MANAGEMENT OF BACTERIAL WILT OF BRINJAL BY USING SOME CHEMICALS AND BIO-AGENT

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CERTIFICATE

This is to certify that the thesis entitled**,** **"MANAGEMENT OF BACTERIAL WILT OF BRINJAL BY USING SOME CHEMICALS AND BIO-AGENT"** submitted to the **DEPARTMENT OF PLANT PATHOLOGY,** Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN PLANT PATHOLOGY,** embodies the result of a piece of bona fide research work carried out by **RUBIYA KHANAM** bearing **REGISTRATION NO. 18-09152,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma elsewhere in the country or abroad.

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

Dated: December, 2020
Place: SAU, Dhaka, Bangladesh

.....
Prof. Dr. Nazneen Sultana
Supervisor

Dedicated
TO
My Beloved Parents

ABBREVIATIONS AND ACRONYMS

Abbreviation	Full word
R. solanacearum	*Ralstonia solanacearum*
WP	Wetable Powder
et al.	And others
Cm^3	Centimeter cube
Cm^2	Centimeter square
Cm	Centimeter
CFU.	Colony Forming Unit
°C	Degree centigrade
SP	Solid powder
EC	Emulcifiable concentrate
@	At the rate
G	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
LSD	Least Significant Difference
DAT	Days after transplanting
%	Percent
RCBD	Randomized Completely Block Design
ANOVA	Analysis of variance
SAU	Sher-e-Agricultural University
Viz.	Namely
Var.	Variety
SDW	Sterilized Distilled Water
TTC	Triphenyl Tetrazolium Chloride
NA	Nutrient Agar

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December, 2020 **The Author**

SAU, Dhaka

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ABSTRACT

The experiment was conducted to evaluate the efficacy of different chemicals and bio-agent for the management of bacterial wilt disease of brinjal caused by *Ralstonia solanacearum* during the period of 2019-2020. Seven treatments viz. T_0= *R. solanacearum* (control), T_1= *R. Solanacearum* + Goldton 50WP @ 0.2%, T_2= *R. Solanacearum* + Tricost 1%WP @ 0.3%, T_3= *R. Solanacearum* + Nirvoy 10SL @ 0.15%, T_4= *R. Solanacearum* + Bactroban 20WP @ 0.2%, T_5= *R. Solanacearum* + Kasumin 2% liquid @ 0.05%, T_6= *R. Solanacearum* + krosin AG10SP @ 0.05% were used as soil drenching. All the transplanted seedlings were inoculated with cell suspension of *R.Solanacearum* (10^8cfu/ml). The effect of the different treatments have shown significant variation compared to control in respect of wilt incidence, yield and yield contributing characters. The disease incidence differed significantly among the treatments ranged from 13.9- 80.6%. Minimum disease incidence 13.9 % was observed in treatment T_6 (Krosin AG10SP). However, the highest disease incidence (80.6%) was observed in T_0 (Control). In case of all the plant growth parameters, treatment T_6 i.e Krosin AG10SP (Streptomycin sulphate 9% + Tetracyclin Hydrochloride 1 %) was found as the best one. The highest yield 27 kg/plot was recorded in case of treatment T_6 (krosin AG10SP) followed by T_2 (Tricost 1%WP) 16.7 kg/plot. The lowest yield was noted in T_0 (control) 5.2 kg/plot. Among the treatments T_6 (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) was found to be effective for the management of bacterial wilt of brinjal that increased yield by 410.9 % over control followed by T_2 (Tricost 1%WP) that increased yield by 216.2 %.

CHAPTER I

INTRODUCTION

Brinjal (*Solanum melongena* L), of the family solanaceae is a common and popular warm weather vegetable crop. It is a staple food in Bangladesh, India and other countries in South and Southeast Asia, where in Bangladesh, it is the third most important vegetable in terms of production and grown on about 50,000 hectares across the country (Choudhary *et al*., 2014). The cultivation area for brinjal production is 124 thousand acre and annual production is 475 thousand ton in 2015-16 (BBS, 2016). Brinjal is one of the most nutritious vegetable. According to Choudhary and Gaur (2009) brinjal consists of almost 95 percent of water and is superior in terms of fiber, folic acid etc. It has very small amount of fat and supplies 25 calories per serving. It contains good amounts of many essential B-complex groups of vitamins such as vitamin B_5, vitamin B_6, vitamin B_1 and B_3. These vitamins are essential in the sense that body requires them from external sources to replenish and required for fat, protein and carbohydrate metabolism. Further, this vegetable is also an excellent source of minerals like manganese, copper, iron and potassium. The antioxidant enzyme, superoxide dismutase utilizes manganese as a co-factor. Potassium is an important intracellular electrolyte that helps counter pressing (hypertension) effects of sodium. In our country one of the major constraints of brinjal production is diseases and insects. Among them wilt disease that caused by bacteria is one of the most devastating for brinjal production. Bacterial wilt caused by *Ralstonia solanacearum* (Yabuchi *et al*., 1995) is a lethal disease of brinjal in tropical, subtropical and warm temperate regions of the world. The bacterial wilt disease is wide spread, affecting many solanaceous vegetable crops in Bangladesh.

R. solanacearum is an important soil borne pathogen globally (Wicker *et al*., 2007 and Hayward, 1991). *Ralstonia solanacearum* is a soil-borne, aerobic,

rod-shaped, non-spore forming, non-capsulated, gram-negative bacterium. Virulent *Ralstonia* colonies produce pink or light red colour or characteristics red center and whitish margin and avirulent colonies are smaller, off-white and non-fluidal using in TTC medium (Kelman and Person, 1954). Minimum, optimum and maximum temperature of strains of *R. solanacearum* is about 10, 35 and 41°C respectively.

According to Nishat *et al.* (2015) in the field, bacterial wilt is the most destructive disease with a severity of 10 - 90% in Bangladesh. Bacterial wilt caused by *R. solanacearum* is known to infect more than 450 plant species in 54 families (Wicker *et al*., 2007) and is widely distributed in the tropical and subtropical regions in the world. Aliye *et al*.(2008) reported that *R. solanacearum* has a large host range of more than 200 species in 50 families. The causal agent of this devastating disease is *R. solanacearum* which was previously called as *Pseudomonas solanacearum*. Bacterial wilt caused by *R. solanacearum*, one of the important disease affecting brinjal and yield loss ranges from 11.67 to 96.67% in India (Bainsla *et al*., 2016). Coelho-Netto *et al*. (2004) reported losses of up to 40% due to bacterial wilt in a commercial eggplant field in the state of Amazon. Nahar *et al*. (2018) observed that, damping-off appears in the nursery within a week after emergence and continues for another week reaching a severity up to 80%. This disease is caused by various soil and seed pathogen ultimately resulting in a poor stand of less vigorous seedlings.

The most frequent external symptoms of the bacterial infected plants are wilting, stunting and yellowing of the foliage. Other symptoms are leaves bent downward showing leaf epinasty, adventitious roots growing in the stems, and the observance of narrow dark stripes corresponding to the infected vascular bundles beneath the epidermis. The most frequent internal bacterial symptoms are progressive discoloration of the vascular tissue, mainly the xylem, at early stages of infection, and of portions of the pith and cortex, as disease develops, until complete necrosis. The symptoms of bacterial wilt including leaf drooping

followed by wilting of whole plants within a few days, leading to total plant collapse. Infected plants may recover temporarily in the evening, when temperatures are cooler. A few days later, a sudden and permanent wilt occurs. The roots and lower portion of the stems have a browning of their vascular system. The invaded roots may rot due to infection from secondary bacteria (McCarter, 1991).

Control of *Ralstonia* is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and its extremely wide host range (Doan and Nguyen, 2005). Not much efforts has been directed towards the production of plant bactericides and as a result very few effective ones are available today for managing plant bacterial disease that increased crop yield and improved plant growth. Some researcher suggested chemical and some suggested biological control measure for this disease. Among those chemicals Copper Oxychloride (Kennelly *et al*., 2007; Pawar *et al*., 2004 and Lee *et al*., 2015), *Trichoderma* (Yendyo *et al*., 2017; Konappa *et al*., 2018), Validamycin (Ishikawa *et al*., 2005; Ishikawa *et al*., 1996 and Date and Nasu, 2001), Bismerthiazol (Guo *et al*., 2020 and Zhou *et al*., 2018), Kasugamycin (Verma *et al*., 2014 and Lee *et al*., 2012), Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1% (Mohsin *et al*., 2016; Verma *et al*., 2014 and Milijašević *et al*., 2009) are some control agents suggested by researchers.

There is no single mean that would successfully control the disease, provide an absolute cure to protect the host plant against the pathogens. However, only a few reports are available in respect of prevalence, isolation, identification and management of this disease in the country. However, consecutive works for accommodation of viable components in an integrated management program have not yet been initiated. Therefore, attempt should put forward to investigate management strategies of the disease.

Considering the above facts, the present research work has been designed to achieve the following objectives:

- To isolate and identify the causal agent of bacterial wilt of brinjal.
- *In vivo* and *in vitro* evaluation of some chemicals and bio-agent against the bacterial wilt pathogen.

CHAPTER II

REVIEW OF LITERATURE

Eggplant (*Solanum melongena L*.) is a popular solanaceous vegetable crop. It suffers from many diseases caused by fungi, bacteria, virus, nematode and mycoplasma. Bacterial wilt of eggplant caused by *Ralstonia solanacearum* is known to be one of the most destructive disease wherever this crop is grown extensively. Research works regarding the management of bacterial wilt of eggplant are very limited in Bangladesh. However, some available and important findings on various aspects of management of bacterial wilt have been compiled and presented below.

2.1 Economic importance of brinjal

Brinjal is an important source of income to many Asian farmers. In Bangladesh, brinjal cultivation rate are increasing day by day. The cultivation area for brinjal production is 124 thousand acre and annual production is 475 thousand ton in 2015-16 (BBS, 2016).

Brinjal is a staple food in Bangladesh, India and other countries in South and Southeast Asia where in Bangladesh, it is the third most important vegetable in terms of production and grown on about 50,000 hectares across the country (Choudhary *et al*., 2014).

Choudhary and Gaur (2009) stated that brinjal consists of almost 95 percent of water and is superior in terms of fiber, folic acid etc. It has very small amount of fat and supplies 25 calories per serving. It contains good amounts of many essential B-complex groups of vitamins such as vitamin B5, vitamin B6, vitamin B1 & B3. These vitamins are essential in the sense that body requires them from external sources to replenish and required for fat, protein and carbohydrate metabolism. Further, this vegetable is also an excellent source of

minerals like manganese, copper, iron and potassium. The antioxidant enzyme, superoxide dismutase utilizes manganese as a co-factor. Potassium is an important intracellular electrolyte that helps counter pressing (hypertension) effects of sodium.

2.2 Importance of bacterial wilt disease

Nahar *et al*.(2018) said that in the nursery, damping-off appears within a week after emergence and continues for another week reaching a severity up to 80%. This disease is caused by various soil and seed pathogen ultimately resulting in a poor stand of less vigorous seedlings.

Bainsla *et al*. (2016) stated bacterial wilt caused by *Ralstonia solanacearum*, one of the important disease affecting brinjal and yield loss ranges from 11.67 to 96.67% in India.

According to Nishat *et al*.(2015) in the field, bacterial wilt has been reported to be the most destructive disease with a severity of 90% in Bangladesh.

Verma *et al*.(2014) stated that *Ralstonia solanacearum* is one of the important dreaded soil's borne bacterial phytopathogen which causes enormous losses in the crop plants in tropical, subtropical and temperate region of the world. In India, the disease is highly prevalent and active throughout the year where the soil is acidic in the Eastern Plateau and Hill Region. Once the disease is established in the field, it cannot be controlled by chemical means.

Milling and Haque (2011) reported that bacterial wilt caused by *R. solanacearum* is deemed to be one of the most important plant diseases in tropical agriculture.

Gilles and Ono (2011) reported that the *R. solanacearum* species complex includes *R. solanacearum, R. syzygii,* and the blood disease bacterium. All colonize plant xylem vessels and cause wilt diseases, but with significant biological differences. *R. solanacearum* is a soil borne bacterium that infects

the 5 roots of a broad range of plants. *R. syzygii* causes Sumatra disease of clove trees and is actively transmitted by cercopoid.

Mandal. (2011) brinjal, tomato and potato were affected by the disease in West Bengal and percentages of damage were 9.86 to 86.45%, 10.54 to 85.63% and 10.72 to 73.82% respectively.

Gou-Gen Hua *et al.*(2008) reported that bacterial wilt, caused by *R. solanacearum* (Smith) is an economically important disease on tomato in many provinces of China.

Aliye *et al.* (2008) reported that *R. solanacearum* has a large host range of more than 200 species in 50 families.

Bacterial wilt caused by *R. solanacearum* (Smith) Yabuuchi is known to infect more than 450 plant species in 54 families (Wicker *et al*., 2007) and is widely distributed in the tropical and subtropical regions in the world.

