INTEGRATED MANAGEMENT OF WILT COMPLEX DISEASE OF EGGPLANT (*Solanummelongena*)

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INTEGRATED MANAGEMENT OF WILT COMPLEX DISEASE OF EGGPLANT (*Solanummelongena)*

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DEDICATED
TO MY BELOVED
PARENTS, HUSBAND
AND DAUGHTER

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CERTIFICATE

This is to certify that the thesis entitled**"INTEGRATED APPROACH FOR THE MANAGEMENT OF WILT COMPLEX DISEASE OF EGGPLANT(*Solanummelongena)*"**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 in PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by**NahidAkter,**Registration No.**11-04480**under my supervision and guidance. No part of the thesis has been submitted anywhere for any other degree or diploma.

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

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INTEGRATED MANAGEMENT OF WILT COMPLEX DISEASE OF EGGPLANT (*Solanummelongena)*

ABSTRACT

The *in vitro* experiments were conducted in the central laboratory and net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka and the field experiment was conducted in the research field of Plant Pathology Division, BARI, Joydebpur, Gazipur to evaluate the efficacy of different treatments alone and in combination with a view to formulate an integrated approach for the management of wilt complex disease of eggplant caused by*Fusariumoxysporum*, *Ralstoniasolanacearum* and *Meloidogyne spp.* during 2017-2018. Sixteen treatments were explored in this experiment. The effect of the different treatments varied significantly from control in respect of wilt incidence, yield and yield contributing characters. The disease incidence differed significantly among the treatments ranged from 0- 60%. No disease incidence (0%) was observed in treatment T_4(*Trichoderma* formulation), T_7(Furadan 5 G + *Trichoderma* formulation), T_8(Krosin10 SP + *Trichoderma* formulation), T_{13}(Autostin 50 WP + *Trichoderma* formulation + Poultry waste), T_{14}(Furadan 5 G + *Trichoderma* formulation + Krosin10 SP) and T_{15}(Krosin10 SP + Furadan 5 G + *Trichoderma* formulation + Poultry waste). However, the highest disease incidence (60%) was observed in control. In case of all the plant growth parameters, treatment $T_{15}$was found as the best one except plant height. The highest yield (32.10 ton/ha) was recorded in case of treatment T_{15}followed by T_{14}(28.24 ton/ha). The lowest yield was noted in control (8.02 ton/ha). The highest Benefit Cost Ratio (BCR) was recorded in treatment T_{15}(5.68) followed by T_{14}(5.56) and T_8 (5.51). The integrated application of Furadan5G, Krosin10 SP, *Trichoderma* formulation and Poultry waste (T_{15}) was found to be effective for the management of wilt complex of eggplant that increased yield by 300% over control followed by T_{14}(Furadan + *Trichoderma* formulation + Krosin) that increased yield by 251.84%.

CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATED TERMS

Abbreviation	Full word
BARI	Bangladesh Agricultural Research Institute
HRC	Horticultural Research Center
et al.	And others
Cm^3	Centimeter cube
Cm^2	Centimeter square
Cm	Centimeter
CFU.	Colony Forming Unit
°C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
G	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
LSD	Least Significant Difference
Ml	Milliliter
%	Percent
RCBD	Randomized Completely Block Design
Res.	Research
SAU	Sher-e-Agricultural University
Viz.	Namely
Var.	Variety
SDW	Sterilized Distilled Water
TTC	TriphenylTetrazolium Chloride
NA	Nutrient Agar

INTRODUCTION

Eggplant (*Solanum melongena L*.), belongs to the solanaceae family, is also known as aubergine, brinjal, melongene, garden egg, or guinea squash in respect of different locations in the world (Yiu, 2006). It is ranked 4th position as vegetables in the world. It is also called 'king of vegetables'. Besides it has been used as fresh nutritious vegetable, eggplant has also good medicinal values. It is highly beneficial for regulation of blood sugar levels and also helps to control the absorption of glucose. It is also recommended for the remedy of liver problems (Shukla and Naik, 1993).

Eggplant is cultivated as a popular and commercial vegetables throughout the tropical and sub-tropical regions of the world. In tropical climate, eggplant can be grown throughout the year as perennial crop and in sub-tropical, it is grown as summer vegetables. It is popular in many countries *viz.*, Central, South and South East Asia, some parts of Africa and Central America (Harish *et al*., 2011). It has been originated in South East Asia especially in India, as Subcontinent people are used to grow eggplant since last 4000 years (Dunlop, 2006). The leading eggplant growing countries of the world are the China, India, Egypt, Iran, Turkey, Indonesia, Iraq etc. (FAO, 2010). Currently, it is extensively grown in Bangladesh, India, Pakistan, Nepal, U.A.E, Sri Lanka, Egypt and other warm countries of the world. Asia possess the largest eggplant production status which comprises more than 90% of the world production area and 87% of the world production (Chawdhary and Gaur, 2009). Global production of eggplant was 51.3 million metric tons in about 1.8-million-hectare area in the world **(**FAO, 2018).

In Bangladesh, eggplant is locally called *begoon*. It is the second most important vegetable crop, next to potato in Bangladesh in respect of acreage and production (BBS, 2017). Currently, eggplant production in Bangladesh is at 5.08 million metric tons from 1.26million acre land (BBS, 2017). Important eggplant growing districts in Bangladesh are Bogura, Chitagang, Kumilla, Dhaka, Dinajpur,

Faridpur and Jamalpur. It can be grown at homestead areas and kitchen gardens because of its popularity especially among urban people. The vegetables can be cultivated year round even during the scarcity period of vegetables. It is the primary source of income for eggplant growers because it gives instant cash to them by continuous harvesting. In Ramadan the demand and market price of eggplant becomes higher compared to other vegetables which brings solvency to the farmers. Thus, it plays a vital role to boost our national economy.

However, sustainable production of this potential vegetable is greatly constrained by a number of diseases caused by fungal, bacterial, nemic, viral and mycoplasmal species which cause adverse effect on the yield and yield contributing characters. This potential crop suffers from about 13 different diseases, so far recorded in Bangladesh (Das *et al.* 2000; Rashid, 2000 and Khan *et al.* 1998).

Eggplant cultivation in Bangladesh is severely impaired by three important wilt pathogens viz. *Ralstonia solanacearum, Fusarium oxysporum* and *Meloidogyne spp.* which are the causal agents of Bacterial wilt, Fungal wilt and Nemic wilt, respectively causing devastating damage of eggplant (Timm and Ameen, 1960; SThe pathogens are also recorded as the major limiting factors for eggplant production throughout the world (Hinata, 1986). Wilt problems are especially severe in the humid tropics. In some cases, 100% of the plants are found to die in kitchen gardens of Bangladesh due to this complex problem (Ali *et al.*, 1994) and root knot nematode solely may cause as much as 27.2% loss in fruit yield of eggplant (Bari, 2001).

In case of bacterial wilt**,** *Ralstonia solanacearum* generally invades plant roots from the soil through root injury or any natural openings to colonize the vascular system thus blocking translocation of water and nutrients. Consequently, wilt starts in the upper leaves followed by complete collapse of the plant vascular system within a few days. Soil is the main source of the pathogen; However, seeds and seedlings may also carry the pathogen (Ramesh, 2008; Vanitha et al.,

2009; Tahat and Sijam, 2010; Sharma and Sharma, 2014). In the field, bacterial wilt has been reported to be the most destructive disease with a severity of 10–90% (Ramesh, 2008; Vanitha *et al*., 2009; Nishat *et al*., 2015). *R. solanacearum* is difficult to manage due to its' genetic diversity and aggressiveness of the pathogen, ability to survive in the varied and adverse environmental conditions, modes of dissemination and large number of weed hosts (Ramesh and Phadke, 2012).

The *Fusarium* wilt or fungal wilt is expressed by partial wilting symptoms in the plant. The fungal growth blocks the xylem vessel of the affected plant and interrupts with the water and nutrient translocation. Thus the plant becomes wilted and ultimately died.

The nemic infection is expressed by gall formation in the root system and ultimately the plant becomes weaken due to interruption in nutrient and water translocation. Moreover, the root system is injured which frequently facilitates easy entry of other wilt causing bacteria and fungi into the plant root system.

There is a strong relationship between nemic wilt, bacterial wilt and fungal wilt to cause infection.

These three pathogens make wilt complex which can be seen even in the same plant/plot. Farmers often face substantial plant loss before fruiting which results in severe economic losses for the wilt complex disease. In the field, this complex may appear soon after transplanting and continues up to fruiting or even final harvest**.**

The common control measures against wilt complex of eggplant include the use of resistant varieties, healthy seeds, crop rotation, agronomic practices, biological control and integrated management (Elphinstone and Aley, 1993). Cultural practices and crop rotation may provide limited control of *Ralstonia solanacearum* (Kucharek, 1998; Pradhanang *et al*., 2003).

Rapid early detection of bacterial wilt pathogens in plant debris, soil or soil related habitats is essential for disease management in the field to prevent losses

and further pathogen spread (Janse *et al*., 1998). Wide host range and broad geographical distribution make *Ralstonia solanacearum* an economically significant pathogen in the world (Cook *et al*., 1989).

Plant health management is now considered as a young approach which aimed at the proper use of IPM components emphasizing on environment, economics and social acceptance (Cook, 2000). Uses of chemical pesticides are discouraged now-a-days for its harmful effect on environment and ecology. Moreover, Improper use of pesticides has led to residue accumulation on eggplants (Chowdhury *et al*., 2013). This made importing countries to consider restrictions on vegetables, especially eggplants from Bangladesh (Rahman, 2016). But it might be incorporated in the disease management program as the last option. Conventional management by using single method are not effective to control this disease. So, integration of different methods will be a good option to control this complex disease.

Chemical control of wilt complex disease is not only very difficult but also practically not available (Hillocks and Waller, 1997a; Martin, 1981 and Mondal *et al.* 1991). Antibiotics are essential for controlling bacterial diseases of plants (Stockwell and Duffy, 2012).

Furadan 3G (Carbofuran) is nowadays very effective to control root knot nematode and other soil borne pathogens of eggplant (Hillocks and Waller, 1997a and Hossain *et al*., 2003). Carbofuran followed by bioagents *S. marcescens* and *T. harzianum* generally decreased nematode development and reproduction parameters compared to the untreated control (Mahfouz *et al.*, 2010).

Organic soil amendments act against soil borne pathogens by developing suppressive soil which is considered as an ecofriendly approach (Palti and Katan, 1997; Singh and Sitaramaiah, 1971).

Bio-control agent like *Trichoderma harzianum* is reported to have great effect against soil borne pathogen by antagonism, mycoparasitism, competition with pathogens for nutrient and space, and induction of systemic resistance in plants (Yedidia *et al*.,2001; Benitez *et al.*, 2004; Harman *et al*., 2004; Howell, 2006; Hermosa *et al*., 2012). Biological control of wilt can be a sustainable and ecofriendly strategy.

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:

1. To isolate and identify the wilt complex pathogens of eggplant;
2. To evaluate the efficacy of the selected chemicals against targeted pathogens; and
3. To formulate an integrated approach for the management of wilt complex of eggplant.

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. The most devastating disruption in sound eggplant production, usually farmers suffer, is the management of fusarium wilt caused by *Fusarium oxysporum* f. sp. *melongenae.* Some 20-30% killing of eggplant in general due to *fusarium* wilting is a regular report from the farmers (Begum, 2007); Bacterial wilt of eggplant caused by *Ralstonia solanacearum* is known to be one of the most destructive disease wherever this crop is grown extensively. *Meloidogyne incognita* and *R. solanacearum* complex contributes to increase in wilt development (Swain *et al.*,1987). Because of evidence that the resistance is broken down in the presence of root-knot nematode, *M. incognita* plays a major role in the etiology of the bacterial wilt in eggplant. Research works regarding the management of wilt complex of eggplant are very limited in Bangladesh. However, some available and important findings on various aspects of management of fungal wilt, bacterial wilt and nemic wilt have been compiled and presented below.

2.1. Symptoms of *Fusarium* wilt disease

Miller *et al*. (2011) and Mueller and Beckman (1988) stated that the general symptoms of wilts caused by *Fusarium spp.* are stunted growth, yellowing and wilting of the leaves, reddish discoloration of the xylem vessels, visible inside the stem as lines (if the stem is cut open lengthways) or dots (if it is cut across). Others are white, pink or orange fungal growth on the outside of affected stems, particularly in wet conditions, root or stem decay.

Elliot (2009) and Hutmacher *et al*. (2003) described that at the seedling stage or in young plants, cotyledons and leaves are wilted and dropped, leading to bare

stems. Early detection of *Fusarium* wilt will be difficult because early symptoms may resemble some types of seedling disease and symptoms developing much later which may be similar to those of other diseases. For example, symptoms are easily confused with those of crown or root-rot, stem cankers, pest injury, drought, nutrient deficiency, bacterial and *Verticillium* wilts.

Burgess *et al.* (2008) reported that, brown vascular discoloration can be observed for this disease in cross sections of stem tissue near the soil line even though these stems remain firm and green on the outside. In some cases, affected plants that do not die are often stunted and their yields are quite reduced. Where the inoculum pressure is high, seedlings may damp off as they emerge from the soil.

Khan and Khan (2002) observed that, *Fusarium spp.* invades the xylem of the pseudo stem by the microconidia where it obstructs the upward movement of water and nutrients. Sieve cells in the stem hinder the conidia from further spread. For this, the spores germinate and grow through the sieve cells where they continue to germinate until the whole vascular system is blocked. As the vessels are plugged and collapse, the water supply to the leaves is blocked, thus causing wilt symptoms. In most cases, the first sign of wilt appears on one and a half-month-old plants and intensifies gradually. Wilting is seen on the lower leaves first and this later extends to the upper leaves. Leaves, twigs or even the whole plant turns brown and later dies and dries up.

According to Armstrong and Armstrong (1975) and Burgess (1981), *Fusarium spp.* is generally classified as soil borne fungus that causes various vascular wilts, root rots and stem rots of cultivated plants.

2.2. Isolation of *Fusarium oxysporum*

Ramaiah and Garampalli (2015) isolated *F. oxysporum* f. sp. *lycopersici* from tomato plant by surface sterilizing of the infected parts of plants with 70% ethanol, immersing the plant parts in 0.3% NaOCl for 10 minutes, rinsing in

sterile distilled water, transferring to potato dextrose agar (PDA) medium in Petri dishes and incubating in dark at 28 ± 1 °C for 7 days.

Joshi *et al.* (2013) isolated *Fusarium spp.* from soil and root sample of tomato plants by washing under tap water, chopping into 2 cm small pieces, surface sterilizing in 0.5% Sodium hypochlorite (NaOCl) solution for 2 minutes, then rinsing twice with triple distilled water and placing on Potato Dextrose Agar medium (PDA) and finally keeping in an incubator at 27 ± 1 °C under dark conditions. After incubation of five days, small colonies of fungus appeared, which were picked with a sterilized toothpick and transferred to fresh, sterilized PDA plates.

Sundaramoorthy *et al.* (2013) collected tomato cultivar (PKM 1) and showed wilt symptoms from a farmer's field. Then they isolated *Fusarium oxysporum* f. sp. *lycopersici* from infected vascular tissues of stem and root regions by surface sterilizing tissue bits with 10 % NaOCl for 5-10 minutes and subsequently washing three times with sterile distilled water, placing on PDA medium separately. Moreover, they incubated at the laboratory conditions at 25 ± 3° C for five days. The fungi were purified separately by transferring the tip of the mycelia into sterilized PDA slants.

2.3. Pathogenic description of *Fusarium oxysporum*

Arif *et al.* (2011), Wang *et al.* (2011) and Balali and Iranpoor (2006) described that different species of *Fusarium* are considered to be some of the most important plant disease pathogens, with some species producing mycotoxins (such as fumonisins, zearalenones and trichothecenes) on plants which contaminate the seeds and enter the food chain, affecting human and animal health, and are thus hazardous to agricultural products, wildlife, livestock and humans.

Barnett and Hunter (1972) reported details about *Fusarium spp.* Mycelium of *Fusarium* is extensive and cottony in culture, conidiophores variable, slender,

simple, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia; conidia hyaline, variable, principally of two kinds, macroconidia several-celled slightly curved or bent at the pointed ends, microconidia 1-celled, ovoid to oblong, borne singly or in chains.

2.4. 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.

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.

2.5. Isolation of *Ralstonia solanacearum*

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^{o}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.

2.6. Pathogenic description of *R. solanacearum*

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

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 (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 (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.

2.7. Symptoms of Nemic wilt (Root Knot) disease

Manju and Sankari (2015) reported that the disease is expressed by gall formation in the root system, and ultimately the plants become weak due to interruption in nutrient uptake from the soil, at severe infection the plants may die. Plant parasitic nematodes are ubiquitous around the world, affects mostly crops causing substantial yield loss to the farmers.

Talukder (1974), Ahmed and Hossain (1985) and Mian (1986) described that root-knot caused by *Meloidogyne incognita* is an important and widely distributed disease in Bangladesh.

