# ORGANIC MANAGEMENT OF WILT COMPLEX AND *TOMATO YELLOW LEAF CURL VIRUS* IN SUMMER TOMATO USING BIO-AGENTS, BOTANICAL AND AGRO BIO-PRODUCTS FORTIFIED SOIL

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**DECEMBER- 2021**

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*A Thesis*

*Submitted to the faculty of Agriculture,*

*Sher-e-Bangla Agricultural University, Dhaka*

*In partial fulfillment of the requirements*

*for the degree of*

**MASTER OF SCIENCE**

**IN PLANT PATHOLOGY.**

**SEMESTER: JULY-DECEMBER, 2021.**

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# CERTIFICATE

This is to certify that thesis entitled, "**ORGANIC MANAGEMENT OF WILT COMPLEX AND *TOMATO YELLOW LEAF CURL VIRUS* IN SUMMER TOMATO USING BIO-AGENTS, BOTANICAL AND AGRO BIO-PRODUCTS FORTIFIED SOIL**" Submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY** embodies the result of a piece of bona fide research work carried out by a student, REGISTRATION NO. **19-10294,** under my supervision and guidance. No part of this thesis has been submitted for any other degree.

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

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Dated:

Dhaka, Bangladesh.

**DEDICATED**

**TO MY**

**BELOVED PARENTS**

# *ACKNOWLEDGEMENT*

*Alhamdulillah, all praises are due to the almighty Allah Rabbul Al-Amin for His gracious kindness and infinite mercy in all the endeavors the author to let his successfully complete the research work and the thesis leading to the degree Master of Science. The author expresses his cordial gratitude, deep sense of respect and enormous indebtedness to my research supervisor* ***Dr. Md. Belal Hossain*** *Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his educational supervision, incessant encouragement, positive suggestion and unvarying inspiration all through the research work and for taking massive care in preparing this manuscript. His insight and professional skill have made a distinctive contribution to complete this piece of research. The author is grateful and indebted to his research Co-supervisor* ***Dr. Fatema Begum*** *Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his sincere guidance and helpful suggestions for the completion of the research work. The author expresses his immense respect and indebtness to* ***Prof. Dr. Md. Rafiqul Islam, Prof. Abu Noman Faruq Ahmmed, Prof. Dr. M. Salahuddin M. Chowdhury, Prof. Dr. Nazneen Sultana, Prof. Dr. F. M. Aminuzzaman, Prof. Dr. Khadija Akhter, Assistant Professor Shukti Rani Chowdhury, Assistant Professor Hosna Ara Chowdhury Nisha,*** *and all other respective teachers of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their valuable teaching, cooperation and encouragement during the entire period of the study. The author thankfully remembers all the staffs of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their cooperation during the research work. The author is especially thankful to* ***Yobaida Afrin Ara Eti, Rifat Ara Sultana****,* ***Golam Sharowar****,* ***A.K.M. Arif-uz-zaman, Md. Humayun kabir (Ph.D fellow)*** *for their cordial support, cooperation and encouragement during the laboratory work. The author is deeply indebted and grateful to his parents, family members, relatives and friends who continuously prayed for his success and without whose love, affection, inspiration and sacrifice this work would not have been completed.*

*The Author*

# Organic management of wilt complex and *Tomato Yellow leaf Curl Virus* in summer tomato using bio-agents, botanical and agro bio-products fortified soil


## ABSTRACT

A pot and a lab experiment was conducted to evaluate the efficacy of selected bio-agents (*Metarhizium anisopliae, Trichoderma harzianum, Verticillum lecanii, Beauveria bessiana*), botanicals [*Clerodendrum infortunatum* (Vat) leaf extract, *Lantana camara* leaf extract, *Azadiracta indica* (Neem) leaf extract] and agro bio-products (Tea wastage, Garlic powder, Mustard oil cake) against the wilt complex and *Tomato yellow leaf curl virus (TYLCV)* in summer tomato cultivation. The experiment was conducted at net house in the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, during the period of July to October, 2021. The experiment was carried out in a complete randomized design (CRD) with three replications and each of the treatment contains 3 pots. The total number of unit pots was 30. Summer variety 'BARI hybrid tomato-4' was used as a tested cultivar. Data on disease incidence and disease severity of wilt complex and *TYLCV* was recorded at 30, 45 and 60 DAT. Fungal pathogen was isolated by tissue planting method. Bacterial pathogen was isolated by dilution and streaking method. In case of wilt complex, among the selected bio–agents, the lowest disease incidence (33.33%) and severity (15%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*) and the highest disease incidence (66.67%) and severity (20%) was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*). In case of *TYLCV*, the lowest disease incidence (66.67%) and severity (20%) was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*) and the highest disease incidence (100%) and severity (30%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*). From the present study, it was found that the wilt complex of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* and *Ralstonia solanacearum* that was confirmed by morphological characteristics of macro and micro conidial structures and biochemical tests viz. gram staining, motility, KOH solubility, catalase and simon's citrate test, respectively. The performance of the treatments in respect of yield and yield contributing characters against wilt complex and *TYLCV* of summer tomato varied significantly. However, from the present study it may be concluded that mustard oil cake and neem leaf extract can be used as ecofriendly approach for effective management of wilt complex and *Tomato Yellow Leaf curl Virus (TYLCV)* in summer tomato cultivation.

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# CHAPTER I

# INTRODUCTION

# INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a major contributor to the fruit vegetable diet of humans. It is cultivated in essentially all countries either in fields or in protected culture. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates. It is one of the most important vegetable crops in Iran (Abd-El Kareem *et al.,* 2006). It is one of the most important, popular and nutritious vegetables grown in Bangladesh. Present world production of tomato is about 170.8 million tons and total tomato growing area is 4.9 million hectares (FAOSTAT, 2016). As a cash crop, it has great demand in the international market (Solieman *et al.,* 2013). The best tomato growing areas are Chittagong, Comilla and Rajshahi. The recent statistics shows that tomato was grown in 67535 acres of land and the total production was approximately 368121 metric tons during the year 2015-2016 and the average yield of tomato was 5451 kg/acre in winter season (BBS, 2016). It is used both as salad and to prepare curry. It is also used to make soups, pickles, conserves, ketchup's, juices, sauces etc. It is widely grown in both winter and summer season around the country (Haque *et al.,* 1999). It also contains a large quantity of water, calcium and niacin all of which have great importance in the metabolic activities of human. It is also a good source of vitamin A, C, E and minerals (potassium, calcium, phosphorus, iron and zinc) that are very good for body and protect the body against the diseases (Taylor, 1987). It is an excellent source of lycopene, carotenoids and polyphenolic compounds which are a powerful source of antioxidant and reduces the risk of prostate cancer (Hossain *et al.,* 2004). Vegetables are inevitable for human's diet and tomato crop gain importance because it has abundant use in everyday life. Like other crops tomato is also under the attack of many diseases caused by different pathogens. Among them, viral diseases are major constraints in tomato production (Chavan *et al.,* 2015). Characteristic symptoms of tomato viruses are stunting, crinkling, rolling mosaic etc. Die back has been found in severe cases (Raj *et al.,* 2005).

