted to the **DEPARTMENT OF PLANT PATHOLOGY**, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) IN PLANT PATHOLOGY**, embodies the results of a piece of bona fide research work carried out by **MD. MAHMUDUR RAHMAN KHAN**, **Registration No. 10-04089**, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

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

Dated: 25th May, 2017
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Dedicated To

My Beloved Parents

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STUDY ON THE POST HARVEST DISEASES OF POTATO IN THE WHOLE SALE MARKETS OF DHAKA

ABSTRACT

A study was conducted to observe the prevalence of post harvest diseases of potatoes and to identify their causes in the whole sale markets of Dhaka. The experiment was conducted in the MS Laboratory of Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during the period of July, 2016- May, 2017. Samples were collected from five noted whole sale markets of Dhaka viz. Karwan bazar, Town hall market, Mirpur-1, Mirpur-10 and Khilkhet bazar. During each visit 5 kgs of potatoes were collected randomly from each of the market places. Total 400 potato samples were investigated and diseased potatoes were sorted out. The most frequent diseases found in the collected samples were common scab, brown rot, soft rot, Fusarium dry rot etc. Pathogens were isolated by tissue planting method and dilution plate method for fungi and bacteria respectively. Several biochemical tests like Gram's staining, Levan test, KOH solubility, Starch hydrolysis, Oxidase, Catalase, Gelatin, liquification and potato soft rotting test were conducted to identify and determine the characteristics of disease causing agents of collected potatoes. The study revealed that collected samples were infected by *Fusarium* spp. causing Fusarium dry rot (3.97%), *Ralstonia solanacearum* causing brown rot (4.27%), *Streptomyces scabies* causing common scab of potato (5.90%), *Pectobacterium* sp causing soft rot of potato (3.68%) and the prevalence of physiological disorder were recorded (5.48%). This study reveals the fact that potatoes are subject to various disease in the markets of Dhaka.

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LIST OF SYMBOLS AND ABBREVIATIONS

No. = Number

% = Percentage

et al. = And others

^{o}C = Degree Celsius

@ = At the rate

WP = Wettable Powder

EC = Emulsifiable Concentrate

etc. = Etcetra

J. = Journal

Viz. = Namely

Cm = Centimeter

Cfu = Colony forming unit

df. = Degrees of freedom

& = And

ppm = Parts per million

Kg = Kilogram

G = Gram

ml = Milliliter

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

hr = Hour (s)

i.e. = That is

T = Treatment

cv. = Cultivar (s)

var. = Variety

mm = Millimeter

µl = Microliter

µm = Micrometer

SAU = Sher-e-Bangla Agricultural University

BBS = Bangladesh Bureau of Statistics

USA = United States of America

NA = Nutrient Agar (media)

NB = Nutrient Broth (media)

TTC = Triphenyl Tetrazolium Chloride

ANOVA = Analysis of Variances

LSD = Least Significant Difference

CV% = Percentages of Co-efficient of Variance

Chapter I

INTRODUCTION

Bangladesh is mainly an agro based country. It is a thickly populated small country with a cultivable area of 14.48 million ha. The per capita net cultivable land would reduce from 0.066 ha in 2000 to 0.047 ha in 2025 (Bhuiyan *et al*., 2002). In Bangladesh Potato is one of the most important vegetables as well as cash crop. Potato (*Solanum tuberosum* L.) is a starchy tuberous crop. It belongs to the solanaceae or nightshade family. It contributes alone as much as 54% of the total annual vegetable production of Bangladesh (Anonymous, 2006). In Bangladesh the cultivation of potato was started in the late 19th century. Bangladesh ranks 11th in the world in terms of potato production in 2008 (Hossain and Miah, 2010). In fact, short cycle of potato frees the land for cultivating other crops. In 2008-2009, about 5166.7 thousands metric tons of potatoes have been produced from 395.6 thousands hectares (2.9% of total cultivated area) of land in Bangladesh (BBS, 2009). The area and production of potatoes are increasing day by day due to its higher demand and profitability and it has increased by 5 percent per annum. The annual growth rates of area, production and yield of potato were estimated at 7.14%, 9.90% and 2.76% during 1999-2000 to 2008-2009, respectively (Miah *et al.*, 2011). But Potato crop has huge production constraints in the field, in the storage and market of which soft rot, Fusarium dry rot, common scab, brown rot and hollow heart and black heart are of highest importance. The host pathogen interaction is influenced by environment acting on either the potato or the pathogen or on both.

The diseases which develop on harvested parts of the plants like seeds, fruits and tubers are called the post harvested diseases. The harvested products may get infected on the way to storage or to market or even before their final consumption. The plant parts may get infected in the field, but expression of symptoms may take place later, at any stage before final consumption. In Bangladesh so far as many nine post harvest diseases in potato have been recorded (PRA, 2015). Sclerotium rot, wilt, common scab, potato leaf roll, brown rot, mosaic, dry rot and

soft rot of potato are the most important diseases (Ahmed *et al*., 2000). The quality of potatoes depends not only on the variety and maturity but also on how they are harvested and handled immediately afterward. When tubers are badly cut and bruised during harvest, they discolor, wither and decay much more rapidly than uninjured tubers, particularly early potatoes harvested during hot weather. Heat injury or scald of tubers left exposed in the field before picking up often greatly damages the early potato. Extreme heat causes death of cells with subsequent breakdown and decay by various organisms. Sub lethal temperatures predispose the tubers to infection.

An estimated 22% of potatoes are lost worldwide per year due to viral, bacterial and fungal diseases which is equivalent to an annual loss of over 65 million tones (Ross, 2008). A report indicated that 0.187 million tons of potato were lost in Bangladesh due to post harvest diseases (Anonymous, 2006). In Bangladesh, considerable works have been done by Bangladesh Agricultural University (BAU) and Bangladesh Agricultural Research Institute (BARI) scientist on different aspects of diseases of potato but no systematic work has been done on the market diseases of this crop, except recording the occurrence of a few diseases and only a limited research work has been done.

Various types diseases of potato occurs in the market places of Dhaka, among them Fusarium dry rot is one of the most common and severe disease of potato. Stevenson *et al*. (2011) estimated yield losses due to dry rot in cold storage that ranges from 6% - 25%, with up to 60% of tubers infected in various cases. Kamaluddin (1970) and Rahman (1969) reported that 2% - 9% losses of tubers occurs every year in market places because of different fungal diseases. It was also reported by them that among of all the diseases in the market places, dry rot caused by *Fusarium calmorum*, is very common and causes most of the damage, but no extensive work has been carried out to assess the loss due to dry rot in the market places of Bangladesh. Bacterial soft rot is considered as one of the most destructive disease of potato in storage, transit conditions and market places (Hossain, 2011). *Erwinia carotovora.* pv. *atroseptica* , *Erwinia carotovora*pv.

carotovora. and *Erwinia chrysanthemi*are the three causal organisms of soft rots of potato (Perombelon and Kelman, 1980). Soft rot can cause heavy losses in stored potatoes if not properly managed, creating a perception of poor quality in export seed potato markets. Common scab is another common disease of potato in the market places. Common scab is a soil dwelling plant pathogenic bacterial species. Although scab does not usually affect total yields, since the economic losses are greatest when tubers infected for table stock and processing varieties, since appearance of potato is important for the market (Wanner, 2009 and Loria *et al*., 2007).

Brown rot is another most severe disease of potato, but still actual losses due this disease in Bangladesh is not reported. Russia halted the potato export from Bangladesh due to the presence of brown rot in stored potato for which Bangladesh had to face a loss of about 9 million doller in 2014 (EPB 2015).

Considering the above facts, the present research programme has been designed with following objectives :

1. To assess the prevalence of different post harvest potato diseases in noted whole sale markets of Dhaka in Bangladesh.

2.To identify different causal organisms responsible for the post harvest loss of potato in whole sale markets of Dhaka.

Chapter II
REVIEW OF LITERATURE

2.1. Present status of potato in Bangladesh

Potatot is being cultivated on an area of 520 thousands ha and the total production is 89,50,024 metric tonnes with an average yield of 19.371 metric tonnes/ha BBS (2014).

According to FAO (2014) annual report, annual consumption of potato has been growing rapidly, from around 34 kg per capita to 38 kg per capita.

Miah *et al*. (2011) stated that the annual growth rates of area, production and yield of potato were estimated at 7.14%, 9.90% and 2.76% during 1999-2000 to 2008-2009, respectively .

Ali and Haque (2011) reported that potato is the world's fourth largest and third largest food crop in Bangladesh and has recently occupied an important place in the list of major food and cash crops in Bangladesh.

Hossain and Miah (2010) stated that Bangladesh ranks 11th in the world in terms of potato production in 2008.

Fakir (2010) stated that an amount of Taka 8 crores approximately was lost annually due to storage disease.

Khan *et al*. (2010) conducted a survey on potato production in some selected areas of Bangladesh. This study showed that potato production is highly profitable, and it can provide cash money to farmers. In terms of profitability, potato production was more attractive than any other winter vegetables.

BBS (2009) reported that; In 2008-2009, about 5166.7 thousands metric tons of potatoes have been produced from 395.6 thousands hectares (2.9% of total cultivated area) of land in Bangladesh .

Fakir (2009) stated that; a few studies on economic aspect of cold storage have been conducted so far in Bangladesh. In a preliminary survey of the diseases of potatoes in cold storage in Bangladesh it was found that 2-9 percent of cold stored potatoes were lost in every year due to disease.

