PREVALANCE OF BROWN ROT OF POTATO IN SOME SELECTED LOCATION OF BANGLADESH AND *IN-VITRO* MANAGEMENT OF *RALSTONIA SOLANACEARUM*

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ABSTRACT

An experiment was conducted in the Disease Diagnostic Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during the period of 2014- 2015 to observe the prevalence of brown rot disease in stored potato tuber collected from different locations of Bangladesh and to find out suitable antibacterial chemicals and antagonists against *Ralstonia solanacearum*, the causal organism of brown rot of potato. Potato samples were collected from eight potato growing region viz. Munshigong, Rangpur, Lalmonirhat, Joypurhat, Pabna, Bogra, Manikgonj and Panchagarh. The highest disease incidence (40 %) was observed in potato tuber collected from Munshigong at six month storage period and the lowest incidence (3.33%) was found in potato tuber collected from Panchagarh at two month storage period. Disease incidence varied significantly from location to location and storage period. The causal organism of brown rot of potato was identified as *Ralstonia solanacearum* on the basis of morphological, biochemical and cultural characteristics. The bacterium was gram negative, rod shaped and showed positive results in KOH solubility test, catalase test, starch hydrolysis test, levan production test, pecteolytic test, gelatin liquefaction test and negative result on oxidase test. It produced highly fluidal, slightly raised and creamy white colonies with light pink or pinkish red centre and irregular margin after 48 hrs of incubation at 30 ^{0}C on TTC medium. Four agrochemicals viz. Kasumin, Bactrol, Copperoxichloride and copper+mancozeb and two bioagents such as *Bacillus subtilis* based formulation (PRH) and *Trichoderma harzianum* were tested against *Ralstonia solanacearum* in the laboratory. Among them the highest inhibition zone (14.44 mm) was recorded when Kasumin used against *Ralstonia solanacearum* wheres Bactrol, *Bacillus subtilis*, *Trichoderma harzianum* and Copperoxychloride showed moderate inhibition zone. However, copper+mancozeb did not show any effect against *Ralstonia solanacearum*.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	= Percentage
et al.	= And others
spp.	= Species
J.	= Journal
No.	= Number
viz.	= Namely
df.	= Degrees of freedom
&	= And
etc.	= Etcetera
0C	= Degree Celsius
@	= At the rate of
cm	= Centimeter
cfu	= Colony forming unit
ppm	= Parts per million
NaCl	= Sodium chloride
Kg	= Kilogram
g	= Gram
ml	= Milliliter
WP	= Wettable Powder
hr	= Hour (s)
cv.	= Cultivar (s)
i.e.	= That is
T	= Treatment

ft = Feet (s)

pv. = Pathovar

var. = Variety

mm = Milimiter

µl = Microliter

µm = Micrometer

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

SAU	=	Sher-e-Bangla Agricultural University
BAU	=	Bangladesh Agricultural University
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
BER	=	Bangladesh Economic Review
USA	=	United States of America
NA	=	Nutrient Agar (media)
PDA	=	Potato Dextrose Agar (media)
PSI	=	Per Square Inch
ANOVA	=	Analysis of variances
LSD	=	Least Significant Difference
CV%	=	Percentages of Co-efficient of Variance

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosome*) popularly known as 'The king of vegetables' is a widely grown nutritious tuber crop throughout the world as well as in Bangladesh belongs to the solanaceae family. Potato is the third most important crop of Bangladesh followed by rice and wheat (Hussain, and Rashid, 1974). Nutritionally, the tuber is rich in carbohydrates or starch and is a good source of protein, vitamin C and B, potassium, phosphorus, and iron (Ensminger *et al*., 1983). Being a carbohydrate rich crop, potato can partially substitute rice, which is our main food item. The major potato growing areas of Bangladesh are Munshigonj, Jamalpur, Nilphamari, Jessore, Bogra, Pabna, Rangpur and Panchagorh. It contributes alone as much as 54% of the total annual vegetable production of Bangladesh (BBS, 2014). Potato production in Bangladesh in the fiscal year (FY) 2012-2013, hit a new record of 8.603 million tones surpassing the record of 8.38 million tones in FY' 2011. On the other hand, potato export saw a quantum leap in the recently concluded fiscal year. In Bangladesh, exports crossed the one lakh tones mark for the first time in the fiscal 2013-14, rising threefold from only 28,416 tones in the previous year (EPB, 2015). Potato production and export is being hampered due to many diseases. In Bangladesh twelve diseases occurred in potato in which brown rot is most important bacterial disease (DAE, 2015). This newly emerging but century old disease sabotaging the potato production and export in Bangladesh. This disease is caused by soil living bacterium *Ralstonia solanacearum.* It is an aerobic, non-sporing, gram-negative plant pathogenic bacterium. *R. solanacearum* is soil-borne and motile with a polar flagellar tuft. It colonizes the xylem, causing bacterial wilt in a very wide range of potential host plants (Terblanche and Villiers, 2013; Agrios, 2008; Paret *et al*., 2008 and Andersona and Gardner, 1999). Brown rot of potato caused by *Ralstonia solanacearum* race 3 biovar 2 is among the most serious disease of potato worldwide, which is responsible for estimated $950 million world wide losses each year. Moreover Race 3 biovar 2 is cold tolerant and classified as a quarantine pathogen for most of the European country (Milling *et al*., 2009; Ephinstone, 2005;). Although the actual losses due to this bacterium in Bangladesh is still not reported. However potato export from Bangladesh to Russia was halted due to the presence of this bacteria in stored potato, which caused a loss about $9million in 2014 (EPB,

2015). The bacterium can maintain infectious populations in soil over several years. It possesses a wide variability in environments, geographic spread and its host range, including several hundred plant species representing 50 families (Hayward and Hartman 1994; Hayward 1992; and Shekhawat *et al.*, 1992). The most severely affected crops as stated by Hayward and Hartman (1994) are tomato (*L. esculentum L.*), potato (*Solanum tuberosum* L.), eggplant (*S. melongena* L.), chilli (*Capsicum* spp.) and groundnut (*Arachis hypogaea* L.). *R. solanacearum* is a soil borne bacteria and once it infects the soil it easily spreads within the field as well as to adjacent fields not only affecting the crop but also rendering the farm unusable to produce of any solanaceous crops (Sequeira, 1993). The disease is known to spread very quickly through furrow irrigation as well as rain water (Taylor *et al.,* 2011). Several strategies have been introduced to manage the bacterial wilt disease. The main control strategy for bacterial wilt has been the use of resistant varieties. Although this disease is incurable, some cultural, chemical and biological practices were found effective against bacterial wilt disease of potato. Integrated approaches showed best performance against this pathogen rather than single approach (Champoiseau, 2008; Anonymous, 2004; Basan, 2002 and Aspiras *et al*., 1985). Copper and its derivatives showed good performance against many plant pathogens (Singh *et al*., 2005; Canteros, 2004; Graham and Leite, 2004; Das and Shyam, 2003; Dixon *et al*., 2000 and Helling *et al.*, 2000.) Copper ions denature proteins, thereby destroying enzymes that are critical for cell functioning. Copper can kill pathogen cells on plant surfaces, but once a pathogen enters host tissue, it will no longer be susceptible to copper treatments. Thus, copper sprays act as protectant fungicide/bactericide treatments, but lack post-infection activity (Behlau et al., 2008). Bactrol a newly emerging chemical belongs to Bismerthiazol is effective against bacterial pathogen of plants (Liang *et al.,* 2016; Xiao-bing, *et al*., 2014). It strengthen the extent of lipid peroxidation, stimulate the O^{-2} production inhibit cell growth and extracellular polysaccharide formation. Kasumin belongs to Kasugamycin group is another antibiotic which acts against many pathogens (Milijasevic *et al*. 2009; and Wyatt and Lun, 1981). It is an aminoglycoside antibiotic that was originally isolated in 1965, from *Streptomyces kasugaensis.* Like many of the known natural antibiotics, kasugamycin inhibits proliferation of bacteria by tampering with their ability to make new proteins, the ribosome being the major target. Kasugamycin inhibits protein synthesis at the step of translation initiation (Schluenzen *et al.,* 2006, Schuwirth *et al*., 2006 and

Okuyama *et al*., 1971). The huge and indiscriminate use of chemical has been triggered the resistance of pathogen (Akinpelu, 2001, Kaur and Arora, 1991). Moreover, the residual effect of these chemicals is harmful not only for environment but also for other living organisms. As a result, the interest to use antagonistic microbial agents against plant pathogens have increased. The use of microbial antagonists has been noted as a promising control strategy. Many fungal and bacterial pathogens have been examined over a period of time for their potential as biocontrol agents (Bonev *et al*. 2008). A large number of Plant growth promoting rhizobacterium were reported to promote plant growth and to control plant diseases (Basan and De-Basan, 2002). The antagonistic microorganisms through antibiosis, competition and exploitation control the population of other microorganism including plant pathogenic ones i.e biological control may possibly become an ideal disease control method (Cook and Baker, 1983). Among them *Bacillus* is one of the important bio-control agent. (Jacobsen *et al*., 2004). *Bacillus subtilis* shows biological activity against phytopatogenic bacteria by producing peptide antibiotics (Backman *et al.*, 1997 and Kloepper, 1997). *Trichoderma* spp. have played a considerable role as bio-control agent (Papavizas, 1985) and is recognized as an effective bio-control agent against soil-borne plant pathogen (Chet and Inbar, 1994). *Trichoderma* produces chemicals called trichodermin which is responsible for its antagonistic properties (Tverdyukov *et al*., 1994). *T. harzianum* may be used as an eco-friendly option to save many beneficial micro-organisms in the nature. This bio-control agent would be potential to protect seedlings against diverse soil borne pathogenic fungi (Goswami and Islam 2002; Monaco et al., 1991). *Trichoderma* is recognized as potential bio-control agent against *Ralstonia solanacearum* (Murthy and Srinivas, 2012; Sharma *et al*., 2010*;* Ceballos *et al*., 2014; Maketon *et al*., 2008). Considering the above facts, the present study was under taken with the following objectives:

1. To study the prevalence of brown rot disease of potato in selected areas of Bangladesh
2. To isolate and identify the causal organism of brown rot of potato collected from selected parts of Bangladesh
3. To determine the efficacy of some selected chemicals and bioagents against *Ralstonia solanacearum* in *in-vitro* condition

CHAPTER II

REVIEW OF LITERATURE

Ralstonia solanacearum constitutes a serious obstacle to the cultivation of many solanaceous plants in both tropical and temperate regions. The greatest economic damage has been reported on potatoes, tobacco and tomatoes. It can sometimes cause total crop losses. Hence, the literature pertaining to the brown rot of potato along with information on related crops disease and pathogen are reviewed here as under.

2.1. Causal organism of brown rot disease of potato and its transmission

Brown rot was transmited readily in seed pieces; plants that appeared resistant were latently infected thus spreading the pathogen. Disease transmission in greenhouses was occured from root system to root system through water movement especially in re-circulating sub-irrigation systems. Outdoors, infected potato seed is responsible for initiating disease, but disease transmission was occured from plant to plant in the soil usually by water movement (Elphinstone and Aley, 1993)

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

Denny and Hayward (2001) reported that *Ralstonia* was positive in H_2S production, starch hydrolysis, KOH solubility, gelatin liquefaction, , sucrose utilization, indole production, Levan Test and Kovac's Oxidase Test and was able to utilize Dextrose, Maltose, Lactose, Sorbitol Manitol, Dulsitol.

Elphinstone (2005) reported that brown rot was mainly tuber-borne as it could latently infect tubers and survived in seed tubers during storage, causing disease when planted in the next season. The bacterium was spread on machinery and in irrigation water. The disease was persisting in fields where infected ground keepers were present.

Kelman (1953) observed that after 48-72 h of incubation at 28^{o}C *Ralstonia* gave circular, smooth, convex and viscous bacterial colonies with pink center and whitish margin on 2,3,5 Triphinyl Tetrazolium Chloride (TTC) medium. On NA medium the bacteria produce watery whitish or off white or cream color irregular colonies.

Rahman *et al*. (2010) reported that virulent isolates produce pink or light red colour colonies or colonies with characteristic red centre and whitish margin and avirulent isolates produce smaller, off-white and non-fluidal or dry on TZC medium after 24 hours of incubation.

2.2. Symptoms of brown rot disease of potato

Disease development occurred at different rates in different varieties, but was favored by warm temperatures (above 15^{0}C with optimum of 27^{0}C) and high soil moisture levels. Brown rot was primarily spread by the planting of infected seed potatoes, but can also spread in soil and in irrigation water stated by French (1994).

Fahmy and Mohamed (1990) reported that symptoms appeared at any plant age and included wilting and yellowing of leaves and stunting of plants. Disease was severe in young, succulent plants and that appeared as rapid wilting of leaves and collapse of stems. Initially, only one stem was wilted. Stems were appeared streaked as infected vascular bundles become visible. Tuber vascular tissue was usually a distinct grayish brown, and the discoloration was expanded into the pith or cortex. Tuber eyes turned grayish brown, and sticky exudates were formed at the eyes or where the stolon is attached to the tuber. Eventually, infected tubers left in the ground had turned into a slimy mass surrounded by a thin layer of outer tissue and periderm.

Hayward (1991) reported that *Ralstonia solanacearum* bacterium was the causal agent of wilting of the potato plant but the symptoms were expressed in the tuber: The initial symptom was brown staining of the vascular ring (hence the name "brown" rot) that started from the stolon (heel) end. Pale, creamy bacterial exudates were oozed from the cut vascular tissues. In severe infections the vascular tissues were rot away completely. Bacterial exudates were oozed from the eyes and the heel end (where the tuber was attached to the stolon).

Janse (1988) reported that the most effective means of spread of brown rot worldwide was through distribution and planting of infected seed potatoes. In some case the

bacteria could latently infect tubers without causing noticeable symptoms and could survive in seed tubers during storage and caused disease when planted in the next season. Symptom expression was occured at different rates in different varieties and was favored by warm temperatures (above 15^0C with optimum around 25^0C) and other environmental conditions (especially high soil moisture).

Janse *et al*. (1998) observed that symptoms were most obvious in the tuber; initially a brown staining of the vascular ring (hence brown rot) started at the stolon end, with further disease progression the vascular tissue were rot away completely and a pale colored sticky ooze was appeared at the eyes lenticels and/or stolon end of the tuber. Mulder and Trukensteen (2005) observed that symptoms in the tuber were very specific: brownish-grey areas were seen on the outside, especially near the point of attachment of the stolon. Cut tubers showed pockets of white to brown pus or browning of the vascular tissue which exuded dirty white globules of bacteria. As the disease progresses bubbly globules of bacteria were exuded through the eyes; soil were adhered to the exuded bacteria, hence the name 'sore eyes' or 'jammy eyes'.

Wullings *et al*. (1998) reported that the first visible symptom of the disease in potato crops was wilting of the leaves at the ends of the branches during hot days with recovery at night. A streaky brown discoloration of the stem an inch or more above the soil line were observed as the disease developed and the leaves had a bronze tint. External symptoms were visible on tubers, depending on the state of development of the disease. Bacterial ooze were emerged from the eyes and stem end attachment of infected tubers.

2.2. Management

Ascarrunz *et al.* (2011) reported that Cells of *Ralstonia solanacearum* were exposed to Cu in distilled water, and the resulting Cu-stressed non-culturable cells were inoculated to natural (non-pasteurized) and pasteurized soils in order to examine their culturability and recovery. Exposing the cells to 20 µm $CuSO_4$ produced transitory non-culturable cells, which exhibited a remarkable recovery in culturability after incubation in the solution for 36 h. Additionally, fresh non-stressed cells were exposed to $CuSO_4$ in the presence of nalidixic acid by adding the antibiotic at different times after the onset of Cu stress to verify any cell multiplication during the

population increase. The results revealed that the non-cultural cells surviving Cu toxicity adapted very quickly to Cu and began multiplying within 12 h.

Bing *et al*. (2014) reported that kasugamycin·bismerthiazol 25% WP 50 mg/kg had a better control effect against bacterial canker disease than armure 4% WP 50 mg/kg and bismerthiazol 20% WP 50 mg/kg. The results showed that kasugamycin·bismerthiazol 25% is efficient fungicide against c. bacteria canker disease, and it was worthy to be popularized in citrus-producing areas.

Ceballos *et al*. (2014) reported that *In vitro,* crude extracts of two strains and two commercial products of *Trichoderma* spp. inhibited 100% of *Ralstonia solanacearum. T. viride* and Ecoterra treatments showed low levels of disease severity by *R. solanacearum* in plants (0.63 and 1.88% respectively).

Different control methods was evaluated for disease management of tomato bacterial wilt caused by *Ralstonia solanacearum* by Lee *et al.,* 2012. All six chemical pesticides applied to the bacterial suspension showed in vitro bactericidal activities against *R. solanacearum*. Minimal inhibitory concentrations (MICs) of copper hydroxide (CH), copper hydroxide-oxadixyl mixture (CH+O), and copper oxychloride-dithianon mixture (CO+D) were all 200 µg/ml; MIC of copper oxychloride-kasugamycin (CO+K) mixture was 100 µg/ml; MICs of both streptomycin-validamycin (S+V) and oxine copper-polyoxine B mixture (OC+PB) were 10 µg/ml.

Horton (1987) reported that utilization of antagonistic rhizosphere bacteria such as *Bacillus spp., P. fluorescens* and *P. putida* significantly increased the survival rate of tomato by 60–90% against bacterial and fusarium wilt disease

Koller *et al.* (2006) reported that the natural control of several phyto-pathogens is based on the presence of suppressive soils where several bio-control microorganisms belonging to *Trichoderma, Pseudomonas* and *Bacillus* genera are detected.

