POST HARVEST DETERIORATION OF POTATO AND ITS CAUSES IN COLD STORAGE OF MUNSHIGONJ

MYNUL HASSAN

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA -1207, BANGLADESH

JUNE, 2016

POST HARVEST DETERIORATION OF POTATO AND ITS CAUSES IN COLD STORAGE OF MUNSHIGONJ

By

MYNUL HASSAN

Reg. No.:10-04174

A Thesis

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

Semester: Jan-Jun, 2016

Approved By:

……..
Supervisor
(Prof.Dr. Nazneen Sultana)
Department of Plant Pathology
Sher-e-Bangla Agricultural University,
Dhaka-1207

……
Co-supervisor
Prof. Dr. M. Salahuddin M. Chowdhury
Department of Plant Pathology
Sher-e-Bangla Agricultural University,
Dhaka-1207

……
Chairman
Associate Prof. Dr. Md.Belal Hossain
Department of Plant Pathology
Sher-e-Bangla Agricultural University,
Dhaka-1207

Dr. Nazneen Sultana
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Mobile: +880-1733955171
E-mail: nazneensau@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled**" POST HARVEST DETERIORATION OF POTATO AND ITS CAUSES IN COLD STORAGE OF MUNSHIGONJ"** 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.) INPLANT PATHOLOGY,** embodies the results of a piece of bona fide research work carried out by **Mynul Hassan**. Registration No. **10-04174**, under my supervision and guidance. No part ofthis 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.

(Prof.Dr. Nazneen Sultana)
Supervisor
Department of Plant Pathology
Sher-e-Bangla Agricultural University,
Dhaka-1207

Dated:25th May, 2017

SAU, Dhaka, Bangladesh

DEDICATED TO

MY

BELOVED PARENTS,

ACKNOWLEDGEMENTS

All praises are due to the Almighty Allah, the great, the gracious, merciful and supreme ruler of the universe to complete the research work and thesis successfully for the degree of Master of Science (MS) in Plant Pathology.

The author expresses the deepest sense of gratitude, sincere appreciation and heartfelt indebtedness to his reverend research supervisor, **Dr. Nazneen Sulatana**, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for her scholastic guidance, innovative suggestion, constant supervision and inspiration, valuable advice and helpful criticism in carrying out the research work and preparation of this manuscript.

The author deems it a proud privilege to acknowledge his gratefulness, boundless gratitude and best regards to his respectable co-supervisor **Dr. M. Salahuddin M. Chowdhury,** Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his valuable advice, constructive criticism and factual comments in upgrading the research work and the documents.

The author would like to express his deepest sense of respect to Associate Professor and Chairman, **Dr. Md. Belal Hossain** for his valuable suggestion during the entire study period.

The author would like to express his deepest respect and boundless gratitude to all the respected teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable teaching, sympathetic co-operation, and inspirations throughout the course of this study and research work.

The author is deeply indebted to his respectful parents for their moral support, encouragement and unquantifiable love with cordial understanding.

Finally the author appreciate the assistance rendered by the staffs of the Department of Plant Pathology and Plant Pathology Laboratory, Sher-e-Bangla Agricultural University, Dhaka, who have helped him during the period of study.

Dated: June, 2016
Place: SAU, Dhaka, Bangladesh The Author

POST HARVEST DETERIORATION OF POTATO AND IT'S CAUSES IN COLD STORAGE OF MUNSHIGONJ

ABSTRACT

An experiment was conducted to observe the prevalence of different diseases of potato in selected cold storages of Munshiganj and to identify the causal agent that causes potato deterioration at cold storages. The survey was conducted at different cold storages of Munshiganj and *In-vitro* experiments were conducted in the MS Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, during the period of July 2016 to May 2017. Potato samples were collected randomly from different cold storages and 200 potatoes were tested to identify the causal organisms. The study showed that the prevalence of the diseases were Dry rot (3.66%), Brown rot (3.21%), Common scab (5.38%), Soft rot (4.82%) and the prevalence of physiological disorder (5.17%). Five causal organisms were identified namely *Ralstonia solanacearum*, *Pectobacterium*, *Pseudomonas* sp, *Streptomyces scabies* and *Fusarium* spp. Bacteria were identified by growing them on selective and semi selective media and conducting different biochemical tests viz. KOH solubility, Gram's staining, Catalase, Oxidase, Levan production, Gelatin liquefaction, Starch hydrolysis, Potato soft rotting and motility test.

CONTENETS

CONTENTS (cont'd)

CONTENTS (cont'd)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

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

Potato (*Solanum tuberosum* L.) is an important vegetable that plays a vital role in global food and nutritional security and alleviation of poverty, especially in Bangladesh. It is the most important vegetable crop in Bangladesh. It is a tuber crop belonging to the family Solanaceae. Bangladeshi vegetable basket is incomplete without potato. In Bangladesh, 461 thousand hectares of cultivable land is under potato cultivation and the country produced 8.3 million tone of potato in 2010-2011 with an average yield 18.0 tha-1 (BBS, 2012). The average yield of potato in Bangladesh is (18.0 tha-1). It contributes 55% of the total vegetable production in Bangladesh (BBS 2012). It is ranked third among the food crops next to rice and wheat on the basis of production. The average yield and total production of potato is about 14.7 t ha-1 and 9.0 million tons, respectively from 0.5 million hectares of land (MOA 2012). Bogra and Joypurhat districts contribute nearly 8% of the total potato production occupying 10.7% of the land surface. The main producing areas are Kalai, Khetlal, Bogra sadar, Munshigonj, Shibganj and Gabtali upazila (Rahman 1990). Area covers under potato cultivation in Bogra and Joypurhat districts about 77% of the total cultivated area (Uddin *et al*., 2009). Latest information on potato production in northern part of Bangladesh is limited, Because there are a great lack of storages facilities. A short harvest period causes about 1.5 million mt of potato get wasted annually. As a result few months after harvest, demand starts to increase and the local market price for potatoes rises threefold. An study under the PROOFS program showed if we were able to prevent 1.5 million mt of potatoes from going to waste through proper storage, an estimated 1 million people can be properly fed for an entire year.

The trend to increase production of potato in Bangladesh is retarded mainly by postharvest problems among which storage is an important one. Potato crop has huge production constraints in the field, in the storage and market, of which soft

rot, dry rot, potato scab, brown rot, hollow heart and black heart are of highest importance. At present two-thirds of the total produce do not find any space in the cold storage and a part of which is consumed shortly after harvest and the rest is kept in traditional storage at home under room temperature and humidity at farm level. These potatoes lose weight gradually and ultimately get shriveled, rotten or sprouted. Thus the quantity of potato is virtually reduced, in the long run, to less than 70 percent of the original production (Rahman 2007) in his study found 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, As a result of this loss price of potato greatly varies between the season and off season. According to (Ahmad 2007) and (Alauddin 2008) potato production is directly proportional to its preservation space. Therefore, if storage facilities are not increased, the production of potato cannot be increased considerably.

Yield losses attributed to dry rot in cold storage ranged from 6 to 25%, with up to 60% of tubers affected in some cases. Stevenson *et al*., (2001); Rahman (1969) and Kamaluddin (1970) reported that 2 to 9% losses of tubers take place every year in each storage due to these diseases. They also reported that of all the diseases in cold storages, dry rot caused by *Fusarium caeruleum* (lib) sacc. 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 Bangladesh. Bacterial soft rot is considered as one of the most destructive diseases of vegetable in storage and transit conditions (Hossain, 1986). Bacterial soft rot commonly occurs in potato tuber after harvest and during storage. Bacteria belonging to the *Pectobacterium* cause soft rot to potato tuber in potato plant in terms of economic importance (Baghee-Ravari, 2011; Czajkowski, 2011). *Erwinia carotovora* pv. *atroseptica*, *Erwinia carotovora* pv. *Carotovora*, and *Erwinia chrysanthemi* are the three soft rots *Erwinias* (Perombelon and Kelman, 1980). Though hollow heart and black heart of potato is not caused by any pathogen, It is very common at cold storage. Brown rot is another most serious disease of potato, although the actual losses due this bacterium in Bangladesh is still not reported however, potato export from

Bangladesh to Russia was halted due the presence of this bacteria in stored potato which caused a loss about 9 million doller in 2014. (EPB, 2015).

A recent report indicated that 0.187 million tons of potato were lost in Bangladesh due to diseases (Anonymous, 2006). 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 cold storage diseases of this crop, except recording the occurrence of a few diseases and only a limited research work has been done. 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 (Fakir 2009). An amount of Taka 8crores approximately was lost annually due to storage disease. (Khalil *et al.*, 2013). Therefore, the emergence of the development of proper storage facilities for potato in Bangladesh is evident. Accordingly, the present study has been undertaken. It may be argued that this study will not only be helpful from academic and theoretical considerations, this should also have practical usefulness.

Considering the above facts, the present study was undertaken with the following objectives:

1. To assess the prevalence of different diseases of potato in selected cold storages of Munshigonj in Bangladesh.
2. To detect and identify different causal agent responsible for postharvest deterioration of potato at cold storage.

CHAPTER II

REVIEW OF LITERATURE

Potato (*Solanum tuberosum*) is one of the most cultivated crop of temeperate and warmer regions of Bangladesh. There are various diseases of potato, Among them Dry rot, soft rot, brown rot and common scab have been considered as the most important diseases of potato in Bangladesh at cold storage. Literatures available on these diseases are enlisted here.

2.1. Importance of the potato

Ahmed (2013) reported that In Bangladesh, the supply of high quality seed potatoes including import and local multiplication by private sector covers only 5% of the total seed requirements.

Shaheb (2013) studied that Farmers are facing various problems to produce seed potato viz. lack of quality seed, disease and insect problem, high price of quality seed, lack of knowledge on quality seed potato production etc.

BBS (2012) stated that Potato contributes 55% of the total vegetable production in Bangladesh .

BBS (2012) reported that Potato (*Solanum tuberosum* L.) is a popular tuber crop and in terms of production, which ranks third as food crop in Bangladesh.

BBS (2012) reported that In Bangladesh, 461 thousand hectares of cultivable land is under potato cultivation and the country produced 8.3 million tone of potato in 2010-2011 with an average yield 18.0 tha-1.

BADC (2012) studied that Bangladesh Agricultural Development Corporation (BADC) has been supplying only 20,000 tons of quality seed-potato among the farmers as against the annual demand of about 0.6 million tons.

The average yield and total production of potato is about 14.7 t ha-1 and 9.0 million tons, respectively from 0.5 million hectare of land. (MOA, 2012).

Czajkowski (2011) reported that Potato is the fourth most abundant food crop of the world after rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*).

Johnson *et al.* (2010) stated that Potato tuber has been used for processing, mainly frozen chips and dehydrated products.

ISAAA (2010) reported that The country needs around 1 million tons seed potato each year, of which only 2-3% is supplied by the government.
CIP (2009) reported that The global total potato crop production exceeds 300 million metric tons and more than a billion people worldwide consume potatoes, which is also ranked third.

Uddin *et al.* (2009) stated that Area covers under potato cultivation in Bogra and Joypurhat districts about 77% of the total cultivated areas.

Hossain *et al.* (2008) reported that it is also used in processing industries at a small scale and utilizes between 80,000 to 100,000tons of potatoes annually.

The average yield of potato in Bangladesh (18.0 tha-1) is very low compared to those in other potato producing countries (Anonymous, 2008).
FAOSTAT (2008) stated that Potato production in Bangladesh is quite low in comparison to that of the leading potato growing countries of the world such as 70.84 million metric ton in China and 34.66 million metric ton in India.

