INTEGRATED APPROACHES FOR THE MANAGEMENT OF WILT COMPLEX OF TOMATO

BY

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CERTIFICATE

This is to certify that the thesis entitled, **"INTEGRATED APPROACHES FOR THE MANAGEMENT OF WILT COMPLEX OF TOMATO''** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN PLANT PATHOLOGY,** embodies the result of a piece of bona fide research work carried out by **Registration No. 12-04916** under my supervision and guidance. No part of the thesis has been submitted anywhere for any other degree or diploma.

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

Dated: 15.10.2019
Place: Dhaka, Bangladesh

Prof. Dr. Md. Rafiqul Islam
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DEDICATED TO
My Beloved Parents

LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
%	Percent
°C	Degree centigrade
BARI	Bangladesh Agricultural Research Institute
cm	Centimeter
cm^2	Centimeter square
CRD	Completely Randomized Design
CV.	Cultivar
Ed.	Edited
Eds.	Edition
et al.	And others
Etc.	Etcetera
Gm	Gram
HRC	Horticultural Research Center
J.	Journal
LSD	Least Significant Difference
No.	Number
PDA	Potato Dextrose Agar
RCBD	Randomized Completely Block Design
Res.	Research
SAU	Sher-e-Agricultural University
SP	Soluble Powder
Var.	Variety
Viz.	Namely
WP	Wettable Powder

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The Author

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INTEGRATED APPROACHES FOR THE MANAGEMENT OF WILT COMPLEX OF TOMATO

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RUBINA TASNIN

ABSTRACT

The in vitro experiments were conducted in the Central Laboratory and net house of the Department of Plant Pathology and the *in vivo* experiment was conducted in the Central Farm of Sher-e-Bangla Agricultural University, Dhaka-1207 during October'2017 to April'2018 to formulate the integrated approach for the management of wilt complex of tomato. The causal pathogens of wilt complex of tomato were isolated and identified as *Fusarium oxysporum* for fungal wilt, *Ralstonia solanacearum* for bacterial wilt and *Meloidogyne spp*. for nemic wilt. In the *in vitro* evaluation, the Autostin (Carbendazim) 50 WP and Krosin (Streptomycin sulphate) 10 SP showed significant results in reducing the growth of *Fusarium oxysporum* (87.88%) and *Ralstonia solanacearum* (83.33%), respectively. In field experiment, the selected treatments were evaluated alone and in combination as T_1 = Autostin 50 WP, T_2 = Furadan 5G, T_3 = Krosin 10 SP, T_4 = *Trichoderma* formulation, T_5 = Poultry waste, T_6 = Autostine 50 WP + *Trichderma* formulation, T_7 = Furadan 5G + *Trichderma* formulation, T_8 = Krosin 10 SP + Poultry waste, T_{11} = Krosin 10 SP + Poultry waste, T_{12} = *Trichderma* formulation + Poultry waste, T_{13} = Autostin 50 WP + *Trichderma* formulation + Poultry waste, T_{14} = Furadan 5G + *Trichderma* formulation + Krosin 10 SP, T_{15} = Furadan 5G + *Trichderma* formulation + Krosin 10 SP + Poultry waste, T_{16} = Control. The combination of treatment T_{15} showed promising result in reducing the incidence of wilt complex of tomato influencing the yield and yield contributing characters where Furadan 5G, *Trichoderma* formulation, Krosin 10 SP and Poultry waste were applied combinedly. Treatment T_{14} (Furadan 5G + *Trichoderma* formulation + Krosin 10 SP) and treatment T_7 (Furadan 5G + *Trichoderma* formulation) also showed remarkable results. The BCR analysis showed that the highest BCR (6.09) was counted in case of T_{15} followed by T_{14} (5.25) and T_7 (4.93) where investing Tk. 1.00, the tomato growers will grossly earn Tk. 6.09, 5.25 and 4.93, respectively.

CHAPTER 1
INTRODUCTION

Tomato (*Lycopersicon esculentum*) is the most important vegetable and cash crop belonging to the family Solanaceae. It is ranked first within the world and contributed 14% of world vegetable production (FAO, 2010). It is a very familiar and lucrative vegetable because of its delicious taste, enticing colour and high nourishing value (Appendix-1) and also for its diversified benefits (Bose and Som, 1980). Tomato can play a crucial role in providing food throughout the year to solve the nutritional problems. Tomato is consumed in numerous ways, including raw as well as an ingredient in many dishes, salads, sauces, and drinks. It is considered as a vegetable for culinary purposes. Day by day the demand of tomato is increasing in the Agro-food processing industries of Bangladesh because of its high demand as a fresh as well as a processed product both in foreign and local markets. Now it is considered as a potential cash crop in the country. It is placed tops of the list of canned vegetables and cash crops (Chowdhury, 1979).

Africa and Asia constitute more than 80% of the global tomato growing areas with about 70% of world tomato production (FAO, 2012). It is widely grown in almost all countries of the world due to its adaptability to wide range of soil and climate (Ahmad, 1976). Tomato grows well in almost all areas in Bangladesh.

In Bangladesh, the cultivated area of tomato is 67535 acres and the production is 368121 MT and the maximum production in Rajhshahi district around 87,928 MT and in Barishal district minimum production is around 7687 MT (BBS, 2017). This production feature is quite low compared to the other leading tomato producing countries like USA, Canada, India (BBS, 2017).

The word tomato comes from the Aztec word ‘Tomatl’. It is known to be originated in Peru in South America. Migrating birds had spread this crop to North America primarily and then to different countries. Wild tomatoes are found in Mexico. The tomato crop was introduced to Europe around in 1550 by the Spanish

priests. In Europe it was familiar as ‘Poma amoris’-Amorous apple or Love apple. It was also well known as ‘Poma peruviana’ apple of Peru.

The British finally in 1880 concede that tomato is edible. Robert Gibbon Johanson an ordinary farmer first ate tomato in USA on a hot day of August in 1820 to elucidate its edibility. From then onwards, the tomato spreads throughout the world.

Tomato is seasonal crop often grown outdoors in temperate region. An average weigh of common tomato is approximately 95 grams. Plants typically grow to 1-3 meters in height and have a weak stem that often crawl over the ground and vines over other plants. It is cultivated as Rabi crop. But now a day’s advanced farmer cultivates summer tomato in main field as well as kitchen or homestead garden.

Fruits of tomato contain higher amount of lycopene. Tomato has the most potential natural anti-oxidants which improves the skin's ability to protect against harmful ultra violet rays. Tomato consumption helps to decrease risk of heart diseases, head and neck cancers and might be strongly protective against neurodegenerative diseases.

The tomato pulp is acidic, this acidity makes tomatoes especially easy to store in home by making tomato sauce. Juice of tomato is often canned and sold as a beverage, unripe green tomatoes can also be breaded and fried, used to make salsa, or pickle. The fruit is also stored by drying by sun and sold in bags or in jars preserving with oil.

However, diseases of tomato act as the chief limiting factor to its economic production. To affect tomato plants almost 200 diseases have been reported (Watterson, 1986). Among all the tomato diseases, early blight, late blight, fungal, bacterial and nemic wilt, viral leaf curl and mosaic are major.

Tomato has enough potentiality to increase agricultural production in our country. But cultivation of tomato is sometime difficult due to high incidence of the wilt pathogens (Ali *et.al.,* 1994). The cultivation in Bangladesh is severely impaired by three important wilt causing pathogens viz. *Ralstonia solanacearum*, *Fusarium oxysporum* and *Meloidogyne incognita*, the causal agent of Bacterial wilt, Fungal wilt and Nemic wilt respectively. Once the plant is affected by wilting pathogens it does not produce good yield and gradually die.

Fungal and bacterial wilt collapsed the vascular system of plants by their physical presence as well as their by-products. Infection of vascular vessels decrease water transportation resulting in wilting of the plants. It was recorded that wilt is one of the major diseases causing the highest pre-harvest losses of tomato (Ali *et.al.,* 1994).

One of the most prevalent disease of tomato is *Fusarium* wilt wherever tomatoes grows intensively. In temperate regions, fungal wilt is most destructive in warm weather and in sandy soil. The fungus is specific for solanaceaous crop and very long lived in all regions of the world. The disease progressed more rapidly in soils that are low in potassium and high in nitrogen. Singh and Kamal (2012) reported that 10-90% yield loss caused by *Fusarium oxysporum* in temperate region.

Ralstonia solanacearum is a soil borne as well as a waterborne bacteria. Usually the bacterium infects the plants through wounds and also at the points of lateral roots. Root-knot nematode is also a soil borne organisms, can cause disease to plant roots and help to penetrate bacterium. Through stem injuries plant infection can also occur caused by insect damage or cultural practices. If the pathogen attacks the host, it can invade the xylem of the plant and hamper water translocation system resulting hurried wilting and cause death. Symptoms of disease are expressed four days after the infection (Ilankoon *et al.,* 2001). At different stages loss in yield and plant mortality ranged from 10.83-90.62% and 10-100, respectively. Mortality and

loss in yield were more appeared in plants inoculated before 60 days (Kishun. Ram 1987)

Another important and widely distributed disease in the country is root-Knot caused by *Meloidogyne incognita* (Talukder, 1974; Timm and Ameen, 1960 and Mian, 1986). The disease is revealed in the root system by gall formation and at last the plants become weak due to forbearance in nutrient uptake from the soil. The plants may die at severe infection. *Meloidogyne spp.* caused up to 80% yield losses in processing tomato growing regions (Kaskavalci, 2007).

Soil sterilization by chemicals, use of systemic chemical fungicides, soil amendments with organic wastes and use of resistant varieties are the common approach for the management of wilt disease. But very few reports are available concerning wilt resistant varieties of tomato (Goth *et al.*, 1986). Crop rotation, soil amendments, field sanitation and biological control or other control methods are rarely effective. Single control approach may not potential and sustainable effort for controlling the disease. Integration of different methods may not be effective approach for the management of the disease.

Considering the exploration of bio-agents and its mechanism for soil treatment has got importance in our agro-ecosystem for plant health management. Application of bio-agents e.g. *Trichoderma* sp. could be a better option in managing the wilt of tomato. *Trichoderma harzianum* is reported as effective tool of biological control (Cook, 1993; Harman, 1989).

Use of indigenous plant products and other organic amendment are relatively a recent innovation for the management of wilt diseases of tomato. Stirling (1989) used poultry manure and sawdust that increased the yield of tomato and controlled nematode.

Furadan 5G was reported as the most effective nematicides in controlling *Meloidogyne sp.* in tomatoes in warm weather conditions (Yaringano *et al.*, 1977).

Considering the above facts, the present research has been undertaken to formulate an integrated approach for the management of wilt complex of tomato with eco-friendly approach.

Objectives:

1. To isolate and identify the wilt disease causing pathogens of tomato.
2. To evaluate the selected IPM components against the fungal and bacterial wilt pathogens under *in vitro* condition.
3. To integrate the IPM components for the management of wilt complex disease of tomato under *in vivo* condition.

CHAPTER 2
REVIEW OF LITERATURES

Many works on fungal and bacterial wilt disease of tomato and several other crops have been done around the world. Literature related to wilt disease has been reviewed in this chapter.

2.1. Symptomatology of fungal wilt

The most typical symptoms of the *Fusarium* wilt disease appeared on tomato plants in field condition. The symptoms appeared on older plants under warm weather conditions. A typical sign of the disease was leaf chlorosis followed by wilting and drying up of leaves. Generally, one side of the plant was affected first. A cross-section of the stem revealed necrosis of the vascular system (Ignjatov *et al.* 2012).

Fusarium wilt of tomato causes great loss in warm climates and sandy soil of temperate regions (Mushtag, 2011).

The first symptoms of fusarial wilt (*Fusarium oxysporum)* of the veinlets and chlorosis of the leaf was observed (Rangswami, 1988). Soon the petiole and leaves drop off and become wilted. The younger leaves may die in succession and the entire plant may wilt and die in the course of a few days.

2.2. Symptomatology of bacterial wilt

Bacterial wilt symptoms are initially visible on tomato foliage. The youngest leaves at the end of the branches wilt in the hottest day. Gradually the entire plant wilt and desiccate even dried leaves remain green. In severe case, general wilting and yellowing of foliage leads to plant death. Stunting of the plant is also a common symptom and the collapse of the stem may be observed (Champoiseau and Momol, 2009).

Momol *et al.,* (2001), while working on *Ralstonia solanacearum*, stated that the emergence of pathogen to susceptible lateral roots caused infection through microscopic wounds of roots. Transplanting, nematodes, insects, and agricultural equipment may be the reason to wound roots. Bacteria then colonize the cortex and advance towards the xylem vessel, from where it rapidly spreads in the plant. Bacterial masses prevent water flow from the roots to the leaves, resulting in plant wilting.

2.3. Symptomatology of Nemic wilt

Root-Knot caused by *Meloidogyne incognita* is essential and widely distributed disease in the country (Talukder, 1974; Timm and Ameen, 1960; Mian, 1986). 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 ((Manju and Sankari, 2015).

2.4. Isolation of associated pathogens

2.4.1. Isolation of *Fusarium spp.*

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* 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.

Ramaiah and Garampalli (2015) isolated *F. oxysporum* from tomato plant by surface sterilizing 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.