Doan and Nguyen (2005) reported that, in Vietnam, bacterial wilt causes significant damage on many important crops under disease-favorable weather conditions. Control is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and its extremely wide host range. The use of resistant varieties has been used to reduce disease.

Elphinstone, (2005) observed direct yield losses by *R. solanacearum* vary widely according to the host, cultivar, climate, soil type, cropping pattern, and strain. For example, yield losses vary from 0 to 91% in the tomato, 33 to 90% in the potato, 10 to 30% in tobacco, 80 to 100% in the banana, and up to 20% in the groundnut.

Coelho-Netto *et al*. (2004) reported losses of up to 40% due to bacterial wilt in a commercial eggplant field in the state of Amazon.

Lambert (2002) reported *R. solanacearum* is on the list of potential bioterrorism agents in the USA and in the European Union, the pathogen has a quarantine status.

Hayward (1991) reported the bacterium is especially destructive in moist soils at temperatures above 24°C. It is sensitive to high pH (alkaline soils), low soil temperature, low soil moisture and low fertility levels. Bacterial wilt, caused by members of the *Ralstonia solanacearum* species complex, is a key emergent disease in non-tropical regions of the world *R. solanacearum* is distributed in many habitats all over the world and has an unusually broad host range (Hayward, 1991. Denny, 2006). It can infect over 200 plant species representing over 50 botanical families (Hayward, 1991).

Shekhawat *et al*.(1978) reported the disease is more severe in Karnataka, Madhya Pradesh, Eastem Plateau and Eastern Plains as compared to hills. Maximum yield losses due to disease are up to 55% in Kumaon Hills, 50% in Karnataka and 77% in Madhya Pradesh.

2.3 Symptoms of bacterial wilt disease

Agrios (2005) stated that older leaves firstly wilted than the youngest leaves affected due to bacterial wilt disease. Finally, the whole plant wilted and died.

Hayward (2005) observed that bacterial wilt of eggplant caused by *Ralstonia solanacearum* was an important disease. It was one of the most destructive pathogens identified because it induces rapid and fatal wilting symptoms in the host plants. Bacterial wilt caused by *Ralstonia solanacearum* (Smith, 1986) formerly known as *Pseudomonous solanacearum* (Yabuuchi *et al*., 1995) was highly challenging and one of the most destructive diseases of solanaceous crops worldwide.

McCarter (1991) observed symptoms of bacterial wilt of tomato including leaf drooping followed by wilting of whole plants within a few days, leading to

total plant collapse. The infected plants may recover, temporarily, in the evening, when temperatures are cooler. A few days later, a sudden and permanent wilt occurs. The roots and lower portion of the stems have a browning of their vascular system. The invaded roots may rot due to infection from secondary bacteria.

Kelman and Sequeira (1965) found that *Ralstonia solanacearum* entered into the roots through various wounds viz. transplanting, cultivation, nematode, insects and natural opening. Then it started to multiply rapidly in the vascular system. Finally, the xylem elements were filled with bacterial cell and slime. the incidence of the disease infection may range from a very few scattered plants or loci of infection in fields where low or erratic natural infestations occur to the rapid death of the plants.

Kelman (1953) reported several external and internal symptoms characterizing the bacterial wilt disease. The most frequent external symptoms of the infected plants are wilting, stunting and yellowing of the foliage. Other symptoms are leaves bent downward showing leaf epinasty, adventitious roots growing in the stems, and the observance of narrow dark stripes corresponding to the infected vascular bundles beneath the epidermis. In the cases where an infected plant does not show wilting, characteristic external symptoms may be dwarfing and stunting of the plant. The most frequent internal symptoms are progressive discoloration of the vascular tissue, mainly the xylem, at early stages of infection, and of portions of the pith and cortex, as disease develops, until complete necrosis.

2.4 The pathogen and its characteristics

Wicker *et al*.(2007) and Hayward (1991) reported that it was an important soilborne pathogen globally. It causes devastating wilt on over 450 plant species belonging to 54 families, covering both monocots and dicots.

Denny (2006) observed the five races of *Ralstonia solanacearum* have different host ranges and geographic distributions. Race 1 is poorly defined group with a very wide host range and is endemic in Southern United states as well as Africa, Asia and South America. Race 2 principally attacks banana and is found in Central America and Southeast Asia Race 3 is distributed worldwide and has primarily been associated with potato. Race 4 affects Ginger in much of Asia and Hawaii and Race 5 affects Mulberries in China.

According to EPPO (1999) majority of the strains isolated from Asia belong to phylotype 1.

Yabuuchi *et al*. (1995) found *R. solanacearum* causing bacterial wilt of potato, brinjal, tomato and other solanaceous crops including other host plants is formerly known as *Pseudomonas solanacearum* (EF Smith). *R. solanacearum* is a Gram- negative, rod shaped, aerobic bacterium that belongs to the B protcobacteria. The species *R. solanacearum* is highly polymorphic, as it is composed of strains with varying biochemical properties which can infest a large spectrum of possible plant hosts.

According to Kelman (1981), *Ralstonia solanacearum* is a soil-borne, aerobic, rod-shaped, non-spore forming, non-capsulated, gram-negative bacterium. The bacterium was positive to oxidase and negative to arginine dihydrolase test.

Cuppels *et al*.(1978) found that *Ralstonia solanacearum* produces two distinguishable types of colonies in tetrazolium medium (TZC). One is small, flat, red and butyrous while the other colony is large, elevated, mostly white with light pink centers and full of fluid using Casamino Acid Peptone Glucose (CPG).

Kelman and Person (1954) identified the virulent (colonies with pink or light red colour or characteristic red center and whitish margin) and avirulent (smaller, off-white and non-fluidal colonies) strains of *Ralstonia solanacearum* were in TTC medium containing 0.005% TTC.

Kelman (1954) found to grow the bacterium in the medium incubation should be done at 28 °C for at least 24 hours. After isolation, *Ralstonia solanacearum* isolates were purified by streaking a single colony of each isolate on Triphenyl Tetrazolium Chloride (TTC) plate.

Kelman (1953) reported *Ralstonia solanacearum* is an aerobic obligate organism.

2.5 Isolation of the pathogen

Ghosh *et al*. (2015) isolated *R. solanacearum* from samples of infected plants collected from different locations of West Bengal of India. After that they cut plant parts into small pieces, surface sterilized with appropriate surface sterilizing reagent and washed with sterile distilled water (SDW) for three times. Later on, dipped in SDW containing culture tubes to allow oozing. After 15-20 minutes, ooze in sterile water was streaked on *R. solanacearum* semi-selective medium (modified SMSA) supplemented with 0.005% 1, 3, five triphenyl tetrazolium chloride (TZC), following quadric streaking method. Then, inoculated Petri-plates were allowed to incubate at 30°C. *R. solanacearum* produced fluidal colony with a pink center and whitish periphery 48 hours after incubation. The pure culture was isolated from such colonies on SMSA medium without TZC. Pure cultures were maintained in sterile distilled water under room temperature for further investigation.

Kumar and Sarma (2004) isolated *R. solanacearum* from wilted ginger plants collected from different locations of Kerala, Assam, and West Bengal, India following standard procedure. *R. solanacearum* colonies which appeared after 36 hours of incubation at 28°C as typical white fluidal with the spiral pink center were purified. A loopful of bacterial growth was suspended in sterile distilled water and kept at 4 °C for short-term storage, while at 80°C in 20 % glycerol for long-term storage.

Dhital *et al*. (2001) isolated *R. solanacearum* from infected potato stems or tubers collected from different sources and locations in Nepal and Thailand by cutting into small pieces and placing in test tubes containing 5 ml of sterile distilled water. Bacteria were allowed to flow from the vascular bundles for 5 to 10 minutes. One loopful of the bacterial suspension was streaked onto tetrazolium chloride (TZC) agar medium and incubated at 28°C for 48 hours. A single colony of *R. solanacearum* showing virulent, fluidal, irregular and creamy white with pink at the center was selected and multiplied in a TTC medium. After 24- 48 hours of incubation, virulent cultures were maintained in sterile distilled water in screw-capped tubes at room temperature.

Schaad *et al*.(2001) observed that isolation is the best made for early infection stages, small pieces of tissue being excised from the margins preferably of the youngest lesions. These are comminuted in small quantities of sterile water and streaked on TTC medium.

Bacterial isolation was performed by Kelman (1954) in Kelman's culture medium with tetrazolium, in which *R. solanacearum* colonies can be partially identified after cultivation for 48 hours at 28^{o}C. On solid agar media individual bacterial colonies are usually visible after 36 to 48 hours growth at 28°C, and colonies of the normal or virulent type are white or cream-colored, irregularly shaped highly fluidal and opaque. Occasionally, colonies of the mutant or non-virulent type appear, these are uniformly round, smaller, and butyrous, or dry. A Tetrazolium Chloride (TZC) medium (Kelman, 1954) can differentiate the two colony types. On this medium virulent colonies appear white with pink centers and non-virulent colonies are a uniform dark red.

2.6 Physiological and biochemical characterization and identification of *Ralstonia solanacearum*

Pawasker *et al*. (2014) observed the colonies of *R. solanacearum* on nutrient agar medium were smooth circular, raised and dirty white. The optical feature

of the colony was opaque and measured around the average of 3 mm in size. Pawasker *et al.* (2014) also observed Starch hydrolysis test of the bacterium which showed that the bacterium was unable to hydrolyze starch. Pawasker *et al*., (2014) also reported the hydrogen sulphide gas production test of *Ralstonia solanacearum* the bacterium was negative in H2S production.

Sahu *et al*. (2013) conducted temperature tolerance test for evaluating the thermal death point of *Ralstonia solanacearum* and indicated that the pathogen survives well up to 30°C, its growth deteriorates in between 30°C to 40°C temperature range. Beyond this range the pathogen does not shows any growth. The results revealed that 37°C is the thermal death point for *Ralstonia solanacearum*.

Sahu *et al*. (2013) observed both the isolates of *R. solanacearum* Le Rj and R show positive results for Citrate tests, Motility test, Arginine Ornithine tests and Oxidase test and negative results revealed from Indole, Methyl red, VP (Voges Prosker), TSI (Triple sugar iron) and Manitol test.

Fajinmi and Fajinmi (2010) temperature is an important factor which affects the growth and aggressiveness of pathogen and expression of symptoms in the plant *R. solanacearum* causing wilt disease is most severe on plants when temperature ranges between 25°C and 35°C and its aggressiveness decreases, either exceeds 35°C or below 18°C temperature. The disease symptom appears rarely below 18°C temperature.

Biochemical characterization: subjecting the isolated bacterial colonies to various biochemical tests, gram's staining, poly B-hydroxyl butyrate- PHB granules test (Lelliott and Stead, 1987), KOH solubility test (Fahy *et al*., 1983), oxidase test (Hildebrand and Senroth, 1972), gelatin hydrolysis, starch hydrolysis, nitrate reduction (Fahy *et al*., 1983), arginine dihydrolase activity, catalase, H2S production, citrate utilization (Hildebrand *et al*., 1988), triple sugar iron agar test, pectin hydrolysis, tween 80 hydrolysis, urease test, indole production, levan production (Vinh *et al*., 2005).

Van *et al*.(2001) *R. solanacearum* can be stored for many years at room temperature in sterilized tap, distilled or de ionized water. It will also survive long- term at -80°C in liquid culture broth amended to 40% glycerol. This pathogen easily loses virulence if repeatedly transferred on agar plates and loses viability if plates are stored at 4°C. This bacterium is known to enter a viable but not culturable (VBNC) state under some circumstances, such as exposure to low temperatures this may complicate culture-based diagnostic methods.

Heyward (1991) reported the bacterium as aerobic and its colonies on solid media are small, irregularly round, white in reflected light and tan in transmitted light.

Kelman, (1953) reported minimum, optimum and maximum temperature of strains of *Ralstonia solanacearum* is about 10, 35 and 41°C respectively.

Kelman (1953) reported approximate minimal and maximal growth temperature values would be 8-10°C and 37-39°C respectively and regarding pH requirements, in general *R. solanacearum* growth is inhibited in acid media but favored in alkaline conditions.

2.7 Pathogenicity study of *R. solanacearum*

Shahbaz *et al.* (2015) recovered isolates of *R. solanacearum* and performed its pathogenicity test by soil drenching and detached leaf method. They found that in soil drenching method, disease symptoms became visible after four days of inoculation. In most of the inoculated plants, partial wilt symptoms were apparent after eight days (average symptom scores 1.5), complete wilting occurred after 12 days (average symptom scores 2.5), death and collapse of seedlings occurred on the 14th day (average symptom scores 3). In detached leaf method disease, symptoms were evident after one day of inoculation. Most of the leaflets showed partial yellowing after four days of inoculation (average symptom scores 1.5), Complete chlorosis occurred after ten days (Average

symptom scores 2.5), eventually, total withering and collapse of inoculated leaves were apparent on the 12th day (Average symptom scores 3) but some on the 14th day of inoculations. They concluded that in *R. solanacearum* pathogenicity test, detached leaf method was more efficient, followed by soil drenching method.