Potato production is directly proportional to its preservation space. (Alauddin 2008 and Ahmad 2007).

Hossain *et al.* (2008) reported that the national average yield of potato is also very low (19.07 tha-1) compare to its potential yields of 30-40 tha-1 due to lack of quality seed, cultivation of indigenous potato tubers and high price of quality seed potato.

Rahman (2007) stated that the quantity of potato is virtually reduced, in the long run,to less than 70 percent of the original production.

Rahman (1990) reported that The main potato producing areas are Kalai, Khetlal, munshigonj, Bograsadar, Shibganj and Gabtali upazila .

2.2. Effect of diseases on cold storage

EPB (2015) reported that Potato export from Bangladesh to Russia was halted due the presence of this bacteria in stored potato which caused a loss about 9 million doller in 2014.

Czajkowski (2011) stated that Approximately 22% of potatoes are lost per year due to viral, bacterial, fungal, and pest attack to potato tuber and potato plant, incurring an annual loss of over 65 million tones.

Fakir (2009) reported 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 potato in cold storage in Bangladesh it was found that 2-9 percent of cold stored potatoes were lost in every year due to different diseases.

Some diseases cause more severe damage and progress more rapidly than others, especially when secondary bacterial soft rot creates “hot spots” that can rapidly lead to the breakdown of an entire pile of potatoes in storage. (Nora Olsen; Jeff Miller; and Phil Nolte 2006)

A recent report indicated that 0.187 million tons of potato were lost every year in Bangladesh due to different diseases. (Anonymous, 2006).

Stevenson *et al*. (2001) reported that Yield losses attributed to dry rot in cold storage ranged from 6 to 25%, with up to 60% of tubers affected in some cases.

Perombelon and Kelman(1980) reported that *Erwinia carotovora.* pv. *atroseptica*, *Erwinia carotovora* pv. *carotovora* and *Erwinia chrysanthemi* are the three soft rots *Erwinias* in cold storage.

Khan *et al*. (1973) stated that 9.5 to 22% tuber losses occur in cold storage in Bangladesh in which 3 to 11% tuber loss were from only soft rot.

2.3. Soft rot disease

Bacteria belonging to the *Pectobacterium* cause soft rot to potato tuber in potato, in terms of economic importance (Baghee-Ravari 2011; Czajkowski 2011).

Czajkowski a. (2011) reported that Bacterial soft rot commonly occurs in potato tuber after harvest and during storage. Several bacterial species under different genera produce various cell-wall degrading enzymes that allow infiltration and maceration of parenchymatous tissues of a wide range of plants on which they feed.

Czajkowski b. (2011) stated that Identification of causative bacterial strains help significantly take appropriate control measures, develop detection systems and resistant plants against causative strains through conventional breeding as well as genetic modifications schemes.

Although many bacteria possess ability to produce tissue macerating enzymes, only few of them such as *Pectobacterium atrosepticum (Pa)*, *Pectobacterium carotovorum* sub sp. *carotovorum (Pcc),* cause soft rot in potatoes (Baghee-Ravari 2011; Czajkowski 2011; De Boer 2003; van der Wolf and De Boer 2007).

Molecular analysis which is more reliable for soft rot identification, than the conventional biochemical and physiological analysis should be done to draw a solid conclusion (Baghee-Ravari 2011; De Boer and Kelman 2000).

Baghee-Ravari (2011) stated that Biochemical and physiological techniques as the characterization tools to characterize pathogenic strains, Since the molecular characterization tools are more reliable than those of the biochemical and physiological ones.

According to Nabhan *et al*. (2006) and Togashi (1988) the inoculated (slices were maintained in moistened petridishes) and incubated bacterial plate were kept at 30°C for 2 to 3 days.

Although potato is the third crop plant in Bangladesh after rice and wheat, little is known about the characteristics of soft rot bacterial strains. Infact, research reports on soft rot bacteria of potato are very scanty in Bangladesh. (Islam 2004 and Rasul *et al*.1999).

Nourian *et al*. (2002) reported that bacterial soft rot caused by *E. carotovora* (Ecc) is a major disease in stored potatoes.

Mortensen (1997) and Kim *et al*. (2002) stated that soft rot bacterial strains were isolated from different samples of potato by “Streak plate” technique.

Agrios (1997) reported that the soft rot symptoms begin with small water soaked lesions, which gradually become soft, mushy, disintegrated, depressed, and discolored. The affected tissues become creamy and slimy in color and gradually become a mass of disorganized cells. Sometimes the whole tuber turns into a soft, watery, decayed mass within 3 to 6 days.

Pérombelon and Kelman (1980) stated that *Ecc* and *Ech* are the causative agents of blackleg and soft rot in the temperate regions (<25°C).

Kelman (1953) observed that after 48-52 h of incubation at 28°C *Ralstonia* gave circular, smooth, convex and viscous bacterial colonies with pink center and whitish margin on 2,3,5 Tetrazolium Chloride (TTC) medium. On NA medium the bacteria produce watery whitish or off white or cream color irregular colonies.

2.4. Dry rot disease

According to Charles Tortoe *et al.* (2010) *Aspergillus flavus* was the most dominant fungal species in cold storage followed by *Aspergillus niger* and *Fusarium oxysporum*.

According to a report of Charles Tortoe *et al*. (2010) *Aspergillus flavus* was the most dominant fungal species during postharvest storage condition of sweet potato followed by *Aspergillus niger*, *Rhizopus stolonifer*, *Trichoderma viride*, *Fusarium oxysporum*, *Penicillium digitatum*, *Cladosporium herbarum*, and *Aspergillus ochraceus*.

All the diseases in cold storages, dry rot caused by *Fusarium caeruleum* (lib) sacc. 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 Bangladesh. (Stevenson *et al*. 2001; Rahman 1969 and Kamaluddin 1970).

Singh and Singh (2000) reported that The bacteria were sprayed in the drop of sterilized water over the slide and were isolated by streaking onto nutrient agar plate and incubated at a room temperature of 26°C for 48 h.

According to a report of Mandal (1981), *Aspergillus niger* infection on potato started around a wound as small water soaked dull area with white mycelia growth, followed by black sporulation of the fungus. *Geotrichum candidum* infection was characterized by softening of internal tissue with foul odour.

2.5. Common scab

Lehtonen *et al*. (2004) conducted a time-saving and cost-effective polymerase chain reaction (PCR)-based method was developed for species-specific identification of the scab pathogens (*Streptomyces scabies* and *S. turgidiscabies*) prevalent in potato (*Solanum tuberosum*) in northern Scandinavia. Species specificity of primers was verified using a collection of previously characterized *Streptomyces* strains isolated from potato scab lesions in Finland and Sweden. A total of 1245 scab lesions was tested from potato cvs Matilda and Sabina grown in the field in two geographic regions of Finland in 2000 and 2001. Freshly harvested or stored potato tubers were incubated at room temperature (18-21°C) under humid conditions for a few days. Bacterial growth was collected from scab lesions for DNA isolation and PCR. The two scab pathogens were detected in same potato fields, tubers and scab lesions. The relative incidence of *S. scabies* was high in freshly harvested tubers but was much lower than that of *S. turgidis cabbies.*

Banyal (2002) reported that A survey of potato diseases was conducted in Lahaul Valley of Himachal Pradesh, India during potato harvesting in 1997-98. Common scab caused by *Streptomyces scabies* was observed in all locations surveyed the severity of these diseases ranged from 13 to 27% during 1997 and 1998.

Singh *et al*. (2000) reported that The effect of temperature (5, 10, 15, 25, 30, 35, 40, 45, and 50°C) and pH (4-10) were observed on the growth of *Streptomyces scabies* isolated from diseased potato. Optimum growth was observed at 28°C and pH 7.

Genet (1999) and Lehtonen *et al*. (2004) described the characteristics of common scab potato disease caused by *S. scabies.*

Prevalence of *Streptomyces scabies* (*S. scabies*) in major potato growing areas of Varanasi and its neighboring districts showed much variation in different parts of

eastern Uttar Pradesh during 1995-96, with Adalpura and Kushaha in Mirzapur district showing the highest disease incidence and scab index. During 1996-97, disease incidence and scab index were highest at Bhaura (Jaunpur district), followed by Dorva and Pahalupur (Allahabad district). Only potato varieties Kufri Sindhuri and Kufri Lalima showed moderate resistance (Mishra and Srivastava 1999).

Sharma *et al*. (1989) found out the distribution, symptoms, pathogen (*Streptornyces scabies*), host pathogen interaction, and sources of infection, predisposing factors, variation and physiological specialization of this disease.

2.6. Brown rot disease

Janse (2007) reported that Symptoms of brown rot caused by *Ralstonia solanacearum* in a potato tuber was light brown vascular discoloration, from the infected vascular tissues cream-white bacterial slime oozes spontaneously. The black tissues were caused by secondary rotting micro-organisms.

Janse (2006) studied that Small, red colored cells (Gram stain) of *Ralstonia solanacearum* in a spiral vessel (smallest element in vascular tissue) of brown rot affected potato tuber.

The disease brown rot of potato incited by *Ralstonia solanacearum*; was reported as isolated incidents in 1995 from France and from the Netherlands in the early 1990s and several outbreaks in 1995 (EPPO, 2005).

Keshwal *et al*. (2000) reported on the effects of physical soil properties, it was found that sandy loam soil with a high sand content and low silt or clay content, with low water-holding capacity was unfavorable for the pathogen and wilt incidence. Elevated disease levels were expressed in clay soils with high water-holding capacities.

Yabuuchi *et al*. (1995) reported that the bacterium of brown rot disease is placed in the genus *Ralstonia.*

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 different cold storages at Munshigonj namely Bicrompur multipurpose cold storage, Kohinur cold storage, Munshiganj ice and cold storage, Tangibari cold storage. From each cold storage 6 kg of potatoes were collected by random sampling. From the collected samples diseased samples containg common scab, soft rot, *fusarium* dry rot, and brown rot were separated from healthy ones.

3.4. Measurement of prevelance of disease occurrence in cold storage

Disease incidence of infected potato was determined by the following formula (Rai and Mamatha, 2005)

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

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˚-6˚ C for further study.

3.6. Isolation of causal organism

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

The collected disease plant specimens were first washed in tap water to make free from sand and soils. The infected portion along with the healthy portion of the plants were cut into small pieces (0.5-1.0 cm) and surface sterilized with 1% clorox for 2-3 minutes. Then the plant 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. After 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. 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 were identified following the key outlined by Booth (1971) and Singh (1982). *Fusarium* spp were purified by using PDA (Begum *et al.* 1998). The pure culture of the pathogens were preserved in PDA slants at 5$\pm$1˚C in refrigerator as stock culture for future use.

