PREVALENCE OF BACTERIAL WILT OF POTATO CAUSED BY *RALSTONIA SOLANACEARUM* AT MANIKGANJ DISTRICT AND ITS *IN VITRO* MANAGEMENT

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This is to certify that the thesis entitled, **"PREVALENCE OF BACTERIAL WILT OF POTATO CAUSED BY *RALSTONIA SOLANACEARUM* AT MANIKGANJ DISTRICT AND ITS *IN VITRO* MANAGEMENT''** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **MAHI SHAWKAT** bearing **Registration No. 09-03486** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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Dedicated To
My Beloved Parents

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PREVALENCE OF BACTERIAL WILT OF POTATO CAUSED BY *RALSTONIA SOLANACEARUM* AT MANIKGANJ DISTRICT AND ITS *IN VITRO* MANAGEMENT

ABSTRACT

A survey on bacterial wilt disease of potato was conducted in three major potato growing upazilas namely,Saturia, Daulatpur and Ghior of Manikganj district during the growing season of 2015-2016. The isolated pathogen was *Ralstonia solanacearum* which was confirmed by different biochemical test viz. Gram staining reaction, Potassium hydroxide solubility test, Kovac's oxidase test, Levan test, Catalase test and Pathogenecity test. Bacterial wilt disease incidence ranged from 25.81% to 45.16% were recorded in those upazillas. Six treatments viz. control (distilled water), bleaching powder (5.2 mg/kg soil), turmeric powder(10%), magnesium chloride (10 mg/kg soil), cow dung powder (1:1) and Krosin AG (0.5 gm/L) were evaluated in the net house. The highest reduction of PDS (percent disease severity) was observed in cow dung powder treatment (56.25%) which was followed by turmeric powder (37.5%) and magnesium chloride (31.25%), respectively. However, the performance in reducing the disease was identical in Krosin AG (12.5%) and bleaching powder (12.5%), which was found to be lower than magnesium chloride but higher than control (distilled water) in the inoculated pots.

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CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is very important for food and income generation as it produces a high yield per unit land and time. It is the world's fourth largest and third largest food crop in Bangladesh and has recently occupied an important place in the list of major food and cash crops in Bangladesh (Ali and Haque, 2011). Soil borne diseases are considered as a limiting factor of many crops including potato. *Ralstonia solanacearum* (formerly called *Pseudomonas solanacearum*) (Smith, 1896) is a soil borne pathogen generally occurs in low lands in tropical or subtropical areas. It is an extremely destructive potato pathogen, causing bacterial wilt or brown rot of potato in the high land tropics of Africa, Asia, and Latin America (Yabuuchi *et al*., 1995) . It had appeared in Europe, disrupted seed potato production and caused serious quarantine related losses (Elphinstone, 2005, Janse, 1996). It is one of the most destructive plant diseases, which is predominantly distributed in the tropical, subtropical and warm temperate regions of the world (Hayward, 1994). It affects as many as 200 plant species representing more than 50 families of particularly members of solanaceous plants such as potato, tomato, eggplant, pepper and tobacco. For example, it is responsible for yield loss of potato to the extent of 50–80% in Kenya, Burundi and Uganda (Ajanga, 1993) and also up to 70% in India (Sinha, 1986). In Ethiopia, the yield loss caused by the disease is not yet determined, but it occurs in potato growing areas of the country at higher incidences and studies regarding the diversity of the pathogen showed that the strains belong to race 3 of biovar 2 of *R. solanacearum* (Yaynu,

1989; Ketema, 1999). It also infects and causes wilt of tomato, pepper, eggplant,banana and many other crops (Yabuchhi *et al.*, 1996).

R. solanacearum was considered a 'species complex' due to significant variation within the group (Fegan, 2005). It attacks almost 450 plant species in 54 different plant families (Allen, 2005). Besides Solanaceae, several dicotyledonous and monocotyledonous families have members susceptible to *R. solanacearum* (Hayward, 1991). The initial symptom is wilting of terminal leaves, followed by a sudden and permanent wilt. Additional symptoms are vascular browning, water soaking of pith followed by browning and browning of cortex near the soil line during the later stages of infection. Bacterial streaming occurs when a freshly cut stem is suspended in water. The common control measures employed in other countries include the use of resistant variety, crop sanitation, crop rotation, selection of disease free planting material and other cultural practices as single or integrated disease management. But control through the use of resistant varieties alone has showed little success. This is because such kind of resistance is strain specific and liable to break down by virulent and highly polymorphic strains of *R. solanacearum* at an ambient temperature and in nematode infested soil (Prior *et al.*,, 1994). Successful control of the pathogen through crop rotation is also not always effective since rotation practices recommended for one area may not perform well at other locations in addition to differences in the strains involved (Prior *et al.*, 1994).

Reduction of wilt was noted by Chellemi *et al.* (1992) with natural and organic amendments. Because, the pathogen can survive for long periods of time in a nutrient depleted environment (Grey and Steck, 2001). During the recent decades, many natural bioactive compounds have been extensively tested and a good number of reports were documented as effective inhibitors

of phytopathogenic bacteria (Leksomboon *et al.*, 2000). Cow dung and urine has been used as insecticides and has been reported to contain antibiotic agents. A wide range of pharmacological attributes of curcumin from turmeric have been well documented for antimicrobial and protective properties (Nagabhushan and Bhide 1992). Oyarzua, *et al.*, 2014, showed that the magnesium salts in the microbiological experiments typically associated with positive effects. It focused on the usefulness of magnesium (in form of $MgCl_2$) as a stress enhancer against *Escherichia coli*. However, very little work has been done to investigate the antibacterial properties of those natural products and their use in controlling the soil borne pathogens. Therefore, the present study was conducted with the following objectives-

i. Prevalence of bacterial wilt disease of potato in three different potato growing areas of Manikganj district.
ii. To isolate, characterize and identify the pathogen from the collected samples.
iii. To evaluate some soil treatments against the identified bacterial isolates to controlling the wilt diseases in *vitro*.

CHAPTER II

REVIEW OF LITERATURE

Sarkar and Chaudhuri (2016) focused on one of the most devastating diseases called bacterial wilt. Study showed the bacterial wilt caused by *Ralstonia solanacearum* for the management through bactericides and biocontrol agents. Other than physical and chemical methods, management of the disease by use of antagonistic bacteria and fungi had been found to be the recent trend.

Lal *et al.* (2016) stated potatoes were affected by different pathogens, viz. fungi, bacteria, viruses and nematodes. These pathogens may cause significant yield losses of the crop, if proper protection measures have not been applied. Among potato pathogens, *Phytophthora infestans*, *Alternaria solani*, *Rhizoctonia solani* and *Fusarium* spp. are the major pathogens in the fungal group, where as *Ralstonia solanacearum*, *Pectobacterium* spp. and *Streptomyces* spp. are in the bacterial group. For management of these pathogens, various methods, that is, chemical control, biological control, resistant varieties, cultural control and physical control, are applied. Resistant varieties are the best and cheapest method for managing the diseases. Chemical management is the second best option for managing the diseases, due to continuous and irrational use of the chemicals; pathogens have developed resistance against certain class of fungicides/bactericides.

Tinatin and Bobusheva (2016) used efficient methods and bioassay for systematic screening of *R. solanacearum* for identification of its phenotype and biochemical profile, as well as for pathogenicity and virulence. As a

result, an aggressive race Biovar3 was most isolated from the potato fields of the Issyk-Kul region, especially in fields where the Picasso variety was grown.The isolated indigenous strains of *Streptomyces diastatochromogenes* strain sk-6 and *Streptomyces bambergiensis* strain k1-3 has the potential to be used as a biocontrol agent for the management of the bacterial wilt of potatoes, as indicated by the reduced percentage wilt incidence. Root zone and soil application of *Streptomyces* diastatochromogenes strain sk-6 and *Streptomyces bambergiensis* strain k1-3 at a dose of 108 cell/ml significantly reduced disease incidence and increased the growth of potato plants. The disease's progress was reduced by 60% and 56% in plants inoculated with Streptomyces diastatochromogenes strain sk-6 and Streptomyces bambergiensis strain k1-3, respectively.