Ali (1993) reported that the problems of root-knot nematode infestation in eggplant was expressed by gall formation in the root system and ultimately the plants become weak due to interruption in nutrient and water uptake from the soil and ultimately the infected plant might be died. Moreover, due to nematode infection, the root system becomes injured facilitating easy entry of the wilt causing organisms like bacteria and fungi into the plant. Nematode infestation resulted symptoms on root as well as on the above-ground parts of the plant. Root symptoms might appear as root-knots, root galls and as root-rots when nematode infections were accompanied by plant pathogenic or saprophytic bacteria and fungi. The root symptoms were usually accompanied by non-characteristics symptoms in the above-ground parts of plants and appearing primarily as reduced growth, symptoms of nutrient deficiencies such as yellowing of foliage, excessive wilt in hot or dry weather reduced yields and poor quality of products. *Fusarium* wilt of eggplant increases in incidence when the plants are also infected by the root-knot.

2.8. Pathogenic description of *Meloidogyne incognita*

Hillocks and Waller (1997b) reported that sedentary endoparasitic nematodes such as the root-knot nematodes (RKN) (*Meloidogyne spp.*) enter into the root and move through the cortex to the vascular system, where they begin to feed and remain to complete the life cycle. In general, the sedentary endoparasites have the most profound effects on the physiology of their hosts and the most complex effects on disease susceptibility. The cortical feeding nematodes may predispose the root to infection, but the effect is localized, providing entry sites for pathogens or increasing nutrient leakage.

Singh and Sitaramaiah (1994) stated that root-knot nematode *Meloidogyne spp.* were the first plant-parasitic nematode to be recognized. The mature female of *Meloidogyne sp.* were swollen, pear or subspherical in shape. They are sedentary endoparasites. The body will remain soft, white and does not form a cyst. The female stylet is slender with well-developed basal knobs. First molt occurs within the egg. Males were vermiform and migratory. Second stage juveniles were vermiform, migratory and infective. Third and fourth larval stages were swollen.

2.9. Pathogenicity study of associated Pathogens with wilt complex disease of eggplant

2.9.1. Pathogenicity study of *Fusarium oxysporum*

Ramaiah and Garampalli (2015) inoculated tomato seedlings with *Fusarium oxysporum* using spore suspension with a conidial concentration of $1x10^5$ conidia/ml by root dip method, and reported that inoculated plants expressed severe infection with the typical symptom like leaf chlorosis. The diseased leaves wilted and dried up with dropping and wilting of the stem tip. The diseased plants wilted down and dried up completely. Their roots were necrotic and rotten, and the necrosis spread to the lower stem. In contrast, control plants were utterly free from disease.

Sibounnavong *et al.* (2012) confirmed pathogenicity by inoculating the pathogen *Fusarium oxysporum* f. sp. *lycopersici* isolate NKSC02 to 15 days old tomato seedling var. Sida using root-dip method with a conidial suspension of the pathogen at 1×10^7 conidia/ml.

El-Kazzaz *et al.* (2008) isolated 33 *Fusarium* isolates from several diseased host plants and tested for their pathogenicity using the soil infestation technique. Pathogenicity tests showed that two isolates of *Fusarium oxysporum*, one isolated from tomato and the other from cotton were highly pathogenic to their host plants producing typical wilt symptoms. All isolates of different *Fusarium*

species were pathogenic causing various diseases on different economic plants with variable degrees.

2.9.2. 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* ($1x10^9$ $CFUml^{-1}$) 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.

Ramaiah and Garampalli (2015) inoculated tomato seedlings with *Fusarium oxysporum* using spore suspension with a conidial concentration of $1x10^5$ conidia ml^{-1} by root dip method and reported that inoculated plants expressed severe infection with the typical symptom like leaf chlorosis. The diseased leaves

wilted and dried up with dropping and wilting of the stem tip. The diseased plants wilted down and dried up completely. Their roots were necrotic and rotten, and the necrosis spread to the lower stem. In contrast, control plants were utterly free from disease.

Sibounnavong *et al.* (2012) confirmed pathogenicity by inoculating the pathogen *Fusarium oxysporum* f. sp. *lycopersici* isolate NKSC02 to 15 days old tomato seedling var. Sida using root-dip method with a conidial suspension of the pathogen at 1×10^7 conidia ml^{-1}.

2.9.3. Pathogenic study of *Meloidogyne spp.*

McGawley (2001) showed that disease complex involving nematodes and fungal pathogens significantly more crop losses than individually some resistant/ tolerant cultivars to *fusarium* wilt disease lose their characteristics and showed the symptoms of disease when parasitized by plant-parasitic nematodes.

Sitaramaiah and Singh (1976) recognized that *Meloidogyne spp* is the first plant-parasitic nematodes. The shape of the mature female of *Meloidogyne* is swollen, pear, or sub-spherical. Female stylate are slender with developed basal knobs. Male nematodes are vermiform and migratory. Inoculated nematodes enter through the root zone and create a node which shows stunted symptoms and gradually die compared to the non-inoculated plant.

2.10. Interaction studies of *M. incognita* and *R. solanacearum*

Zakir and Bora (2009) described that association of pathogenic and above pathogenic levels of inoculum of both *Meloidogyne incognita* and *Ralstonia solanacearum* increased the severity of wilt on brinjal crops as compared to associations of below pathogenic inoculums levels of both the pathogens. Inoculation of *R. solanacearum* alone and below pathogenic levels of *M. incognita* could not reduce the plant growth parameters of brinjal significantly.

Manila (2006) performed Interaction studies between *R. solanacearum* and *M. incognita* on tomato revealed that inoculation of *M. incognita*, 15 days prior to the inoculation of *R. solanacearum* led to maximum wilt incidence (100%) followed by combined inoculation of both the pathogens (75%). Low wilt incidence was noticed in plants inoculated with bacterium alone, which was on par with that of the inoculation of bacterium prior to nematode inoculation.

Chindo *et al.* (1991) found that the effect of root-knot nematode *M. incognita* Race 1, infection on the severity of two vascular wilts of tomato incited by *P. solanacearum* and *Fusarium oxysporum* f. sp. *lycopersici* on three tomato cultivars, namely Hunkuyi local (susceptible to three parasites), CL-119-1-2-0-0 (susceptible to nematode but resistant to *P. solanacearum*) and Roma VF (susceptible to nematode but resistant to *F. oxysporum* f.sp. *lycopersici*) was studied under greenhouse conditions.

2.11. Interaction studies of *Meloidogyne spp*. with *Fusarium oxysporum*

Avelar *et al*. (2001) found that the root-knot nematodes initiated the attack and the magnitude of the symptoms could be due to the presence of fungi which caused root-rot. In the case of tobacco, *M. incognita* increased Fusarium wilt infection when nematode and fungus were on the opposite root halves. However, when similar work was done with cowpea and cotton, the infection was increased only when the two organisms were inoculated onto the same root half (Hillocks, 1986).

Corazza (1998) observed that presence of *Meloidogyne spp.* reduced or annihilated the resistance of plants to *Fusarium oxysporum* f. sp. *melonis.* Coffee wilt caused by *Fusarium oxysporum* develops vascular wilt symptoms and was associated with nematode damage. In case of Fusarium wilt of sunflower (*Fusarium oxysporum* f. sp. *carthami*), the nematode *Meloidogyne incognita* played a vital role in enhancing disease severity.

Hillocks and Waller (1997b) reported that sedentary endo-parasitic nematodes increased disease caused by vascular wilt fungi. Localized wounding and nutrient sink effects around the nematode feeding site played a role in increased infection by Fusarium wilt pathogens. *Meloidogyne spp*. also exerted a systemic effect on disease susceptibility in some hosts. Mechanism of nematode enhanced susceptibility was shown to be retardation of host defense mechanisms in the xylem.

2.12. Integrated approach for wilt complex disease management under field conditions

Khan and Parvatha Reddy (1993) stated that protection of crop plants from disease-causing agents had been the focal point of the pathologist. In depth analysis and realization about the startling features of microbial ecology had implied workers to replace term "control" with "management". No single method of pest control had given a lasting solution. There was hardly any comprehensive effort to highlight the significance of multi pathogenic scenario and their integrated management.

2.12.1. Management through fungicides (Carbendazim)

Hamini-Kadar *et al.* (2014) investigated the *in vitro* effect of 2 pathogenic fungi of tomato, *Fusarium oxysporum* f.sp. *radicis –lycopersici, F. communae,* and *F. redolens* to determine the effectiveness of the fungicides in reducing fungi growth. Each fungicide was assayed at 0, 100, 200, 400 and 500 mg/l rate in potato dextrose broth and incubated at 28 ^{0}C for seven days. Mycelia weights of the three fungi were significantly reduced at 100mg/l by the two fungicides, and a significant reduction was observed at 400 and 500 mg/l. Application of Trifidan significantly decreased the mycelia weight of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. communae* irrespective of the rate applied. Trifidan completely inhibited *F. redolens*, and its inhibition started at 100mg/L.

Amini *et al.* (2010) tested six fungicides; benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole, and azoxystrobin for their efficacy against *Fusarium oxysporum* under *in vitro* and *in vivo* conditions. Different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 μg/ml) were used to assess their inhibitory activities against the pathogen through mycelia growth inhibition on potato media. Four concentrations of fungicides as mentioned above (0.1, 1, 10, and 100 μg/ml) were successful for controlling *Fusarium* wilt on tomato plants in glasshouse. Fungal radial growth was measured, and the median effective concentration (EC50) values (μg/ml) were determined. The result of glasshouse tests revealed that different range of efficiency among tested fungicides in lessening disease infestation. Prochloraz and bromuconazole were the most effective fungicides in both *in vitro* and *in vivo*, followed by benomyl and carbendazim. Moreover, other fungicides were less effective. On consideration of the fungicide's application date, it was seen that they were less effective when applied seven days after tomato plant infection, compared with one day before infection. No phytotoxic symptoms, especially on seedlings, were observed after prochloraz, bromuconazol, and benomyl application at recommended doses. However, both fludioxonil and bromuconazole were shown to be phytotoxic to tomato seedlings.

Dwivedi and Pathak (1980) observed that the effects of fungicides (Autostin and Difolatan) were sufficient to cheek the pathogen (*Fusarium oxysporum f. sp. lycopersici*) growth. They sprayed a 0.1% concentration of Autostin on plant immediately after the symptom appeared.

2.12.2. Management through Furadan 5G (Carbofuran)

Faruk *et al.* (2001) conducted two separate experiments in Bangladesh to evaluate the efficacy of re-plant soil treatment with poultry refuse, neem leaf powder, neem seed powder and Furadan 5G for the management of root-knot nematodes (*Meloidogyne spp*.) of tomato. Soil inoculated with root-knot nematodes were treated with poultry refuse at 200g and neem leaf and seed

powder at 10g and Furadan 5g at 2g per pot. On the other hand, in the field experiment, soils were treated by neem leaf powder, poultry refuse and Furadan 5G @ 0.5t/ha, 10t/ha and 2g/pit, respectively. Among the treatments, neem leaf powder and its combination with Furadan 5G gave a considerable reduction of root-knot disease. The treatments also improved plant growth (weight and length of shoot and root) and increased significantly yield of tomato in the field.

Hassan (1995) tested Furadan 5G and Miral 3G against root-knot (*Meloidogyne javanica*) of brinjal in granular and liquid forms of application, either alone or in combination. The two chemicals on higher concentration and combination in both types of application gave an excellent response in plant growth characters with corresponding lower number of galls, adult females, and egg masses. Furadan 5G more suppressed larval population.

Kartono (1980) stated that Temik 10G, Furadan 3G, and Nemagon 20G reduced the population of *Meloidogyne sp.* in soil up to the 60^{th} day. Temil 10G appeared to give better results than Furadan or Nemagon.

2.12.3. Management through Krosin 10 SP (Streptomycin sulphate 90% and Tetracycline hydrochloride 10%)

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

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.

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

Milijašević *et al. (*2009) 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.

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

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

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.*

2.12.4. Management through organic manure (Poultry waste)

Vyas *et al.* (2009): Result of three years trials indicated that application of *Paecilomyces lilacinus* @ 25 kg spore dust with carrier/ha [(10^9 conidia/g) [at the time of transplanting+ poultry manure @10 tons/ha (a week prior to transplanting) or mustard cake @ 2 tons/ha (a week prior to transplanting) or *P. lilacinus* @ 25 kg spore dust with carrier/ha [(10^9 conidia/g) [at the time of transplanting] + neem cake @ 2 tons/ha (a week prior to transplanting) or *P. lilacinus* @ 25 kg spore dust with carrier/ha [(10^9 conidia/g) [at the time of transplanting] + carbofuran 3G @ 2 kg/ha in two equal splits [one at the time of transplanting and the other after 2.5 months] improved plant growth and considerably reduced gall index and also gave higher brinjal fruit yield over control.

Galip (2007) stated that, the efficacy of soil solarization, Dazomet, poultry manure, olive processing waste (OPW), and soil solarization in combination with poultry manure or OPW or half doses of Dazomet against *Meloidogyne incognita* on tomato cultivars was investigated in greenhouses in western Anatolia, Turkey, between 2002 and 2004. The maximum soil temperature average was increased 47.1^0C by soil solarization alone at 15 cm soil depth of soil in the first year. Soil solarization alone and in combination with CM increased the mean of maximum soil temperature by 41.2 and 40.9 ^{0}C respectively, at the 15 cm soil depth in the second year. Root galling caused by *M. incognita* in tomato plants in the soil solarization plus organic amendment plots (poultry manure or OPW) was lower than in plots that underwent the other treatments. In addition, tomato yields in plots subjected to soil solarization and soil solarization in combination with organic amendment (poultry manure or OPW) were similar to those in plots subjected to Dazomet and soil solarization plus half doses of Dazomet.

Bora and Phukan (1983) tested four soil amendments (mustard oil cake, poultry manure, sawdust and decaffeinated tea waste) in pots containing 3, 5 kg soil gave significant reductions of *Meloidogyne incognita* populations on jute (as judged

by gall number and egg mass /g root and by the nematode population in soil). Mustard oil cake was the most effective but was also phytotoxic. Sawdust was more effective than tea waste, and poultry manure at the lowest dose was the least effective. Sawdust and to a lesser extent tea waste had the best effect on plant height and the dry and fresh weights of shoots and roots.

Shafique *et al.* (2001) reported that application of neem cake, mustard cake and farmyard manure at 25 g/1kg of soil significantly reduced the incidence of *M. javanica* infesting mung bean cv. MNH-92 and increased plant height and fresh weight of shoot compared to control. These treatments significantly reduced the number of galls and the number of egg masses per plant. The most effective treatment was neem cake followed by mustard cake.

Stirling (1989) used poultry manure and sawdust @ 24, 36 or 48 t/ha were incorporated into the soil with urea (0-1800 kg nitrogen/ha), and their effects on yield of ginger and populations of *M. incognita* were compared with those of nematicide programs involving ethylene-dibromide and Fenamiphos. The pre-plant nematicide treatments proved inadequate, but improved nematode control was achieved when post-plant applications of Fenamiphos followed these treatments. Total yields in soil amended with poultry manure or sawdust plus urea were higher than in non-amended soil and equal to or higher than those in the best nematicide treatments. The yield increased for poultry manure appeared to be due in part to its beneficial effects on soil fertility.

Duhaylongsod (1988) incorporated various organic amendments along the furrows of microplots at rates of 10 tons/ha in soil infested with *Meloidogyne incognita or Rotylenchulus reniformis.* Three-week-old tomato CV. VC-11-1 seedlings were then transplanted to the plots. Fenamiphos at 10k a.i/ha was applied as a comparative control. Fresh chicken waste and composed sawdust caused the most initial and final reductions in *R. reniformis* levels. Composed Gliricidia leaves were ineffective against the nematode. Fresh Gliricidia leaves,

and chicken dung initially reduced *M. incognita* leaves, but only the later remained effective throughout the seasons, and fresh dung was more effective than Fenamiphos. Rice straw and sawdust also reduced *M. incognita* populations.

2.12.5. Management through biocontrol agent (*Trichoderma spp.*)

Hermosa *et al*. (2012), Howell (2006), Benitez *et al*. (2004), Harman *et al*. (2004) and Yedidia *et al.* (2001) described that the mechanisms that were deployed by *Trichoderma spp.* were antagonism, mycoparasitism, competition with pathogens for nutrient and space, and induction of systemic resistance in plants.

Harman (2000) and Shoresh *et al.* (2010) stated that *Trichoderma spp.* were also known to enhance plant growth and productivity.

2.12.5.1. *Trichoderma spp.* against *Fusarium spp.*

Ramaiah and Garampalli (2015) isolated 20 indigenous *Trichoderma* species from tomato rhizosphere soil collected from tomato growing fields in and around Mysore, Karnataka, India among which eight isolates displayed significant activity against the test pathogen. Among the eight isolates two isolates, *T. harzianum,* and *T. viride* exhibited excellent inhibitory effects on the test pathogen *Fusarium oxysporum* f. sp. *Lycopersici* in dual culture technique. The most effective and completely inhibited the mycelial growth at 75% concentration, followed by *A. flavus, T. koningii, T. viride, P. italicum, and P. citrinum.*

Kulkarni (2015), Dubey *et al.* (2011) and Harman (2000, 2006) stated that in the current system, soil amendment with the biocontrol agent *Trichoderma harzianum* might be a good strategy. *Trichoderma spp.* had been well-documented to control a wide range of soil- and seed-borne pathogens.