*Fusarium oxysporum* is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes huge economic losses by inducing necrosis and wilting symptoms in many crop plants (Cotxarrera, *et al.,* 2002). *Fusarium oxysporum f. sp. lycopersici* (FOL) is a known pathogen of tomato plant (Suárez-Estrella *et al.,* 2007). Tomato yield is significantly reduced by *F. oxysporum f. sp. lycopersici* because it can destroy roots of tomatoes at all growth stages. Numerous strategies have been proposed to control this fungal pathogen (Biondi *et al.,* 2004; Ahmed, 2011). Currently, the most effective method in preventing tomato from *Fusarium* wilt is treating tomato seeds with chemical fungicides. However, the use of chemical fungicides can be harmful to other living organisms (Lewis *et al.,* 1996). Several pathogens are known to limit worldwide production of tomato, of which *Fusarium solani f. sp. Eumartii* and *Fusarium oxysporum f. sp. ciceris* (Fusarium wilt) is one of the most important. Management of Fusarium wilt has been primarily through development of resistant cultivars as a part of an integrated management approach. *Fusarium solani* strain (*F. solani (Mart.) Sacc. f. sp.)* is also a worldwide soil-borne fungus attacking a wide range of host plants including citrus (Sherbakoff, 1953) with a great overall impact on productivity. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield (Nemec *et al.,* 1996). *Fusarium* wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici* (FOL) is the major limiting factor in the production of tomato. The disease causes great losses, especially on the susceptible varieties of tomato when soil and air temperature are rather high during the warm season (Agrios, 1997, Mandal *et al.,* 2009). Controlling such diseases mainly depend on fungicidal treatments (Rauf, 2000). However, fungicidal applications cause hazards to human health and increase environmental pollution. Therefore, alternatives eco-friendly approaches treatments for control of plant diseases are prefered (Rojo *et al.,* 2007). The biological control is the best alternative especially against soil borne pathogens. Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature. Among the various

antagonists used for the management of plant diseases, *Trichoderma spp.* play a vital role. Recently, it is suggested that, *Trichoderma* affects induced systemic resistance mechanism in plants against pathogens (Haggag and Amin, 2001, Prasad *et al.,* 2002, Hibar *et al.,* 2007, Jayalakshmi *et al.,* 2009). Among the various isolates of *Trichoderma, T. Asperellum, T. harzianum, T. virens, T. viride, and T. hamatum* are used against the management of various diseases of crop plants caused by soil borne pathogens. These filamentous fungi are very common in nature, with high population densities in soil and plant litters (Samuels, 1996). Many studies have proved the potential of *Trichoderma spp.* as biological agents antagonistic to several plant pathogens (Sivan and Chet, 1993, Naseby *et al.,* 2000, Tondje *et al.,* 2007, Hanssen, *et al.,* 2010).

*Tomato leaf curl virus (TLCV)* is one of the most devastating viruses that causes huge losses in yield of tomato crop around the globe. *TLCV* was firstly reported in the 1980s in Sudan and is transmitted through whitefly (*Bemesia tabaci*) as insect vector in nature (Sugano *et al.,* 2011). It is a circular single stranded DNA Geminivirus belongs to family Geminiviridae (Gottlieb *et al.,* 2010). In our country, the yield of tomato is not satisfactory in comparison to other tomato growing countries (Aditya *et al.,* 1999). Although the total cultivated area and production of tomato in our country have increased gradually over the last few years but the productivity is still very low (6.46 t ha-1) compared to the average world yield (34.86 t ha-1) (FAOSTAT, 2016). Tomato production in our neighboring country India was 7873 kg/acre (Indian Horticulture Database, 2017) where as our production is 5451 kg/acre only. The environment of India and Bangladesh are almost same and thus the variation comes mainly due to pest and diseases infestation. There are many types of diseases occurs in tomato like fungal, bacterial, viral and nemic disease. Globally tomato is susceptible to more than 200 diseases, out of which 40 are caused by viruses (Martelli and Quacquarelli, 1982; Lukyanenko, 1991). However, the incidence and economic impact of virus infections in tomato varies greatly depending upon different factors like country, cropping method and the virus itself (Martelli and Quacquarelli, 1982). In our country 16 different tomato viruses are identified. Among the viral diseases *Tomato*

*Yellow Leaf Curl Virus (TYLCV)* is the most devastating that caused upto loss may be raised upto 100% (Akanda *et al.,* 1991). *TYLCV* is also wide spread in many Mediterranean, Middle Eastern, American, African, and Asian countries. *TYLCV* is an ssDNA plant virus, which belongs to the family Geminiviridae of the genus Begomovirus (Czosnek and Laterrot, 1997). This viral disease is transmitted by whiteflies (*Bemisia tabaci*) and by grafting but not transmitted mechanically. The disease was first reported in Israel and Jordan Valley in the early 1960s and is now economically significant in many countries (Jones *et al.,* 1993). The causal agent was described in 1964 and named as *Tomato yellow leaf curl virus (TYLCV*) by Cohen and Harpaz in 1964. Since then, *TYLCV* has been reported from all over the tropics, subtropics, the Mediterranean, the Caribbean's and the Americas (Czosnek and Laterrot, 1997, and Nakhla *et al.,* 1993). *TYLCV* threatens both commercial tomato productions in the fields and home garden which could be able to infect plants at any stage of plant growth (Gupta, 2000). In Bangladesh *TYLCV* incidence was first reported by (Akanda *et al.,* 1991) based on symptomatology. Symptoms of *TYLCV* include stunted plant growth, chlorotic yellowing of leaves, and distortion of leaflets in a cupped down and inward shape or upward curling of the leaflet margins (Cohen and Lapidot, 2007). The impact of *TYLCV* on tomato production is very severe. If plants are infected at an early stage, they do not bear fruit and their growth becomes severely stunted and 100% yield loss occurs. Preliminary studies regarding incidence, transmission, detection and characterization have been carried out in the different part of the world (Rybicki, 2015). Chemicals and botanicals/plant extracts have been used to control the insect vector for the management of plant viruses. Eco-friendly, Non-chemical approaches including micronutrients and botanicals have been used to manage plant virus (Ali *et al.,* 2014). The incidence of *TLCV* can be reduced through different chemicals using certain practices in the field (Chakraborty *et al.,* 2003). Another approach practiced in protected culture is ultraviolet absorbing plastic films; have presented good results (Antignus *et al.,* 2001). Non-chemical approaches to control the diseases are not only ecofriendly but also the need of time demanding. Continual prevalence of viral diseases is considered to be the major cause of low tomato

production. There is the scarcity of information regarding incidence and management of *TLCV* available in Bangladesh as well as in the world.

## OBJECTIVES

The specific objectives of this study are given below -

-To assess the disease intensity level of wilt complex and *Tomato yellow leaf curl virus* (*TYLCV*) in summer tomato cultivation,

-To evaluate the efficacy of selected bio-agents, botanicals and agro bio-products in controlling wilt complex and *TYLCV*.

# CHAPTER II

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## About Tomato

Tomato was introduced to Europe most probably from Mexico (Blanca *et al.*, 2012) in the 16th century by Spanish conquistadors. Due to its resemblance with toxic Solanum species like belladonna and mandrake, the tomato was long used for ornamental purposes only appearing in cookbooks by the beginning of the 17th century. From Spain, the tomato reached Italy and England, whence British subsequently "exported" tomato to Asia, Middle East and North America (McCue, 1952; Bergougnoux, 2014).

Tomatoes are adapted to a wide range of environmental conditions, but in temperate areas low temperatures and short growing seasons can limit growth. Tomatoes prefer slightly acidic soils with a pH of 6.0 to 6.8. The tomato plant requires significant quantities of water, but not in excess, since tomato roots will not function under water-logged (anaerobic) conditions. (Cox and Tilth, 2009).

Sufficient moisture must be maintained to establish the plant and carry it through to fruit production. When the moisture level surrounding the roots is too high, epinasty, poor growth, late flowering, fewer flowers and lower fruit set occurs. Fruit disorders such as cracking and blossom-end-rot are common when water availability is inconsistent. Even under moderate water stress, photosynthesis is slowed because the movement of gases through the stomata is restricted and the movement of water up the xylem is slowed (Benton, 2008).

## Tomato Morphology

Tomato was classified by the Swedish botanist Carl Linnaeus in 1753 in the genus Solanum with the species *Ephitet lycopersicum*. It belongs to the family Solanaceae, which contains over 3000 plant species, including many economically important plants such as potato, eggplant, peppers, petunia and tobacco. With 1250–1700 species, Solanum is the largest genus in the Solanaceae family. Tomato is botanically classified as the cultivated tomato *S. lycopersicum* and its twelve wild species. Wild tomato species have very small fruit while the modern cultivated

tomatoes have a large variation in fruit size, ranging from less than 20 g for cherry tomato up to 500 g for the beef tomato (Bergougnoux, 2014). Although usually cultivated as an annual crop, tomato is a perennial plant. It has bipinnate leaves, hairy stems and flowers with usually 5–7 petals (Blanca *et al.*, 2012). Tomato is diploid (Nesbitt & Tanksley, 2002) and its genome size is approximately 900 Mb, comprising 12 chromosomes and 34,727 protein-coding genes (The Tomato Genome Consortium, 2012).