Makari *et al*. (2013) inoculated tomato and chilli plants with five ml of inoculums of *R. solanacearum* (1x109CFUml-1) isolated from potato and ginger at root zone by making slight injury to the root with a disposable syringe. It was shown that all the seven isolates from potato and ginger induced wilt symptoms in tomato and chilli plants. *R. solanacearum* isolates exhibited wilting symptoms 3 to 4 days after inoculation and all the inoculated plants wilted within 5 to 10 days.

During plant infestation, *R. solanacearum* produces so-called pathogenicity factors that are essential for disease development. Next to this, many virulence factors are produced that enhance the pathogen's ability to colonize host tissue, allowing it to reach population sizes that often exceed 10^9 CFU/g plant tissues. Important factors that determine the successful invasion of a host include the abilities to circumvent recognition by the plant (HR), to attach to plant cells and to produce cell-wall-degrading enzymes (CWDES). The best-studied pathogenicity factor is probably the T3SS, which is encoded by the hrp genes. The system constitutes a "needle" complex, or translocon, that delivers so-called “effectors” proteins into the host cell. Proteins that are secreted by the conserved type II secretion system (T2SS) also have great impact on the development of wilting. (Valls *et al*., 2006), (Hikich *et al*., 2007).

Aldon *et al*.(2000), Cunnac *et al*.(2004) reported to function in Gram- negative bacteria, the Type 3 secretion system must cross three biological membranes, ie the inner and outer bacterial membranes and the host plasma membrane. This occurs through the proteinaceous needle complex, which allows the translocation of effectors proteins into host cells upon contact with host (plant) tissue.

2.8 Host invasion and development of disease by the pathogen

Yao and Allen (2006) reported the movement of the pathogen towards plant roots is induced by chemotaxis i.e, the presence of diverse amino acids, organic acids and root exudates. Thus, mutants defective in the CheA ar CheV proteins, which are essential chemotaxis signal transduction agents, showed significantly reduced virulence as compared to the wild-type. This suggested that specific directed motility is required for full virulence rather than random motion.

Elphinstone (2005) found *R. solanacearum* multiplies and moves systematically within the plant after invading a susceptible host before occurrence of bacterial wilt symptoms.

Grimoult *et al*. (1994); *Nakaho et al*. (2000) reported *R. solanacearum* cells proliferating at the sites of infection rapidly invade the intercellular spaces of the root cortex, followed by colonization of the intercellular spaces in the inner cortex and the vascular parenchyma. After 4-5 days, bacterial cells invade the xylem vessels, which are probably facilitated by the action of cellulolytic enzymes or by release of tyloses from parenchyma or xylem cells which contribute to vascular dysfunction.

2.9 Management of Bacterial wilt by different means

2.9.1 Management through Copper oxychloride

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

Kennelly *et al*. (2007) recommended that copper compounds were the standard bactericides for controlling many bacterial diseases.

Lee *et al*. (2012) evaluated efficacy of different control methods for disease management of tomato bacterial wilt caused by *Ralstonia solanacearum*. 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. Among these chemical pesticides, treatment of the detached tomato leaves with the 5 pesticides (1 mg/ml), except for OC+PB delayed early wilting symptom development caused by the bacterial inoculation (106 and 107 cfu/ml). Four pesticides, CH, CH+O, CO+K and S+V, showed disease protection in pot analyses.

Lee *et al*.(2015) investigated Control efficacy was with fungicides as 3 copper compound, 3 antibiotic fungicides and one fungicide containing to quinolone against the growth of *Ralstonia solanacearum* on NA medium and the disease occurrence on pepper seedlings. Among 7 fungicides, oxytetracycline was shown the highest activity against a growth of the pathogen in the agar diffusion method, but validamycin showed no activity against the pathogen. With 1000 gmL^{-1} of each copper fungicide as copper hydroxide, copper oxychloride + dithianone and copper sulfate, 2.2, 1.3 and 1.5 mm in size of clear zone only could be found, respectively. Copper fungicides showed the control efficacy lower than antibiotic fungicides.

Mohsin *et al*. (2016) conducted a study at the Molecular Plant Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, to find out suitable antibacterial chemicals against *Ralstonia solanacearum*. Bacterial wilt of tomato is a century old devastating disease caused by the bacteria *Ralstonia solanacearum*. Five chemicals viz: Bleaching powder, Streptomycin, Neomycin, Bactrol and Copper oxychloride were tested both *in-vitro* and *in-vivo* against the causal organism. It was observed that Copper oxychloride did not show any effect against *Ralstonia solanacearum*. Also, Copper oxychloride did not show any inhibition. In field

condition, highest incidence and lowest yield were recorded in control and Copper-oxychloride treatments.

2.9.2 *Trichoderma* spp. against *Ralstonia solanacearum*

In the current system, soil amendment with the biocontrol agent *Trichoderma harzianum* might be a good strategy. *Trichoderma* spp. have been well documented to control a wide range of soil- and seed-borne pathogens (Harman, 2000, 2006; Dubey *et al.*, 2011; Kulkarni, 2015) including P. vexans (Srinivas *et al.*, 2005; Ghosh, 2017) and *R. solanacearum* (Yendyo *et al.*, 2017; Konappa *et al*., 2018).

Adnan (2013) observed that in fields with improved management, *T. harzianum* persisted at the end of the growing season and reduced populations of *R. solanacearum* and *P. vexans*. Also, seed preserved from improved management plots showed a reduction of both pathogens compared to seed from farmers' practice plots. The reduction of pathogens both in field soil and preserved seed for improved management plots may imply that a next crop will start with a 25 lower level of disease pressure. The effect of tomato seed treatments with *Pseudomonas fluorescens* in the control of bacterial wilt under greenhouse conditions revealed that the treatments protected plants against soil-borne infections of the bacterial wilt organism.

2.9.3 Management through Validamycin

Lee *et al*. (2015) evaluated the efficacy of fungicides as 3 copper compound, 3 antibiotic fungicides and one fungicide containing to quinolone against the growth of *Ralstonia solanacearum* on disease occurrence on pepper seedlings. Among 7 fungicides, validamycin showed no activity against the pathogen. Copper fungicides showed the control efficacy lower than antibiotic fungicides except for validamycin.

Ishikawa *et al*. (2005) stated that tomato wilt, is effectively controlled by a foliar spray of validamycin A (VMA) or validoxylamine A (VAA) (≥10 µg/ml); however, neither VMA nor VAA was effective in vitro. In pot tests, the effect of a foliar application of VMA or VAA at 100 µg/ml lasted for 64 days. Plants sprayed with VMA or VAA accumulated salicylic acid and had elevated expression of the systemic acquired resistance (SAR) marker genes P4 (PR-1), Tag (PR-2), and NP24 (PR-5). Foliar spray of VMA also controlled late blight and powdery mildew of tomato. The disease control by VMA and VAA lasted up to 64 days after treatment, was broad spectrum, and induced the expression of PR genes, all essential indicators of SAR, suggesting that VMA and VAA are plant activators.

Date and Nasu (2001) investigated effects of validamycin A on the development of tomato and eggplant bacterial Wilt. Validamycin A application, at 2000, 1000 and 300ppm on aerial part of tomato showed efficacy to tomato bacterial wilt, respectively, and effective application time of the chemical was 7 - 15 days before the pathogen infect. The chemical at 50 - 500ppm sprayed on aerial part of tomato after planting was delayed development of the bacterial wilt of tomato in roof-vent plastic house. The chemical at 500ppm sprayed every 7days a few times after planting on aerial part of eggplant with forcing culture was delayed development of the bacterial wilt of eggplant. The chemical at 125-500ppm showed suppressed severely growth tomato (cv. Ponderoza etc.), but on eggplant.

Ishikawa *et al*. (1996) Stated that validamycin A (VM-A), the active ingredient of Validacin (R), inhibits fungal growth by inhibiting trehalase activity. Therefore, VM-A was tested against *Pseudomonas solanacearum* for its efficacy in controlling tomato bacterial wilt. In media containing trehalose as the sole carbohydrate, VM-A at 50 µg/ml inhibited growth of *P. solanacearum* to rates similar to that of the bacteria in media without carbohydrate for seven days after inoculation. VM-A also gave excellent control of tomato bacterial wilt in greenhouse pot tests, when directly injected into plant stems. In field

tests, foliar sprays of VM-A at 250 µg/ml five days before and two days after inoculation had reduced disease by 47.4% by four weeks after inoculation. In the tomato stem between 0 and 5cm above the soil line, the bacteria population in the non-treated plot reached 3.84x 10^10 cfu/g fresh weight, whereas that in the VM-A (500 µg/ml)-treated plot reached 2.13x10^9 cfu/g fresh weight. Inhibition of bacteria growth by VM-A may delay the appearance of disease symptoms of tomato bacterial wilt.

2.9.4 Management through Bismerthiazol

Guo *et al*. (2020) designed and synthesized a series of trifluoro methyl pyridine amide derivatives containing sulfur moieties (thioether, sulfone and sulfoxide). Their antibacterial activities against *Ralstonia solanacearum* (*R. solanacearum*) evaluated. Notably, the half-maximal effective concentration (EC50) value of sulfone-containing compound F10 is 83 mg L-1 against Xoo, which is better than that of commercial thiodiazole copper (97 mg L-1) and bismerthiazol (112 mg L-1). Thioether-containing compounds E1, E3, E5, E6, E10, E11 and E13 showed much higher activities against *R. solanacearum* with the EC50 value from 40 to 78 mg L-1, which are much lower than that of thiodiazole copper (87 mg L1) and bismerthiazol (124 mg L1).

Zhou *et al.*(2018) stated that bismerthiazol as a very commonly used bactericide induced the biosynthesis of constitutive and/or elicited jasmonic acid (JA), jasmonoyl-isoleucine conjugate (JA-Ile), ethylene and H_2O_2 but not salicylic acid.

Yu *et al*. (2016) stated that the bactericide bismerthiazol has been used to control rice bacterial blight (X. oryzae pv. oryzae). He demonstrate that bismerthiazol can effectively control citrus canker by both inhibiting the growth of X. citri ssp. citri and triggering the plant's host defense response through the expression of several pathogenesis-related genes (PR_1, PR_2, CHI, and $RpRd_1$) and the nonexpresser of PR genes (NPR_1, NPR_2, and NPR_3) in

'Duncan' grapefruit, especially at early treatment times. In addition, he also found that bismerthiazol induced the expression of the marker genes CitCHS and CitCHI in the flavonoid pathway and the PAL_1 (phenylalanine ammonia lyase 1) gene in the salicylic acid (SA) biosynthesis pathway at different time points. Moreover, bismerthiazol also induced the expression of the priming defense-associated gene AZI1.

Li *et al.*(2015) conducted a study where, a series of 2-mercapto-5-substituted-1,3,4-oxadiazole/thiadiazole derivatives were synthesized and evaluated for their antibacterial activities against tomato bacterial wilt caused by *Ralstonia solanacearum* (*R. solanacearum*) via the turbidimeter test *in vitro.* Antibacterial bioassays indicated that most compounds demonstrated appreciable antibacterial bioactivities against *R. solanacearum*. Among the title compounds, compound 4i demonstrated the best inhibitory effect against *R. solanacearum* with half-maximal effective concentration (EC50) values of 14.69 and 15.14μg/mL, respectively, which were even better than those of commercial agents bismerthiazol and thiodiazole Copper. *In vivo* antibacterial activities tests under greenhouse conditions revealed that the control efficiency of compound 4i against rice bacterial leaf blight and tobacco bacterial wilt were better than those of bismerthiazol and thiodiazole Copper.

2.9.5 Management using Kasugamycin

Verma *et al*. (2014) stated that *Ralstonia solanacearum* is one of the important dreaded soil's borne bacterial phytopathogen which causes enormous losses in the crop plants in tropical, subtropical and temperate region of the world. Against these three strains of *R. solanacearum*, four antibiotics were screened through food poison techniques viz. Kasugamycin, Streptomycin, Ceftriaxone and Gentamicin. The different strains of *R. solanacearum* and antibiotic sensitivity showed varied response. He observed that Kasugamycin was less antibacterial as compared to other tested antibiotics and inhibited >50% cfu at 4 ppm.

Lee *et al*.(2012) evaluated efficacy of different control methods was for disease management of tomato bacterial wilt caused by *Ralstonia solanacearum*. 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. MIC of copper oxychloride-kasugamycin (CO+K) mixture was 100 µg/ml could be used for prevention of bacterial wilt disease of tomato plants without any phytotoxicity. Thus, they suggested that copper compounds, antibiotics and essential oils have potency as a controlling agent of tomato bacterial wilt.

2.9.6 Management using Streptomycin sulphate and Tetracycline hydrochloride

Mohsin *et al*. (2016) conducted a study at the Molecular Plant Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, to find out suitable antibacterial chemicals against *Ralstonia solanacearum*. Bacterial wilt of tomato is a century old devastating disease caused by the bacteria *Ralstonia solanacearum*. Five chemicals viz: Bleaching powder, Streptomycin, Neomycin, Bactrol and Copper oxychloride were tested both *in-vitro* and *in-vivo* against the causal organism. Among the chemicals bleaching powder showed best performance against the causal organism while Streptomycin, neomycin and Bactrol showed moderate performance. Streptomycin, Neomycin and Bactrol showed moderate inhibition zone. Streptomycin, Neomycin and Bactrol resulted in the moderate incidence and yield. Besides Bleaching powder provided highest suppression of plant infection compared with other treatments.

