IN-VITRO SCREENING OF SOME CHEMICALS AGAINST *ERWINIA CAROTOVORA* THE CAUSAL AGENT OF SOFT ROT OF POTATO

BY

SANJANA AKTER

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

DHAKA -1207

JUNE, 2017

IN-VITRO SCREENING OF SOME CHEMICALS AGAINST *ERWINIA CAROTOVORA* THE CAUSAL AGENT OF SOFT ROT OF POTATO

REGISTRATION NO. 11-04410

A Thesis

Submitted to the Faculty of Agriculture,

Sher-e-Bangla Agricultural University,

Dhaka, in partial fulfillment

of the requirements

for the degree

of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

SEMESTER: JANUARY-JUNE, 2017

Approved by:

Dr. Nazneen Sultana	**Abu Noman Faruq Ahmmed**
Professor	Associate Professor
Department of Plant Pathology	Department of Plant Pathology
Sher-e-Bangla Agricultural University	Sher-e-Bangla Agricultural University
Supervisor	**Co-Supervisor**

Prof. Khadija Akhter
Chairman
Examination Committee
Department of Plant Pathology

Department of Plant Pathology

Sher-e-Bangla Agricultural University

Sher-e -Bangla Nagar, Dhaka-1207, Bangladesh

CERTIFICATE

This is to certify that the thesis entitled ***"IN-VITRO SCREENING OF SOME CHEMICALS AGAINST ERWINIA CAROTOVORA THE CAUSAL AGENT OF SOFT ROT OF POTATO"*** *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*** *in* ***Plant Pathology*** *embodies the result of a piece of bona-fide research work carried out by* ***Sanjana Akter*** *Registration No. 11-04410 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: *June, 2017*
Dhaka, Bangladesh

Prof. Dr. Nazneen Sultana
Supervisor

ACKNOWLEDGEMENT

Alhamdulillah, all praises are due to the almighty ***Allah Rabbul Al-Amin*** *for His gracious kindness and infinite mercy in all the endeavors the author to let her successfully complete the research work and the thesis leading to the degree Master of Science.*

The author would like to express her heartfelt gratitude and most sincere appreciations to her ***Supervisor, Prof. Dr. Nazneen Sultana****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her valuable guidance, advice, immense help, encouragement and support throughout the study. Likewise grateful appreciation is conveyed to her* ***Co-Supervisor, Associate Professor, Abu Noman Faruq Ahmmed****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his constant encouragement, cordial suggestions, constructive criticisms and valuable advice to complete the thesis.*

The author would like to express her 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 wishes to extend her special thanks to her class mates and friends and roommates for their keen help as well as heartiest co-operation and encouragement during experimentation.

The author is deeply indebted and grateful to her parents, brothers, sister and relatives who continuously prayed for her success and without whose love, affection, inspiration and sacrifice this work would not have been completed.

Finally the author appreciates the assistance rendered by the staff members of the Department of Plant Pathology and central farm, Sher-e-Bangla Agricultural University, Dhaka, who have helped her lot during the period of study.

The Author

IN-VITRO SCREENING OF SOME CHEMICALS AGAINST *ERWINIA CAROTOVORA* THE CAUSAL AGENT OF SOFT ROT OF POTATO

ABSTRACT

In vitro efficacy six selected chemicals were evaluated against *Erwinia carotovora* subsp. *carotovora* the causal agent of potato soft rot disease. The experiment was done at Molecular Plant Pathology laboratory of the department of Plant Pathology of Sher-e- Bangla Agricultural University. *E. carotovora* was isolated from infected potato tubers by dilution plate method and different biochemical tests and pathogenicity test were performed to confirm the bacterial species. Six chemicals viz. Sunvit 50 WP (Copper Oxychloride) @ 0.2%, Dithane M-45 (Mancozeb) @ 0.2%, Boric acid (Boric Powder) @ 0.1%, Kasumin (Kasugamycin) @ 0.02%, Autostin 50 WG (Carbendazim) @ 0.3% and Chlorox (Sodium Hypochlorite) @ 0.2% were tested against *E. carotovora* by well diffusion method. For each treatment there were four replications and tested chemical volume was 100 µl. Data were recorded up to five days of incubation. Among six chemicals only four chemicals viz. Sunvit 50 WP, Dithane M-45, Boric acid and Kasumin showed bactericidal activity against *E. carotovora* subsp. *carotovora*. Maximum zone of inhibition (mm) was obtained after 48 hours of incubation with Sunvit 50 WP (30.35 mm), followed by Dithane M-45 (20.15 mm), Boric acid (19.15 mm) and Kasumin (16.28mm). While the least zone of inhibition was recorded with Autostin 50WG (3.83mm) and Chlorox (2.42mm). Sunvit 50 WP produced the maximum growth inhibition (33.79%) of the pathogen, on the other hand Cholorx (3.47%) did not effectively inhibit the growth of *E. carotovora*. No inhibition was recorded with untreated control/water. Sunvit 50WP proved to be the best chemical followed by Dithane M-45 under *in-vitro* screening test against *E. carotovora* subsp. *carotovora.*

LIST OF CONTENTS

LIST OF CONTENTS (Cont'd)

LIST OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

%	=	Percentage
BBS	=	Bangladesh Bureau of Statistics
CV %	=	Percent Coefficient of Variation
DMRT	=	Duncan's Multiple Range Test
e.g.	=	exempli gratia (L), for example
et al.,	=	And others
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
i.e.	=	that is
Kg	=	Kilogram (s)
L	=	Litre
M.S.	=	Master of Science
mg	=	Miligram
ml	=	MiliLitre
No.	=	Number
^{o}C	=	Degree Celceous
SAU	=	Sher-e-Bangla Agricultural University
USA	=	United States of America
µl	=	Microliter

CHAPTER I
INTRODUCTION

Potato *(Solanum tuberosum* L.) is a starchy tuber crop belonging to the family Solanacae. Potato is considered as world's fourth largest food crop after wheat, maize and rice (Douches *et al*, 1996). It provides a balanced source of starch, vitamins and minerals to many communities and an integral part of global food supply. It is now one of the popular traditional staple foods in Bangladesh. Total area under potato crop in Bangladesh has been estimated 4,30,255 hectares and production was 82,05,470 metric tones in the year 2011-2012 (BBS, 2012). Still potato production is quite low in comparison to that of the leading potato growing countries of the world such as Netherlands 42.99 metric tones/ha, USA 38.40 metric tones/ha and UK 39.64metrictones/ha (FAO, 2008).

Many Harmful bacteria or pathogens can affect potato production which results in great economic losses (Bathily *et al*., 2010). Among many pathogenic bacteria *Erwinia carotovora* subsp. *carotovora c*ausing soft rot of potato is considered as most divesting diseases (Akbar *et al*, 2014). It is an important post-harvest disease which cause huge losses in stored potatoes if not properly managed. *Erwinia carotovora* is a soil borne pathogen mainly survives in clay and heavy soil association with low calcium concentration (Lambert and Manzer, 1991). These bacteria can attack potato tubers from field to storage as they survive in soil, in decaying plant debris and in the seed tubers. Bacteria get enter the seed potatoes through wounds and injuries. Abundant moisture at the surface of the wound tissue, high temperature is needed for infection and continues high humidity create an ideal condition for rapid multiplication and spread of the disease in the potato tubers. The first symptom is the development of a water-soaked area on the tuber around a lenticel or the eye but eventually the whole tuber becomes infected (Rai, 1979). Soft rot bacteria produce of large quantities of extracellular pectic enzymes that bind plant cells together with association a wide range of other cell wall

degrading enzymes, resulting tuber structure to fall apart and may be a foul smelling odour occurs (Olivieri *et al*, 2004).

An estimated 22% of potatoes are lost per year to viral, bacterial and fungal diseases and pests, which is equivalent to an annual loss of over 65 million tonnes and bacterial soft rot contributes alone as much as 50% of the total potato production (Czajkowski *et al*., 2011). The effect of the disease is more pronounced in the countries where appropriate storage facilities are lacking or inadequate. Potato crop has huge production constraints in the field, in the storage and market, of which soft rot, dry rot, potato scab, potato gangrene and hollow heart are of highest importance.

Bacterial soft rot is the second most destructive bacterial disease caused by *Erwinia caorotovora*. It is considered as one of the most destructive diseases of potato in storage and transit conditions. It causes a greater total loss of produce than any other bacterial disease. Masum *et al*. (2011) carried out a survey which revealed that potato tubers were affected with soft rot 3.95%, 3.73%, 3.27% in three different districts Mymensingh, Rajshahi and Dhaka, respectively. They concluded that Diamond cultivar is most susceptible to soft rot 5.55%.

Soft rot is a common and serious post harvest problem of potato tubers. The disease causes severe losses of the crop in storage (Bdliya and Haruna, 2007). Normally chemicals are not recommended for the control of soft rot disease because it has high risk of residual effect on health and impact on the environment.

However, many researchers evaluated various chemicals in order to control the soft rot bacteria. A number of chemical compounds with antimicrobial activity are identified, which increase resistance in potato against soft rot bacteria (Hammerschmidt and Smith, 1997). Among numerous chemicals copper-based compounds effective against soft rot bacterial growth at various cupric ion concentrations *in vitro* (Rashid *et al*., 2013). Suppression of bacterial soft rot in

potato tubers by application of Streptomycin give better result in comparison to *Datura stramonium* leaves extract with highest inhibition zone (Viswanath *et al*, 2018). Chemicals viz. acetic acid, boric acid and bleaching powder significantly decreased the infection rate, loss in weight and increased percentage of disease reduction (PDR) against *Erwinia carotovora* subsp. *carotovorum* of potato (Rahman *et al.*, 2017). The antibiotics had a significant effect on plant pathogenic microorganisms which produce cell wall degrading enzymes (Alice and Sivaprakasam 1995).

Bacteria can multiply very fast and produce disease in favorable condition and the most serious aspect is that there are hardly any possibilities to control bacterial pathogens on potato. So use of chemicals free from health hazards may be an effective and acceptable way to control soft rot bacteria of potato. So, search for such chemicals is necessary. Considering the great economic losses, the present investigation is undertaken to test six chemicals based on their performances and evaluated against soft rot bacteria of potato tubers.