Yang *et al.* (2016) evaluated for the first time the antibacterial activity and mechanisms of action of coumarins against the phytopathogen *R. solanacearum* and investigated the effect of functional group substitution. They first tested the antibacterial activity of 18 plant-derived coumarins with different substitution patterns, and found that daphnetin, esculetin, xanthotol, and umbelliferone significantly inhibited the growth of *R. solanacearum*. Daphnetin showed the strongest antibacterial activity, followed by esculetin and umbelliferone, with MICs of 64, 192, and 256 mg/L, respectively, better than the archetypal coumarin with 384 mg/L. We further demonstrated that the hydroxylation of coumarins at the C-6, C-7 or C-8 position significantly enhanced the antibacterial activity against *R. solanacearum*.

Nasir (2016) studied Late Blight (LB) and Bacterial Wilt (BW) which play an important role in reduction of the yield in Ethiopia, LB occured

throughout the major potato production areas and estimated of losses ranging from 6.5 to 61.7%, depending on level of susceptibility of the varieties.

Yuliar *et al.* (2015) revealed the development of control methods against bacterial wilt diseases caused by *Ralstonia solanacearum* and focused advances in control measures, such as biological, physical, chemical, cultural, and integral measures, as well as biocontrol efficacy and suppression mechanisms. Biological control agents (BCAs) have been dominated by bacteria (90%) and fungi (10%). Avirulent strains of *R. solanacearum*, *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp. are well known BCAs. New or uncommon BCAs have also been identified such as *Acinetobacter* sp., *Burkholderia* sp., and *Paenibacillus* sp. Inoculation methods for BCAs affect biocontrol efficacy, such as pouring or drenching soil, dipping of roots, and seed coatings.

Tita *et al.* (2015) conducted an experiment to evaluate the effects of compost on bacterial wilt of potatoes. Six compost amendments were built using 3 grass species (*Ageratum haustonianum, Pennisetum purpureum,* and *Tithonia diversifolia*) and 2 animal dung (cow dung and poultry manure).The trial had 7 compost types (treatments): 6 treatments with different composts at 50% and sterilized soil (control). Bacterial wilt incidence and severity were significantly reduced with the incorporation of compost irrespective of the compost type. The most suppressive compost to bacterial wilt was *T. diversifolia* and poultry manure (disease incidence: 7.5% and disease severity: 1.3%), with the highest population of fluorescent bacteria (20 x 1011 cfu/ml). The study suggests that *T. diversifolia* and

poultry manure compost could be incorporated to an integrated control strategy of bacterial wilt on potato. The antagonistic bacteria in *T.diversifolia*+ poultry manure compost will be studied later.

Guchi (2015) stated many factors that reduce the yield of the crop among which the diseases like late blight (*Phytophthora infestans*) and bacterial wilt (*Ralstonia* (*Pseudomonas*) *solanacearum*) play an important role. Bacterial wilt of potato was managed by using biological control agents, resistant varieties, intercropping, certified disease free seed, selective bactericides and cultural practices such as destruction of cull piles by freezing or deep burying, destruction.

Deberdt *et al.* (2014) conducted a research with the genetic and phenotypic diversity of *R. solanacearum* strains in French Guiana was assessed using diagnostic polymerase chain reactions and sequence-based (*egl* and *mutS*) genotyping on a 239-strain collection sampled on the families Solanaceae and Cucurbitaceae, revealing an unexpectedly high diversity. Strains were distributed within phylotypes I (46.9%), IIA (26.8%), and IIB (26.3%), with one new endoglucanase sequence type (*egl*ST) found within each group. Phylotype IIB strains consisted mostly (97%) of strains with the emerging ecotype (IIB/sequevar 4NPB).

Mondal *et al.* (2014) performed an experiment throughout West Bengal to evaluate the bacterial wilt disease was confirmed by ooze test in the field and biochemical tests in the laboratory in every case. A sharp relationship was observed between disease intensity and different meteorological factors during the experimental period. In most of the cases, wilting process of wild

plants started from the month of March (Av. Tmax. 32°C and Tmin. 20°C) when temperature gradually rises. The maximum wilt intensity was recorded during August-September (Av. Tmax. 30°C and Tmin. 26°C) and death of such plants ceased at the end of October (Av. Tmax.29°C and Tmin. 22°C) or first week of November (Av. Tmax. 29°C and Tmin. 19°C).

Kalpage and Costa (2014) investigated the effectiveness in controlling bacterial wilt caused by two isolates of *R. solanacearum* (isolate 6 and AB3) was investigated under plant house conditions. Bacteriophage mixtures at a concentration of 2.86x106 pfu/ml were applied to the rhizosphere as a soil drench by several methods. The phage isolates had different lytic patterns on host *R. solanacearum* isolates and varied in their plaque morphology. Percentage wilt incidence by isolate 6 was reduced by 10%, due to application of the phage mixture immediately before the inoculation of the pathogen or when applied three times as a soil drench. Wilt incidence by isolate AB3 was reduced by 20% due to the application of the phage mixture by the two methods. Survival of the bacteriophage in soil treated with pahges ranged from 0.2 x 103 – 3.5 x 104 pfu/g of soil, after 15 days of the last application of phages.

Murthy *et al.* (2013) conducted an experiments to analyze the in vitro antibacterial potential of turmeric plant against ten highly virulent isolates of *R. solanacearum*. The antibacterial activity of the extracts was assayed by agar well diffusion method on Tryptone Soya agar. The results revealed that the average zone of inhibition of the rhizome extract was ranging at 20-26mm against *R. solanacearum*. Various concentrations of the extracts were prepared by dissolving extracts in DMSO. The means and standard

error of triplicate tests were recorded. The minimum inhibitory concentration (MIC) was determined by two-fold micro broth dilution method for the tested pathogens. The MIC of the turmeric extract was 2-20µg ml-1.

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

Janse (2012) evaluated the disease brown rot of potato, caused by the bacterium *Ralstonia*(*Pseudomonas*) *solanacearum*(*Rsol*), Race 3, biovar 2 (R3b2). The disease was found for the first time in 1992 in a potato field in the Netherlands and caused an outbreak in the warm summer of 1995 that appeared to be connected to use of contaminated irrigation water as in other outbreaks in western Europe at that time. The control measures and testing procedures for *Ralstonia solanacearum* in different substrates, laid down in an EU Directive, were followed. The persisting presence of *Rsol* in surface water necessitates an enduring alert and actively maintained control and survey system. The main lessons learned are: stay away, if possible, from surface water; use disease free (tested) and certified seed; apply strict hygiene; handle/grade and store seed and ware/industry potatoes separately; compensate growers or enable them to insure against the disease; invest pro-

actively in emergency plans and in up to date diagnostic expertise, education and advice; maintain an active and statistically meaningful survey and control system; perform a regular survey in ware and industry potatoes, greenhouse host crops and surface water.

Muthoni *et al.* (2012) evaluated the potential of host resistance as an important component of integrated management of bacterial wilt in Kenya. Bacterial wilt has spread to all potato growing areas in Kenya, affecting over 70% of potato farms and causing yield losses of between 50 to 100%. This disease has no effective means of control because crop protection chemicals are ineffective and expensive and biological control agents are ineffective. In addition, phytosanitary methods such as quarantine are either expensive or difficult to apply and cultural methods such as crop rotations are largely impractical because the farms are too small to allow effective rotation, the pathogen has a wide host range, and it persists for long in the soil.

Siri *et al.* (2011) suggested the disease is widespread in Uruguay, characterization of prevalent *R. solanacearum* strains in that country has not been done. In all, 28 strains of *R. solanacearum* isolated from major potato-growing areas in Uruguay were evaluated, including 26 strains isolated from potato tubers and 2 from soil samples. All strains belonged to phylotype IIB, sequevar 1 (race 3, biovar 2). Genetic diversity of strains was assessed by repetitive-sequence polymerase chain reaction, which showed that the Uruguayan strains constituted a homogeneous group. In contrast, inoculation of the strains on tomato and potato plants showed, for the first time, different levels of aggressiveness among *R. solanacearum* strains belonging to phylotype IIB, sequevar 1. Aggressiveness assays were also performed on

accessions of *S. commersonii*, a wild species native to Uruguay that is a source of resistance for potato breeding. No significant interactions were found between bacterial strains and potato and *S. commersonii* genotypes, And differences in aggressiveness among *R. solanacearum* strains were consistent with previously identified groups based on tomato and potato inoculations. Moreover, variation in responses to *R. solanacearum* was observed among the *S. commersonii* accessions tested.