Lal *et al*.(2016) stated that solanaceae crops were affected by variant pathogens, such as. fungi, bacteria, viruses, and nematodes. These pathogens caused significant yield losses of agricultural crop if proper protection measures had not been applied. Among pathogens *Rhizoctonia solani* and *Fusarium* spp. were the major pathogens in the fungal group, whereas *Ralstonia solanacearum* and Streptomyces spp. were in the bacterial group. Various methods, like chemical control, biological control, resistant varieties, cultural control, and physical control, were applied to reduce these pathogens attack. Above all, resistant varieties were the best and cheapest method for managing bacterial diseases. Chemical control came as the second choice for managing the diseases. Due to 20 continuous and irrational use of the chemicals pathogens have developed resistance against a particular class of fungicides/bactericides.

Sarkar and Chaudhuri (2016) focused on one of the most devastating diseases called bacterial wilt. The study showed the bacterial wilt was caused by *Ralstonia solanacearum* for the management through bactericides and biocontrol agents.

Verma *et al*. (2014) stated that *Ralstonia solanacearum* is one of the important dreaded soil's borne bacterial phytopathogen which causes enormous losses in the crop plants in tropical, subtropical and temperate region of the world. Against these three strains of *R. solanacearum*, four antibiotics were screened through food poison techniques viz. Kasugamycin, Streptomycin, Ceftriaxone and Gentamicin. The different strains of *R. solanacearum* and antibiotic sensitivity showed varied response. He observed that Streptomycin showed antibacterial efficacy and inhibited >50% cfu at 3 ppm.

Ramesh and Phadke (2012) said that bacterial wilt is difficult to manage due to the genetic diversity and aggressiveness of the pathogen, its ability to survive in the varied and adverse environmental conditions, its modes of dissemination and large number of weed hosts.

Lee *et al*.(2012) evaluated efficacy of different control methods for disease management of tomato bacterial wilt caused by *Ralstonia solanacearum*. All six chemical pesticides applied to the bacterial suspension showed *in vitro* bactericidal activities against *R. solanacearum*. Among the treatments MICs of both streptomycin-validamycin (S+V) and oxine copper-polyoxine B mixture (OC+PB) were 10 µg/ml. Among these chemical pesticides, treatment of the Four pesticides, streptomycin-validamycin showed disease protection in pot analyses.

Milijašević *et al*. (2009) studied with 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. Streptomycin had the best effect in reducing pathogen population size in all sampling regions. Moreover, this antibiotic completely stopped the spread of *C. michiganensis* subsp. *michiganensis* in the region most distant from the inoculum focus.

Chemical bactericides such as copper compounds and antibiotics have limited impact (Hartman and Elphinstone, 1994).

Murakoshi and Takahashi (1984) developed various control strategies to suppress bacterial wilt of potato. Chemical control through fumigation and antibiotics (streptomycin, neomycin, ampicillin, tetracycline, penicillin) had shown little suppression of *Ralstonia solanacearum*.

CHAPTER III

METHODS AND MATERIALS

3.1 Experiment site and duration

i. *In vitro* experiment was done in Plant Pathology laboratory, Sher-e-Bangla Agricultural University for isolation of causal organism during August- September, 2019.

ii. Pathogenicity test of the isolated pathogens were conducted in the net house of Department of Plant Pathology, Sher-e-Bangla Agricultural University during November 2019- January 2020.

iii. The field experiment was conducted in the research field of Sher-e-Bangla Agricultural University to find out the effect of different treatments for integrated approach for the management of wilt complex of brinjal during September, 2019 to April, 2020.

3.2 Collection, Isolation and identification of causal agents

3.2.1 Preparation of media

The following media was used for the isolation of the targeted pathogens (*Ralstonia solanacearum*).

3.2.1.1 Casamino Acid-Peptone-Glucose (CPG) medium (Denny, 2001)

Table 1. Composition of CPG media

Ingredients	Amount (Per liter)
Casamino acid (casein hydrolysate)	1 g
Peptone	10 g
Glucose	5 g
For solid media (plates) add: Agar	17 g

All the ingredients were added to 1L sterile distilled water to make a mixture. The mixture was sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. It was mixed well before dispensing. pH was adjusted to 6.5-7.0.

3.2.1.2 Triphenyl Tetrazolium Chloride (TTC or TZC) medium

Composition of TTC media: Mixture was similar to CPG medium.

After autoclaving the medium was cooled to 55˚C and added 5 ml of a 1% stock solution of 2, 3, 5-triphenyl tetrazolium chloride. The stock was filter sterilized or autoclaved for 5 minutes at 121˚C, and stored at 4˚C or frozen. Final pH was adjusted to 6.5-7.0. This medium was developed to differentiate between the two colony types: virulent colonies appear white with pink centers and non-virulent colonies appear dark red (Kelman, 1954).

3.2.1.3 Nutrient Agar (NA) Media:

Table 2. Composition of NA media

Ingredients	**Amount (gm Per liter)**
Peptone	10. 00
Beef extract	10.00
Sodium chloride	5.00
Agar	17 .00

Suspending above 25 grams in 1000 ml purified/distilled water. The mixture was heated if necessary to dissolve the medium completely. The mixture was sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. The pH was maintained after sterilization as 7.3±0.1.

3.2.2 Isolation and identification of the bacteria causing bacterial wilt of brinjal

3.2.2.1. Isolation of *Ralstonia solanacearum* from wilt infected brinjal plants

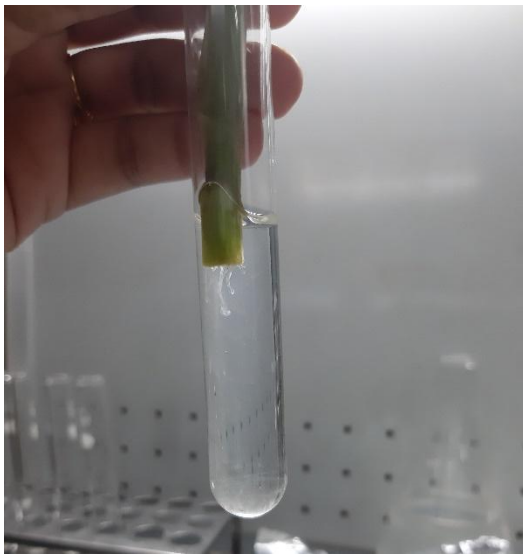
Naturally wilt infected plants were selected based on visible symptoms of bacterial wilt as described by Shekhwat *et al.* (1992) and Ali and Dey (1998). To confirm bacterial wilt infection oozing test was performed (Shekhawat *et al.* 1992). For isolation of *R. solanacearum* from wilt infected plant specimens, streak plate technique was followed using a selective medium, Tetrazolium chloride agar (TZC) as described by Kelman (1954).

Diseased stem of brinjal were washed under tap water and cut into small pieces (2-3cm) from the base. The pieces of infected stem were surface sterilized with 5% chlorox for 1 minute and 70% ethanol for 1 minute and rinsed in sterilized distilled water. The surface sterilized pieces were immersed in 5 ml of sterilized distilled water in a test tube for oozing. The bacterial ooze released from the infected stem was thoroughly mixed in water after discarding the stem pieces. One loopful of suspension was streaked on the TZC agar medium in Petri plates and incubated at 30^0 for 48 hr. Virulent colonies of *R. solanacearum* were selected on the basis of characteristic colony characters on TZC medium (Kelman, 1954).

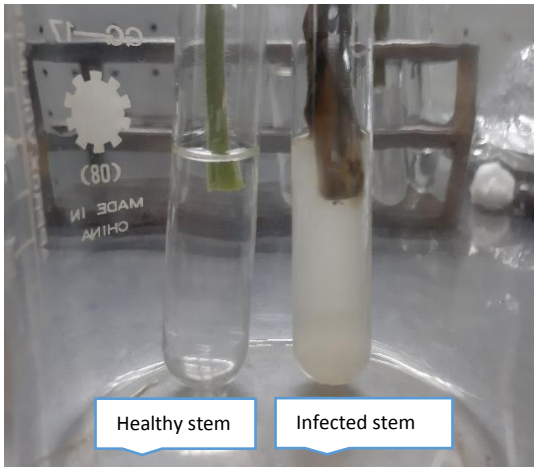
For further study, virulent colonies were identified based on color (Kelman, 1954) and well-isolated fluidal colonies were restreaked on CPG (without the stock solution of TTC) plates and NA plates because some strains are sensitive to the formazan pigment produced from TTC (3.2.2.2). Preserved *R. solanacearum* plates for future use. Two loopful of bacteria from a composite of about six individual 48 to 72 hrs old colonies were transferred to screw capped test tubes containing 5 to 8 ml of sterilized distilled water for storage (Kelman and Person, 1961). The tubes with the cultures were preserved at

room temperature (25-30 C). The isolates preserved in sterile water were recultured on TTC medium at every 3 months.

a.



b.



c.

Plate 1. Images of isolation of causal agent of bacterial wilt of brinjal.

a. wilted brinjal plant; **b.** collection of bacterial ooze in a test tube; **c.** Bacterial streaming discharge of healthy and infected stem.

3.2.3 Biochemical tests for identification of *Ralstonia solanacearum*

For characterization of the isolates of *R. solanacearum* a series of biochemical tests were conducted. The tests were: Gram reaction (Suslow *et al., 1982*), Starch hydrolysis test (Singh, 2015), Oxidase test (Kovacs, 1956), Potassium hydroxide solubility test.

3.2.3.1 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 counterstained with safranin for approximately 10 seconds then washed with water. The slide was dried and observed under a microscope at 100X.

3.2.3.2 Potassium hydroxide solubility test

On glass slide a loopful of bacteria from a well grown young colony was mixed with a drop of 3% aqueous KOH solution. 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 then raised a few centimeters from the glass side. Strands of viscid materials confirmed the bacterium was Gram negative.

3.2.3.3 Oxidase reaction

Aqueous solution of (1%) of tetramethyl -p- phenylene diamine 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 tetramethyl -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.

3.2.3.4 Starch hydrolysis test

The ability of bacterium to hydrolyse starch was studied by growing on nutrient agar containing 1% soluble starch. The sterilized liquefied nutrient agar was poured to sterilize Petri plates and allowed to solidify. The culture was inoculated in the center of the plates and incubated for seven days at room temperature (28 ± 1 0C). The plates were then flooded with Lugol's iodine (Iodine 1g, potassium iodide 2 g and distilled water 300 ml.). Clear zone around bacterial culture indicates positive test.

3.3 *In vivo* assay of treatments against wilt pathogens

3.3.1 Treatments

The following treatments

- T_0= *Ralstonia Solanacearum* (Control)
- T_1= *R. Solanacearum* + Goldton 50WP @ 0.2%
- T_2= *R. Solanacearum* + Tricost 1%WP @ 0.3%
- T_3= *R. Solanacearum* + Nirvoy 10SL @ 0.15%
- T_4= *R. Solanacearum* + Bactroban 20WP @ 0.2%
- T_5= *R. Solanacearum* + Kasumin 2% liquid @ 0.05%
- T_6= *R. Solanacearum* + krosin AG10SP @ 0. 05%

3.3.3 Preparation of treatments

Treatment solution were prepared followed by the application rate of registered agricultural pesticides lists. For T_0 only sterile distilled water was used, for T_1, 2g of Goldton 50WP (Copper oxychloride) was dissolved in 1L of sterile distilled water; for T_2, 3g of Tricost 1%WP (*trichoderma*) was dissolved in 1L of sterile distilled water; for T_3, 1.50ml of Nirvoy 10SL (validamycin) was dissolved in 1L of sterile distilled water; for T_4, 2g of Bactroban 20WP (bismerthiazol) was dissolved in 1L of sterile distilled water; for T_5, 0.5ml of Kasumin 2%liquid (Kasugamycin) was dissolved in 1L of sterile distilled

water; for $T_{6,}$ 0.5ml of Kasumin 2%liquid (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) was dissolved in 1L of sterile distilled water. The solution was mixed well to ensure complete solubilization.

3.3.4 Preparation of pure culture *Ralstonia Solanacearum*

The isolated *R. solanacearum* were purified by repeated sub-culturing at regular intervals on the TTC media. The media were then incubated at 25±2°C. Therefore, the cultures were purified by the single spore isolation technique.

3.4. Pathogenicity test of bacteria isolate under pot culture

3.4.1. Soil sterilization and pot preparation

For soil sterilization 0.4% formalin solution was thoroughly mixed up with soil @ 200ml/cft soil and kept under polythene sheet for 48 hours. Later the soil was exposed to sun for 7 days. After that the soil was filled in surface sterilized pots of 25 cm in diameter.