Tomato is cultivated for its fleshy fruit (Blanca *et al.*, 2012). Botanically, tomato is a fruit berry, and not a vegetable (Bergougnoux, 2014). The fruit is a specialized organ that results from the development of the ovary after successful flower pollination and fertilization. It provides a suitable environment for seed maturation and dispersal (Chevalier *et al.*, 2011). The fleshy fruit corresponds to the ovary and is composed of an epidermis, a thick pericarp (composed of exocarp, mesocarp and endocarp) and the placental tissues, which surround the seeds. The pericarp is the outer wall of the gynoecium, and is composed of at least two carpels, which determine the number of fruit locules (Bergougnoux, 2014).

**About wilt of tomato and *Tomato yellow leaf curl virus (TYLCV)*:**

Fusarium wilt diseases, caused by pathogenic formae speciales of the soil inhabiting fungus *Fusarium oxysporum* can cause severe losses in a wide variety of crop plants. On tomato, two symptomologically distinct forms of the pathogen can cause either a vascular wilt (*F. oxysporum f. sp. lycopersici*) or a crown and root rot (*F. oxysporum f. sp. radicis-lycopersici*). Both of these pathogens occur throughout most tomato growing areas and either can devastate a (Larkin *et al.*, 1998). Tomato yield is significantly reduced by *F. oxysporum f. sp. lycopersici* because it can destroy roots of tomatoes at growth stages. Numerous strategies have been proposed to control this fungal pathogen (Biondi *et al.*, 2004; Ahmed, 2011).

Biological control has potential for the management of these diseases. A variety of soil microorganisms have demonstrated activity in the control of various soilborne plant pathogens, including Fusarium wilt pathogens (Larkin *et al.*, 1998). *Trichoderma* and *Gliocladium spp.*, have been used to control a variety of fungal

pathogens, including *Rhizoctonia, Pythium, Sclerotinia, Sclerotium,* and *Fusarium spp*. (Harman, G. E. 1991; Lewis *et al.,* 1996; Lewis *et al.,* 1985),

Fusarium wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici* (FOL) is the major limiting factor in the production of tomato. The disease causes great losses, especially on the susceptible varieties of tomato especially when soil and air temperature are rather high during the warm season (Agrios, 1997, Mandal *et al.*, 2009). Controlling such diseases mainly depend on fungicides treatments (Rauf, 2000). However, fungicidal applications cause hazards to human health and increase environmental pollution. Therefore, alternatives, eco-friendly approach treatments for control of plant diseases are needed (Rojo *et al.,* 2007). The biological control is the best alternative especially against soil borne pathogens. Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature. Among the various antagonists used for the management of plant diseases, *Trichoderma spp.* plays a vital role. Recently, it was suggested that, *Trichoderma* affects induced systemic resistance mechanism in plants against pathogens (Haggag and Amin, 2001, Prasad et al., 2002, Hibar *et al.,* 2007, Jayalakshmi *et al.,* 2009).

Biological control of *Fusarium oxysporum f. sp. lycopersici (FOL)* causing wilt disease of tomato was studied in vitro as well as under pot conditions. Dual culture technique showed that *Aspergillus niger, Penicillium citrinum, Penicillium sp*. and *Trichoderma harzianum* inhibited the radial colony growth of the test pathogen (Hend *et al.,* 2012). Numerous studies have demonstrated reduced incidence of diseases in different crops after supplementing the soils with fungal or bacterial antagonists (Mukhopadhyay, 1987; Smith *et al.,* 1990; Bashar and Rai, 1994; Singh *et al.,* 2002; Akrami *et al.,* 2011; Ahmed, 2011). Recent developments in commercialization of biological control products have accelerated the approach of fungal antagonists (Fravel *et al.,* 2003).

Plant associated microorganisms may make better biocontrol agents because they are already closely associated with and adapted to the plant or plant part as well as the particular environmental conditions in which they must function (Larkin *et al.*, 1998).

Specific nonpathogenic isolates of *F. oxysporum* and *F. solani* collected from a Fusarium wilt-suppressive soil were the most effective antagonists, providing significant and consistent disease control (50 to 80% reduction of disease incidence) in several repeated tests (Larkin *et al.*, 1998).

Several fungal and bacterial isolates collected from the roots and rhizosphere of tomato plants also significantly reduced Fusarium wilt of tomato. Combinations of antagonists, including multiple Fusarium isolates, Fusarium with bacteria, and Fusarium with other fungi, also reduced disease, but did not provide significantly better control than the nonpathogenic Fusarium antagonists alone (Larkin *et al.*, 1998).

The virus belongs to genus Begomovirus and has a single-stranded DNA (ssDNA). The genomes are encapsidated in about 20X30 nm geminate particles (Goodman, 1977).

Among the viruses infecting tomato, *TYLCV* has the highest economic impact (Czosnek, 2007) and it is considered as one of the most devastating plant viruses worldwide (Hanssen *et al.,* 2010; Péréfarres *et al.,* 2012). Currently, 10 different Begomovirus species and their strains are associated with *Tomato yellow leaf curl disease (TYLCD)* (Brown *et al.,* 2015). Among them, *TYLCV* is the most dominant species and it is divided into different strains, among which the Israel (*TYLCV*) and mild (*TYLCV-Mld*) strains are most prevalent (Hanssen *et al.,* 2010; Lefeuvre *et al.*, 2010; Navas-Castillo *et al.,* 2011). *Tomato yellow leaf curl virus (TYLCV)* is a geminivirus transmitted by whitefly (*Bemisia tabaci*). It causes most destructive disease of tomato throughout the Mediterranean region, the Middle East and the tropical regions of Africa and Central America. It is also reported from Japan, Australia and the USA. In many cases yield loss can be up to 90% reported by (Gafni, 2003).

Symptoms of *TYLCV* were first observed in the Jordan Valley in 1929 (Cohen & Lapidot, 2007). It took about 30 years before the virus was first described and found to be circulative and persistent in the insect vector (Cohen & Harpaz, 1964). During the 1970´s, the first electron micrographs (EM) were produced showing the novel geminate particle morphology of geminiviruses (Goodman, 1981) and it was

discovered that the virions of Begomoviruses contain a genome of ssDNA (Goodman, 1977). EM observations of thin sections of *TYLCV* infected tomato leaves also indicated that geminate particles are located in the nuclei of phloem parenchyma cells (Russo *et al.,* 1980; Cherif & Russo, 1983). In the following decade, *TYLCV* virions were isolated and purified (Czosnek *et al.,* 1988) and in 1991, the genome sequence of *TYLCV* was published (Navot *et al.,* 1991).

*TYLCV* has a wide host range with more than 30 plant species in over 12 families, including vegetables and ornamentals as well as wild plants and weeds. The reservoirs for *TYLCV* vary among regions and because infection of other hosts than tomato can be symptomless, reservoirs may not be obvious (Polston & Lapidot, 2007).

In tomato, *TYLCV* can cause yield losses of up to 100% and induce symptoms such as upward curling, reduction and yellowing of leaves as well as flower abortion and overall reduction in growth (Díaz-Pendón *et al.,* 2010; Navas-Castillo *et al.,* 2011).

*Tomato yellow leaf curl virus (TYLCV)* is a group of whitefly-transmitted geminiviruses (Cohen and Harpaz, 1964; Czosnek *et al.,* 1988), causing an extensive yield loss to tomato crops in many tropical and subtropical regions worldwide (Czosnek and Laterrot, 1997).

Tomato leaf symptoms include chlorotic margins, small leaves that are cupped, thick rubbery. The majority (90%) of flowers abscises after infection and therefore few fruits are formed. *TYLCV* is considered as a phloem limited virus (Gafni, 2003).