Considering the above discussion, the following objectives were taken:

Objectives of the research work

- To isolate and identify of causal organism of soft rot disease of potato; and
- To evaluate the efficacy of six different chemicals against *Erwinia carotovora subsp. carotovora* under *in-vitro* condition.

CHAPTER II

REVIEW OF LITERATURE

2.1. Present status, incidence, severity and distribution of soft rot disease

Mehedi (2014) surveyed market diseases of potato cv. Diamant in five different markets at Mymensingh in May and September 2013. From each shop 40kg potatoes were taken. Potatoes were grouped in different category. Survey results indicated that the average soft rot incidence was 0.36% in May and 0.99% in September. The average loss of potato tubers due to soft rot was found 0.48% in May and 1.31% in September.

Czajkowski *et al*. (2011) reported that, approximately 22% of potatoes are lost per year due to viral, bacterial, fungal, and pests attack. potato tuber and potato plant, incurring an annual loss of over 65 million tones and bacterial soft rot alone accounts for 30–50% of this huge loss. He added that bacterial soft rot causes substantial losses in transit and storage, particularly in the warm regions where temperatures are high and there are no facilities available for cold storage

Masum *et al.* (2011) reported that, among the cultivars, maximum loss incurred within the three months namely July, August and September, where losses were caused by soft rot (3.97%), dry rot (0.88%), and scab (0.70%).

Hajhamed *et al* (2007) concluded that, bacterial soft rot causal agent, *Erwinia carotovora* sub sp. *carotovora* (Van Hall) Dye is one of the most common potato diseases in the warm region and induces quick and heavy spoilage losses. It is one of the most important and widespread bacterial disease of a variety of plants either in the field or in storage.

Olsen *et al*. (2006) *Erwinia carotovora* subsp. *carotovora* is one major group of bacteria that causes soft rot in potatoes. During bulk storage, soft rot is commonly

a result of a favorable micro climatic conditions within the potato pile producing localized “hot spots”. The pathogen can spread quickly to healthy tubers located below such hot spots in the pile, which is facilitated by intense respiration activity and the heat released from rotting tubers.

Smadja *et al*. (2004) stated that *Erwinia carotovora* subsp. *atroseptica* is responsible for potato blackleg disease in the field and tuber soft rot during crop market. The process leading to the disease occurs in two phases: a primary invasion step followed by a maceration step. Bacteria communicate with a quorum- sensing (QS) process based on the production of N-acylhomoserine lactones (HSL). The role of HSL throughout plant infection was analyzed. HSL produced by a specific *E. carotovora* wild-type strain, which was particularly virulent on potato, were identified.

Esfahani (2003) reviled a 2-year investigation report regarding to determine the various losses of potato tubers after harvesting and during the market periods, that was conducted in Isfahan, Iran. Due to various factors including diseases and/or environment approximately 23.02% of the harvested tubers go to waste. The components of these losses individually were: soft rot *Erwinia carotovora* (3.43%), Fusarium dry rot (4.89%), brown rot *Ralstonia solanacearum* (2.30%), tuber greening (2.44%), internal bruising (1.27%), physiological disorders (4.38%), deep injuries due to unsuitable harvesting (1.72%) and pests (1.65%). Statistical analysis showed no any significant effects in years but there was a high correlation coefficient. The losses of potato tubers in were 25.66, 24.76, 23.34, 21.24, 20.50, 18.56 and 17.20%, respectively. Potato cultivars differed in their degree of losses

Okonkwo *et al*. (2003) conducted a study at Kuru, Jos Plateau, Nigeria to assess six market methods for the market of seed potato. The methods were: Market in perforated polythene bags, jute bags, baskets, racks, crates and floor. Potato varieties used were Nicola (medium to long dormancy) and B7716-2 (short

dormancy). The seed tubers were stored for six months during which daily maximum and minimum temperatures of the market environment, and the relative humidity were recorded. Tuber weight loss, loss due to rots, sprout number and length per tuber at the end of the market were also recorded. Results showed that only polythene bag can reduce tuber weight loss, but resulted in highest tuber rots due to soft rot disease of potato.

Perombelon (2002) reported that soft rot is a seed-borne disease. He added that all species easily cause soft rot of tubers during storage if they found favorable environmental conditions for disease progression. In the same year he worked with three sub specie soft rot *Erwinia, Erwinia carotovora* subsp. *carotovora, E. carotovora* ssp. *atroseptica* and *E. chrysanthemi* associated with potatoes causing tuber soft rot and black leg (stem rot). Soft rot *Erwinia* tend to out compete other bacteria in tuber rots because of their ability to produce larger quantities of a wider range of cell wall degrading pectic enzymes.

Garcia (2000) showed that, species and subspecies characterization was conducted using the biochemical tests of acid production from sugars, sensitivity to erythromycin, growth at different temperatures, and in 5-10% NaC1, and the phosphates reaction. The results showed *Erwinia* ssp. to be present in all the samples. Of the 15 pure isolates, 13 were *Erwinia carotovora* subsp. *carotovora,* one each of *Erwinia carotovora* subsp. *atroseptica* and *Erwinia chrysanthemi*.

Rajeh *et al*., (2000) reviled a survey of vegetables in fields and market which indicated that soft rot disease occurred in different areas in Jordan, including Jordan Valley and the Upland. Eighty-seven bacterial isolates were collected from various crops. Isolates were biochemically and physiologically tested. Results of the different tests indicated that the causal agent of soft rot disease in Jordan was *Erwinia carotovora* pv. *carotovora*. The obtained bacterial isolates were grouped into five groups based on their capacity to produce acid from different carbon sources.

Rasul *et al*. (1999) studied the behaviour of 14 exotic varieties, 3 recommended varieties and 6 advanced lines of potato, following market of tubers in a netted wooden box under natural conditions for up to 195 days. Much variation was observed among the genotypes for all the characters studied. Percentage weight loss was higher in the exotic varieties (10.02-54.45%) after 135 days of market than in the other lines and varieties (12.89%-35.52%). Sprouting was earlier in the recommended varieties and lines (96.0 days) than in the exotic varieties (118.7 days). On average, tubers of the recommended varieties and lines shrank earlier than those of the exotic 1st generation material. Rotting of tubers by bacterial soft rot (*Erwinia* spp.) during market varied from 31.3% to 36.8%. Recommended varieties Kufri Sindhuri, Cardinal and Multa, advanced line P93 and exotic varieties Granola, Mondial, Producent and Vital performed best in market.

Haynes *et al*. (1997), conducted experiments and reported on susceptibility to soft rots *(Erwinia* spp) in *Solanum tuberosum* can be determined by inoculation of tuber slices. Slices from 15 tubers of Atlantic, Norchip and superior were inoculated with *Erwinia carotovora* subsp. *atroseptica. Erwinia carotovora* subsp. *carotovora* and incubated at 20^0C and 30^0C for 48 hours.

Obradovic (1994) tested *Erwinia* originating from different localities of Yugoslavia was collected and tested. Of them, 19 strains were studies for their pathogenic, morphological, culture, biological and physiological characterizes. According to the results obtained, 3 strains were identified as *Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora*.

Kumar *et al*. (1992) observed when 5 methods of inoculating potato tubers with *Erwinia carotovora* subsp. *carotovora* were compared, the most consistent result were obtained by injecting with a bacterial suspension. The reactions of 7 cultivars were determined by the injection method, Kufri Lalima and Kufri Sindhuri were resistant and the others were moderately resistant to highly susceptible.

Jaggi *et al*. (1991) reported *Erwinia carotovora* subsp. *carotovora* and subsp. *atroseptica* (soft rot) are present in potato soils and enter tubers when the lenticels are open in early August. Harvesting at this time presented the greatest risk of infection. The infection risk is also high when tubers are exposed to high humidity during market.

Morales and Suarez (1989) observed that, potatoes are affected by soft rot disease caused by *Erwinia carotovora* and incidence was 8% recorded in some markets in Tamil Nadu in the summers of 1983 and 1984. In inoculated tests on 9 commonly grown cultivars, only Multa, Diamant and Cardinal were found susceptible.

Turkensten and Mulder (1996) made a detailed survey on bacterial and fungal diseases of potato in the hilly areas of Pakistan. They collected samples and analyzed those samples using organism-specific antibodies and other techniques. They concluded that most important bacterial disease in Khyber Pakhtunkhwa causing losses over 30% followed by *Erwinia carotovora* subsp. *atroseptica* (potato blackleg) causing losses up to 30% and by *Erwinia carotovora* ssp. *carotovora* (potato soft rot) causing losses up to 10%.

Cromarty and Easton (1973) stated that, soft rot causes substantial losses in transit and storage, particularly in the warm regions where temperatures are high and there are no facilities available for cold storage.

2.2. Pathogen characterization and host-pathogen interactions

Accurate and timely Biochemical test is a must for the identification of soft rot causing bacteria, because it is nearly impossible to identify the causal agent by looking at symptoms. Different methods are available for the detection and identification of soft rot bacteria. Identification of soft rot *Erwinias* are usually done on the basis of their biochemical and phenotypic characteristics.

Priou *et al*. (1999) described that when two drops of 3% potassium hydroxide (KOH) is placed on the ooze and mixed using a laboratory loop or a wooden toothpick for 10 seconds. The formation of a milky thread upon lifting the toothpick indicates the presence of *R. solanacearum* (a Gram-negative bacterium), whereas with *C. michiganensis* subsp. *Sepedonicus* (a Gram positive bacterium) the thread is not produced.

Suslow *et al* (1982) examined the, Gram-stain reactions of pathogenic and saprophytic bacteria isolated from plant parts. A loopful inoculum of Bacterial cells from an agar medium were aseptically removed with a toothpick and placed on a glass slide in a 2-3 drops of 3% KOH with rapid circular agitation. With Gram-negative strains the suspension became viscous as revealed by a mucoid thread that formed when the toothpick was lifted. Gram-positive bacteria dispersed into the drop and did not have this reaction.

De Boer *et al*. (1979) reported that, some *Ecc* strains do vary in the ability to produce reducing substances from sucrose and acids from α-methyle glucoside

Kovacs (1956) observed that when a loopful inoculum from pure culture of bacteria was smeared with a sterilized platinum loop over the area of filter paper containing oxidize reagent and previously impregnated with 1% aqueous solution of Nitrogen, (Nitrogen tetra methyl-p-phenolin-diaminedihydrochloride) develop deep blue or purple color within ten seconds indicating the oxidation of the reagent.