Ross (2008) reported that, An estimated 22% of potatoes are lost worldwide per year to viral, bacterial and fungal diseases which is equivalent to an annual loss of over 65 million tones.

Report indicates that 0.187 million ton of potato were lost in Bangladesh due to post harvest diesaese (Anonymous 2008)

Anonymous (2007) showed that potato contributes alone as much as 54% of the total annual vegetable production of Bangladesh .

Rahman (2007 reported that about 3 lakh tons of potato which is one third of the total annual production is wasted every year a mounting a loss of more than Taka 60 crores annually.

According to New World; Encyclopedia (2007), Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) and provides a balanced source of starch, vitamins and minerals to many communities in the global village (Rowe, 1993).

Moazzem and Fujita (2007) observed that; Adequate supply of potato stabilizes the vegetable market all round the year.

2.2. Potato diseases and their effect on post harvest

In Bangladesh, so far as many as nine diseases in potato have been recorded PRA (2015).

Russia halted the potato export from Bangladesh due to the presence of brown rot in stored potato for which Bangladesh has to face a loss of about 9 million doller in 2014 (EPB 2015).

Nadia *et al*. (2013) carried out a survey in some selected potato growing districts in Bangladesh to know the status of bacterial wilt disease caused by *Ralstonia solanacearum* in terms of its incidence and severity. The results showed that the highest wilt incidence was recorded in Munshigonj (22.65%); followed by Nilphamari (19.98%) and the lowest incidence was recorded in Jamalpur (9.07%). The highest bacterial wilt severity was recorded in Munshigonj (3.80), while the lowest wilt severity was recorded in Jamalpur (2.90).

Stevenson *et al*. (2011) reported that, In Bangladesh, considerable works have been done by Bangladesh Agricultural University BAU and BARI scientist on different aspects of diseases of potato but no systematic work has been done on the market diseases of this crop, except recording the occurrence of a few diseases and only a limited research work has been done.

Hossain (2011) mentioned that, Bacterial soft rot is considered as one of the most destructive diseases of potato in storage, transit conditions and market places.

Wanner (2009) and Loria *et al*. (2007) observed that, Although common scab does not usually affect total yields, since the economic losses are greatest when tubers infected for table stock and processing varieties, since appearance of potato is important for the market.

Ahmed *et al*. (2008) stated that, late blight, stem rot/sclerotium rot, wilt, common scab, potato leaf roll, mosaic and brown rot are the most important diseases.

Wang and Lin (2008) reported that, worldwide major hosts of *Ralstonia solanacearum* are: *Solanum tuberosum* (potato), *Lycopersicon esculentum* (tomat), *Capsicum annuum* (sweet, pepper), *Solanum melongena* (aubergine), *Nicotiana tabacum* (tobacco), *Arachishy pogaea* (groundnut), *Musa paradisiacal* (banana and plantain) and *Heliconia*s pp.

Wharton *et al*. (2008) reported that, Fusarium dry rot of potato can reduce crop establishment by killing developing potato that crop losses can be up to 25%, while more than 60% of tubers can be infected in storage.

Wicker *et al*. (2007), Hayward, (1991) stated that, *Ralstonia solanacearum* is an important soil borne pathogen globally. It causes devastating wilt on over 450 plant species belonging to 54 families, covering both monocots and dicots.

Rouson (2004) reported that various diseases due to high temperature (28.13-29.35 ^{0}C) and high humidity (85-86.23%) infect potatoes in the market places.

2.3. Symptomology of different potato diseases

Goyer *et al*. (2010) observed that, The occurrence of scab and its severity varies from cultivar to cultivar, field to filed and year to year.

Wanner (2009), Hooker (1998) found out tuber potatoes become infected through wounds during storage or preparation for planting by *Fusarium* dry rot disease all over the world.

Perombelon (2009), Tsror *et al*; (1997), observed that, the soft rot bacteria can also interact with other pathogens, especially vascular ones such as *Ralstonia solanacearum*, *Fusarium* spp, *Verticillium* spp. *and Rhizoctonia solani.*
Milling *et al*. (2009) and Ephinstone (2005) stated that, brown rot of potato caused by *Ralstonia solanacearum* race 3 biovar 2 is among the most serious disease of potato worldwide, which is responsible for estimated $950 million worldwide losses each year. Moreover Race 3 biovar 2 is cold tolerant and classified as a quarantine pathogen for most of the European country.

The pathogen is distributed by infected seed tubers or soil and survives well in the absence of host plants (Wang and Lazarovits, (2009) and Loria *et al*. (1997).

Champoiseau (2008) identified that *Ralstonia solanacearum* developed two types of colonies on tetrazolium chloride (TZC) medium on which virulent colonies appear as white with pink centers and non-virulent colonies appear as small off-white colonies. On this medium, typical bacterial colonies appear fluidal, irregular in shape, and white with pink centers after 2 to 5 days incubation at 28ºC.

Elphinstone (2008) reported that brown rot was mainly tuber-borne as it could latently infect tubers and survived in seed tubers during storage, causing disease when planted in the next season. The bacterium was spread on machinery and in irrigation water. The disease was persisting in fields where infected ground keepers were present.

Decaying of the seed tuber before emergence or infection of emerging sprouts, results in non emergence, poor stand, stunting and missing hill. All these symptoms are common manifestations of the disease. (Shroeder 2008); (Pérombelon and Kelman 1980).

Janse *et al*. (1998) observed that symptoms were most obvious in the tuber; initially a brown staining of the vascular ring (hence brown rot) started at the stolon end, with further disease progression the vascular tissue were rot away completely and a pale colored sticky ooze was appeared at the eyes lenticels and/or stolon end of the tuber.

Khan *et al*. (1997) described the symptoms to Fusarium dry rot as the infections generally begin at wound sites once infection occurs, it slowly enlarges in all directions. The skin over the infected area sinks and wrinkles, sometimes in concentric rings, due to the fungus drying out the contents of the tuber.

Lorang *et al*. (1995) reported that; depending on pathogen strain, cultivar susceptibility, environmental conditions, symptoms of scab can appear as lesions of variable sizes and depth on tuber surfaces.

The type of lesion formed on a tuber due to common scab is thought to be determined by a combination of host resistance, aggressiveness of the pathogen strain, time of infection, and environmental conditions. (Babcock *et al*. 1993); (Hooker, 1986) and (Labruyere, 1971).

Pérombelon and Kelman (1980) stated that, soft rot pathogens cause maceration and decaying of parenchymatous tissue in the infected tissues.

Pérombelon and Kelman (1980), repoted that, the most characteristic symptom of potato blackleg caused by both *Pectobacterium* and Dickeya species is a slimy, wet, black rot lesion spreading from the rotting mother tuber up the stems,

especially under wet conditions. However, when conditions are dry, symptoms tend to be stunting, yellowing, wilting and desiccation of stems and leaves.

Coplin (1980), Tegene and Korobko (1985) stated that, Bacterial soft rot of potato caused by *Pectobacterium* is known mainly as a post harvest disease.

2.4. Isolation and Identification of Pathogen

Razi (2012) in his study showed that gram negative bacteria are rod shaped with rounded ends and they show red or pink stain due to retaining the counter staining dye called Safranin.

Chater *et al.* (2010) and Chater (2006) stated common scab of potato is caused by soil borne, Gram positive, filamentous bacteria in the genus *Streptomyces*. This saprophytic genus is well known for production of antibiotics.

Milling *et al*. (2009) reported that brown rot of potato, a quarantine disease caused by the bacterium *Ralstonia solanacearum* (biovar 2, race 3, phylotype IIB), was diagnosed with certainty for the first time in The Netherlandsin 1992.

Cullen *et al*. (2009) observerd that there are estimated to be thirteen species of *Fusarium* causing dry rot of potato worldwide.

Khan *et al*. (2007) studied that bacterial soft rots are caused by several types of bacteria, but most commonly by species of Gram-negative bacteria, *Erwinia* spp. , *Pectobacterium* spp. , and *Pseudomonas* spp.

Sutton (2006) stated that, the KOH test is done using a drop of 3% potassium hydroxide on a glass slide. A visible loopful of cells from a single, well-isolated colony is mixed into the drop. If the mixture becomes viscous and “strings out” then the bacteria is gram negative.

Cullen *et a*l. (2005) and Choiseul *et al*. (2001); stated that isolated outbreaks of disease have been caused by the highly pathogenic species *F. sambucinum* (formerly *F. sulphureum*), but workers have also noted a high incidence of *F. avenaceum*in Scotland associated with post-storage rotting of potato.

Lambert (2002) reprted that, In 2002, the subgroup of *R. solanacearum* known as race 3 biovar 2 (R3B2) was listed in the United States as a bioterrorism select agent.

Shashirekha *et al.* (1996) confirmed from the experimentally induced bacterial pathogen, bacteria that was reisolated and compared with the original isolate of *Erwinia carotovora.*

According to Drayford (1993) dry rot symptoms (four pieces per rot) should be transferred onto quarter strength potato dextrose agar (Merck) plus 200 mg L–1streptomycin and incubated at 16°C. *Fusarium* spp. should be transferred onto potato dextrose agar (PDA) then grown on synthetic nutrient agar and identified using characteristics of colony morphology.