The various prominent symptoms of tomato leaf curl virus such as upward curling of leaf margins, stunting, reduction of leaf size, corrugated leaf, shortening of internodes and severe reduction in fruit yield, had been observed from Middle East (Makkouk and Laterrot, 1983). The upward leaf curling and interveinal and marginal chlorosis in tomato plants due to tomato leaf curl virus is reported by (Zhang *et al.,* 2008).

In Greece, *TYLCV* disease symptom as leaf curling, reduced leaf size, yellowing, shortened internodes and a bushy appearance. Mechanical inoculation was unproductive while transmission was obtained by grafting on to healthy tomato plant (Avgelis *et al.,* 2001).

It was reported that symptoms of stunting, curling and yellowing of leaf margins, and marked reductions in the number of fruits were observed in some greenhouse-grown tomato cv. Naxos plants in the province of Bari in Apulia, Italy, were observed in the being an isolate of *TYLCV-Sar*. The nucleotide sequence of the 580 bp amplicon shared 99.5% homology with a clone from a Sicilian isolate and 97.5% with a clone from a Sardinian isolate of *TYLCV-Sar*. This is the first report of *TYLCV* in Apulia, Italy (Sialer *et al.*, 2001).

*TYLCV* was present in almost all fields of Belgaum, Dharward, Haveri districts of Karnataka with percent disease incidence of 4 to 100 % in rabi and 60 to 100 % during summer season. (Reddy *et al.*, 2011).

*TYLCV* is quite general in the tropics. In Africa, it has been reported from South Africa, Senegal, Tanzania, Malawi, Zambia, Zimbabwe, Nigeria, Ivory Coast, Egypt and Sudan (Yassin *et al.*, 1982; AVRDC, 1987; Czosneck et al.1990; Nakhla *et al.,* 1993; Nono-Womdim, 1994; Chiang *et al.,* 1996). It is also widespread in the rest of the Old World and in the New World, e.g., in South East Asia and East Asia, the Americas and the Mediterranean (Green and Kallo, 1994; Chiang *et al.,* 1996; Polston and Anderson, 1997; Czosnek and Laterrot, 1997).

A survey of tomato and pepper viruses was conducted in Sudan during the last ten years. It covered central, northern, eastern, southeastern and western regions of Sudan. The results revealed the presence of many mosaic - inducing virus and virus like agents. *Cucumber mosaic virus (CMV), Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Tomato yellow leaf curl virus (TYLCV) and Potato virus Y (PVY)* were all found to infect both tomato and pepper (Elshafie *et al.*, 2005).

In the semi-tropical climatic zone of Egypt, indicated that at the beginning of Spring and early Summer (February - April), when tomato plants have just established, *TYLCV* incidence is very low (Moustafa, 1991). The latter becomes high towards the end of summer (September – mid-October), and then coincides with peak whitefly population density (Riley *et al.,* 1995).

This is followed by high *TYLCV* incidence and severe damage in the fall (Autumn) when production losses rise to 80% and almost all plants are infected. Similarly, Cohen and Antignus (1994) observed that in the Jordan Valley, the spread of

*TYLCV* was significantly correlated with *B. tabaci* population size. As in Egypt, peak whitefly population occurred between the first week of September and Mid-October. In Tanzania, *TYLCV* symptoms and whitefly vector presence are reported to be most common during November to February (Nono-Womdim *et al.,* 1996).

Water deficits improved the quality of fruits, increased soluble solids and acidity and that water stress throughout the growing season significantly reduced yield and fruit size, but plants stressed only during flowering showed fewer but bigger fruits than completely non-stressed plants (Nuruddin *et al.,* 2003).

The *Tomato yellow leaf curl virus* (*TYLCV*) is one of the most devastating viral disease of cultivated tomato (*Lycopersicon esculentum*) in tropical and subtropical regions of worldwide causing the losses up to 100 per cent (Moriones and Navas, 2000). It has been reported that water deficit stress increases the flower abortion, thus affects the fruits settings. The low marketable fruit yield obtained for some tomato varieties might be due to non-development of flowers. It was observed that only 50% of the flowers produced developed into fruits, thus sink size was a limiting factor to fruit production in tomato (Olaniyi *et al.,* 2010).

# CHAPTER III

# MATERIALS AND METHODS

# MATERIALS AND METHODS

## 3.1 Experimental site

The experiment was conducted at Net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh, during the period of July 2021 to October 2021. The experimental area was situated at 23°46' N latitude and 90°22'E longitude at an altitude of 8.6 meter above the sea level (Anon. 1988).

## 3.2 Soil characteristics

The soil characteristics of the experiment field was a medium high land which belongs to the Modhupur tract, Agro Ecological Zone no 28. The soil texture was silt loam, Low level of nutrients, non-calcareous, acidic, brown or red soil of Tejgaon soil series with a pH 6.7. Before conducting the experiment, soil samples were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU) at a depth of a 0 to 30 cm and analyzed in the Soil Resources Development Institute (SRDI), Farmgate, Dhaka.

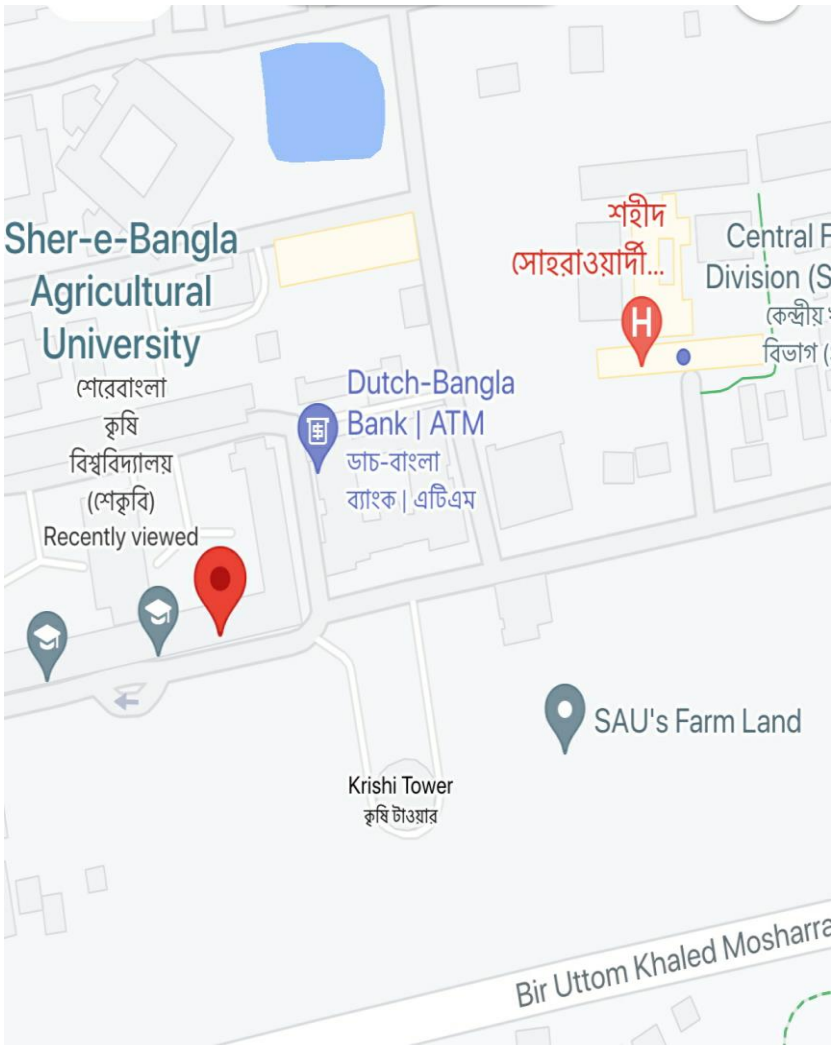
## 3.3 Climatic condition

The climate of the Modhupur Tract varies slightly from north to south, the northern reaches being much cooler in winter. Average temperatures vary from 28℃ to 32℃ in summer, falling to 20℃ in winter, with extreme lows of 10℃. Rainfall ranges between 1000 mm and 1500 mm annually, heavy rainfall in Kharif season (May-September) and scanty in Rabi season (October-March). Severe storms are unusual but tornadoes have struck the southern areas. During the month of December, January and February there was no rainfall. During the period of investigation, the average maximum temperature was 32℃ and average minimum temperature was 20℃. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment was collected from Bangladesh Meteorological Department, Agargaon, Dhaka.