Algam *et al.* (2010) evaluated the effects of chitosan and sixteen *Paenibacillus* strains against the wilt pathogen *Ralstonia solanacearum* were evaluated *in vitro* and under greenhouse conditions. Chitosan and two *Paenibacillus* strains, in particular *Paenibacillus polymyxa* MB02-1007, were found to have strong *in vitro* antibacterial activities against *R. solanacearum.* In addition, chitosan applied as soil drench or seed treatment significantly reduced wilt incidence by 72% and 48%, respectively while *P. polymyxa* MB 02-1007 as a soil drench or a seed treatment significantly reduced wilt incidence by 82% and 88%. In general, regardless of the application method, plant growth parameters as well as the activities of chitinase and b-1,3-glucanase in potato plants were significantly increased by chitosan and *P. polymyxa* MB02-1007 as compared to the corresponding control, both in the absence and presence of *R. solanacearum*. The growth of potatoes, however, was promoted by chitosan more as a soil drench than as a seed treatment, while *P. polymyxa* MB02-1007 as a seed treatment was more effective than as a soil drench.

Yadessa *et al.* (2010) conducted a research to evaluate *R. solanacearum* race 3 biovar 2 (phylotype II), the causal agent of bacterial wilt of potato, the

most destructive bacterial disease of this crop in Ethiopia for which no effective control measures are available. In this study, the effects of amending topsoil with three different levels (1, 5 and 10%) of cocopeat, farmyard manure (FYM) compost and green compost, and two levels of bacterial inoculations were tested on infection of potato by *R. solanacearum* compared to non-inoculated treatments. Absence of disease at the highest rate of FYM was supported by a lower number of culturable *R. Solanacearum* bacteria recovered from rhizosphere soil two months post-inoculation in soil amended with 10% FYM. Soil amended with 10% FYM gave higher root and aboveground dry weight. Among the amendments tested, FYM at 5 or 10% would be an interesting option to manage *R. solanacearum* in the major potato growing regions of Ethiopia.

Ghosh et al. (2009) investigated to reveal that T1 (TPS whole tuber planting) and T4 (supervised management – cowdung @ 40 ha^{-1} at land preparation + seed piece tuber treatment with carbendazim 2.5 gL^{-1} and Streptocycline @ 1 gL^{-1} + stable bleaching powder drenching without removal of affected plant at 40 Days After Planting (DAP) @ 10 gL^{-1}, along with protective banding with well decomposed cowdung + oilcake + Single Super Phosphate + Muriate of Potash mixture at 20:5:3:1 in each bacterial wilt affected plant + mancozeb spray @ 2.5 gL^{-1} at 50, 57 and 60 DAP) were the best treatment in terms of their responses to yield, disease management and higher return per rupee investment.

Vailleau *et al.* (2007) described the soilborne pathogen *R. solanacearum*, the causal agent of bacterial wilt which attacks more than 200 plant species, including some legumes and the model legume plant *Medicago truncatula*.

They demonstrated that *M. truncatula* accessions Jemalong A17 and F83005.5 were susceptible to *R. solanacearum* and, by screening 28 *R. solanacearum* strains on the two *M. truncatula* lines and differential interactions were identified. *R. solanacearum* GMI1000 infected Jemalong A17 line, and disease symptoms were dependent upon functional hrp genes. An in vitro root inoculation method was employed to demonstrate that *R. solanacearum* colonized *M. truncatula* via the xylem and intercellular spaces. *R. solanacearum* multiplication was restricted by a factor greater than 1 × 105 in the resistant line F83005.5 compared with susceptible Jemalong A17.

Kuarabachew *et al.* (2007) evaluated 50 fluorescent pseudomonas were collected from different potato growing areas in Ethiopia isolated and characterized, and evaluated on king's B medium for their antibiosis towards *Ralstonia solanacearum* the pathogen of bacterial wilt of potato. Out of the 50 isolates only three i.e., Pf S2, Pf Wt3 and PfW1 showed inhibition against the growth of the pathogen. The potato tubers were dipped into 48 hrs old culture suspension of the three isolates i.e., Pf S2, Pf Wt3, PfW1 and Pfri (Indian reference strain) for 1 hr and planted in pots containing sterilized soil. Bacterization of tubers with isolates Pf S2, Pf Wt3, and PfW1, significantly reduced by 59.83% the incidence of bacterial wilt compared to the pathogen-inoculated control and increased plant growth (plant height and dry weight) by 59.83%, 76.89% and 28.44%, respectively.

Yabuuchi *et al.* (2007) observed that plants with tops killed by *R. solanacearum* may bear healthy and diseased tubers, while plants that show no signs of the disease in their tops may sometimes produce diseased tubers.

Henok *et al.*, (2007) three isolates of *Pseudomonas fluorescens* i.e., PfS2, PfWt3 and PfW1 showed inhibition against the growth of the pathogen. Bacterization of tubers with isolates Pf S2, Pf Wt3, and PfW1, significantly reduced by 59.83% the incidence of bacterial wilt compared to the pathogen inoculated control.

Pawar *et al*. (2004) tested the efficacy of different fungicides like mancozeb, copper oxychloride, copper hydroxide in controlling bacterial diseases.

Bekele and Hailu (2001) had done a research on the efficacy and economics of fungicide spray in the control of late blight of potato in Ethiopia. The result showed that, Ridomil MZ - 63.5% WP which is both systemic and protectant in action gave the best control (78.8%). On the other hand Chlorothalonil, Mancozeb and Brestan 10 did not differ significantly in respect to disease control, and gave 59.3, 43.0 and 46.8% control, respectively. However, the three fungicides significantly ($P< 0.05$) controlled late blight when compared to the control plot. They conclude that, the fungicides Chlorothalonil 50% EC and Brestan 10 can be used to control late blight. Overall, Ridomil MZ 63.5% WP gave effective control of late blight and the best return.

Momol *et al.*, (2001) evaluated the *Ralstonia solanacearum* and stated that the infection of undisturbed roots of susceptible hosts through microscopic wounds caused by the emergence of lateral roots. Transplanting, nematodes, insects and agricultural equipment are also able to wound roots. Bacteria then colonize the cortex and advance towards the xylem vessel, from where

it rapidly spreads in the plant. Bacterial masses prevent water flow from the roots to the leaves, resulting in plant wilting.

Hartman *et al.* (1994) reported that there are no bactericides available for chemical control of the bacterial wilt disease; while others reported that it is difficult to control bacteria with chemicals (Grimault *et al*., 1992).

CHAPTER III

MATERIALS AND METHODS

Three experiments were conducted on survey the disease prevalence, identify the causal pathogen and *in vitro* management of the bacterial wilt disease of potato. The methodologies of the three experiments are as follows:

3.1. Prevalence of bacterial wilt disease of potato in three different potato growing locations of Manikganj district

3.1.1 Sample collection

Bacterial wilt samples were collected from three different potato growing areas of Manikganj district (Saturia, Daulatpur and Ghior Upazila).

Figure 1. Location of bacterial wilt sample collection from three different potato growing fields of Manikganj district (Saturia, Daulatpur and Ghior Upazila).

A survey was carried out for collection of the infected samples in three different potato fields of Manikganj (Saturia, Daulatpur and Ghior Upazila @ at least 3 infected fields per Upazila) during the potato season of 2015-2016. The infected samples were collected randomly from the infested field during the investigation. Percentage incidence of the disease in potato plants were recorded based on their symptomatology through ooze streaming and calculated the averages of three different Upazilas.

3.1.2. Survey and sampling procedure

Survey was done through interviewing of the farmers on the field prevalence of the disease. Three infected fields from each Upazila were selected. Total nine samples were collected during the survey. Infected potato plants were collected based on typical symptoms and put in a polyethylene bags and brought to the laboratory of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka for bacterial isolation, identification and confirmation for further study.

3.1.3. Data Collection

Data were collected on the percent disease incidence in three location from Saturia, Daulatpur and Ghior Upazila of Manikganj district; and disease scores (0~4 scale) (Swanson *et al.*, 2005), PDI (Percent Disease Index), percent disease reduction etc. were mesurement of the inoculated plants.