Lugtenberg and Kamilova (2009) provided an environmentally friendly *Fusarium* spp. control by using of antagonistic microorganisms represented an alternative disease management strategy

Chakraborty and Chatterjee (2008) evaluated biological control agent for wilt disease of eggplant (*Solanum melongena* L.) caused by *Fusarium solani.* Application of five *Trichoderma* species, *T. harzianum, T. viride, T. lignorum, T. hamatum* and *T. reesei* was carried out. The effect of volatile and non-volatile antibiotics of *Trichoderma* origin on growth inhibition of the wilt pathogen was studied. *T. harzianum* showed maximum growth inhibition (86.44 %) of the pathogen through mycoparasitism. The non-volatiles produced by the *Trichoderma* species exhibited 100 % growth inhibition of the pathogen under *in vitro* condition Treatments with two most efficient *Trichoderma* species, *T. harzianum* and *T. viride* resulted in the decreasing population of *Fusarium solani* in the soil thereby deterring disease incidence in field condition.

2.12.5.2. *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

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.

2.12.5.3. *Trichoderma spp.* against *Meloidogyne spp.*

Mahfouz *et al.* (2010) stated that the efficacy of carbofuran at 1 mg a.i./kg soil, *Serratia marcescens* (1 x 109 bacterium cells/ml water) at 2 ml of the suspension/kg soil, and three different *Trichoderma harzianum* isolates each separately added at 50 ml/kg soil against the root-knot nematode *Meloidogyne incognita* on two tomato cultivars Super Strain B and Alisa, was assessed in the glasshouse. The fresh and dry weights of shoots were higher in nematode-free plants of the two cultivars than both *M. incognita*-infested plants and the above-mentioned treatments. Carbofuran followed by *S. marcescens* and *T. harzianum* generally decreased nematode development and reproduction parameters compared to the untreated control. Although chemical nematicide viz. carbofuran showed a significant effect in the increase of growth parameters and in the suppression of *Meloidogyne incognita* multiplication, it can be replaced to some extent by microbial antagonists viz. *Serratia marcescens* and *Trichoderma harzianum* isolates to comply with environmental issues confronting the use of chemicals.

Dhawan and SinghSatyendra (2009) stated that the effect of antagonistic fungus, *Pochonia chlamydosporia*, a parasite of nematode eggs, neem cake and carbofuran alone and in combinations were evaluated in micro plots infested with *Meloidogyne incognita* on okra under net house conditions.

Zakir and Bora (2008) assayed on population dynamics of *Pseudomonas fluorescens* and *Trichoderma harzianum* from two different substrates *viz.* vermicompost and wheat bran after different days of storage revealed that both

the substrates, the population density of *Pseudomonas fluorescens* significantly increased up to 45 days of storage (Liza Barua and Bora, 2009). The highest reduction of *Meloidogyne incognita* and *Ralstonia solanacearum* population in soil was observed in combined application of *T. harzianum* and *P. fluorescens* when applied against the complex. *P. fluorescens* was proved *to be more promising followed by T. harzianum in suppressing the population of R. solanacearum.* The treatment effectively improved all the plant growth parameters and yield of the crop with the corresponding decrease in the nematode reproductive rate. The treatment also produced a minimum final bacterial population in the soil along with less per cent wilt incidence.

Rangaswamy *et al.* (2000) *Pasteuria penetrans* alone or in combination with neem cake, parasitized the *M. incognita* juveniles and adults whereas, *T. viride,* alone or in combination with either neem or castor cake, was most effective in parasitizing the egg masses of the nematode in tomato

Zakir and Bora (2009) stated that the integration of summer ploughing, half recommended dose each of carbofuran 3G, neem cake, streptocycline and a full dose of *Trichoderma harzianum* were found superior treatments against *Meloidogyne incognita* and *Ralstonia solanacearum* complex in brinjal under field conditions.

CHAPTER III

METHODS AND MATERIALS

3.1. Experiment site and duration

1. *In vitro* experiment was done in Plant Pathology laboratory, Sher-e-Bangla Agricultural University for evaluating the efficacy of chemicals during August- September, 2017.

2. Pathogenicity test of the isolated pathogens were conducted in the net house of Department of Plant Pathology, Sher-e-Bangla Agricultural University during September- October, 2017.

3. The field experiment was conducted in the research field of Plant Pathology Division of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur to find out the effect of different treatments for integrated approach for the management of wilt complex of eggplant during September, 2017 to April, 2018.

3.2. Collection, Isolation and identification of causal agents

3.2.1. Preparation of different types of media

The following media were used for the isolation of the targeted pathogens.

3.2.1.1. Potato Dextrose Agar (PDA) media

Composition of PDA media

Ingredient	**Amount (Per liter)**
Potato slices	200 gm
Dextrose	20 gm
Agar	20 gm
Water	1 L

200 gm sliced, peeled potatoes were boiled in 1 liter distilled water to make potato infusion for 30 min. Potato infusion was Filtering through cheesecloth, saving effluent, Dextrose, Agar and Water (if needed to fill 1 L) was Mixed and boiled to dissolve. The mixture was sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. pH was adjusted to 5.5 ± 0.2.

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

Composition of CPG media

Ingredient	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 1 L 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.

3.2.1.3. Triphenyl Tetrazolium Chloride (TTC or TZC) medium

Composition of TTC media

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

The mixture was sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. It was mixed well before dispensing. After autoclaving the medium was cooled to 55℃ and added 5 ml of a 1% stock solution of 2, 3, 5-tripheny

tetrazolium chloride. The stock was filter sterilized or autoclaved for 5 minutes at 121℃, and stored at 4℃ or frozen. Final pH should be 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).

3.2.1.4. Nutrient Agar (NA) and Nutrient Broth (NB) Media:

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. pH after sterilization7.3±0.1.

Nutrient Broth (NB) media

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

For 1 L of NB media, it was prepared according to the composition of NA media where agar was not used.

3.2.2. Collection of diseased samples

Samples were collected from the infected plants of eggplant. For isolation of fungus (*Fusarium oxysporum*) and bacteria (*Ralstonia solanacearum)* infected stem was collected while for isolation of nematode (*Meloidogyne spp*.) infected root sample was chosen.

3.2.2.1. Isolation and identification of *Fusarium oxysporun f. sp. melongena* causing fungal wilt of eggplant

The experimental plots were inspected routinely to observe the *Fusarium* wilted plant. To identify the pathogen, the diseased plants were collected from the field and were taken to the laboratory, the diseased stem were cut into small pieces (about 0.5-1cm) and surface sterilized by dipping in 10% Sodium Hypochlorite solution for 2-3 minutes. The cut pieces were then washed in water for three times and were dried by soaking the extra water with sterilized tissue paper. The pieces were placed into PDA media and moist chamber with help of sterile forceps and incubated at 25 ± 1^0 C for 10-15 days. Later the pathogen was purified using hyphal tip culture method and grown on PDA media at 25 ± 1^0 C for 2 weeks. The organism was identified based CMI description. (Plate. 1)

For maintenance, pure culture of *Fusarium oxysporum* was transferred to PDA media and maintained at 4^0C and subculture was done periodically within 2 months for further use.

3.2.2.2 Isolation and identification of the bacteria causing bacterial wilt of eggplant

Infected eggplant showing typical symptoms of bacterial wilt were collected and cut obliquely at the base of the stem and surface sterilization was done. The bacterial wilt was confirmed by the bacterial ooze test. The stem was cut into small pieces (5-6 cm) and placed in sterile distilled water. The stem was allowed to secrete milky white thread like ooze from the cut ends of the diseased tissue in water which made the water turbid. This ooze (turbidity) confirmed the presence of bacteria causing bacterial wilt (Fig. 6). The pathogen was isolated on Triphenyl Tetrazolium Chloride (TTC) media by serial dilution of the ooze suspension followed by spreading method (Kelman, 1954). One hundred microliter of the diluted bacterial suspension was poured onto the surface on the solidified TTC media in sterilized Petri plates. The bacterial suspension was

spread onto the surface of TZC medium with a sterilized spreader. The inoculated plates were incubated at 32^0 C for 48 hours. The isolated bacterium was identified as *Ralstonia solanacearum* by morphological and colony characters (Plate 2).

The isolated bacterium was identified as *Ralstonia solanacearum* under refrigeration at 20^oC for maintenance of virulence.

3.2.2.3. Isolation and identification of nematode

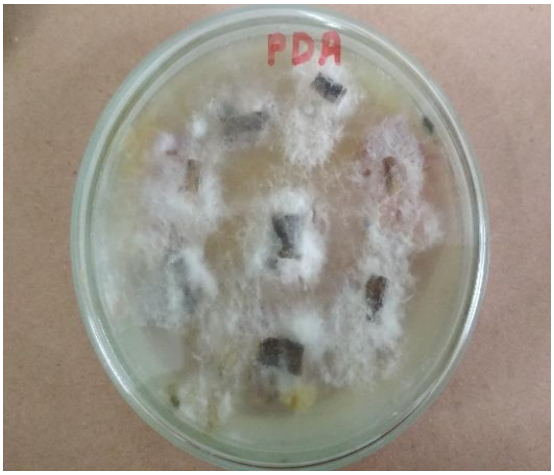
A plant showing nemic wilt symptom was uprooted and washed the root gently under running tap water. Egg masses were picked and a single egg mass of the root knot was crushed in distilled water and studied under compound microscope (Plate 3).

3.2.2.4. Maintenance of *Meloidogyne spp.*

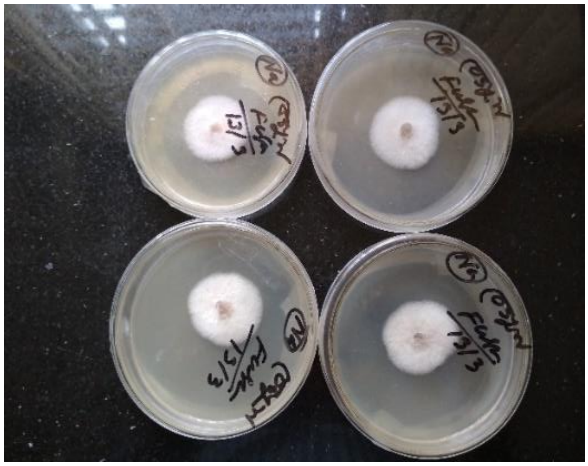
Egg masses were picked up and kept for hatching in water in petri dish. After 24 to 36 hours hatched out second stage (J2) juveniles inoculated in twenty-one days old seedlings grown in sterilized soil and maintained in net house. Some infected root was also mashed in blender and eggs are separated from upper solution after precipitation of 30 minutes. Eggs were also inoculated in 21 days old seedlings of eggplant for maintaining nematode in pot culture. After 100 to 150 days the nematode allowed to complete 2-3 generations, the plants were depotted carefully and root system was washed free of soil. The galls containing egg masses were again used to inoculate 21 days old seedlings grown in earthen pots containing sterilized soil for mass multiplication of the nematode under aseptic conditions.

A. Infected *Fusarium* wilted plant

B. Putting the infected stem on moist chamber



C. Growth of *Fusarium* after 10 days on PDA media

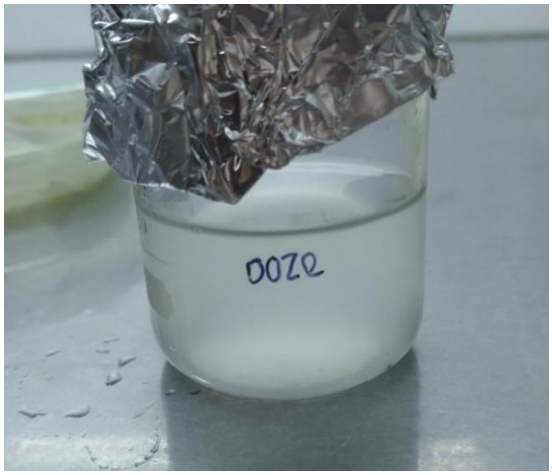


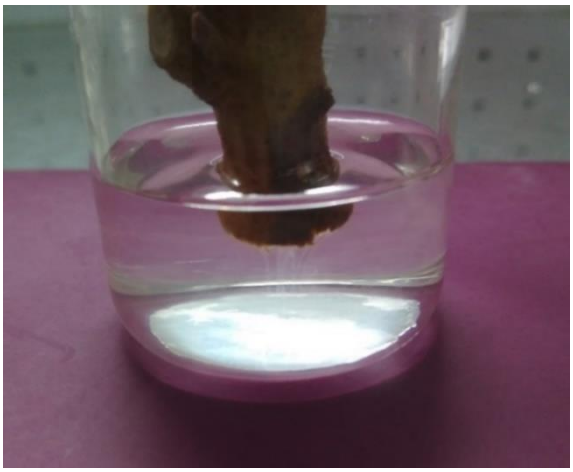
D. Pure culture of *Fusarium oxysporum*

Plate 1: Isolation and preparation of pure culture of *Fusarium oxysporum* from infected stem.

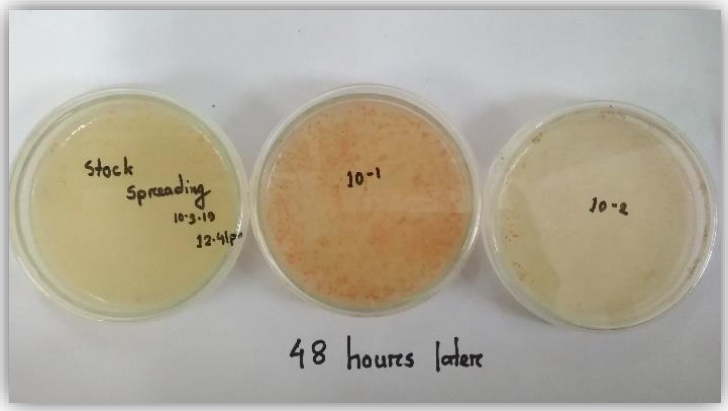
(a) Symptom of bacterial wilt

(b) Brown discoloration at vascular system with ooze





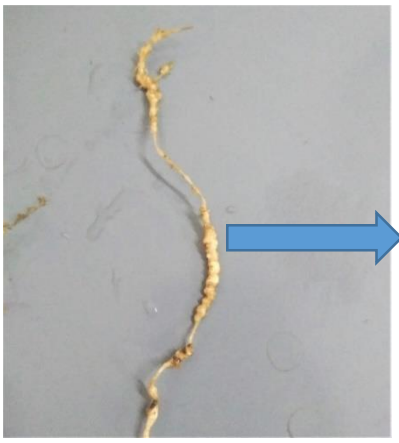
(c) and (d) Ooze from infected stem collected in SDW



(d) Pure culture of *Ralstonia solanacearum* prepared from the ooze by serial dilution

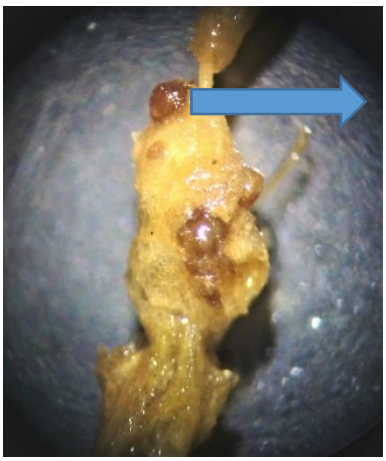
Plate 2. Isolation and preparation of pure culture of *Ralstonia solanacearum* on TTC media from infected stem by dilution plate technique.

a) Plant root heavely infected by Nematode

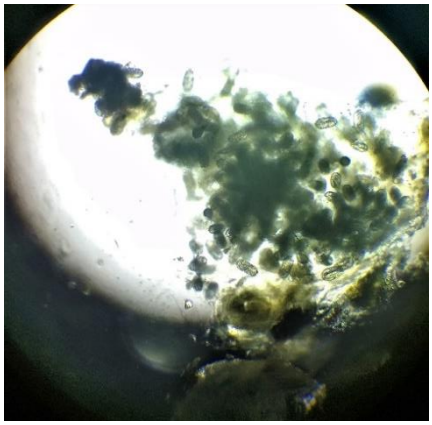


b) Typical symptom of root knot

c) Egg mass under compound microscope



d) Egg mass on knot under sterio microscope



e) Egg release from egg mass

Plate 3. Isolation of nematode from infected plant

3.3. *In vitro* assay of Autostin 50WP and Krosin 10 SP against wilt pathogens

3.3.1. Methods

The selected chemicals in this research (Autostin 50 WP and Krosin AG) were tested for their *in vitro* inhibitory activity against *Fusarium oxysporum* and *R. solanacearum*, respectively by poisoned food technique of Islam (2005). In case of *in vitro* assay, the efficacy of chemicals was assayed by following food poison techniques.

i. Cup/ Groove method
ii. Disc / Zone inhibition method

3.3.2. Preparation of chemicals

0.1% Autostin 50 WP and 0.05% Krosin 0 SP were prepared by dissolving 1 g and 0.5 g of the respected chemicals in 1 L Sterile Distilled Water (SDW). The solution was mixed well to ensure complete solubilization.