2.3. Disease management via chemicals

Rahman *et al.*, (2017) investigated, bactericidal effect by using some chemicals against potato soft rot bacteria *in vitro* and in storage. The chemicals were acetic acid, boric acid, bleaching powder, lactic acid, calcium hydroxide, calcium

chloride, potassium chloride and sodium hypo-chloride. Among eight chemicals only three chemicals viz. acetic acid, boric acid and bleaching powder showed bactericidal activity against potato soft rot bacteria *Pectobacterium carotovorum* subsp. *carotovorum (E. carotovora subsp. carotovora)* P-138 *in vitro*. Based on the results of *in vitro* experiment three chemicals were used to control soft rot of potato in storage. Boric acid was the most effective in controlling the soft rot disease of potato in storage followed by acetic acid and bleaching powder.

Makhlouf and Abdeen (2014) screened one antimicro biolchemical compound chitosans (CS) with concentration (1, 3, 5 %) combine with three biocontrol agents (Bacillus subtilis, *Pseudomonas fluorescens* and *Trichoderma virdi*) each *in vitro* and in storage against the growth of *Erwinia carotovora* subsp *carotovora*. All bio-control agents combined with Cs reduced the bacterial soft rot disease to various degrees. The stronger antagonistic activity against *Erwinia carotovora* was found in treatment Cs 5% with biocontrol agents (*T. virdi, P. fluorescens* and *B. subtilis* respectively). All treatments reduced the soft rot infection to 20-week storage with two types of potatoes (Spunta and Cara) varieties by (91, 86, 83.6 and 77.3% respectively in Spunta c.v.) and (88.6, 86.4 and 79.8% in Cara c.v.). The lowest antagonistic activity against *Erwinia carotovora* was found in treatment CS 1% with biocontrol agents (*T. virdi, P. fluorescens* and *B. subtilis* respectively) varieties by (64.2, 58.6 and 43.7% respectively) compared with the treatments of biocontrol agents individually.

Rashid *et al.*, (2013) screened, five chemicals viz. Cupravit 50 WP (Copper oxychloride), Sulcox 50 WP (Copper oxychloride), Champion 77 WP (Copper hydroxide), Indofil M- 45 (Mancozeb) and Bavistin 50 WP (Carbendazim) in vitro against the growth of *Erwinia carotovora* subsp. *carotovora* by well diffusion method measuring the inhibition zone. Among the chemicals, Sulcox 50 WP (Copper oxychloride) was highly effective against it with 31.00 mm inhibition zone after 48 hours of incubation at 0.2% concentration when 100µl/well was used.

Garica *et al*. (2010) studied the efficacy of various copper-based compounds, viz Phyton-27, Champ 2, fixed copper, and analytical copper. They found that copper-based compounds suppressed bacterial growth at various cupric ion concentrations *in vitro* and Champ 2 or fixed copper were also found effective in reducing soft rot.

Hajhamed *et al.*, (2007) investigated, the management of the disease using salicylic acid and BION as resistance inducing factors, potassium sulfate, ammonium phosphate and calcium chloride as salt compounds, under artificial inoculation condition. All tested agents decreased the disease compared with the control. Disease severity was completely reduced when salicylic acid was applied at 0.9 mM, before or at the same time or after inoculation with the pathogen. Efficiency of the inducer agent and salt compounds were increased against the disease by increasing their rates. Salicylic acid, calcium chloride and potassium sulfate were the most effective against the disease compared with BION and ammonium phosphate.

Abd El-Khair and Karima (2007) applied bactericides, i.e., streptomycin sulfate, Starner and Micronite Soreil for controlling the soft rot disease causing by *Erwinia carotovora* subsp. *carotovora in vitro* and in field. In vitro, results showed that the Starner reduced the pectolytic enzymes (PG and PME enzymes), while Starner and streptomycin sulfate reduced the cellulolytic enzyme (Cx). The tested materials were also powerful bactericide against the bacterial soft rot pathogen. Streptomycin sulfate prevents the soft rot disease in daughter potato tubers and increased the vegetative characters, plant height and number of leave per plant. Starner and Micronite Soreil gave a moderate effect in reducing the incidence of soft rot disease, while a positive effect on tuber weight and plant tuber yield has been recorded than control.

Ashok *et al.,* (2003), reported that Mancozeb at concentration of 400 g/kg reduced the bacterial attack and improved plant yield as well as chlorinated water minimized the wounding during harvesting and storage.
Garza *et al.,* (2002), observed when copper compounds were applied in closed irrigation system it reduced the population of *Ecc.*

Chen and Lin (2000) screened 8 bactericides and 12 microbial pesticides, including a chemical mixture of 10% streptomycin+tetracycline, 63% copper oxychloride+mancozeb and 12.5% streptomycin. The chemical mixture showed high efficacy in inhibition of the growth of the soft-rotting bacterium and promoted tuber budding and plant growth to calla lily in the field.

Jerry (1999) reported that, suppression of bacterial soft rot in potato tubers can be obtained by application of kasugamycin. An immersion in kasugamycin at 10 to 320 mg/L (ppm) prevented the development of soft rot on inoculated (*Erwinia carotovora* subsp.*carotovora**)***. When stored tubers were inoculated with *E. c. carotovora* and incubated up to 5 days in fog chamber at 20 C, immersion treatments in 20 to 400 ppm kasugamycin either had no effect or increased soft rot development. By contrast, when the stored tubers were cut into sections before inoculation and treatment, immersion in 300 ppm reduced soft rot development on the cut surfaces from 83% (inoculated controls) to 3.2%.

Blom and Brown (1999) compared four sterilants–bactericides (Physan-20, Fixed Copper, Phyton- 27, and Virkon) as preplanting dips of calla lillis rhizomes to reduce plant losses due to latent field infected *Erwinia carotovora* soft rot during greenhouse. The copper based compounds (Fixed Copper or Phyton-27) provided better control of bacterial soft rot than either Physan- 20 or Virkon only during the first 6 weeks of forcing.

Alice and Sivaprakasam, (1995), found maximum inhibition zone (27.66 mm) against *Erwinia carotovora* using Streptocycline consisting of streptomycin sulphate 90% and tetracycline hydrochloride 10%.

Harris (1979) described a method for assessing the potential of chemicals in preventing bacterial degeneration of wounded potato tubers. He conducted an experiment using a wide range of potential bactericides, more often in small laboratory-scale experiments than in the field. In his experiment the organic compounds such as hydroxyquinoline and 5-nitro-8-hydroxyquinoline were effective for control of soft rot in wounded potato tubers.

CHAPTER III
MATERIALS AND METHODS

3.1. Collection of sample

Experiment was carried out to evaluate the efficacy of different chemicals against *Erwinia carotovora* subsp. *carotovora* in vitro causing soft rot of potato. The potato tubers were collected from various places in Dhaka district. The tubers from kawran bazar, Anondo bazar and local market to isolate the bacterium. The tubers were collected and washed with tap water to remove external dirt and mud and stored at 4 ^{0}C prior to the experiment.

3.2. Experimental site and period.

The experiment was carried out in the Molecular Plant Pathology Laboratory of Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during the period of January to December 2017.

3.3. Isolation and purification of the pathogen

3.3.1. Preparation of NA media

In a 1000 ml conical flask 15g bacterial agar powder, 3 g beef extract, 5 g peptone and 5 g sodium chloride were taken, then the flask was volumed with 1000 ml distilled water. Then the mixture was shaken carefully to mix the ingredients in the distilled water. It was then autoclaved at 121 ^{0}C under 15 PSI pressure for 15 minutes. After autoclaving about 12 ml of nutrient agar medium was poured in sterile 9 cm petri plates and was allowed to solidify. Nutrient agar media preparation method was followed by Schaad (1988).

3.3.2. Isolation of bacteria

The pathogen was isolated by dilution plate method. Totally twelve potato tuber samples were taken to isolate the pathogen. Diseased tubers were first washed with tap water then surface sterilized with 95% ethanol for 3 min and rinsed thoroughly 3 times with sterilized distilled water to remove sterilent. The rotted tissues of tuber were macerated in sterile water to make a bacterial suspension. Tenfold serial dilution was made from the stock solution. 0.1 ml of each dilution was placed on the surface of a nutrient agar plate and distributed using a sterile, L-shaped glass rod at three replications as described by Goszczynska and Serfontein (1998). The inoculated NA plates were incubated at 30^{o}C and observed after 24 hrs and 48 hrs. A part of a well isolated typical colony was taken using a sterile wire loop and streaked on fresh NA plate to get pure culture.

3.3.3. Preservation of bacteria

A slant culture of purified bacteria was done on NA media in small screw-cap test tubes in order to preserve the bacteria for future use and kept it in refrigerator at 4^{0}C.

3.4. Identification of the bacteria

Bacteria were identified based on morphological, cultural and biochemical features as per standard microbiological procedures and grew the bacteria over selective media.

3.4.1. Morphological characterization

Morphological and cultural characteristics of the pathogen such as cell shape, cell size, pigmentation were studied following standard procedures described by Schaad (1992); Gerhardt (1981).

3.5. Biochemical characterization

Biochemical tests such as oxidase test, gelatine liquefaction test, starch hydrolysis test, catalase test were done following methods described by Pastor *et al* (2010), Schaad (1992) and Salle (1961).

3.5.1. Gram staining

A small drop of distilled water was placed on a clean microscope slide. a small part of young colony was removed with the help of sterile loop from agar medium and then the bacterial was smeared on the slide. The bacterial was air dried and heat fixed by passing it four times through the Bunsen flame. Then the slide was flooded with crystal violet solution for 1 minute followed by rinsing under with tap water for a few seconds and excess water was drained off. Then the slide was flooded with Lugol's iodine solution for 1 minute. After that decolourization was done with 95% ethanol for 30 seconds and again rinsed with water and air dried. Then counter-stain with safranine for 10 seconds was done. It was then rinsed with water and dried. Then the glass slide was observed at 100x magnification using oil immersion The Gram negative bacteria appeared red in color and Gram positive bacteria appeared violet in color (Pastor *et al.*,2010).