## 3.4 Map of Dhaka City


Map 1. Dhaka city

### 3.5 Experimental site Map




Map 2. Experimental Site

### 3.6 Experimental Design

The experiment was carried out in a completely randomized design (CRD) with three replications and each of the treatment contains 3 pots. The total number of unit pots was 30.

### 3.7 Planting Material

Tomato variety 'BARI hybrid tomato-4' were selected to conduct the research. Seeds were collected mainly from Bangladesh Agricultural Research Institute (BARI), Gazipur.

### 3.8 Treatments of the Experiment

In total ten (10) treatments were considered in this experiment. These were as Follows:

$T_0$ =Control

$T_1$= *Metarhizium anisopliae* + *Trichoderma harzianum* ($10^8$ + 20 ml/litre )

$T_2$= *Metarhizium anisopliae* + *Verticillum lecanii* ($10^8$ + $10^8$ )

$T_3$ = *Metarhizium anisopliae* + *Beauveria bessiana* ($10^8$ + $10^{10}$ )

$T_4$= Vat leaf extract (20%)

$T_5$= Lantana leaf extract (20%)

$T_6$= Neem leaf extract (20%)

$T_7$= Tea wastage (500 gm/pot)

$T_8$= Garlic powder (35 gm/pot)

$T_9$= Mustard oil cake (200 gm/pot)

### 3.9 Seedlings preparation

For the seedlings preparation, seeds were soaked overnight in distilled water. Seedlings were grown in a seed bed of the experimental field of SAU. The soil was mixed with desired amount of fertilizers and cowdung. Finally, the seeds were sown in individual row and proper care was taken for better germination and seedling development. Some seedlings were found damping off diseased then cupper oxychloride (Semco) was treated in the tray @ 1g/L water.

### 3.10 Pot preparation and transplanting of Seedlings

At first soil was prepared and mixed with proper amount of fertilizer and cowdung. The pot was filled with the soil and seedlings from the tray were transferred in the pot.

### 3.11 Intercultural operations

#### 3.11.1 Gap filling

Gap filling was done after one week of transplanting. The seedlings were taken from the same source and gap filling was done where it was necessary.

#### 3.11.2 Weeding

Three hand weeding was done. First one was done at 20 DAT (Days after Transplanting) and second and third weeding were done at 40 and 60 DAT respectively.

#### 3.11.3 Manure and Fertilizer management

| Manure/fertilizer | Dose/ha |
|---|---|
| Cowdung | 2kg |
| Urea | 20 gm |
| TSP | 10 gm |
| MP | 10 gm |
| Gypsum | 5 gm |
| Zinc Sulphate | 5 gm |
| Boric Acid | 5 gm |

#### 3.11.4 Irrigation and drainage water

Irrigation was done according to the need. The pots were irrigated by a watering can.

### 3.11.5 Staking

When the plants were well established, staking was given to each plant by bamboo sticks to keep plants erect.

### 3.12 Parameters assessed

All experimental plants were selected and mean data of the following parameters were recorded. The following parameters were assessed:

a. Disease incidence (%)
b. Disease severity (%)
c. No of flowers/plant
d. No of fruits/plant
e. Fruits diameter
f. Individual fruit weight
g. Shoot length
h. Root length
i. Yield/plant
j. Root weight

### 3.12.1 Disease incidence (%)

Disease incidence, which measures the extent of proportion of a disease within a given field (Agrios 2005), was estimated by using the following formula:

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

Disease was identified by visual basis, observing the typical symptoms of wilt and *TYLCV*.

### 3.12.2 Disease severity (%)

Symptom development was evaluated according to the symptom severity scale described by Lapidot and Friedmann, (2002). Disease severity was calculated by the following formula and following disease rating scale Table 1.

$$\text{Disease severity (\%)} = \frac{\text{Sum of total disease rating}}{\text{Total No of observation} \times \text{Maximum grade in the scale}} \times 100$$

**Table 1. Disease severity rating scale of *TYLCV* to determine disease severity**

| Grading Scale | Symptoms |
|---|---|
| 0 | No visible symptoms, healthy plant. |
| 1 | Very slight yellowing of leaflet margins on apical leaf. |
| 2 | Some yellowing and minor curling of leaflet ends |
| 3 | A wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop |
| 4 | very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops |

### 3.12.3 No of flowers/plant

The number of flowers of each plant was counted at an age of 30, 45 and 60 DAT. Only the healthy flowers were considered and the data was recorded.

### 3.12.4 No of fruits/plant

The number of fruits of each plant was counted and mean number of tomato fruits of each variety were recorded.

### 3.12.5 Fruits diameter

Mean diameter of collected tomatoes from each plant as per variety were measured by a slide calipers in centimeter (cm).

### 3.12.6 Individual fruit weight

Individual fruit weight was measured by a digital balance meter in gram (g). A mean weight was taken of collected fruits from each plant as per variety.

### 3.12.7 Shoot length

Shoot length of the plant of was measured by a meter scale from the ground to longest tip of the plant in centimeter (cm) at a last harvesting time stage.

### 3.12.8 Root length

Root length of the plant of was measured by a meter scale in centimeter (cm) while the plant was uprooted.

### 3.12.9 Yield/plant

Every time tomato was harvested followed by measuring the weight and diameter and data were recorded. Total yield per plant was measured in kg and the diameter was measured in cm.

### 3.12.10 Root weight

Root weight of the plant of was measured by a digital balance meter in gram (g), while the plant was uprooted.

## 3.13 Isolation of Causal organism

### 3.13.1 Isolation and Identification of fungi

For fungal isolation from plant, the stem was washed under tap water, chopped into 2 cm small pieces and surface sterilized in 0.5% NaOCl for two minutes then rinsed twice with triple sterilized water and placed on blotter paper and finally kept in an incubator at 25°C ± 2°C under dark conditions. After incubation of 3 days the colony morphology was studied.

After that PDA (potato dextrose agar) media was prepared for pure culture of the isolates. In the preparation of PDA media (potato dextrose agar). 50 gm. of potato was peeled and sliced for 250ml of media. Now 250ml distilled water was taken on a pan and the potato slices was taken in the water after that the pan was kept on a stove for boiling. After 15 minutes the water was filled with the starch of the potato slices that was taken on a conical flask. On that 5 gm. agar powder and 5 gm. dextrose powder were mixed with it and sealed with cotton and foil paper. After that autoclaved for 45 minutes under $121^0$ c temperature 15 psi pressure.

**Figure 1. Plant sample for fungal isolation**

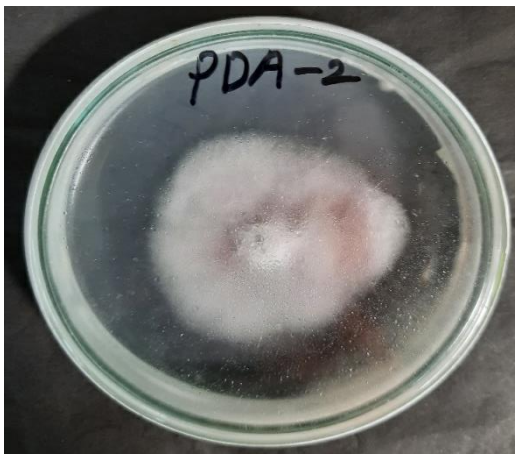



**Figure 2. Pure culture of *Fusarium***

### 3.13.2 Isolation and Identification of Bacteria:

Field identification of infected plant samples was done by analytically observing the bacterial wilt symptoms which involved wilting, yellowing of leaves, stunting of growth and observance of narrow dark stripes corresponding to the diseased vascular bundles beneath the epidermis.