3.3.3. Preparation of pure culture *Fusarium oxysporum* and *Ralstonia solanacearum*

The isolated *Fusarium oxysporum* and *R. solanacearum* were purified by repeated sub-culturing at regular intervals on the PDA media and TTC media, respectively. The media were then incubated at 25±2°C. Therefore, the cultures were purified by the single spore isolation technique. 7 Days and 2 days old culture was needed for bioassay of *Fusarium oxysporum* and *R. solanacearum*, respectively.

3.3.4. Bioassay for *Fusarium oxysporum* by cup method

After autoclaving the media all the work was done in Laminar air flow cabinet. Five milimeter disc of PDA media was scooped from three places maintaining an equal distance from the centre by a sterilized disc cutter. One milileter

fungicidal solution was put into each hole and stored overnight for diffusion of the chemical. The next day 5 mm mycelial discs of *Fusarium oxysporum* (seven-day-old) were taken and aseptically inoculated at the center of the PDA plates. Holes filled with SDW was served as control (Islam, 2005). All the Plates were incubated at 25 ± 2° C for seven days and mycelial growth of the pathogen was recorded and % inhibition of mycelial growth was calculated using the formula of Islam (2005).

$$\text{Inhibition of mycelial growth\%} = \frac{A - B}{A} \times 100$$

Where,

A = Mycelial growth in control (cm)

B = Mycelial growth in treatment (cm)

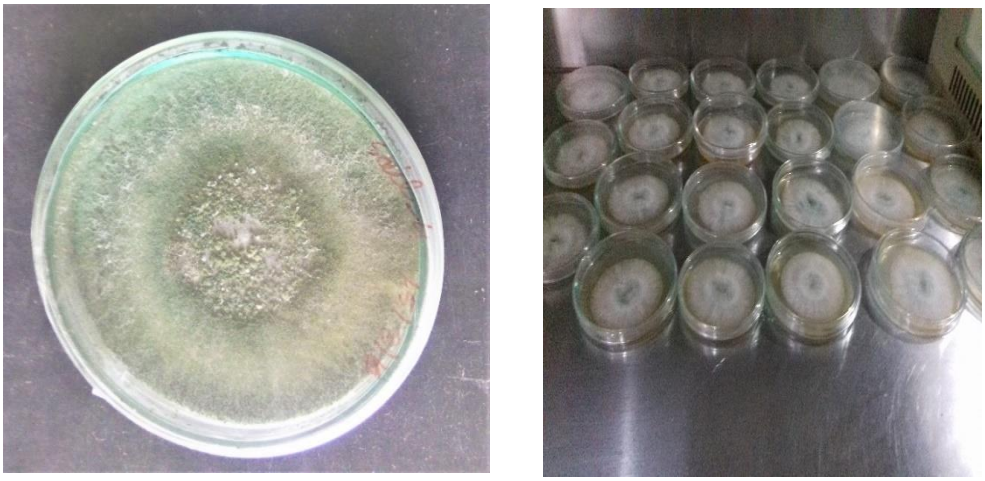
3.3.5. Krosin 10 SP against *R. solanacearum* by Disc method

Bioassay of antibacterial chemicals against the bacteria was done by well diffusion method measuring the inhibition zone. At first, two test tubes each containing 10 ml nutrient broth were taken which were inoculated with 48 hours old pure culture of bacteria grown on NA plate. Then the test tubes were transferred in shaker incubator machine maintaining 30^{o}C temperature and 150 rpm for 24 hours. After shaking, the broth culture was spread uniformly on it with the help of sterile cotton swabs. Then 5 mm disc of filter paper (whatman-2) was soaked in chemical suspension @0.05% and placed at the centre of the inoculated plates. In case of control only sterile water was used instead of chemical. It was replicated 3 times. The plates were incubated at 30^{o}C in incubation chamber for 48 hours. Diameter of inhibition zone was then measured around the disc (Islam, 2005).

3.2.6. Mass multiplication of *Trichoderma spp.*

Pure culture of *Trichoderma harzianum* was collected from laboratory of Plant Pathology Division, BARI. Then it was mass multiplied on black gram bran *in*

vitro. The sterilized substrate was allowed to cool down at room temperature and then inoculated with 5 mm disc of *Trichoderma harzianum*. Ten (10) disc for each 500 ml flask were used for inoculation. It was then incubated at room temperature 25± 2^0c. After incubation for 25 days the substrate was taken out from the flask, shade dried in Laminar air flow cabinet and grinded in a blender. Then it was treated as *Trichoderma* formulation Islam (2005). Mass multiplication was carried out according to the necessity of the field experiment (Plate 4).



a) Mother culture of *Trichoderma harzianum* b) Sub culturing on PDA media

(c) Mass multiplication of *Trichoderma harzianum* on black gram substrate

Plate 4: Mass multiplication of *Trichoderma hazianum*

3.4. Pathogenicity test of fungus, bacteria and nemic isolates 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 (Plate 5). 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.

a) Soil thoroughly mixed with formalin

b) Soil covered with polythene sheet

Plate 5. Soil sterilization

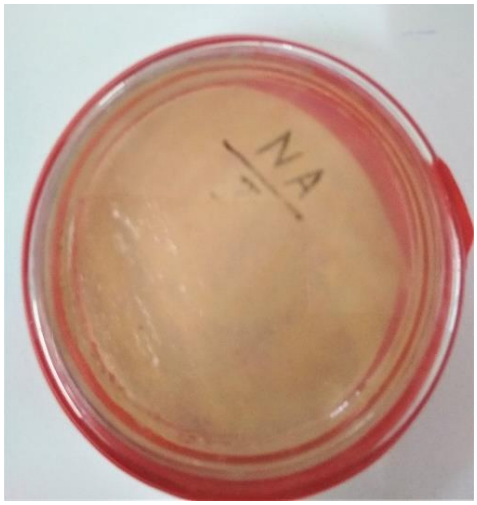
3.4.2. Seedling preparation

Eggplant seedlings were raised in plastic bags containing sterilized soil having fertilizers as per the package of practices. The seedlings were watered and monitored regularly.

3.4.3. Pathogenicity test of bacterial wilt of eggplant

Twenty-five days old seedlings of eggplant were treated with 5 ml spore suspension of *Ralstonia solanacearum* by injecting into the vascular system of the seedlings where inoculum density was 10^5 CFU ml^{-1} solution. Uninoculated plants served as positive control. The plants were watered regularly and observed

for appearance of wilt symptom. The plant expressing wilt symptoms were selected, the bacterium was re-isolated and compared with the original culture of *Ralstonia solanacearum* to satisfy the Koch's postulates (Plate 6).



a) Pure culture of *Ralstonia solanacearum*

b) Ioculation of spore suspension by injection

d) Uninoculated pot served as control.

e) Ioculation of spore suspension by injection

Plate 6: Pathogenicity test for bacterial isolates

3.4.4. Pathogenicity test of fungal wilt of eggplant

Seedlings of six-leaf were selected for pathogenicity test using the root-dip assay modified from that of Ramaiah and Garampalli (2015). Spore suspension was obtained from 7 days old culture of *Fusarium oxysporum* on PDA media and seedlings with wounded roots was submerged for 10 min with 50 ml of the conidial suspension (1×10^6 conidia ml^{-1}) in sterile H_2O. Control plants were dipped in SDW. After treatment the seedlings were transplanted into pots and kept in shade house. The plants were watered regularly and observed for appearance of wilt symptom. The plants expressing wilt symptoms were selected, the fungus was re-isolated and compared with the original culture of *Fusarium oxysporum* to satisfy the Koch's postulates.

a) Root clipping of eggplant

b) Root dipping in fungal spore suspension

d) Inoculated plant

d) Uninoculated plot served as control

Plate 7: Pathogenicity test for *Fusarium oxysporum* by root-dip assay method

3.4.5. Pathogenicity test of nemic wilt disease of eggplant

Egg masses were picked up and kept for hatching in water in petri dish. After 24 to 36 hours hatched out second stage (J2) juveniles inoculated in twenty-one days old seedlings grown in sterilized soil and maintained in net house. Some infected root was also mashed in blender and eggs are separated from upper solution after precipitation of other material after 30 minutes. Eggs were also inoculated in 21 days old seedlings of eggplant. After 100 to 150 days the nematode allowed to complete 2-3 generations, the plants were depotted carefully and root system was washed free of soil.

3.5. Integration of treatments for management of wilt complex under field conditions

3.5.1. Experimental site and Duration

The experiment was conducted in the research field of Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during September, 2017 to April, 2018.

3.5.2. Soil type

The soil of the experimental plot was loam to clay loam in texture belonging to the Madhupur Tract (AEZ-28). Medium high land, fairly good drainage field with above flood leveled condition.

3.5.3. Climate

The climate of the experimental area was tropical to sub-tropical. 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 weather station of Bangladesh Rice Research Institute, Joydebpur, Gazipur 1701 which was shown in (**Appendix 1**).

3.5.5. Variety

BARI hybrid Begoon-3 (oblong) was used for the experiment.

3.5.6. Collection of seeds

Ten gram of healthy, matured and disease free seeds of eggplant variety BARI hybrid begoon 3 were collected from HRC, BARI, Joydebpur, Gazipur (Fig. 1).

Fig. 1: Collection of seed

3.5.7. Raising of seedlings

Five gram of BARI Hybrid Begoon -3 was sown in lines on a well prepared fertile seedbed. Seedlings were monitored regularly and watering was done upto transplanting in the field as per need (Plate 8). Weeding and thinning were carried out as per necessity in the seedbed.

a) Eggplant seeds were shown in line

b) Eggplant seedlings were ready to transplant

Plate 8: Raising of seedlings in seedbed

3.5.8. Treatments

Treatment no.	Treatment and their combination
T_1	Autostin 50 WP (seedling treatment + foliar spray @ 0.1%)
T_2	Furadan 3 G (soil treatment) @ 10 g/ pit
T_3	Krosin 10 SP (seedling treatment + drenching of rhizosphere soil @ 0.05%)
T_4	*Trichoderma* formulation (Soil application) @ 20g/ m^2 (1×10^8 cfu/g of soil)
T_5	Poultry waste (soil amendment) @ 2 ton / ha
$T_6 = T_1 + T_4$	Autostin + *Trichoderma* formulation
$T_7 = T_2 + T_4$	Furadan + *Trichoderma* formulation
$T_8 = T_3 + T_4$	Krosin + *Trichoderma* formulation
$T_9 = T_1 + T_5$	Autostin + Poultry waste
$T_{10} = T_2 + T_5$	Furadan + Poultry waste
$T_{11} = T_3 + T_5$	Krosin + Poultry waste
$T_{12} = T_4 + T_5$	*Trichoderma* formulation + Poultry waste
$T_{13} = T_1 + T_4 + T_5$	Autostin + *Trichoderma* formulation + Poultry waste
$T_{14} = T_2 + T_4 + T_3$	Furadan + *Trichoderma* formulation + Krosin
$T_{15} = T_2 + T_3 + T_4 + T_5$	Krosin+ Furadan + *Trichoderma* formulation + Poultry waste
T_{16}	Control

3.5.9. Collection of test materials

Autostin 50WP, Krosin 10 SP, Furadan 5G and Poultry waste were purchased from the local market of Joydebpur, Gazipur. Pure culture of *Trichoderma harzianum* was collected from culture stock of Plant Pathology laboratory, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. Later it was mass multiplied on black gram bran in Plant Pathology laboratory, SAU, Dhaka.

3.5.10. Application of manure and fertilizers

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

Doses of fertilizer

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.

3.5.11. 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 sixteen unit plots of 3.0m x 1.0m. Forty-eight plot was made with maintaining 0.6m × 0.6m planting distance. Each bed was designed with five pit for transplantation. The total land area was 24.5m x 11.0m =269.5 m^2. Every treatment combination put once at each block **(Appendix 2)**.

3.5.12. Application of treatments

3.5.12.1. Foliar spray and drenching with Autostin 50 WP

Roots of seedlings were treated with Autostin 50 WP @ 2g /L of water during transplanting and foliar spraying 3 times at 10 days' interval after transplantation.

Details of Fungicides

Trade name	Chemical name	Active ingredient	Mode of action	Conc. (ppm)/(Pg ml^{-1})
Autostin 50 WP	Methyl-2-Benzimidazole Carbamate	50% Carbendazim	Systemic	50, 100

(Rashid, 2000)

3.5.12.2. Soil treatment with Furadan 5 G

Five gram of furadan was applied and mixed well with the rhizosphere soil that are assigned for furadan during transplanting of eggplant seedlings. The specification of nematicide is described below

Details of Nematicide

Trade name	Chemical name	Active ingredient	Mode of action
Furadan 5G	Carbamic acid, methyl-2,3-dihydro-2,2-dimethyl-7-benzofuranyl ester	Carbofuran	Systemic

(Rashid, 2000)

3.5.12.3. Application of Krosin 10 SP / Bactericide

Roots of seedlings were treated with Krosin 10 SP @ 0.5gm/L of water during transplanting and drenching of root zone was done 3 times at 10 days' interval after transplantation.

3.5.12.4. Soil treatment with Poultry waste

Poultry waste was applied @ 2 kg / plot as soil amendment before 21 days of transplanting in the allotted plots and mixed properly with the soil.

3.5.12.5. Application of *Trichoderma* formulation

Spore suspension was prepared by scraping the 10-12 days old culture maintaining the concentration $1x10^8$ conidia / ml solution. The roots of seedlings were soaked with *Trichoderma* suspension. The soil of the specific plot was treated @ 200gm/pit of black gram based *Trichoderma* formulation. This treatment was given at the time of transplanting (Plate 9).

a) *Trichoderma* formulation

b) *Trichoderma* suspension

c) Seedling treatment with *Trichoderma* suspension

d) Application of *Trichoderma* formulation in pit.

Plate 9 . Application of *Trichoderma* formulation

3.5.13. Seedling Transplantation

After preparation of the experimental field, 35 days old seedlings were uprooted from the seedbed and transplanted in the experimental plots on the 14th December, 2017 in the afternoon (Fig: 2). The seedbed was watered two hours before transplanting to minimize root damage during uprooting of seedlings from the seedbed. Seedlings were transplanted maintaining 60 cm × 60 cm planting distance in the plot and each plot contain five plants. Just after transplantation the seedlings were sufficiently watered with the help of a bucket sprinkler. Each pit received one seedling where the pit was previously prepared according to treatment. Watering was continued till the seedlings were established in the field.

Fig. 2. Seedling transplantation in field

Fig. 3: Field view of experimental plot

3.5.14. Intercultural operations

After transplantation, gap filling was done where seedlings were not established. The plants were monitored regularly. At 15 to 20 days after transplanting weeding was done which followed by split application of fertilizer. After split application of fertilizer flood irrigation was given where excess water was allowed to be drained out. Channel irrigation was done as per requisition. General field sanitation was maintained throughout the growing period by removing infected and blighted leaves, wilted and dead plants.

a) Irrigation in experimental field

b) Symptom of the infected shoot atatck by brinjal shoot and fruit borer

c) Insecticide (Ripcord 10EC & Pegasus) d) spraying of insecticide for controlling brinjal shoot and fruit borer and Aphid

Plate 10: Intercultural Operation in experimental field

3.5.15. Collection of Data

A) Disease incidence (DI)

a) Number of infected plant by *Fusarium* sp.
b) Number of infected plant by *Ralstonia solanacearum*
c) Number of infected plant by *Meloidogyne* sp.

B) Yield and yield contributing characters

- Plant height (cm)
- Number of branch plant $^{-1}$
- Number of leaf plant^{-1}
- Number of fruits plant^{-1}
- Fruit length
- Yield plant^{-1}
- Yield plot^{-1}
- Yield ha^{-1}

3.5.16. Calculation of Disease Incidence (DI)

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

The disease incidence was calculated by the following formula:

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

3.5.17. Termination of the experiment

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

3.5.18. Cost-Benefit Analysis and Calculation of Benefit Cost Ratio (BCR)

Costing of application of management of wilt of eggplant was done based on the current market price of input, rate of hiring labour 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:

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

3.5.19. 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

The results of the experiments were presented in this chapter for evaluation of the efficacy of treatments to manage the wilt complex of eggplant, caused by *Fusarium oxysporum*, *Ralstonia solanacearum* and *Meloidogyne spp*.

4.1. Identification of causal organisms

4.1.1 Identification of fungi

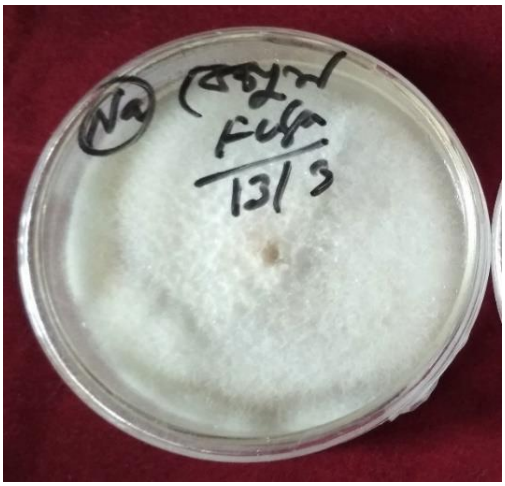
The cultural and morphological appearances viz. colony colour, pigmentations, mycelium and conidia) were observed on potato dextrose agar (PDA) media. The isolated fungus was identified using Methuen handbook of colour chart (Kornerup and Wancher, 1978) (Table 1).