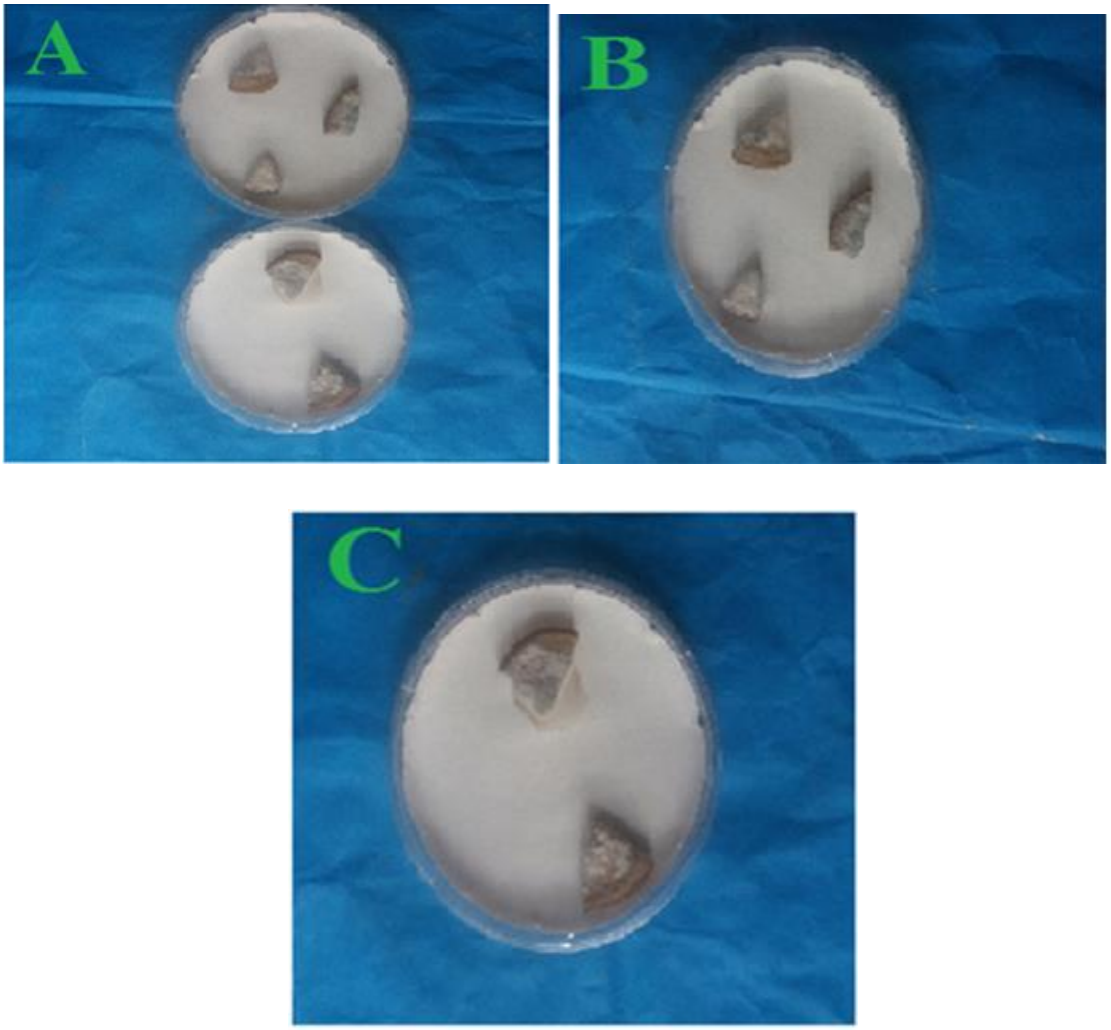


Fig. 1. Isolation (A, B and C) of *Fusarium* dry rot

3.6.2. Preparation of Potato Dextrose Agar (PDA) media

Potato dextrose agar (PDA) medium was prepared as described by Islam (2009). 200 g peeled potato extract, 20 g dextrose and 17 g agar were 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˚C 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 20 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. Then it was 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 they 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 grew over NA plate was re-streaked on fresh NA plate with the help of a loop to get pure colony.

3.7.3. Preparation of Triphenyltetrazolium chloride (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. Then 1% stock solution of TTC medium 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°C for future use.

3.7.4. Preparation of CPG media

CPG media was prepared by adding 1 g casamino acid, 10 g peptone, 5 g glucose, and 17 g agar in 1000 ml distilled water. The mixture is than 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 into several petridish.

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 solanesiarum* were selected on the basis of characteristic colony charter on TTC medium (Kelman 1954).

3.7.7. Preparation of Citrimide agar

In an 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 to mix the elements properly. After that, it was autoclaved at 121°C 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°C. Viruent colonies of *Pseudomonous* were selected on the basis of growth of bacteria on Citrimide Agar medium.

3.7.9. Growth of *Streptomyces* on water agar

Water agar is the medium of choice for isolation of *Streptomyces* 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, a piece of the tissue was removed from 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 was 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 al.* 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 on 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 on drawing it with a loop and 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 loop-full bacterial culture was stab inoculated into the tube containing 12% (w/v) gelatine with the help of a sterile transfer loop. Then it was incubated at 30˚C 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 loop-full bacterial culture was streak inoculated with the help of a sterile transfer loop. Then it was incubated at 30˚C 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 and they were cut 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.

CHAPTER IV

RESULTS

4.1. Visual symptoms of collected samples

4.1.1. *Fusarium* dry rot

Dry rot causes the skin of the tuber to wrinkle (Fig. 2). The rotted areas of the potato were brown. Rot depressions in the surface was seen on the potato tuber. Tubers were shriveled to form “mummies”.

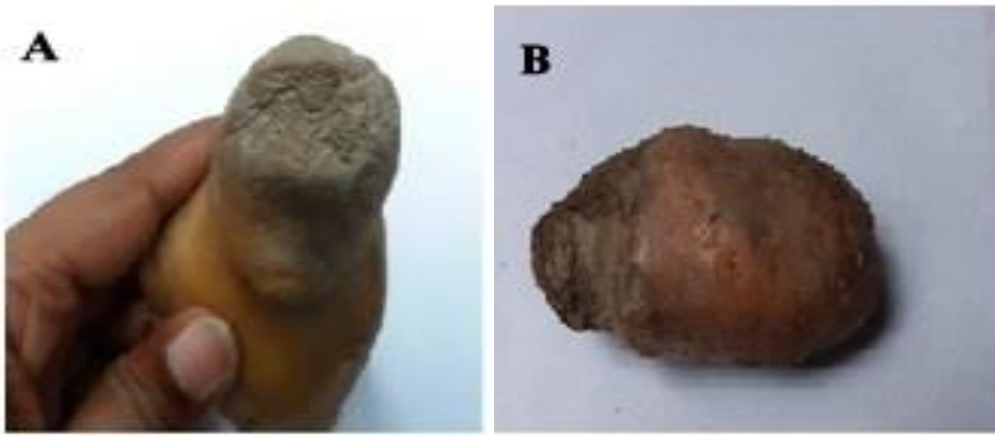


Fig. 2. (A and B) *Fusarium* dry rot.

4.1.2. Soft rot

Water soaked soft tissues were observed on infected tubers (Fig. 3). The healthy part of a tuber was clearly distinguishable from the macerated, creamy infected part. There was a foul smelling odour as the potato is broken down by the bacteria.

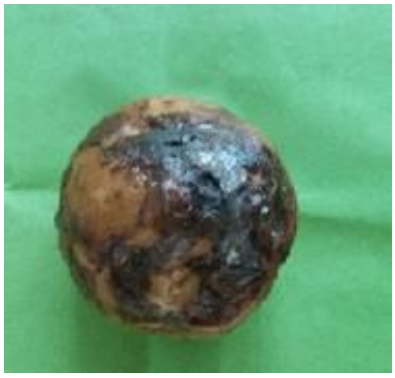


Fig. 3. Soft Rot

4.1.3.Common scab

Affected tubers were found either shallow or deep scabs (Fig. 4) or a combination of both. A shallow scab was seen causing a superficial, roughened and russeted area on the tuber. A deep scab was observed consisting of lesions which was 1mm to 10mm deep, roughly circular, upto 10mm in diameter and surrounded by corky tissue.

Fig. 4. Common Scab (A. Shallow Scab B. Deep Scab)

4.1.4.Brown rot

Grey lesion was observed on infected potato tuber (Fig.5). After cutting the tuber it was observed that a milky-white sticky exudates (ooze) deposited on the lesion which indicated the presence of bacterial cell in the infected tuber.

Fig. 5. Brown Rot of Potato

4.2. Measurement of prevalence of disease occurance in cold storage

The collected samples were infected by *Fusarium* spp. causing dry rot of potato (3.66%), *Ralstonia solanacearum* causing brown rot (3.21%), *Streptomyces scabies* causing common scab of potato (5.38%), *Pectobacterium* causing soft rot of potato (4.82%) and physiological disorder hollow heart and black heart were found (5.17%).

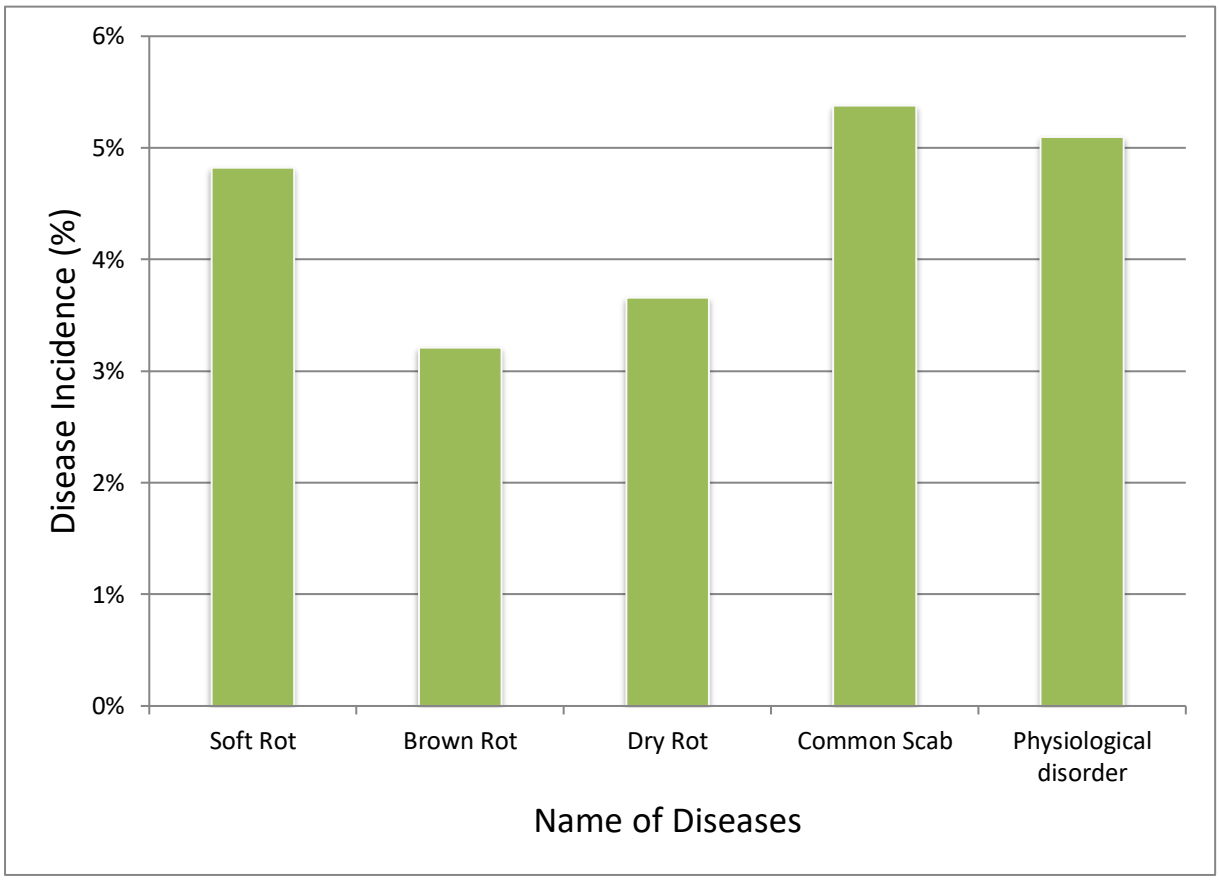


Fig. 6. Incidence of Different Potato Diseases in cold storages.