Liang *et al.* (2016) observed that the photodegradation of bismerthiazol was investigated after 4 and 8 h of irradiation in a solar simulator. Inhibition of *Xanthomonas oryzae* pv. *oryzae* (Xoo) was greater with a photolysed solution than with a non-photolysed solution of bismerthiazol. Inhibition of Xoo was significantly

greater with bismerthiazol and 2-amino-5-mercapto-1, 3, 4-thiadiazole than with 5-amino-1, 3, 4-thiadiazole. In addition, Xoo strain 2-1-1 was bismerthiazol- and 2-amino-5-mercapto-1, 3 , 4-thiadiazole resistant in vivo, In vitro assays indicated that the sulfhydryl group was crucial for the inhibition of Xoo by bismerthiazol and its photoproducts. Bismerthiazol and 2-amino-5-mercapto-1, 3, 4-thiadiazole might have a similar mode action *in vivo* and *in vitro.*

Louwes *et al.* (2001) conducted a study to determine the sensitivity of *Ralstonia* to copper as well as to a mixture of copper with mancozeb. The highest copper concentration where *Ralstonia* grew was 50 µg/ml. However, 45.5% of the bacterial strains from orchards with regular sprays of copper compounds grew in the presence of 50 µg copper/ml. In contrast, only 13.4% of the strains that never received copper sprays grew in such a copper concentration. Mixing mancozeb with copper increased the tolerance of *Ralstonia* to copper.

Murthy *et al.* (2013) reported that *Trichoderma asperellum* was used as a biological control agent against bacterial wilt disease caused by *Ralstonia solanacearum*.. Two isolates of *Trichoderma asperellum* (T4 and T8) exhibiting high antagonistic activity against a virulent strain of *Ralstonia solanacearum* (RS. Seed treatment with *T. asperellum* isolates significantly improved the quality of seed germination and seedling vigor. Efficacy of T4 and T8 isolates, evaluated under greenhouse conditions exhibited suppression by about 50% in disease incidence of bacterial wilt and promoted growth in tomato plant.. The seed application with T4 and T8 isolates induced a significant increase in the activities of peroxidase (POX), Polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and β−1, 3-glucanase in treated tomato plants. Increased activities of phenylalanine ammonia lyase, peroxidase, Polyphenol oxidase, β-1, 3-glucanase were observed in T4 and T8 pretreated tomato plants challenged with *Ralstonia solanacearum*. *Trichoderma asperellum* treatment also triggered the defense related enzymes involved in synthesis of phenols. Higher accumulation of phenolics was noticed in plants pre-treated with T4 and T8 challenged with *Ralstonia solanacearum.*

Neither *Bacillus. subtilis* nor *Trichoderma harzianum* alone could control the bacterial wilt, but when combined, their controlling capabilities were as effective as a chemical treatment (Maketon *et al.*, 2008).

Posas *et al*. (2007) reported that bacterial wilt caused by *R. solanacearum* is a serious threat for agricultural production. In this study, *Bacillus* amyloliquefaciens strains CM-2 and T-5 were found antagonistic to *R. solanacearum.*. The possible mechanism of resistance inducement by the antagonistic bacteria was also evaluated.

Sharma *et al.* (2010) found that twenty one days old seedlings of resistant (Swarna Lalima) and susceptible (*Pusa Ruby*) varieties of tomato grown under pot conditions were inoculated with pathogen *R. solanacerum* (OD_{600nm} = 0.3abs unit) simultaneously with the suspension cultures of *T. harzianum* and *T. viride*. Higher content of total carbohydrate, total phenols, total soluble proteins and total free amino acids in the bacterial wilt resistant plants were recorded indicating their efficacy to be used as marker for resistance against bacterial wilt. The marked increase in the content of phenol in the stem of *R. solanacerum* infected plants was due to response of *T. viride.*

Shekhawat *et al.* (1992) stated that potato bacterial wilt incidence reduced up to 79% by *Bacillus* spp. both under greenhouse conditions. Similarly *P. flurescens* isolates reduced incidence of bacteria by 43 to 75% and actinomycetes up to 79%.

Three copper-based compounds (copper hydroxide, copper oxychloride, copper sulphate), two antibiotics (streptomycin and kasugamycin) and a plant activator (ASM) significantly reduced population sizes and spread of *C. michiganensis* subsp. *michiganensis* among tomato seedlings in the greenhouse. Kasugamycin significantly limited pathogen population size, compared to copper bactericides, but it was less effective than the other antibiotic compound, i.e. streptomycin (Milijasevic *et al.,* 2009).

Wyatt and Laund 1981 found that In vitro application of kasuagamycine inhibited the growth of soft ror pathogen of potato. Inhibition activity further increased when streptomycine was added with it

Yu *et al*. (2016) reported that the bactericide bismerthiazol has been used to control rice bacterial blight (*X. oryzae* pv. *oryzae*). Bismerthiazol can effectively control citrus canker by both inhibiting the growth of *X. citri* sp. *citri* and triggering the plant's host defense response through the expression of several pathogenesis-related genes (*PR1, PR2, CHI,* and *RpRd1*) and the nonexpresser of PR genes (*NPR1, NPR2,*

and *NPR3*) in ‘Duncan’ grapefruit, especially at early treatment times. In addition, we found that bismerthiazol induced the expression of the marker genes *CitCHS* and *CitCHI* in the flavonoid pathway and the *PAL1* (phenylalanine ammonia lyase 1) gene in the salicylic acid (SA) biosynthesis pathway at different time points. Moreover, bismerthiazol may serve as an alternative to copper bactericides.

CHAPTER III

MATERIALS AND METHODS

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3.1. Experimental Site

The experiment was carried out in the Molecular Disease Diagnostic Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.2. Time of Experiment

The experiment was conducted from December, 2014 to January, 2016.

3.3. Sample collection

Potato samples were collected from eight potato growing districts of Bangladesh viz. Munshigong, Manikgong, Pabna, Bogra, Rangpur, Lalmonirhat, Panchagarh, and Joypurhat. Five kilogram medium sized potatoes (Diamond) were collected from each district. Samples were collected randomly. Two months stored potatoes were collected directly from the farmer's storage

3.4. Observation of visual symptoms of brown rot disease and determination of incidence of brown rot

Collected potato samples were observed visually for brown rot symptoms. Twenty medium sized two months stored potatoes from each sample were cut to observe the brown rot symptoms. The potatoes were cut at three locations viz. stem end portion, middle portion and seed end portion to observe the symptoms. On the basis of symptom development disease incidence were calculated.

Every potato along with brown rot infected, was counted in the laboratory for calculating the incidence of the disease and then it was expressed in percentage. Disease incidence of brown rot of 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. Storage of potato samples for further analysis

Rest of the potatoes from each sample were stored at room temperature and 70-80% relative humidity. Disease incidence was calculated after four and six month of storage from this potato samples by the same procedure as described in 3.4 section.

3.6. Experimental design

The experiment was laid out in a complete randomized design (CRD) with three replications. There were 8 treatments combinations. The total numbers of unit plates were 24.

3.7. Isolation and identification of causal organism of brown rot of potato

3.7.1. Preparation of Nutrient Agar (NA)

Nutrient agar media (Appendix-I) was prepared according to the method followed by Schaad (2001). At first 15 g bacto agar was taken in an Erlenmeyer flask containing 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. For mixing properly the nutrient agar was shaken thoroughly for few minutes. It was then autoclaved at 121^0C under 15 PSI pressure for 15 minutes.

3.7.2. Isolation of causal organism on NA media

The causal organism of brown rot of potato was isolated by dilution plate method.The diseased potatoes were washed under running tap water. And then was cut into small pieces. Surface sterilize were done by dipping them in 5% sodium hypochlorite solution for 2-3 minutes. Then it was washed three times with sterile water. After surface sterilization the cut pieces were kept in a test tube containing 3-4 ml of sterile water and kept for 30 minutes for bacterial streaming and getting stock. One ml of this stock solution was transferred with the help of sterile pipette into the second test tube containing 9 ml sterile water and shaken thoroughly resulting 10^{-1} dilution. Similarly, final dilution was made up to 10^{-4}. Then 0.1 ml of each dilution was spread over NA plate previously dried (to remove excess surface moisture) at three replications as described by Goszczynska and Serfontein (1998). Glass-rod was used for spreading. The inoculated NA plates were kept in incubation chamber at 30^0C. The plates were

observed after 24 hrs and 48 hrs. Then single colony grown over NA plate was restreaked on another plate with the help of a loop to get pure colony.

3.7.3. Isolation on TTC medium

For isolation of causal organism from infected potato specimens, streak plate technique was followed using a selective medium, Tetrazolium chloride agar (TZC) as described by Kelman (1954). The medium contained peptone 10 g, casein hydrolysate 1 g, glucose 5 g, and agar 20 g in 1000 ml of distilled water. The mixture was cooked, pH was adjusted to 7.0 using 0.1N KOH and autoclaved at 121^0 C under 1.1 kg/cm2 pressure for 20 minutes. Aqueous solution of 2, 3, 5- triphenyltetrazolium chloride (TTC) was prepared by dissolving 1g of the chemical in 100 ml of distilled water in an Erlenmeyer flask. The 1% stock solution of TTC solution was separately sterilized by passage through 0.45µm pore size filters (Millipore). The sterilized TTC solution was poured into the sterilized medium at the rate of 5 ml/1000 ml before solidification and mixed thoroughly. The medium was poured into petri plates (9 cm) at the rate of 20 ml/plate. The TTC was kept in a colored bottle and was wrapped with aluminum foil to avoid light and preserved in a refrigerator at $4\ ^0$C for future use.