Table 1. Cultural and morphological characteristics of *Fusarium oxysporum*

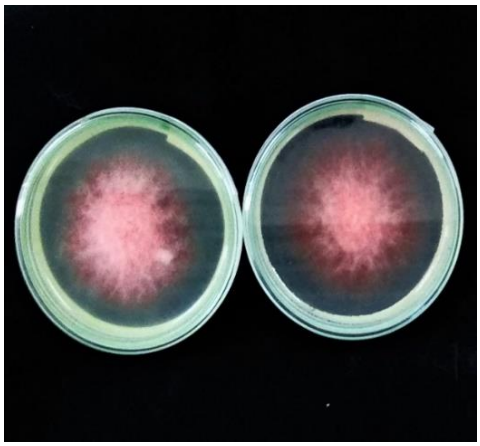
Colony propagule	Charecteristics	Septation	Color
Colony	Luxuriant with regular cottony growth		White later causing peach-violet discoloration of medium
Mycelium	Smooth and branched	Septate	Hyaline
Macro-conidia	Relatively slender in shape with curved apical cell and foot shaped basal cell.	Septate	Hyaline

Barnett and Hunter (1972) reported details about *Fusarium spp.* which was in agreement with the findings above. Moreover, they found micro conidia of *Fusarium spp.* which was 1-celled, hyaline, ovoid to oblong.

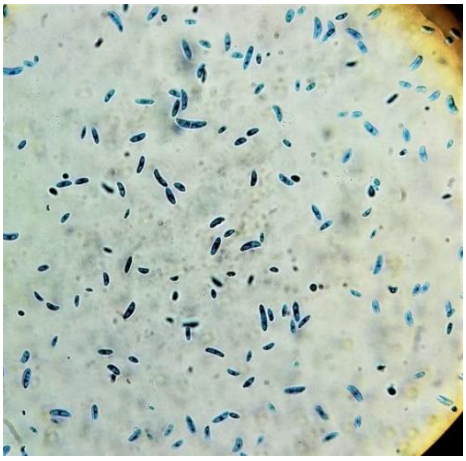
Gerlach and Nirenberg (1982) and Nelson *et al.* (1983) described the colony appearances of *F. oxysporum* on PDA as highly variable. The peach-violet colony colour and pigmentations observed in isolates which were consistent with the description by Booth (1971). Therefore, in the present study, isolates of *F. oxysporum* were clearly identified based on cultural and morphological characteristics.



(a) and (b) Pure culture of *Fusarium oxysporum* showing whitish mycelial growth on PDA media;



c) Pure culture of *F. oxysporum* showing peach violet discoloration on PDA media in reverse side of pertiplate



d) Macroconidia and microconidia of *Fusarium oxysporum* under compound microscope 40X.

Plate 11: Physical features of *Fusarium oxysporum*.

4.1.2 Identification of bacteria

4.1.2.a. Morphological characters

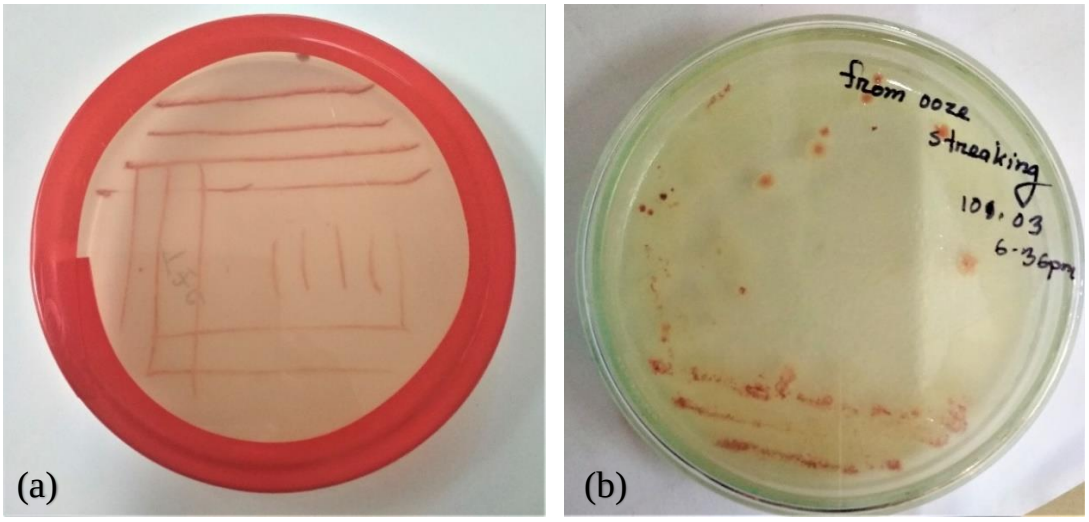
The bacterium was rod shaped with rounded ends, gram negative (red colour) and capsulated under the compound microscope at 125X magnification with oil immersion objectives.

The findings were in agreement with the findings of Kelman (1981) who reported that *Ralstonia solanacearum* is a soil-borne aerobic, rod shaped, non-spore forming, gram-negative bacterium. The bacterium is positive in oxidase and negative arginine dihydrolase reaction.

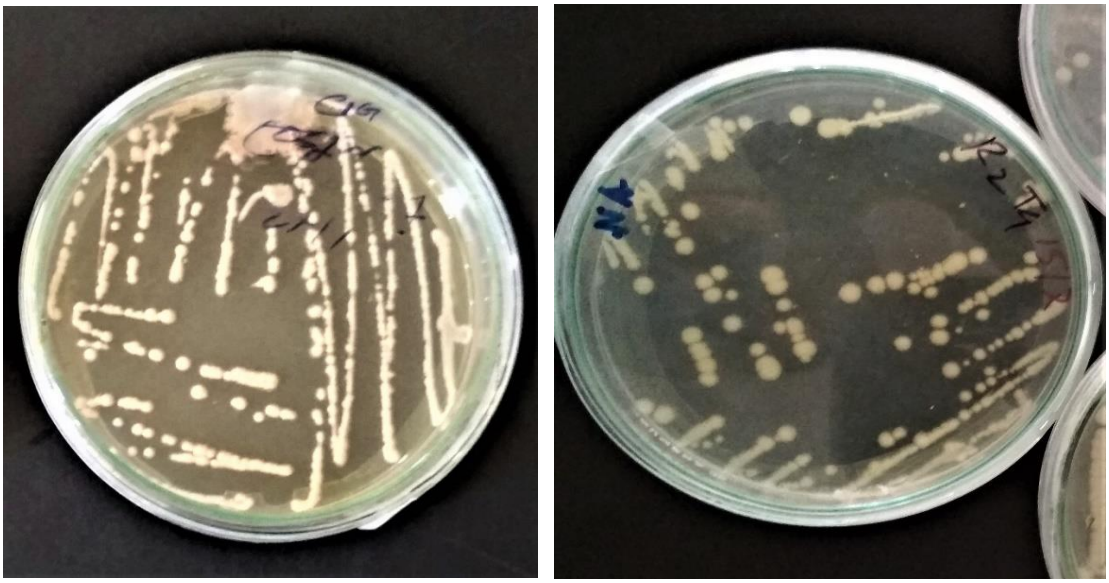
4.1.2.b. Colony morphology on different growth media

Colonies of *Ralstonia solanacearum* of some samples on TTC medium appeared as red coloured with whitish margins (virulent). Circular, mucoid, convex, lucid coloured colonies were found on NA medium in respect to some samples (virulent) (Plate. 12).

These findings were matched with the report of Khan et al., (1974). He stated that *R. solanacearum* produced colonies on TZC medium which were highly fluidal, white colour with slight pink center, round to irregular in shape. Further, Shoba (2002) and Prasanna Kumar (2004) observed similar characters of isolates on TZC medium.



(a) and (b) *Ralstonia solanacearum* growing on semi selective TTC media giving pink coloured colonies with whitish margin)



(c) Pure culture of *Ralstonia solanacearum* on CPG media

(d) Pure culture of *Ralstonia solanacearum* on NA media

Plate 12: Physical features of *Ralstonia solanacearum*

4.1.3 Identification of nematode

The egg masses and female nematode were observed in stereomicroscope where pear shaped female surrounded by egg masses and was identified as *Meloidogyne spp.* Eggs and larvae were studied in semi-permanent slide under compound microscope which was prepared from root gall (Plate 13).

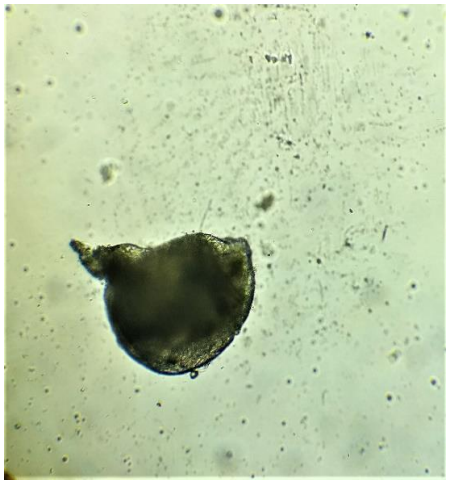
Table 2: Morphological characteristics of *Meloidogyne spp.*

Characters	**Original description (Goodey *et al*., 1965)**	**Observed**
Body	Male: Elongated larvae Female: Typical saccade, spheroid with a distinct neck.	Male: Elongated larvae Female : pear shaped, spheroid with a distinct neck.
Stylet	Male: Strong with rounded Knob. Female: Slenderer than in males or larvae but with strong basal knob.	Male: Strong rounded knob.
Oesophagous	With very large median bulb followed by short isthmus.	Large median bulb followed by short isthmus
Vulva and anus	Female: Typically, opposite to neck and surrounded by a pattern of fine lines resembling human finger prints.	Female: Opposite to neck and surrounded by a pattern of fine lines.
Spicules	Male: Very near to the terminus.	Male: Very near to the terminus.

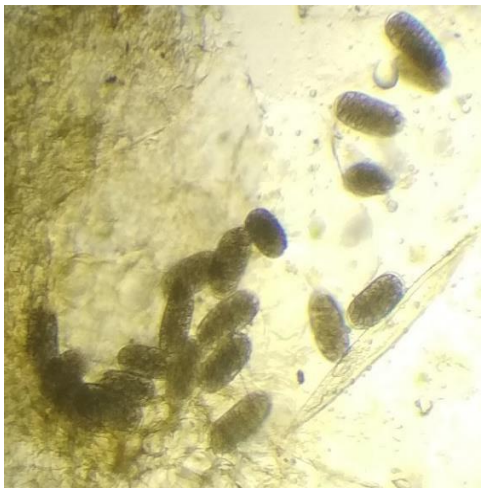
From the above table it could be told that the morphological features that were studied were alike to the report of Goodey *et al*. (1965).

The pear shaped female nematode of *Meloidogyne* sp. produced huge number of knots or galls in the root system and a remarkable number of egg masses were found along with the knots. This result was same with the report of Ali (1993) and Singh and Sitaramaiah (1994) in case of nematode infestation.

(a) Female nematode under steriomicroscope



(b) Pear shaped female nematode under compound microscope

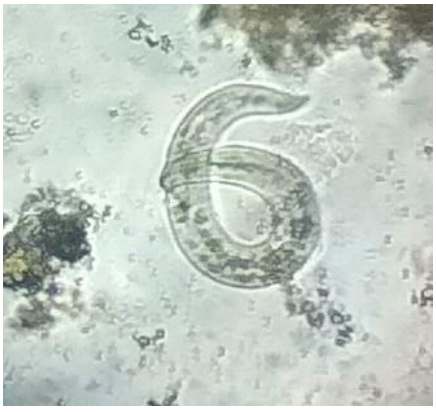


(c) Eggs after crushing of egg mass

(d) Juvenile developed in egg

(e)

(e) Juvenile under compound microscope



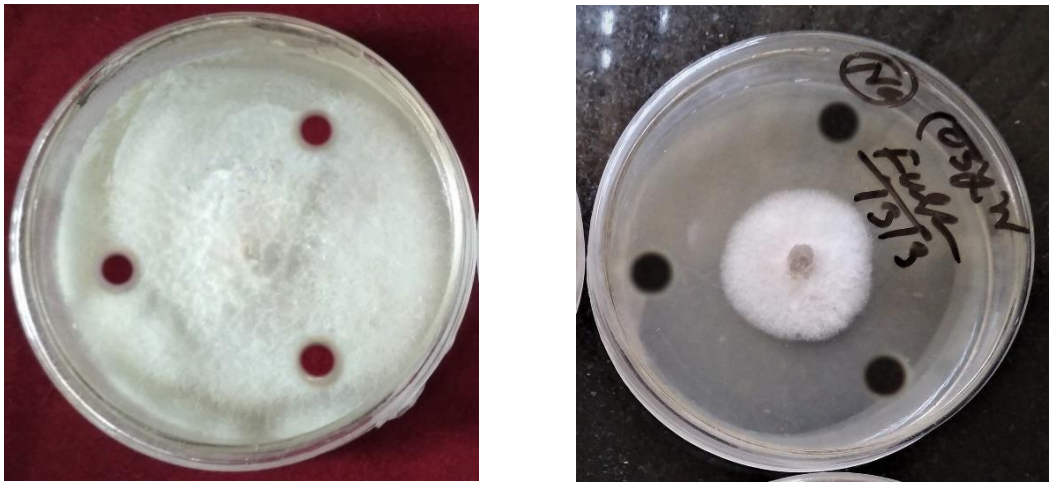
(f) adult male nematode under compound microscope

Plate 13: Particulars of *Meloidogyne spp.*

4.2. *In vitro* evaluation of chemicals against isolated pathogens

4.2.1. Bioassay of Autostin 50WP against *Fusarium oxysporum*

The fungicide Autostin 50 WP (Carbendazim) @ 0.1% was assayed *in vitro* against the *Fusarium oxysporum* causing wilt of eggplant and found promising in reducing the mycelial growth of the fungus. The redial mycelial growth was recorded 2.03 cm while it was 8.5 cm in control. The inhibition of radial mycelial growth was 87.88% over control.



A = Control plate B= Treated plate

Plate 14. Evalution of the efficacy of fungicide by food poisoning method (Cup method)

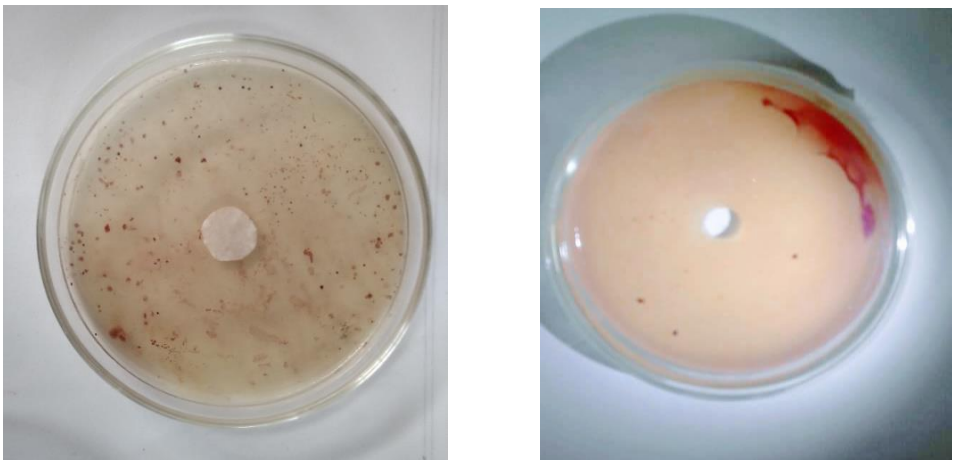
Table 3: Result of bioassay of Autostin 50 WP against *Fusarium oxysporum*

Treatment	Redial mycelial growth (cm)	% Inhibition over control
Autostin 0.1% (Carbendazim)	1.70	80.89
Control	8.90	--

In *in vitro* evaluation, Autostin 50 WP was found to be promising in reduction of the growth of *Fusarium oxysporum.* Amini *et al.*, (2010) reported that Carbendazim (Autostin 50 WP) found to be effective in controlling *Fusarium oxysporum* in *in vitro* and *in vivo* evalution.

4.2.2. Bioassay of Krosin 10 SP against *Ralstonia solanacearum*

The bactericide Krosin 10 SP (Streptomycin sulphate and Tetracycline hydrochloride) @ 0.05% was assayed in *in vitro* against the *Ralstonia solanacearum* causing wilt of eggplant and found promising in reducing bacterial growth. The redial of inhibition zone was recorded 7.5 cm while it was 0.0 cm in control. The inhibition of radial mycelial growth was 83.33% over control (Plate 15).



A = Control plate B= Treated plate

Plate 15. Evalution of the efficacy of bactericide by food poisoning method (disc method)

Table 4: Result of bioassay of Krosin 10 SP against *Ralstonia solanacearum*

Treatment	Redial mycelial growth (cm)	% Inhibition over control
Krosin 0.05% (Streptomycin sulphate)	7.5	83.33
Control	0.0	--

Murakoshi and Takahashi (1984); Svetlana Milijasevic *et al.* (2009) reported that antibiotics like Streptomycin found to be very effective in controlling *Ralstonia solanacearum* causing bacterial wilt.