4.3. Identification of *Fusarium* spp.

4.3.1. Identification of *Fusarium oxysporum*

Characteristic on PDA medium

Mycelia was floccose and abundant. Mycelial color was seen white on PDA. Some isolates of *F. oxysporum* produced dark magenta pigment in the agar and some isolates produced no pigment at all.

Characteristics of Macroconidia

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.

Apical Cell morphology: Tapered and curved.

Basal Cell morphology: Foot shaped

Number of septa: Usually 3-septate.

Abundance: Abundant sporodochia was found.

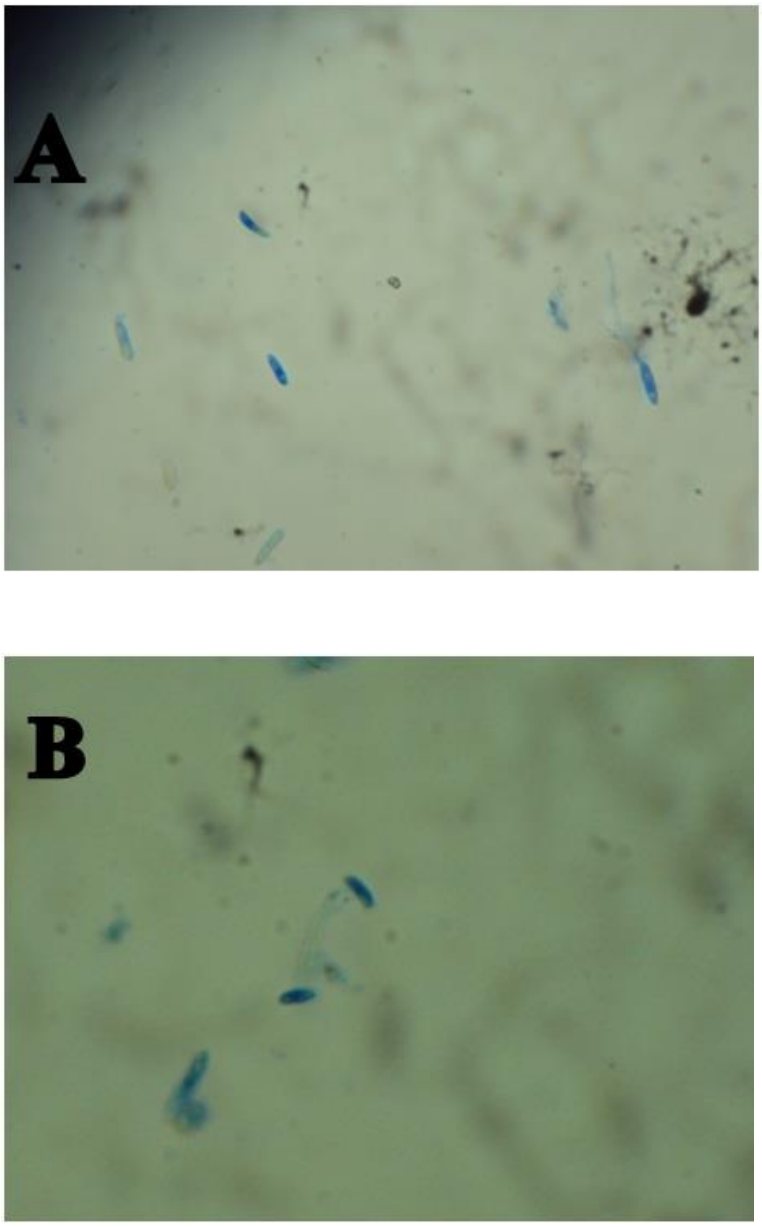


Fig. 7. Macroconidia (A and B) of *Fusarium oxysporum* (at 40 objective).

4.3.2. Identification of *Fusarium culmorum*

Characteristics on PDA medium.

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

Characteristics of Macroconidia

General morphology: Robust and thick walled. Wider at the midpoint of the macroconidium. The dorsal side was somewhat curved, but theventral side was almost straight.

Apical Cell morphology: Rounded and blunt.

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

Number of septa: Usually 3- or 4-septate

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

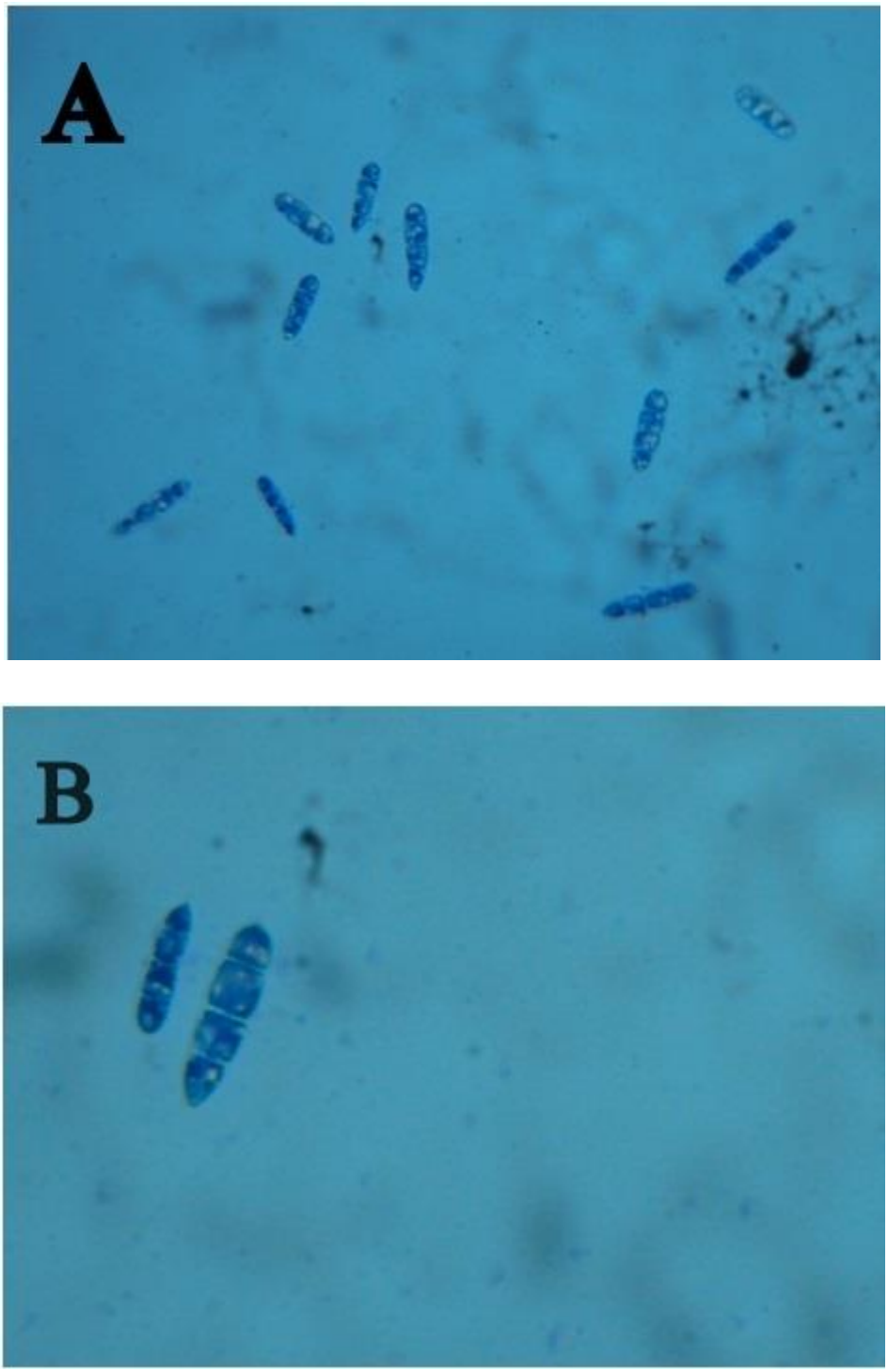


Fig. 8. Macroconidia (A and B) of *Fusarium culmorum* (at 40 objective).

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.

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.

Characteristics of Microconidia

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

Aerial mycelium presentation: False heads

Abundance: Abundant in the aerial mycelia.

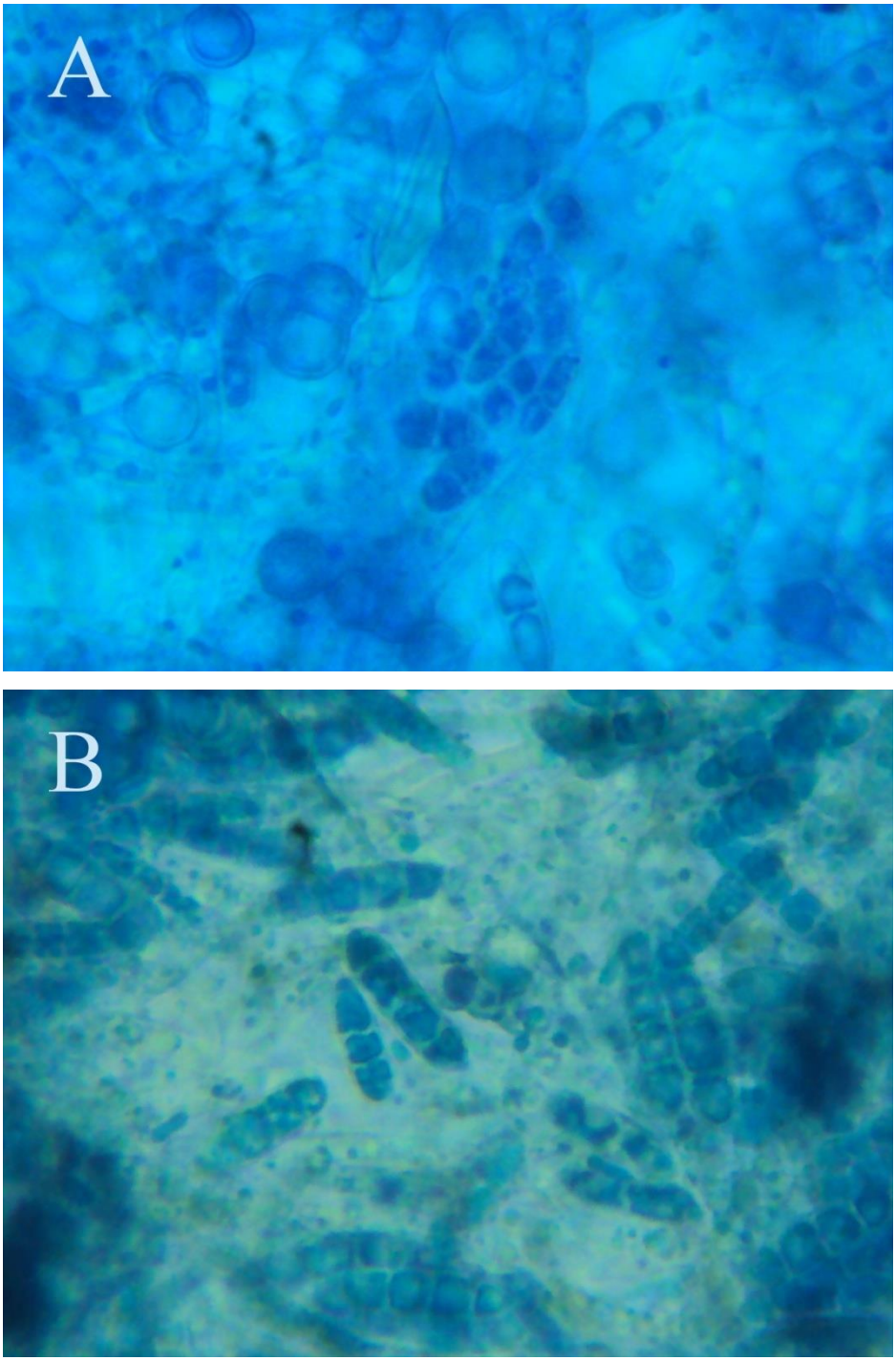


Fig. 9. Micro and Macroconidia (A and B) *Fusarium solani* (at 40 objective).