#### 3.13.2.1 Isolation of Bacteria:

Collected tomato plant materials were surface sterilized with 1% Sodium Hypochlorite (NaOCl) solution for 1 to 2 min, followed by three repeated washings with sterilized distilled water and blow dried according to procedure by (Singh *et al.,* 2015). The plant sections (0.5-1 cm) were taken in a mortar to crash them with some sterile distilled water. Then the extract was diluted for $10^4$ times with sterile distilled water using sterile test tubes. From that 0.1ml water was taken with a micropipette and plated onto Nutrient Agar (NA) medium. (NA media was prepared by mixing 7.5gm of NA media powder in 250ml sterilized water and then autoclaved.)

**Figure 3. Pure culture of *Ralstonia***

### 3.13.2.2: Identification of Bacteria:

Different biochemical tests were carried out to identify and characterize the bacteria are described below.

### 3.13.2.2.1 Gram staining test:

**Slide preparation:**

For the gram staining procedure at first the slide was prepared. a clean sterile slide was taken on that a clean sterile loop was used to place a small amount of bacterial isolate accompained with a drop of clean water. By moving the loop round and round on the slide a thin layer of bacterial smear was prepared. Chemical preparation:

1. Crystal violet dye: to prepare 0.5% (aq) solution of crystal violet in a glass container 100ml distilled water was taken. In that water 0.5gm of crystal violet powder was mixed.

2. Safranin preparation: to prepare 0.5 % (aq) solution of safranin in a glass container 100ml distilled water was taken and in that 0.5 gm safranin powder was mixed.

3. Iodine solution: Lugol's Iodine solution was taken

4. Dicolorizing agent: for dicolorization 70% ethanol was used.

**Procedure of gram staining:**

On the bacterial smear prepared before, 0.5% crystal violet was poured in a flooding amount. After 1 minute the slide was rinsed under running tap water. Then iodine solution was poured again in flooding amount, kept for 1 minute and washed under running tap water. Next the 70% ethanol was poured for dicoloring and kept for 30-60seconds. Again washed under tap water. Now 0.5% safranin was poured for counter staining, kept for 30 seconds and washed under tap water. Then the slide war dried by the burner.

In the next step, a drop of immersion oil was taken on the stained bacterial smear and observed under compound microscope through the 100X objectives. Here the slide was taken so close to the lens that it was dipped into the oil of the slide and made the bacteria visible.

**3.13.2.2.2 Motility test:**

This test is done to observe weather the bacterial isolate is motile of non-motile. In this test a number of sterile test tubes were taken according to the total bacterial isolate number. First in a beaker 1L distilled water was taken and the beaker was placed on a stove After the water was warm 30gm of SIM (Sulfide Indole Motility) media powder was dissolved in that warm water by stirring with a glass rod. After some time the mixture started boiling and after 5-7 minutes of boiling a transparent solution was ready. Then that hot transparent solution was poured immediately in sterile test tubes at a height of half. As per the number of bacterial isolates prepared, sterile test tubes were taken. Then the opening of test tubes was sealed with cotton and foil paper. After that they were autoclaved. When the autoclaving is done test tubes are taken out from the machine and placed straight in a test tube holder in the laminar air flow cabinet to cool down. After cooling a golden semi solid media was developed. After that the bacterial isolates were placed with the help of a long needle by stabbing into the media. A long sterile needle was taken and a small amount of culture was touched with it and stabbed in the semi-solid media into the test tube. The tubes were properly labeled. And incubated at $30^{o}$ C temperature for 4 days.

After incubation a few drops of triphenyl tetrazolium chloride (TTC) was dropped (10ml distilled water + 0.1gm TTC) into the test tubes, after 15 minutes red marks were visible. In the media where the bacterial growth was existing, a reaction happened and a red sign was formed. The non-motile bacteria gave only a straight line following their growth area of stabbing

(negative) and the motile bacteria turned the whole media red as the bacteria moved all around the media (positive).

### 3.13.2.2.3 KOH solubility test:

It is a quick method for testing weather the bacteria is gram positive or negative. For this method a sterile slide was taken, on that a drop of 3% KOH solution was dropped. Now by a sterile loop or a sterile toothpick a small amount of 18hrs old bacterial isolate was macerated. After that when the loop or toothpick was put upside from the solution if a thin thread was seen then the bacteria was gram negative and test was positive. And if thread not formed the vice versa.

### 3.13.2.2.4 Catalase test:

For these test 3% $H_2O_2$ solution was prepared. In 10ml distilled water 0.3ml $H_2O_2$ was mixed and prepared in a sterile screw cap test tube. On a sterile glass slide a pinch of bacterial isolate was macerated with a sterile loop. After that a drop of prepared $H_2O_2$ solution was dropped.

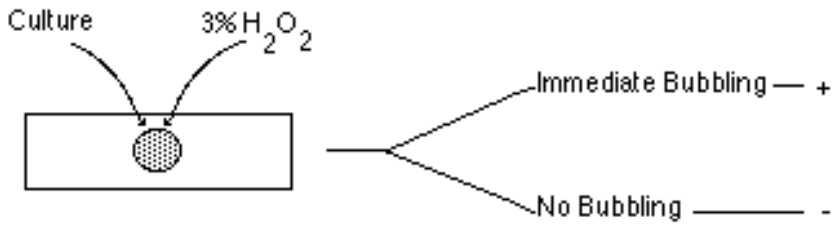



**Figure 4. Procedure of catalase test.**

### 3.13.2.2.5 Simon's citrate:

First in a beaker 1L distilled water was taken and the beaker was placed on a stove. After the water was warm 24.28gm of Simon's Citrate Agar powder was dissolved in that warm water that immediately turned green. After some time the mixture started boiling and after 57 minutes of boiling a green solution was ready. Then that

hot solution was poured immediately in sterile test tubes. As per the number of bacterial isolates prepared, sterile test tubes were taken.

Then the opening of test tubes was seal with cotton and foil paper. After that they were autoclaved. When the autoclaving was done test tubes were taken out from the machine and placed in a $25^0$-$30^0$ angle in the laminar air flow cabinet to cold and solidify. When the slants were become cold and coagulated the bacterial isolated were placed in a zigzag pattern or in a straight line with the help of a sterile loop. Here also the inoculating loop was sterilized after each use and then incubation for 4days.

After incubation it was found that some slants turned into blue color from green. It was because the bacteria grew, absorbed the citrate of the media (positive result). Where the citrate is not absorbed the color remained green as before (negative results).

### 3.14 Statistical analysis of data

The data were analyzed by using the “Statistix 10” Software. The mean value was compared according to LSD range test at 5% level of significance. Tables, bar diagram, linear graphs and photographs were used to present the data as and when necessary.