Plant pathogenicity in the genus is based on production of the toxin thaxtomin which induces the characteristic symptoms of CS. Bignell *et al.*(2010); Healy *et al*.(2000); Lawrence *et al*.(1990); King *et al.*(1989).

Lambert and Loria (1989) stated that, *Streptomyces scabies*, *S. turgidi scabies* and *S. europaei. Scabieis* are amongst the most well known common scab causing species world wide. *Streptomyces scabies* was the first described species causing CS on potatoes.

Smith (1986) observed that, Bacterial wilt is caused by *Ralstonia solanacearum* formerly called *Pseudomonas solanacearum.*

Suslow *et al*. (1982) stated that Gram differentiation test is a method for gram differentiation of plant pathogenic bacteria without staining.

Pectolytic clostridia causes soft rot in potato (*Solanum tuberosum)* (Campos *et al.* 1982; Perombelon *et al.* 1979; Lund, 1972)

Yabuuchi *et al*. (1976) reported that *Ralstonia solanacearum* is a soil borne gram negative bacterium which is a recognized pathogen in over 200 families of Plants.

Kelman and Person (1954), recommended using TTC (2,3,5-triphenyltetrazolium chloride) media and 523 media for isolation and maintenance of pathogenic isolates of *Ralstonia solanacearum.*

Kelman and Person (1954) observed that, In TTC medium *Ralstonia solanacearum* develops medium or largely unmixed mucoid and magenta to deep red coloured colonies with whitish margins.

Chapter III
MATERIALS AND METHODS

3.1. Experimental site

The experiment was conducted in the MS Laboratory of Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

3.2. Experimental period

The experiments were conducted during the period of July 2016 to May 2017.

3.3. Collection of samples

Potato samples were collected from five different whole sale markets of Dhaka namely Karwan bazar, Town hall market, Mirpur-1, Mirpur-10 and Khilkhet bazaar during the time of July-August 2016. During each visit 5 kgs of potatoes were collected by random sampling from each market place. Total 400 samples were investigated carefully and diseased samples were separated from healthy ones.

3.4. Measurement of prevelance of disease occurance in whole sale markets of Dhaka

According to Rai and Mamatha (2005). Disease incidence of infected potato was determined by the following formula-

$$\text{Percent potato tuber infection} = \frac{\text{Number of infected potato tuber}}{\text{Number of total potato tuber observed}} \times 100$$

3.5. Preservation of samples

The collected samples were washed properly to remove the soils and dust from

the potatoes then dried and kept in a poly bag and stored in the refrigerator at 5^0-6^0 C for further study.

3.6. Isolation of Causal Organism

3.6.1. Isolation of *Fusarium* spp. from dry rot infected potato

The collected diseased specimens were first washed in tap water to make them free from sand and soils. The infected portion along with the healthy portion of the tubers were cut into small pieces (0.5-1.0 cm) and surface sterilized with 1% Clorox for 2-3 minutes. Then the pieces were washed with sterilized water thrice and placed on sterilized filter paper to remove excess water adhering to the pieces. Three pieces were plated in acidified PDA plates and also in moist chambers aseptically maintaining equal distance. The plates were incubated for 7 days at 25$\pm$ 1^0C. Afetr incubation period, the fungual mycelia that grew over PDA were taken with the help of sterile needle and transferred on new PDA plates in three replications. *Fusarium* spp. were purified by using PDA (Begum *et al.* 1998). Then the plates were incubated for 7 days for 12 hours alternating cycles of light and darkness at 25$\pm$ 1^0C temperature. After incubation pure culture of the fungus was identified following the key outlined by Leslie *et al.* (2006). The pure culture of the pathogens was preserved in PDA slants at 5$\pm$ 1^0C in refrigerator as stock culture for future use.

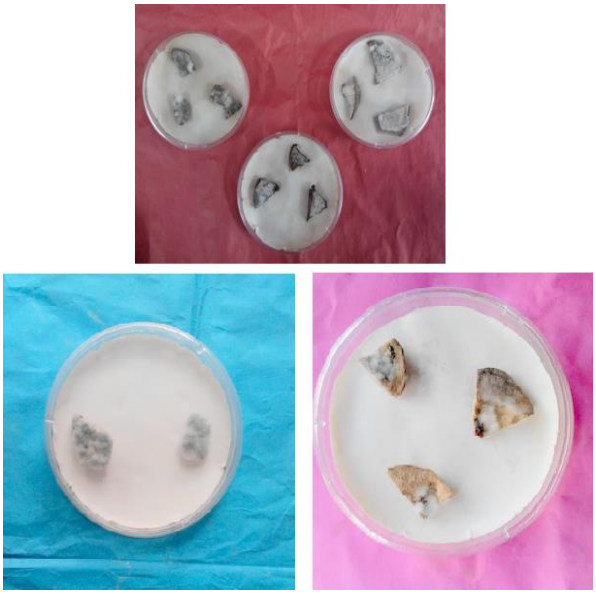

Fig.1. Isolation of fusarium dry rot of potato in moist chamber

3.6.2. Preparation of Potato Dextrose Agar (PDA) media

Potato dextrose agar (PDA) medium was prepared as described by Hussain *et al.* (2012). 200 g peeled potato extract, 20 g dextrose and 17 g agar was taken in a Conical flask containing 1000 ml distilled water and mixed well for the preparation of 1 liter PDA medium. Then it was autoclaved for 20 minutes at 121^0C under 15 PSI pressure. For 250 ml medium 20 drops of 50% lactic acid was added to avoid the contamination of bacteria.

3.7. Isolation and identification of Bacteria from diseased potato

3.7.1. Preparation of Nutrient Agar (NA)

In a conical flask 15 g bacto agar was added in 1000 ml distilled water 5 g peptone and 3 g beef extract were then added to it for the preparation of 1 liter NA medium. To mix the components properly the medium was shaken thoroughly for few minutes. It was then autoclaved at 121^0 C under 15 PSI pressure for 15 minutes.

3.7.2. Isolation of Bacteria on NA media

The diseased potatoes were washed properly with water. Then they were cut into small pieces. For surface sterilization of the diseased samples they were kept in 95% ethanol solution for 1 minute. Then they were washed three times with distilled water. After surface sterilization the cut pieces were kept in a petri dish containing 3-4 ml of distilled water. Then were chopped into very small pieces using a sterile sharp blade. One ml of this stock solution was transferred with the help of sterile pipette into a test tube containing 9 ml distilled water and shaken thoroughly resulting 10^{-1} dilution. Similarly, final dilution was made up to 10^{-3}. Then 0.1 ml of each dilution was spread over NA plate at three replications as described by Goszczynska and Serfontein (1998). The inoculated NA plates were kept in an incubation chamber at 30^0C. The plates were observed after 24 hrs and

48 hrs. Then single colony grown over NA plate was restreaked on fresh NA plate with the help of a loop to get pure colony.

3.7.3. Preparation of TriphenyltetrazoliumChloride (TTC)

Aqueous solution of 2, 3, 5- triphenyltetrazolium chloride (TTC) was prepared in an erlenmeyer flask by dissolving 1g of the chemical in 100 ml of distilled water. The 1% stock solution of TTC solution was separately sterilized by passage through 0.45μm pore size filters (Millipore). The TTC was kept in a colored bottle and was wrapped with aluminum foil to avoid light and preserved in a refrigerator at 4^0C for future use.

3.7.4. Preparation of CPG media

CPG media was prepared by adding 1g casamino acid, 10 g peptone, 5 g glucose, and 17 g agar in 1000 ml distilled water. The mixture is then taken in an erlenmeyer flask and was then autoclaved for 20 minutes at 121^0 C under 15 PSI pressure.

3.7.5. Preparation of TTC medium

The sterilized TTC solution was poured into the sterilized CPG medium at the rate of 5 ml/1000 ml before solidification and it was mixed thoroughly. For solidification, the CPG media with TTC was poured in to several petri dish.

3.7.6. Growth of Bacteria on TTC medium

The pure colony that grew over NA medium was transferred on TTC medium by streak plate method. The plates were kept in an incubation chamber at 30^0C after inoculating them on TTC medium. Viruent colonies of *Ralstonia solanacearum* were selected on the basis of characteristic colony charter on TTC medium (Kelman, 1954). (Fig. 13).

3.7.7. Preparation of Citrimide Agar

In a Erlenmeyer flask 46.5 g Citrimide Agar was taken in 1000 ml water. Then 10 ml glycerin was added in it. The mixture was boiled for to mix the elements properly. After that, it was autoclaved at 121^0C under 15 PSI pressure for 20 minutes.

3.7.8. Growth of Bacteria on Citrimide Agar

The pure colony that grew over NA medium was transferred on Citrimide agar medium by streak plate method. After inoculation the plates were kept in an incubation chamber at 30^0C. Virulent colonies of *Pseudomonous* were selected on the basis of growth of bacteria on Citrimide Agar medium. (Fig. 12)

3.7.9. Growth of *Streptomyces* on Water Agar

Water agar is the medium of choice for isolation of *Streptomyces scabies* from lesions on potato tubers. Most pathogenic species grow and sporulate on water agar and observation of the filamentous nature of the non sporulating colonies is easiest on this relatively clear medium. After cutting away the brown lesion, apice of the tissue is removed form beneath and macerated in a small volume of sterile water with a sterile blade and suspended in 5ml sterile water. Using a sterile Pasteur pipette, a drop of this suspension is spread by streaking onto water agar. Alternatively the whole tissue pieces can be placed in a tube of sterile water and immersed in a water bath for 30 minutes at 60^0C than placed onto water agar.(O'Brien *et a.,* 1984)

3.8. Biochemical test

3.8.1. KOH solubility test or Gram differentiation test

It is a rapid method for gram differentiation of plant pathogenic bacteria without staining (Suslow *et al*., 1982). Two drops of 3% KOH solution were placed at the centre of a clean glass slide. One loopful colonies of bacterial pathogen

(grown NA medium) were added to the KOH solution and homogenized with a nichrome loop with rapid circular movement of about 10 seconds. Viscous strand formation was observed and on drawing it with a loop it formed a fine thread of slime, 0.4 to 2.5 cm in length.