3.4.2. Seedling preparation

BT Brinjal 2 seedlings were raised in plastic bag. Sterilized soil having fertilizers as per the package of practices was used for Seedbed preparation. The seedlings were watered and monitored regularly.

3.4.3. Pathogenicity test of bacterial wilt of brinjal

Thirty-five days old seedlings of brinjal were treated with spore suspension of *Ralstonia solanacearum* by sterile hypodermal syringe into the vascular system of the seedlings where inoculum density was 10^8 cfu/ml solution. The plants were watered regularly and observed for appearance of wilt symptoms. The plant expressing wilt symptoms after 10 days of inoculation. Symptoms were selected, the bacterium was re-isolated and compared with the original culture of *Ralstonia solanacearum* to satisfy the Koch's postulates.

3.5. Efficacy of different treatments in controlling wilt of brinjal under field conditions

3.5.1 Location of experiment

The experiment was conducted during September, 2019 to April, 2020 in the research field of Sher-e-Bangla Agricultural University situated at 23077′ N latitude and 90033′ E longitude at an altitude of 8.6 meter above the sea level (Anon., 2004).

3.5.2 Agro-Ecological Region

The experimental site belongs to the Agro-ecological zone of "The Modhupur Tract", AEZ-28 (Anon., 1988a). This was a region of complex relief and soils developed over the Modhupur clay, where floodplain sediments buried the dissected edges of the Modhupur Tract leaving small hillocks of red soils as 'islands' surrounded by floodplain (Anon., 1988).

3.5.3 Climate

Experimental site was located in the subtropical monsoon climatic zone, set a parted by winter during the months from November to February (Rabi season). Plenty of sunshine and moderately low temperature prevails during experimental period, which is suitable for brinjal growing in Bangladesh. The annual average maximum and minimum temperature was 30.33^0C and 21.25^0C. The average annual rainfall was 2036 mm. The relative humidity varied from 73.50% to 81.22%. The day length varied from 1 to 2 hours where there was scanty rainfall during the experimentation.

3.5.4. Weather

The average of monthly maximum, minimum and average temperature, relative humidity, total rainfall and sunshine hours received at the experimental site during the period of the study have been collected from the mini weather station of SAU which was shown in (Appendix 1).

3.5.5 Planting material

BT Brinjal 2 was used as the planting materials.

3.5.5.1 Seed collection

The seeds of BT Brinjal 2 was collected from Bangladesh Agricultural Development Corporation (BADC), Gabtoli, Dhaka.

a. Seeds of Brinjal

b. Eggplant seeds were shown in line

c. Eggplant seedlings were ready to transplant

Plate 2: Raising of brinjal seedlings in seedbed

3.5.5.2 Collection of chemicals and bio-agent used as treatments

Goldton 50WP (Copper oxychloride) was collected from farm, Tricost 1%WP (*trichoderma*), Nirvoy 10SL (validamycin), Bactroban 20WP (Bismerthiazol), Kasumin 2%liquid (Kasugamycin), Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) were purchased from local market, savar, Dhaka.

Table 3. Details of chemicals

Trade name(Treatments)	Active ingredient	Common name	Dosage rate/ha(ml/gm)
Goldton 50WP	Copper Oxychloride 50% WP	Copper Oxychloride (50%)	2g/l of water
Tricost 1%WP	2×10^6CFU/ml *Trichoderma spp*	*Trichoderma*	3g/l of water
Nirvoy 10SL	Validamycin 3% SL	Validamycin	1.50 ml/l of water
Bactroban 20WP	Bismerthiazol (Thiadiazole group)	Bismerthiazol	2g/l of water
Kasumin 2% liquid	Kasugamycin 3% SL	Kasugamycin	0.5 ml/l of water
Krosin AG 10SP	Streptomycin sulphate (9%) + Tetracycline hydrochloride (1%) (9:1SP)	Streptomycin sulphate (9%) + Tetracycline hydrochloride (1%)	o.5 ml/l of water

3.5.5.3 Land preparation

Power tiller was used for the preparation of the experimental field. Then it was exposed to the sunshine for 7 days prior to the next ploughing. Thereafter, the land was ploughed and cross-ploughed to obtain good tilth. Deep ploughing was done to produce a good tilth, which was necessary to get better yield of this crop. Laddering was done in order to break the soil clods into small pieces followed by each ploughing. All the weeds and stubbles were removed from

the experimental field. The plots were spaded one day before planting and the whole quantity of fertilizers were incorporated thoroughly before planting according to fertilizer recommendation guide (Islam, 2006).

3.5.5.4 Fertilizer and manure application

After opening the land, well decomposed cowdung was applied and thoroughly mixed up with soil. Before final land preparation, inorganic fertilizers were applied.

Table 4. Doses of fertilizers

Manures and fertilizers were applied as per standard recommendation of Hand book of Agricultural Technology, BARI. (BARI, 2017)

Doses of fertilizers and manures	**Rate (Kg / ha)**
Cowdung	10,000
Urea	375 (300+25+25+25)
TSP	150
MoP	250 (125+50+75)
Gypsum	100

The total amount of cow dung, TSP, gypsum and a half of urea, MP were applied during final land preparation. Urea and MP were applied in two installments as top dressing after 15 days of transplanting and just after fruiting. Last split application of urea was given at the middle of harvesting.

3.5.5.5 Design and layout of the experiment

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The main land was divided into three blocks each containing seven unit plots of 2.5m x 2.5m. Total twenty one plot was made with maintaining 0.5 m space between plots and 0.75 m space between blocks. Plant to plant distance were maintained as 60cm x 40cm. Each bed was designed with nine pit for transplantation. The total land area was 21.5m x 10.0m =215 m^2. Every treatment combination put once at each block (Figure 1)

1. T_0= *Ralstonia Solanacearum* (Control)
2. T_1= *R. Solanacearum* + Goldton 50WP @ 0.2%
3. T_2= *R. Solanacearum* + Tricost 1%WP @ 0.3%
4. T_3= *R. Solanacearum* + Nirvoy 10SL @ 0.15%
5. T_4= *R. Solanacearum* + Bactroban 20WP @ 0.2%
6. T_5= *R. Solanacearum* + Kasumin 2%liquid @ 0.05%
7. T_6= *R. Solanacearum* + krosin AG10SP @ 0.05%

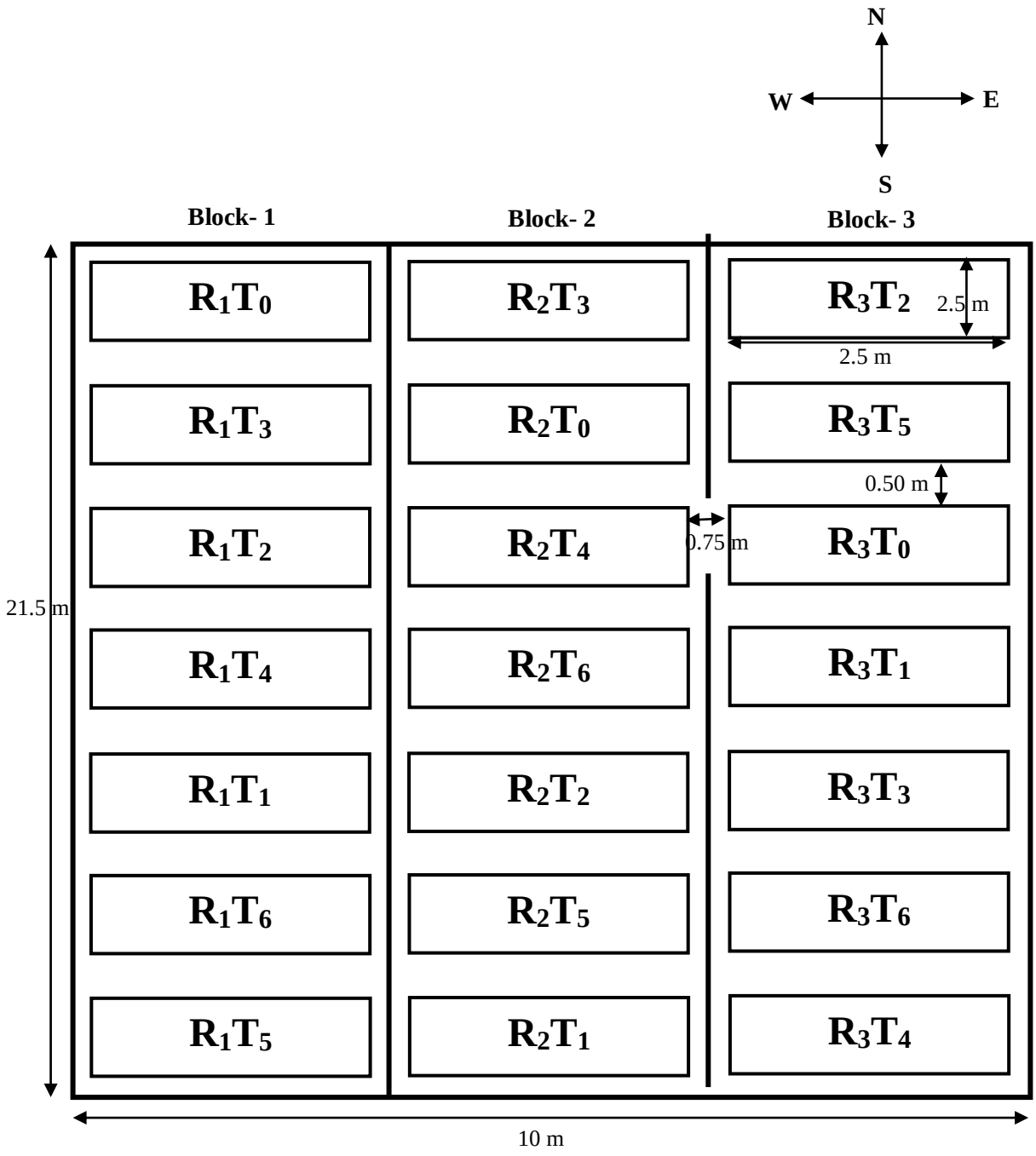


Figure 1: Showing the layout of the experiment

3.5.5.6 Transplanting of seedlings

Healthy seedlings were grown in seed bed with intensive care. 25 days old healthy and uniform sized seedlings were transplanted in the field during afternoon followed by watering. Nine healthy seedlings were transplanted in each plot of each block maintaining plant to plant distance 75 cm and line to line 75 cm (Islam, 2006).

The seedbeds were watered before uprooting the seedlings. At the time of uprooting care was taken so that root damage was minimum and some soil remained with the roots. Transplantation was done in 1 December, 2019.

a.

b.

Plate 3: Field Experiment **a.** Seedlings transplantation in field; **b.** Field view of experimental plot

3.5.5.7 *Ralstonia* inocula Preparation and Inoculation procedure

Stock cultures were streaked on TTC medium and incubated at 30^0c for 48 hrs. A single virulent colony was recultured in CPG medium for multiplication at 30^0c for 48 hrs. After incubation, bacterial cells were harvested with loop in 250ml sterile distilled water and inoculum suspension was adjusted to 10^8cfu/ml. All the plants were inoculated with bacterial cell suspension by using sterile hypodermal syringe (Winstead *et al*.,1952). It was done at the evening just 10 days after transplant.

Plate 4: Spore Suspension (10^8 cfu/ml) of *Ralstonia*

3.5.5.8 Application of treatments

Treatment suspensions were applied using a knapsack sprayer at the evening period just 13 days after transplanting. All the plants are treated by soil drenching method. Treatments were sprayed on the root zone and to the whole plant. Sprayer were labeled and shaken thoroughly before use.

3.5.5.9 Intercultural operations

Fifteen days after transplanting one third Urea and Muriate of Potash were applied following ring method followed by weeding and irrigation. Remaining was applied after 35 and 55 days after transplanting. General field sanitation was maintained throughout the growing period by removing infected and blighted leaves, wilted and dead plants.

Weeding

The plants were kept under careful observation. Weeding was done as and when necessary to keep the plots clean. Weeding was done at every 15 days interval from planting to the peak flowering stage. As the land was covered by plant canopy by that time weeding was discontinued. Spading was done from time to time specially to break the soil crust and keep the land weed free after each irrigation.

Irrigation

Irrigation was given a when necessary by observing the soil moisture condition. Irrigation was given throughout the growing period. The first irrigation was done 40 days after planting followed by irrigation at 20 days after the first irrigation. Each fertilizing was followed by irrigation.

Earthing Up

Earthing up was done when required by drawn up the soil from the space between the rows.

Insect Pest control

As prevention measure against the insect pest like cutworm, shoot and fruit borer, leafhooper etc. Actara 2gm per litter was applied to reduce the attack in the field. Many Cleaning practices were also done to reduce the insect attack. Ripcord @ o.1ml/L was also applied to control the insect pest.

a.

b.

c.

d.

Plate 5: Images showing diseases and pests of brinjal plant.

a. little leaf disease; **b.** root knot disease; **c.** mealy bug; **d.** whitefly attack .