The surface sterilized pieces of potato tuber were immersed in 5 ml of sterilized distilled water in a test tube for oozing. The bacterial ooze released from the infected tuber was thoroughly mixed in water after discarding the tuber pieces. One loopful of suspension was streaked on the TZC agar medium in Petri plates and virulent colonies were identified on the basis of characteristic colony characters on TZC medium (Kelman, 1954).

3.8. Biochemical test

3.8.1. Gram's staining

A small drop of sterile water was placed on a clean microscope slide. Part of a young colony (18-24 hrs old) was removed with a cold, sterile loop from the nutrient agar medium and the bacteria were smeared on to the slide that was very thin. The thinly spread bacterial film was air dried. Underside of the glass slide was heated by passing it four 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.

3.8.2. KOH solubility test

It is a rapid method for gram differentiation of plant pathogenic bacteria without staining (Suslow et al., 1982). Two drops of 3% KOH solution were placed at the centre of a clean glass slide. One loopful colonies of bacterial pathogen (grown NA medium) were added to the KOH solution and homogenized with a nichrome loop with rapid circular movement of about 10 seconds. Viscous strand formation was observed and on drawing it with a loop it formed a fine thread of slime, 0.4 to 2.5 cm in length.

3.8.3. Starch hydrolysis test

For starch hydrolysis test, pure colony of bacterium was spot inoculated on nutrient agar plate containing 0.2% soluble starch. Then 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.

3.8.4. 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.

3.8.5. Oxidase test

This test is particularly valuable for differentiating *Pseudomonalds* from certain other gram negative rods (Shekhawat et. al., 1992). Aerobic or facultative anaerobic bacteria, i. e., those with respiratory activity are divisible into two groups, those which are oxidase positive and those which are oxidase negative. An oxidase positive reaction transport is indicative of the presence of a cytochrome- C-Oxidase in the respiratory electron chain. Among *Pseudomonalds,* the test has important differential value because isolates of *R. solanacearum* give positive reaction. Tetramethyl-p-

phenyl diamine is oxidised by the cytochromecytochrome oxidase system of the bacterium to a purple compound. Aqueous solution of (1%) of tetramethyl-p-phenylenediamine 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 (TTC medium) of each isolate was rubbed separately on the impregnated surface of the filter paper stripe by a platinum loop. Purple color develops within 10 seconds, which indicated positive reaction of oxidase test.

3.8.6. Gelatine liquefaction test

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

3.8.7. Levan test

One loop-full bacterial culture was streak inoculated into NA plate containing 5% (w/v) sucrose with the help of a sterile transfer loop. Then it was incubated at 30 ^{0}C for 24 hours to observe whether levan is produced or not.

3.8.8. Pecteolytic test

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

3.9. *In vitro* evaluation of some agrochemicals and bio-agent against *Ralstonia solanacearum*

3.9.1. Preparation of agrochemicals

Four agrochemicals viz Bctrol, Kasumin, copperoxichloride and copper+mancogeb were used for *in vitro* test against brown rot disease.

3.9.1.1. Preparation of Bactrol solution

0.2 gram bactrol powder (Figure1) were weighted and dissolved in 100 ml sterile distilled water to made 0.2% concentration of the solution. Then it was filter sterilized by 0.45 micron miliporefilter.

3.9.1.2. Preparation of kasumin solution

0.3 ml kasumin liquid (Figure1) were taken and mixed in 100 ml sterile distilled water and thus made 0.3% concentration of the solution. Then it was filter sterilized by 0.45 micron miliporefilter.

3.9.1.3. Preparation of copperoxichloride solution

0.3 gram copperoxichloride powder (Figure1) were weighted and dissolved in 100 ml sterile distilled water and thus made 0.3% concentration of the solution. Then it was filter sterilized by 0.45 micron miliporefilter.

3.9.1.4. Preparation of copper+mancogeb solution

0.3 gram copper+mancogeb powder were weighted and dissolved in 100 ml sterile distilled water and thus made 0.3% concentration of the solution. Then it was filter sterilized by 0.45 micron miliporefilter

Figure 1: Antibacterial chemicals used against *Ralstonia solanacearum*

A. Kasumin liquid
B. PRH (*Bacillus* suspension)
C. Sulcox powder(copperoxychloride)
D. Bactrol powder.

3.9.2. Preparation of bioagents

3.9.2.1. *Bacillus subtilis*

Bacillus suspension based formulation (PRH) was collected from Sidique market of Dhaka.

3.9.2.2. Preparation of *Trichoderma* culture

Trichoderma harzianum culture was obtained from Department of Plant Pathology, Sher-e- Bangla Agricultural University, Dhaka (Figure 2).

3.9.2.3. Purification of *Trichoderma* fungi

A small colony from collected *Trichoderma harzianum* was transferred on PDA plate with the help of sterile needle. Then it was incubated for 3-7 days at room temperature.

3.9.2.4. Preparation of Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) medium (Appendix-I) was prepared as described by Islam (2009). 200 g peeled potato extract, 20 g dextrose and 17 g agar were taken in an Erlenmeyer flask containing 1000 ml distilled water and mixed well for the preparation of 1 liter PDA medium. Then it was autoclaved at 121^0C under 15 PSI pressure for 15 minutes. 20 drops of 50% lactic acid was added per 250 ml medium to avoid the contamination of bacteria.

3.9.2.5. Preparation of Potato Dextrose broth

Potato dextrose broth (Appendix-I) was prepared as described by Islam (2009). 200 g peeled potato extract and 20 g dextrose were taken in an Erlenmeyer flask containing 1000 ml distilled water and mixed well for the preparation of 1 liter potato dextrose broth. Then it was autoclaved at 121^0C under 15 PSI pressure for 15 minutes. 20 drops of 50% lactic acid was added per 250 ml medium to avoid the contamination of bacteria.

3.9.3. Screening of antagonistic organisms against *Ralstonia solanacearum*

3.9.3.1. Antagonism of *Trichoderma harzianum* against *Ralstonia solanacearum*

Biocontrol agents were screened for their efficacy against the growth of *Ralstonia*

solanacearum by well diffusion method measuring the inhibition zone (Yenjerappa, 2009). *Trichoderma harzianum* were grown on potato dextrose broth in incubating shaker machine maintaining 30ºC temperature and 150 rpm for 24 hours. 1 ml of each culture was taken in eppendroff (EP) tubes which were then centrifuge in centrifuge machine at 1000 rpm for 15 minutes. Pellets were found in lower part and supernatant were found in upper part and of EP tubes (Plate 2). These supernatant were transferred to the fresh eppendroff tubes. Then the supernatant were dropped on the hole of previously swabbed plate with the pathogenic bacteria each at three replications. Only sterile water was used as control. Then the plates were incubated at 30^0C in incubation chamber. Zone of inhibition around the holes were measured and recorded after every 24 hours for 5 days.

3.9.3.1. Antagonism of *Bacillus subtilis* against *Ralstonia solanacearum*

Antagonism study was done by following the section of 3.9.3.1.

3.9.4. Estimation of percent inhibition zone

Inhibition diameter at every plate under each treatment was measured by scale. Percent inhibition zone was estimated by the following formula (Bonev *et al.*, 2008)

$$\text{Percent inhibition zone} = \frac{\text{Control diameter}}{\text{Control diameter - treatment diameter}} \times 100$$

3.10. Statistical analysis

Data collected during experimental period were tabulated and analyzed following Statistical package MSTAT-C. Treatment means were compared with Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). .