3.2. Isolation, identification and characterization of the pathogen from the collected samples.

Total nine infected potato samples of bacterial wilt were collected from three different locations of three Upazilas of Manikganj district and samples were

tested for isolation, identification and biochemical characterization of the pathogens.

3.2.1. Preparation of Triphenyl Tetrazolium Chloride (TTC) Medium

TTC is the standard selective media for the proper growth of *R. solanacearum* (Kelman, 1954 and Schaad, 1988). The medium was prepared by dissolving peptone 10gm, glucose 10gm, casamino acid (i.e., casein hydrolysate) 1gm and agar 18gm in 1000 ml of distilled water. The p^H of the medium was adjusted to 7.0 using 0.1N KOH and cooked on hot plate. After cooking, the medium was autoclaved for 20 minutes at 121^0C under 15 PSI/1.1kg/cm^2 pressure. The aqueous solution of Triphenyl Tetrazolium Chloride (TTC) agar was prepared by dissolving 1gm of chemical in 100 ml (i.e. 1% w/v) distilled water in Erlenmeyer flask. The TTC solution was separately sterilized in an autoclave at 121^0C under 15 PSI for 20 minutes. The sterilized TTC solution was poured into the sterilized medium at the rate of 5ml/1000ml and mixed thoroughly. The medium was poured in petridishes at the rate of 20ml/plate. One loopful of the suspension was streaked in each plate on the surface of the solidified selective TTC medium and incubated at 30^0c for 48 hours.

3.2.2. Isolation and purification of bacterial wilt pathogen of potato

The collected potato plants were washed under running tap water. The diseased portions were cut into small pieces. Surface sterilization were done by dipping them in 5% sodium hypochlorite solution for 2-3 minutes. They were washed three times with sterile water. After surface sterilization 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}. After preparing different dilution, 0.1 ml of each dilution was spreaded over NA plate thrice to remove excess surface moisture as described by Serfontein (1998). Spreading was done with the help of a glass-rod. The inoculated NA plates were kept in incubation chamber at 30^{o}C. The plates were observed after 24 hours and 48 hours. Then single colony grown over NA plate was restreaked on another plate with the help of a loop to get pure colony.

3.2.3. Biochemical characterization and confirmation of bacterial wilt pathogen (*R. solanacearum*) for artificial inoculation

Identification of the pathogen causing bacterial wilt of potato was done by biochemical tests and cultural features of the pathogen as per standard microbiological procedures described by Hayward (1991).

3.2.4. Biochemial tests of isolated bacteria

3.2.4.1. Gram staining test

A loop full of the bacterium was spread on a glass slide and fixed by heating on a very low flame. Aqueous crystal violet solution (0.5%) was spread over the smear for 60 seconds and then washed with running tape water. It was then flooded with iodine solution for one minute, rinsed in tape water and decolorized with 95% ethanol. After washing the specimen was counter-stained with safranin for approximately 10 seconds then washed with water. The slide was dried and observed under a microscope at 100X.

3.2.4.2. Potassium hydroxide solubility test

On glass slide a loopful of bacteria from a well grown colony was mixed with a drop of 3% KOH aqueous. Mixing was continued for less than 10 seconds. A toothpick was used for picking bacteria from a colony as well as for mixing it. The toothpick was raised a few centimeters from the glass side. Strands of viscid materials confirmed the bacterium was Gram negative.

3.2.4.3. Kovac's oxidase test

A 24 hrs old bacterial colony on nutrient agar augmented with 1% glucose was used in this test. A loopful of inoculums was rubbed on filter paper pervaded with 15%(w/v) freshly prepared aqueous solution of Nitrogen tetramethyl-p-phenylene diamine dihydrochloride (Kovacs, 1956). The inoculums was smeared over the area of filter paper containing oxidase reagent to develop deep blue or purple color within ten seconds indicating the oxidation of the reagent.

3.2.4.4. Levan test

The bacterium (*R. solanacearum*) was inoculated on Nutrient agar with 5% sucrose and incubated at 30^0c for 48 hrs. Levan sucrase which catalyzes the synthesis of Levan form sucrose, is produced by a number of bacteria including *R. solanacearum*. When the bacteria were grown on a medium containing sucrose, the production of an extracellular enzyme (levan sucrase) was induced and sucrose was converted to levan and glucose. During the fermentation process, the bacteria also utilize sucrose for maintenance and growth.

3.2.4.5. Catalase test

This test was performed with isolates to check their liveliness. Standard cultures were used for control purpose. One milliliter of 3% hydrogen peroxide (H_2O_2) was placed on the microscope slides. The bacterial isolates collected from seed samples were subjected to catalase test. Bacterial isolates developed in NA medium for 24 hrs and a loopful of bacteria cells were added in the drop of hydrogen peroxide. Bubbles arising from the solution were recorded positive reaction.

3.2.4.6. Pathogenicity test of wilt pathogen (*R. solanacearum*)

In the pathogenicity test, the isolates of *R. solanacearum* isolated from the different potato plants caused bacterial wilt symptoms in the inoculated potato plants. Bacterial wilt isolates of *R. solanacearum* were multiplied in TTC medium (Casamino acid 1 g/l, Peptone 10 g/l, Glucose 10 g/l pH 7.2) to inoculate on potato plants in *vitro* conditions. Stock cultures of the isolate in water were streaked on TTC meadium and incubated at 30^0C for 48 hrs. A single virulent colony of *R. solanacearum* was transferred to individual culture plates containing TTC medium. After 24 hrs of incubation the bacterial cells were harvesterd in sterile distilled water and incubation suspension was prepared. The bacterial suspension was adjusted to a concentration of 10^8cfu/ml. The potato seedlings were grown in greenhouse under *vitro* conditions. Thirty days old seedlings of potato were pulled out gently, washed free of soil and a few tertiary roots were clipped with sterilized scissors and dipped in the bacterial culture for 10 minutes (Kelman, 1952). The inoculated seedlings were transplanted to plastic case/busket containing sterilized soil. *In situ* inoculation of potato plants was

carried out by pinching off the stem at the base of the stem by clipping the needle into 20 ml of bacterial suspension with each samples isolate and then all was covered with soil. Plants similarly inoculated with sterile water served as the control.

3.2.5. Morphological characters

Morphological characteristics of the pathogen such as cell shape, gram's reaction and pigmentation were studied as per standard procedures described by Gerhardt (1981).

3.3. Effect of soil treatments on the management of bacterial wilt pathogen (*R. solanacearum*)

3.3.1 Treatments

T_1 - Control

T_2 - Bleaching powder solution (5.2mg/kg Soil)

T_3 - Turmeric powder solution (10%) for tuber treatement (30 seconds)

T_4 - Magnesium chloride solution (10mg/kg Soil)

T_5 - Cowdung powder (1:1)

T_6 – KrosinAG Bactericide (0.5gm/L)

3.3.2. Plastic case Preparation

The experiment was carried out in the net house of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka. Potato tubers were grown in plastic case (60×30 cm in rectangular shape) containing sandy loam soil at 22–25^{O}C and RH 75–80%. The usual agricultural practices of irrigation were followed. The case was filled with soil and

polythene sheet was used under the lower surface of the plastic case and the polythene sheets were leaked to remove the unnecessary water during applying water as per requirements. The cracked bricks were put into the lower regions of soil in plastic case. The control treatments of plastic case was also prepared with the ratio of soil:cowdung (1:1). The others treatments were applied to the plastic case as per recommended doses.

3.3.3 Application of Treatments

All the treatments were applied into the busket at the time of soil preparation for planting of tubers. The treatments were applied at the rate of Bleaching powder solution (5.2mg/kg Soil), Turmeric powder solution (10%), Magnesium chloride solution (10mg/kg Soil), Cowdung powder (1:1) and Krosin AG Bactericide (0.5gm/L).

3.3.4 Potato tubers placing in case

The tubers were sliced by taking the 2/3 buds. The tubers were treated by the turmeric powder solution for 30 sec. and then placed into the plastic case. The tubers were covered with the applied treatments into soil.