3.8.2. Gram's staining

At first on a clean microscope slide a small drop of distilled water was mounted. Small Part of a young colony (24 hrs old) was removed with the help of a sterile loop from the nutrient agar medium and then the bacterial smeare was made on the slide. The thinly spread bacterial film was air dried. Underside of the glass slide was heated by passing it two times through the flame of a sprit lamp for fixing the bacteria on it. Then the slide was flooded with crystal violet solution for 1 minute. It was rinsed under running tap water for a few seconds and excess water was removed by air. Then it was flooded with lugol's iodine solution for 1 minute. After that it was decolorized with 95% ethanol for 30 seconds and again rinsed with running tap water and air dried. Then it was counterstained with 0.5% safranine for 10 seconds. It was rinsed under running tap water for a few seconds and excess water was removed by air. Then the glass slide was examined at 40x and 100x magnification using oil immersion.

3.8.3. Catalase test

A few drops of freshly prepared 3% H_2O_2 (Hydrogen peroxide) was added with 48 hours old pure culture of bacterium grown on NA plate and observed whether it produced bubbles within a few seconds or not.

3.8.4. Oxidase test

For this test Aqueous solution of (1%) of tetra methyl-p-phenylene-diamine dihydrochloride is used as test reagent. A strip of Whatman filter paper (No. 2) was soaked with 3 drops of 1% aqueous solution of freshly prepared tetra methyl-p- phenylene-diamine dihydrochloride (color indicator). A loopful of young

bacterial culture (24 hours) of each isolate was rubbed separately on the surface of the filter paper by a platinum loop. Purple color develops within 10 seconds, which indicated positive reaction of oxidase test.

3.8.5. Gelatine liquefaction test

One loopful bacterial culture was stab inoculated into the tube containing 12% (w/v) gelatin with the help of a sterile transfer loop. Then it was incubated at 30^0C for 24 hours. Gelatin liquefied microorganism was determined by the formation of liquid culture after keeping it at 5^0C in refrigerator for 15 minutes**.**

3.8.6. Levan test

Into a NA plate containing 5% (w/v) sucrose one loopful bacterial culture was streak inoculated with the help of a sterile transfer loop. Then it was incubated at 30^0C for 24 hours to observe whether levan test is positive or not.

3.8.7. Starch hydrolysis test

For starch hydrolysis test, pure colony of bacterium was spot inoculated on nutrient agar plate containing 2% soluble starch. After that it was incubated at 30^0C for at least 48 hours in incubation chamber. After incubation the plate were flooded with lugol's iodine solution and observed whether a clear zone appeared around the colony or not.

3.8.8. Potato soft rotting test

Potato tubers were disinfected with 99% ethanol, cut up into slices of about 7-8 mm thick, and then placed on moistened sterile filter paper in sterile Petri dishes. Bacterial cell suspension was pipetted into a depression cut in the potato slices. One potato slice pipetted with sterile water was treated as control. Development of rot on the slices was examined 24–48 h after incubation at 25 °C. Examination was done for 5 days after inoculation. Two slices were inoculated for each isolate.

Chapter IV

RESULTS

4.1. Visual symptoms of collected samples

4.1.1. Dry rot of potato

Wrinkled skin of the diseased potato were observed visually. The rotted areas of the potato were brown, and grayish. There were also some whitish and pinkish fungal growth on the surface area of the diseased potato (Fig. 2). In some diseased potatoes extensive rotting caused the tissues to shrink and collapse, leaving dark sunken area on the outside of the tuber.

Fig. 2. Dry rot of potato

4.1.2. Soft rot of potato

After cutting some portion of the infected tuber a foul smelling odour came out because of the break down of cells by the bacteria (Fig. 3). When finger was pressed against the soft and rotted portion of the infected tubers watery exudates came out.

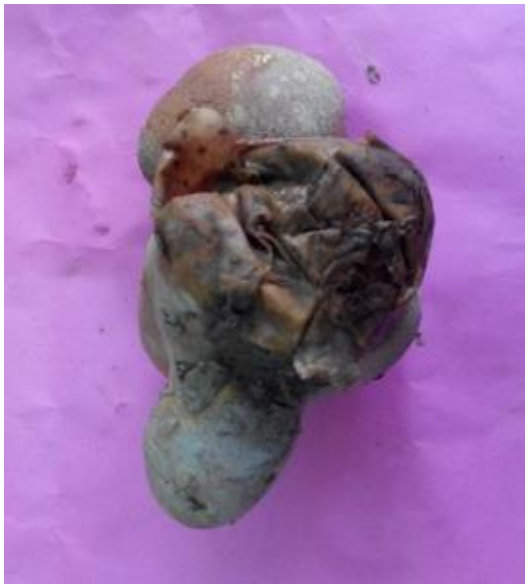

Fig. 3. Soft rot of potato

4.1.3. Brown rot of potato

A pale, creamy and milky white ooze like sticky exudates were observed visually after cross sectioning of the diseased potato (Fig. 4). The discoloration extended into the cortex of the tuber which indicated the presence of bacterial cell in the infected tubers.

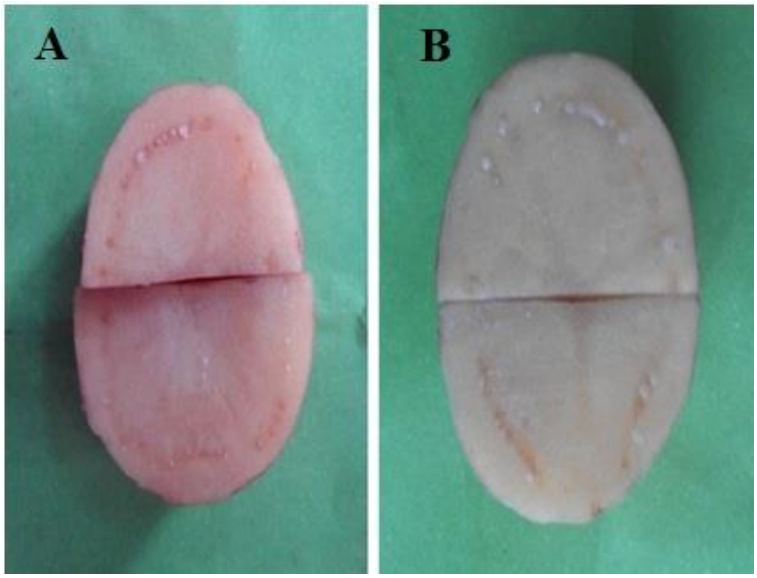

Fig. 4. A and B showing brown rot symptom of potato

4.1.4. Common Scab

Roughly circular, raised, brown, corky lesions of varying size developed randomly across the tuber (Fig. 5). Some of the lesions are raised and some are warty in appearance level with the surface, some lesions are sunken into the tuber. Superficial russetting was seen and a layer of corky tissue covered large areas of the tuber.

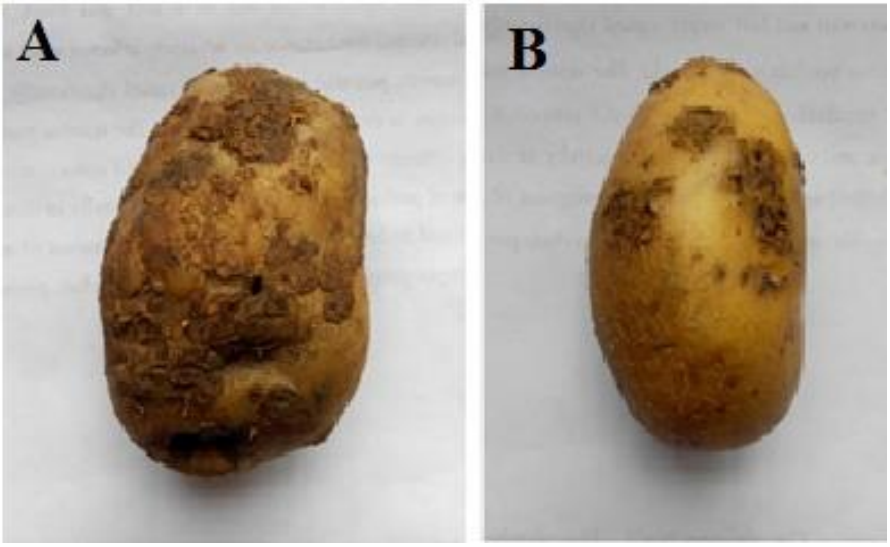

Fig. 5. A. Deep scab of potato, B. Shallow scab of potato

4.2. Measurement of prevalence of disease occurance in market places of Dhaka

The collected samples were infected by *Fusarium* spp. causing dry rot of potato (3.97%), *Ralstonia solanacearum* causing brown rot (4.27%), *Streptomyces scabies* causing common scab of potato (5.90%), *Pectobacterium* causing soft rot of potato (3.68%) and physiological disorder was found (5.48%).(Fig. 6)