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.

Were to determine the cultural characteristics 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 were used as the basis for separating bacteria into taxonomic group.

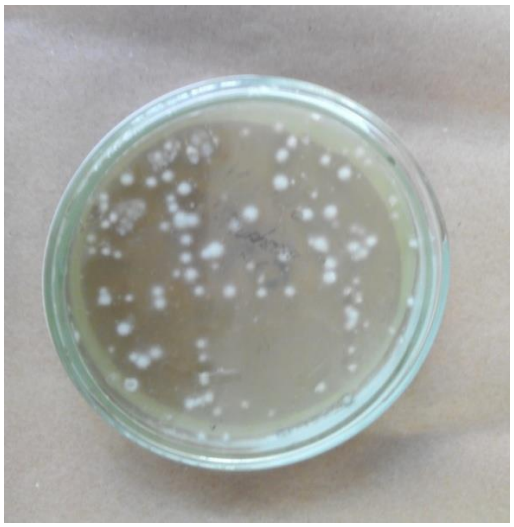


Fig. 10. Isolation of bacteria by spread plate method

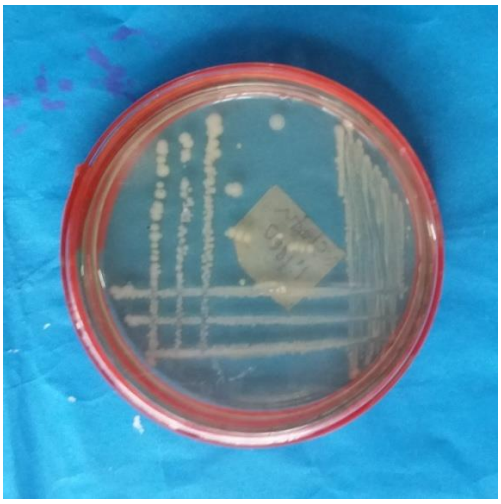


Fig. 11. Isolation of bacteria by streak plate method

Table 1. Cultural Characterization of different bacteria on NA plates

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

- Isolates 1-6: *Pectobacterium*
- Isolate 7: *Pseudomonas* sp.
- Isolates 8-10: *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 softening test
Pectobacterium	+	_	+	_	+	+	_	+
Pectobacterium	+	_	+	_	+	+	_	+
Pectobacterium	+	_	+	_	_	+	_	+
Pectobacterium	+	_	+	_	_	+	_	+
Pectobacterium	+	_	+	_	_	+	_	+
Pectobacterium	+	_	+	_	+	+	_	+
Pseudomonas sp	+	_	_	+	+	+	+	_
R.solanecearum	+	_	+	+	+	+	_	_
R.solanecearum	+	_	+	+	_	+	_	+
R.solanecearum	+	_	+	+	_	+	_	_

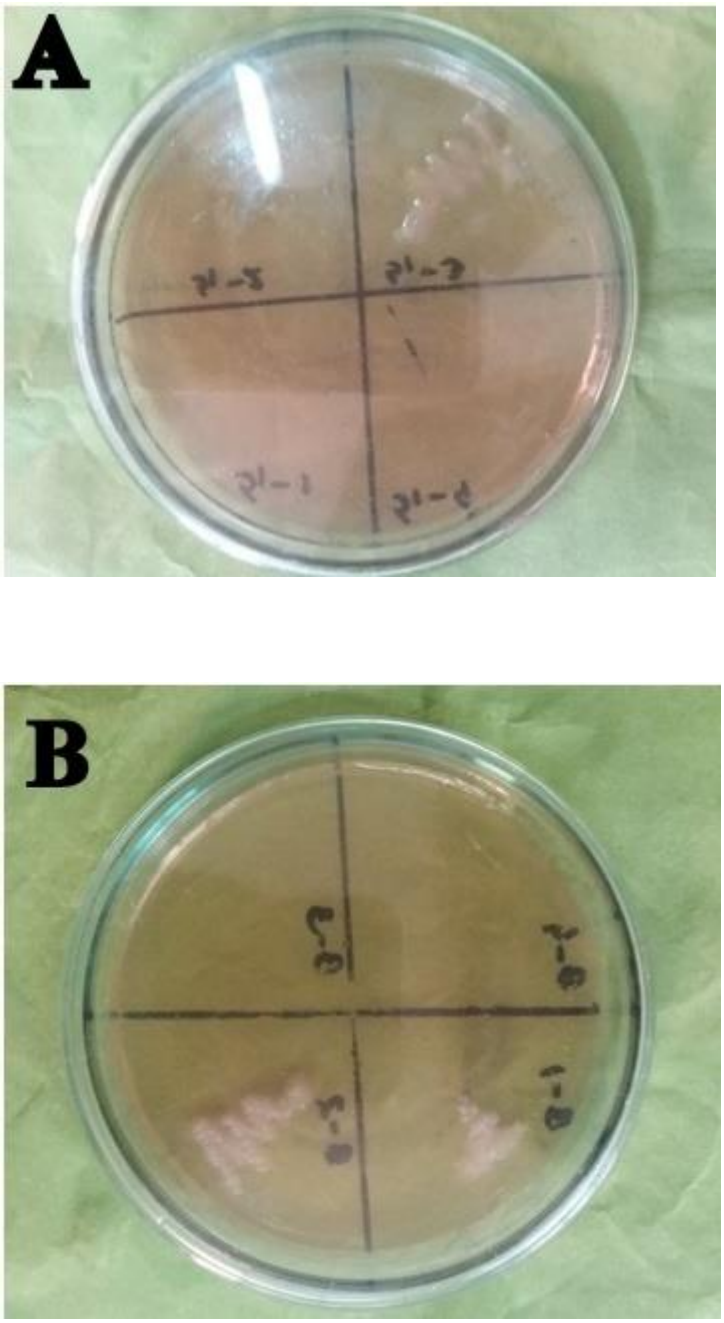


Fig. 12. Isolation of *Pseudomonas* spp (A and B) on Cetrimide agar medium

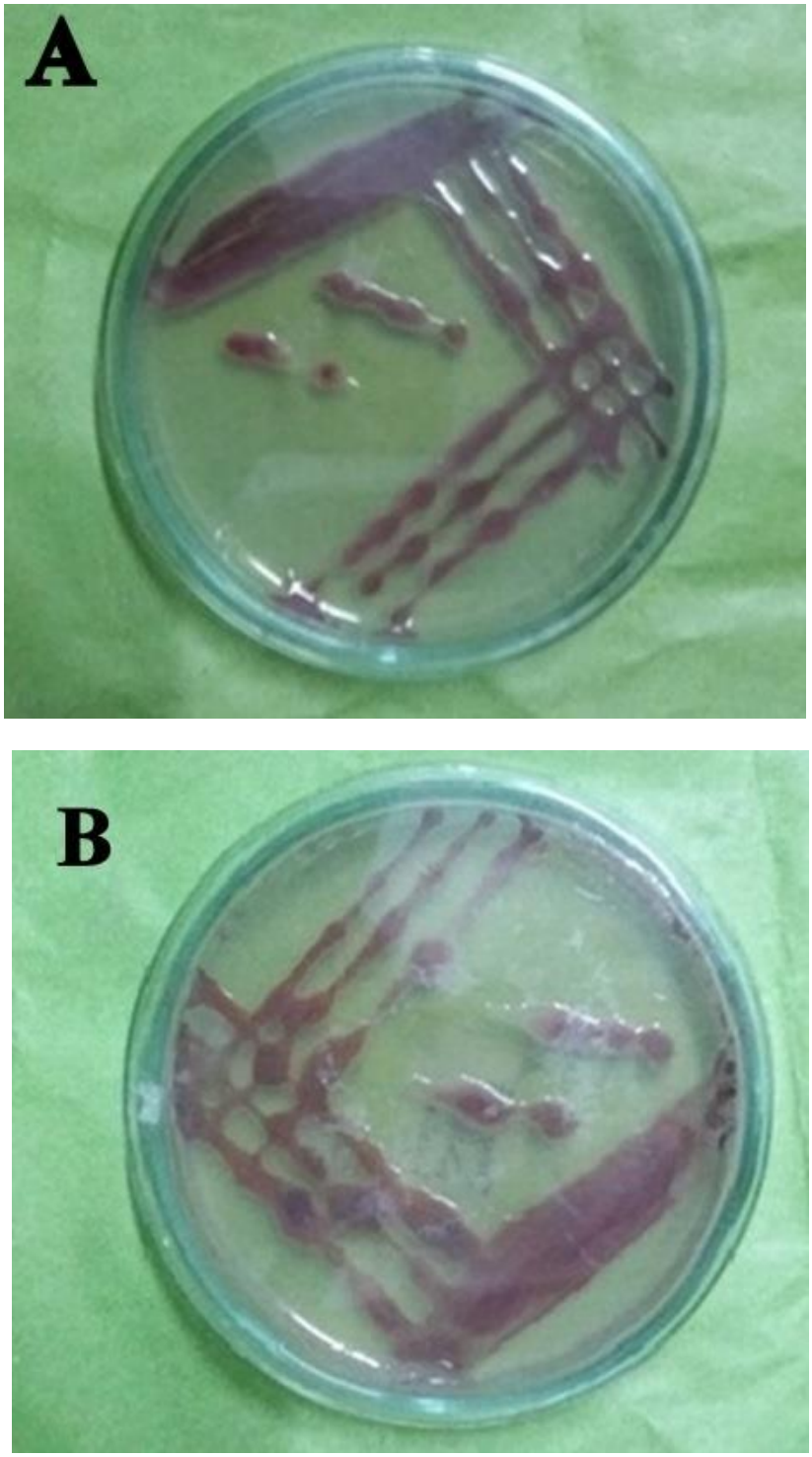
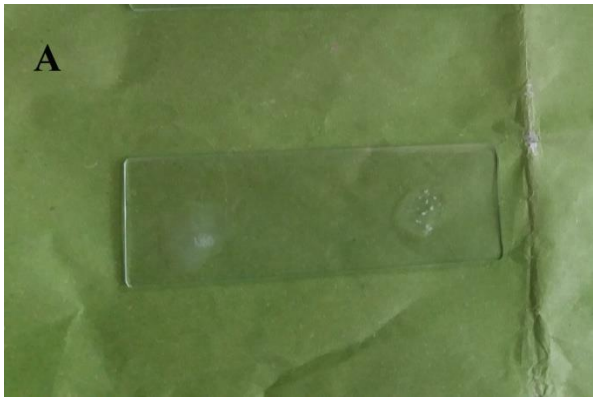
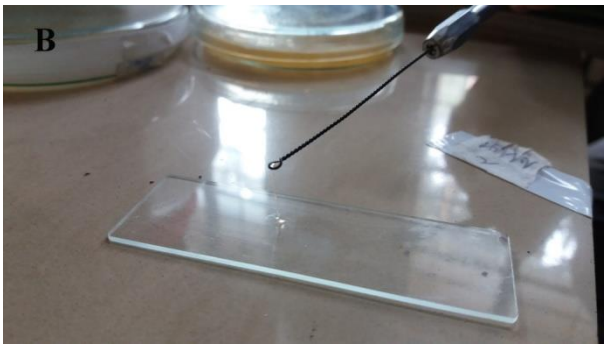