3.5.2. KOH solubility test

Few drops of 3% KOH solution were placed at a clean glass slide. A loopful of well isolated bacterial colony was mixed to the 3% KOH solution with a sterial toothpick of about 10 seconds. Gram-negative bacteria became gummy and produced a mucoid thread when lifted with the toothpick while Gram-positive bacteria will not (Suslow *et al.*,1982).

3.5.3. Catalase test

A few drops of freshly prepared 3% H_2O_2 (Hydrogen peroxide) was placed at the center of a clean glass slide. 48 hours old pure culture of bacterium grown on NA

plate was added with it and observed whether the bacteria formed bubbles within a few seconds or not (Schaad, 1988).

3.5.4. Oxidase test

1% aqueous (w/v) solution of NNN'Ntetramethyl-p-phenylene-diamine-dihydrochloride solution was used as test reagent. Whatman filter paper was soaked with reagent and the paper was placed on a petri dish. Then young colony of bacteria was picked with a sterile tooth pick and rubbed onto the moistened filter paper and observed up to 60 seconds whether it changed color to dark purple or not, Kovacs, (1956).

3.5.5. Gelatin liquefaction test

Nutrient broth was prepared containing 12% (w/v) gelatin and filled in to the test tubes. One loopful bacterial culture was stab inoculated into the tube with the help of a sterile transfer loop. Then it was incubated at 30^{o}C for 1-2 days. It was observed whether the bacteria liquefied gelatin after keeping it at 5^{o}C in refrigerator for 15 minutes or not (Salle, 1961).

3.5.6. Starch hydrolysis test

For starch hydrolysis test, pure colony of bacterium was streak inoculated on nutrient agar plate containing 2% soluble starch. Then it was incubated at 30^{o}C for at least 2 days in incubation chamber. After that the plates were flooded with lugol's iodine solution. The positive result of starch hydrolysis test was mentioned by clear zone surrounding the bacterial colony. The zone showed that starch in the media could be hydrolyzed because of an ezymatic reaction, *i.e.*, amylase, secreated by the antagonist (Karkalas, 1985).

3.6. Pathogenicity test:

Potato tubers were disinfected with 99% ethanol, cut up into slices of about 1cm thick with sterile blade, and then placed on moistened sterile filter paper in sterile Petri dishes. Bacterial culture was suspended into sterial distilled water to make bacterial cell suspension. 0. 5 ml suspension was pipetted into a depression cut in the healthy potato tuber 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. Softening of the inoculated tuber slices was taken as a positive reaction. . From the softened/macerated slice tissue, bacteria were re-isolated and compared with the original isolate of inoculated pathogen (Shashirekha *et al.,* 1987).

3.7. Preparation of chemicals at specific concentration

All the chemicals were available either in wettable powder or in liquid form. For the experiment the concentrations of chemical were prepared based on their performances against the bacterial soft rot of potato according to literature with little adjustment. Treatments were as follows**:**

T_1 – Sunvit 50WP at 0.2%, T_2 –Dithane M-45 at 0.3%, T_3-Boric Acid at 0.1%, T_4-Kasumin at 0.02%, T_5- Autostin 50WG at 0.2% and T_6 - Chlorox at 0.2%,

Table 1. Trade name, common name and specific concentration of six selected chemicals.

SI	Trade Name	Common Name	Concentration
01	Sunvit 50WP	Copper oxychloride	0.2%
02	Dithane M-45	Mancozeb	0.3%
03	Boric Acid	Boric powder	0.1%
04	Kasumin	Kasugamycin	0.02%
05	Autostin 50WG	Carbendazim	0.2%
06	Chlorox	Sodium hypochlorite	0.2%

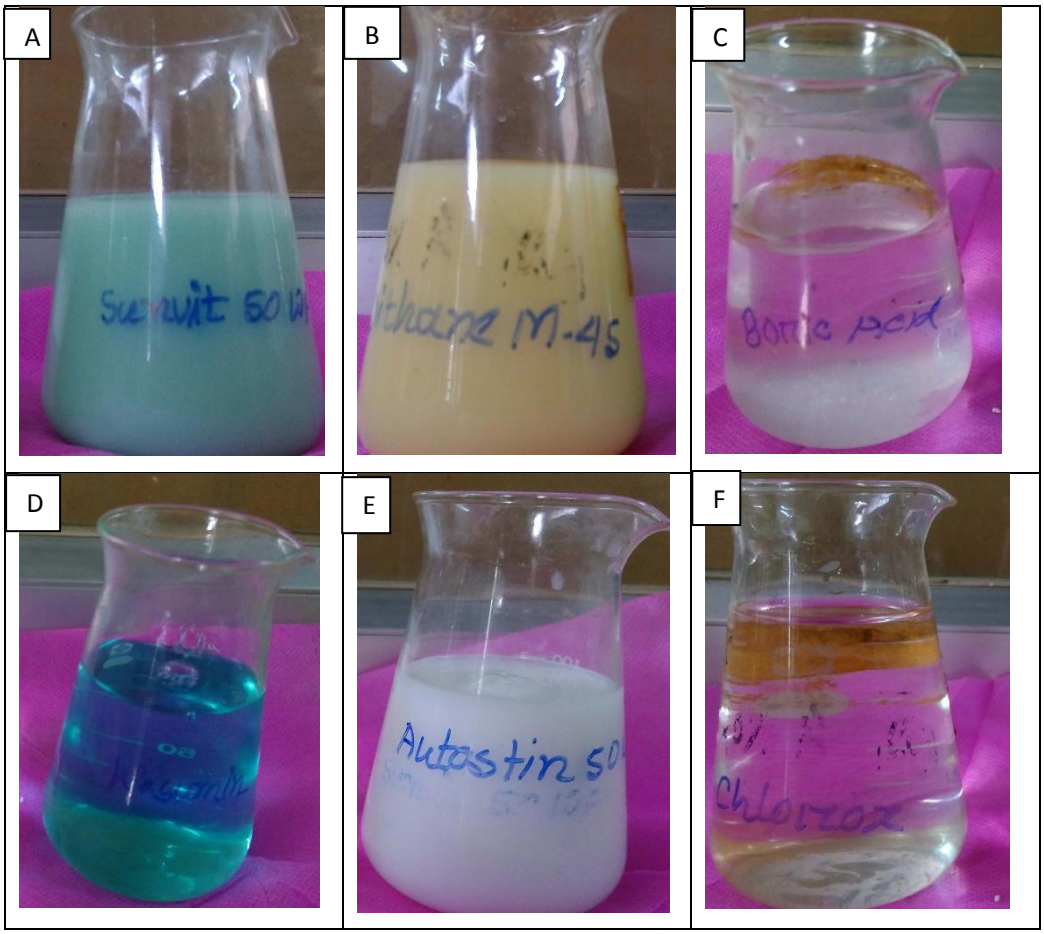

**Figure-1: A- Sunvit 50WP @ 0.2%; B- Dithane M-45 @ 0.3%
C- Boric acid @ 0.1%; D- Kasumin @ 0.02%
E- Autostin 50WG @ 0.2%; F- Cholorx @ 0.2%**

3.8. *In-vitro* screening of antibacterial activity of six different chemicals

Six selected chemicals viz. Sunvit 50 WP (Copper oxychloride) at 0.2%, Dithane M-45 (Mancozeb) at 0.3%, Boric acid (Boric powder) at 0.1%, Kasumin (Kasugamycin) at 0.02%, Autostin 50WG (Carbendazim) at 0.2% and Chlorox (Sodium hypochlorite) at 0.2% and were screened against the test bacterium *Erwinia carotovora* by well method measuring the inhibition zone. Two wells of 5 mm in diameter were made in the same NA plate maintaining equal distance and the pure culture of *Erwinia carotovora* subsp. *carotovora* was streaked uniformly on it with sterile loop. One well was filled with definite concentration chemical

suspension with 100 µl volume and other well was filled with sterile water as negative control. Each combination of pathogen, chemical and sterile water was replicated four times and plates were incubated at 30±1ºC. Zone of inhibition around the wells were measured by observing the radial growth of bacterial averaging two diameters of colony at right angle to one another and recorded after every 24 hours for 5 days.

The percentage (%) growth inhibition was determined using the formula modified by Amadioha (2004) as:

$$\% \text{ inhibition} = \frac{dc-dt}{dc} \times 100$$

Here,

dc = Colony diameter of control.

dt = Colony diameter of treatment

Data analysis

The collected data during experimental period were tabulated and analyzed following Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

CHAPTER IV
RESULTS

4.1. Isolation and identification of different bacteria

Several cultural, physiological and biochemical tests were performed to identify *E. carotovora* subsp. *carotovora*.

4.1.1. Identification of bacteria from colony morphology

Isolation was done to determine the cultural and morphological characteristics of bacteria.

Colonies of bacterium on NA medium were found after 48 hours of incubation at 30^0C (Figure 2). Colonies were purified by restreaking on another nutrient agar plate in order to identify from colony morphology (Figure 3). Cultural characteristics of bacteria is presented in Table 1.