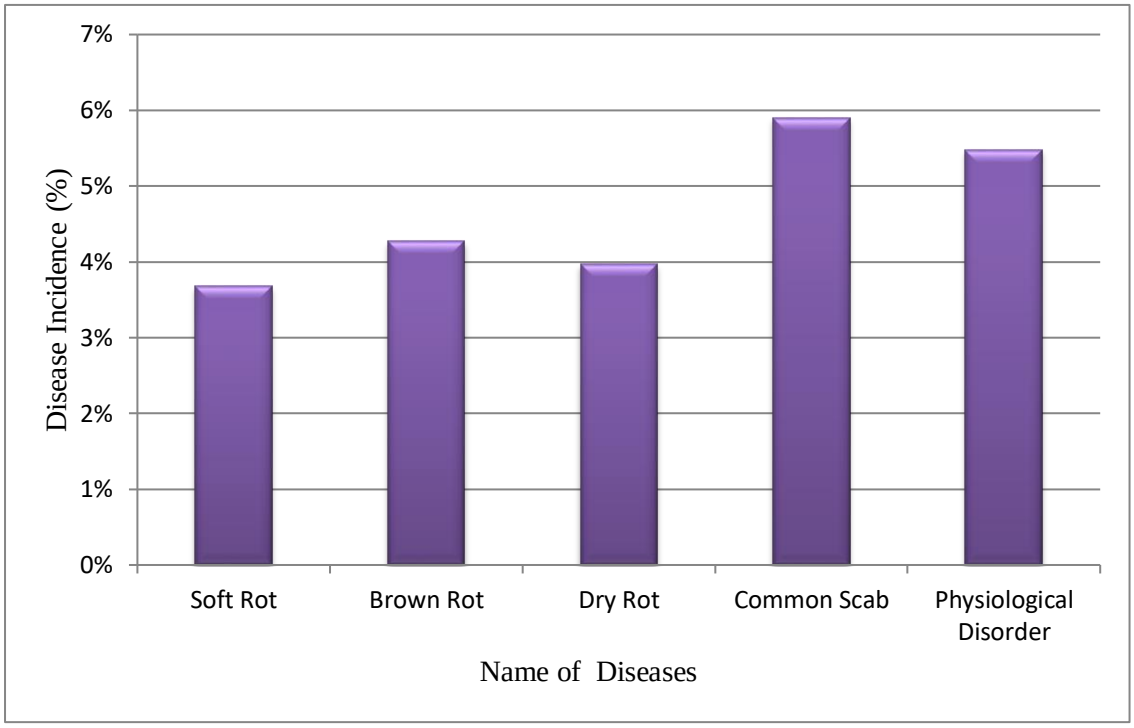

Fig. 6. Prevalence of different potato diseases in the whole sale markets of Dhaka

4.3.Identification of Fusarium spp.

Three species of *Fusarium* were identified namely *Fusarium oxysporum*, *Fusarium solani*, *Fusarium culmorum*, Theses species of *Fusarium* were identified based on their cultural characteristics on PDA medium and the conidia (macro and micro) produced.

4.3.1. Identification of *Fusarium oxysporum*

Characteristicsof *Fusarium oxysporum* on PDA medium

Mycelia was floccose and abundant. White mycelial color was observed on PDA medium. Some isolates of *F. oxysporum* produced dark magenta pigment in the agar medium and some isolates produced no pigment at all.

Characteristics of Macro conidia of *Fusarium oxysporum*

Sporodochia: In some isolates abundant sporodochia was found present and in some isolates sporodochia was found absent.

General morphology: Short to medium in length, straight to slightly curved, relatively slender shaped and thin walled.(Fig. 7)

Apical cell morphology: Tapered and curved.

Basal cell morphology: Foot shaped

Number of septa: Usually 3-septate.

Abundance: Abundant sporodochia was found.

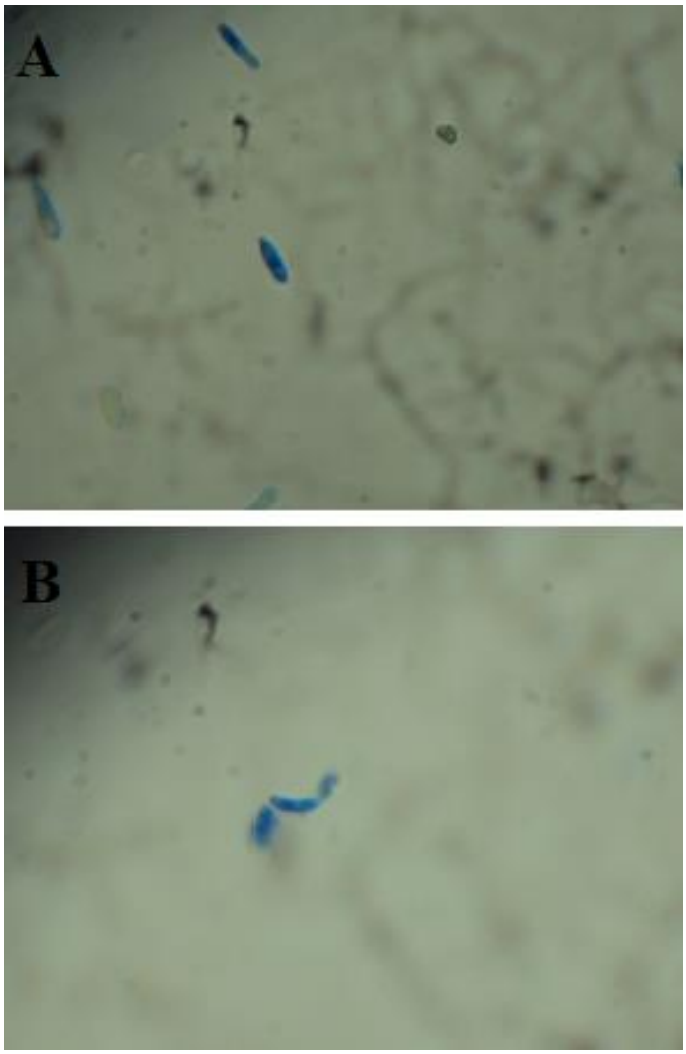

Fig. 7. A and B showing macroconidia of *Fusarium oxysporum* at 40x magnification

4.3.2. Identification of *Fusarium culmorum*

Characteristics on PDA medium.

Fusarium culmorum grows rapidly producing abundant sporodochia. Olive brown mycelium and olive brown pigment in the agar was observed.

Characteristicsof Macroconidia

General morphology: Robust and thick walled. Widest at the mid point of the macroconidium. The dorsal side is somewhat curved, but the ventral side is almost straight.(Fig. 8)

Apical cell morphology: Rounded and blunt.

Basal cell morphology: Notched and without a distinct foot shape.

Basal cell morphology: Notched and without a distinct foot shape.

Number of septa: Usually 3-4 septate.

Abundance: Usually abundant in sporodochia. These macroconidia usually are uniform in shape and size.

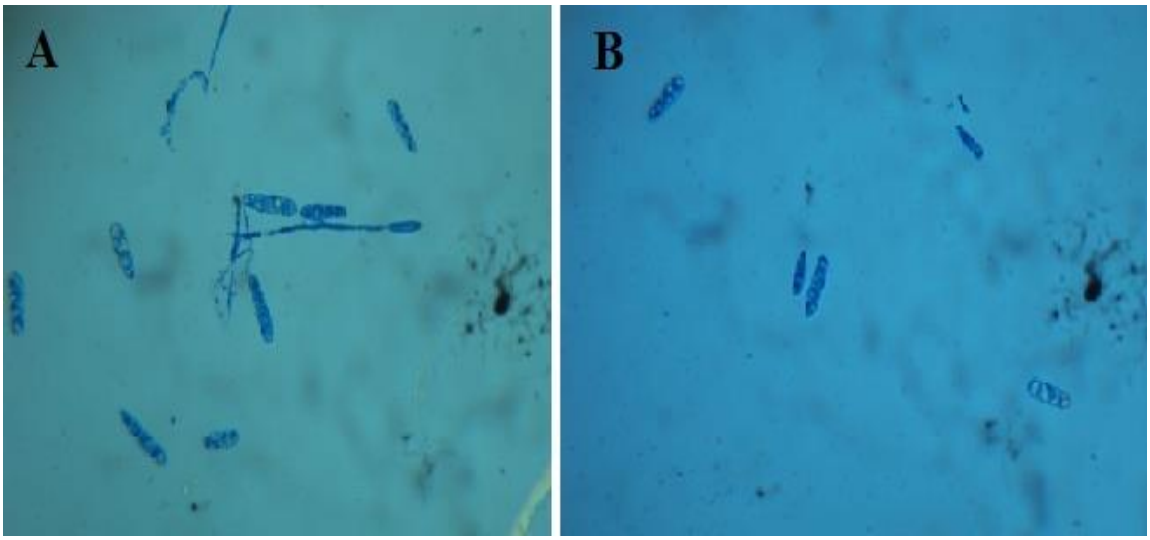

Fig. 8. A and B showing macroconidia of *Fusarium culmorum* at 40x magnification

4.3.3. Identification of *Fusarium solani*

Characteristics on PDA medium.