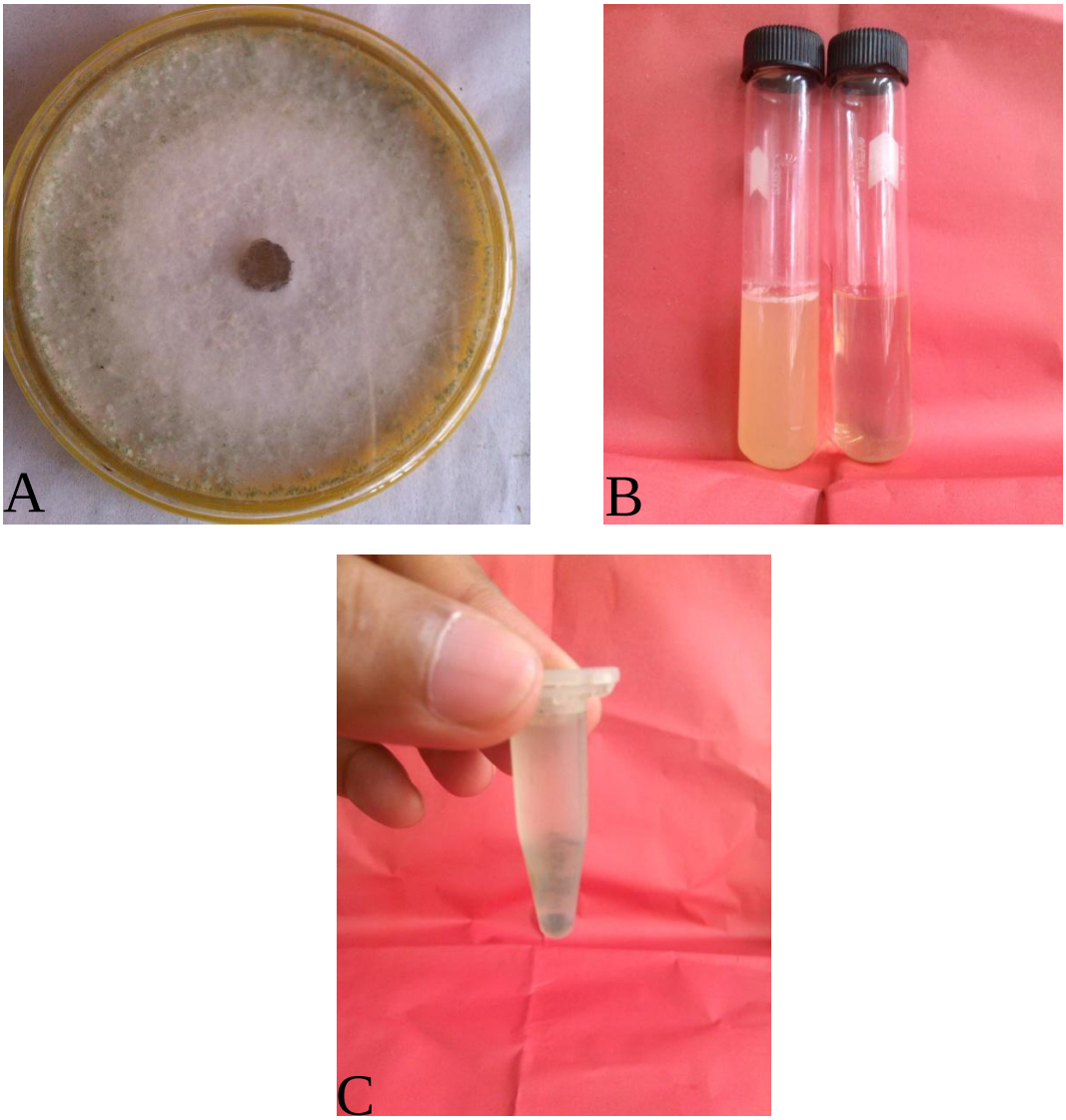

Figure 2: Culture and preparation of *Trichoderma* antagonist

A. Culture of *Trichoderma* on PDA
B. Culture of *Trichoderma* on PDB
C. Centrifuged suspension of *Trichoderma*

CHAPTER IV
Results

This chapter includes the experimental results. Potatoes collected from different zones were assessed against brown rot pathogen. Results were compiled based on disease incidence at different storage period.

4.1. Incidence of brown rot disease on collected potato sample

Result showed that disease incidence of brown rot of potato varied from location to location and ranged from 3.33% to 40% at different storage period (Table 1, Figure 3). After two months storage period highest incidence (25%) of brown rot of potato was found in potato samples collected from Munshigong district followed by Rangpur (23.33%), Lalmonirhat (21.67%) and Joypurhat (21.67%). Moderate incidence (16.67 and 18.33%) was found in the potato samples collected from Pabna and Bogra District respectively. At the same time lowest incidence (3.33%) was found in the potato sample collected from Panchagarh district followed by Manikgong district (8.33%).

After four months storage period, disease incidence were increased in the potato sample collected from different districts. Highest incidence (30%) of brown rot of potato was found in potato samples collected from Munshigong district followed by Rangpur (28.33%), Lalmonirhat (26.00%) and Joypurhat (26.67%). Moderate and statistically similar incidence (21.33 and 21.67%) was found in the potato samples collected from Pabna and Bogra District respectively. Lowest incidence (8.00%) was found in the potato sample collected from Panchagarh district followed by Manikgong district (16.00%).

After six months storage period highest incidence (40%) of brown rot of potato was found in potato samples collected from Munshigong district followed by Rangpur (38.67%), Lalmonirhat (36%) and Joypurhat (37.67%). Moderate incidence (31.67 and (30.33%) was found in the potato samples collected from Pabna and Bogra District respectively. At the same time lowest incidence (16%) was found in the potato sample collected from Panchagarh district followed by Manikgong district (23.33%).

Table 1: Disease incidence of brown rot of potato at different storage period collected from different districts of Bangladesh

Districts	Disease Incidence (%)		
	After 2 month	After 4 month	After 6 month
Munshigong	25.00 a	30.00 a	40.00 a
Rangpur	23.33 ab	28.33 a	38.67 ab
Lalmonirhat	21.67 ab	26.00 ab	36.00 abc
Joypurhat	21.67 ab	26.67 ab	37.67 abc
Pabna	16.67 b	21.33 bc	31.67 bc
Bogra	18.33 ab	21.67 bc	30.33 cd
Manikgong	8.33 c	16.00 c	23.33 d
Panchagorh	3.33 c	8.00 d	16.00 e
CV (%)	23.61	14.93	12.79
$s_{\bar{x}}$	2.357	1.919	2.342
Level of Significance	**	**	**

Each data represents the mean value. Values followed by the same letter within a column are not significantly different ($p \leq 0.01$) according to Duncan's multiple range test

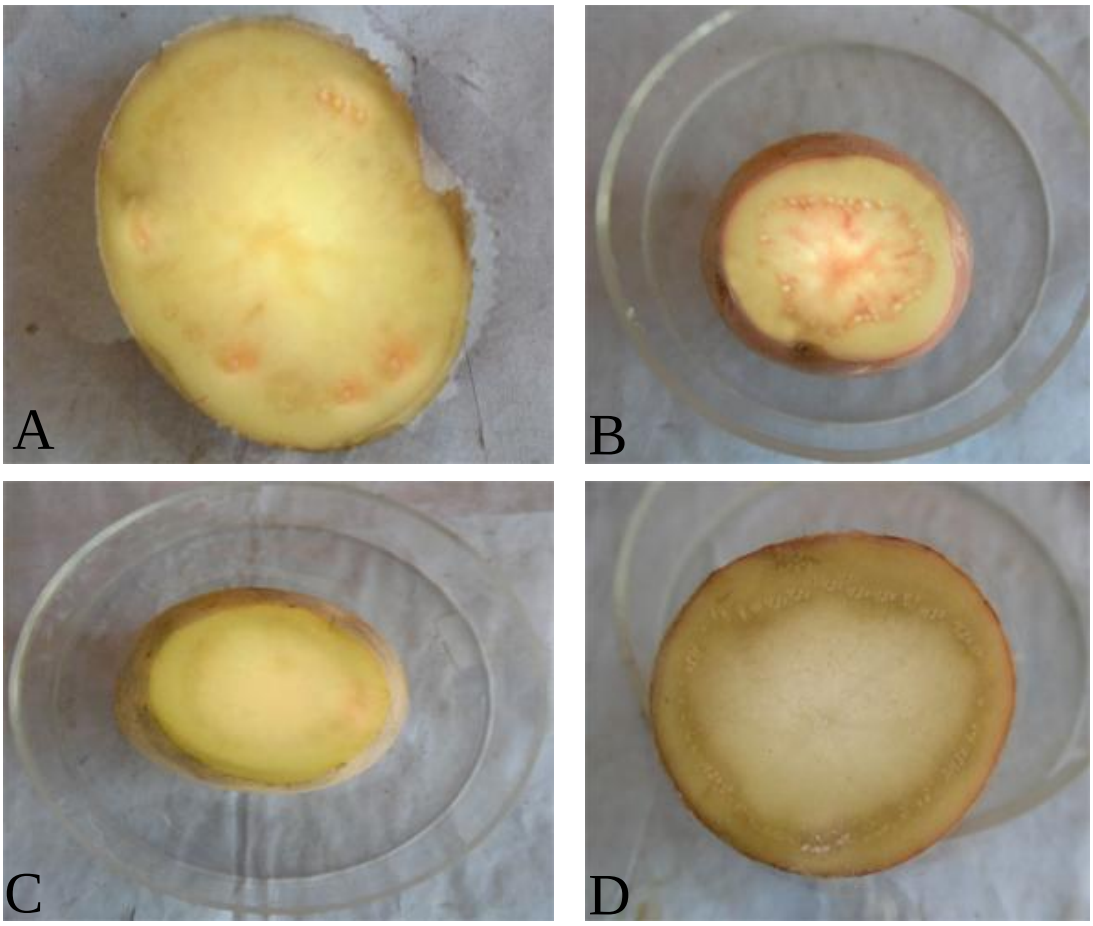

Figure 3: Disease incidence of brown rot of potato collected from different location of Bangladesh

A. Brown rot infected potato from Munshigong after two months of storage
B. Brown rot infected potato from Munshigong after six months of storage
C. Brown rot infected potato from Panchagarh after two months of storage
D. Brown rot infected potato from Panchagarh after six months of storage

4.2. Isolation of brown rot pathogen from potato

Ralstonia solanacearum isolated from infected tuber which yielded well separated, typical, white, convex, mucoid, irregular watery colonies of bacterium on nutrient agar medium after 48 hours of incubation at 30 ^{0}C (Figure 4.). Colonies were purified by restreaking the isolated colony on nutrient agar plate. The bacterial pathogen produced highly fluidal, slightly raised and creamy white colonies with light pink or pinkish red centre and irregular margin after 48 hrs of incubation at 30^{0}C on TTC medium (Figure 5). Colonies were purified by restreaking the isolated colony on TTC plate**.**

4.3. Preservation of brown rot pathogen of potato

Purified bacterium was kept in refrigerator at 4 ^{0}C in small screw-cap test tubes on NA slant, which served as a stock culture for further studies.