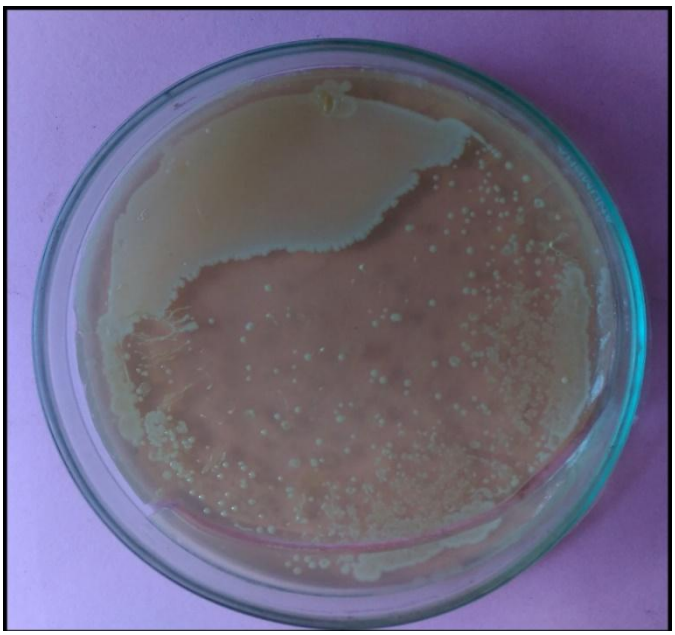

Figure 2: Pure culture of *E. carotovora* subsp. *carotovora*. obtained by spread plate method on NA

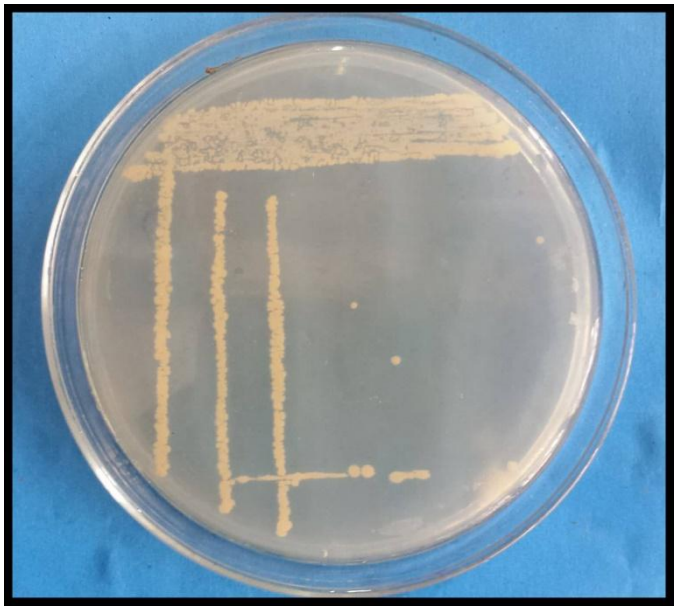

Figure 3: Pure culture of *E. carotovora* subsp. *carotovora*. obtained by streaking method on NA

Table 2. Cultural characterization of *Erwinia carotovora* on NA plates

Name of cultural character	Observation
Colony size	Small to moderate large
Form	Circular
Pigmentation	Creamy white
Elevation	Convex

4.2. Preservation of bacteria

Purified bacteria on NA slant separated and were kept in refrigerator at 4^0C in test tubes. It was served as a stock culture for further studies (Figure 4).

Figure 4: Culture of *E. carotovora* subsp. *carotovora* on NA slant

4.3. Identification of bacteria from biochemical characters

The isolated bacteria *Erwinwia carotovora* was confirmed by different biochemical tests. The bacteria were gram negative as they resulting red colour after a series of Gram reaction test. In Gram differentiation test or KOH solubility test, the bacteria produced a mucoid thread when lifted with the help of toothpick. The bacteria formed bubbles resulting positive catalase test when a few drops of freshly prepared 3% H_2O_2 added with pure culture of bacterium grown on NA plate. The bacteria formed dark purple colour when smeared on filter paper containing NNN'Ntetramethyl-p-phenylene-diamine-dihydrochloride in oxidase test. In gelatin liquefaction test the bacteria liquefied gelatin after keeping it at 5^oC

in refrigerator for 15 minutes, resulting gelatin liquefaction test positive**.** A clear zone appeared around the colony, in starch hydrolysis test.

The results of biochemical tests for *E. carotovora* subsp. *carotovora* are presented in figure 05-06 and in the Table 2.

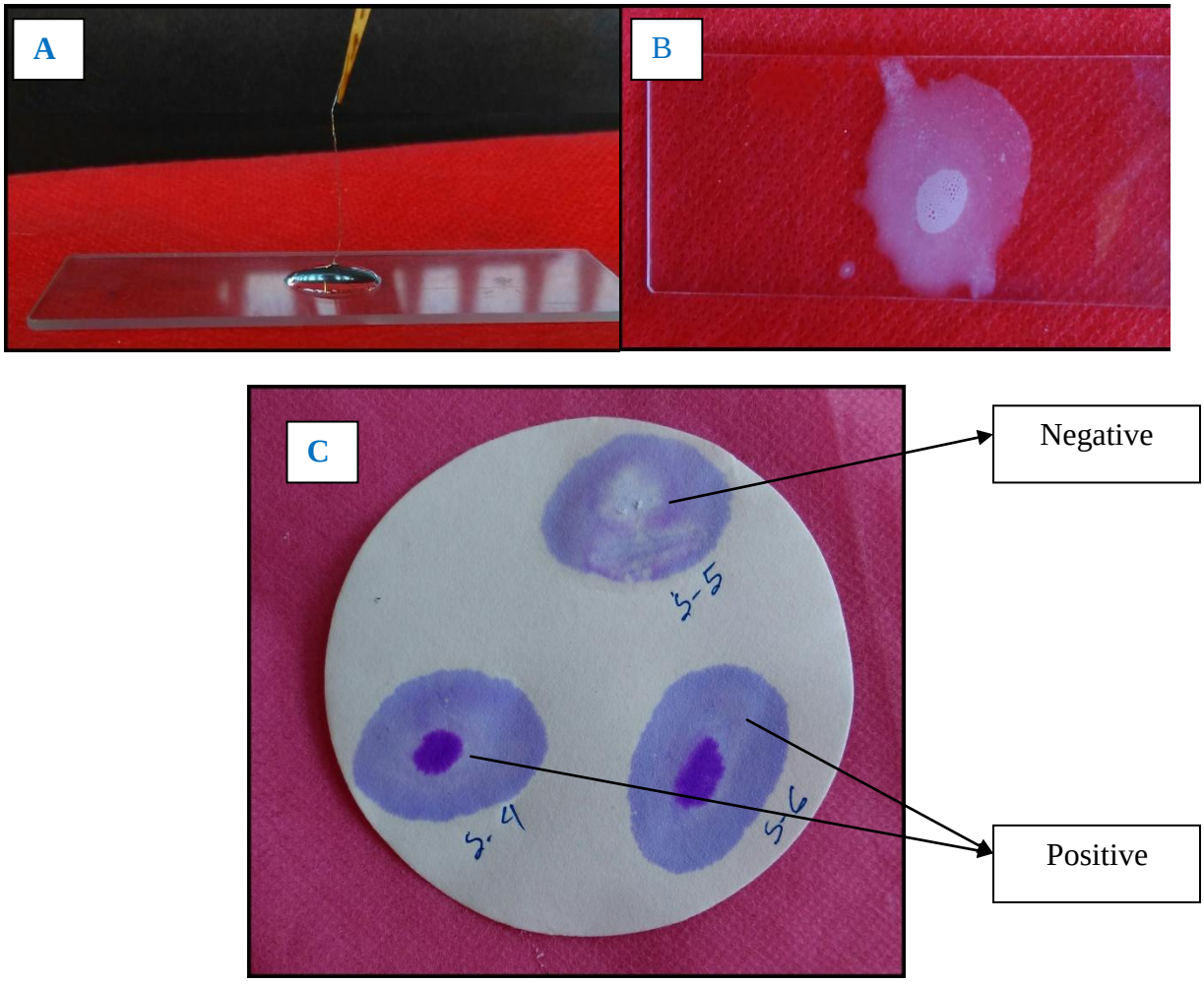

Figure 5: Biochemical tests- A. KOH solubility test. B. Catalase test. C. Oxidase test.

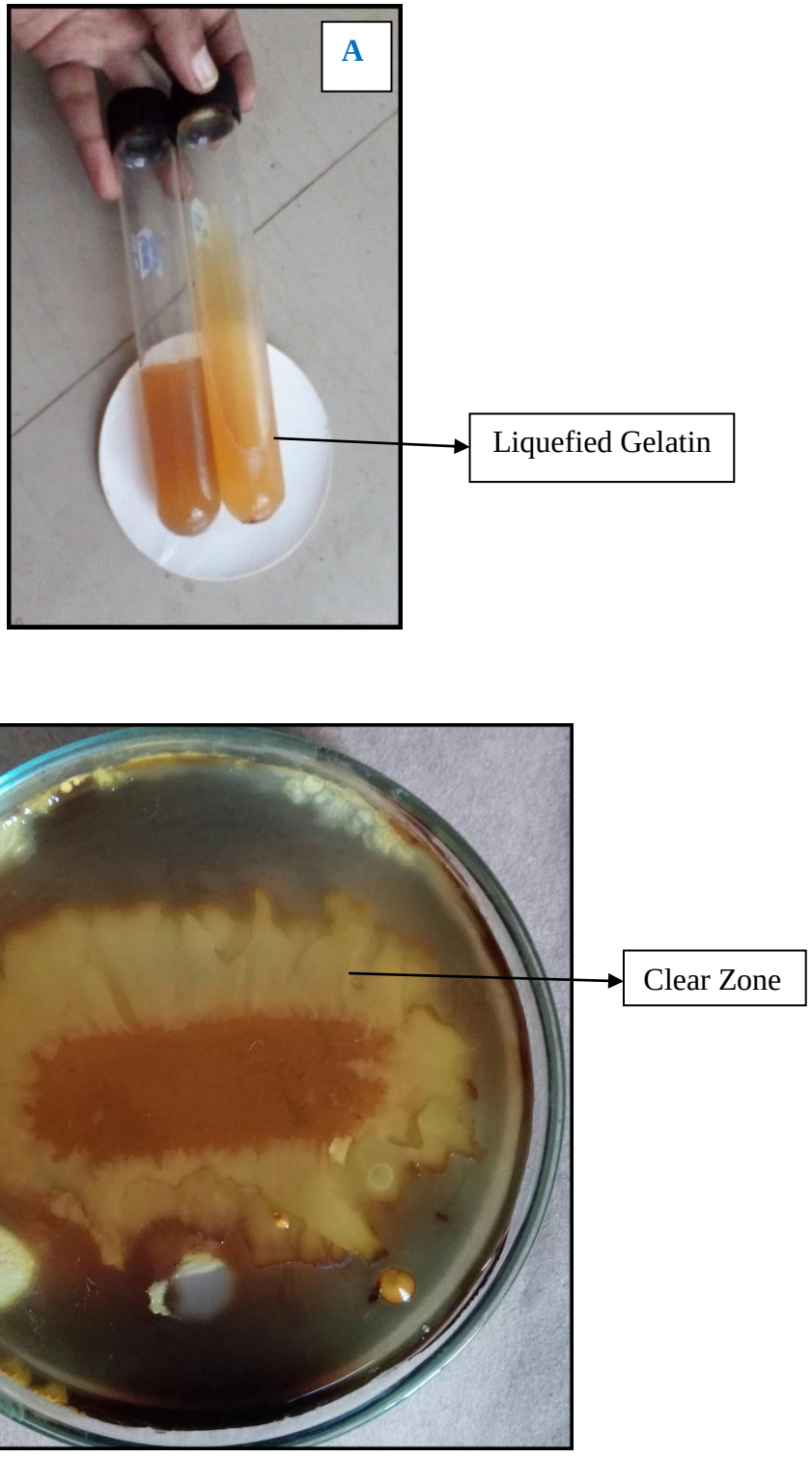

Figure 6: Biochemical tests- A. Gelatin liquification test. B. Starch hydrolysis test.