3.3.5 Inoculation method of *R. solanacearum*

In the axil puncturing method the inoculation was carried out with sterile dissection needle, by dipping the needle with the bacterial suspension and inserting at the axil of the leaf along with a drop of inoculum and gently pressing to ensure the inoculum reached the vascular tissues (Snedecor and Cochran, 1957). The whole experiment was carried out under net house condition in a controlled randomized block design with six treatments of

three replications each with six plants (Total 36 plants). Periodical observations were made on bacterial wilt symptom expression and per cent disease incidence was recorded in all the three methods of inoculation. The data on percentage bacterial wilt were transferred to arc sine values and analysed statistically.

3.3.6 Assessment of Disease severity

The disease severity of plants scoring the percentage of wilted leaves with an arbitrary scale (Swanson et al., 2005) where:

0 = no leaves wilted (healthy plant),

1 = 1-25% wilted (tolerant plant),

2 = 26-50% wilted (moderately tolerant plant),

3 = 51-75% wilted (susceptible plant) and

4 = all leaves wilted or dead (highly susceptible)

Wilted plants were evaluated every second day till the 28th day post inoculation.

$$\text{PDI} = \frac{Sum\ of\ all\ rating}{Total\ no.of\ observation\ \times Highest\ scale} \times 100$$

3.3.7. Statistical analysis

The recorded data were compiled, tabulated and subject to statistical analysis. Analysis of variance was done with the help of computer package programme M-STATC. The mean differences were adjudged by Ducan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984)

CHAPTER IV

RESULTS

4.1. Field prevalence of the potato fields during the survey of three Upazilas of Manikganj district

Prevalence of bacterial wilt in the farmers fields was recorded from different infected potato fields of Manikganj district (Figure 2.). The observed incidence was recorded in Saturia 29.03%, Daulatpur 15.16 % and Ghior 25.81 % and the location were having a medium high land, sandy to sandy loam soil type and a mixed cropping pattern under the AEZ no. 12 (Table 2). Five potato varieties namely-Sheel, Bilatee, Cardinal, Du Hajari and Diamond etc. were found to be mostly cultivated (Table 2).

4.1.1 Prevalence of bacterial wilt disease in different potato growing locations of Manikganj district

Figure 2. Field prevalence of bacterial wilt disease of potato in Manikganj district.

Table 1. Field condition of the potato fields and prevalence of wilt disease during the survey of three Upazilas of Manikganj district

Location	AEZ	Soil topography	Cropping pattern	Variety cultivated	(%)Incidence in the field
Saturia	12. Low Ganges River floodplain	Medium high land, sandy to sandy loam soil	Mixed	Sheel, LalSheel, Du Hajari, Diamond	29.03
Daulatpur				Sheel, Bilatee, LalPakri, Du Hajari, Diamond	15.16
Ghior				Sheel, Bilatee, Diamond	25.81

4.1.2. Field Symptoms of bacterial wilt of potato caused by *Ralstonia solanacearum*

Typical symptoms (Figure 3.) were wilting, yellowing and rapid death of the plants.. Wilting was first seen as a drooping of the tip of some of the lower leaves similar to that caused by a temporary shortage of water. Symptoms of the disease were studied and confirmed by visual observation as per standard procedure by Champoiseau *et al*. (2009).

Figure 3. Showing typical symptoms of bacterial wilt (*R. solanacearum*) in the field.

4.2. Isolation, identification and characterization of the pathogen from the collected samples of potato

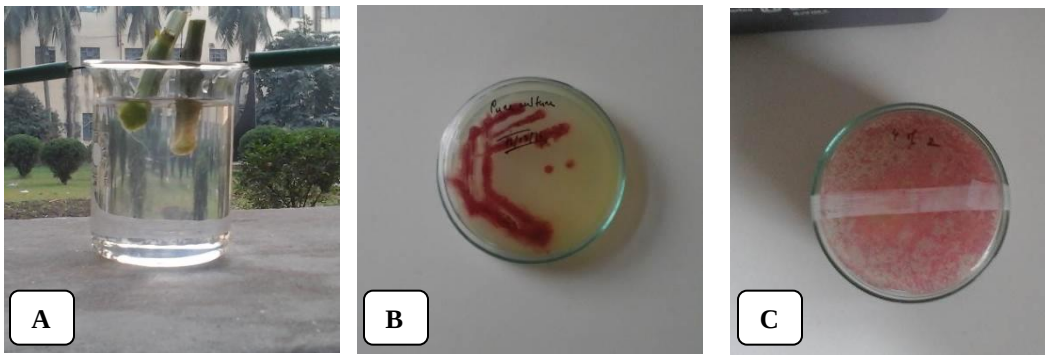

Figure 4. Bacterial streaming test (A), Growth and morphology of *R. solanacearum* on TTC medium (Streak plate method) (B), Growth and morphology of *R. solanacearum* on TTC medium (Spread plate method) (C).

4.2.1. Biochemical tests and characterization of bacteria isolated from different samples of potato

The samples were observed to stream oozes from the stems and colonies of *R. solanacearum* produced irregularly round or typical, smooth surface, fluidal colonies with red centers on TTC media (Figure 4) during the characterization.

A series of biochemical tests such as Gram staining test, Potassium hydroxide solubility test, Kovac's oxidase test, Levan test and catalase test, Pathogenecity test, especially selective for *Ralstonia solanacearum* were performed.

4.2.1.1. Gram staining test

The Gram staining reaction was performed using crystal violate. The microscopic results showed that all of the isolates of *R. solanacearum* did not retain violet colour i.e. the isolates retained counter stain(pink colour). Therefore, all isolates of *R. solanacearum* representing each group are gram negative and straight or curved rod shaped which is the characteristic feature of any plant pathogenic bacteria (Table. 2).

4.2.1.2. Potassium hydroxide solubility test

All of the plant pathogenic bacteria are usually Gram negative except *Clavibacter* and *Streptomyces.* The Gram negative test of bacterium was also confirmed by Potassium hydroxide solubility test. The result revealed that an elastic thread or viscious thread was observed when loop raised from the bacterial solution by toothpick a few centimeters from glass slides in case of all group of tested bacterial isolates of indicating that all groups of bacterial isolates are Gram negative (Table. 2) .

4.2.1.3. Kovac's Oxidase test

In Kovac's oxidase test positive isolates produce purple blue color when mass of bacterial growth is rubbed on filter paper impregnated with oxidase reagent. This test is used for differentiation between aerobic and anaerobic bacteria (Kovacs, 1956). In our studies, the tested isolates of all group of *Ralstonia solanacearum* isolates showed positive response in development of color within few seconds which indicated that the result of the test was positive for *Ralstonia solanacearum* isolates (Table. 2).

4.2.1.4. Levan test

The test isolates of collected bacterial groups were negative for levan production and levan formation results from the enzymatic activity of levan sucrase on sucrose. The glucose is metabolized and the fructose is polymerized. Alginates may also be formed on sucrose agar (Fett *et al*., 1986),(Gross,1984 and Rudolph,1987). Levan formation is responsible for the production of mucoid colonies by some *Pseudomonas*. Production of levan as an extra cellular capsule or slime layer results in colonies which are characteristically raised, convex and dome shaped in appearance (Schaad, 1980) (Table. 2).

4.2.1.5. Catalase test

The bacterial isolates collected from different samples of potatoes were tested for catalase test. This is used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism . Catalase is a hemi enzyme capable of decomposing hydrogen peroxide to water and oxygen (Klement *et al*., 1964). All Gram negative bacteria produce gas bubbles when these are mixed with a drop of H_2O_2 on glass slide. Production of gas bubbles give a clue for presence of aerobic and facultative anaerobic bacteria (Schaad, 1980). Results of this test showed that all the tested isolates were able to raised bubbles on the slide and indicated as positive reaction that these might be *Ralstonia solanacearum* (Table. 2).

4.2.1.6. Pathogenecity test of isolated bacteria

The findings of the pathogenecity test of all the isolates collected from different infected fields of three upazillas of Manikganj district showed positive (Table 2.) on 30 days old potato seedlings. Three potato varieties namely- Diamond, Cardinal and Sheel were tested.