4.4. Identification of the pathogen

Brown rot pathogen was identified by studing on morphological, biochemical and cultural features of the pathogen as per standard microbiological procedures.

4.4.1. Morphological characters

The bacterium was rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative (red color) and capsulated under the compound microscope at 100x magnification with oil immersion. The cells were readily stained with common stains such as crystal violet (Figure 6 A).

In KOH solubility test, a mucoid thread was produced by the bacteria (Figure 6 B). Therefore the test was positive i.e., the bacterium was gram negative that supports the result of gram's staining test.

Figure 4: Culture of *Ralstonia* on NA medium

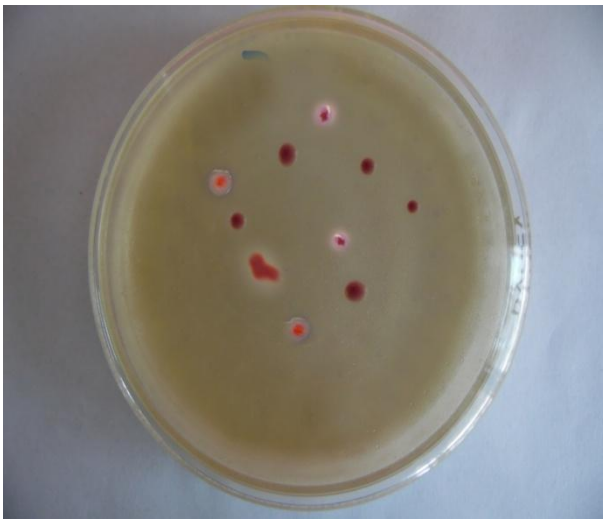

Figure 5: Culture of *Ralstonia* on TTC medium

4.4.2. Biochemical characters

Results obtained on various biochemical tests for the pathogen are presented in Table 2.

Table 2. Biochemical characteristics of *Ralstonia solanacearum*

Biochemical tests	Results
Catalase test	Positive
Oxidase test	Negative
Starch hydrolysis test	Positive
Gelatine liquefaction test	Positive
Levan test	Positive
Pecteolytic test	Positive

In starch hydrolysis test, a clear zone was formed after adding lugol's iodine around the bacterial colony indicated starch hydrolysis (amylase activity) i.e., the test was positive (Figure 7B).

In catalase test, bubbles were formed after adding 3% H_2O_2 onto the colony of the bacterium within a few seconds ((Figure 7C), which revealed that the test was positive.

In oxidase test, after rubbing the bacterium onto the moistened whatman filter paper, it did not form any color ((Figure 7A), which revealed that the test was negative.

In gelatine liquefaction test, after 15 minutes of refrigeration at 5^oC, gelatin was liquefied ((Figure 7E). Thus the bacterium showed the positive result.

In levan test after incubated at 30^0c the bacteria produced levan thus the bacterium showed positive result((Figure 7F).

In pecteolytic test the bacteria showed positive result. After incubation for 48 hours the bacterium was able to rot the potato ((Figure 7D).

On the basis of morphological, biochemical and cultural characteristics the causal organism of brown rot of potato was identified as *Ralstonia solanacearum*

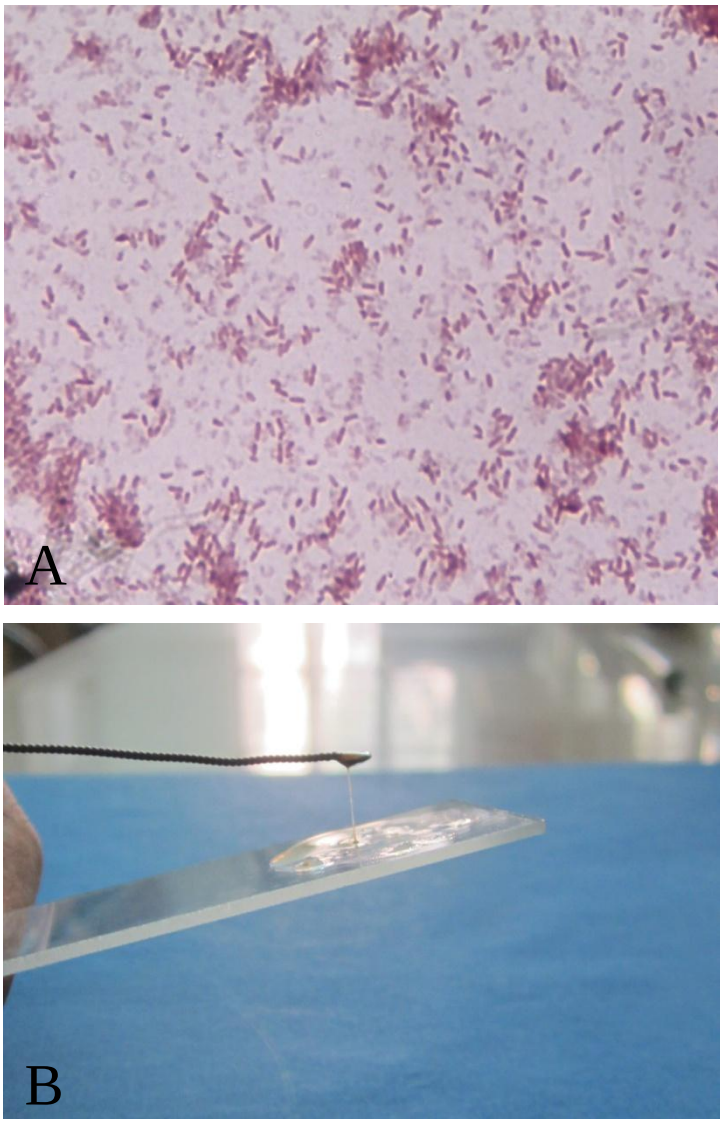

Figure 6: Morphological characteristics of causal organism

A. Gram staining test
B. KOH solubility test

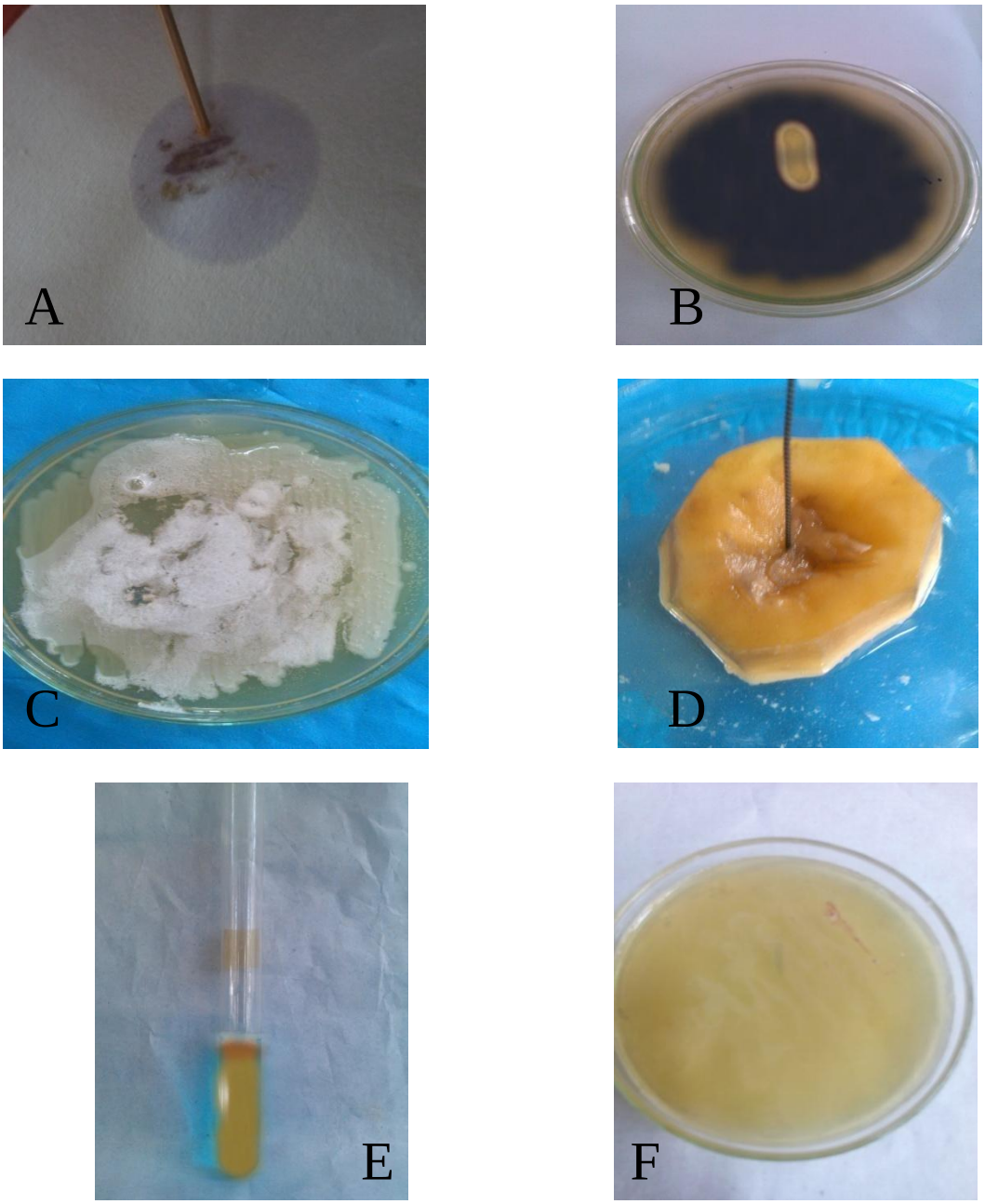

Figure 7: Biochemical test result for identification of causal organism

A. Oxidase test
B. Starch hydrolysis test
C. Catalase test
D. Pecteolytic test
E. Gelatin liquefaction test
F. Levan test