# CHAPTER IV

# RESULTS AND DISCUSSIONS

# RESULTS AND DISCUSSION

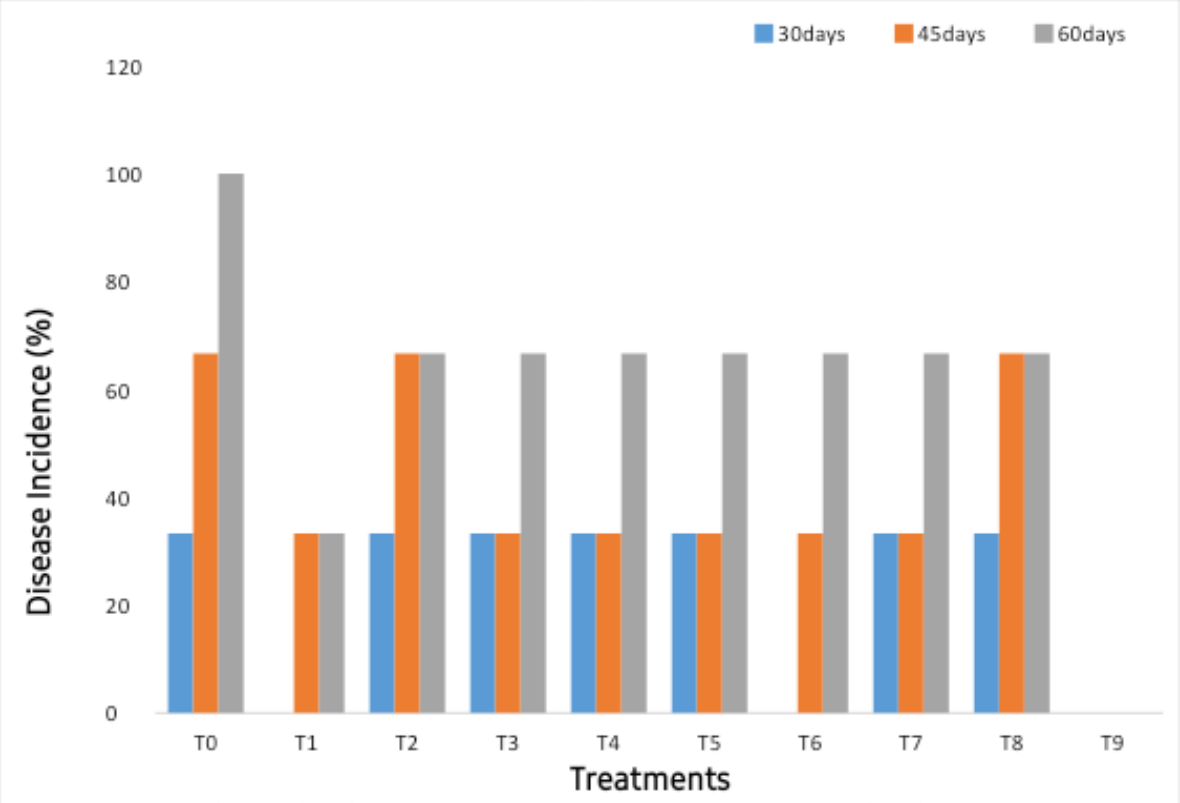
The present study was conducted to evaluate the combined effect of selected bio-agents, botanicals and agro bio-products against the wilt complex and *Tomato yellow leaf curl virus (TYLCV)* in summer tomato cultivation under pot condition. The Bio-management of wilt complex and *TYLCV* in response to selected different treatments, percent disease incidence and disease severity on the basis of visible symptoms, identification of disease causing agents, yield and yield contributing characters were recorded. The results have been presented in graphs, tables and figures under headings and subheadings.

## 4.1. Effect of selected bio-agents, botanicals and agro bio-products on disease incidence (%) of wilt complex at 30, 45 and 60 days after transplanting (DAT)

The effect of different treatments on disease incidence (%) of wilt complex was observed based on visible symptoms. Disease incidence was recorded three times at 30, 45 and 60 DAT.

At 30 DAT, the disease incidence was found in $T_0$ (Control), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage) and $T_8$ (Garlic powder). No disease symptom was appeared in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake). At 45 DAT, the highest disease incidence (66.67%) was found in $T_0$ (control) similar results was recorded in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*) and $T_8$ (Garlic powder) treatments. The lowest disease incidence (33.33%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage) treatments. No disease symptom was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease incidence (100%) was found in $T_0$ (control). The moderate disease incidence (66.67%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* +

*Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage) and $T_8$ (Garlic powder). The lowest disease incidence (33.33%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*). No disease symptom was found in $T_9$ (Mustard oil cake) upto the last observation. Results are presented in figure 5 and figure 9.



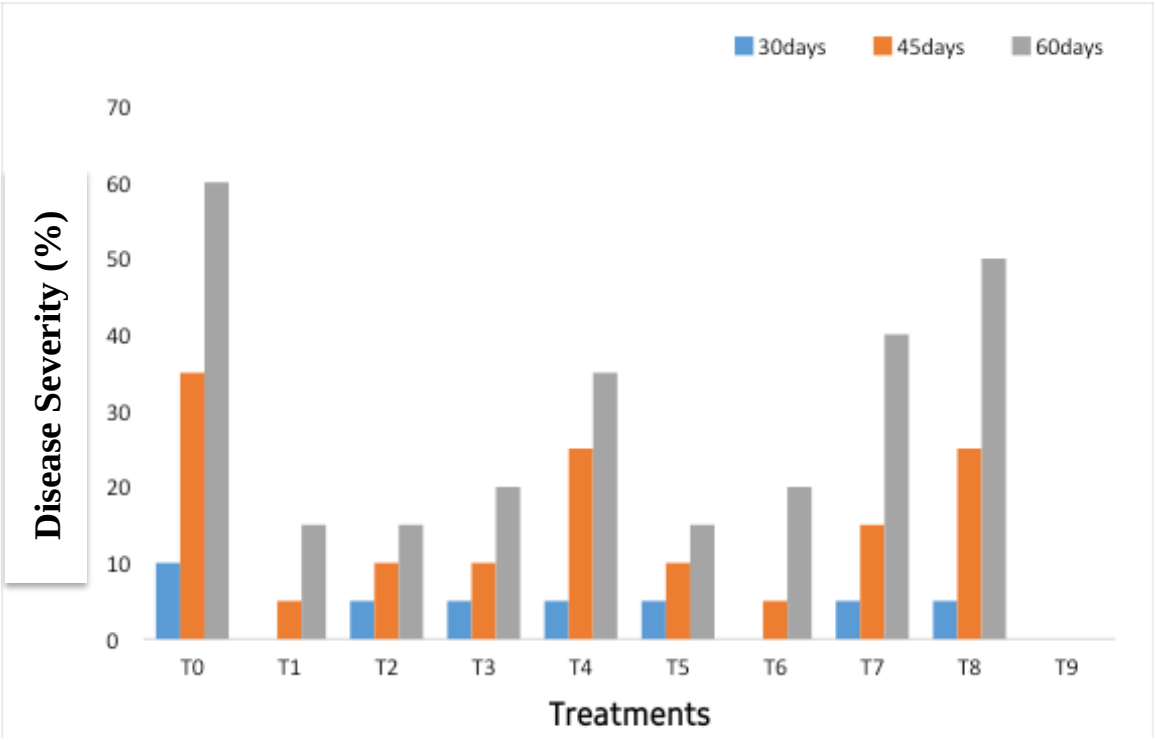

**T0 (Control), T1 *(Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 5. Presentation on disease incidence (%) of wilt complex at 30, 45 and 60 DAT**

## 4.2. Effect of selected bio-agents, botanicals and agro bio-products on disease severity (%) of wilt complex at 30, 45 and 60 days after transplanting (DAT)

The effect of different treatments on disease severity (%) of wilt complex was observed based on visible symptoms. Disease severity was recorded three times at 30, 45 and 60 DAT.

At 30 DAT, the highest disease severity (10%) was found in $T_0$ (control) .The lowest disease severity (5%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage), $T_8$ (Garlic powder). No disease symptom was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_6$ (Neem leaf extract), $T_9$ (Mustard oil cake). At 45 DAT, the highest disease severity (35%) was found in $T_0$ (Control) followed by $T_8$ (Garlic powder), $T_4$ (Vat leaf extract), treatments. The lowest disease Severity was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage) treatments. No disease severity was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease severity (60%) was found in $T_0$ (Control) followed by $T_8$ (Garlic powder). The moderate disease severity was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage). The lowest disease Severity was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_5$ (Lantana leaf extract). No disease severity was found in $T_9$ (Mustard oil cake) upto the last observation. Results are presented in figure 6 and figure 9.