Table 2. Results of Biochemical tests of *R. solanacearum* of potato collected from different locations of Manikganj district

Sample/ isolate No.	Gram's staining test	KOH test	Kovac's oxidase test	Levan test	Catalase test	Pathogenicity test	Inference
Isolate 1	+	+	+	-	+	+	*R. solanacearum*
Isolate 2	+	+	+	-	+	+	*R. solanacearum*
Isolate 3	+	+	+	-	+	+	*R. solanacearum*
Isolate 4	+	+	+	-	+	+	*R. solanacearum*
Isolate 5	+	+	+	-	+	+	*R. solanacearum*
Isolate 6	+	+	+	-	+	+	*R. solanacearum*
Isolate 7	+	+	+	-	+	+	*R. solanacearum*
Isolate 8	+	+	+	-	+	+	*R. solanacearum*
Isolate 9	+	+	+	-	+	+	*R. solanacearum*

4.3. Effect of treatments on the PDI at different DAI of bacterial wilt pathogen (*R. solanacearum*) on potato plants

Significantly the highest PDI was observed 2.77, 4.94, 12.03 and 19.75 in case of control in 16^{th}, 20^{th}, 24^{th}& 28^{th} DAI respectively. The lowest PDI was observed 0.62, 0.92, 2.46, 2.46 and 2.46 in case of cow dung treated plants in 16^{th}, 20^{th}, 24^{th}& 28^{th} DAI level respectively . However, turmeric powder solution treated plants were showed the second lowest PDI significantly which was followed by magnesium chloride solution treated plants. But, the Krosin AG (commercial bactericide) and bleaching powder solution as traditional farmer's practices in controlling the bacterial wilt disease showed the significantly the lowest disease reduction and the second highest PDI in artificially inoculated potato plants in the net house (Table. 3).

Table 3. Effect of treatments on the PDI at different DAI of bacterial wilt pathogen (*R. solanacearum*) on potato plants

	PDI of the inoculated potato plant													
Treatment	4th DAI		8thDAI		12thDAI		16thDAI		20thDAI		24thDAI		28thDAI	
	DSS	PDI	DSS	PDI	DSS	PDI	DSS	PDI	DSS	PDI	DSS	PDI	DSS	PDI
T1	1.25 a	0.31	1.50 a	0.92	2.50 a	3.08	2.50 a	2.77	4.00 a	4.94	3.50 a	12.03	4.00 a	19.75
T2	1.50 a	0.30	1.50 a	0.62	2.00 b	2.46	2.50 a	3.08	3.50 b	4.56	3.00 b	11.11	3.50 b	13.88
T3	0.00 b	00	0.25 b	0.30	0.50 d	0.61	1.25 c	0.92	2.50 c	3.08	2.00 c	3.71	2.50 c	3.08
T4	0.50 b	00	0.50 b	0.30	1.25 c	0.30	1.50 b	0.61	2.75 c	2.46	2.25 c	3.71	2.75 c	4.46
T5	0.00 b	00	0.25 b	0.62	0.50 d	0.62	1.00 d	0.92	1.75 d	2.46	1.50 d	2.46	1.75 d	2.46
T6	0.00 b	00	0.50 b	0.30	1.00 c	0.62	1.25 c	2.78	3.50 b	3.71	3.00 b	6.71	3.50 b	12.03
LSD (0.05)	0.5811		0.2599		0.3084		0.2349		0.3227		0.3275		0.322	
CV (%)	72.16		73.70		50.80		30.00		32.98		27.43		22.91	
PDI = Percent Disease Index; DAT=Days After Inoculation; DSS = Disease Severity Score														

T_1 - Control

T_2 - Bleaching powder solution

T_3 - Turmeric powder solution

T_4 - Magnesium chloride solution

T_5 - Cowdung powder

T_6 – Krosin AG Bactericide

4.4. Effect of different treatments on PDI at the 28th DAI against bacterial wilt (*R. solanacearum*) of potato

Due to the effect of different treatments significantly different PDI was recorded and the lowest PDI (percent disease index) was observed in case of cow dung powder solution 2.46% which was followed by turmeric powder solution 3.08% and $MgCl_2$ solution 4.46%. Whereas, the highest PDI (percent disease index) was observed in case of control which was 19.75% which was followed by bleaching powder (13.88%) and Krosin AG (12.03%).

4.5. Effect of different treatments on the mean disease severity of bacterial wilt (*R. solanacearum*) of potato

The effect of different treatments on the mean disease severity of bacterial wilt (*R. solanacearum*) of potato was represented in (Figure 6) and significant differences were observed in case of mean disease severity.The lowest score were observed in case of cow dung treatment (1.75) which was followed by turmeric powder solution (2.5) and magnesium chloride solution (2.75) treated plants. But significantly the highest disease score was observed in case of control (4.0) which was followed by bleaching powder (3.5) as traditional farmer's practices and commercial bactericide Krosin AG (3.5).

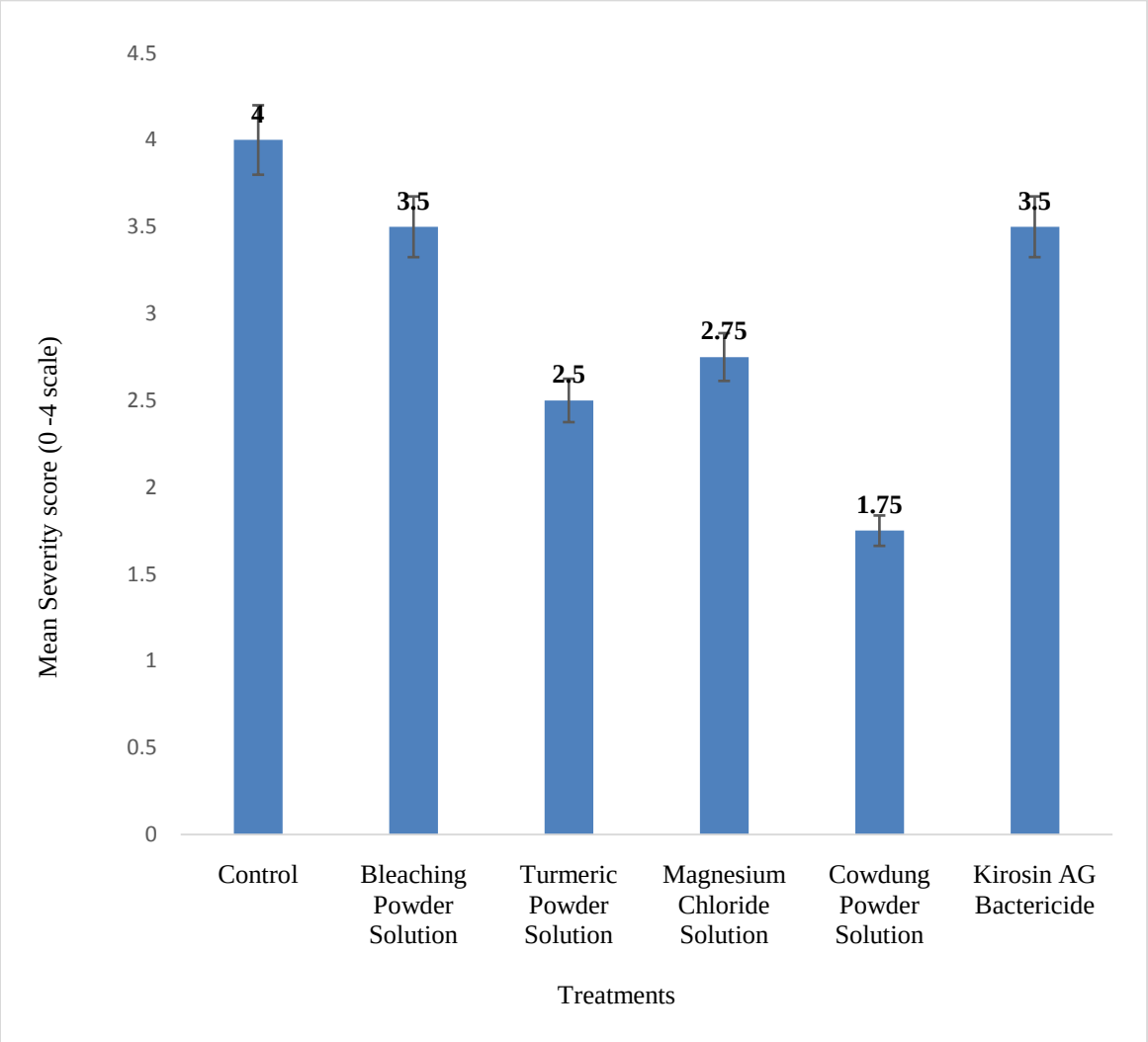

Figure 5. Effect of different treatments on the mean disease severity of bacterial wilt (*R. solanacearum*) of potato

4.6. Effect of different treatments in percent reduction of disease severity of bacterial wilt (*R. solanacearum*) in potato plants at 28th day after inoculation

Significant differences were observed due to the effect of different treatments in case of percent reduction of disease severity of bacterial wilt (*R. solanacearum*) in inoculated potato plants at 28th DAI (day after inoculation). It was showed that the percent reduction of disease severity was the highest in case of cow dung (56.25%) treated plants which was followed by turmeric powder (37.5%) and magnesium chloride (31.25%) treated plants (Fig. 7). But, the lowest percent reduction (12.50%) was observed in case of both traditional farmer's practice (bleaching powder) and commercial bactericide (Krosin AG).