Cultures of *Fusarium solani* were white with sparse mycelium. No pigmentation was found on agar medium .

Characteristics of Macroconidia

General morphology: Relatively wide, straight and stout.(Fig. 9)

Apical Cell morphology: Blunt and rounded.

Basal Cell morphology: Foot shaped, straight to almost cylindrical.

Number of septa: 4 to 6 septate.

Abundance: Usually abundant in sporodochia.

Characteristicsof Microconidia

Shape/septation: Oval, ellipsoid, and fusiform with 1 to 2 septa.

Aerial mycelium presentation: False heads

Abundance: Abundant in the aerial mycelia.

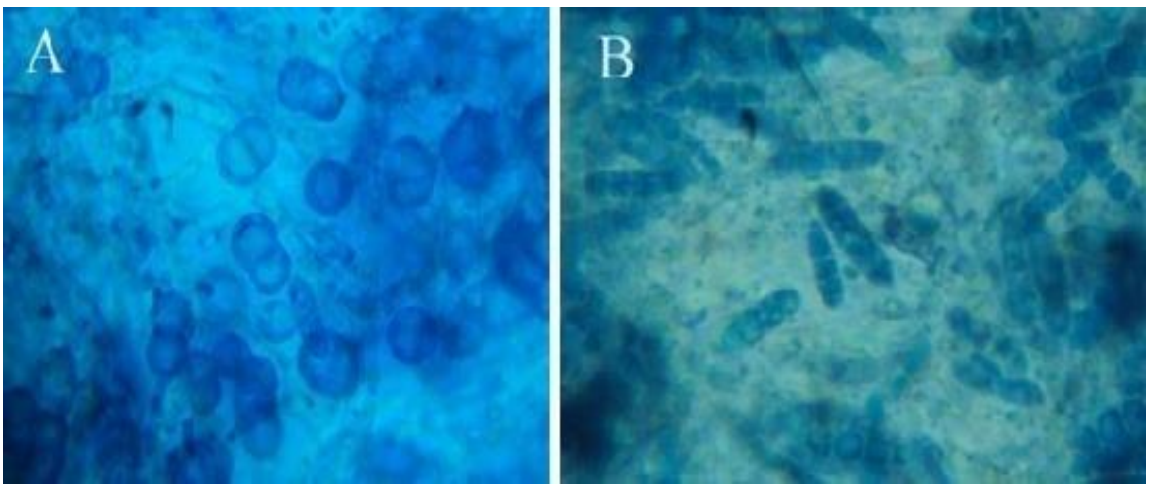

Fig. 9. A. Microconidia of *Fusarium solani* at 40x magnification, B. Macroconidia of *Fusarium solani* at 40x magnification

4.4. Isolation and Identification of Different Bacteria

Several cultural, physiological and biochemical tests were conducted and some selective and semi-selective media were also used to identify and differentiate the bacteria.

4.5. Cultural Characterization of different bacteria on NA plates and different biochemical test results

Cultural characteristics are tools of bacteria as an identifying and classifying bacteria into taxonomic groups. When grown on a variety of media, bacteria exhibit differences in the microscopic appearance of their growth. These differences, called cultural characteristics are used as the basis for separating bacteria into taxonomic group. Growth of different bacteria on NA plates are shown in (Fig. 10) and (Fig. 11) and cultural characteristics of different bacteria are showen in Table. 1 and different biochemical test results are shown in Table. 2 and in (Fig. 14) and (Fig. 15).

Fig. 10. Isolation of bacteria by spread plate method

Fig. 11. Isolation of bacteria by streak plate method

Table 1. Cultural Characterization of different bacteria on NA plates

Isolates	Size	Pigment	Form	Margin	Elevation
Pectobacterium sp	Small	Yellowish	Circular	Entire	Convex
Pectobacterium sp	Small	Yellowish	Circular	Entire	Convex
Pectobacterium sp	Moderate	Whitish	Irregular	Serrate	Raised
Pectobacterium sp	Moderate	Yellowish	Irregular	Serrate	Convex
Pectobacterium sp	Moderate	Whitish	Irregular	Serrate	Raised
R. solanacearum	Moderate	Creamish	Circular	Undulate	Convex
R. solanacearum	Large	Whitish	Irregular	Undulate	Raised
R. solanacearum	Large	Whitish	Irregular	Undulate	Flat
R. solanacearum	Moderate	Creamish	Circular	Undulate	Convex
R. solanacearum	Moderate	Whitish	Irregular	Undulate	Flat

Isolate No.1. *Pectobacterium* sp

Isolate No.2. *Pectobacterium* sp

Isolate No.3. *Pectobacterium* sp

Isolate No.4. *Pectobacterium* sp

Isolate No.5. *Pectobacterium* sp

Isolate No.6. *Ralstonia solanacearum*

Isolate No.7. *R. solanacearum*

Isolate No.8. *R. solanacearum*

Isolate No.9. *R. solanacearum*

Isolate No.10. *R. solanacearum*

Table 2. Biochemical tests for identification of different bacteria

Name of Isolates	KOH solubility test	Gram Staining	Catalase Test	Oxidase Test	Gelatin liquefaction Test	Levan Test	Starch Hydrolysis Test	Potato soft rotting test
Pectobacterium	+	_	+	_	_	+	_	+
Pectobacterium	+	_	+	_	+	+	_	+
Pectobacterium	+	_	+	_	+	+	_	+
Pectobacterium	+	_	+	_	_	+	_	+
Pectobacterium	+	_	+	_	+	+	_	+
R. solanecearum	+	_	+	+	_	_	_	+
R. solanecearum	+	_	+	+	_	_	_	+
R. solanecearum	+	_	+	+	+	+	_	+
R. solanecearum	+	_	+	+	+	+	_	+
R. solanecearum	+	_	+	+	_	_	_	+