Fig. 13. Isolation of *Ralstonia solanacearum* (A and B) on TTC medium





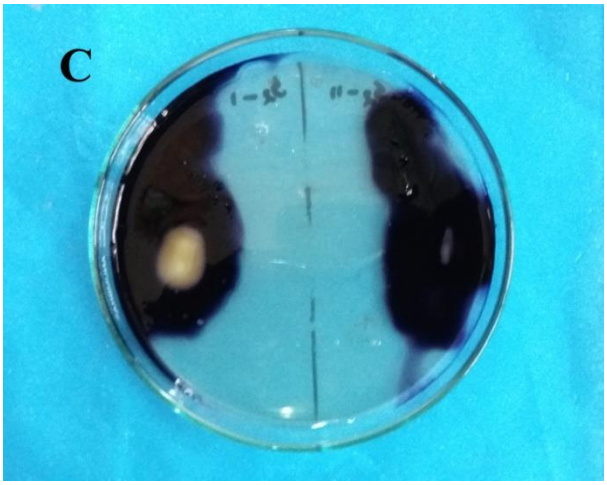


Fig. 14. Biochemical tests A. Catalase test B. KOH test C. Starch hydrolysis

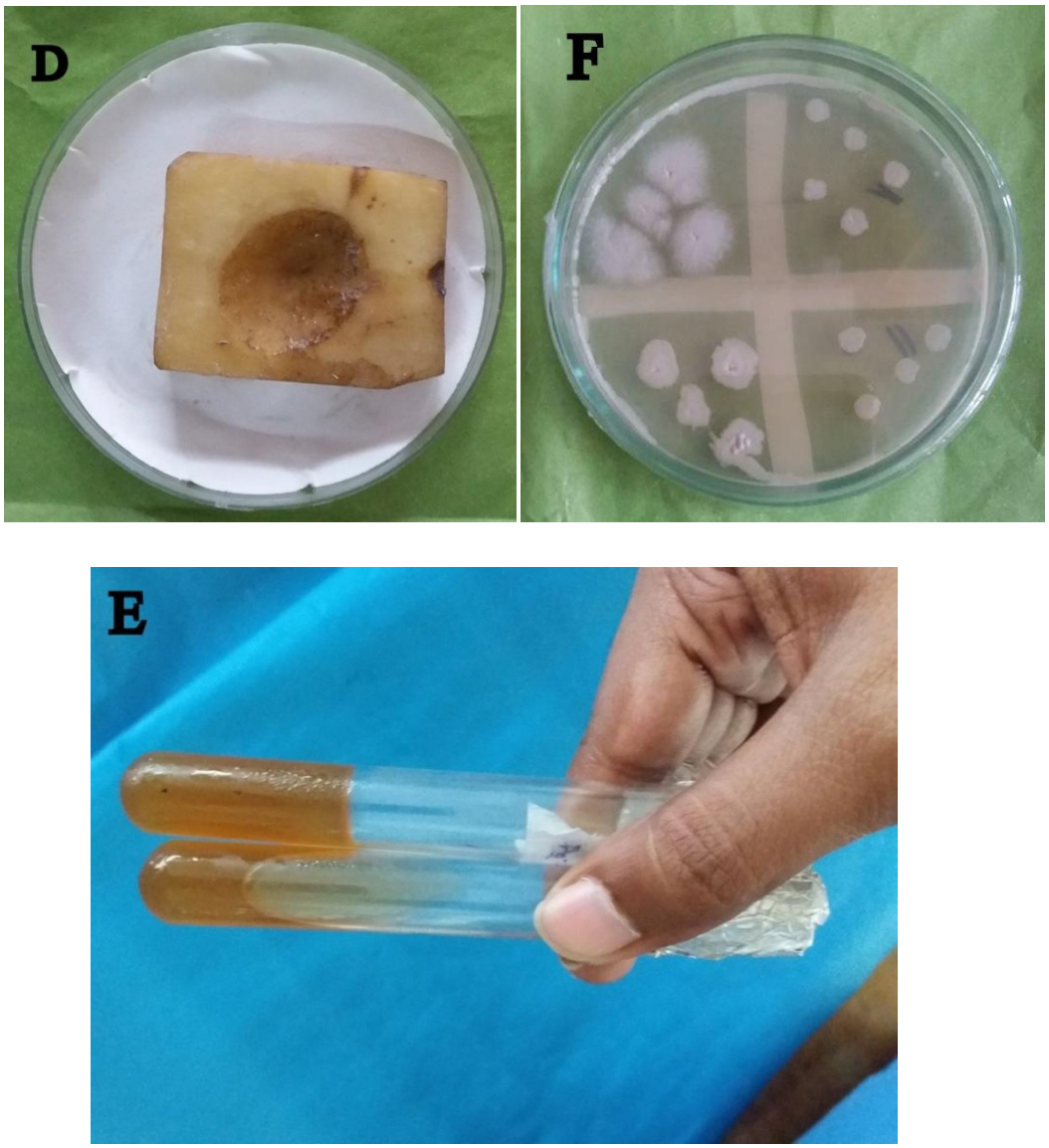


Fig. 14. Biochemical tests D. Potato soft rotting test E. Gelatin liquefaction test F. Levan test

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

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. The colonies formed after 4-5 days of inoculation. *Streptomyces* are easily differentiated form fungi by their smaller hyphae and spores.

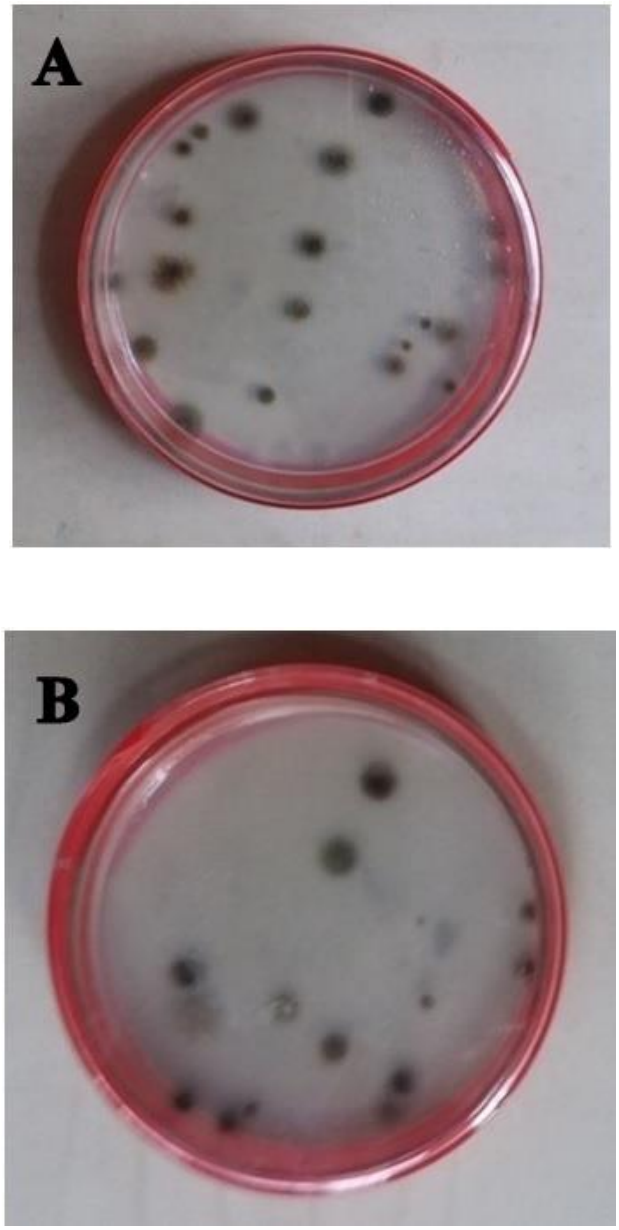


Fig. 15. Isolation of *Streptomyces scabies* (A and B) on water agar medium

CHAPTER V
DISCUSSION

An study was conducted in the cold storage of the district of munshigonj to observe the prevalence of diseases in stored potato. Common scab, Brown rot, *Fusarium* dry rot, soft rot of potato, hollow heart and black heart were more frequent potato diseases in these cold storages. The diseases recorded in the present study based on visual symptoms following the description of Brunings and Gabriel (2003). Prevalence of different postharvest disease of potato in cold storages were varied in respect of infection. Some diseases cause more severe damage and progress more rapidly than others, especially when secondary bacterial soft rot creates "hot spots" that can rapidly lead to the breakdown of an entire pile of potatoes in storage.

In the present study isolation and identification revealed that three fungal species and four genera of bacteria were isolated and identified. Three species of *Fusarium* were identified namely *Fusarium oxysporum*, *Fusarium culmorum* and *Fusarium solani.* Partially similar result found by Choiseul *et al.* (2007). Their macro and micro conidial characteristics was identified following the description of Leslie *et al.* (2006). For isolation NA medium was used by following dilution plate method. After isolation different bacterial genera were separated by growing them on selective and semi-selective media. Cultural characteristics also recorded as diagnostic features of the bacteria. *Ralstonia solanacearum* formed a large amount of colonies on NA medium after 48-72 hours of incubation. *R. solanacearum* formed a satisfactory number of colonies on TTC after 48-72 hours of incubation. In TTC medium, largely unmixed mucoid magenta deep red coloured colonies of *R. solanacearum* were developed with whitish margins colonies of bacterium. It was confirmed according to the description of Kelman and Person, (1954). 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. After the gram's staining under the compound microscope at 100x magnification with oil immersion, the bacterium was rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative (red colour) and capsulated (Murray *et al*., 2007). 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). *R. solanacearum* also showed positive result in leavn test. Same test result was found by Rahman *et al*. (2010). Bacterial soft rot of potato caused by *Pectobacterium* is known mainly as a postharvest disease (Coplin, 1980; Tegene&Korobko, 1985). In the present study Cetrimide agar medium was used to differentiate *Pectobacterium* from *Pseudomonous*. Following the process described by Alder and Burrow (2006). *Pectobacterium* was found to be rod shaped creamy white slightly raised and glistening colonies, a Gram negative rod was seen after staining. Similar result of gram staining was found by Gupta and Thind, (2006). 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 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 for catalase tests. It showed positive result in levan test and pectolytic test, they are unable to utilize starch what was reported by Bradbury (1986). *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).

The present study was based on only the prevalence of the postharvest diseases and their causal organisms in the stored potato. From the result, it could be concluded that several bacteria and fungi are responsible for post harvest disease of potato in cold storages that causes qualitative and quantitative losses of potato. Further studies are promising enough to reveal and characterize the pathogen responsible for postharvest disease.