2.4.2. Isolation of *Ralstonia solanacearum*

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

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

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.

2.4.3. Isolation of *Meloidogyne incognita*

Puncturing aerial organs (stems and leaf mid-ribs) of susceptible plants with capillary glass tubes containing a known number of *Meloidogyne incognita* larvae or eggs. The nematodes mature in the stem or leaf and produce offspring that have not been exposed to contamination with other soil-borne nematode species. This technique is a simple, inexpensive method for maintaining an abundant supply of *Meloidogyne incognita* in the laboratory. By utilizing capillary glass tubes for inoculations, single egg sacs can be isolated to establish and perpetuate pure nematode cultures.

2.5. Pathogenicity Study of Associated Pathogens

2.5.1. Pathogenicity study of *Fusarium spp.*

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. Consequently, such isolates were identified as *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *vasinfectum,* respectively. On the other hand, all isolates of other different *Fusarium* species were pathogenic causing various diseases on different economic plants with variable degrees.

Nirmaladevi and Srinivas (2012) studied the pathogenicity of 114 isolates on five susceptible varieties by root cut and dip inoculation method. The isolates were categorized into four groups viz., highly pathogenic, moderately pathogenic, weakly pathogenic, and non-pathogenic based on the symptomatically variations in the test tomato varieties, whereas non-inoculated tomato seedlings showed no symptoms.

Ignjatov *et al.* (2012) recovered seven isolates from tomato xylem and three isolates from tomato fruit collected from different production region and storage and warehouses of Vojvodina respectively and tested the pathogenicity of the isolates of the fungi by sowing tomato seeds in artificially infected soil mixture made of the substrate and sterile sand at a ratio of 3:1. They confirmed the pathogenicity of isolates TFW1-TFW12 after 14 days. Infected soil had no significant influence on seed germination, but it caused plant wilting after emergence. However, TFM1-TFM7 isolates did not have a significant influence on emergence or growing. No significant differences were found related to the uninfected control. Only isolate TFM7 caused root rot similar to "damping off" symptoms.

Ramaiah and Garampalli (2015) inoculated tomato seedlings with *Fusarium oxysporum* f. sp *lycopersici* 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. Sidausing root-dip method with a conidial suspension of the pathogen at 1×10^7 conidia/ml.

2.5.2. Pathogenicity study of *R. solanacearum*

Makari *et al.* (2013) inoculated tomato and chili plants with five ml of inoculums of *R. solanacearum* ($1x10^9$ CFU/ml) 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 chili plants. *R. solanacearum* isolates exhibited wilting symptoms 3 to 4 days after inoculation and all the inoculated plants wilted within 5 to 10 days.

Shahbaz *et al.* (2015) recovered isolates of *R. solanacearum* and performed it is 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.

2.5.3. Pathogenicity study of *Meloidogyne incognita*

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.

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 (McGawley, 2001).

2.6. Interaction of *Fusarium* spp., *R. solanacearum* and *Meloidogyne spp.*

Pitcher (1965) stated that in addition to disease-causing fungi, several plant-parasitic nematodes are found associated with sugar beet seedlings suffering from various kinds of root disorders and wilt. Nematodes are known as initiators of wounds through which fungal and bacterial pathogens can enter into plant tissues making them more vulnerable for invasion and multiplication of the disease-causing agents.

2.7. Management of wilt diseases of tomato

2.7.1. Management through Furadan 5G

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.

Yaringano and Villalba (1977) reported that among the nematicides, Furadan 5G was the most effective in controlling *Meloidogyne spp.* in tomatoes in the dry tropics.

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.

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 @ 0.5 t/ha, 10 t/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.

2.7.2. Management through different fungicides

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.

Amini *et al.* (2010) evaluated six fungicides; benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole, and azoxystrobin, for their efficacy against *Fusarium oxysporum* f. sp. *lycopersici in vitro* and *in vivo*. Different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 μg/ml) was 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 (50 EC) values (μg/ml) was 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 fungicides 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.

Déo (2013) evaluated Carbendazim (50% SC) at four rates (0.1, 0.3, 1 and 3 mg/mL) *invitro* on radial growth of mycelia of *Fusarium oxysporum* f. sp. *lycopersici* "strain F20". Mycelia were isolatedfrom tomato (*Lycopersicon esculentum*) and *Colletotrichum capsici* "strain C226.3" isolated from chili (*Capsicum annum* L). It was observed that the fungicide at all the concentrations tested inhibited mycelia radial growth of the fungi. Inhibitory effect of the carbendazim tested on mycelia radial growth was most significant at 3 mg/ml. Carbendazim 50 EC is highly toxic with no growth of *F. oxysporum* f. sp. *lycopersici* "strain F20". Where, *C. capsici* "strain C226.3" in media containing respectively 0.55 and 0.35 mg/L. That inhibitory effect is fast to strain C226.3 than to strain F20

at the lowest carbendazim concentrations (0.1 and 0.3 mg/L) while the situation tends to reverse at the highest carbendazim concentrations (3 and 1 mg/L).

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 28^0C for seven days. Mycelia weights of the three fungi were significantly reduced at 100 mg/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.

2.7.3. Management through different bactericides

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

Lal *et al.* (2016) stated that Solanaceae crops were affected by various pathogens, such as. Fungi, Bacteria, Viruses, and Nematodes. These pathogens cause significant yield losses of agricultural crop if proper protection measures have not been applied. Among pathogens *Rhizoctonia solani* and *Fusarium* spp. are the major pathogens in the fungal group, whereas *Ralstonia solanacearum*, and *Streptomyces* spp. are in the bacterial group. Various methods, like chemical control, biological control, resistant varieties, cultural control, and physical control, are applied to reduce these pathogens attack. Above all, resistant varieties are the best and cheapest method for managing diseases. Chemical control comes 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.

Yuliar *et al.* (2015) revealed the development of control methods against bacterial wilt diseases caused by *Ralstonia solanacearum.* He made progress in biological, physical, chemical, cultural, and integral control measures, also in biocontrol and suppression mechanisms. Bacteria (90%), dominates among all biological control agents (BCAs), whereas *solanacearum*, *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp. are also known BCAs. New or uncommon BCAs are *Acinetobacter* sp., *Burkholderia* sp., and *Paenibacillus* sp. BCAs inoculation method affects biocontrol efficacy, such as pouring or drenching soil, dipping of roots, and seed coatings.

Mondal *et al.* (2014) surveyed West Bengal to evaluate the bacterial wilt disease was confirmed by ooze test in the field and biochemical tests in the laboratory in every case. A sharp relationship was observed between disease intensity and different meteorological factors during the experimental period. In most of the cases, the wilting process of wild plants started from March (Average maximum temperature 32°C and minimum temperature 20°C) when temperature gradually rises. The maximum wilt intensity was recorded during August-September (Average maximum temperature 30°C and minimum temperature 26°C), and death of such plants ceased at the end of October (Average maximum temperature 29°C and minimum temperature 22°C) or first week of November (Average maximum temperature 29°C and minimum temperature 19°C).

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

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

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

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

2.7.4. Management through *Trichoderma harzianum*

2.7.4.1. *Trichoderma spp.* antagonistic to *Fusarium spp.*

Ramezani (2010) investigated the mycoparasitism inhibitory effects of five *Trichoderma spp.* (*T. harzianum*, *T. koningi*, *T. longiconis*, *T. hamatum* and *T. viride*) on the growth of the causal agent of tomato *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lycopersici*) by dual culture in laboratory condition and reported that the maximum and minimum inhibitory effect was caused by *T. harzianum* and *T. viride* respectively. In the greenhouse, the comparison of the efficacy of disease reduction was carried out between soil and seed treatments treated with *T. harzianum* spores. Results showed that seed treatment did not cause disease reduction, but soil treatment caused disease reduction by 92%.

Ramaiah and Garampalli (2013) 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.*

Sundaramoorthy and Balabaskar (2013) reported that under *in vitro* conditions, *Trichoderma harzianum* (ANR-1) isolate effectively inhibits the radial mycelia growth of the pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL) causing *Fusarium* wilt of tomato (by 53%) when compared to all other 15 isolated native isolates. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%). Also, tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62im) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolates and untreated control.

Sivan and Chet (1989) investigated the possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. They found that the addition of conidia of *Trichoderma harzianum* in soil or seed significantly reduced the chlamydospore germination rate of both *F. oxysporum* f. spp. *vasinfectum* and *F. oxysporum* f. spp. *melonis*. They further added that the inhibition of germination of chlamydospores might be due to the competition.

Chet and Inbar (1994) found *Trichoderma harzianum* as an effective biocontrol agent against soilborne plant pathogenic fungi. Lectins were found to be involved in recognition between *Trichoderma* and its host fungi; whereas Chitinase is involved in the degradation of the host wall.

Mikorva (1982) studied the antagonistic activity of *Trichoderma spp*. against some soil pathogens and reported that among 5 *Trichoderma spp.*, three isolates of *Trichoderma harzianum* were most antagonistic.

Parveen and Ghaffar (1991) observed that seed treatment with *Trichoderma harzianum* gave complete control of *Fusarium oxysporum* on 30 and 120 days old tomato plants.

Quarles (1993) used seed treatment with *Trichoderma* as an alternative to methyl bromide and reported that in some crops, *Trichoderma spp*. protected plants as effectively as chemical seed treatments, resulting in improved yields.

2.7.4.2. *Trichoderma spp*. antagonistic to wilt complex

Scarselletti and Faull (1994) reported that the compound 6-pentyl-α-pyrone (6-p-p) produced by *Trichoderma harzianum* when added to agar at 0.3mg/ ml caused a 69.6% and 31.7% reduction in growth of *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici,* respectively after 2 days and at a concentration of 0.45 mg/ml 6-p-p completely inhibited *Fusarium* spore germination.

Saxena and Mukhopadhyay (1987) found that *T. viride* adversely influenced the hatching of *M. incognita* larvae with the highest inhibition of hatching occurring in the standard concentration of filtrate.

Parveen and Ghaffar (1991) studied the comparison of *Trichoderma harzianum, T. koningii, Gliocladium virens, Paecilomyces lilacinus, Bradyrhizobium japonicum* and *Rhizobium meliloti* with Carbofuran for control of *M. javanica* on tomato and okra in soil naturally or artificially infested with *F. oxysporum*. An artificial infestation of soil with *F. oxysporum* significantly reduced gall formation. *T. harzianum, T. koningii,* and *G. virens* showed better control of *M. `javanica* in naturally infested soil than in *Fusarium* infested soil on both the plant species.

P. lilacinus, B. japonicum, and Carbofuran significantly controlled gall formation on tomato and okra roots in nature and *Fusarium* infested soil.

2.7.5. Management through soil amendment with organic substances

Alam (1987) stated the pollution free control of plant-parasitic nematodes by soil amendment with plant wastes. Results from experiments proved that chopped plant leaves when incorporated into infested soil effectively controlled populations of plant parasitic nematodes and improved tomato growth.

Bora and Phukan (1983) tested 4 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.

Chindo and Khan (1986) observed that growth and fruit yield of tomato increased and nematode damage lessened with increasing level of poultry manure; yield increase was significant at 4 t/ha. The nematode population declined considerably by mid-season but increased towards harvest. The optimum rate of manure for nematode control and crop growth was about 4 t/ha; the highest level; did not increase yield further and resulted in more vegetative growth and delayed fruiting.

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 10 kg a.i/ha was applied as a comparative control.

Duque (1988) conducted a pot experiment outside the green house to compare the effectiveness of two rates of each of chicken dung (8.11 and 16.22 g/pot), urea (0.42 and 0.68 g/pot) and BIOACT (10 ml of 10 g/40 litre and 10 ml of g/25 litre suspension which are equivalent to 1 million and 2 million spores per ml, respectively) in controlling nematodes.

Mesfin *et al.* (1987) experimented in December 1986-1987 to determine the effectivity of five control strategies in controlling *Meloidogyne incognita* on potted kenaf fertilized with three levels of urea. The control strategies used were the application of chicken dung (5 t/ha), sawdust (5 t/ha), neem cake (294 kg/ha), *Paecilomyces lilacinus* (Ton.) samson (50,000 spores/ml) and Phenamiphos 10 g (5 kg a.i./ha). Pots treated with urea at the equivalent rates of 72 (N_1) and 132 kg/ha (N_3) were used as control. All control strategies reduced root-knot nematode population, egg mass number per root, and root galling based on the comparison with control.

Stirling (1989) used poultry manure and sawdust @ 24, 36 or 48 t/ha were incorporated into the soil with urea (0-1800kg 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.

CHAPTER 3
MATERIALS AND METHODS

The present experiments on the wilt disease complex in tomato ***(****Lycopersicon esculentum*) and its management were carried out in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, during 2017-2018. Materials as well as methodology followed in the experiments were discussed detail in this chapter.

3.1. Experimental site

3.1.1. Experimental location

1. **Lab experiment:** The *in vitro* experiment was conducted in the Central Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.
2. **Pot experiment:** The *in vitro* experiment was conducted in the net house of Department of Pant Pathology, Sher-e-Bangla Agricultural University, Dhaka.
3. **Field experiment:** The *in vivo* experiment was conducted in the central farm of Sher-e-Bangla Agricultural University, Dhaka.