4.4. Pathogenicity test

The characteristic symptoms were observed on potato tuber slice after five days of inoculation as softening of the tissue. Re isolation was carried out from these tissue and comparison was made with the original culture to confirm the identity of the pathogen. Artificially inoculated potato tubers yielded the bacterial colonies similar to the original ones.

Based on the morphological, biochemical and pathogenicity test, the pathogen was identified as *Erwinia carotovora.*

Table 3. Characteristics of isolated *E. carotovora* subsp. *carotovora* to different tests are listed below.

Name of tests	Reaction
Gram staining	-
Gram differentiation test (KOH solubility test)	+
Gelatin liquification test	+
Starch hydrolysis test	+
Catalase test	+
Oxidase test	+
Pathogenicity test	+

4.5. *In-vitro* screening of chemicals against *E. carotovora* subsp. *carotovora*

The efficiency of six selected chemicals T_1= Sunvit 50WP (Copper Oxychloride) at 0.2%, T_2= Dithane M-45 (Mancozeb) at 0.3%, T_3= Boric Acid (Boric Powder) at 0.1%, T_4=Kasumin (Kasugamycin) at 0.02%, T_5= Autostin 50WG (Carbendazim) at 0.2% and T_6= Chlorox (Sodium Hypochlorite) at 0.2% were evaluated against *E. carotovora* subsp. *carotovora* and the results were presented in table 4 and figure 7.

Table 4. *In-vitro* screening of chemicals against *Erwinia carotovora* subsp .*carotovora*

Chemicals	Con.	Volume µl	Inhibition Zone				
			24h	48h	72h	96h	120h
Sunvit50WP	0.2%	100	27a	30.35a	28.25a	26.05a	22.28a
Dithane M-45	0.3%	100	18.4b	20.15b	18.48b	16.18b	13.65b
Boric acid	0.1%	100	16.83c	19.15b	17.63c	15.83b	12.83b
Kasumin	0.02%	100	14.88cd	16.28c	13.88d	11.98c	9.68c
Autostin	0.2%	100	2.39d	3.83d	1.28e	0d	0d
Chlorox	0.2%	100	1.15d	2.42d	0.675e	0d	0d
Control		100	0d	0d	0e	0d	0d
CV%			2.88	4.30	3.52	2.74	2.50

Each data represents the mean of four replications.

Values followed by the same letter within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

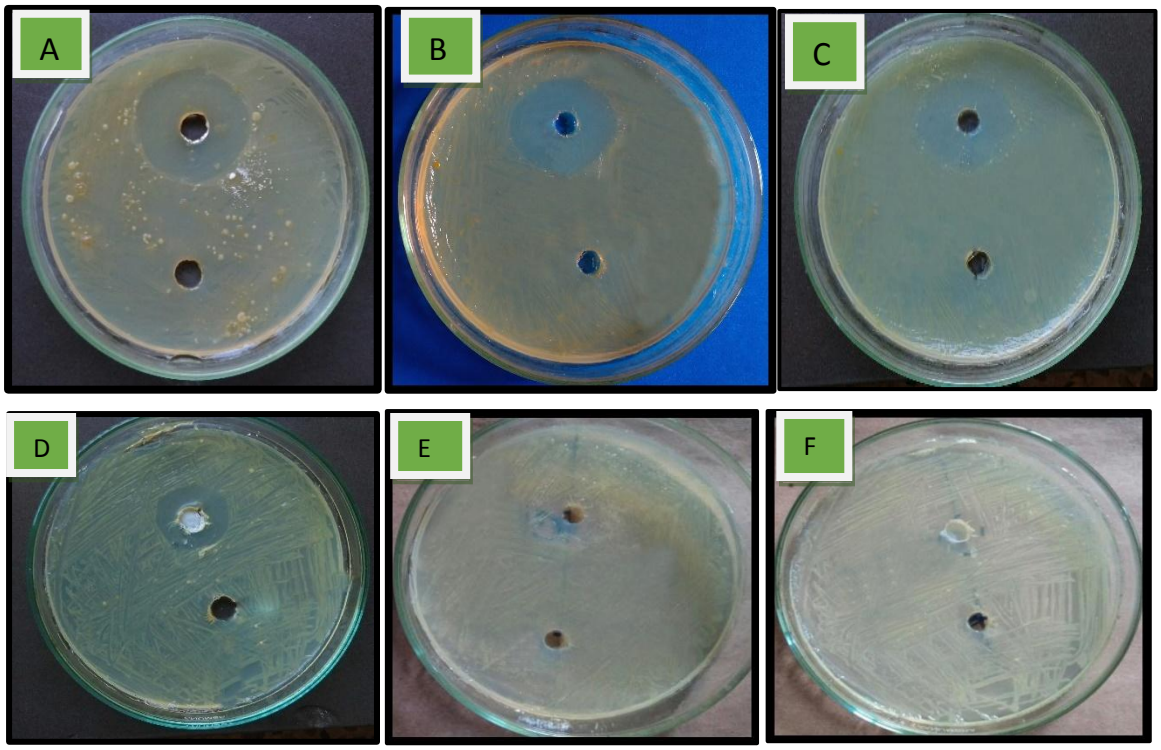

Figure 7: Screening of different chemicals against *Erwinia carotovora* subsp. *Carotovora* (A) Sunvit 50 WP (B) Dithane M-45 (C) Boric Acid (D) Kasumin (E) Autostin 50 WG and (F) Cholorx after 24 hours of incubation.

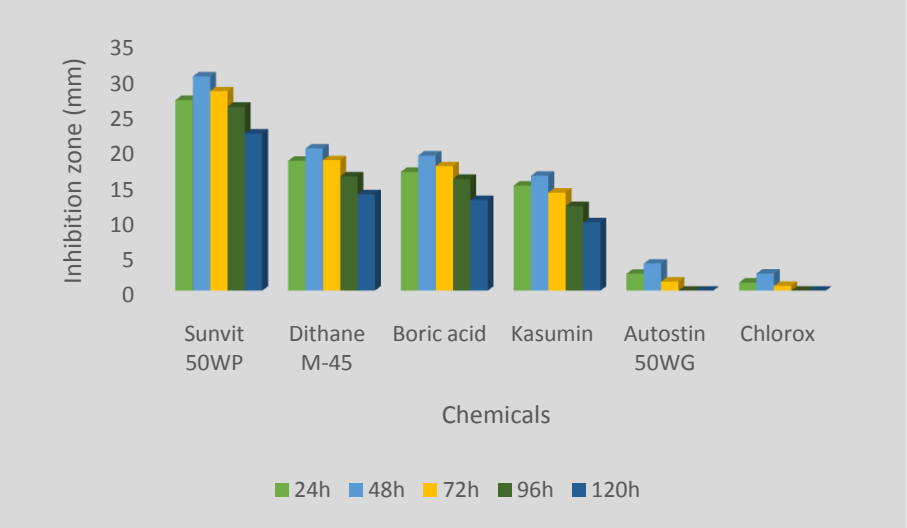

Figure 8: Effect of six chemicals on growth inhibition of *E. carotovora* at different time of incubation.

In-vitro screening of six different chemicals against *Erwinia carotovora*, were studied and found significant variations in terms of inhibition zone of isolated bacteria (Table 4 and Figure 7). Among the six chemicals, Sunvit 50 WP at 0.2% showed the highest inhibition zone (30.35 mm) after 48 hours of incubation followed by Dithane M-45 (20.15mm). Boric acid at 0.1% and Kasumin at 0.02% showed moderate inhibition zone 19.15mm and 16.18mm, respectively. Cholox (Sodium hypochlorite) at 0.2% showed the lowest inhibition zone 2.42mm. Best results were obtained against the growth of *E. carotovora* after 48 hours of chemicals incubation, the similar trend was found for every treatment (Figure 8). May be over time chemicals reduced their efficacy compare to progression of disease resulting reduced inhibition zone.

The results presented in Table 4 and Figure 9 revealed that Sunvit 50 WP produced the maximum growth inhibition (33.79%) of the pathogen after two days of incubation and was statistically superior over rest of the chemicals tested. Other chemicals viz., Cholorx (3.47%) did not effectively inhibit the growth of *E. carotovora*.