CHAPTER VI

SUMMARY AND CONCLUSION

Potato is an important tuber crop in Bangladesh which is grown all over the world. It belongs to the family of Solanaceae. Though the demand of potato is increasing day by day, it's production is not satisfactory in terms of area and yield, due to different diseases of potato. Tuber of potato is succeptible to attack by various diseases in Bangladesh in which common scab, *Fusarium* rot, brown rot, soft rot and physiological disorder hollow heart and black heart are very common. However least concrete information regarding their distribution, incidence, severity, epidemiology and management are available. Therefore, the present study was designed to study the prevalence of the diseases and to identify the causal agent of different diseases of potato in selective cold storage at munshigonj. Samples were collected from four selected cold storage at Munshigonj namely Bicrompur multipurpose cold storage, Kohinur cold storage, Munshiganj ice and cold storage, Tangibari cold storage.

Result of the survey showed that the prevalence of potato diseases in cold storage were *Fusarium* spp. causing dry rot of potato (3.66%), *Ralstonia solanacearum* causing brown rot (3.21%), *Streptomyces scabies* causing common scab of potato (5.38%), *Pectobacterium* causing soft rot of potato (4.82%) and physiological disorders were found (5.17%).

The causal organism of fungual diseases were isolated from the infected tuber using moist chamber followed by inoculation on PDA. The causal agent of different postharvest bacterial diseases of potato were isolated from the infected tuber by following dilution plate technique method using nutrient agar medium, water agar medium (Only for *Streptomyces*),TTC medium and Cetrimide agar medium were used to separate bacteria. In TTC medium *Ralstonia solanacearum* produced largely unmixed mucoid magenta Deep red coloured colonies.

The causal organism of soft rot disease of potato was identified as *Pectobacterium.* In NA medium which produced white, smooth colonies, domed

shaped, shining, mucoid colonies with entire and serrate edges. *Pectobacterium* is a gram negative bacteria. This bacteria showed positive results in KOH solubility test, Catalase test, pectolytic test 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 bacteria *Streptomyces scabies* is the causal organism of scab of potato which was isolated from collected samples on water agar medium. The colour of the colonies were dark oblivious green. It is easy to differentiate *Streptomyces* form fungi by their smaller hyphae and spores.

The present study was based on only the prevalence of the postharvest diseases and their causal organisms in the cold storage of munshigonj. Further studies needed to investigate the storage conditions that favors postharvest disease development and investigation should be carried out to find out an effective method to prevent or minimize postharvest deterioration of potato in cold storages.

CHAPTER VII

REFERENCES

Agrios, GN. (1978). Plant Pathology, Academic Press, London. 2nd edition, pp. 473-474.

Alam, S. M.K., Tigashi, J., Ohtomo, T. and Namai, T. (1999). Bacterial soft rot, a new disease of Pak-Choi (Brassica campestris L. chinensis group) caused by *Erwinia carotovora. Ann. Phytopathol. Soc. Jpn.* **65**(1): 46-48.

Anonymous, (2006). Commission Directive 2006/63/EC of 14 July 2006: amending Annexes II to VII to Council Direc-tive 98/57/EC on the control of *Ralstonia solanacearum* (Smith) Yabuuchi *et al*. Official *Journal of the European Communities*, **206**: 36-106.

Baghee-Ravari, S., Rahimian, H., Shams-Bakhash, M., Lopez-Solanilla, E., Antunez-Lamas, M. and Rodriguez-Palenzuela, P. (2011). Characterization of *Pectobacterium* Species from Iran using biochemical and molecular methods. *Eur. J. Plant Pathol.* **129**: 413-425.

Breukers, A. (2006). Bio-economic modelling of brown rot in the Dutch potato production chain. PhD Thesis Wageningen University, Wageningen, The Netherlands.

Breukers, A., van der Werf, W., Mourits, M. and Lansink, A.O. (2007). Improving cost-effectiveness of brown rot control: the value of bio-economic modelling. *EPPO Bulletin/Bulletin OEPP* **37**: 391-394.

Buddenhagen, I. W., Sequeira, L. and Kelman, A. (1962). Designation of races of *Pseudomonas solanacearum*. *Phytopathology*, **52**: 726.

Caruso, P., Palomo, J. L., Bertolini, E., Álvarez, B., López, M. M. and Biosca, E. G. (2005). Seasonal variation of *Ralstonia solanacearum* biovar 2 populations in a Spanish river: Recovery of stressed cells at low

temperatures. *Journal of the Applied and Environmental Microbiology,* **71**: 140-148.

Castillo, J. A. and Greenberg, J. T. (2007). Evolutionary dynamics of *Ralstonia solanacearum*. *Journal of the Applied and Environmental Microbiology*, **73**: 1225-1238.

Cellier, G. and Prior, P. (2010). Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. *Phytopathology*, **100**: 1250-1261.

Cook, D. and Sequeira, L. (1994). Strain differentiation of *Pseudomonas solanacearum* by molecular genetic methods. In: Hayward, A.C., Hartman, G. L. (eds). Bacterial wilt: the Disease and its Causative Agent, *Pseudomonas solanacearum*, pp. 77-93.

Choiseul, J., Allen, L. and Carnegie, S. F. (2007). Fungi Causing Dry Tuber Rots of Seed Potatoes in Storage in Scotland. *Potato Res.* **49**: 241-253.

Czajkowski, R., Perombelon, M. C. M., van Veen, J. A. and van der Wolf, J. M. (2011). Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and Dickeya species: a review. Plant Pathol., doi: 10.1111/j.1365-3059.2011.02470.x

De Boer. (2003). Characterization of pectolytic erwinias as highly sophisticated pathogens of plants. *Eur. J. Plant Pathol.* **109**: 893-899.

De Boer, S. H. and Kelman, A. (2000). Gram-negative bacteria: *Erwinia* soft rot group. In Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd edn. ed. Schaad, N.W., Jones, J.B. and Chun, W. pp. 56–72.

Dickey, R.S. and Kelman, A. (1988). *Erwinia carotovora* or soft rot group. In: Schaad NW Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd edn. pp 44- 59.

Dye, D. N. (1969). A taxonomic study of the genus *Erwinia*. 1. "The amylovora group. *New Zealand J. Sci.* **11**: 590-607.

Elphinstone, J. G., Stanford, H. M. and Stead, D. E. (1998). Survival and transmission of *Ralstonia solanacearum* in aquatic plants of Solanum dulcamara and associated surface water in England. *Bulletin OEPP/EPPO Bulletin* **28**: 93-94.

Elphinstone, J. G., Stead, D. E., Caffier, D., Janse, J. D., Lopez, M. M., Mazzucchi, U., Müller, P., Persson, P., Rauscher, E., Sousa Santos, M., Stefani, E. and van Vaerenbergh, J. (2000). Standardization of methods for detection of *Ralstonia solanacearum* in potato. *Bulletin OEPP/EPPO Bulletin***, 30**: 391-395.

Fegan, M. and Prior, P. (2005). How complex is the "*Ralstonia solanacearum* species complex"? In: Allen, A. Prior, P. and Hayward, A. C. (eds). Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex, pp. 449-461.

Grey, B. E. and Steck, T. R. (2001). The viable but non culturable state of *Ralstonia solanacearum* may be involved in long-term survival and plant infection. *Applied and Environmental Microbiology*, **67**: 3866-3872.

Haynes, K. G., Potts, W. J. E. and Goth, R. W. (1997). Evaluation of the reliability of determining soft rot resistance in potatoes by the tuber slice method. *Am. Potato J.* **74**(4): 265-275.

Hayward, A. C. (1992). Identification of *Pseudomonas solanacearum*. In SAVERNET Bacterial Wilt Training Course held on October 5 to November 16 AVRDC. P. 101.

Hossain, M. (1986). Survey of Northern Ireland seed potato stocks for Assessment of Bacterial Soft Rot. *Bangladesh J. Agril.* **11**(4): 65-72.

Hugh, R. and Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram- negative bacteria. *J. Bacteriol.*, **66**: 24-26.

Islam, A. K. M. S. (2004). Characterization of soft rot bacterial pathogen of some vegetables. A MS Thesis. 69 pp. Department of Plant Pathology. Bangabandhu Sheikh Mujibur Rahman Agricultural University. pp 69

Karnjanarat, S., Tsuchiya, K., Matsuyama, N. and Wakimoto, S. (1987). Physiological, biochemical and pathological differentiation among strains of *Erwinia carotovora* isolated from Japan and Thailand. *Ann.Phytopathol. Soc. Jpn.* **53**: 460–469.

Islam, M. S. (1995). Investigation into bacterial storage diseases of potato of some markets of Mymensingh district. M. Sc. Thesis. Plant Pathol. Dept.. Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh. pp. 18-25

Kamaluddin, G. (1970). A study of fungal diseases of potato in East Pakistan with special reference to those occurs in storage. M. Sc.Thesis. Plant Pathol. Dept., East Pakistan Agricultural University, Mymensingh. pp. 6-13.

Khan, A. A,, Rahman, S. and Kamaluddin, G. (1973). A preliminary survey of the diseases of potatoes in cold storage in Bangladesh. *J. Biol. Agil. Sci.* **2**: 15-21.

Klement, A. and Goodman, R. (1967). The hypersensitive reaction to infection by bacterial plant pathogens. *Ann. Rev. Phytopathol*. **5**: 17-44.

Kovacs, N. (1956). Identification of *Pseudomonas solanacearum* by the oxidase reaction. Nat., 178: 703.Kreig, N. R., Holt, J. G. Eds (1984). Bergeey‟s manual of systematic bacteriology. Vol. I. Williams and Wilkins, London. pp 141-177.

Kumar, A., Chauhan, S. K., Pundhir, V. S. and Singh, R. S. (1992). A method of testing tuber resistance against soft rot bacterium. *J. Indian Potato Assoc.* **19**(3-4): 157-161.

Lelliot, R. A., Billing, E. and Hayward, A. C. (1966). A determinative scheme for the Fluorescent Plant Pathogenic Pseudomonads. *J. App. Bacteriol.* **29**:70-489.

Lelliot, R. A. and Dickey, R. S. (1984). Genus VII. *Erwinia*. Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209AL, pp. 469–476.

Leslie,J. F. and Summerell, B. A. (2006). The Fusarium laboratory manual.blackwell publishing professional, Ames, IA, USA, pp. 388.

Lund, B. M. (1979). Bacterial soft rot of potatoes. In oc. App. 1. Acteriol Tech. Ser. 12. Plant pathogens. Ed. D. W. Lovelock, R. R. Davies, 14-49 pp. London: Academic. Not cited Ma B, Hibbing ME, Kim HS et al.(provide full names) (2007) Host range and molecular phylogenies of the soft rot entero bacterial genera *Pectobacterium* and Dickeya. *Phytopathol.* **97**: 1150-1163

Messiha, N. A. S., van Diepeningen, A. D., Farag, N. S., Abdallah, S. A., Janse, J. D. and van Bruggen, A. H. C. (2007). Stenotrophomonasmaltophilia: a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. *European Journal of Plant Pathology*, **118**: 211-225.

Messiha, N. A. S., van Diepeningen, A. D., Franz, E., Janse, J. D., Schoeman-Weerdesteijn, M. E., Termorshuizen, A. J. and van Bruggen, A. H. C. (2009). Survival of *Ralstonia solanacearum* is affected more by soil type than by management. *Applied Soil Ecolog,y* **43**: 206-215.