3.1.2. Experimental period

The experiment were carried out during the winter season October' 2017 to April' 2018.

3.1.3. Soil type

The soil of the experimental site belongings to the "Madhupur Tract" of AEZ-28. The detail of the experiment site have been shown in Appendix-2.

3.1.4. Climate

The climate of the experimental area was of sub-tropical in nature characterized by heavy rainfall along with high temperature during the *Kharif* (April

to September) season and moderately low temperature with scanty rainfall during the Rabi (October to March) season, (Anonymous, 1997).

3.1.5. Weather

The monthly mean of daily maximum, minimum, average temperature, relative humidity at the experimental site have been collected during the period of the study (Appendix 3).

3.2. Experimental Details

3.2.1. Collection of test materials

Furadan 5G, Autostin 50 WP, Krosin 10 SP were purchased from the market. Poultry waste was collected from the Mohammadpur Poultry Farm, Dhaka. *Trichoderma harzianum* was collected from the Plant Pathology Division of BARI.

3.2.2.Variety used

Seeds of tomato variety BARI 14 was collected from HRC, BARI and prepared seedlings in the seedbed of Central Farm, SAU.

3.2.3. Treatment

T_1 = Autostin 50 WP (Foliar spray @ 0.2%)

T_2 = Furadan 5G (Soil treatment)

T_3 = Krosin 10 SP (Foliar spray @ 0.5%)

T_4 = *Trichoderma* formulation (Soil treatment)

T_5 = Poultry waste (Soil amendment)

T_6 = Autostine 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_{10} = Furadan 5G + Poultry waste

T_{11} = Krosin 10 SP + Poultry waste

T_{12} = *Trichoderma* formulation + Poultry waste

T_{13} = Autostin 50 WP + *Trichoderma* formulation + Poultry waste

T_{14} = Furadan 5G + *Trichoderma* formulation + Krosin 10 SP

T_{15} = Furadan 5G + *Trichoderma* formulation + Krosin 10 SP + Poultry waste

T_{16} = Control

3.3. Cultivation of tomato

3.3.1. Raising of seedlings

Tomato seedlings were raised in seedbed of SAU Central Farm. A seedbed was prepared which was 1.5 m length and 1 m breadth and filled with fertile soil. About five gm of seed was sown on seedbed in lines on 14 November 2017. Seedlings were observed regularly and watering was done as per requirement up to transplanting in the main field.

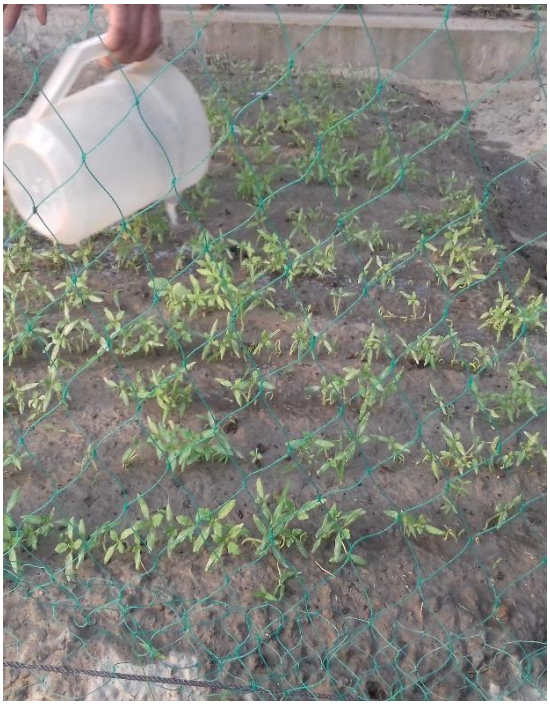
3.3.2. Land preparation

The land was firstly ploughed with a power tiller in the first week of December 2017 and the soil left exposed for 7 days to sunlight. Then the land was ploughed and cross-ploughed by a country plough until the soil had a proper tilth. Land required five to six times ploughing and every ploughing was followed by laddering to level the land and break up clods. After each ploughing weeds and rubbish was removed. Finally spade was used to prepare plots and drains.

3.3.3. Design and layout

The *in vitro* experiment was laid out in CBD (Completely Randomized Design) and the field experiments were laid out in RCBD (Randomized Complete Block Design) with three replications. In RCBD the whole plot was divided into three blocks each containing sixteen (16) plots of 3.5m x 1.0m size, giving 48 units plots. Each of the treatment combinations put once at each block. One meter wide space was kept between the blocks and 0.5m between plots (Appendix-4).

A



B

Plate 1: Raising Seedling on Seedbed

A) Sowing of seeds in seed bed

B) Watering of seedlings

3.3.4. Manure and fertilizers application

Fertilizers and manure were applied as per following standard recommendation to carry out the field experiments (Anonymous, 1997).

Manures /Fertilizers	Rate /ha
Cow dung	10 tones
Urea	226 kg
TSP	222 kg
MoP	250 kg

A half of the total amount of cow dung and TSP were applied during final land preparation and remaining half was applied in the pits before transplanting. MoP and Urea were applied in two installments as ring dressing after 15 and 35 days of transplanting.

3.3.5. Transplanting Seedlings

30 days old tomato seedlings were transplanted in main field of Central Farm on 15 Dec, 2017. Plant to plant distance was maintained at 75 cm. Bamboo sticks were used to keep seedlings upright. Plants were observed regularly and irrigation was done as per needed.

3.3.6. Cultural Operation

Gap filling, earthing up, flood irrigation, netting, spraying Actara for insect killing is also done as per requirement. After transplantation gap filling was done in case of any seedling died. In 20 days after planting (DAP) weeding was done which followed split doze fertilizer application. On the 15th January 2018 1st split

application of fertilizer was done and the 2nd split application was done on 4th February 2018 as treatment of double dose nitrogenous fertilizer.

3.4. Application of treatment

3.4.1. Application of Autostin 50 WP

Autostin 50 WP (0.2%) solution was applied on root zone as well as surface of plant body at 10 days interval.

3.4.2. Application of Furadan 5G in soil

Furadan 5G was applied in the soil during transplantation of seedlings for those plot that are assigned for Furadan 5G application. 5gm Furadan 5G was put in each pit and mixed up the adjacent soil before transplanting the seedlings. The specification of nematicide is mentioned in Table 1

Table 1. Details of nematicide (Furadan 5G)

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

3.4.2.1. Preparation and application of fungicides solution

Fungicidal solutions were prepared dissolving required amount of fungicide in water for each concentration in 100 ml Erlenmeyer flask. Flasks were labeled appropriately and shaken thoroughly before use. Then the fungicide solution was applied in assigned plots for soil drenching. Flasks were labeled appropriately and shaken thoroughly before use of fungicide solutions. The particulars of fungicide used in this study is given in Table.

Table 2. Details of fungicides

Common name	Chemical name	Active ingredient(a.i)
Autostin 50 WP	Methyl-2-Benzimidazole Carbamate	50% Carbendazim

3.4.3. Application of Krosin 10 SP

Bactericide, Krosin 10 SP (0.5%) was applied with 10 days interval at the root zone by spraying with hand sprayer so that the rhizosphere soil drenched well.

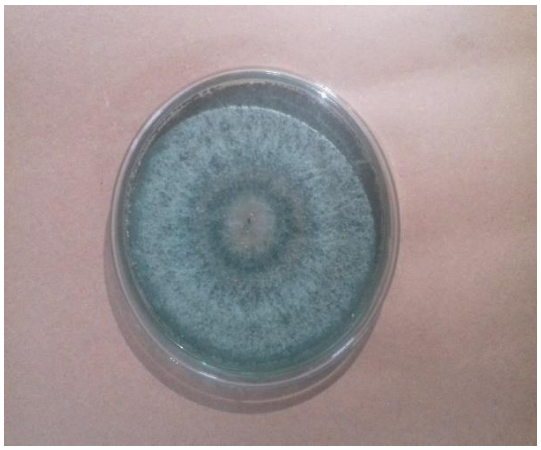
3.4.4. Application of Poultry waste

Poultry waste at the rate of 5 kg /plot was applied to the soil in specific plots at twenty days before transplanting and mixed with soil properly.

3.4.5. Mass multiplication of *Trichoderma* formulation

Peat soil, Black gram bran and water were used in 1:1:2 ratio for mass multiplication of *Trichoderma harzianum* (Islam, 2005). The requisite amount of materials were mixed thoroughly in a conical flax and autoclave at 121^{o} C for 15 minutes for sterilization. The sterilized substrate allowed to cool down at room temperature and then inoculated with 5 mm diameter mycelium disc of 7 days old culture of *Trichoderma harzianum*. 10 discs for each 500 ml flasks were used for inoculation. Inoculated flaxes were then incubated at room temperature 25± 2^{o} C.

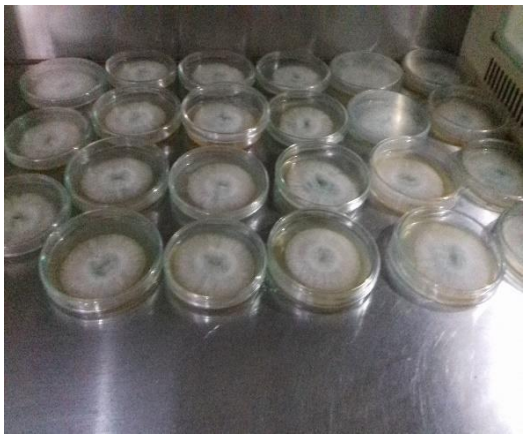
After incubation for 25 days the substrates were taken out from the flask, shade dried in laminar air flow cabinet and grinded in a blender. The grinded materials were kept in polythene beg with labeling and treated as *Trichoderma* formulation.



A) Mother culture of *Trichoderma spp.*

B) Mass Culturing on PDA media

C) Sub Culture of *Trichoderma harazianum*



D) Mass Production of *Trichoderma harazianum*

E) *Trichoderma* mass production in substrate

Plate 2. Mass multiplication of *Trichoderma harazianum*

A) Ploughed land

B) Transplanting seedling

C) Irrigation of field

D) Tagging of plants

E) Netting of experimental field

F) Collecting fruits

Plate 3. Views of field experiment

3.5. Identification of Diseased Plant

3.5.1. Identification of *Fusarium* wilt disease

The fusarial wilt was identified observing the partial (one sided) wilt of the infected plants. Chlorosis of leaves followed by slow wilting and drying up of leaves of one side of the plant considered as the typical fungal wilt of tomato (Ignjatov *et al.* 2012).

Necrosis of the stem in the vascular system was revealed by cross-section also taken into consideration for the identification.

3.5.2. Identification Bacterial wilt disease

Sudden wilting and collapse of the whole plant within few days were considered to determine the bacterial wilt (Champoiseau & Momol, 2009). The entire plant readily died including the youngest leaves and twigs.

3.5.3. Identification Nemic wilt (Root knot disease)

Nematode infection of plants determined by observing the roots as well as the symptoms of above ground parts of the affected plants. The below ground symptoms appeared with root knots in infected plants and above ground symptoms appeared as stunted growth for deficiencies of nutrients, yellowing of leaves, wilting in sun light or cloudy weather and reduced the yields by hampering the quality of fruits.

3.6. Data Collection

1) Disease Incidence for Fungal wilt, Bacterial wilt and Nemic wilt
2) Yield contributing characters
    a) Plant height (cm)
    b) Number of branch/plant
    c) Number of leaf/plant
    d) Total weight of fruit/plant
    e) Single weight of fruit/plant
3) Fruit yield

3.7. Calculation of disease incidence

Disease incidence was calculated by using the formula -

$$\text{Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Number of total inspected plants}} \times 100$$

3.8. Cost-benefit analysis and Benefit-Cost Ratio

Costing of application of treatments for management of wilt of tomato was done based on the current market price of inputs, rate of labour wage and agricultural equipment. Price of the gross return was determined on the basis of current market price of 2018 (Appendices 8 and 9).

Estimation of Cost-Benefit Ratio (BCR) was done according to (Islam *et al.*, 2005) using the following formula-

$$\text{Benefit Cost Ratio} = \frac{\text{Gross return (Tk. /ha)}}{\text{Cost of Production (TK. /ha)}}$$

3.9. Analysis of data

The relevant data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments by Statistics 10 software.

3.10. Isolation and Identification of pathogen

3.10.1. Sterilization of materials

All glass wares were wrapped with brown paper or aluminum foil paper and were sterilized for two hours in hot air oven at 160-180^{0}C. For media sterilization, it was poured in to conical flask followed by plugging in non-absorbent cotton and then they were sterilized at 15 P.S.I (121.6^0 C) for 20 minutes in an autoclave. The sterilized media in conical flasks were poured in to sterilized petridishes for conducting some experiments. These were stored in a refrigerator at 5-8^{0}C for further use.

The inoculating needle, glass rods, loops, forceps etc. were sterilized by dipping them in 70% ethanol followed by flaming over spirit lamp. For isolation, the affected plants were collected and affected portion of samples were cut into small bits (2-3 mm) followed by surface sterilization with 1:1000 (0.1%) Mercuric chloride (HgCl2) solution for 30 seconds or 0.5% Sodium hypochlorite (NaOCl) solution for 2 minutes and then washed in sterile distilled water for 2-3 times to remove traces of the chemicals.