Table 5. Efficacy of six chemicals in inhibition of growth of *E. carotovora* subsp. *carotovora*

SL. No	Chemicals	Growth inhibition (%) *E. carotovora* at 2 DAI*
1	Sunvit 50WP	33.79
2	Dithane M-45	23.17
3	Boric Acid	21.36
4	Kasumin	18.91
5	Autostin 50WG	5.22
6	Chlorox	3.47
7	Control	0.0

*In column, DAI = Days after inoculation

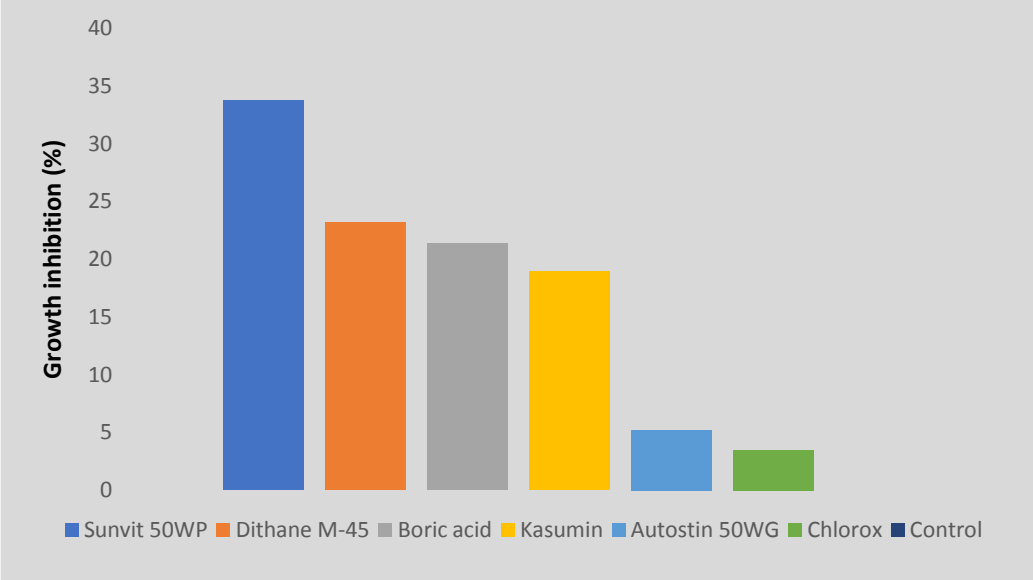

Figure 9. Growth inhibition pattern for *Erwinia carotovora* subsp. *carotovora*

CHAPTER V
DISCUSSION

The present study was conducted to isolate the bacteria *Erwinia carotovora* from soft rot infected potato tubers and evaluation of efficacy of six selected chemicals viz; Sunvit 50WP (Copper oxycloride) at 0.2%, Dithane M-45 (Mancozeb) at 0.3%, Boric Acid (Boric powder) at 0.1%, Kasumin (Kasugamycin) at 0.02%, Autostin 50WG (Carbendazim) at 0.2% and Chlorox (Sodium hypochlorite) at 0.2% against isolated bacteria.

The colonies of *Erwinia carotovora* subsp. *carotovora* was found creamy white, round, slightly raised, smooth with entire edges, small to moderate large on NA media. Similar types of colonies were found by Opara and Agugo 2014, and Rashid *et al*, 2013.

The bacteria *E. carotovora* was gram negative as they resulting red colour after a series of Gram staining test. Similar results have been also supported by Gerhardt (1981). It gave positive result in KOH solubility test, starch hydrolysis test, catalase test, gelatin liquefaction test and pathogenicity test. These results agreed with various tests as follows. Bacteria smeared with oxidating reagent develop deep blue or purple color (Kovacs, 1956). A Gram-negative bacterium formed milky thread when 3% (KOH) is mixed with it (Suslow *et al*., 1982). Nutrient broth containing 12% gelatin with bacterial *E. carotovora* culture when incubated at 30^{o}c for 1-2 days followed by 5^{o}C in refrigerator for 15 minutes, gelatin liquefied Plates containing bacterium with 2% soluble starch when flooded with lugol's iodine solution, clear zone surrounding the bacterial colony mentioned positive result (Cowan, 1974).

Sunvit 50WP, Dithane M-45, Boric Acid and Kasumin showed antibacterial activity against *E. carotovora* subsp. *carotovora*.

Among the tested chemicals best result was found in case of Sunvit 50 WP (Copper oxychloride) @ 0.2% with 30.35 mm inhibition zone followed by Dithane M-45 (Mancozeb) with 20.15 mm inhibition zone. Boric acid @ 0.1% showed moderate efficacy with 19.15 mm inhibition zone. Kasumin (Kasugamycin) @ 0.02% showed bactericidal activity against *E. carotovora in vitro* with 16.28 mm inhibition zone. Autostin 50WG (Carbendazim) @ 0.2% and Cholorx (Sodium hypochlorite) @ 0.2% exhibited least inhibition zone 3.83 mm and 2.42 mm, respectively. These results agreed with various test results could be summarized as follows. Rashid *et al*, (2013) showed that Copper oxychloride and Mancozeb had antibacterial activity against *E. carotovora* with 31.00 mm and 18.67 mm inhibition zone, respectively. Rahman *et al.*, (2017) reported that Boric acid at 0.05% and 0.10% concentration showed bactericidal activity against *E. carotovora* subsp. *carotovora in vitro*. Jerry (1999) and Viswanath *et al* (2018) in their separate work reported antibacterial activity of Kasugamycin. Rashid *et al*, (2013) and Rahman *et al*, (2017) in their separate work agree with the findings on Autostin 50WG and Cholorx.

Best results were obtained against the growth of *E. carotovora* after 48 hours of chemicals incubation. Sunvit 50 WP (Copper oxychloride) @ 0.2% showed inhibition zone after 24 (27.00 mm), 48 (30.35 mm), 72 (28.25 mm), 96 (26.05 mm) and 120 hours (22.28 mm). The similar trend was found for every treatment. Many researchers in their experiment reported the similar trend. Rashid *et al*, (2013) and Adamu *et al*, (2017) in their separate work revealed the negative relationship between treatment efficacy and time duration.

Under *in-vitro* screening of six chemicals Sunvit 50WP have the strong bactericidal effect followed by Dithane M-45, Boric acid and Kasumin. These four chemicals have given encouraging results, indicating their potential in the control of *E. carotovora* subsp. *carotovora* causing potato soft rot disease.

CHAPTER VI

SUMMARY AND CONCLUSION

The experiment was conducted in the Molecular Plant Pathology Laboratory of the department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka, during the period of January, 2017 to December, 2017 to isolate, identify and evaluate the efficacy of six selected chemicals against *Erwinia carotovora* subsp. *carotovora*. The bacteria, *E. carotovora* was identified by various biochemical tests and pathogenicity test. Total twelve isolate of *E. carotovora* were isolated and identified.

E. carotovora subsp. *carotovora* produce creamy white, round, slightly raised, smooth with entire edges, small to moderate large colony on nutrient agar media. The bacteria resulted Gram negative in Gram staining test. Positive in KOH solubility test, starch hydrolysis test, catalase test, gelatin liquefaction test and pathogenicity test. These above result revealed that, the bacteria was *E. carotovora* subsp. *carotovora.*

Six selected chemicals were tested *in-vitro* against *E. carotovora* subsp. *carotovora* by well diffusion method. Among the chemicals Sunvit 50WP (Copper oxychloride) @ 0.2% had the most capability to inhibit the growth of bacteria with a 30.35 mm inhibition zone. Dithane M-45 (Mancozeb) @ 0.3% also inhibited the growth of *Erwinia carotovora* with a 20.15 mm inhibition zone. Boric acid (Boric powder) @ 0.1% and Kasumin (kasugamycin) @ 0.02% showed moderate efficacy to inhibit the growth of bacteria with inhibition zone of 19.15 mm and 16.18 mm, respectively. Autostin 50WG (Carbendazim) and Chlorox (Sodium hypochlorite) had less capability to inhibit the growth of *E. carotovora* subsp. *carotovora.*

The present study clearly indicated that *E. carotovora* subsp. *carotovora* responsible for soft rot of potato. Among the chemicals tested Sunvit 50WP (Copper oxychloride) @ 0.2% was found most effective against the bacteria *in vitro*. Other chemicals also have moderate effect against *E. carotovora* subsp. *carotovora*. These chemicals have significant effects on the production and activity of cell wall degrading enzymes produced by plant pathogenic bacteria. However, mechanism of acting the chemical compounds and their harmful activities have not determined. In addition, phytotoxicity and the environmental effects of the chemicals on epiphytic beneficial microorganisms need to be addressed before transfer the technology to farmers. A through and wide scale investigation is needed to find out an effective method to control soft rot of potato in storage.

REFERENCES

Ashmawy, N.A., Jadalla, N.M., Shoeib, A.A. and Bebany, A. (2015). Identification and genetic characterization of *Pectobacterium* spp. and related *Enterobacteriaceae* causing potato soft rot diseases in Egypt. *J. of Agric. Sci.* **41**(1): 66-70.

Akbar, A., Din, S., Ahmad, M., Khan, G., Alam, S.(2014). Effect of Phytobiocides in controlling soft rot of tomato. *J. Nat. Sci. Res*. **4**:11.

Afek, U and Orenstein J, (2002). Disinfecting potato tubers using steam treatments. *Canadian Journal of Plant Pathology* **24**: 9-36.

Amadioha, A.C. (2004). Control of black rot of potato caused by *Rhizoctonia bataticola* using some plant leaf extracts. *Arch. Phytopathol. Plant Prot*, **37**:111-117.

Adamu, S.H., Lal, A.A., and Simon, S. (2017). *In vitro* efficacy of certain botanicals against bacterial soft rot of tomato (*Solanum lycopersicum* L.). *African Journal of Agricultural Research*. **12**(23): 2049-2055.

Abd-El-Khair, H. and Karima, H.E.H. (2007) Application of some bactericides and bio agents for controlling the soft rot disease in potato. *Research Journal of Agricultural Biological Science*, **3**(5):463-473.

Alice, D. and Sivaprakasam, K. (1995). Antibiotics and garlic clove extract inhibitory agents of cell wall degrading enzymes. *Hindustan Antibiotics Bulletin.* **37**: 44-47.

BBS (2012) Statistical Year Book of Bangladesh. Statistics Division, Agriculture Wing, Ministry of Planting, Govt.of The People's Republic of Bangladesh, Dhaka. P.60

Bathily, H., Babana, A.H and Samaké, F. (2010). *Bacillus pumilus* a new pathogen on potato tubers in Mali. *Afr. J. Microbiol. Res*. **4**(20): 2067 – 2071.

Bdliya, B.S and Haruna, H.U. (2007). Efficacy of solar heat in the control of bacterial soft rot of potato tubers caused by *Erwinia carotovora* subsp. *carotovora*. *J. Plant Protec. Res*. **47**: 1.

Blom, T.J. and Brown, W. (1999). Pre plant copper-based compounds reduce Erwinia soft rot on *calla lilies*. *Horticulture Technology*. **9**(1): 56-59.

Czajkowski, R., Perombelon, M.C.M., vanVeen, 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 Pathology*. **10**: 1365-3059.

Cromarty, R.W. and Easton, G.D. (1973). The incidence of decay and factors affecting bacterial soft rot of potatoes. *Am. Potato J*. **50**: 398–407.