3.5.5.10 Harvesting

First harvesting was started on the 20 January, 2020 and continued to 28 February, 2020. At each harvest, the number of fruits, individual weight of fruit (g), total weight of the fruits (kg) and individual fruit diameter (cm) was taken plot wise.

3.5.5.11 Collection of Data

A) Disease incidence (DI)

- Number of infected plants by *Ralstonia solanacearum*.

B) Yield and yield contributing characters

- Plant height (cm)
- Number of branches plant^{-1}
- Number of leaves plant^{-1}
- Number of fruits plant^{-1}
- Fruit length
- Yield plant^{-1}
- Yield plot^{-1}
- Yield ha^{-1}

3.5.6 Calculation of Disease Incidence (DI)

The observations like percent wilt incidence bacteria was recorded and the susceptible, tolerant and resistant levels of varieties were assessed.

$$\% \text{ Disease incidence} = \frac{\text{Number of infected plant(s)}}{\text{Number of total plants}} \text{ X } 100$$

3.5.7 Termination of the experiment

The experiment was terminated by uprooting of plants from the field when fruit setting had no economic value (140 days).

3.5.8 Cost-Benefit Analysis and Calculation of Benefit Cost Ratio (BCR)

Costing of application of management of wilt of brinjal was done based on the current market price of input, rate of hiring labor and agricultural machineries. Price of the field product was determined on the basis of current market value (Appendices 4 & 5). Estimation of Benefit Cost Ratio (BCR) was done according to Islam (2005) using the following formula:

$$\text{BCR} = \frac{\text{Gross return (Tk./ha)}}{\text{Total cost of production (Tk./ha)}}$$

3.5.9 Statistical analysis

The data were statistically analyzed by using computer-based software Statistix 10 software. The data were analyzed by using analysis of variance to find out the variation of results from experimental treatments. Treatment means were compared by LSD value.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Identification of causal organism

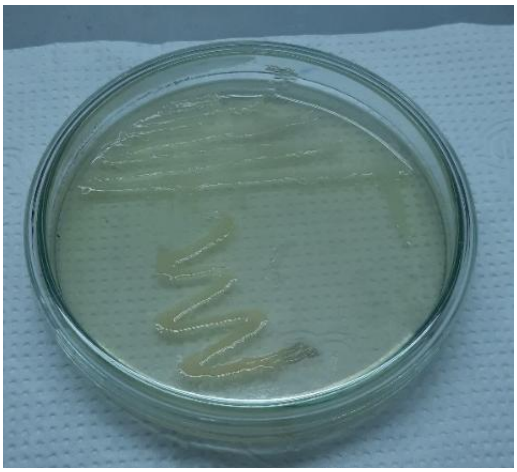
4.1.1 Morphological characters

The bacterium was rod shaped with rounded ends, gram negative (red color) and capsulated under the compound microscope at 125X magnification with oil immersion objectives.

The findings agreed with the findings of Yabuuchi *et al*. (1995), Heyward (1991) and Kelman (1981) who reported that *Ralstonia solanacearum* is a soil-borne aerobic, rod shaped, nonspore forming, and gram-negative bacterium.

4.1.2 Colony morphology on different growth media

Colonies of *Ralstonia solanacearum* of some samples on TTC medium appeared as red colored with whitish margins (virulent) (Plate 6.c). Circular, mucoid, convex, lucid coloured colonies were found on NA medium in respect to some samples (virulent) (Plate 6.b). These findings were matched with the report of Cuppels *et al*. (1978) and Khan (1974). They stated that *R. solanacearum* produced colonies on TZC medium which were highly fluidal, white color with slight pink center, round to irregular in shape. Further, Shoba (2002) and Prasanna Kumar (2004) observed similar characters of isolates on TZC medium. White or cream coloured, irregularly round, fluidal and opaque colonies were found on CPG medium (Plate 6.a) (Sharma and Singh, 2019).



a. Pure culture of *R. solanacearum* on CPG medium

b. Pure culture of *R. solanacearum* on NA medium

c. Pure culture of *R. solanacearum* on TTC medium

Plate 6: Growth of *R. Solanacearum* on different media

4.1.3 Biochemical analysis

The bacteria was found gram negative in gram staining test, positive for Potassium hydroxide solubility, oxidase and starch hydrolysis test. In oxidase test, after rubbing the bacterium onto the moistened oxidase disk, it formed violet color which revealed that the test was positive. In starch hydrolysis test, after adding lugol's iodine a clear zone was formed around the bacterial colony indicated starch hydrolysis (amylase activity) i.e., the test was positive. Results obtained on various biochemical tests for the pathogen are presented in Table 5

Table 5. Biochemical test results of *Ralstonia solanacearum*

Biochemical test	Result
Gram staining test	Negative (-)
Potassium hydroxide solubility test	Positive (+)
Oxidase reaction test	Positive (+)
Starch hydrolysis test	Positive (+)

Kelman (1981) stated that the bacterium was positive to oxidase test. Sahu *et al*. (2013) stated that *R. solanacearum* show positive results for Oxidase test, Potassium hydroxide solubility, and starch hydrolysis test and negative results gram staining test.

4.2 Pathogenicity test for *Ralstonia solanacearum*

The isolates of *R. solanacearum* was inoculated to 35 days old brinjal seedlings by stem puncture method (Winstead *et al*.,1952). The inoculated plants lost turgidity, leaves started drooping and plants wilted suddenly (Plate 7.b). The first symptom of disease was observed within 7 days after inoculation. Re-isolation of the bacterium was made from the artificially inoculated infected plants on the TZC medium, the colonies were found to be similar to that of *R. solanacearum* inoculated previously. Thus on the basis of various morphological and cultural characteristic and the result of pathogenicity test the bacterium was identified as *Ralstonia solanacearum*. Kelman (1954),

Buddenhagen *et al.* (1962), Hayward (1964), Schaad (1992) and Hayward (1991) observed the similar happenings. This was in conformity with the results obtained by Subhalaxmi (1999) and Shoba (2002) by re-isolating the bacterium on TZC medium thus proving the Koch's postulates of the isolated bacterium.

wilted plant

Plate 7. Pathogenicity test for *Ralstonia solanacearum*

4.3. Effect of different treatments on number of infected plant and disease incidence of bacterial wilt of brinjal at different days after transplanting

4.4. Number of infected plant and Disease incidence at 10 days after transplanting

The treatments applied in the management of wilt of brinjal differed significantly in respect of disease related parameters at 10 days after transplanting (Appendix V). Results are presented in table 6.

Effect of different treatments on disease incidence of bacterial wilt of brinjal was observed in the field study. After 10 days of transplanting, the number of infected plant was minimum (1) for T_6 (Krosin AG10SP) whereas the

maximum number of infected plant (5) was recorded in the T_0 (control) treatment which was statistically identical with T_4 (Bactroban 20WP) (4.7%). The second lowest infected plant (2) was recorded in T_3 (Nirvoy 10SL) and T_5 (Kasumin 2%liquid) where the value statistically identical. Foliar spray of Nirvoy 10SL(validamycin 3% SL) also delayed and reduced disease symptoms of tomato bacterial wilt (Ishikawa *et al*.,1996).

All the treatments significantly reduced bacterial wilt incidence compared to control except for T_4 (Bactroban 20WP) ranged from 8.3% to 41.7%. The highest disease incidence (41.7%) of bacterial wilt was recorded in control which was statistically identical with Bactroban 20WP T_4 (38.2%). The lowest disease incidence (8.3%) was observed from T_6 which was application of Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochlorid 1%). The second lowest disease incidence (16.7) was observed from T_3 (Nirvoy 10SL) and T_5 (Kasumin 2%liquid).

Disease incidence was 80% reduced than control by T_6 which is application of Streptomycin Sulphate 9% + Tetracyclin Hydrochlorid 1%. T_3 (Nirvoy 10SL) and T_5 (Kasumin 2%liquid) reduced 60% disease incidence than control. T_1 (Goldton 50WP) and T_2 (Tricost 1%) reduced 40% disease incidence than control. T_4 (Bactroban 20WP) reduced only 6.7% disease incidence over control.

Table 6. Number of infected plant and Disease incidence at 10 days after Transplanting

Treatments	Number of infected plant per plot	Disease Incidence (DI) (%)	DI reduced over control (%)
T_0	5.0 a	41.7 a	--
T_1	3.0 b	25.0 c	40.0
T_2	3.0 b	25.0 c	40.0
T_3	2.0 c	16.7 d	60.0
T_4	4.7 a	38.2 a	6.7
T_5	2.0 c	16.7 d	60.0
T_6	1.0 d	8.3 e	80.0
LSD (0.05)	0.4	3.7	--
C.V. (%)	7.4	5.3	--

T_0= *Ralstonia Solanacearum* (Control) ; T_1= *R. Solanacearum* + Goldton 50WP ; T_2= *R. Solanacearum* + Tricost 1%WP ; T_3= *R. Solanacearum* + Nirvoy 10SL ; T_4= *R. Solanacearum* + Bactroban 20WP ; T_5= *R. Solanacearum* + Kasumin 2% liquid and T_6= *R. Solanacearum* + Krosin AG10SP

4.4.1 Number of infected plant and disease incidence at 20 days after transplanting

After 20 days of transplanting the number of infected plant (1.3) was minimum for T_6 (Krosin AG10SP) whereas the maximum infected plant (8) was recorded in the T_0 (control) treatment which was statistically identical with T_4 (Bactroban 20WP) (7.7). The second lowest infected plant (3.7) was recorded in T_2 (Tricost 1% WP) which was statistically identical with T_3 (Nirvoy 10SL) (4) (Table 7).

All the treatments significantly reduced bacterial wilt incidence compared to control except for T_4 (Bactroban 20WP) ranged from 11.1% to 66.7%. The highest disease incidence (66.7%) of bacterial wilt was recorded in T_0 (control)

which was statistically identical with T_4 (Bactroban 20WP) (63.9%). The lowest disease incidence (11.1%) was observed from T_6 which is application of (Krosin AG10SP) Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%. The second lowest disease incidence (30.6%) was observed from T_2 (Tricost 1%WP) which was statistically identical with T_3 (Nirvoy 10SL) (Table 7).

83.3% disease incidence was reduced over control incase of T_6 (Krosin AG10SP) followed by 54.2% in case of T_2 (Tricost 1%WP) reduced,T_3 (Nirvoy 10SL) reduced 50% disease incidence over control. T_1 (Goldton 50WP) and T_5 (Kasumin 2% liquid) reduced 37.5% disease incidence than control. T_4 (Bactroban 20WP) reduced only 4.2% disease incidence over control (Table 7).

The T_6 treatment which was the application of Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) showed the best performance in terms of reducing disease incidence. Krosin AG10SP is well known antibacterial agent.

Table 7. Number of infected plant and disease incidence at 20 days after transplanting

Treatments	Number of infected plant per plot	Disease Incidence (DI %)	DI reduced over control (%)
T_0	8.0 a	66.7 a	--
T_1	5.0 b	41.7 b	37.5
T_2	3.7 c	30.6 c	54.2
T_3	4.0 c	33.3 c	50.0
T_4	7.7 a	63.9 a	4.2
T_5	5.0 b	41.7 b	37.5
T_6	1.3 d	11.1 d	83.3
LSD (0.05)	0.6	5.4	--
C.V. (%)	6.2	6.8	--

T_0 = *Ralstonia Solanacearum* (Control); T_1 = *R. Solanacearum* + Goldton 50WP; T_2 = *R. Solanacearum* + Tricost 1%WPm; T_3 = *R. Solanacearum* + Nirvoy 10SL; T_4 = *R. Solanacearum* + Bactroban 20WP; T_5 = *R. Solanacearum* + Kasumin 2% liquid and T_6 = *R. Solanacearum* + Krosin AG10SP

4.4.2 Number of infected plant and disease incidence at 30 days after transplanting

The treatments applied in the management of wilt of brinjal differed significantly in respect of disease related parameters at 30 days after transplanting (Appendix V).

After 30 days of transplanting the number of minimum infected plant (1.7) was found for T_6 (Krosin AG10SP) whereas the maximum infected plant (9.7) was recorded in the T_0 (control) treatment which was statistically identical with T_4 (Bactroban 20WP) (9.3). The second lowest infected plant (5) was recorded in T_2 which was Tricost 1% WP (Table 8).

All the treatments significantly reduced bacterial wilt incidence compared to control except for T_4 (Bactroban 20WP) ranged from 13.9% to 80.5%. The highest disease incidence (80.5%) of bacterial wilt was recorded in control which was statistically identical with T_4 (Bactroban 20WP) (77.8%). The lowest disease incidence (13.9%) was observed from T_6 which is application of Krosin AG10SP. The second lowest disease incidence (41.667%) was observed from T_2 (Tricost 1% WP) (Table 8).

Disease incidence was 82.8% reduced over control by T_6 which was application of Krosin AG10SP. T_2 (Tricost 1%WP) reduced 48.27% disease incidence over T_0 (control) and T_3 (Nirvoy 10SL) and T_5 (Kasumin 2% liquid) reduced 31.0% disease incidence over control. T_1 (Goldton 50WP) reduced 17.2% disease incidence than control. T_4 (Bactroban 20WP) reduced only 3.4% disease incidence over control (Table 8).