3.10.2. Preparation of medium

3.10.2.1. Preparation of PDA medium

Peeled and sliced potato (200 g) was boiled in 500 ml of distilled water till softening of the potato tissues. Then the potato extract was filtered through a clean sieve or clean cloth. Twenty grams of agar powder was boiled in 500 ml of distilled water till it was melted completely. Both the solutions were gradually mixed. Dextrose (20 g) was added with constant stirring and the final volume was made up to 1000 ml with distilled water and autoclaved at 1.1 Kg/cm^2 (15 psi) for 15 minutes.

Table 3: Composition of PDA media

Sl. No.	Ingredient	Composition
1	Peeled and sliced potato	220 g
2	Dextrose	20 g
3	Agar powder	20 g
4	Distilled water	1000 ml

3.10.2.2. Preparation of CPG, TTC and NA media

CPG contains four ingredients. TTC media was used as a selective medium for the isolation of *Ralstonia sp*. To make TTC medium 1% of sterilized aqueous solution of 2, 3, 3-Triphenyl-o-tetrazolium Chloride (TTC) was added aseptically to the CPG medium before solidification and poured in petriplates. The virulent isolates of *Ralstonia sp.* will be appeared as small, round, slimy, white with pink center.

Table 4: Composition CPG and TTC media

Sl. No.	Ingredients	Composition
1	Casamino acid (Casein hydrolyzed)	1.0 g
2	Peptone	10.0 g
3	Glucose	5.0 g
4	Agar powder	17.0 g

Table 5. Composition of Nutrient Agar (NA) medium

Sl. No.	Ingredients	Composition
1	Nutrient broth	13.0 gm
2	Agar	17 gm
3	Water	1000

3.11. Isolation of wilt pathogens

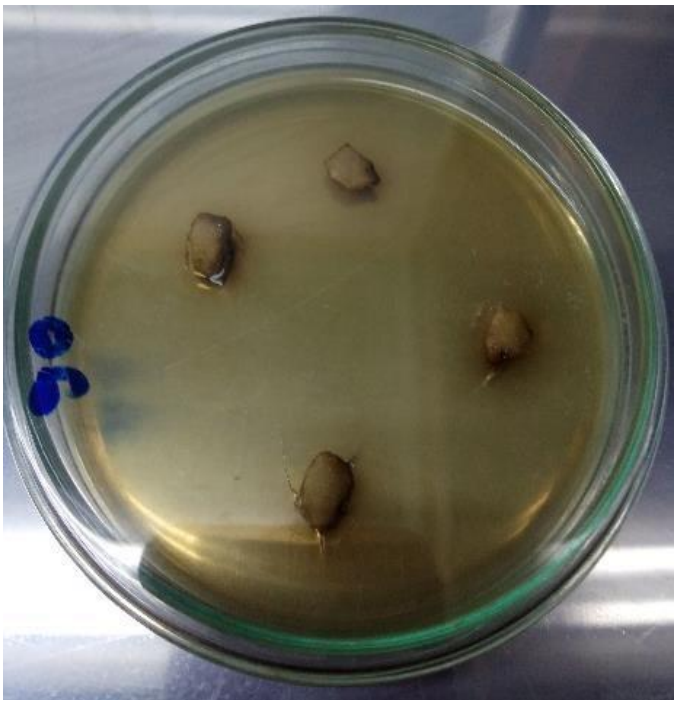
3.11.1. Isolation of wilt pathogens in PDA media

The tissue culture technique was used for isolation of pathogens described by 'Riker and Riker' (1936). The infected tomato plants were collected from field and the roots and plants were washed in running tap water to remove all dirt and other foreign materials. Affected portion of the stem was cut into small bits (2-3 mm). These bits were surface sterilized with 1:1000 (0.1%) Mercuric chloride ($HgCl_2$) solution for 30 seconds and were washed thoroughly in sterile distilled water for three times to remove traces of $HgCl_2$ solution. Finally with the help of a sterilized inoculating needle the disinfected bits were transferred to the petriplates (4 bits per petriplate) containing PDA. All these operations were carried out inside aseptic inoculation chamber, which was previously disinfected by 70% ethanol.

Then the plates were incubated at room temperature (27 ± 1^0C) and the growth was observed periodically.

3.12. Maintenance of pure culture of the isolated fungus

After having the pure culture of relevant fungus it was sub cultured on PDA media and allowed to grow at 27 ± 1^0C for 10 days and such slants were preserved in a refrigerator at 5^0C and renewed once in 30 days.



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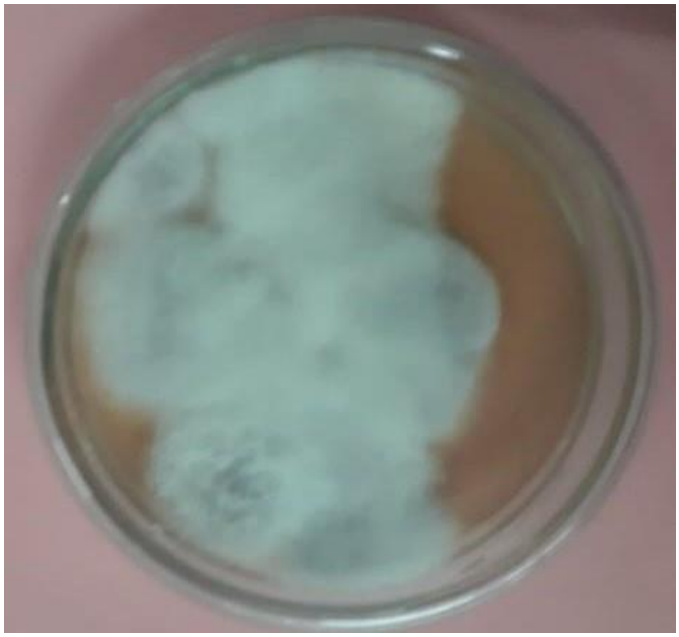


B

Plate 4. Isolation of *Fusarium oxysporun*

A) Diseased Tissue placed on PDA

B) Whitish mycelial growth of *Fusarium oxysporum*

A

B

Plate 5. Isolation of *Ralstonia solanacearum*

A) Completely wilted tomato plant

B) Infected vascular zone

3.13. Isolation of bacterium on TTC media

Isolation of bacterium was done following streak plate method on TTC medium. For this purpose, a loopful of the bacterial suspension was taken with an inoculating needle and touched at one end of the medium surface in the plate. A set of 3-4 lines was drawn on the upper side of the plate. Then the needle was sterilized in flame and a second set of 4-5 lines was drawn from the first set at right angles to the first set. Same procedure was followed to draw a third set of line from the second set. The plates were then incubated in an upside down position in incubator for 24-48 hours maintained at $30^0C \pm 1^0C$.

3.14. Maintenance of bacterium on TTC media

The plates were observed for the growth of the bacterial colonies after 24 hours of incubation. The bacterial colonies usually appeared within 48 hours of incubation. Bacterial colony showing distinct character was re-streaked in another media plate as was done for isolation of bacteria as a part of purification procedure. The colonies were selected from the final plate and each was transferred to separate slant surface containing TTC medium and incubated for 48 hours. After the desirable growth of the bacterium on the slant surface, the plate was labeled and kept in a refrigerator for further use.

3.15. Isolation of Nematodes (Root knot nematodes)

Root zone was inspected and diseased plants were uprooted by using spade. Roots were observed carefully. Egg mass found in the infected root were observed for nematodes under microscope.

A

B

C

D

Plate 6. Identification of *Meloidogyne spp*.

A. Uprooting infected plant with spade

B. Infected Rootzone

C. Root knot on root

D. Searching egg under Microscope 40x

3.16. Pathogenicity test under pot culture experiment

3.16.1. Soil Sterilization

For soil sterilization 0.4% formaline at the rate of 200 ml/cft soil thoroughly mixed up and kept under polythine cover for 48 hours. The soil exposed for 7 days and then ready to sowing or planting.

3.17. Inoculation by fungal and bacterial isolates

The isolates of *Fusarium oxysporum* and *Ralstonia solanacearum* and *Meledogyne sp.* were tested for their pathogenic activities on the tomato crop under pot culture experimentation.

Spore solution of ten days old culture of *Fusaruim oxysporum* was inoculated by drenching the rootzone of the tomato plants in pots containg sterilized soil.

Isolates of *Ralstonia solanacearum*as spore solution was inoculated by injecting the solution in the vascular system of tomato seedlings in pots containg sterilized Soil.

3.18. Morphological studies of isolated pathogens

3.18.1. Morphological studies of the isolated fungal pathogen

A small loop of culture of *Fusarium oxysporum* lifted up from ten days old culture and placed on cotton blue to obtain clear stained spores. A cover slip was placed over it. Microphotographs of macro-conidia were taken for morphological study.

Collected Soil for sterilization

Spraying Formalin on Soil

Soil covered with polythen sheet

Plate 7. Events of soil sterilization

A

B

C

D

Plate 8. A view of pot experiments for pathogenic test

A. Non inoculated tomato plant and
B. Inoculated plant
C. Prepared isolates solution
D. Inoculation on rootzone

3.18.2. Morphological studies of the isolated bacterium

3.18.2.1. Gram staining

The procedure for Gram staining as follows-

A thin smear of bacterial suspension was made on a clean sterilized glass slide and dried in air. Bacteria cells were fixed by gently heating over the flame for few seconds. Then it was flooded with Gram's crystal violet for 1 min. Then it was washed with water and flooded with Gram's iodine solution for 45 sec. It was again washed with water and flooded with Gram's de-colourizer until no further violet colour came off. It was then washed with water and counterstained with 0.1% safranin for about 20 seconds. It was finally washed with water, dried in air and observed under oil emersion objective. Observation was taken on colour of the bacterial cells and its size (length and breadth) were measured. The cells retaining purple to violet colour were marked as Gram positive and others were as Gram negative.

3.19. *In vitro* evalution

3.19.1. *In vitro* evalution of Fungicide

The efficacy of Autostin 50 WP (Carbendazim) was evaluated by food poisoning method (modified cup method). PDA plate was prepare with 15 ml media. After solidification, 5 mm discs of the media were scooped from 3 places of plate maintaining an equal distance from each other and center of the plate using sterilized disc cutter. One ml of fungicidal solution (0.1%) was put into each hole and the plate were stored overnight in refrigeration (normal) for diffusion of fungicide into the medium around the hole. The next day one 5mm PDA blocks of 7 days old culture of *Fusarium oxysporum* were cut with sterilized disc cutter and one block was placed at the center of the plate. The plates were incubated in an incubation chamber at 25^{o} C. The redial mycelial growth of *Fusarium oxysporum* was recorded

at 24 hour interval until the colony covered the whole plate of control (Islam et. al, 2005)

3.19.2. *In vitro* evaluation of bacteriocide

The efficacy of bacteriocide (Krosin 10 SP) was evaluated by food poisoning technique (modified cup method). Petri-plates were prepared with 15 ml NA media. After solidification, the surface of the media softly rinse with spore solution of *Ralstonia solanacearum.* Then a 5mm disc of the media was scooped from the center of the plate. One ml of Krosin 10 SP (0.1%) was poured into the hole and the plates were incubated in an incubation chamber at 25^0 C for 7 days. The scooped of the control plate was poured with 1 ml plain sterile water. After 7 days the growth less zone of the treated plate was measured.

3.20. Measurement of inhibition of pathogen

After incubation, while the control plates were fully covered with pathogen growth. The redial colony growth (in case of *Fusarium oxysporum*) and growth less zone (in case of *Ralstonia solanacearum*) was measured by taking the average of the two diameters taken right angles for each (Islam *et. al* 2005).

Inhibition percentage of the pathogens was computed by the following formula-

$$\text{Percent Inhibition} = \frac{A - B}{B} \times 100$$

A = Radial growth in control plates

B = Radial growth in treated plates

CHAPTER 4
RESULTS AND DISCUSSION

4.1. Symptomatology study of wilt disease of tomato

Initially yellowing and wilting of lower leaves was observed in *Fusarium* wilt. In the beginning of this disease one side of the plant got wilted. The yellowing and wilting gradually progressed up. Then the yellowed and wilted leaves dried up and dropped prematurely. Finally, the entire plant become wilted. In case of bacterial wilt, the plant got wilted suddenly without yellowing of foliage. When the stem was cut, brown discoloration of the internal tissues was revealed. In case of nemic wilt or root knot disease, knots were found in the root system of the uprooted diseased plant. The diseased plant found to be wilted in the day light and become normal in the night or cloudy day. The plants gradually got weaken with time.