4.3. Pathogenicity test

4.3.1. Pathogenicity test for *Fusarium oxysporum*

The eggplants were inoculated with inocula of *F. oxysporum* by root dip method (Sibounnavong *et al.* 2012) to test the capability of the fungus producing disease symptoms in eggplants. The inoculated plants were wilted after 20 days of inoculation. Reisolation of the fungus was made from the artificially inoculated infected plants on the PDA media. On the basis of various morphological and cultural characteristic and the result of pathogenicity test the fungus was identified as *Fusarium oxysporum.*

The result is supported by the findings of Adhikary *et al.* (2017) who observed that *F. oxysporum* f. sp. *melongenae* was able to produce wilting symptoms in eggplants. The finding is also supported by the finding of Begum (2007) and Altinok (2005) who reported wilting in eggplants in Turkey by *F. oxysporum* f. sp. *melongenae.*

(a) Fusarium wilted plant (b) Healthy Plant

Plate 16. Pathogenicity test for *Fusarium oxysporum*

4.3.2. Pathogenicity test for *Ralstonia solanacearum*

The isolates of *Ralstonia solanacearum* was inoculated to 25 days old eggplant seedlings by injection method. The inoculated plants lost turgidity, leaves started drooping and plants wilted suddenly (Plate 16). The first symptom of disease was observed within 7 days after inoculation. Reisolation 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 *Ralstonia 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).

This was in conformity with the results obtained by Subhalaxmi (1999) and Shobha (2002) by re-isolating the bacterium on TZC medium thus proving the Koch's postulates of the isolated bacterium.

a) Wilted plant inoculated with *Ralstonia solanacearum*)

b) Uninoculated healthy plant in case of control

Plate 17: Pathogenicity test for *Ralstonia solanacearum*

4.3.3. Pathogenicity test for *Meloidogyne spp.*

After 100 to 150 days the nematode allowed to complete 2-3 generations, the plants were depotted carefully and root system was washed free of soil. Knot was found on the root which initially confirmed the presence of *Meloidogyne spp.* Egg mass, juvenile, female nematode were similar to the organisms that were artificially inoculated. This finding was matched with Zakir and Bora (2009). He described that association of pathogenic and above pathogenic levels of inoculum of *Meloidogyne incognita* reduce the plant growth parameters of brinjal significantly.

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(a) Wilted plant inoculated with *Meloidogyne spp*. (b) showing knot on root

(b) Uninoculated healthy plant in case of control**.**

Plate 18: Pathogenicity test for *Meloidogyne spp.*

4.4. Effect of different treatments on Disease Incidence (DI) of wilt complex of eggplant

The effect of different treatments on disease incidence of wilt was recorded and data was presented in Table 5.

4.4.1. Effect of different treatments on disease incidence of fungal wilt of eggplant

The effect of different treatments applied in the integrated approach varied significantly on the incidence of fungal wilt compared to control. The disease incidence of fungal wilt was ranged from 0 to 13.33 %. Among the treatments, the highest disease incidence was recorded in control (13.33%). The second highest wilt incidence (6.67%) was recorded in case of treatment T_2 (Furadan 5G), T_3 (Krosin 10 SP), T_{10} (Furadan 5G+ Poultry waste), T_{11} (Krosin 10 SP + Poultry waste) while no wilt incidence was noticed in case of rest of the treatments viz. treatment T_1 (Autostin 50 WP), T_4 (*Trichoderma* formulation), T_5 (Poultry waste), T_6 (Autostin 50 WP + *Trichoderma* formulation), T_7 (Furadan 5G + *Trichoderma* formulation), T_8 (Krosin 10 SP + *Trichoderma* formulation), T_9 (Autostin 50 WP + Poultry waste), T_{12} (*Trichoderma formulation* + Poultry waste), T_{13} (Autostin 50 WP + *Trichoderma* formulation + Poultry waste), T_{14} (Furadan 5G + *Trichoderma* formulation + Krosin10 SP) and T_{15} (*Trichoderma* formulation + Furadan 5G + Krosin 10 SP + Poultry waste).

4.4.2. Effect of different treatments on disease incidence of bacterial wilt of eggplant

All the treatments significantly reduced bacterial wilt incidence compared to control, ranged from 0 to 33.33%. The highest disease incidence (33.33%) of bacterial wilt was recorded in control. The second highest disease incidence (13.33%) was recorded in case of T_5 (Poultry refuse) followed by T_1 (Autostin),

T_6 (Autostin + *Trichoderma* formulation), T_9 (Autostin + Poultry waste). No wilt incidence was noticed in term of treatment T_2 (Furadan), T_3 (Krosin), T_4 (*Trichoderma* formulation), T_7 (Furadan + *Trichoderma* formulation), T_8 (Krosin + *Trichoderma* formulation), T_{12} (*Trichoderma formulation* + Poultry waste), T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_{14} (Furadan + *Trichoderma* formulation + Krosin) and T_{15} (Krosin + Furadan + *Trichoderma* formulation + Poultry waste).

4.4.3. Effect of different treatments on nemic wilt incidence

Nemic wilt incidence was ranged from 0 to 13.33%. The highest incidence (13.33%) was observed in control. Disease incidence 6.67 % was found in treatment T_1 (Autostin), T_6 (Autostin + Trichoderma formulation), T_9 (Autostin + Poultry refuse) and T_{12} (Trichoderma formulation + Poultry refuse). No nemic wilt incidence was observed in case of treatment T_2 (Furadan), T_4 (*Trichoderma* formulation), T_7 (Furadan + *Trichoderma* formulation), T_8 (Krosin + *Trichoderma* formulation), T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_{14} (Furadan + *Trichoderma* formulation + Krosin) and T_{15} (*Trichoderma* formulation + Furadan + Krosin + Poultry waste).

4.4.4. Effect of different treatments on disease incidence of wilt Complex of eggplant

The cumulative of wilt complex differed significantly compared to control where disease incidence was ranged from 0- 60.0 %. The highest disease incidence of wilt complex was recorded in control (60%). The second highest disease incidence (20%) was recorded in case of T_5 (Poultry waste) followed by (13.33%) in T_1 (Autostin), T_6 (Autostin + *Trichoderma* formulation), and T_9 (Autostin + Poultry waste). The lower (6.67%) incidence was recorded in the treatment of T_2 (Furadan), T_3 (Krosin), T_{10} (Furadan + Poultry waste), T_{11} (Krosin + Poultry waste), T_{12} (*Trichoderma formulation* + Poultry waste). The highest performance to control wilt complex was noticed in case of treatment T_4

(*Trichoderma* formulation), T_7 (Furadan + *Trichoderma* formulation), T_8 (Krosin + *Trichoderma* formulation), T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_{14} (Furadan + *Trichoderma* formulation + Krosin) and T_{15} (*Trichoderma* formulation + Furadan + Krosin + Poultry waste) with no wilt incidence.

In case of wilt complex of eggplant, the treatment combinations showed better result than individual application of each of the management components. In most cases, the combination of Autostin 50 WP, Furadan 5G, *Trichoderma* formulation and Poultry waste contributed remarkable effort in controlling the wilt complex. It might be due to the combined effect of fungicide (Autostin 50 WP), bacteriocide (Krosin 10 SP), nematicide (Furadan 5G) and the suppressive action of poultry waste as soil amendment. It should be mentioned here that the combination of treatments where the Autostin 50 WP was absent also showed better results in reducing the fungal wilt incidence. It might be due to the presence of *Trichoderma* formulation that acted against *Fusarium oxysporum* and consequently controlled the fungal incidence (Adhikary *et el.,* 2017). It attributed that nematicide controlled nematode as well as bacteria. For the reason, in absence of bacteriocide Furadan 5G was enabled to combat the bacteria as the nemic infection facilitates the penetration of bacteria through wounds created by the stylet of the plant parasitic nematodes. Zakir Hussain and Bora (2008) reported that combined application of Carbofuran 3G, Neem cake, Streptocyclin and *Trichoderma harazianum* found to be effective in controlling *Meloidogyne incognita* and *Ralstonia solanacearum* causing wilt complex of Brinjal under field condition. Pavithra (2012) reported that integration of Streptocylin @0.5 g/L + COC 50% WP (1g/L) intercropped with Mustard reduced bacterial wilt incidence by 23.35%, reduction of knots by 72.4%.

A number of research reports are available on *Trichoderma harazianum* against soil born pathogen (*Harman et al., 1989; Hoque et al., 1990, Odhikary et al.,* 2017). Odhikary *et al.,* 2017 while working on 'Recovery of *Fusarium* wilt infected eggplant reported that pre- inoculation by *Trichoderma harazianum* significantly reduced the severity of fungal wilt caused by *Fusarium oxysporum.*

Table 5. Effect of different treatments against wilt incidence of eggplant

Treatments	Disease incidence (DI) (%)			Total DI (%)
	Fusarium Wilt	Bacterial wilt	Nemic wilt	
T_1	0.00 (0.71) b	6.67 (1.96) bc	6.67 (1.96) ab	13.33(3.21) bc
T_2	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc
T_3	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc
T_4	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c
T_5	0.00 (0.71) b	13.33 (3.22) b	6.67 (1.96) ab	20.00 (3.83) b
T_6	0.00 (0.71) b	6.67 (1.96) bc	6.67 (1.96) ab	13.33(3.21) bc
T_7	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c
T_8	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c
T_9	0.00 (0.71) b	6.67 (1.96) bc	6.67 (1.96) ab	13.33(3.21) bc
T_{10}	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc
T_{11}	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc
T_{12}	0.00 (0.71) b	0.00 (0.71) c	6.67 (1.96) ab	6.67 (1.96) bc
T_{13}	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c
T_{14}	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c
T_{15}	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c
T_{16}	13.33 (3.21) a	33.33 (5.71) a	13.33 (3.21) a	60.00 (7.45) a
CV %	104	72.80	105.43	80.06

*Values in a column with same letter (s) do not differ significantly (p=0.05)

*Figures in parenthesis are the mean of square root transformed values.

Treatments:

T_1 = Autostin 50 WP (seedling treatment + foliar spray @ 0.1%)	T_9 = Autostin + Poultry waste
T_2 = Furadan 3G (soil treatment)	T_{10} = Furadan + Poultry waste
T_3 = Krosin 10 SP (seedling treatment + drenching of rhizospheric zone @ 0.05%)	T_{11} = Krosin + Poultry refuse
T_4= *Trichoderma* formulation (soil treatment)	T_{12} = *Trichoderma* formulation + Poultry waste
T_5 = Poultry waste (soil amendment)	T_{13} = Autostin + *Trichoderma* formulation + Poultry waste
T_6 = Autostin + Poultry waste	T_{14} = Furadan + *Trichoderma* formulation + Krosin
T_7= Furadan + *Trichoderma* formulation	T_{15} =Krosin + Furadan + *Trichoderma* formulation + Poultry waste
T_8 = Krosin + *Trichoderma* formulation	T_{16} = Control

4.4.2. Effect of different treatments on plant growth parameters of eggplant

The treatments applied in the management of wilt complex of eggplant differed significantly in respect of yield and yield contributing characters (Table 6)

In case of plant height, the highest shoot length (76.40 cm) was observed in T_5 (Soil amendment with Poultry waste) whereas the lowest was recorded in the control (53.97 cm) treatment. The second highest shoot length (73.63 cm) was recorded in T_{15} (*Trichoderma* formulation combined with Krosin, Furadan and Poultry waste) that were statistically identical with treatment T_8 (72.67 cm) and T_{13} (72.33 cm).

The highest number of branches (17.03) was found in case of T_{15} where *Trichoderma* formulation, Krosin, Furadan and Poultry waste were applied combinedly. The second highest number of branches (16.93) was recorded in treatment T_{14} (*Trichoderma* formulation + Furadan + Krosin) that were statistically similar with T_8 (16.57) and T_{13} (16.27). The lowest number of branches was noted (12.93) in case of control.

Considering number of leaf per plant, the maximum result (179.0) was found in treatment T_{15} where *Trichoderma* formulation, Krosin, Furadan and Poultry waste were applied combinedly. The second highest number of leaf per plant (174.33) was recorded in treatment T_{13} (Autostin +*Trichoderma* formulation+ Poultry waste) that was statistically similar with treatment T_{14} (172.00). The lowest number of leaf per plant (139.00) was found in the control.

The maximum number of fruit per plant (37.33) was observed in case of T_{15} where *Trichoderma* formulation, Krosin, Furadan and Poultry waste were applied combinedly. The second highest number of fruit per plant (33.43) was recorded in T_{13} (Autostin, *Trichoderma* formulation and Poultry waste).

The treatment T_{14}, T_{11} and T_8 were statistically similar in case of number of fruit, respectively. The lowest no. of fruit per plant (23.37) was found in the Control.

In terms of length of fruit, the highest fruit length (23.00 cm) was observed in case of treatment T_{15} where *Trichoderma* formulation, Krosin, Furadan and Poultry waste were applied combinedly. The treatment T_{13}, T_{14} and T_1 showed similar trend of result in case of fruit length. The lowest fruit length (15.07 cm) was found in control.

Table 6. Effect of different treatments on plant growth parameter of eggplant

Treatments	Plant height	No. of branches	No. of leaf plant^{-1}	No. of fruit plant^{-1}	Fruit length
T_1	63.63 h	13.40 e	163.33 c-e	30.10 c-f	20.47 b-d
T_2	64.07 gh	14.93 cd	162.33 c-e	28.13 fg	19.20 c-e
T_3	67.00 e-h	14.87 d	163.67 b-e	28.53 e-g	19.80 b-e
T_4	69.00 c-f	15.80 a-d	162.67 c-e	28.77 d-g	17.00 f-h
T_5	**76.40 a**	15.10 cd	161.67 c-e	30.70 b-f	19.13 c-f
T_6	71.53 b-d	16.13 a-d	156.33 e	28.87 c-g	18.07 e-g
T_7	65.80 f-h	15.03 cd	158.00 de	26.47 g	18.53 d-g
T_8	**72.67 a-c**	**16.57 ab**	163.67 b-e	31.57 b-e	17.00 f-h
T_9	65.57 f-h	16.13 a-d	162.33 c-e	30.53 b-f	15.73 h
T_{10}	68.33 c-g	15.70 a-d	162.00 c-e	28.47 e-g	16.60 gh
T_{11}	67.07 e-h	16.23 a-c	168.00 b-d	31.97 b-d	18.07 e-g
T_{12}	67.27 d-h	15.57 b-d	170.67 a-c	29.50 c-g	19.93 b-e
T_{13}	**72.33 a-c**	**16.93 a**	**174.33 ab**	**33.43 b**	**21.47 ab**
T_{14}	70.57 b-e	16.27 a-c	172.00 a-c	**32.07 bc**	**20.93 a-c**
T_{15}	**73.63 ab**	**17.03 a**	**179.00 a**	**37.33 a**	**23.00 a**
T_{16}	**53.97 i**	**12.93 h**	**139.00 f**	**23.37 h**	**15.07 h**
CV %	3.90	5.16	4.03	6.55	7.00

*Values in a column with same letter (s) do not differ significantly (p=0.05)

Treatments:

T_1 = Autostin 50 WP (seedling treatment + foliar spray @ 0.1%)	T_9 = Autostin + Poultry waste
T_2 = Furadan 3G (soil treatment)	T_{10} = Furadan + Poultry waste
T_3 = Krosin 10 SP (seedling treatment + drenching of rhizospheric zone @ 0.05%)	T_{11} = Krosin + Poultry refuse
T_4= *Trichoderma* formulation (soil treatment)	T_{12} = *Trichoderma* formulation + Poultry waste
T_5 = Poultry waste (soil amendment)	T_{13} = Autostin + *Trichoderma* formulation + Poultry waste
T_6 = Autostin + Poultry waste	T_{14} = Furadan + *Trichoderma* formulation + Krosin
T_7= Furadan + *Trichoderma* formulation	T_{15} =Krosin + Furadan + *Trichoderma* formulation + Poultry waste
T_8 = Krosin + *Trichoderma* formulation	T_{16} = Control

4.4.3. Effect of different treatments on yield of eggplant

The treatments applied for the management of wilt complex of eggplant differed significantly in respect of fruit yield. (Table 7)

The highest yield per plant (3.12 kg) was recorded in case of T_{15} where Krosin, Furadan, *Trichoderma* formulation and Poultry waste were applied combinedly. Treatment T_{14} (Furadan + *Trichoderma* formulation + Krosin) produced the second highest yield (3.12 kg) followed by treatment T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_8 (Krosin + *Trichoderma* formulation), T_4 (*Trichoderma* formulation) yielding 3.10 kg, 3.07 kg, 2.90 kg, respectively. Treatment T_{14}, T_{13}, T_8 and T_4 were statistically similar in terms of yield. The lowest yield per plant (0.9 kg) was noted in control.