Chen, C.W. and Lin, C.Y. (2000). Control of *Erwinia* soft rot disease of Calla lily. *Plant Pathology Bulletin.* **9**(3): 107-114.

Cowan, S.T. (1974). Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd ed. Cambridge University Press, Cambridge. pp. 67-83.

Douches, D.S., Maas D., Jastrzebski, K. and Chase, R.W. (1996). Assessment of potato breeding progress in the USA over the last century. *Crop Science*. **36**: 1544–52.

De Boer, S.H., Allan, E. and Kelman, A. (1979). Survival of *Erwinia carotovora* in Wisconsin soils. *Am. Potato J*. **56**: 243 - 53.

Dickey and R. S. (1979). Erwinia chrysanthemi: A comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. *Phytopathol*. **69**: 324 - 29.

Esfahani, M.N (2003) Study on potato losses in the stores of Farydan, Isfahan. Plant Pests and Diseases Research Department, Agricultural Research Center of Isfahan, Isfahan, Iran. Research.**19**(2): 191-208.

FAO (2008) Production Yearbook. Vol. 52. FAO Statistical Series No. 125. Food and Agricultural Organization of theUnited Nations.Rome.Italy**10**(5):26

Garcia, R. (2000) Species and subspecies of Erwinia causing soft rot and black leg of cultivated potato in Merida State, Venezuela. *Revista Forestal Venezolana*. **34** (44-1): 107-114

Garza, J.A.G., Allen, W., Blom, T.J and Brown, W. (2010). Pre- and post-plant applications of copper-based compounds to control *Erwinia* soft rot of calla lilies, *Canadian Journal of Plant Pathology*, **24**(3): 274-280

Gerhardt, P. (1981). Manual of methods of general bacteriology. American Society of Microbiology Washington, D.C. Pp. 127.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedures for agricultural research (2 ed.). pp. 34-35.

Goszczynska, T. and Serfontein, J.J. (1998). Milk tween agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae* pv.

syringae, Pseudomonas syringae pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*. *J. Microbiol. Method*. **32**(1): 6572.

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. *American Potato Journal*, **74**(4): 265-275.

Hammerschmidt, R and Smith, J.B. (1997). Acquired resistance to disease in plants, *Hort. Rev*. **18**: 247-289.

Harris, R. (1979). Chemical control of bacterial soft-rot of wounded potato tubers. *Potato Research*. **22**: 9- 245

Hajhamed, A.A., Abd El-Sayed, W.A., Abou El-Yazied, A and Abd El-Ghaffar, N.Y. (2007) Suppression of bacterial soft rot disease of potato**.** *Egypt. J. Phytopathol.* **35**(2): 69-80.

Jerry, A.B. (1999) Suppression of bacterial soft rot in potato tubers by application of kasugamycin. *American journal of potato*. **76** (3):127-136.

Jaggi, W., Oberholzer, H., Winger, F.A (1991) Effect of harvest and market conditions of potato infection by *Erwinia carotovora*. *Land wirtschaft Schweiz.* **4**(8): 421-426.

Karkalas, J. (1985). An improved enzymatic Method for the Determination of Native and activity from the rhizosphere of beans. *Appl. Microbiol*. **11**: 533-538.

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

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature*. **178**: 703.

Lambert, D.H and Manzer, F.E. (1991) Relationship of calcium to potato scab. *Phytopathology*. **81**:632–636.

Masum, M.M.I., Islam, S.M.M., Islam, M.S., and Kabir, M.H. (2011). Estimation of loss due to post harvest diseases of potato in markets of different districts in Bangladesh. *African Journal of Biotechnology*. **10**(56): 11892-11902

Mehedi, H.R. (2004). Study on market diseases of potato and effect of some plant extracts on *Fusarium caeruleum*. M.S thesis. Department of Plant Pathology, Bangladesh Agricultural University.

Morales, G.Y., Suarez, N (1989) Resistance of varieties of potato to *Erwinia carotovora* PV. *carotovora*. Centre Agricola. **16**(2):14-19.

Makhlouf, A.H and Abdeen, R (2014). Investigation on the Effect of Chemical and Biological Control of Bacterial Soft Rot Disease of Potato in Storage. *Journal of Biology, Agriculture and Healthcare*. **4**(10)????

Opara, E.U and Agugo, B.A. (2014) Control of posthavest rot of sweeetpotato (*Ipomoea batatas* Lam.) tuber *in vitro* and *in vivo. Journal of Agriculture and Sustainability*. **6** (1): 50-68.

Olivieri, F.P., Maldonada, S., Tonon, C.V. and Casalongue, C.A. (2004). Hydrolytic activities of *Fusarium solani* and *Fusarium solani* f. sp *eumartii* associated with the infection process of potato tubers. *J. Phytopathol*. **152**(6): 337-344

Olsen, N., Miller, J and Nolte, P. (2006). Diagnosis and Management of Potato Storage Diseases.

Obradovic, A. (1994). A pathogenic, biochemical and physiological characteristics of the bacteria *Erwinia carotovora* group, the potato pathogen. Center for Vegetables crops. Smedervaska palanka, Yugoslavia.

Okonkwo, J.C., Amadi, C.O., Nwokoch, H.N., William, J.O and Okoye, W.I. (2003). Assessment of six market methods for seed potato market in Nigeria. National Root Crops Research Institute, Umudike, Umuahia,Abia State, Nigeria. **34**: 91-96.

Priou,S., Aley, P., Chujoy, E., Lemaga, B., & French, E. R. (1999).Integrated management of bacterial wilt of potato, pp. 111-122.

Pérombelon, M.C.M. (2002). Potato diseases caused by soft rot *Erwinia*: An overview of pathogenesis. *Plant Pathology*, **51**:1-12

Pastor, N.A., Reynoso, M.M., Tonelli, M.L., Masciarelli, O., Rosaso, S.B., Tonelli, M.L., Masciarelli,D.,Rosas, S.B. and Rovera, M. (2010). Potential bio control *Pseudomonas* sp. Pc12 against damping-off of tomato caused by *Sclerotium rolfsii*. *J. Plant Pathol*. **92:** 737– 745.

Rashid, M., Chowdhury, M.S.M., and Sultana, N. (2013). *In-vitro* Screening of some chemicals and biocontrol agents against *Erwinia carotovora* subsp. *carotovora*, the causal agent of soft rot of potato (*Solanum tuberosum*).*The Agriculturists,* **11**(2): 1-9.

Rahman, M.M., Khan, A.A., Mian, I.H., Akanda, A.M. and Alam, M.Z. (2017). Effect of some chemicals on incidence of potato soft rot disease in Bangladesh. *Bangladesh J. Sci. Ind. Res.* **52**(2): 135-140.

Rai, R.P. (1979). Fusarium equiseti (Corda) Sacc. causing dry rot of potato tubers-a new report. *Current Science*, **23**: 1043 – 1045.

Rajeh, O.N., Hamed and Khlaif. (2000). Soft rot disease of vegetables in Jordan: host range, reaction of some potato cultivars to the infection and effect of planting date. Horticulture and Plant Protection Department, Faculty of Agriculture University of Jordan. Jordan Research. **27**(2): 149-157.

Rasul. M.G., Islam, M.S., Sheikh, M.H.R. (1999) Storability of different potato varieties under natural condition. Department of Genetics and Plant Breeding, IPSA, Gazipur- 1703, Bangladesh. **34**(1): 86-90

Salle, A.J. (1961). Fermentation of carbohydrates and related compounds. In: Laboratory manual on fundamental principles of bacteriology. 5th ed. McGraw Hill Book Company, Inc. New York. pp. 94-98.

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. Phytopathology, **72**: 917-918.

Schaad, N.W. (1988). Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. *American Phytopathological Society*, St. Paul, Minnesota. pp. 185-197.

Schaad, N.W. (1992). *Xanthomonas*. In: Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. International Book Distributing Co. Lucknow. pp. 165.

Shashirekha, M.N., Karanth, N.G.K and Narasimham, P. (1987). Surface microflora of seed potatoes (Solanum tuberosum L., Kufri Jyoti): Isolation

and identification of organisms responsible for spoilage of potatoes grown at Devanahalli. *Journal of Food Science and Technology*, **24**:261-263.

Smadja, B., Latour X., Faure, D., Chevalier, S., Dessaux, Y. and Orange, N (2004). Involvement of N-acylhomoserine lactones throughout plant infection by *Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium atrosepticum*). Laboratoire de Microbiologie du Froid-UPRES 2123, Universite de Rouen **17**(11): 1269-1278.

Turkensteen, L.J., Mulder, A (1996) market diseases of potatoes.Part 1: dry rot. Trockenfaiile.Kartoffelbau **47**(11):381-383.

Viswanath, H.S., Bhat, K.A., Bhat, N.A., Wani, T.A. and Mohammad, N.M. (2018). Antibacterial efficacy of aqueous plant extracts against storage soft rot of potato caused by *Erwinia carotovora Int.J.Curr.Microbiol.App.Sci* **7**(1): 2630-2639.

APPENDIX- I

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

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-diaminedihydrochloride was prepared from the absolute solution.

Gram 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

Gelatine Liquefaction Media

Beef extract	3.0 g
Peptone	5.0 g
Gelatine	120 g
Distilled water	1000 ml

APPENDIX- II

PREPARATION OF AGROCHEMICALS

Preparation of Sunvit 50WP (Copper oxychloride) solution at 0.2%

Sunvit 50WP (copper oxychloride)	0.2g
Sterile Distilled Water	100ml

Preparation of Dithane M-45(Mancozeb) solution at 0.3%

Dithane M-45(Mancozeb)	0.3g
Sterile Distilled Water	100ml

Preparation of Boric acid solution at 0.1%

Boric Powder	0.1g
Sterile Distilled Water	100ml

Preparation of Kasumin (kasugamycin) solution at 0.02%

Kasumin liquid	0.02ml
Sterile Distilled Water	100ml

Preparation of Autostin 50WG (carbendazim) solution at 0.2%

Autostin 50WG (carbendazim)	0.02g
Sterile Distilled Water	100ml

Preparation of Cholorx (Sodium hypochloride) solution at 0.2% solution

Cholorx (Sodium hypochlorite)	0.2ml
sterile distilled water	100ml