Mishra, B. B. and Srivastava, J. S. (1991). Anatomical studies in common scab of potato. *Inst. Agril. Sci., India.* **2**(2): 113-114.

Mishra, K. K. and Srivastava, J. S. (1999). Severity and prevalence of common scab of potato in eastern U. P. *J. Indian Potato Assoc*. **26**(3-4): 143-155.

Moraes, M. A. (1947). A vascular disease of potato (Bacterium solanacearum). Smith, E. F. *Agronomia Lusitana* **9**: 277-328.

Mortensen, C. N. (1997). Seed Bacteriology laboratory guide. Danish Govt.Ins. Seed Pathol (DGISP) for developing countries.Copenhagen,Denmark. pp. 1-2.

Nabhan, S., Al-Chaabi, S. and Abu-Ghorrah, M. (2006). Evaluation of pathogenicity of different *Erwinia* isolates causal agents of potato soft rot and blackleg, and assessment of susceptibility of some potato cultivars under laboratory conditions. The Ninth Arab Congress of Plant Protection, 19-23 November 2006, Damascus, Syria.

Nourian, F., Kushalappa, A. C. and Ramaswamy, H. S. (2002). Physical, physiological and chemical changes in potato as influenced by *Erwinia carotovora* infection. *J. Food Process. Preserv.* **26**(5): 339-360.

O'Brien, J. G., Blanchette, R. A. and Sutherland, J. B. (1984). Assessment of Streptomyces spp. from elms for biological control of Dutch elm disease. Plant Dis. 68:104-106.

Rasul, M. G., Islam, M. A. and Sheikh, M. H. R. (1999). Storability of different potato varieties under natural conditions. *Bangladesh J. Sci. and Indust. Res.* **34**(1): 86-90.

Perombelon, M. C. M. and Kelman, A. (1980). Ecology of the soft Erwinias. *Ann. Rev. Phytopathol.* **18**: 361-387.

Persson, P. (2008). Successful eradication of *Ralstonia solanacearum* from Sweden. *Bulletin OEPP/EPPO Bulletin* **28**: 113-119.

Pingsheng, J., Allen, C., Sanchez-Perez, A., Yao, J., Elphinstone, J., Jones, J. B. and Momol, M. T. (2007). New diversity of *Ralstonia solanacearum*

strains associated with vegetable crops in Florida. *Plant Disease*, **91**: 195-203.

Poussier, S., Trigalet-Demery, D., Vanderwalle, P., Goffinet, B., Luisetti, J. and Trigalet, A. (2000). Genetic diversity of *R. solanacearum* as assessed by PCR-RFLP of the hrp gene region, AFLP and 16S rRNA sequence analysis, and identification of an African subdivision. *Microbiology* , **146**: 1679-1692.

Rahman, S. (1969). Study of storage diseases of potato in East Pakistan. M. Sc. Thesis. Plant Pathol. Dept., East Pakistan Agricultural University, Mymensingh. pp. 19-34.

Rich, E. E. (1983). Potato Diseases, Academic Press, New York. London. Initial identification of common genera. In A Laboratory guide for identification of plant pathogenic bacteria". 2nd edition, Ed. N. W. Schaad. APS Press, St. Paul, MN, USA., pp 1-58.

Saddler, G. S., Trigalet, A. and Feuillade, R. (1998). Genetic diversity of *Ralstonia solanacearum* Race 3 in western Europe determined by AFLP, RC-PFGE and Rep-PCR. In: Prior P.H.,Allen C., Elphinstone J. (eds). Bacterial Wilt Disease: Molecular and Ecological Aspects, pp. 44-49.

Seo, S. T., Furuya, N., Lim, C. K., Tsuchiya, K. and Takanami, Y. (2001). Variation of *Erwinia carotovora* subsp. carotovora isolated from Korea. *J. Fac. Agril. Kyushu Univ.* **45**: 431–436.

Schaad, N. W., Jones, J. B. and Chun, W.(2001). Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul. Third edition, pp: 236-249.

Sherf, A. F. and Macnab, A. A. (1986). Vegetable diseases and their control. 2ndedi. A Wiley Interscience Publication, John Wiley and Sons, Inc. NewYork.

Shing, R. S. (2001). Plant Disease. Oxford and IBA Publishing Company Pvt. Ltd. New Delhi, pp. 86-90.

Singh, A. and Singh, A. K. (2000). Effect of temperature and pH on growth of *Streptomyces aureofaciens* causing common scab of potato Regional Res. Station, India. *Crop Res. Hisar.* **20**(3): 544-545.

Smith, C. and Bartz, J. A. (1990). Variation in the pathogenicity and aggressiveness of isolates of *Erwinia carotovora* subsp. Carotovora isolated from potato plant. *Plant Dis.* **74**: 505-509.

Suslow, T. V., Schroth, M. N. and Isaka, M. (1982). Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathol*. **72**: 917-98.

Tamburic-Iincic, L. (1996). Species identification and potato tuber resistance to *Fusarium* spp. ZastitaBilja (Serbia), 47(2): 167-177.Potato brown rot, in field and microcosm soils in temperate climates. *Phytopathology,* **90:** 1358-1366.

Timms-Wilson, T. M., Bryant, K. and Bailey, M. J. (2001). Strain characterization and 16S-23S probe development for differentiating geographically dispersed isolates of the phytopathogen *Ralstonia solanacearum*. *Environmental Microbiology*,**3**: 785-797.

Togashi, J. (1988). Identification of the organisms causing the soft rot disease in potatoes in the fields of Tsuruaka district. Bull. Yamaga *Univ. Agric. Sci.*, **10**(3): 473-478.

Tomlinson, D. T., Elphinstone, J. G., El-Fatah, H. A., Agag, S. H. A., Kamal, M., Soliman, M. Y., El-Aliem, M. M. A., El-Ghany, H. A., El-Haddad, S. A., Fawzi, F. G. and Janse, J. D. (2005). Survival of the potato brown rot bacterium (*Ralstonia solanacearum* biovar 2) in Egyptian soils. Potato Conference, Emmeloord, the Netherlands pp. 233-238.

Toth, I. K., Kenneth, S. B., Maria, C. H. and Paul, R. J. B. (2002). Soft rot Erwiniae: from genes to genomes. *Mol. Plant Pathol.* **4**(1): 17-30.

Anonymous. (1998). Council Directive 98/57/EC of 20 July 1998 on the control of *Ralstonia solanacearum*. Annex II-test scheme for the diagnosis, detection and identification of *Ralstonia solanacearum*. Official *Journal of the European Communities*, **235**: 8-39.

Toukam, G. M. S., Cellier, G., Wicker, E., Guilbaud, C., Kahane, R., Allen, C. and Prior, P. (2009). Broad diversity of *Ralstonia solanacearum* strains in Cameroon. *Plant Disease*, **93**: 1123-1130.

Van Beuningen, A. R., Tax, M., Voogd, J. G. B., van Overbeek, L. S. and Janse, J. D. (2005). Brown rot of potato: breakthrough in prevention of reintroduction as caused by irrigation and spraying. *Gewas-bescherming* **36**: 248-252.

Van der Wolf, J. M., Bonants, P. J. M., Smith, J. J., Hagenaar, M., Nijhuis, E., van Beckhoven, J. R. C. M., Saddler, G. S., Trigalet, A. and Feuillade, R. (1998). Genetic diversity of *Ralstonia solanacearum* Race 3 in western Europe determined by AFLP, RC-PFGE and Rep-PCR. In: Prior P.H., Allen C.,Elphinstone J. (eds). Bacterial Wilt Disease: Molecular and Ecological Aspects, pp. 44-49.

Van Elsas, J. D., Kastelein, P., de Vries, P. M. and van Overbeek, L. S. (2001). Effects of ecological factors on the survival physiology of *Ralstonia solanacearum*bv. 2 in irrigation water *Canadian Journal of Microbiology*, **47**: 1-13.

Van Elsas, J. D., Kastelein, P., van Bekkum, P., van der Wolf, J. M., de Vries, P. M. and van Overbeek, L. S. (2000). Survival of *Ralstonia solanacearum* biovar 2, the causative agent of postharvest. *Journal of the European Communities*, **93**: 1123-1130.

Villa, J. E., Tsuchiya, K., Horita, M., Natural, M., Opina, N. and Hyakumachi, M. (2007). Phylogenetic relationships of *Ralstonia solanacearum* species complex strains from Asia and other continents based on 16S rDNA, endoglucanase, and hrpB gene sequences. *Journal of General Plant Pathology*, **71**: 39-46.

Virtanen, E., Vihlman, K. and Valkonen, J. P. T. (2004). Occurrence and survival of potato scab pathogens (Streptomyces species) on tuber lesions: quick diagnosis based on a PCR-based assay. *Plant Pathol*. **53**(3): 280-287.

Wastie, R. L., Stewart, H. E. and Brown, J. (1989). Comparative susceptibity of some potato cultivars to dry rot caused by *Fusarium sulphureum* and *F. solani* var. caerulem. *Potato Res.* **32**(1): 49-55.

Weber, J., Rober, K. C. and Papenhagen, F. (1989). Screening of potato breeding material for susceptibility to soft and dry rot. German United Democrat. Repub. Kartoffelforschung-Aktuell. pp. 38-41.

Chowdhury, H. C. (1956). Storage tests for the control of diseases and insect pests. *Am. Potato J.* **4**: 6-14.

Weller, S. A., Elphinstone, J. G., Smith, N. C., Boonham, N. and Stead, D. E. (2000). Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluoro-genic PCR (TaqMan) assay. *Applied and Environmental Microbiology* **66**: 2853-2858.

Wenneker, M., van Beuningen, A. R., van Nieuwenhuijze, A. E. M. and Janse, J. D. (1998). Survival of the brown rot bacterium (*Pseudomonas solanacearum*) in and on diverse substrates and effectiveness of some disinfecting substances for the decontamination of surface water. *Gewas-bescherming* **29**: 7-11.

Wenneker, M., Verdel, M. S. W., Van Beuningen, A. R., Derks, J. H. J. and Janse, J. D. (1999). *Ralstonia* (Pseudomonas) *solanacearum* race 3 (biovar 2) in

surface water and natural weed hosts: first report on stinging nettle (Urticadioica). *European Journal of Plant Pathology* ,**105**: 307-315

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D. and Prior, P. (2009). Epidemiological evidence for the emergence of a new pathogenic variant of *Ralstonia solanacearum* in Mar-tinique (French West Indies). *Plant Pathology,* **58**: 853-861.

Wang , (1998). Isolation and physiological characterization of the pentachlorophenol degrading bacterium . *chemosphere* **62**:709-714.

Wicker, E., Lefeuvre, P., de Cambiaire, J. C., Lemaire, C., Poussier, S. and Prior, P. (2011). Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. *The ISME Journal,* doi:10.1038/ismej.2011.160.

Williamson, L., Nakaho, K., Hudelson, B. and Allen, C. (2002). *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. *Plant Disease* , **86**: 987-991.

Wullings, B. A., Beuningen, A. R., van Janse, J. D. and Akkermans, A. D. L. (1998). Detection of *Ralstonia solanacearum*, which causes brown rot of potato, by fluorescent in situ hybridization with 23S rRNA-targed probes. *Applied and Environmental Microbiology,* **64:** 4546-4554.

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°C for 15 minutes at 15 lb pressure.

Nutrient Agar (NA)

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

Nutrient Broth (NB)

Beef extract (Difco)	3.0 g
Peptone (Difco)	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