4.2. Isolation and identification of the associated fungus

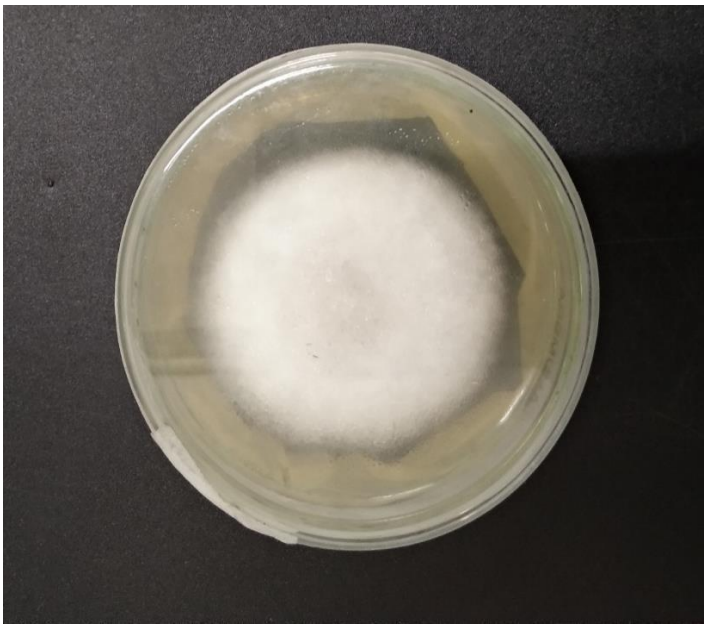
In *Fusarium oxysporum* macro-conidia were observed when fresh samples were observed under microscope. The culture obtained from affected stems of tomato plant was identified as *Fusarium oxysporum* based on the morphological and cultural characters and by pathogenicity test. The culture produced pure white to creamy white cottony colony on Potato Dextrose Agar.

4.3. Isolation and identification of the associated bacteria

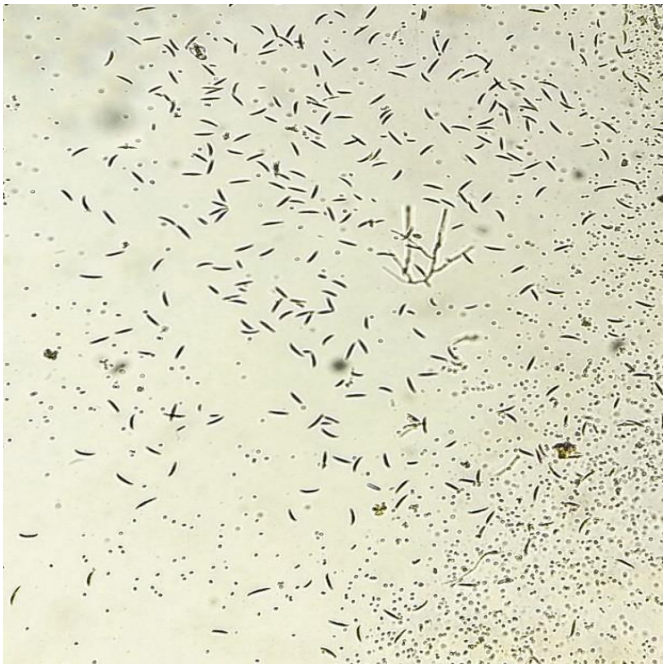
The isolated bacteria cultured on TTC media showed white colony with spiral pink center appeared after 48 hours of incubation. Based on the colony color on TTC media and gram staining test and results of the pathogenicity test, the isolated bacteria is identified as *Ralstonia solanacearum*.

4.4. Isolation and identification of the associated nematode

Numerous root knots were found in the root system and enormous egg mass was observed while investigated under microscope. Thus, the nematode was confirmed as *Meloidogyne spp.*



A



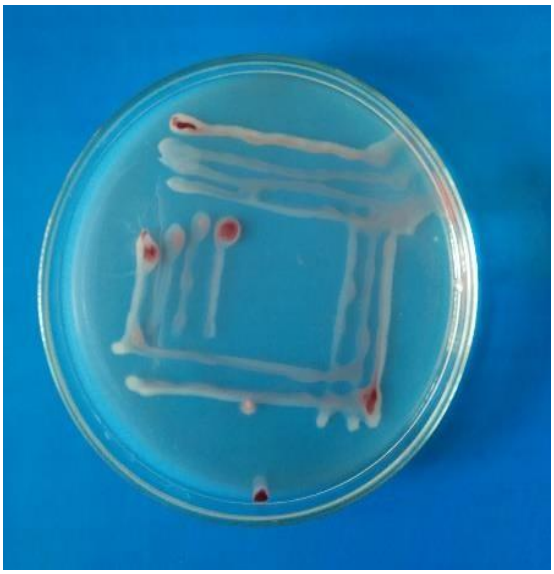
B

Plate 9. Isolation and Identification of *Fusarium oxysporum*

A. Pure culture of *Fusarium oxysporum* on PDA

B. Macro and micro conidia observed under compound microscope (10 x)

A

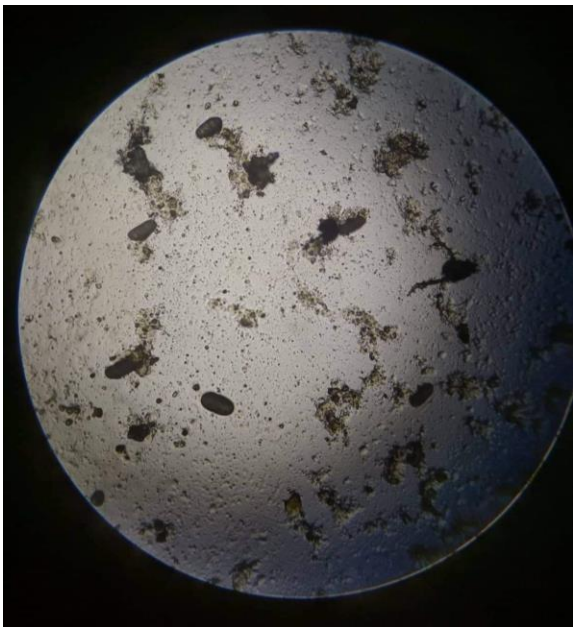


B

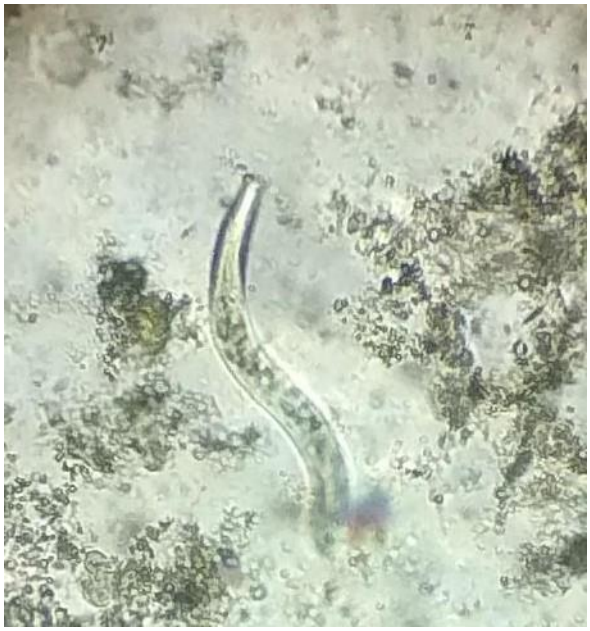
Plate 10. Isolation and Identification of *Ralstonia solanacearum*

A. Bacterial growth from diseased tissue

B. Pure culture of *Ralstonia solanacearum*



A



B

Plate 11. Isolation and Identification of Nematode under compound microscope

A. Egg mass of nematode of *Meloidogyne spp.*

B. Juvenile of male nematode, *Meloidogyne spp.* observedunder Microscope

4.5. *In vitro* evaluation of fungicide (Autostin 50 WP)

The fungicide Autostin 50 WP (Carbendazim) was assayed in *vitro* against the *Fusarium oxysporum* causing wilt of tomato and found promising in reducing the mycelial growth of the fungus. The redial mycelial growth was recorded 1.03 cm in case of Autostin 50 WP while it was 8.5 cm in control. The inhibition of radial mycelial growth was counted 87.88% over control.

Table 6. *In vitro* Bioassay of Autostin 50 WP against mycelial growth of *Fusarium oxysporum*

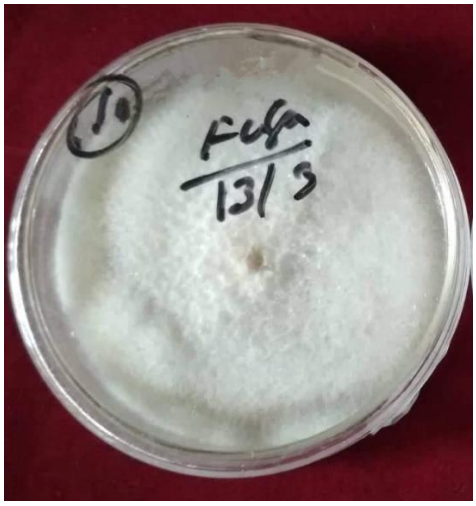
Treatment	Redial mycelial growth (cm)	% Inhibition over control
Autostin 0.2% (Carbendazim)	1.03 a	87.88
Control	8.50 b	--
LSD value	1.17	
CV value	7.01	

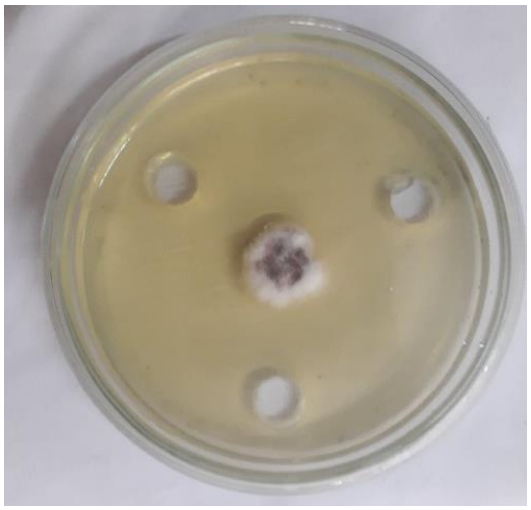
4.6. *In vitro* evaluation of bacteriocide (Krosin 10 SP)

The bacteriocide Krosin 0.5% (Streptomycin sulphate) was assayed in *vitro* against the *Ralstonia solanacearum* causing wilt of tomato and found promising in reducing growth of the bacteria. The redial inhibition zone was recorded 7.5 cm while it was 0.0 cm in control. The inhibition of bacterial growth was 83.33% over control.

Table 7. *In vitro* Bioassay of Krosin against mycelial growth of *Ralstonia Solanacearum*

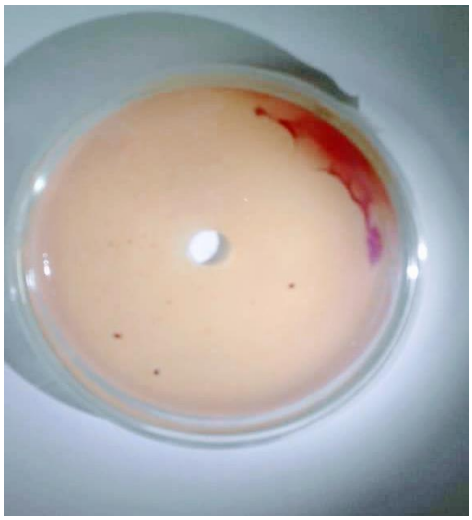
Treatment	Redial inhibition zone (cm)	% Inhibition over control
Krosin 10 SP (0.5%) (Streptomycin sulphate)	7.5 a	83.33
Control	0.0 b	--
LSD value	2.48	
CV value	18.86	





Control plate Treated plate

Plate 12. Evaluation of the efficacy of fungicide by food poisoning method (modified cup method)



Control plate Treated plate

Plate 13. Evaluation of the efficacy of bacteriocide by food poisoning method (modified cup method)

4.7. Effect of different treatments on growth parameter and yield of tomato

The effect of treatments applied for the management of wilt complex of tomato differed significantly in respect of yield containing characters and yield (Table 8).

In case of plant height, the highest height (41cm) was recorded in case of treatment T_{15} where the application of Krosin 10 SP, Furadan 5G, *Trichoderma* formulation and Poultry waste was combined. The lowest plant height was found in the control treatment (31cm). The treatment T_{14} where Furadan 5G, *Trichoderma harazianum* and Krosin 10 SP were applied was noted as the second highest performer regarding plant height which was statistically alike with that of treatment T_{13} (Autostin 50 WP + *Trichoderma harazianum* + Poultry waste), T_7 (Furadan 5G + *Trichoderma* formulation*)* and T_8 (Krosin 10 SP + *Trichoderma* formulation) that resulted 40 cm plant height.

In case of plant branch, the highest number of branches (11.33) was recorded in case of treatment T_{15} where the application of Krosin 10 SP, Furadan 5G, *Trichoderma* formulation and Poultry waste were combined which was statistically similar with T_{13} where Autostin, *Trichoderma harazianum*, Krosin 10 SP was combined. The treatment T_7 where Furadan 5G with *Trichoderma harazianum* was applied that was the second highest branch (9.33). The lowest branches were counted in the control treatment (3.33).

In case of number of leaf, the highest number of leaf per plant (45) was found in the treatment T_{15} which was statistically alike with the treatment T_{13} where Autostin 50 WP, *Trichoderma harazianum* and Poultry waste were applied combinedly. The second highest number of leaf (42) was counted in treatment T_{14}

where Furadan 5G, *Trichoderma harazianum* and Krosin 10 SP were applied combinedly and that was statistically alike with T_{13}. The lowest leaf (25) was found in the control treatment.