This is a clear outcome that in the field study Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) effectively controlled the disease incidence in all counting i.e. 10 DAT, 20 DAT, 30 DAT whereas T_2 (Tricost 1%WP) moderately controlled the bacterial disease. This outcome is partially supported by Verma *et al*., 2014. They used four antibiotics and screened through food poison techniques viz. Streptomycin, kasugamycin, Ceftriaxone and Gentamycin. They found streptomycin showed antibacterial efficacy and inhibited >50% cfu at 3 ppm, but Kasugamycin was found less antibacterial as compared to other tested antibiotics and inhibited >50% cfu at 4 ppm. Chemical i.e. streptocycline + copper oxychloride was drenched at 30 days after transplanting results in reduce the further spread of disease the finding was also supported by singh *et al*. (2012) Nirvoy 10SL, Kasumin 2% liquid and Goldton 50WP had minimal effect on control of bacterial wilt disease. Bactroban 20WP was ineffective against bacterial wilt disease in the field study. This results also partially supported by, Mohsin *et al*., 2016. They used five chemicals viz: Bleaching powder, Streptomycin, Neomycin, Bactrol and Copper Oxychloride against *R.Solanacearum*. They observed

Streptomycin, Neomycin and Bactrol showed moderate performance. Dutta and Verma (1969) studied the efficacy of streptomycin in controlling of bacterial wilt of eggplant and found best results.

Streptomycin had the best effect in reducing pathogen (*C.michiganensis* sub sp. *michiganensis*) among tomato seedlings in the greenhouse were supported by Milijašević *et al.*,(2009). They used 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 pathogen. The antibiotic completely stopped the spread of *C.michiganensis* subsp. *michiganensis* in the region most distant from the inoculum focus.

Table 8. Number of infected plant and disease incidence at 30 days after transplanting

Treatments	Number of infected plant per plot	Disease Incidence (DI %)	DI reduced over control (%)
T_0	9.7 a	80.6 a	--
T_1	8.0 b	66.7 b	17.2
T_2	5.0 d	41.7 d	48.3
T_3	6.7 c	55.6 c	31.0
T_4	9.3 a	77.8 a	3.4
T_5	6.7 c	55.6 c	31.0
T_6	1.7 e	13.9 e	82.8
LSD (0.05)	0.9	6.8	--
C.V. (%)	7.6	6.2	--

T_0 = *Ralstonia Solanacearum* (Control) ; T_1 = *R. Solanacearum* + Goldton 50WP ; T_2 = *R. Solanacearum* + Tricost 1%WP; T_3 = *R. Solanacearum* + Nirvoy 10SL ; T_4 = *R. Solanacearum* + Bactroban 20WP ; T_5 = *R. Solanacearum* + Kasumin 2% liquid and T_6 = *R. Solanacearum* + Krosin AG10SP

a. **b.** **c.**

Plate 8: Bacterial infected plants **a.** Wilted plant at 10 DAT**; b.** Wilted plant at 20 DAT**; c.** Wilted plant at 30 DAT

4.4.3 Effect of different treatments on plant growth parameters of brinjal

The treatments applied in the management of wilt of brinjal differed significantly in respect of plant growth parameters (Table 9) (Appendix IV).

In case of plant height, the maximum plant height (73.5 cm) was observed in T_6 (Krosin AG10SP) whereas the minimum was recorded in the T_0 (control: 50.5 cm) treatment which was statistically similar with T_3 (Nirvoy 10SL) (50.9 cm). The second highest shoot length (61.7 cm) was recorded in T_5 (Kasumin 2% liquid) that were statistically identical with treatment T_2 (Tricost 1%WP) (60.3 cm). *Trichoderma spp.* were known to enhance plant growth and productivity which was reported by Harman (2000) and Shoresh *et al.* (2010).

Considering number of leaf per plant, the maximum result (112.5) was found in treatment T_6 (Krosin AG10SP) which was statistically identical with T_2 (Tricost 1%WP (110.1). The third best was T_3 (Nirvoy 10SL) (98.2) which was statistically similar with T_4 (Bactroban 20WP) (94.1) and T_5 (Kasumin 2% liquid) (96.2). The lowest number of leaf per plant (79.3) was found in the control.

The highest number of branches (12.01) was found in case of T_6 where Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) were

applied. The second highest number of branches (10.2) was recorded in treatment T_2 (Tricost 1%WP) that were statistically identical with T_3 (Nirvoy 10SL) (9.5). The lowest number of branches was noted (12.93) in case of control.

The maximum number of fruit per plant (35.1) was observed in case of T_6 where Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) were applied. The second highest number of fruit per plant (31.6) was recorded in T_2 (Tricost 1%WP) which was statistically similar with T_3 (Nirvoy 10SL) (30.1). The lowest number of fruit per plant (23.37) was found in the Control.

In terms of length of fruit, the highest fruit length (12.1 cm) was observed in case of treatment T_6 where (Krosin AG10SP) were applied. Similar result also obtained by Das *et al*.(1995). They tested 14 antibiotics and amongst them Tetracycline was the most effective against *Ralstonia Solanacearum*. The treatment T_2 (Tricost 1%WP) (9.7) produced second best number of fruits which was statistically identical with T_3 (Nirvoy 10SL) (8.8 cm) and similar with T_5 (Kasumin 2%liquid) (8.7 cm). The lowest fruit length (15.07 cm) was found in control. This findings were similar with the result of Revathi *et al.*2018. They used one botanical (neem cake), two bio agents (*Trichoderma harzianum* and *Pseudomonas fluorescens*) and one chemical (Streptocycline + copper oxychloride) in single as well as combination of three were used in integrated disease management of bacterial wilt. They found that plant height, number of flowers and number of branches was recorded more in *P. fluorescens* treatment followed by neem cake , + *T. harzianum* + *P. fluorescens*, Streptocycline + copper oxychloride.

Table 9. Effect of different treatments on plant growth parameters of brinjal

Treatments	Plant height (cm)	No. of leaves per plant	No. of branches per plant	No. of fruits per plant	Fruit length (cm)
T_0	50.5 D	79.3 d	4.3 e	17.1 f	5.8 e
T_1	55.2 C	90.0 c	7.6 d	25.4 d	7.6 cd
T_2	60.3 B	110.1 a	10.2 b	31.6 b	9.7 b
T_3	50.9 cd	98.2 b	9.5 b	30.1 bc	8.8 b
T_4	45.4 E	94.1 bc	7.5 d	20.6 e	7.5 d
T_5	61.7 B	96.2 bc	8.7 c	28.4 c	8.7 bc
T_6	73.5 A	112.5 a	12.1 a	35.1 a	12.1 a
LSD (0.05)	4.4	6.9	0.8	2.4	1.2
C.V. (%)	4.3	4.2	5.0	4.9	7.6

T_0 = *Ralstonia Solanacearum* (Control) ; T_1 = *R. Solanacearum* + Goldton 50WP ; T_2 = *R. Solanacearum* + Tricost 1%WP ; T_3 = *R. Solanacearum* + Nirvoy 10SL ; T_4 = *R. Solanacearum* + Bactroban 20WP ; T_5 = *R. Solanacearum* + Kasumin 2% liquid and T_6 = *R. Solanacearum* + Krosin AG10SP

4.5 Effect of different treatments on the yield of brinjal

The treatments applied for the management of bacterial wilt of brinjal differed significantly in respect of fruit yield (Appendix VI). Results are presented in table 10.

The highest yield per plant (2.9 kg) was recorded in case of T_6 where Krosin AG 10SP were applied. Treatment T_2 (Tricost 1%WP) produced the second highest yield (2.39 kg) which was statistically similar with T_3 (Tricost 1%WP) (2.26 kg), T_5 (Kasumin 2% liquid) (2.25 kg) and T_1 (Goldton 50WP) (2.20 kg). The lowest yield per plant (1.88 kg) was noted in control which was statistically similar with T_4 (Bactroban 20WP) (2.05 kg).

In case of yield (kg) per plot the highest yield per plot (27.0 kg) was recorded in case of T_6 where Krosin AG10SP were applied. Treatment T_2 (Tricost 1% WP) produced the second highest yield (16.7 kg) followed by T_3 (Nirvoy 10SL) (12.1 kg) which was statistically identical with T_5 (Kasumin 2%liquid) (12.0 kg). After that it was T_1 (Goldton 50WP) (2.20 kg) which performed third lowest yield per plot. The lowest yield per plot (5.2 kg) was noted in control which was statistically identical with T_4 (Bactroban 20WP) (5.5 kg) (Table 10).

The yield of brinjal per hectare differed significantly among the treatments. The highest yield (43.25 ton) was recorded in case of treatment T_6 where Krosin AG10SP were applied. Treatment T_2 (Tricost 1%WP) was the second highest (26.8 ton) performer for yield per hectare followed by treatment T_3 (Nirvoy 10SL) (19.3 ton) which was statistically identical with T_5 (Kasumin 2% liquid) (19.2 ton). Treatment T_1 (Goldton 50WP) (14.1 ton) was fifth best performer in case of yield per hectare. The lowest yield (8.47 ton) was noted in

treatment T_0 which was control and it was statistically identical with T_4 (Bactroban 20WP) (8.8 ton).

The treatment T_6 (Krosin AG10SP) showed promising results in each and every case of yield and yield contributing characters. Treatment T_6 (Krosin AG10SP) produced 410 % yield increase over control. The second best result was observed from T_2 (Tricost 1%WP) which increased 216% yield over control followed by T_3 (Nirvoy 10SL), T_5 (Kasumin 2% liquid) and T_1 (Goldton 50WP). T_4 (Bactroban 20WP) showed no yield increase over control. So it can be concluded that among the seven treatments T_6 (Krosin AG10SP) was the best against *Ralstonia solanacearum* which was followed by T_2 (Tricost 1%WP) and T_3 (Nirvoy 10SL), T_5 (Kasumin 2%liquid) respectively. On the other hand T_1 (Goldton 50WP) had slight advantage against the pathogen whereas T_4 (Bactroban 20WP) had no significant difference.

The most promising measure was T_6 treatment which was the application of Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%). Krosin AG10SP is well known antibacterial agent. The similar result was partially supported by Revathi *et al.*2018. They found that yield was recorded more in neem cake , + *T. harzianum* + *P. fluorescens,* Streptocycline + copper oxychloride.

Treatments with pathogen + antibiotics streptomycin, cefixin and tetracyclin were found very effective in reducing the population of *R. Solanacearum* of soil (Rafi.,2014). This results partially supported by Mohsin *et al*.,(2016). They found that Streptomycin, Neomycin and Bactrol showed moderate yield.

Table 10. Effect of different treatments on yield of brinjal

Treatments	Yield (kg) Plant^{-1}	Yield (kg) Plot^{-1}	Yield (ton) ha^{-1}	Yield increased over control (%)
T_0	1.9 d	5.2 e	8.5 e	--
T_1	2.2 bc	8.8 d	14.1 d	66.9
T_2	2.4 b	16.7 b	26.8 b	216.2
T_3	2.3 bc	12.1 c	19.3 c	127.8
T_4	2.1 cd	5.5 e	8.8 e	3.7
T_5	2.3 bc	12.0 c	19.2 c	126.8
T_6	2.9 a	27.0 a	43.3 a	410.9
LSD (0.05)	0.2	1.5	2.2	--
C.V. (%)	8.8	7.4	6.8	--

T_0 = *Ralstonia Solanacearum* (Control) ; T_1 = *R. Solanacearum* + Goldton 50WP ; T_2 = *R. Solanacearum* + Tricost 1%WP ; T_3 = *R. Solanacearum* + Nirvoy 10SL ; T_4 = *R. Solanacearum* + Bactroban 20WP ; T_5 = *R. Solanacearum* + Kasumin 2% liquid and T_6 = *R. Solanacearum* + Krosin AG10SP

4.6 Correlation regression study between disease incidence and yield

Correlation study was done to determine the relationship between yields per hectare with disease incidence of eggplant. Result showed that significant and negative correlation existed between disease incidence of wilt and yield of the corresponding plot. Yield was decreased with the increase of disease incidence of bacterial wilt of eggplant.