In case of yield (kg) per plot treatment T_{15} (Krosin + Furadan + *Trichoderma* formulation + Poultry refuse) was given the highest result (18.62 kg) where the second highest yield (16.03kg) was found in case of treatment T_{14} (Furadan + *Trichoderma* formulation + Krosin) followed by treatment T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_8 (Krosin + *Trichoderma* formulation), T_4 (*Trichoderma* formulation) giving yield 15.93 kg, 15.47 kg,14.63 kg, respectively. Treatment T_{14} was statistically similar to treatment T_{13}, T_8 and T_4. The lowest yield per plot (4.80 kg) was recorded in control.

The yield of eggplant per hectare differed significantly among the treatments. The highest yield (32.10 ton) was recorded in case of treatment T_{15} where Krosin, Furadan, *Trichoderma* formulation and Poultry waste were applied combinedly. Treatment T_{14} (Furadan + *Trichoderma* formulation + Krosin) was given the second highest (28.24 ton) yield followed by treatment T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_8 (Krosin + *Trichoderma* formulation), T_4 (*Trichoderma* formulation) produced 27.56 ton, 27.34 ton, 25.86 ton, respectively. Treatment T_{14}, T_{13}, T_8 and T_4 was statistically similar in case of yield per hectare. The lowest yield was noted in treatment T_{16} (8.02 ton).

The treatment T_{15} showed promising results in each and every case of yield and yield contributing characters. Treatments T_{15} contained Krosin, Furadan, *Trichoderma* formulation and Poultry waste. This result was supported by the efficacy of single components on the plant. *Trichoderma spp.* were known to enhance plant growth and productivity which was reported by Harman (2000) and Shoresh *et al*. (2010). The mechanisms acted by *Trichoderma spp.* were antagonism, mycoparasitism, competition with pathogens for nutrient and space and induction of systemic resistance in plants which were responsible for reduction of disease and account for increased yield. The findings were keeping with the results of Yedidia *et al*., 2001; Benitez *et al*., 2004; Harman *et al*., 2004; Howell, 2006; Hermosa *et al*., 2012. Carbofuran also showed a significant effect in influencing the growth parameters and suppression of *Meloidogyne spp.* (Mahfouz *et al.,* 2010). The yield increased in case of poultry manure appeared to be due the positive effects on soil fertility and increasing the suppressing nature of soil. These findings were supported by Stirling (1989). Krosin was responsible for inducing resistence in plants against *Ralstonia solanacearum.* Thus the integration of Krosin, Furadan, *Trichoderma* formulation and Poultry waste showed the highest performances in yield and yield contributing characters. Treatment T_{14} (Furadan + *Trichoderma* formulation + Krosin), T_{13} (Autostin + *Trichoderma* formulation + Poultry waste), T_8 (Krosin + *Trichoderma* formulation), T_4 (*Trichoderma* formulation) also gave satisfactory yield due to the treatments effects. Though treatment T_{13} comprised of *Trichoderma* formulation and Poultry waste it gave lower yield than T_{14}. It might be due to the presence of Autostin 50 WP which have antifungal properties.

Table 7. Effect of different treatments on yield of eggplant

Treatments	Yield (kg) plant^{-1}	Yield (kg) plot^{-1}	Yield (ton) ha^{-1}
T_1	2.26 d-f	11.40 d-f	20.21 d-f
T_2	2.13 ef	10.43 e-f	19.02 ef
T_3	2.20 ef	10.57 d-g	19.62 ef
T_4	**2.90 bc**	**14.63 bc**	**25.86 bc**
T_5	2.03 f	8.97 g	18.13 f
T_6	2.33 d-f	10.93 d-g	20.80 d-f
T_7	2.13 ef	10.67 d-g	19.32 ef
T_8	**3.07 b**	**15.47 b**	**27.34 b**
T_9	2.23 d-f	10.13 fg	19.91 d-f
T_{10}	2.06 f	9.80 fg	18.43 f
T_{11}	2.60 cd	12.47 c-e	23.18 cd
T_{12}	2.36 d-f	11.83 d-f	21.10 d-f
T_{13}	**3.10 b**	**15.93 b**	**27.56 b**
T_{14}	**3.12 b**	**16.03 b**	**28.24 b**
T_{15}	**3.60 a**	**18.62 a**	**32.10 a**
T_{16}	0.9 g	4.80 h	8.02 g
CV %	9.73	11.18	9.61

*Values in a column with same letter (s) do not differ significantly (p=0.05)

Treatments:

T_1 = Autostin 50 WP (seedling treatment + foliar spray @ 0.1%)	T_9 = Autostin + Poultry waste
T_2 = Furadan 3G (soil treatment)	T_{10} = Furadan + Poultry waste
T_3 = Krosin 10 SP (seedling treatment + drenching of rhizosphere zone @ 0.05%)	T_{11} = Krosin + Poultry refuse
T_4= *Trichoderma* formulation (soil treatment)	T_{12} = *Trichoderma* formulation + Poultry waste
T_5 = Poultry waste (soil amendment)	T_{13} = Autostin + *Trichoderma* formulation + Poultry waste
T_6 = Autostin + Poultry waste	T_{14} = Furadan + *Trichoderma* formulation + Krosin
T_7= Furadan + *Trichoderma* formulation	T_{15} =Krosin + Furadan + *Trichoderma* formulation + Poultry waste
T_8 = Krosin + *Trichoderma* formulation	T_{16} = Control

(a) Vegetative growth of T_{15}

(b) Growth of T_{15} after flowering

(c) Vegetative growth of control

(d) Growth of control after flowering

(e) Fruit yield of T_{15}

(f) Fruit yield of control

Plate 19: Growth parameters and yield of the best treatment and control

(a) Individual fruit weight of treatment T_{15}

(b) Individual fruit weight of control

(c) Fruit yeild from one plant in treatment T_{15}

(d) Fruit yeild from one plant in control

Plate 20. Comparative study of yield data of best treatment and control

Fig. 4. Pictorial view of yield data from one pick

4.7. Effect of different treatments on yield increased over control (%)

The maximum fruit yield increased over control (300%) was shown in case of the treatment T_{15} (Krosin + Furadan + *Trichoderma* formulation + Poultry waste) followed by T_{14} (Furadan + *Trichoderma* formulation + Krosin), T_{13} (Autostin + *Trichoderma* formulation + Poultry waste) and T_8 (Krosin + *Trichoderma* formulation) resulting 251.84%, 243.64% and 240.74%, respectively. The lowest result was observed in Poultry waste (125.92%) preceded by Furadan 3G combined with Poultry waste (129.63%).

Table 8. Effect of different treatments on yield of eggplant over controls

Treatments	Yield (ton) ha^{-1}	Yield increased over control
T_1	20.21	151.85
T_2	19.02	137.04
T_3	19.62	144.44
T_4	25.86	222.21
T_5	18.13	125.92
T_6	20.80	159.26
T_7	19.32	140.73
T_8	27.34	240.74
T_9	19.91	148.14
T_{10}	18.43	129.63
T_{11}	23.18	188.89
T_{12}	21.10	162.96
T_{13}	27.56	243.64
T_{14}	28.24	251.84
T_{15}	32.10	300.00
T_{16}	8.02	-

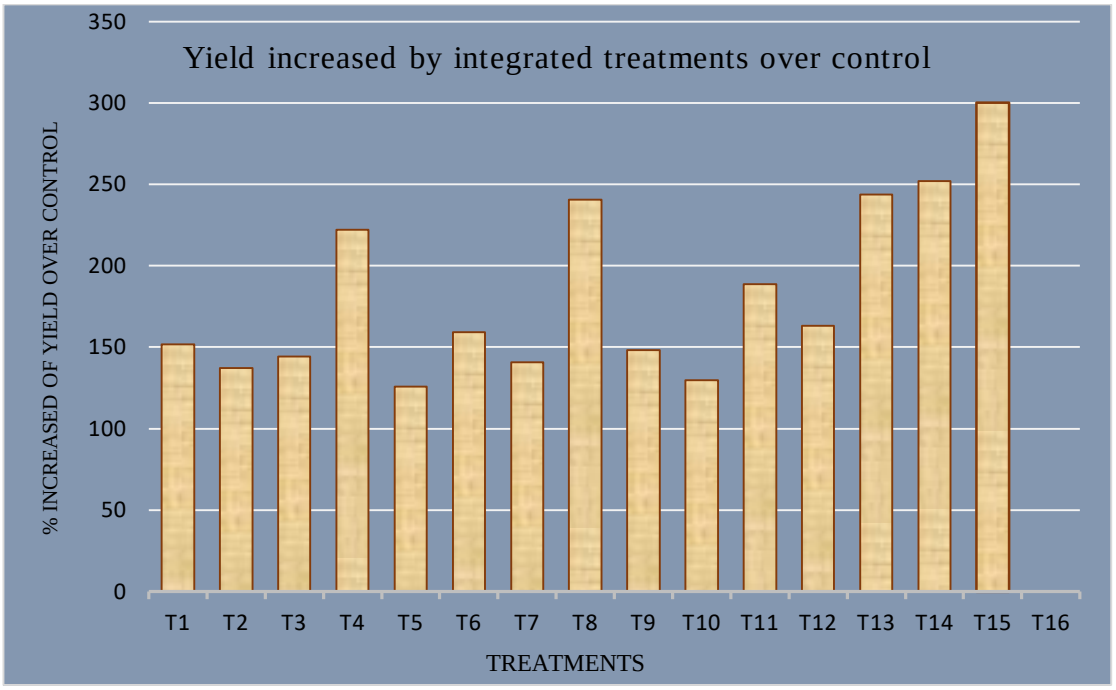

Fig. 5. Increased of yield over control by different treatments

4.8. Benefit Cost Ratio (BCR) for the different treatments to manage wilt complex

For management of wilt complex of eggplant, cost analysis of the treatments applied was analyzed (Appendix- 4). The benefit cost ratio (BCR) of treatment T_{15} (Krosin + Furadan + *Trichoderm a* formulation + Poultry waste) was the highest (5.68) where the farmers could earn Tk. 5.68 by investing Tk 1.0 only. The higher BCR was 5.56 for the application of treatment T_{14} where Furadan 5G, *Trichoderma* formulation and Poultry waste were applied in combination. The BCR value was 5.51 for treatment T_8 (Krosin + *Trichoderma* formulation) because of the lower price of krosin and *Trichoderma* formulation.

Besides, application of *Trichoderma* formulation and Poultry manure were not only suppressed the soil borne pathogens but also improved soil properties. For this, treatment T_{15} (Krosin + Furadan + *Trichoderma* formulation + Poultry waste) has given highest yield despite of the higher treatment cost. The lower BCR for the application of other treatments are due to the lower yield of those treatments.

Table 9. Benefit Cost Ratio (BCR) of sixteen different treatments for controlling wilt complex

Treatments	Yield (ton)ha^{-1}	Gross income (Tk ha^{-1})	Total cost of production (Tk ha^{-1})	Net return (Tk ha^{-1})	BCR
T_1	20.21	707495	91720 + 5700 = 97420	404280	4.14
T_2	19.02	665877	91720 + 9000 = 100720	380500	3.77
T_3	19.62	686686	91720 + 4920 = 96640	392400	4.06
T_4	25.86	905177	91720 + 2600 = 94320	517240	5.48
T_5	18.13	634664	91720 + 11600 = 103320	362660	3.51
T_6	20.80	728303	91720 + 5700 = 100020	416180	4.16
T_7	19.32	676282	91720 + 11600 + 2600 = 103320	386440	3.74
T_8	27.34	957199	91720 + 11600 + 4920 = 99240	546980	5.51
T_9	19.91	697090	91720 + 5700 + 11600 = 109020	398340	3.65
T_{10}	18.43	645069	91720 + 9000 + 11600 = 112320	368620	3.28
T_{11}	23.18	811538	91720 + 4920 + 11600 = 108240	463740	4.28
T_{12}	21.10	738708	91720 + 11600 + 2600 = 105920	422120	3.98
T_{13}	28.24	988412	91720 +5700 +11600 + 2600 = 111620	555120	4.97
T_{14}	22.29	780325	91720 + 9000 + 2600 + 4920 = 108240	602100	5.56
T_{15}	32.10	1123668	91720 + 4920 + 2600 + 11600 + 9000 = 119840	681400	5.68
T_{16}	8.02	280917	91720	160520	1.75

4.9. Correlation and regression study between wilt incidence and yield

Correlation study was done to determine the relationship between yield per plot with wilt 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 wilt complex of eggplant.

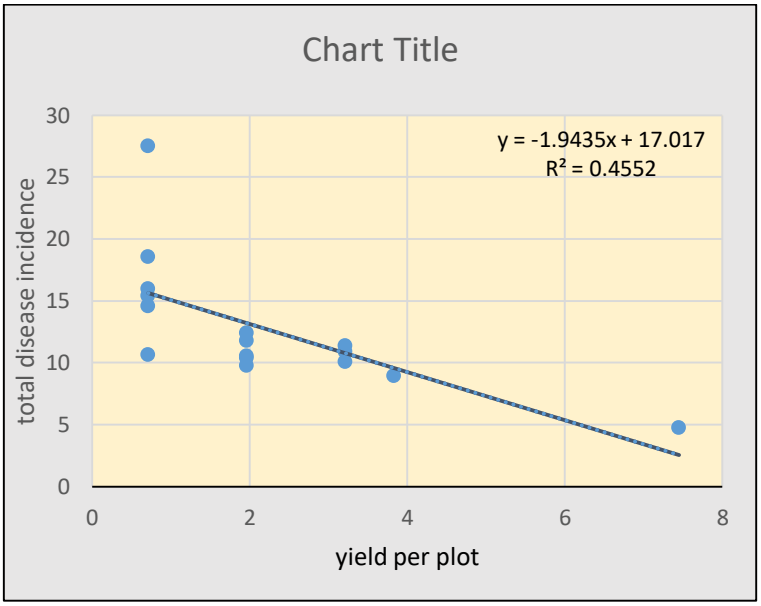

Fig. 6: Relationship between DI and yield of eggplant

CHAPTER – V

SUMMERY AND CONCLUSION

Among the vegetables grown in Bangladesh, eggplant (*Solanum melongena* L.) is most important in terms of year round availability, food value, taste, farmer's income perspective and as an export item. It is quite popular as the poor man's crop (Sammaiah, 2011). Successful production of eggplant is mainly hindered by wilt diseases caused by fungus, bacteria and nematode which are the widest spread and destructive disease, causing huge losses to crop growers.

R. solanacearum, F. oxysporum and *Meloidogyne* sp. were isolated from eggplant plants showing typical symptoms of bacterial wilt, fungal wilt and nemic wilt, respectively. The bacterium and fungus were identified on the basis of morphological, cultural characters while, nematode on the basis of morphological characters and perennial pattern. Final confirmation was done by testing Koch's postulates. In *in vitro* evaluation, Autostin 50WP (Carbendazim) and Krosin 10 SP (streptomycin sulphate and tetracycline hydrochloride) was found effective in retardation of physical growth of *F. oxysporum* and *R. solanacearum by* 87.33% *and* 83.33%, respectively.

The present investigation was also attempted with a view to identify the most efficient and economic management of *F. oxysporum, R. solanacearum and M. incognita* causing wilt complex of eggplant with integrated approach. Three chemicals viz. Autostin 50WP (Carbendazim) Furadan 5G (Carbofuran), and Krosin 10 SP (streptomycin sulphate), one bio-agent *Trichoderma harzianum*, a soil amendment poultry waste and their different combinations were evaluated against *F. oxysporum*, *Ralstonia solanacearum* and *Meloidogyne incognita* causing wilt complex of eggplant in the field condition. The cost analysis for BCR was done to select the best treatment which gives the farmers the most benefit.

In field evaluation, the effect of the treatments, alone and in combinations, for management of wilt complex of eggplant were determined by recording data in terms of wilt incidence, yield and yield contributing characters against wilt disease.

In case of fungal wilt, the effect of different treatments on the disease incidence were differed significantly compared to control. The lowest 6.67% wilt incidence was found in Furadan and Krosin alone and Furadan and Krosin combined with poultry waste. The highest (13.33%) fungal wilt incidence was observed in control. The other treatments showed no wilt incidence.

In case of bacterial wilt, the disease incidence was significantly reduced by the application of the treatments compared to control. The lowest bacterial wilt incidence (6.67%) was noted in autostin, autostin combined with *Trichoderma* formulation or poultry waste. The highest (33.33%) bacterial wilt incidence was recorded in control which was statistically similar with poultry waste (13.33%). The other treatments effectively control the bacterial wilt with no wilt incidence.

The effect of different treatments on the nemic wilt incidence were differed significantly compared to control. The highest (13.33%) nemic wilt incidence was found in control which was statistically similar to Autostin and Poultry waste alone and Autostin combined with *Trichoderma* formulation or Poultry waste. Though rest of the treatments (Furadan, Krosin, *Trichoderma* formulation, Poultry refuse alone and with combination) showed no wilt incidence.

For the disease incidence (DI) of wilt complex, the effect of different treatments on the disease incidence were differed significantly compared to control. The lowest DI was recorded in the treatment where *Trichoderma* formulation was combined with either Furadan or Krosin or Autostin and Poultry waste or Furadan and Krosin and Poultry waste. The highest (80%) DI was found in control.