In case of number of fruit, the highest fruit number per plant (157) counted in the treatment T_{15} where Krosin 50 WP, Furadan 5G, *Trichoderma* formulation and Poultry waste were used in combination. The second highest number of fruit (148.33) was recorded in case of treatment T_{14} followed by treatment T_{13} (142.33) and these two treatments remained statistically alike. The lowest number of fruit (75) was found in the control treatment.

In case of single fruit weight, significantly the highest single fruit weight per plant (130 gm) was counted in the treatment T_{15} followed by treatment T_{14} (100 gm) which was statistically identical with that of the treatment T_7 (100) where Furadan 5G and *Trichoderma* formulation were used.

In case of yield of tomato, treatment T_{15} gave the highest yield (41.80 ton/ha) where the application of Krosin 10 SP, Furadan 5G, *Trichoderma* formulation and Poultry waste were combined (Table 9). The second highest yield was recorded in case of T_{14} (33.47 ton/ha) where Furadan 5G, *Trichoderma harazianum* and Krosin 10 SP were applied together. The third highest yield was recorded in case of T_7 (29.81 ton/ha) where Furadan 5G and *Trichoderma* formulation were used together which was statistically identical with T_{13} (29.81 ton/ha). The lowest yield was recorded in case of control treatment T_{16} (10.1 ton/ha). Based on the yield performances of the treatments, it was calculated that the yield increased by 313.86 % in case of the treatment T_{15} followed by T_{14} (231.38%), T_{13} (195.14%) and T_7 (195.14%) over control (T_{16}).

Table 8. Effect of different treatments on yield and yield contributing characters of tomato

Treatment	Height plant^{-1} (cm)	Branch/ Plant	Leaf plant^{-1}	Fruit plant^{-1}	Single Fruit wt. (gm)	Yield Kg plot^{-1}	Legend
T_1	39 a-c	8 c	32 de	133 c	85 cd	15.5 d	T_1 = Autostin (seedling treatment + foliar spray @ 0.2%)
T_2	38 bc	7 c	32 de	128 cd	82 c-e	16 d	T_2 = Furadan (soil treatment)
T_3	34 e	7.67 c	32 de	122 de	75 e	14.6 d	T_3 = Krosin 10 SP (seedling treatment + foliar spray @ 0.5%)
T_4	35 de	9 a-c	32 de	100 f	77 de	15.6 d	T_4= *Trichoderma* formulation (soil treatment)
T_5	37 cd	8.67 a-c	36 cd	100 f	80 de	15 d	T_5 = Poultry waste (soil amendment)
T_6	38 bc	8.67 a-c	35 c-e	104 f	80 de	15 d	T_6 = Autostin + *Trichoderma* formulation
T_7	40 ab	9.33 a-c	38 bc	147 b	100 b	**21.9 bc**	T_7= Furadan + *Trichoderma* formulation
T_8	40 ab	8.33 bc	37 b-d	134 c	90 c	18.5 b-d	T_8 = Krosin 10 SP + *Trichoderma* formulation
T_9	37 cd	7.33 c	32 de	104 f	84.33 c-e	15 d	T_9 = Autostin + Poultry waste
T_{10}	37 cd	8 c	32 de	117 e	80 de	16.5 d	T_{10} = Furadan + Poultry waste
T_{11}	39 a-c	9 a-c	36 cd	125 d	82 c-e	17 d	T_{11}= Krosin 10 SP + Poultry waste
T_{12}	39 a-c	8.33 bc	36.33 cd	126 de	82 c-e	17.5 cd	T_{12} = *Trichoderma* formulation + Poultry waste
T_{13}	40 ab	11.33 a	44 a	142.33 b	85 cd	19 b-d	T_{13} = Autostin + *Trichoderma* formulation + Poultry waste
T_{14}	40 ab	8 c	42 ab	148.33 b	100 b	**22 b**	T_{14} = Furadan + *Trichoderma* formulation + Krosin 10 SP
T_{15}	**41 a**	**11.33 a**	**45 a**	**157 a**	**130 a**	**26.6 a**	T_{15} = Krosin + Furadan + *Trichoderma* formulation + Poultry waste
T_{16}	**31 f**	**3.33 d**	**25 f**	**75 g**	**60 f**	**9.4 e**	T_{16} = Control
LSD value	2.98	2.80	5.05	6.34	1.6848	4.46	* Values in a column with same letter (s) do not differ significantly.
CV value	4.74	20.23	8.59	3.10	6.98	15.56	

Table 9. Effect of different treatments on fruit yield of tomato against wilt complex diseases

Treatment	Yield/plant (kg)	Yield/plot (kg)	Yield (ton/ha)	Yield increased over control (%)
T_1	2.64 b-d	15.5 d	29.04 b-d	187.52
T_2	2.29 b-d	16 d	25.19 b-d	149.40
T_3	2.05 cd	14.6 d	22.55 cd	123.26
T_4	2.09 b-d	15.6 d	22.99 b-d	127.62
T_5	2.14 b-d	15 d	23.54 b-d	133.06
T_6	2.14 b-c	15 d	23.54 b-d	133.06
T_7	2.71 b	21.9 bc	29.81 bc	195.14
T_8	1.95 d	18.5 b-d	21.45 d	112.27
T_9	2.14 b-d	15 d	23.54 d	133.06
T_{10}	2.36 b-d	16.5 d	25.96 b-d	157.03
T_{11}	2.21 b-d	17 d	24.31 b-d	141.48
T_{12}	2.5 b-d	17.5 cd	27.5 b-d	172.2
T_{13}	2.71 bc	19 b-d	29.81 bc	195.14
T_{14}	2.77 b	**22 b**	33.47 b	231.38
T_{15}	**3.8 a**	**27.6 a**	41.80 a	**313.86**
T_{16}	**0.9 e**	9.4 e	10.1 e	-
LSD value	0.70	4.46	7.76	
CV value	18.12	15.50	18.56	

Legend

T_1 = Autostin (seedling treatment + foliar spray @ 0.2%)

T_2 = Furadan (soil treatment)

T_3 = Krosin 10 SP (seedling treatment + foliar spray @ 0.5%)

T_4= *Trichoderma* formulation (soil treatment)

T_5 = Poultry waste (soil amendment)

T_6 = Autostin + *Trichoderma* formulation

T_7= Furadan + *Trichoderma* formulation

T_8 = Krosin 10 SP + *Trichoderma* formulation

T_9 = Autostin + Poultry waste

T_{10} = Furadan + Poultry waste

T_{11}= Krosin 10 SP + Poultry waste

T_{12} = *Trichoderma* formulation + Poultry waste

T_{13} = Autostin + *Trichoderma* formulation + Poultry waste

T_{14} = Furadan + *Trichoderma* formulation + Krosin 10 SP

T_{15} = Krosin + Furadan + *Trichoderma* formulation + Poultry waste

T_{16} = Control

* Values in a column with same letter (s) do not differ significantly.

A. Infested field

B. Partially wilted plant (Fungal wilt)

C. Completely wilted plant (Bacterial wilt)

D. Plant wilted on day light but normal at night cloudy day (Nemic wilt)

Plate 14. Symptoms of wilted plant observed in main field

A

B

Plate 15. Effect of treatments comparison in main field

A. Healthy plant growth in treated plot

B. Diseased plant in Control plot

A

B

Plate 16. Tomato fruit quality

A) Healthy tomato fruits harvested from T_{15} (Treated field)

B) Healthy tomato fruits in standing plant in T_{15} (Treated pot)

A. Healthy tomato plants on pot experiment (non-inoculated)

B. Wilted tomato plants on pot experiment (inoculated by bacteria)

Plate 17. A view of pot experiments

A. Non inoculated healthy plant

B. *Fusarium* inoculated plant

C. *Ralstonia solanacearum* inoculated plant

D. Nematode inoculated plant

Plate 18. Non inoculated and Inoculated tomato plant under pathogenicity test

4.8. Effect of different treatments on disease incidence of wilt complex of tomato

The effect of different treatments on disease incidence was recorded and data was presented in Table 10.

4.8.1. Effect of different treatments on the incidence of fungal wilt

The effect of different treatments on the incidence of fungal wilt differed significantly. The highest wilt incidence (9.85%) was recorded in control treatment. The second highest wilt incidence was recorded in treatment T_2 (Krosin 10 SP) which was statistically similar to the treatments T_3 (Krosin 10 SP), T_{10} (Furadan 5G + Poultry waste), T_{11} (Krosin 10 SP + Poultry waste) where wilt incidence was 5.09%. No wilted plant at all were observed in case of T_1 (Autostin 50 WP), T_4 (*Trichoderma* formulation), T_5, (Poultry waste), T_6 (Autostin 50 WP + *Trichderma* formulation), T_7 (Furadan 5G + *Trichderma* formulation), T_8 (Krosin 10 SP + *Trichderma* formulation), T_9 (Autostin + Poultry waste), T_{12} (*Trichderma* formulation + Poultry waste), T_{13} (Autostin 50WP + *Trichderma* formulation + Poultry waste), T_{14} (Furadan 5G + *Trichderma* formulation + Krosin 10 SP) and T_{15} (Krosin 10 SP + Furadan 5G + *Trichderma* formulation + Poultry waste).

4.8.2. Effect of different treatments on the incidence of bacterial wilt

In case of bacterial wilt, some treatments showed promising effect in reducing the incidence of bacterial wilt. The highest wilt incidence was (9.68%) recorded in control treatment. The second highest wilt incidence was recorded in treatment T_1 (Autostin) which was statistically similar to the treatments T_5 (Krosin 10 SP), T_6 (Furadan 5G + Poultry waste), T_9 (Krosin 10 SP + Poultry waste) and T_{13} (Autostin 50WP + *Trichoderma* formulation + Poultry waste) where bacterial

wilt incidence was 5.09%. No bacterial wilted plants were observed in case of T_2 (Furadan 5G), T_3 (Krosin 10 SP), T_4 (*Trichoderma* formulation), T_7 (Furadan 5G + *Trichderma* formulation), T_8 (Krosin 10 SP + *Trichderma* formulation**)**, T_{10} (Furadan 5G + Poultry waste), T_{11} (Krosin 10 SP + Poultry waste), T_{12} (*Trichderma* formulation + Poultry waste)**,** T_{14} (Furadan 5G + *Trichderma* formulation + Krosin 10 SP)**,** T_{15} (Krosin 10 SP + Furadan 5G + *Trichderma* formulation + Poultry waste).

4.8.3. Effect of treatments on the incidence of nemic wilt

In case of nemic wilt, some treatments showed promising effect in reducing the incidence of nemic wilt. The highest wilt incidence (9.68%) was recorded in control treatment. The second highest wilt incidence was recorded in treatment T_5 (Krosin 10 SP), which was statistically similar to the treatments T_6 (Furadan 5G + Poultry waste), T_9 (Krosin 10 SP + Poultry waste) and T_{12} (*Trichoderma* formulation + Poultry waste) where wilt incidence was 5.09%. No nemic wilted plant were observed in case of T_1 (Atostin 50 WP), T_2 (Furadan 5G), T_3 (Krosin 10 SP), T_4 (*Trichoderma* formulation), T_7 (Furadan 5G + *Trichderma* formulation), T_8 (Krosin 10 SP + *Trichderma* formulation**)**, T_{10} (Furadan 5G + Poultry waste), T_{11} (Krosin 10 SP + Poultry waste), T_{13} (Autostin 50WP + *Trichderma* formulation + Poultry waste)**,** T_{14} (Furadan 5G + *Trichderma* formulation + Krosin 10 SP)**,** T_{15} (Krosin 10 SP + Furadan 5G + *Trichderma* formulation + Poultry waste).

4.8.4. Effect of treatments on cumulative disease incidence

In case of cumulative disease incidence, the highest incidence (28.56%) was recorded in control treatment followed by treatment T_5 (Poultry waste) which was statistically similar to the treatments T_6 (Furadan 5G + Poultry waste), T_9 (Krosin + Poultry waste) where wilt incidence was 14.45%, 9.85%, 9.85%, respectively. No

wilt incidence was observed in case of T_1 (Atostin 50 WP), T_2 (Furadan 5G), T_3 (Krosin 10 SP), T_4 (*Trichoderma* formulation), T_7 (Furadan 5G + *Trichderma* formulation), T_8 (Krosin 10 SP + *Trichderma* formulation), T_{10} (Furadan 5G + Poultry waste), T_{11} (Krosin 10 SP + Poultry waste), T_{12} (*Trichderma* formulation + Poultry waste)**,** T_{13} (Autostin 50 WP + *Trichderma* formulation + Poultry waste), T_{14} (Furadan 5G + *Trichderma* formulation + Krosin 10 SP), T_{15} (Krosin 10 SP + Furadan 5G + *Trichderma* formulation + Poultry waste).