4.5. *In vitro* evaluation of antibacterial chemicals and bioagents

4.5.1. Efficacy of antibacterial chemicals and bioagent against *Ralstonia solanacearum* at different days after incubation

Statistically significant difference in case of inhibition zone was found among different treatments (Table 3; Figure 8, 9)**.** After 24 hours of incubation the highest inhibition zone (3.83 mm) was formed by Kasumin at 0.2% concentration followed by Bactrol (3 mm) at the same concentration. Moderate and statistically same inhibition zone (1.1, 1.1 and 1 mm) was formed by *Bacillus*, copperoxichloride and *Trichoderma* respectively At the same time no inhibition zone (00 mm) was formed by copper+mancozeb at the same concentration.

At 48 hours of incubation the highest inhibition zone (7.77 mm) was formed by Kasumin at 0.2% concentration followed by Bactrol (6.64 mm) at the same concentration. Moderate and statistically same inhibition zone (3.33, 3.00 and 2.22 mm) was formed by *Bacillus*, copperoxichloride and *Trichoderma* respectively. At the same time no inhibition zone (00 mm) was formed by copper+mancozeb.

At 72 hours of incubation the highest inhibition zone (14.4 mm) was formed by Kasumin at 0.2% concentration followed by Bactrol (11.11mm) at the same concentration. Moderate and statistically same inhibition zone (6.66, 5.5 and 4.4 mm) was formed by *Bacillus*, copperoxichloride and *Trichoderma.* At the same time lowest inhibition zone (00 mm) was formed by copper+mancozeb.

Inhibition zone significantly increased from 24 hours to 72 hours by each treatments except copper+mancozeb treatments. It remained stable from 24 to 72 hours of incubation.

Table 3: *In vitro* evaluation of antibacterial chemicals and antagonist fungi against *Ralstonia solanacearum*

Treatments	Inhibition Zone At		
	24 hour	48 hour	72 hour
Kasumin @ 0.3 %	3.83 a	7.77 a	14.44 a
Bactrol @ 0.2 %	3.00 b	6.64 b	11.11 b
Bacillus subtilis based formulation	1.11 c	3.33 c	6.66 c
Copperoxichloride @ 0.3%	1.11 c	3.00 c	5.50 cd
Trichoderma harzianum	1.00 c	2.22 c	4.44 d
Copper+mancozeb @ 0.3 %	0.00 d	0.00 d	0.00 e
CV (%)	15.81%	11.86%	14.38%
s_ x	0.1798	0.2456	0.5444
Level of Significance	**	**	**

Each data represents the mean value. Values followed by the same letter within a column are not significantly different ($p \leq 0.01$) according to Duncan's multiple range test

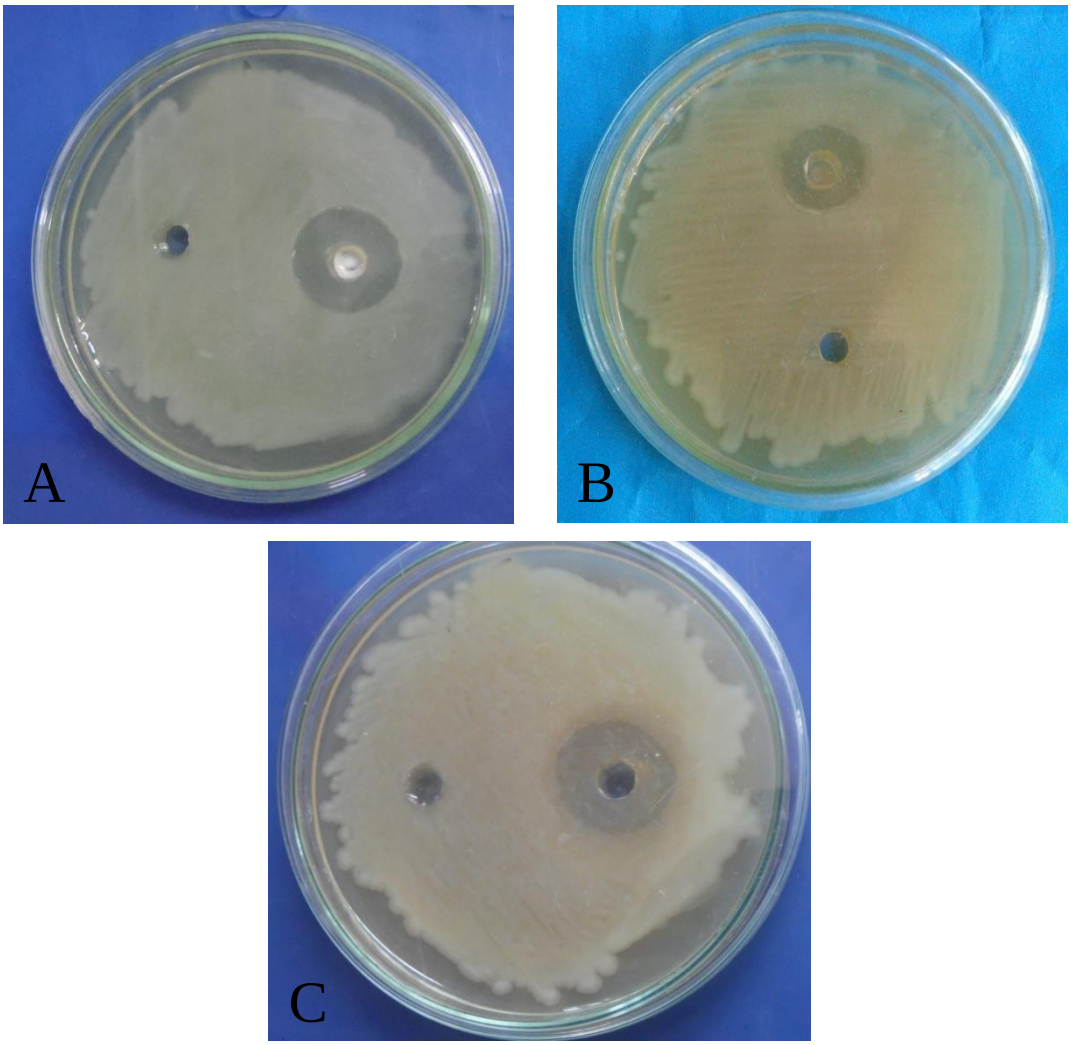

Figure 8: *In vitro* evaluation of antibacterial chemicals and bioagents against *Ralstonia solanacearum*