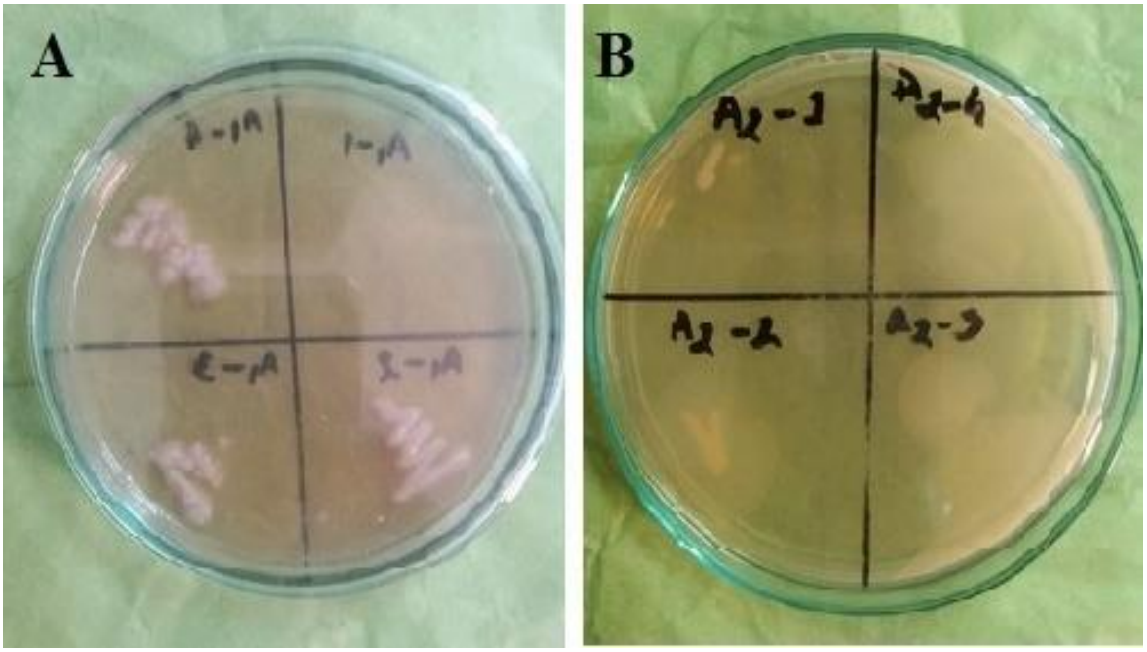

Fig. 12. A and B showing isolation of *Pseudomonas* sp. on Cetrimide agar medium

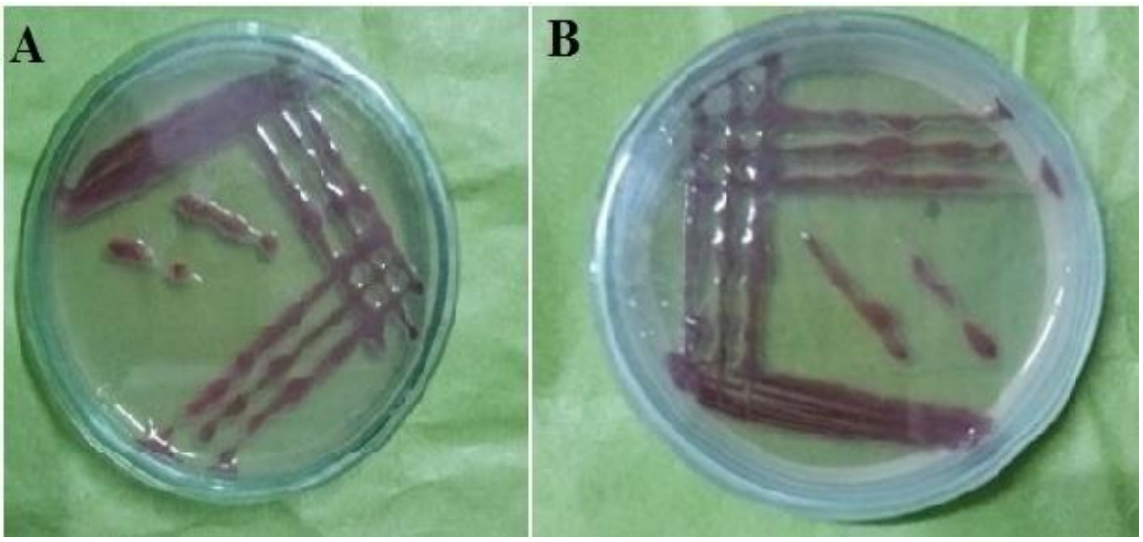

Fig. 13. A and B showing isolation of *Ralstonia solanacearum* on TTC medium

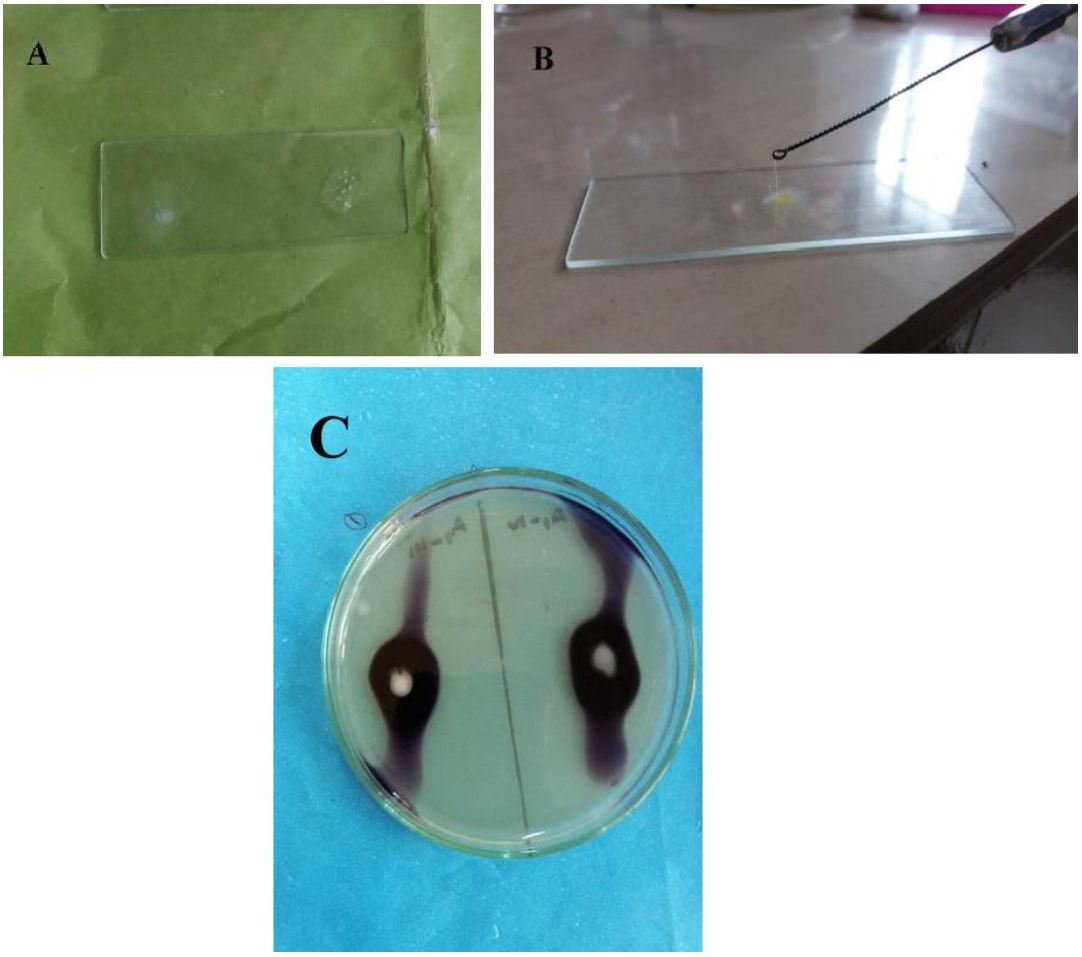

Fig. 14. Biochemical tests- A. Catalase test, B. KOH test, C. Starch Hydrolysis

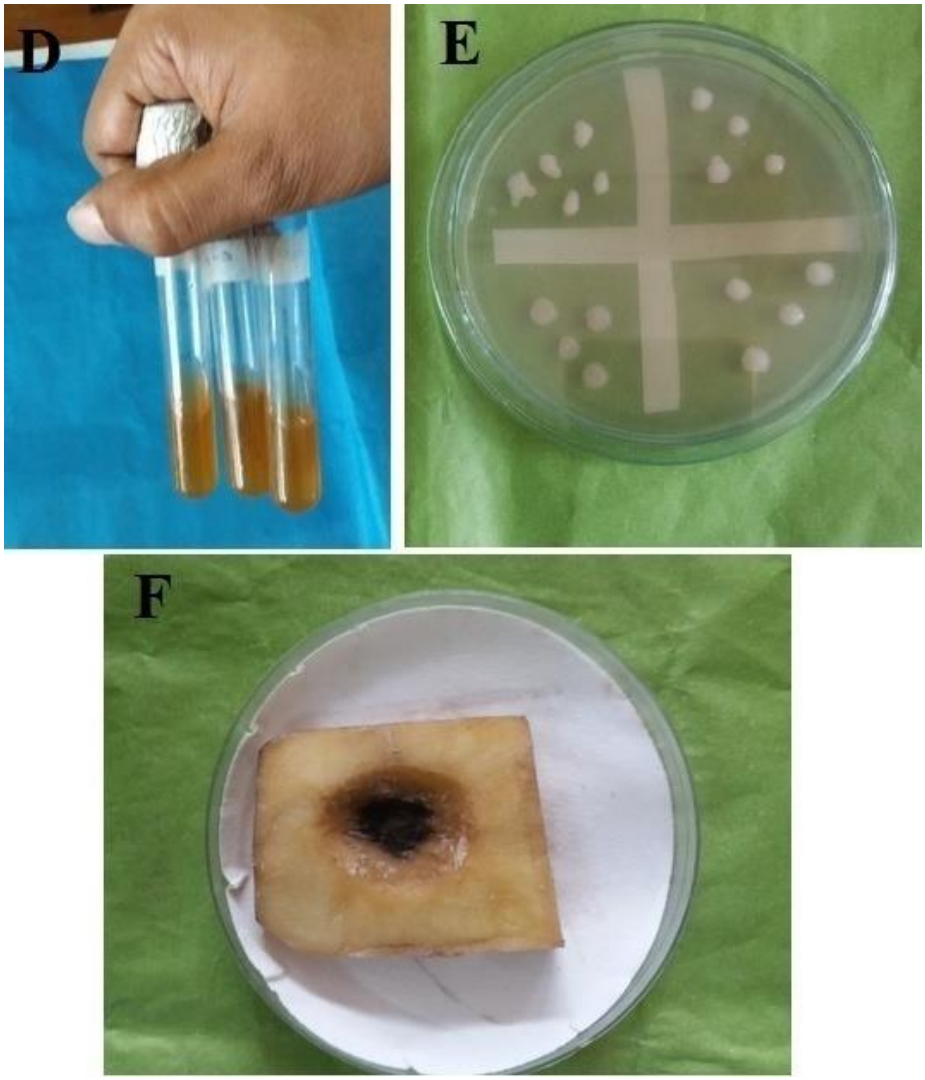

Fig. 15. Biochemical tests- A. Gelatin liquefaction test, B. Levan test, C. Potato soft rotting test

4.6. Isolation and Identification of *Streptomyces scabies* from common scab of potatao.

Streptomyces scabies produced filamentous colonies on solid agar media. Initially colonies consists of substrate mycelium and were smooth and firm to rubbery in texture. The colour of the colonies were dark oblivious green (Fig. 16) The colonies formed after 4-5 days of inoculation. *Streptomyces* are easily differentiated form fungi by their smaller hyphae and spores.