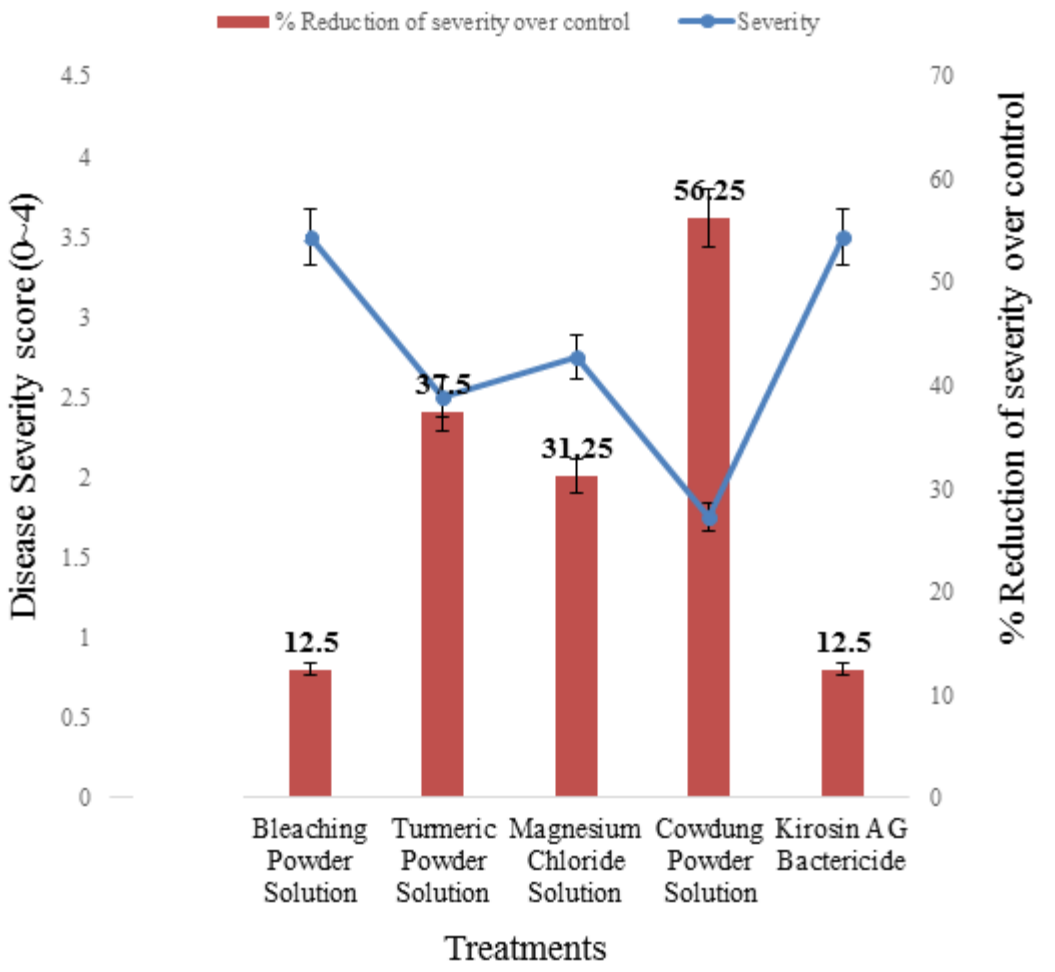

Figure 6. Effect of different treatments in % reduction disease severity of bacterial wilt (*R. solanacearum*) in potato plants at 28th day after inoculation

4.7. Effect of soil treatments on the management of bacterial wilt pathogen (*R. solanacearum*) *in vitro*

The Potato tubers were planted after washing the tubers with the sterilized water (Control)(7 A). The highest disease severity of bacterial wilt was appeared with the PDI value of 19.75 in control after 28 DAI which was damaged the potato plant significantly. But the severity decreased (13.88) by the application of Bleaching Powder. The disease severity was reduced significantly by using Bleaching Powder over control (Figure 7 B),

In case of turmeric powder solution (Figure 7 C), The disease severity of bacterial wilt was appeared with the PDI value of 3.08 at 28 days after planting. The disease severity was reduced significantly by using turmeric powder solution over control. The Magnesium Chloride ($Mgcl_2$) Solution reduced the bacterial wilt of Potato over control (Figure 7 D). The disease severity of bacterial wilt was appeared with the PDI value of 4.46 at 28 days after planting. The Cowdung mixture with the soil (1:1) reduced the bacterial wilt of Potato over control (Figure 7 E). The disease severity of bacterial wilt was appeared with the PDI value of 2.46 at 28 days after planting. In case of Krosin AG (Figure 7 F) solution, The disease severity of bacterial wilt was appeared with the PDI value of 312.03 at 28 days after planting.,