A. Kasumin @ 0.3%
B. Bactrol @0.2%
C. *Bacillus subtilis* based formulation

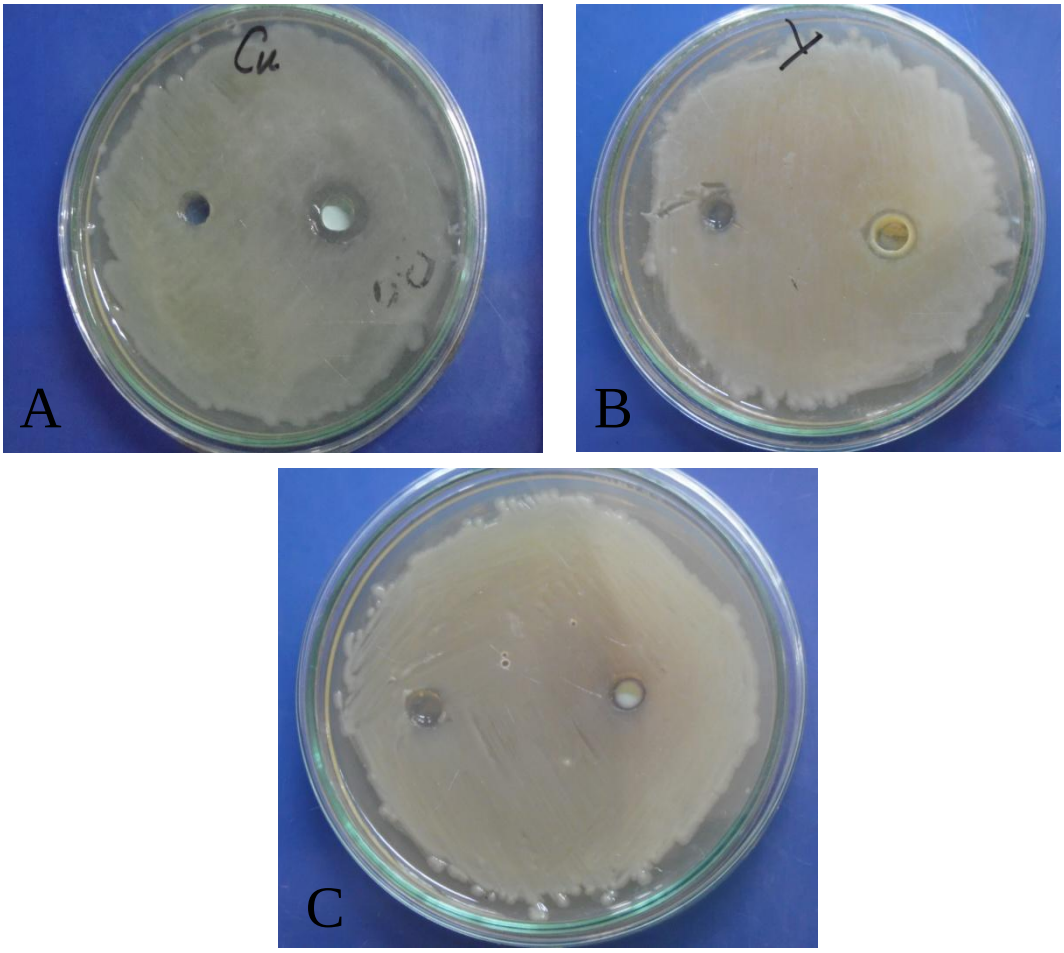

Figure 9: *In vitro* evaluation of antibacterial chemicals and bioagents against *Ralstonia solanacearum*

A. Copper oxichloride @ 0.3%
B. *Trichoderma harzianum*
C. Copper+mancozeb @ 0.3%

CHAPTER V

DISCUSSION

Potato is now an important tuber crop of Bangladesh. Potato sample collected from eight different locations of Bangladesh viz. Munshigong, Manikgong, Pabna, Bogra, Rangpur, Lalmonirhat, Panchagarh, and Joypurhat. showed brown rot of potato as a common disease. The disease recorded in the present study based on visual symptoms following the description of Brunings and Gabriel (2003). Prevalence of brown rot in potato varied in respect of location and storage. Similar variation was recorded by Rahman *et al*., 2010. In the present study, it was also observed that the incidence of brown rot in potato varied from location to location. These variations may be due to effect of environment in different agro-ecological zone and soil condition. In the present study highest incidence of brown rot of potato was found in Munshigonj district and lowest incidence was found in Panchagarh district. Incidence of brown rot of potato is significantly influenced by average temperature, relative humidity. The study showed that disease incidence was also varied with the increase of storage period. Disease incidence increased with the increase of storage period. The highest incidence was found after six month of storage. Long term storage increased this disease significantly. In the present study the causal organism of brown rot of potato (*Ralstonia solanacearum*) was isolated from infected potatoes collected from different location following standard dilution plating technique using nutrient agar medium and TTC selective medium. Typical, whitish, watery convex, mucoid, colonies of bacterium were produced on nutrient agar medium after 48 hours of incubation at 30^0C (Schaad *et al*., 2001). The bacteria produced small whitish with pink centered colony on TTC medium (Kelman, 1954). The causal agent of brown rot of potato (*Ralstonia solanacearum*) was identified by conducting studies on its morphological, biochemical and cultural features as per standard microbiological procedures. The bacterium was rod shaped with rounded ends gram negative (red color) and capsulated, after gram's staining under the compound microscope at 100x magnification with oil immersion. A mucoid thread was produced in KOH solubility test that supports the result of gram's staining test i.e., the bacteria was gram negative. Similar result in KOH solubility test was found by Schaad, (1992), Kishun and Chand, (1991); and Celino et al., (1952). In the present study the bacterium (*Ralstonia solanacearum*) showed positive results in starch hydrolysis test, catalase

test, levan production test, pecteolytic test and gelatine liquefaction test and negative result in oxidase test. The result of the present study was in agreement with the report Denny and Hayward, (2001) Dhital *et al*., (2001) and Christ, (1998).

Brown rot of potato is devastating disease of potato. There is no available effective management strategy against this disease. In this study four agrochemicals viz.Kasumin, Bactrol, Copperoxychloride and copper+mancozeb and two bioagent such as *Trichoderma* and *Bacillus* suspension were used *in vitro* evaluation against *Ralstonia solanacearum*. Kasumin (14.44 mm) and Bactrol (11.11 mm) exhibited significantly superior efficacy in inhibiting the growth of *Ralstonia solanacearum* while *Bacillus subtilis* (6.66 mm), copperoxychloride (5.55 mm) and *Trichoderma harzianum* (4.44 mm) showed moderate inhibition zone against the pathogen. No inhibition zone (00 mm)was produced by copper+mancozeb. Kasumin deteriorate protein synthesis during translation stage and thus inhibit the bacterial growth. Bactrol inhibit exopolysacchiride formation and cell proliferation and thus reduce bacterial population. Toder (2005) found that *B. subtilis* produced many kinds of antibiotics which suppress soil borne bacteria. Tverdyukov *et al*., (1994) reported that *Trichoderma* produced chemicals called trichodermin which is responsible for antagonistic properties. From the above study it has been found that among the chemicals and bioagents used Kasumin was the best followed by Bactrol. On the other hand *Bacillus subtilis* showed moderate performance regarding growth inhibition of *R. solanacearum. Trichoderma harzianum* had least potentiality. The application of agrochemicals were found better as compared to bioagents. May be the strains of *Bacillus* and *Trichoderma* were weakly active against *Ralstonia solanacearum.* Though agrochemicals were found the best but those have environmental hazards. Control of plant pathogen through biological means is no doubt a safe technique, hence more work is required to find out suitable and safe management option to control brown rot disease of potato.

CHAPTER VI

SUMMARY AND CONCLUSION

Potato belongs to the family Solanaceae is an important tuber crop grown all over the world. Though the demand of potato is increasing day by day, it's production in terms of area and yield is not satisfactory due to different diseases of potato. Tuber of potato is vulnerable to attack by various diseases in Bangladesh especially brown rot. However least concrete information regarding their distribution, incidence, severity, epidemiology and management is available. Therefore, the present study was designed to study the occurrence and prevalence of brown rot on tubers of potato collected from eight different locations of Bangladesh viz. Munshigonj, Manikgonj, Pabna, Bogra, Rangpur, Lalmonirhat, Panchagarh, and Joypurhat and to study the *in vitro* effective management strategies of the disease. The initial symptom of the disease was brown staining of the vascular ring (hence the name "brown" rot) that started from the stolon (heel) end. Pale, creamy bacterial exudates were oozed from the cut vascular tissues. In severe infections the vascular tissues were rot away completely. Bacterial exudates were also oozed from the eyes and the heel end (where the tuber was attached to the stolon).

The causal organism of brown rot of potato was isolated from the infected leaf by following dilution plating technique using nutrient agar medium and TTC medium. The causal organism was purified by restreaking on nutrient agar medium with single colony. The pathogen was identified by its morphological, biochemical and cultural features as per standard microbiological procedures. The bacterium was gram negative, rod shaped with rounded ends. It showed positive result to KOH solubility test, starch hydrolysis test, catalase test, levan production test, gelatine liquefaction test and pecteolytic test but negative result to oxidase test. The bacteria produced small whitish with pink centered colony on selective TTC medium. The study revealed that, incidence of brown rot of potato varied from location to location and storage period. The highest incidence of brown rot of potato was found in Munshigong and lowest incidence was recorded in Panchagarh district. In case of storage period the highest incidence was found after six month of storage and the lowest incidence was recorded after two months of storage. Incidence of brown rot of potato increased with the increase of storage duration. *In-vitro* evaluation of agrochemicals and bioagents was done by measuring the inhibition zone of the causal

organism. *In-vitro* evaluation of antibacterial chemicals indicated that, Kasumin and Bactrol were highly effective against *Ralstonia solanacearum*. Copper+mancozeb showed lowest performance against *Ralstonia solanacearum.* Among two bioagents, *Bacillus subtilis* based formulation showed best performance than *Trichoderma harzianum.* Therefore, further study is required to understand the proper epidemiology of the disease and to develop effective management strategies. *Bacillus subtilis* and *Trichoderma.* should be incorporated in brown rot management system as eco-friendly alternatives.

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APPENDICES

Appendix I. Preparation of culture media and reagents

The compositions of the media used in this thesis work are given below: Unless otherwise mentioned all media were autoclaved at 121^0C 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

Potato Dextrose Agar (PDA)

Peeled potato	200 g

Dextrose	20 g
Agar	17 g
Distilled water	1000 ml

Potato Dextrose Broth

Peeled potato	200 g
Dextrose	20 g
Distilled water	1000 ml

Gelatine Liquefaction Media

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

Starch hydrolysis media and reagent

Culture medium

Nutrient broth (Difco)	8.0 g
Soluble potato starch	10.0 g
Bacto agar (Difco)	15.0 g
Distilled water	1000 ml

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

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

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.