Table 10. Effect of different treatments on disease incidence and yield of tomato

Treatment	Disease incidence (DI) (%)			Total disease incidence (%)	Yield kg plot^{-1}	Legend
	Fungal wilt	Bacterial wilt	Nemic wilt			
T1	0.00 (0.71) b	5.09 (1.73) ab	0.00 (0.71) b	0.00 (0.71) b	15.5 d	T_1 = Autostin (seedling treatment + foliar spray @ 0.2%)
T2	5.09 (1.73) ab	0.00 (0.71)	0.00 (0.71) b	0.00 (0.71) b	16 d	T_2 = Furadan (soil treatment)
T3	5.09 (1.73) ab	0.00 (0.71)	0.00 (0.71) b	0.00 (0.71) b	14.6 d	T_3 = Krosin 10 SP (seedling treatment + foliar spray @ 0.5%)
T4	0.00 (0.71) b	0.00 (0.71)	0.00 (0.71) b	0.00 (0.71) b	15.6 d	T_4= *Trichoderma* formulation (soil treatment)
T5	0.00 (0.71) b	9.68 (2.75) a	5.09 (1.73) ab	14.45 (3.27) ab	15 d	T_5 = Poultry waste (soil amendment)
T6	0.00 (0.71) b	5.09 (1.73) ab	5.09 (1.73) ab	9.85 (2.25) b	15 d	T_6 = Autostin + *Trichoderma* formulation
T7	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	**21.9 bc**	T_7= Furadan + *Trichoderma* formulation
T8	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	18.5 b-d	T_8 = Krosin 10 SP + *Trichoderma* formulation
T9	0.00 (0.71) b	5.09 (1.73) ab	5.09 (1.73) ab	9.85 (2.25) b	15 d	T_9 = Autostin + Poultry waste
T10	5.09 (1.73) ab	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	16.5 d	T_{10} = Furadan + Poultry waste
T11	5.09 (1.73) ab	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	17 d	T_{11}= Krosin 10 SP + Poultry waste
T12	0.00 (0.71) b	0.00 (0.71) b	5.09 (1.73) ab	0.00 (0.71) b	17.5 cd	T_{12} = *Trichoderma* formulation + Poultry waste
T13	0.00 (0.71) b	5.09 (1.73) ab	0.00 (0.71) b	0.00 (0.71) b	19 b-d	T_{13} = Autostin + *Trichoderma* formulation + Poultry waste
T14	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	**22 b**	T_{14} = Furadan + *Trichoderma* formulation + Krosin 10 SP
T15	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	0.00 (0.71) b	**26.6 a**	T_{15} = Krosin + Furadan + *Trichoderma* formulation + Poultry waste
T16	9.85 (2.25) a	9.68 (2.75) a	9.68 (2.75) a	28.56 (5.22) a	**9.4 e**	T_{16} = Control
Lsd value	9.30 (1.82)	7.06 (1.57)	6.5 (1.46)	14.62	4.46	* Values in a column with same letter (s) do not differ significantly.
CV (%) value	103.15	77.44	80.59	90.79	15.56	** Figures of parenthesis are the mean of square root transformed values.

4.8.5. Correlation and regression study

Correlation and regression study were done to determine the relationship between total disease incidence and yield of tomato. Significant and negative correlation was observed between total disease incidence and yield ton/ha (Fig. 1). The constructed regression equation was y = -0.5826x + 35.032 and r^2= 0.39. The study revealed that the total disease incidence (%) was significantly and negatively correlated with yield of tomato.

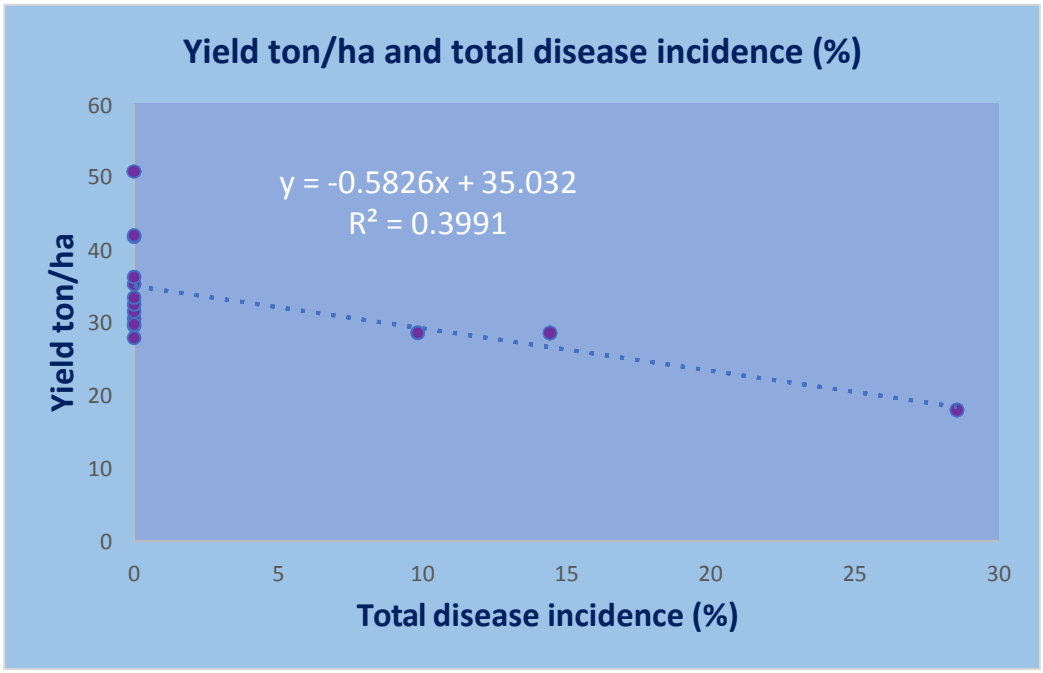


Fig 1. Relation between yield ton/ha and total disease incidence (%)

Table 11. Benefit cost Ratio (BCR) of different treatments against wilt complex of tomato

Treat-ments	Yield (ton/ha)	Gross Retun (Tk/h)	Total cost of production (Tk/ha)	Gross income (Tk/ha)	BCR
T_1	29.04	580800	89695+9000=98695	482105	**4.8**
T_2	25.19	503800	89695+8600=98295	405505	**4.1**
T_3	22.55	451000	89695+6600=96295	354705	**3.6**
T_4	22.99	459800	89695+2200=91895	367905	**4**
T_5	23.54	470800	89695+10800=100495	370305	**3.68**
T_6	23.54	470800	89695+9000+2200=100895	369905	**3.66**
T_7	29.81	596200	89695+8600+2200=100495	495705	4.93
T_8	21.45	429000	89695+6600+2200=98495	330505	**3.35**
T_9	23.54	470800	89695+9000+10800=109495	361305	**3.29**
T_{10}	25.96	519200	89695+8600+10800=109095	410105	**3.75**
T_{11}	24.31	486200	89695+6600+10800 =107095	379105	**3.54**
T_{12}	27.5	550000	89695+2200+10800=102695	447305	**4.35**
T_{13}	29.81	596200	89695+9000+2200+10800=111695	484505	**4.33**
T_{14}	33.47	669400	89695+8600+2200+6600= 107095	562305	5.25
T_{15}	41.8	836000	89695+8600+6600+2200+10800= 117895	718105	6.09
T_{16}	**10.1**	**202000**	**89695**	**112305**	**1.25**

Details of BCR in Appendix 7 & 8.

Price: Tomato Tk 20/kg.

4.9. Cost-benefit analysis and estimation of Benefit-Cost Ratio (BCR) of the treatments used for management of wilt complex of tomato

4.9.1. Cost-benefit analysis

Cost-benefit analysis of different treatments has been estimated and shown in Table 11. The highest gross return of Tk 709105/ha was obtained in treatment T_{15} application which was 313.86% higher yield over control. The higher gross return of taka 498105/ha was obtained in in case of T_{14}.

4.9.2. Benefit-cost ratio (BCR)

Benefit-cost ratio for all the treatments were estimated and presented in Table 11. It has been found that use of treatment T_{15} (Krosin 10 SP + Furadan 5G + *Trichderma* formulation + Poultry waste) resulted 6.09 BCR in comparison to untreated control. Out of other treatments, treatments T_{14} yielded higher BCR (5.25) in comparison to untreated control followed by use of T_7 (4.93), T_1 (4.8), T_2 (4.1), T_3 (3.6), *Trichoderma harzianum* T_4 (4.0), and T_5 (3.68), respectively.

The symptom of fungal wilt of tomato was initial yellowing and wilting of lower leaves caused by *Fusarium oxysporum*. The plant got wilted partially, wilting progressed up gradually. Leaves of the infected plant dried prematurely. The whole plant died soon. It has been reported by many researchers that fungal wilt of tomato caused by several species of *Fusarium* (Ignjatov *et al.* 2012). In case of bacterial wilt, the plant got wilted suddenly and collapsed soon after infection. Discoloration of internal tissue raveled brown in color. The present findings were kept in with the findings of Momol *et al.*, (2001) and Champoiseau and Momol, (2009). In case of nemic witl, the plant was observed wilted in sun light and became normal in the night and gradually weakened. Manju and Sankari, 2015 found similar symptoms in case nemic infection.

The associated pathogens with fungal, bacterial and nemic wilt were isolated by standard procedures. On PDA medium, the fungus produced pure white to

creamy white cottony mycelia. A huge number of macro-conidia were produced by 10 days old culture of the fungus. Slightly curved or sickle shaped and four to five celled macro-conidia were observed. Nirmaladevi and Srinivas (2012) reported that the colour of *Fusarium oxysporum* was varied between white to creamy white and light to pink. The bacteria when inoculated by streaking on Tetrazolium chloride (TZC) agar medium produced white creamy colony with pink to reddish center after 48 hours of incubation. Ghosh *et al*., 2015; Kumar and Sarma, 2004; and Dhital *et al*., 2011 found similar results in case of wilt pathogen *Ralstonia solanacearum*. In case of nemic infection, huge number of egg masses was observed. Vermiform, juvenile male nematodes were also observed. The present findings were matched with the findings of Sitaramaiah and Singh, 1976.

In *in vitro* evaluation, both Autostin 50 WP and Krosin 10 SP were found to be promising in reduction of the growth of *Fusarium oxysporum* and *Ralstonia solanacearum*, respectively. 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. Several researchers (Murakoshi and Takahashi, 1984) reported that antibiotics like Streptomycin found to be very effective in controlling *Ralstonia solanacearum* causing bacterial wilt.

In integrated management of wilt complex of tomato, 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), bactericide (Krosin 10 SP), nematicide (Furadan 5G) and the suppressive action of poultry waste as soil amendment. It to be mentioned here that the combination of treatments was the bacteriocide (Krosin 10 SP) was absent but showed better results in reducing the bacterial wilt incidence. It might be due to the presence of Furadan 5G that acted against nematode and consequently

controlled the bacterial incidence. It attributed that nematicide controlled nematode as well as bacteria. As the nemic infection facilitates the penetration of bacteria through wounds created by the stylet of the plant parasitic nematode. 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. Integration of Streptocyline @ 0.5 g/L + COC 50% WP (1 g/L) intercropped with Mustared reduced bancterial wilt incidence by 23.35%, knots reduction by 72.4%.

Several research reports are available on *Trichoderma harazianum* against soil born pathogen (Harman *et al.,* 1989; 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.*

Regarding the costing of the experiment, it was found that treatment combination T_{15} found to be cost effective (BCR: 6.09) where tomato growers will earn Tk. 6.09 by investing Tk. 1 only. Treatment T_{14} also found to be effective (BCR: 5.25) followed by T_7 (BCR: 4.93). It was noticed that despite of highest cost of production (Tk. 117895) the T_{15} yielded desired BCR 6.09 because of the highest yield (41.8 y/ha).

CHAPTER 5
SUMMARY AND CONCLUSION

Tomato (*Solanum lycopersicum*) is one of the most lucrative vegetable crops across the world. Successful cultivation of tomato is hindered by various diseases among which wilt diseases caused by fungus (*Fusarium oxysporum),* bacteria (*Ralstonia solanacearum)* and nematode (*Meloidogyne spp.)* are the most widespread and destructive disease, causing huge losses to crop growers.

The severely wilted tomato plants were collected from the infested fields of Sher-e Bangla Agricultural University Horticultural farm and pathogens involved viz. fungus, bacteria and nematode were isolated and identified as *Fusarium oxysporum, Ralstonia solanacearum* and *Meloidogyne sp.*, respectively on the basis of key characteristics and final confirmation was done by testing the pathogenicity. The tomato plants were inoculated individually with the pathogen.

In the present investigation, sixteen (16) different selected treatments were explored to formulate an integrated approach for the management of wilt complex of tomato. Three chemicals viz. Furadan 5G (Carbofuran), Autostin 50 WP (Carbendazim) and Krosin 10 SP (Streptomycin sulphate), one bio-agent *Trichoderma harazianum*, a soil amendment with Poultry waste and their different combinations were evaluated against *Fusarium oxysporum, Ralstonia solanacearum* and Meloidogyne *spp.* causing fungal wilt, bacterial wilt and nemic wilt, respectively. The cost analysis for BCR (Benefit Cost Ratio) calculation was done for implication of management components.

In *in vitro* evaluation, Autostin 50 WP and Krosin 10 SP found to be very effective in retardation of the physical growth of *Fusarium oxysporum* and *Ralstonia solanacearum* by 87.88 % and 83.33 %, respectively.

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

In case of fungal wilt incidence, most of the treatments showed significantly better results in comparison to control. No fungal wilt incidences were recorded in case of those treatments where Autostin 50 WP, *Trichoderma sp*., and poultry waste were combined with different permutations. The highest fungal wilt incidence (9.85%) was observed in control treatment.