Treatments effects were differed significantly in respect of plant growth characters viz. plant height, number of branches, number of leaf per plant, number of fruit per plant and length of fruit compared to control. Most of the treatments showed similar trend of resuts in case of yield and yield contributing characters. The highest performance was found in case of the treatment combination of *Trichoderma* formulation with Furadan 5 G, Krosin 10 SP, Autostin 50WP and Poultry waste. The lowest result was found in control. The highest yield per plant (18.62 kg) was recorded in case of *Trichoderma* formulation combined with Furadan 5G, Krosin 10 SP, and Poultry waste. On the contrary, the lowest result was found in control.

Based on the market price of the produce and contemporary production cost, the highest BCR (5.68) was observed in the treatment where *Trichoderma* formulation was combined with Furadan 5G, Krosin 10 SP, and Poultry waste.

Considering the overall performance of the treatments, it can be concluded that the wilt complex of eggplant could be controlled successfully and cost effectively by the integrated application of Krosin 10 SP (Bacteriocide), Furadan 5G (Nematicide) along with *Trichoderma* formulation (Bioagent) and Poultry waste (Soil amendment) in different permutations. However, further study was suggested to find out the alternatives of chemicals and their combinations to avoid environmental hazards. 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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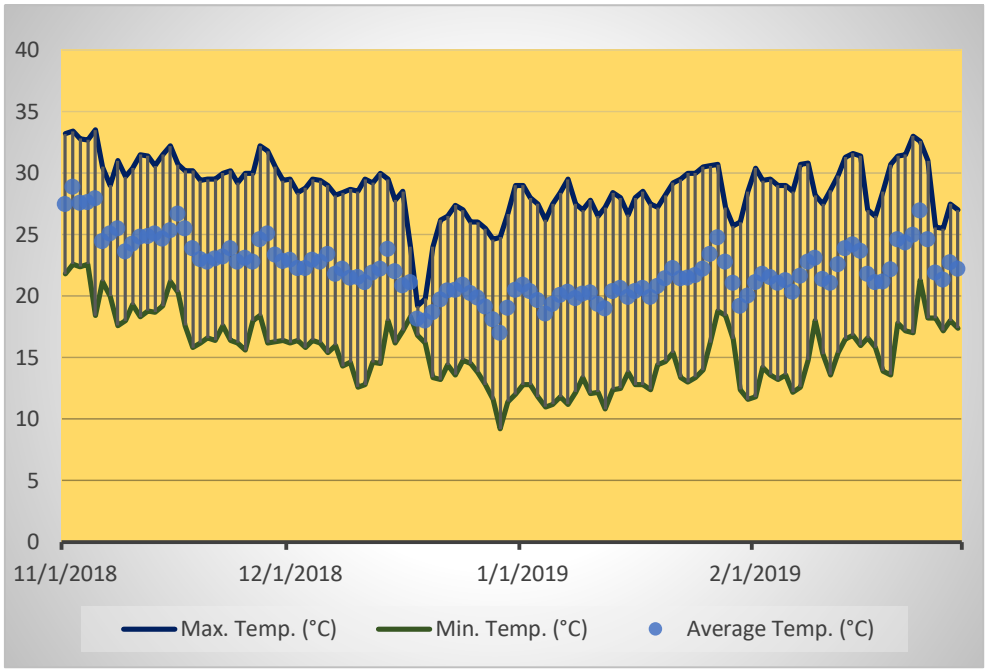
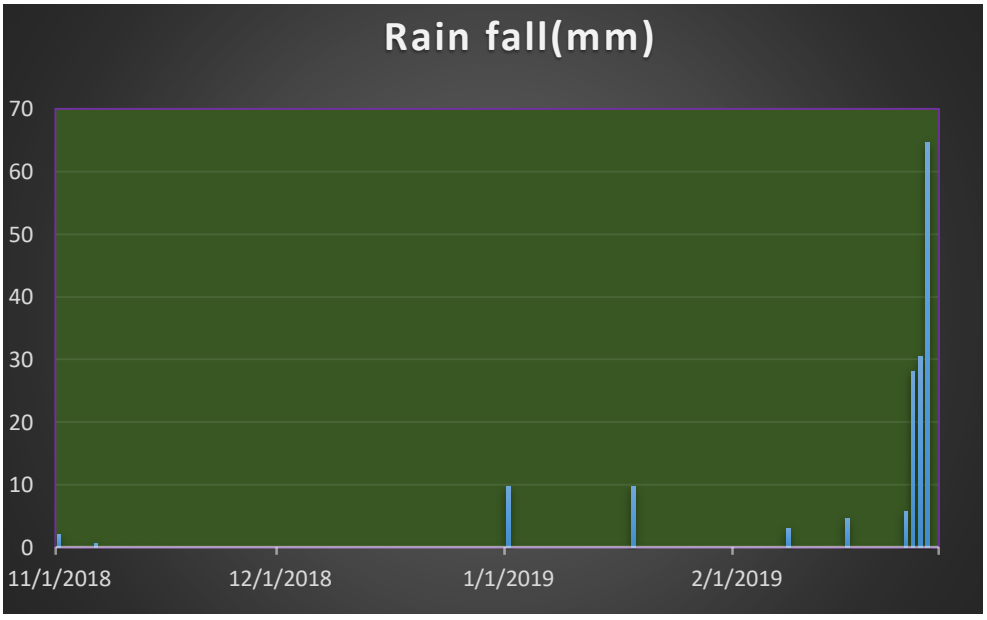
APPENDICES

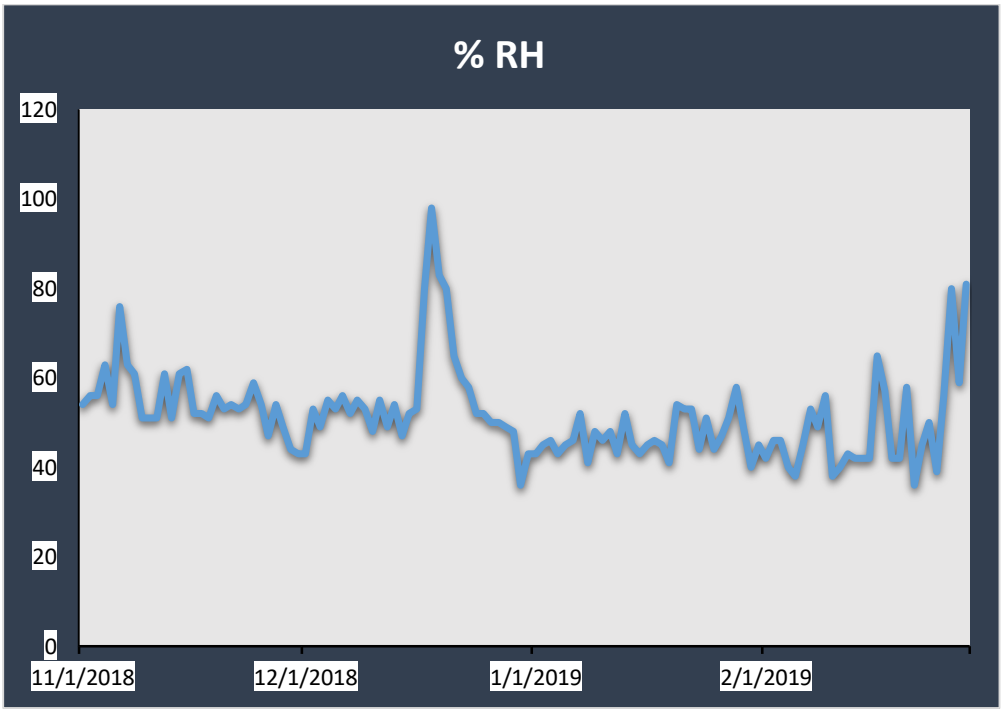
APPENDIX-1: Daily data of weather parameter during November/2017 to March/2018



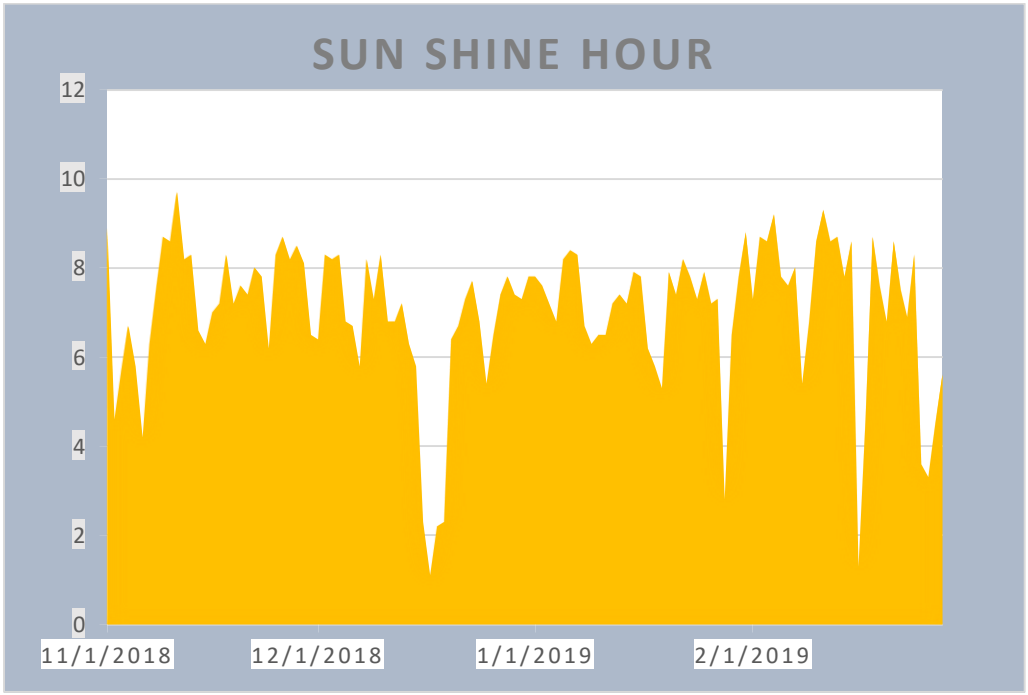
(a) Daily maximum temperature, minimum temperature and average temperature



(b) Daily rainfall (mm) during experimental period



(c) Daily relative humidity (% RH) during experimental period

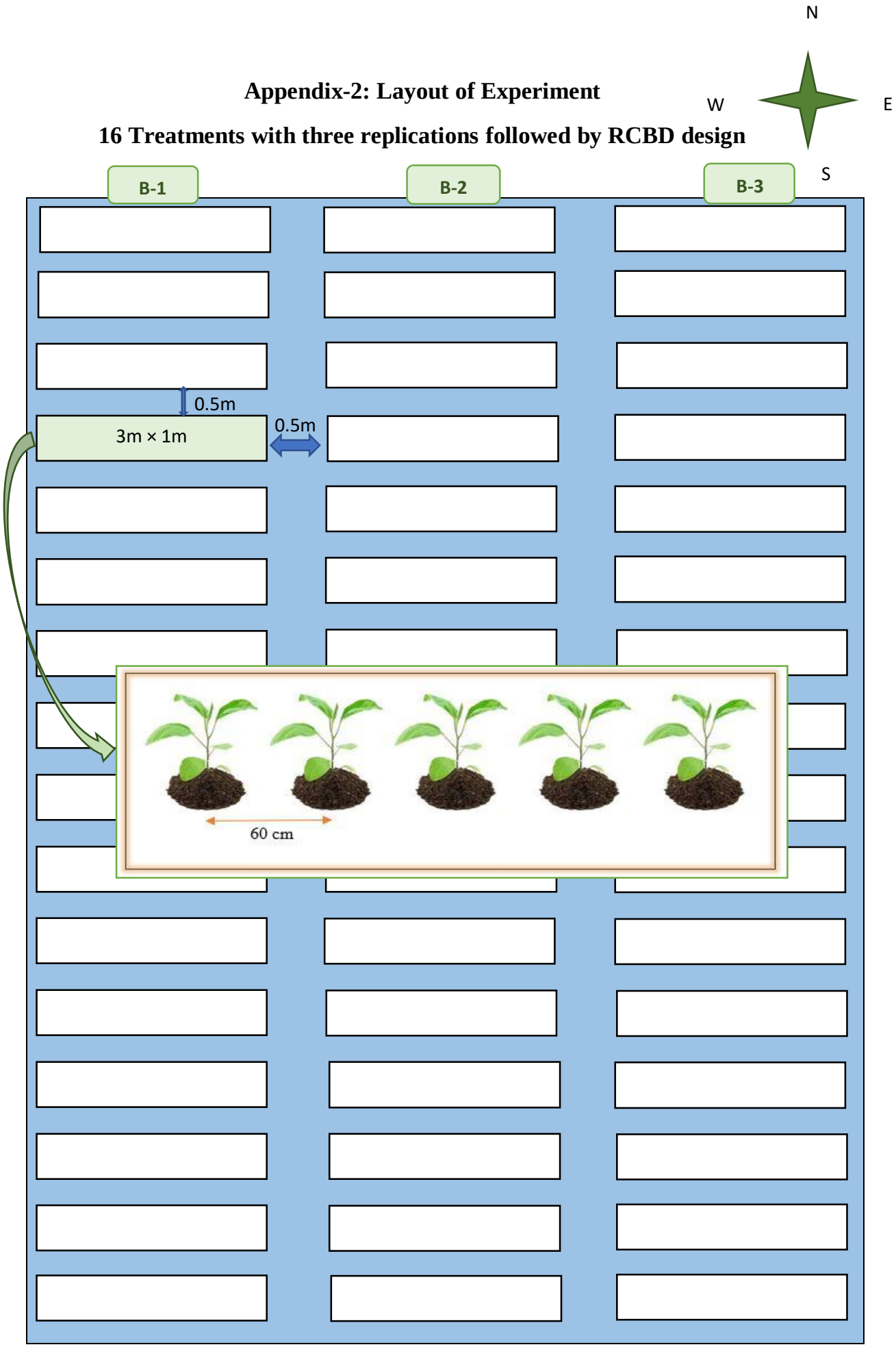


(d) Daily sun shine hour during experimental period

N.B. (Data were collected from the weather station of Bangladesh Rice Research Institute, Joydebpur, Gazipur.)

Appendix-2: Layout of Experiment

16 Treatments with three replications followed by RCBD design



Appendix 3: Photographs of wilted plants at different days after transplanting


(a) 23 December, 2017 at 10 days after transplanting


(b) 7 January, 2018 at 25 days after transplanting


(c) 16 January,2018 at 35 days after transplanting


(d) 26 January,2018 at 45 days after transplanting

(e) 18 February, 2018 at 65 days after transplanting

(f) 18 February, 2018 at 65 days after transplanting

(g) 22 February, 2018 at 70 days after transplanting

Field visit by scientists of Bangladesh Agricultural Research Institiute, Joydebpur, Gazipur.



(h) 15 march, 2018 at 95 days after transplanting


Differential photograph between Fungal and Bacterial wilt in field condition

Appendix 4. Analysis of cost of application of cultural practices in production of eggplant

Cost items	Per hectare cost in taka				
	Unit	Quantity	Cost unit^{-1}	Times	Total cost
Seed	gm	300	5	1	1500/-
Land preparation					
• Ploughing (diesel for tractor)	• Liter	25	65	3	4875/-
• Tractor hired	• Day^{-1}	1	6000	1	6000/-
• Human labour	• Man day^{-1}	1	400	3	1200/-
Seedling plantation					
• Human labour	• Man day^{-1}	10	400	1	4000/-
Fertilization and manuring					
• Urea	• Kg	300	20		6000/-
• TSP	• Kg	200	19		3800/-
• MoP	• Kg	175	21		3675/-
• Gypsum	• Kg	10	15		150/-
• Cowdung	• Ton	10	4000	1	40000/-
Weeding					
• Human Labour	• Man day^{-1}	8	400	3	9600/-
Irrigation					
• Human labor	• Man day^{-1}	1	400	3	1200/-
• Shallow machine hired	• Hour	2	600		3600/-
Insecticide spraying					
• Ripcord	• Liter	0.3	1500	3	1350/-
• Pegasus	• Liter	0.3	1300	3	1170/-
• Human labour	• Man day^{-1}	2	400	3	2400/-
• Sprayer hired	• hour	4	100	3	1200/-
Total (a)					**91720/-**

Appendix-5: Analysis of cost of application of Treatments in production of eggplant

Cost items	Per hectare cost in taka				
	Unit	Quantity	Cost unit^{-1}	Times	Total cost
Autostin 50WP	kg	0.5	2200	3	3300/-
Human Labor	Man day^{-1}	2	400	3	2400/-
Total (b)					**5700/-**
Krosin	kg	0.3	2800	3	2520/-
Human Labor	Man day^{-1}	2	400	3	2400/-
Total (c)					**4920/-**
Furadan 5G	kg	60	130	1	7800/-
Human Labor	Man day^{-1}	3	400	1	1200/-
Total (d)					**9000/-**
Trichoderma formulation	kg	70	20	1	1400/-
Human Labor	Man day^{-1}	3	400	1	1200/-
Total (e)					**2600/-**
Poultry waste	kg	2000	5	1	10000/-
Human Labor	Man day^{-1}	4	400	1	1200/-
Total (f)					**11600/-**
Sprayer hired	hour	4	100	3	1200/-