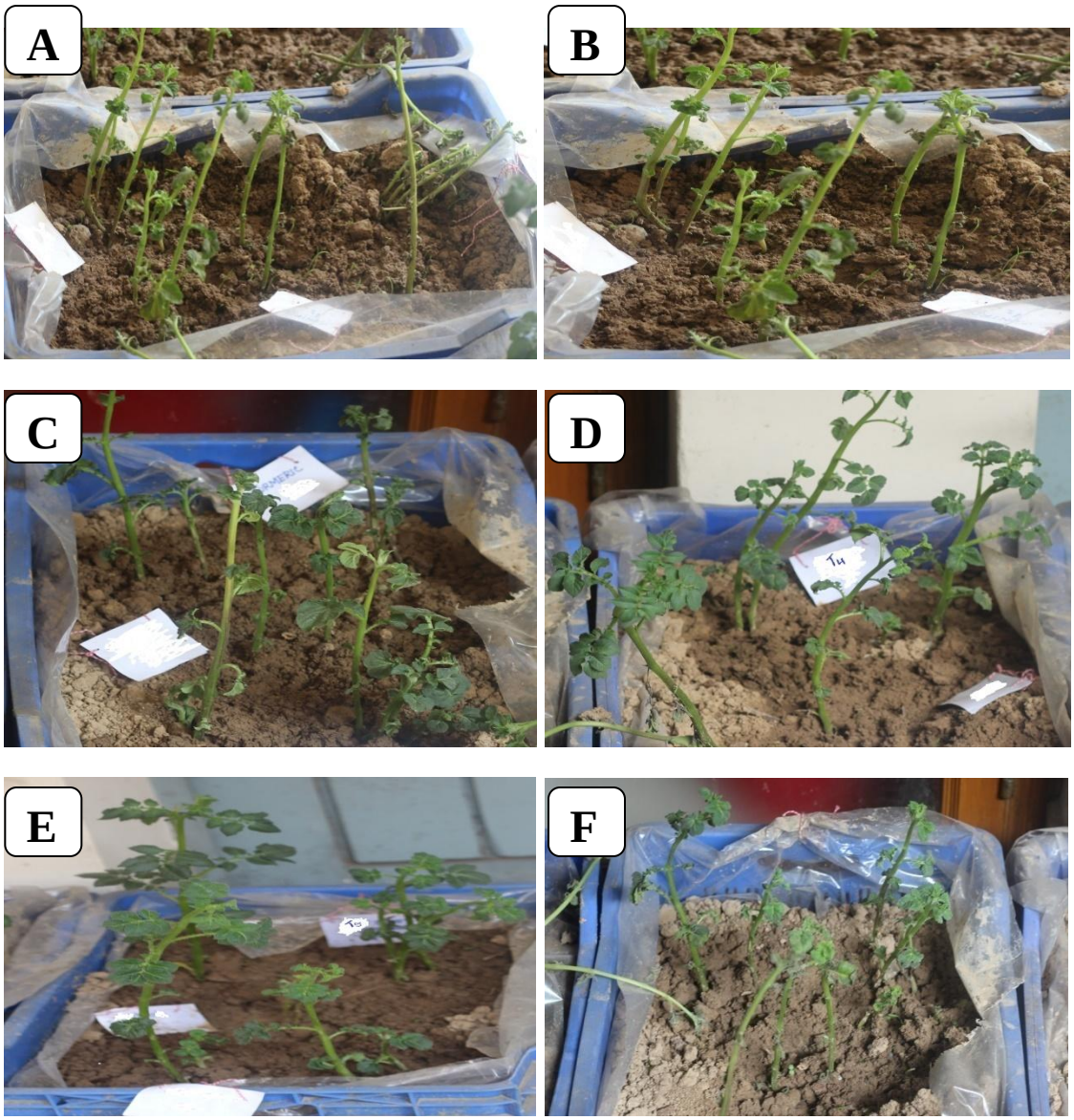

Figure 7. Symptoms of Seedling with the application of Control (A), Bleaching Powder (B), Turmeric powder (C), $Mgcl_2$ (D), Cowdung (E), Krosin AG (F)

CHAPTER V

DISCUSSION

The bacterial wilt (*R. solanacearum*) diseases of potato were recorded with the typical symptoms from Manikgonj district. Typical symptoms were observed during field investigation. The symptoms were wilting, yellowing and rapid death of the plants. Wilting was first seen as a drooping of the tip of some of the lower leaves similar to that caused by a temporary shortage of water. At first only one branch in a hill showed wilting. Affected leaves later became permanently wilted and rolled upwards and inwards from the margins. The wilting then extended to leaves further up the stem and was followed by a yellowing of the leaves. The leaves finally turned brown and fall off, beginning at the base of the stem and continuing upwards. The symptoms were observed similar to the observation of Champoiseau *et al*. (2009).

Isolation from the infected stem yielded well separated, typical, lucid, convex, mucoid colonies of bacterium on nutrient agar medium after 48 hours of incubation at 30^{o}C. Isolation and identification of *R. solanacearum* was done using the protocol described by Hayward (1991). The Gram's stained slide observed under the compound microscope at 100 X magnification with oil immersion, the bacterium was rod shaped with rounded ends, cells appeared singly and also in pairs, Gram negative (red colour) and capsulated which was supported the studies done by Murray *et al*., 2007.

Results showed that the highest PDI was observed in case of control in all 16^{th}, 20^{th}, 24^{th}& 28^{th} DAI and the lowest PDI was observed in case of cow dung treated plants in case of all of the DAI level. However, turmeric

powder solution treated plants were showed the second lowest PDI which was followed by magnesium chloride solution treated plants. But, the Krosin AG (commercial bactericide) and bleaching powder solution as traditional farmer's practices in controlling the bacterial wilt disease showed the second highest PDI in artificially inoculated potato plants in the pot house. Therefore, the effect of five different treatments on PDI at the 28^{th} DAI (Days after inoculation) of bacterial wilt (*R. solanacearum*) of inoculated potato was observed the lowest by cow dung powder solution which was mostly followed by turmeric powder and $MgCl_2$. But the highest disease scores at the same DAI was observed in case of control which was mostly followed by bleaching powder as traditional farmer's practices and commercial bactericide Krosin AG. Because, the effect of different treatments on the mean disease severity of bacterial wilt (*R. solanacearum*) of potato was observed the lowest in case of cow dung treatment (1.75) which was followed by turmeric powder solution (2.5) and magnesium chloride solution (2.75) treated plants. But the highest disease score was observed in case of control (4.0) which was followed by bleaching powder (3.5) as traditional farmer's practices and commercial bactericide Krosin AG (3.5).

The bactericide Krosin AG had an active ingredient of 9% streptomycin sulphate and tetracyclin showed lower effect on disease reduction as compared to other which was supported by the study of Farag *et al.*, 1982; Farag *et al.*, 1986 and also supported by CABI, 2017. Because, it was found that antibiotics such as streptomycin, ampicillin, tetracycline and penicillin showed hardly any effect (Farag et al., 1982); in fact, streptomycin application increased the incidence of bacterial wilt in Egypt (Farag et al., 1986). In the study, cow dung was observed to show the highest reduction of

pathogen and it was supported by Shrivastava and Pal (2014), Research conducted on water, ethanol and n-Hexane extract of whole cow dung against *E. coli* and *Pseudomonas* by Shrivastava and Pal, 2014 revealed their antimicrobial properties against Gram negative type bacteria . It might be due to the encouragement of plant growth promoting. In case of turmeric powder it was also observed to reduce the wilt disease significantly which was supported by the study of Narasimha *et al.,* 2015 and it was partially supported by Balan *et al.,* 2016. Magnesium chloride was also found to be effective against the disease which was supported by the study of Oyarzua *et al.,* 2014. Bleaching powder and Krosin AG (bactericide) was found to be suggested by some of the Agriculture Extension officers (AEO) to some farmers against the disease and those were found to reduce the disease as compared to control. However, differences in severity of wilt might be varied with the diversity of host plants, phenotype, genotype, geographical distribution of the pathogen and the range of environmental conditions conducive to bacterial wilt disease (Chatterjee *et al*., 1997).

CHAPTER VI

SUMMARY AND CONCLUSION

The present study was conducted to find out the prevalence of bacterial wilt disease of potato in three different potato growing locations of Manikganj district and to isolate, identify and characterize the pathogen from the collected samples and to evaluate soil treatment against the collected bacteria pathogen for controlling the wilt diseases *in vitro*.

In the present study, twenty seven samples were collected from nine infected farmers field. The symptomatology were studied and samples were brought to the laboratory of Department of Plant Pathology at Sher-e-Bangla Agricultural University, Dhaka. Different biochemical test viz. Gram's staining test, Potassium hydroxide solubility test, Kovac's oxidase test, Levan test, Catalase test and Pathogenecity test were carriedout for the characterization and identification of the isolated bacteria. The findings of all biochemical tests of all isolates were showed positive results and finally wilt disease were confirmed, caused by *Ralstonia solanacearum.*

Six different treatments were conducted to know the effect of soil treatment on the management of bacterial wilt pathogen in a net house of Department of Plant Pathology at Sher-e-Bangla Agricultural University, Dhaka. Significantly the highest PDI was observed 2.77, 4.94, 12.03 and 19.75 in case of control in 16^{th}, 20^{th}, 24^{th}& 28^{th} DAI respectively. The lowest PDI was observed 0.62, 0.92, 2.46, 2.46 and 2.46 in case of cow dung treated plants in 16^{th}, 20^{th}, 24^{th}& 28^{th} DAI level respectively. In case of disease severity, cowdung powder showed the lowest score(1.75) and the highest score was observed from the control(4.0). In case of disease reduction, cow

dung powder gave the best results (56.25%) in reducing the disease score against *R. solanacearum* in inoculated potato plants significantly. Turmeric powder (37.5%) and $MgCl_2$ (31.25%) solution also found significantly better as compared to other treatments and significantly lower efficacy were found in case of bleaching powder and Krosin AG in case of reducing the disease severity score.

Results of this study have shown comparably high levels of latent infection of *R. solanacearum* in both treatments and control and samples were tested, indicating the potential treatments as well as the economic implications. So, the incidence and severity of potato could differ in the different locations of Dhaka as well as the whole country. We recommend the use of cowdung as well as the Turmeric powder and $MgCl_2$ solution could be the better treatments against *R. solanacearum.*

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APPENDIX

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 121^0C 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

TriphenylTetrazolium Chloride (TTC):

2,3,5triphenyltetrazolium chloride (Soluble) 10.0 g

Distilled water 1000 ml 66

Gram staining reagents

Gram 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 67

KOH solubility reagents

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