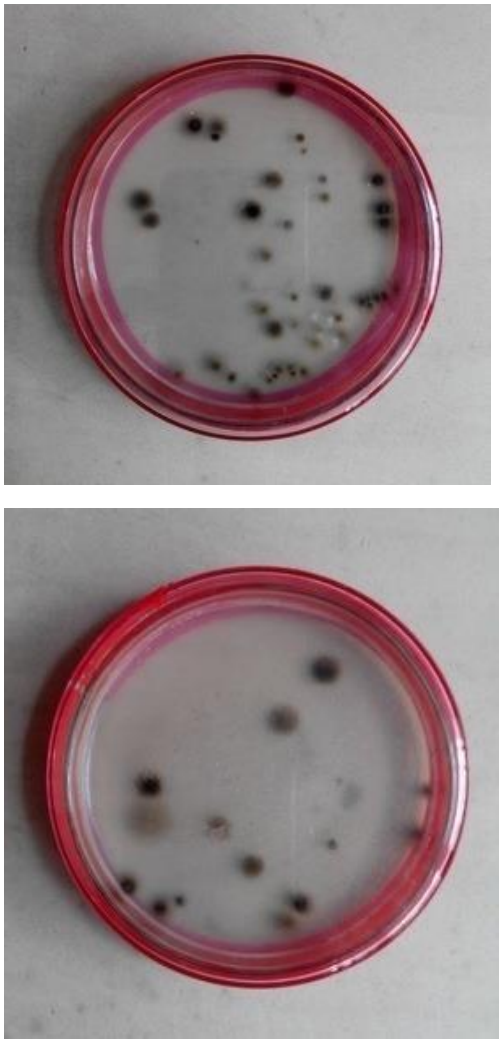

Fig. 16. Isolation of *Streptomyces scabies* on water agar medium

Chapter V
DISCUSSION

The present study was conducted to assess the prevalence of different potato diseases and to identify their causal organisms in selected whole sale markets of Dhaka. Brown rot, Fusarium dry rot, common scab and soft rot of potato were found most common potato diseases in the market places. Physiological disorder hollow heart and black heart was also very frequent. In the present study the disease symptoms were identified by investigating them visually and then the disease causing agents were isolated and several biochemical tests were conducted to identify them.

In the present study three fungal species and four different types of bacteria were isolated and identified. Moist chamber was used to isolate *Fusarium* spp. from diseased potato. *Fusarium* spp. were purified by using PDA medium according to Begum *et al.* (1998). Three species of *Fusarium* was identified namely *Fusarium oxysporum, Fusarium culmorum* and *Fusarium solani*. Their macro and micro conidial characteristics were determined by following the descriptions of Leslie *et al*. (2006). For isolation of bacteria NA medium was used following dilution plate method. After isolation, different bacterial genera were separated by growing them on selective and semi selective media. Cultural characteristics of bacteria were also recorded as they were isolated on NA medium. Bacterial soft rot of potato caused by *Pectobacterium* is known mainly as a post harvest disease (Coplin 1980); Tegene and Korobko (1985). In the present study *Pectobacterium* was differentiated from *Pseudomonous* using cetrimide agar medium following the protocol described by Kumar *et al.*, (2007). The colonies of *Pectobacterium* was found to be creamy white slightly raised and glistening. Under the compound microscope at 100x pinkish rod shaped bacteria with rounded ends was observed, it was confirmed according to the description of Razi (2012). In KOH solubility test a mucoid thread was lifted

with the loop that supports the result of Gram's staining test i.e., the bacteria was gram negative. Olivieri *et al*. (2004) and Mahmoud *et al*. (2008) in their separate works reported the similar result. Smear culture with a drop of hydrogen peroxide (H_2O_2) produced bubbles indicating positive result for catalase tests and on levan test and pectolytic test *Pectobacterium* showed positive results in both cases. Similar biochemical test results were found by Ashmawy *et al*., (2015). *Pectobacterium* were unable to utilize starch what was reported by Bradbury (1986). *Ralstonia solanacearum* is the causal organism of brown rot of potato (Dhital *et al.,* 2001), Large size of fluidal colonies were formed on NA medium and TTC medium after 48-72 hours of incubation by *R. solanacearum*. In TTC medium *R. solanacearum* developed midium, largely unmixed mucoid, magenta and deep red coloured colonies with whitish margins. Same description was stated by Kelman and Person (1954), After the Gram's staining under the compound microscope at 100x magnification with oil immersion, the bacterium was seen rod shaped with rounded ends, cells appeared singly and also in pairs (red colour) and capsulated this indicates the bacterium was Gram negative. Razi (2012) recorded the same result of gram negative bacteria under compound microscope. A mucoid thread was lifted with the loop in KOH solubility test that supports the result of Gram's staining test i.e. the bacteria was Gram negative this result is supported by the research work of Sutton (2006). Positive results were recorded in catalase and oxidase test in case of *R. solanacearum.* Same result was recorded by Dhital *et al.* (2001). *R. solanacearum* showed positive result in leavn test. This result is supported by Ahmed *et al*. (2013). In pectolytic test the causal agent of brown rot of potato (*R. solanacearum*) was confirmed as avirulent strain as it was found negative for some samples and some samples showed positive result and confirmed as virulent strain. It was confirmed on the basis of colony morphology Kelman (1954). Potato common scab, caused by *Streptomyces scabies* (Tajul *et al.* 2013). *Streptomyces scabies* produced filamentous colonies on solid agar media. Initially colonies consists of substrate mycelium and were smooth and firm to rubbery in texture. The colour of the colonies were dark oblivious green.

Streptomyces scabies are easily differentiated form fungi by their smaller hyphae and spores. This result was partially supported by Schaad *et al*. (2000).

In Bangladesh so far as many nine diseases in potato have been recorded, PRA (2015). In the present study three species of *Fusarium* and four types of bacteria were identified, so there might be some other pathogens present in the market places which was not indentified through this experiment. That is for further study is required to identify those pathogens and diseases caused by them.

Chapter VI

Summary and Conclusion

Potato (*Solanum tuberosum* L) is annual, herbaceous vegetable crop of family Solanaceae. It is the 4th most important food crop of the world. In Bangladesh, potato is one of the major food items. Though the demand of potato is increasing day by day, but the whole sellers and the retailers of potato has to face losses every year due to different diseases of potato. Tuber of potato is vulnerable to attack by various diseases. In the market places common scab, Fusarium rot, brown rot, soft rot and physiological disorder hollow heart causes product loss of potato. Therefore, the present study was designed to study the prevalence of the diseases and to identify the causal organisms of different diseases of potato in the market places of Dhaka. Diseased potatoes were collected from five whole sale markets of Dhaka namely Karwan Bazar, Mirpur-1, Mirpur-10, Town hall market and Khilkhet Bazar.

Result of the study showed that the incidence of potato diseases were *Fusarium* spp. causing dry rot (3.97%), *Ralstonia solanacearum* causing brown rot (4.27%), *Streptomyces scabies* causing common scab of potato (5.90%), *Pectobacterium* causing soft rot of potato (3.68%) and physiological disorder hollow heart and black heart were found (5.48%).

The causal organisms of Fusarium dry rot diseases were isolated from the infected tubers using moist chamber followed by inoculation on PDA. Three species of *Fusarium* were identified namely *Fusarium oxysporum, Fusarium culmorum* and *Fusarium solani*.

The causal agent of soft rot of potato was identified as *Pectobacterium* that produced white, smooth, domed shaped, shining, mucoid colonies with entire and serrate edges in NA medium. *Pectobacterium* is a Gram negative bacteria. This

bacteria showed positive results in KOH, Catalase, pectolytic and levan test and they showed negative results in starch hydrolysis and oxidase test. Different isolates of pectobacterium showed both positive and negative results in Gelatin liquefaction test.

The causal organisms of post harvest bacterial diseases of potato were isolated from the infected tuber by following dilution plate technique using nutrient agar medium, water agar medium (Only for *Streptomyces*) TTC medium and Cetrimide agar medium were used to separate bacteria. In TTC medium *R. solanacearum* produced largely unmixed mucoid magenta deep red coloured colonies.

Streptomyces scabies is the causal agent of common scab of potato. The bacteria were isolated on water agar medium from collected samples. The colour of the colonies were dark oblivious green. *Streptomyces* are easily differentiated form fungi by their smaller hyphae and spores.

The present study was based on only the prevalence of the post harvest diseases and their causal organisms in the whole sale markets Dhaka. Further studies will be needed to determine the factors that influence the post harvest diseases of potato in the market places.

Chapter VII

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APPENDICES

Preparation of culture media

The composition of the media used in this thesis work are given below : Unless otherwise mentioned all media were autoclaved at 121^{o}C for 20 minutes at 15 lb pressure.

Nutrient Agar (NA)

Beef extract	3.0 g
Peptone	5.0 g
Bacto agar	15.0 g
Distilled water	1000 ml

Nutrient Broth (NB)

Beef extract	3.0 g
Peptone	5.0 g
Distilled Water	1000 ml

Triphenyl Tetrazolium Chloride (TTC)

2,3,5 triphenyl tetrazolium chloride (Soluble)	10.0 g
Distilled water	1000 ml

KOH solubility reagent

3% aqueous solution of KOH was prepared from the KOH granules.

Catalase reagent

3% aqueous solution of H_2O_2 was prepared from the H_2O_2 absolute solution.

Oxidase reagent

1% aqueous solution of NNN'N-tetramethyl-p-phenylene-diamine dihydrochloride was prepared from the absolute solution.

Gram's staining reagents

Gram's Crystal violet (Hucker's modification)

Solution A : Crystal violet (90% dye content)	2.0 g
Ethyl alcohol	20.0 ml
Solution B : Ammonium oxalate	0.8 g
Distilled water	80.0 ml

Solution A and B in equal volume to prepare crystal violate solution.

Gram's Iodine (Gram's modification of Lugol's solution)	
Iodine	1.0 g
Potassium iodide (KI)	2.0 g
Distilled water	300.0 ml

Add iodine after KI is dissolved in water to prepare Gram's Iodine solution.

Gram's alcohol (decolorizing agent)	
Ethyl alcohol (95%)	98 ml
Acetone	2 ml
Safranin (counter stain)	
Safranin (2.5% solution in 95% ethanol) 10 ml	
Distilled water	100 ml