In case of bacterial wilt incidence, all the treatments significantly reduced bacterial wilt incidence compared to control. No bacterial wilt incidences were noticed in case of those treatments where Krosin 10 SP, Furadan 5G, *Trichoderma spp*. and Poultry waste were incorporated with different combinations. The highest bacterial wilt incidence (9.68%) was recorded in control which was statistically similar with Poultry waste.

In case of nemic wilt incidence, no nemic incidences were recorded where Furadan 5G, *Trichoderma spp*. and soil amendment with Poultry waste were combined with different permutations. The highest (9.68%) nemic wilt incidence was counted in control which was statistically similar to the application of Poultry waste, Autostin 50 WP and *Trichoderma* formulation singly.

In case of cumulative incidence of wilt complex, the treatments where the Fungicide (Autostin 50 WP), Bacteriocide (Krosin 10 SP) and Nematicide (Furadan 5G) were combined along with *Trichoderma* and Poultry waste completely controlled the wilt complex of Tomato.

Treatments were differed significantly in respect of plant growth characters viz. plant height, number of branches, number of leaves per plant, number of fruits per plant, single fruit weight and yield compared to control. Most of the treatments showed similar trend of results in case of yield and yield contributing characters.

The highest performances were found in case of the treatment combination of *Trichoderma* formulation with Krosin 10 SP, Furadan 5G and Poultry waste. The lowest results were found in control. The highest yield per plot (27.6kg) was counted in case of the combination of *Trichoderma* formulation, Krosin 10 SP, Furadan 5G and Poultry waste followed by the combination of *Trichoderma* formulation, Krosin 10 SP and Furadan 5G (22 kg). The lowest result was found in control (9.04 kg). Based on the market prices of produce and contemporary production cost, the highest BCR (6.09) was calculated in case of T_{15} where the tomato growers will get net return TK.6.09 by investing TK. 1.00 only.

Considering the overall performances of the treatments applied in the experiment, it can be concluded that the wilt complex (Fusarial, Bacterial and Nemic wilt) of tomato could be controlled successfully as well as cost effectively by the application of Autostin 50 WP (Fungicide), Krosin 10 SP (Bacreriocide) and Furadan 5G (Nematicide) along with *Trichoderma harzianum* (Bio-agent) and Poultry waste (Soil amendment) combinedly in different permutations. However, further study are suggested to carry out for a consecutive years in different Agro-Ecological Zones (AEZs) to formulate a sustainable approach.

CHAPTER 6
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APPENDICES

Appendix 1: Nutritive value of tomato (Per 100 gram)

Energy	75 kJ (18 kcal)
Carbohydrates	4.0 g
Sugars	2.6 g
Dietary fiber	2.6 g
Fat	0.2 g
Protein	1.0 gm
Water	95.0 g
Vitamin C	22.0 mg
Vitamin A	300 IU

Appendix 2:

Soil type:

Agro-ecological region: Madhupur Tract (AEZ-28).

Land Type	: Medium high land.
General soil type	: Non-Calcareous Dark gray floodplain soil
Soil series	: Tejgaon
Topography	: Up land
Elevation	: 8.45
Location	: SAU Farm, Dhaka.
Field level	: Above flood level.
Drainage	: Fairly good.
Firmness (consistency)	: Compact to friable when dry.

The physical and chemical characteristics of the soil collected from Soil Resource Development Institute (SRDI), Farmgate, Dhaka is presented bellow (For 0-14 cm depth):

Particle size distribution:

Sand : 34%

Silt : 46%

Clay : 20%

Soil texture : Loam to clay loam.

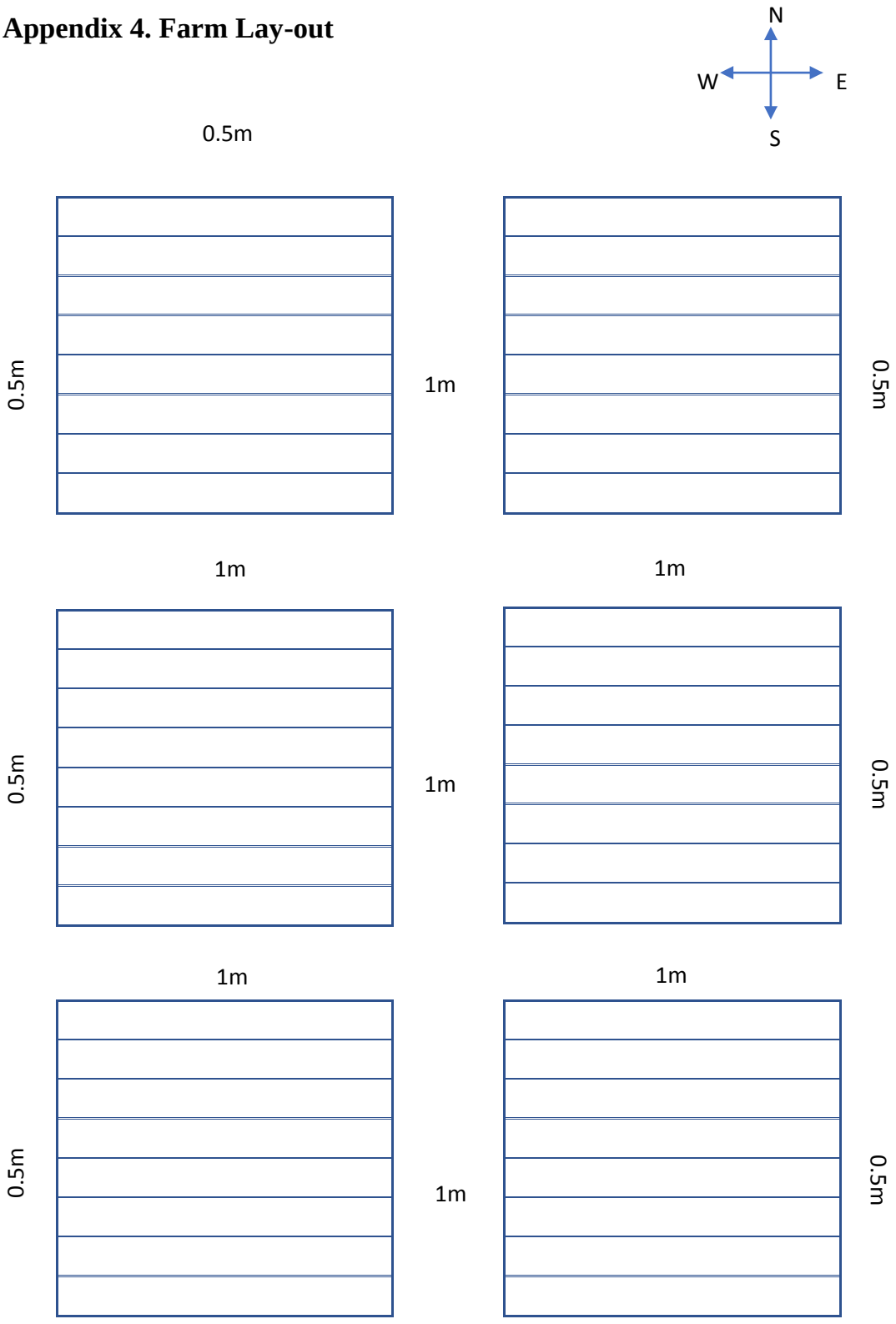
Appendix 3. Monthly mean of daily maximum, minimum and average temperature, relative humidity during October/2017 to April/2018

Month	**Temperature(^{0}C)			**Relative Humidity (%)
	Max.	**min.**	**Ave**	
October, 2017	32	23	27.5	**79**
November, 2017	30	17	23.5	**65**
December, 2017	25	13	24	**74**
January, 2017	24	11	17.5	**68**
February,2017	28	14	21	**57**
March, 2017	32	20	26	**57**
April	**34**	**23**	**28.5**	**66**

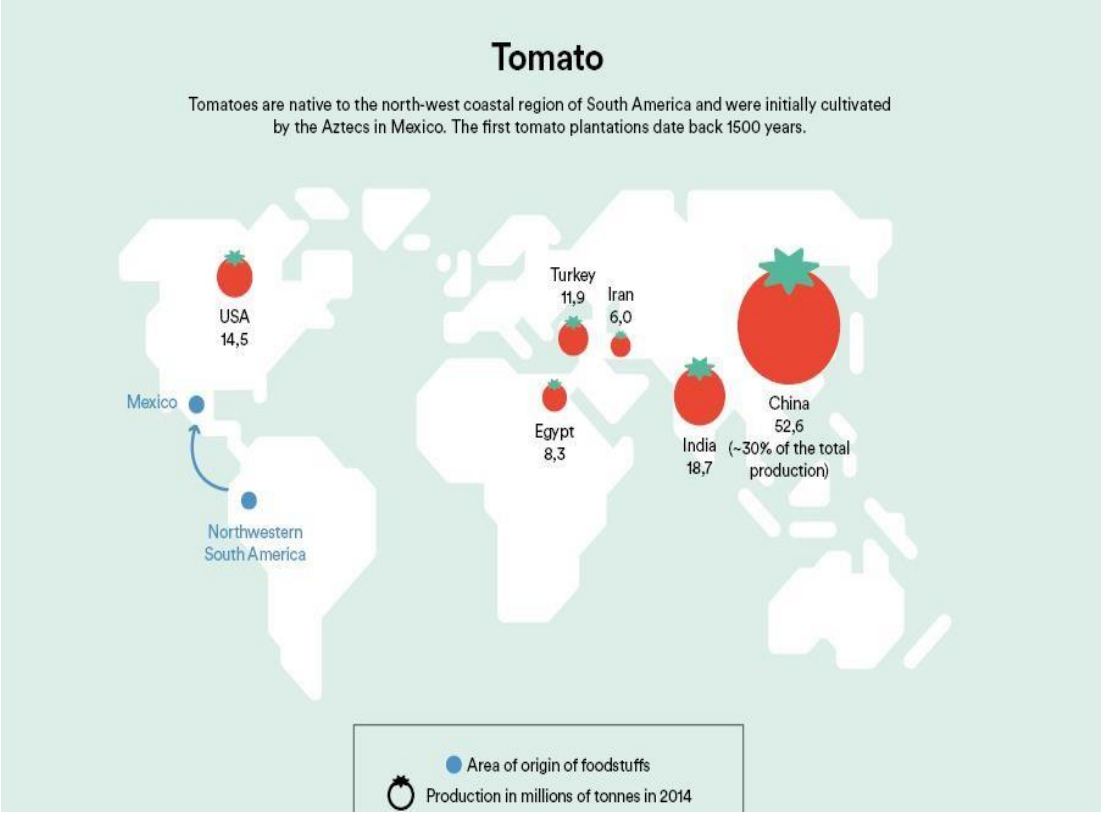
Source: www.holiday-weather.com/Bangladesh/Dhaka

**=Monthly average

Appendix 4. Farm Lay-out



Appendix 5. Map showing contribution of tomato (Worldwide)



A. Tomato production in millions of tonnes in 2014

Appendix 6. View of field experiment

A. Birds eye view of field experiment

B. View of experimental field in SAU Central Farm

Appendix 7. Details of cost of production of tomato excluding treatment cost

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)** • **Tractor hired** • **Human labour**	• Liter • Day^{-1} • Man day^{-1}	22 1 2	65 6000 400	3 1 3	4290/- 6000/- 2400/-
Seedling plantation • **Human labour**	• Man day^{-1}	10	400	1	4000/-
Fertilization and manuring • **Urea** • **TSP** • **MoP** • **Gypsum** • **Cowdung**	• Kg • Kg • Kg • Kg • Ton	300 200 175 10 10	20 19 21 15 4000	1	6000/- 3800/- 3675/- 150/- 40000/-
Weeding **Human Labour**	• Man day^{-1}	6	400	3	7200/-
Irrigation • **Human labor** • **Shallow machine hired**	• Man day^{-1} • Hour	3 2	400 600	3 3	3600/- 2400/-
Insecticide spraying • **Actara** • **Human labour** • **Sprayer hired**	• Liter • Man day^{-1} • hour	0.3 2 4	1200 400 100	3 3 3	1080/- 2400/- 1200/-
Total(a)					89695/-

Appendix 8. Cost of application of treatments for production of tomato

Cost items	Per hectare cost in taka				
	Unit	**Quantity**	**Cost unit^{-1}**	**Times**	**Total cost**
Autostin	Kg	1	2200	3	6600/-
50WP	Man day^{-1}	2	400	3	2400/-
Human					
Labor					
Total (b)					9000
Krosin	Kg	0.5	2800	3	4200/-
Human	Man day^{-1}	2	400	3	2400/-
Labor					
Total (c)					6600
Furadan 5G	Kg	60	130	1	7800/-
Human	Man day^{-1}	2	400	1	800/-
Labor					
Total (d)					8600
Trichoderma	Kg	70	20	1	1400/-
Formulation	Man day^{-1}	2	400	1	800/-
Human					
Labor					
Total (e)					2200/-
Poultry waste	Kg	2000	5	1	10000/-
Human	Man day^{-1}	2	400	1	800/-
Labor					
Total (f)					10800/-
Sprayer	Man day^{-1}	4	100	3	1200/-
Hired					