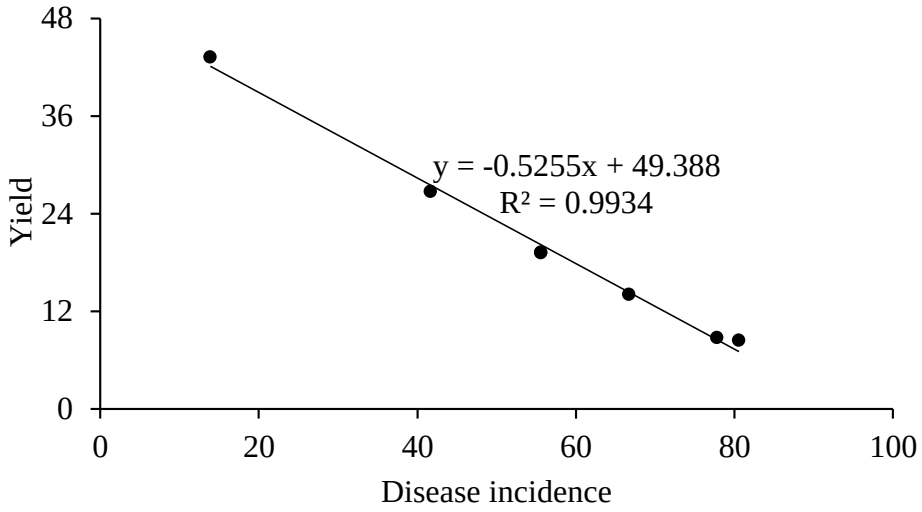


Figure 2. Relation between disease incidence and yield

CHAPTER V

SUMMARY AND CONCLUSION

In Bangladesh, brinjal (*Solanum melongena* L.) is one of the most important vegetables in terms of year round availability, nutritional value, taste, farmer's income perspective and as an export item. It is very popular as called the poor man's crop. Successful production of brinjal is greatly hampered by the wilt diseases caused by *R. solanacearum* which is one of the widest spread and destructive disease, causing huge losses to crop growers.

The present investigation was carried to find out the efficacy of chemicals and bio-agents in controlling wilt disease of brinjal caused by *R.solanacearum*. Seven treatments were used to manage bacterial wilt in field condition. The treatments viz. T_0 = *Ralstonia Solanacearum* (Control); T_1 = *R. Solanacearum* + Goldton 50WP; T_2 = *R. Solanacearum* + Tricost 1%WP; T_3 = *R. Solanacearum* + Nirvoy 10SL; T_4 = *R. Solanacearum* + Bactroban 20WP; T_5 = *R. Solanacearum* + Kasumin 2%liquid and T_6 = *R. Solanacearum* + Krosin AG 10SP were evaluated against *Ralstonia solanacearum* causing wilt of brinjal in the field condition.

R. solanacearum was isolated from naturally wilt infected brinjal plants showing typical symptoms of bacterial wilt. Causal organism of bacterial wilt of brinjal was purified by restreaking on nutrient agar medium with single colony. The colonies of *R. solanacearum* highly fluidal, slightly raised white creamy colonies with pinkish red center, have irregular margin after 48 hrs of incubation at 30^0c on TTC medium. The bacteria was identified on the basis of morphological and cultural characters. Final confirmation was done by testing Koch's postulates.

BT Brinjal 2 variety was used as planting materials. Seeds were sown in seedbed i.e prepared with sterilized soil. Monitoring and watering was done

regularly. Twenty five days old healthy and uniform sized seedlings were transplanted in the field during afternoon. Thirty five days old transplanted seedlings were inoculated with cell suspension (10^8 cfu/ml) following stem puncture method by using sterile syringe. Thirty eight days old seedlings were treated with seven treatments including one control by soil drenching method.

The minimum number of infected plant (1.7) was found in T_6 (Krosin AG10SP) whereas the maximum infected plant (9.7) was recorded in the T_0 (control) treatment which was statistically identical with T_4 (Bactroban 20WP) (9.3). In field evaluation, the effect of the treatments for management of bacterial wilt of brinjal were determined by recording data in terms of number of infected plant, wilt disease incidence (%), yield and yield contributing characters against wilt disease.

All the treatments significantly reduced bacterial wilt incidence compared to T_0 (control) except for T_4 (Bactroban 20WP) that ranged from 13.9% to 80.5%. The minimum disease incidence (13.9%) was observed from T_6 which is application of Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%. On the other hand the highest disease incidence (80.5%) of bacterial wilt was recorded in control which was statistically identical with T_4 (Bactroban 20WP) 77.8%. Disease incidence was 82.8% reduced over control by the application of T_6 (Krosin AG10SP), T_2 (Tricost 1%WP), T_3 (Nirvoy 10SL), T_5 (Kasumin 2% liquid) had moderate effects against wilt disease whereas T_1 (Goldton 50WP) was less effective. T_4 (Bactroban 20WP) was ineffective against wilt disease which reduced only 3.4% disease incidence over control.

Treatments effects were differed significantly in respect of plant growth characters viz. plant height, number of leaves per plant, number of branches, number of fruits per plant and length of fruit compared to control. Most of the treatments showed similar trend of results in case of yield and yield contributing characters except for T_4 (Bactroban 20WP). The highest performance was found in case of the T_6 (Krosin AG10SP) treatment. The lowest result was found in T_0 (control).

The highest yield (2.9 kg/plant, 27.0 kg/plot and 43.25 ton/ha) was recorded in case of Krosin AG10SP (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) treatment. The lowest yield (1.88 kg/plant, 5.2 kg/plot and 8.47 ton/ha) was noted in T_0 (control) was statistically identical with T_4 (Bactroban 20WP) (2.05 kg/plant, 5.2 kg/plot and 8.8 ton/ha).

Considering the overall performance of the treatments, it can be concluded that the bacterial wilt of brinjal could be controlled successfully and cost effectively by the application of T_6 (Krosin AG10SP) which is (Streptomycin Sulphate 9% + Tetracyclin Hydrochloride 1%) in different permutations. However, further study is suggested to find out the alternatives of chemicals. It was also suggested to carry out the study for consecutive years in different Agro Ecological Zones (AEZs) to formulate a sustainable approach.

CHAPTER VI

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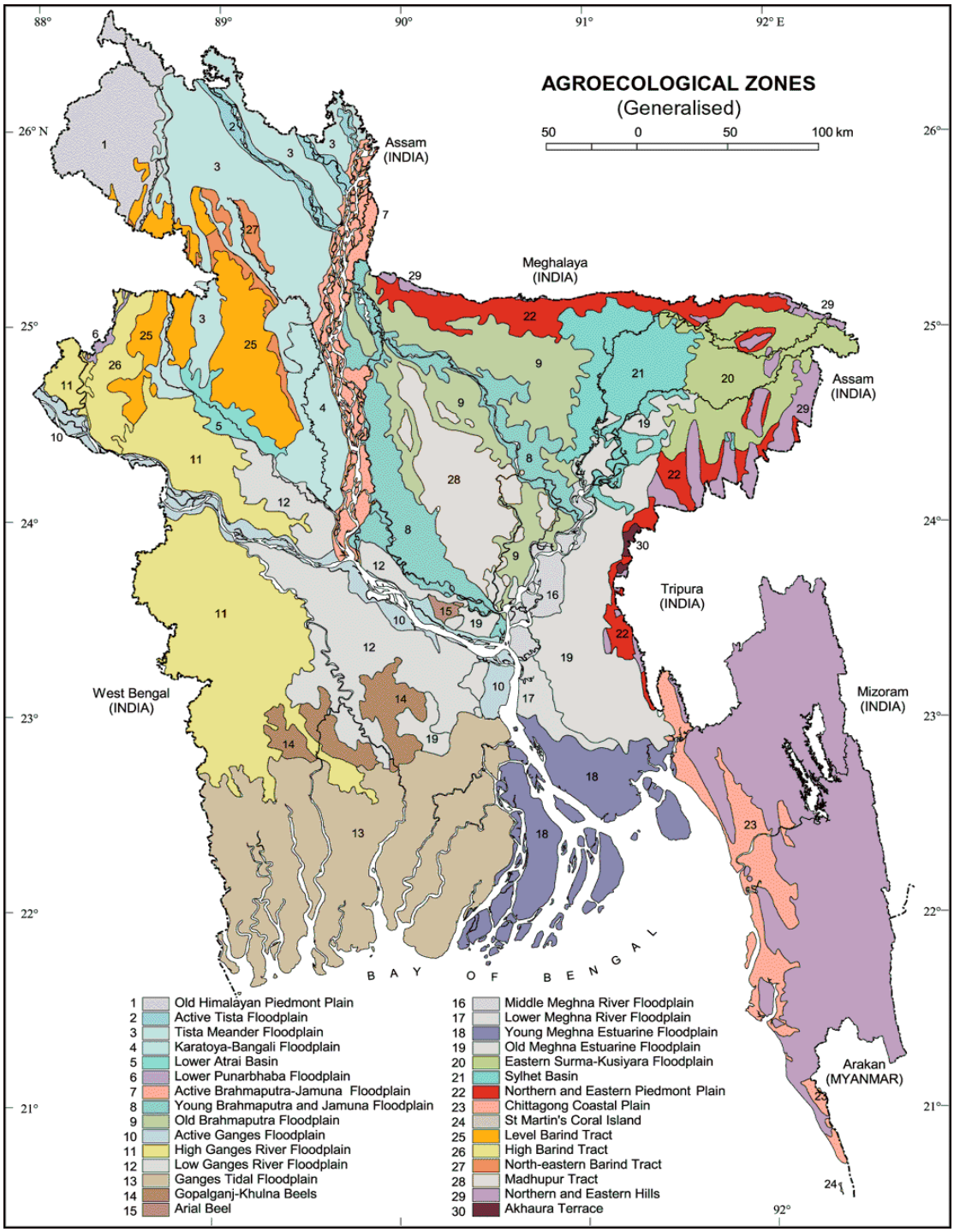
APPENDICES

Appendix I: Map showing the location of the site of the experiment.



Appendix II: Morphological characteristics of the experimental field.

Morphology	Characteristics
Location	SAU Farm. Dhaka
Agro-ecological zone	Madhupur Tract (AEZ 28)
General Soil Type	Deep Red Brown Terrace Soil
Parent material	Madhupur clay
Topography	Fairly level
Drainage	Well drained
Flood level	Above flood level

Appendix III: Physical and chemical properties of the soil

Characteristics	Value
Particle size analysis	
% Sand	30
% Silt	40
% Clay	30
Textural class	Clay loam
Consistency	Granular and friable when dry
pH	5.6
Bulk Density (g/cc)	1.45
Particle Density (g/cc)	2.53
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.06
Available P (ppm)	20.0
Exchangeable K (meq/100g soil)	0.12

Source: SRDI, 2015

Appendix IV: Analysis of variances of the data on different attributes of brinjal

Source of variation	Degrees of freedom	Mean Squares				
		Plant Height	Number of leaves	Number of branches	Number of fruits	Fruit length
Replication	2	0.756	4.2	0.0914	10.321**	0.9505
Treatment	6	261.084**	1300.19**	18.1586**	119.9**	11.6871**
Error	12	6.046*	15.09	0.1814	1.785	0.4255

* significant at 0.05 level
** significant at 0.01 level

Appendix V: Analysis of variances of the data on different attributes of brinjal

Source of variation	Degrees of freedom	Mean Squares					
		Number of infected plant			Disease Incidence		
		10 DAT	20 DAT	30 DAT	10 DAT	20 DAT	30 DAT
Replication	2	0.04762	0.4286	0.0476	10.566**	18.49**	23.77**
Treatment	6	6.38095*	15**	19.6508**	389.62**	1122.22**	1585.33**
Error	12	0.04762	0.0952	0.2698	4.402*	9.24*	14.53**

* significant at 0.05 level
** significant at 0.01 level

Appendix VI: Analysis of variances of the data on different attributes of brinjal

Source of variation	Degrees of freedom	Mean Squares		
		Yield per plant	Yield per plot	Yield per hectare
Replication	2	0.00464	0.76	2.096
Treatment	6	0.30264	172.142**	440.684**
Error	12	0.01464	0.72	1.556

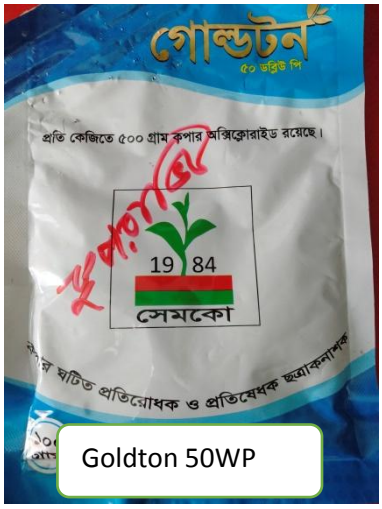
* significant at 0.05 level
** significant at 0.01 level

Appendix VII: Different Chemicals and Bio-agent used in the study against *R. Solanacearum*.

Bactroban 20WP

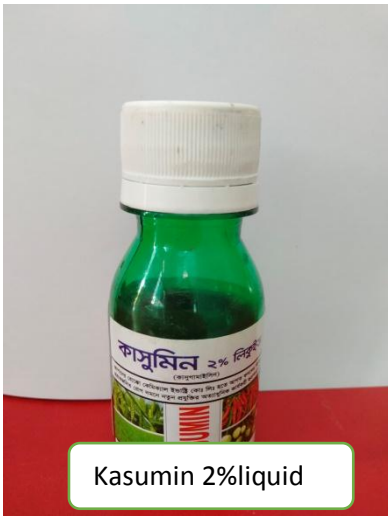
Krosin-AG10SP

Tricost 1%WP



Goldton 50WP

Nirvoy 10SL



Kasumin 2%liquid

Appendix VIII: Different photographs during the period of research work