**T0 (Control), T1 *(Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

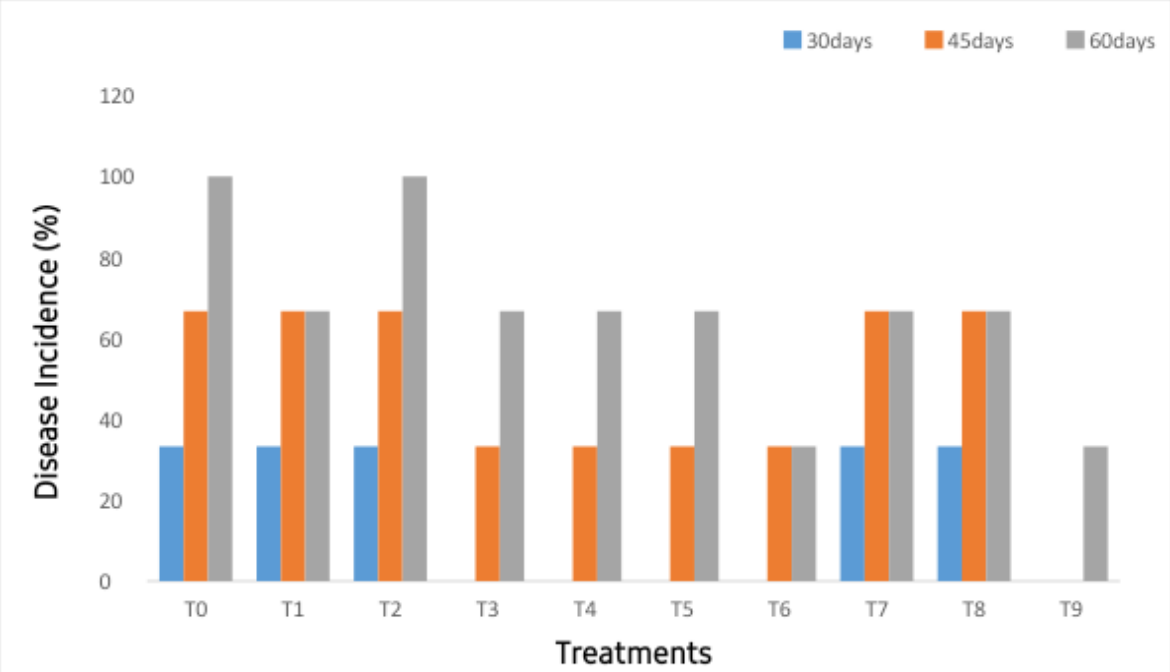
**Figure 6. Presentation on disease severity (%) of wilt complex at 30, 45 and 60 DAT**

### 4.3. Effect of selected bio-agents, botanicals and agro bio-products on disease Incidence (%) of *TYLCV* at 30, 45 and 60 days after transplanting (DAT)

The effect of different treatments on disease incidence (%) of *TYLCV* was observed based on visible virus and virus like symptoms. Disease incidence was recorded three times at 30, 45 and 60 DAT.

At 30 DAT, the viral disease symptoms was appeared in $T_0$ (Control), $T_1$ (*Metarhizium anisopliae + Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae + Verticillum lecanii*), $T_7$ (Tea Wastage) and $T_8$ (Garlic powder). No disease symptom was appeared in $T_3$ (Metarhizium + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake). At 45 DAT, the highest disease incidence (66.67%) was found in $T_0$ (Control), similar results was recorded in $T_1$ (*Metarhizium anisopliae + Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae + Verticillum lecanii*), $T_7$ (Tea Wastage),

$T_8$ (Garlic powder) treatments. The lowest disease incidence (33.33%) was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract) treatments. Again no disease symptom was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease incidence (100%) was found in $T_0$ (Control) and $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*). The moderate disease incidence (66.67%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage), $T_8$ (Garlic powder).The lowest disease incidence (33.33%) was found in $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake). Results are presented in figure 7 and figure 9.



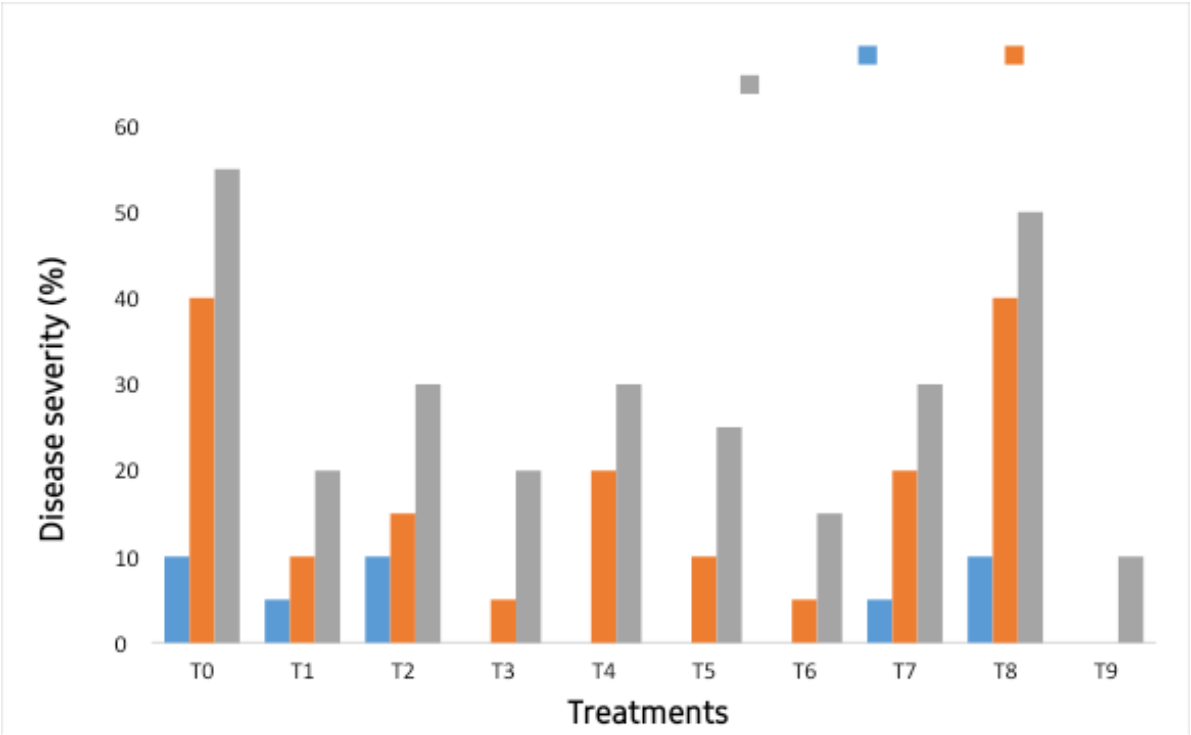

**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 7. Presentation on disease incidence (%) of *TYLCV* at 30, 45 and 60 DAT**

### 4.4. Effect of selected bio-agents, botanicals and agro bio-products on disease Severity (%) of *TYLCV* at 30, 45 and 60 days after transplanting (DAT)

The effect of different treatments on disease severity (%) of *TYLCV* was observed based on remarkable visible symptoms. Disease Severity was recorded three times at 30, 45 and 60 DAT.

At 30 DAT, the highest disease Severity (10%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_8$ (Garlic powder). The lowest disease Severity (5%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_7$ (Tea Wastage) treatments. No disease symptom was found in $T_0$ (Control), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_9$ (Mustard oil cake). At 45 DAT, the highest disease Severity (40%) was found in $T_8$ (Garlic powder) treatments. The moderate disease Severity was found in $T_0$ (Control), $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage) treatments. The lowest disease Severity was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_6$ (Neem leaf extract). No disease symptom was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease Severity was found in $T_0$ (Control) followed by $T_8$ (Garlic powder). The moderate disease Severity was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage). The lowest disease Severity was found in $T_9$ (Mustard oil cake). Results are presented in figure 8 and figure 9.




**T0 (Control), T1 *(Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 8. Presentation on disease severity (%) of *TYLCV* at 30, 45 and 60 DAT**

(A) (B)

**Figure 9. (A) Healthy summer tomato plant where soil bio-fortified with mustard oilcake**

**(B) Control (Non fortified soil)**

## 4.5. Isolation, identification and characterization of causative agents responsible for wilt complex in summer tomato cultivation

### 4.5.1 Isolation, identification and characterization of *Fusarium oxysporum* f.sp. *lycopersici*

On the basis of colony morphology and characteristics of macro and micro conidia, fungal isolates were identified as *Fusarium*. On further microscopic study, isolates were identified as *F. oxysporum* on the basis of macro conidia characteristics which were thin walled generally 3-5 septate, fusoid falcate macro conidia with somewhat hooked apex and pedicillate base (Booth, 1971) as clearly shown in figure 11.

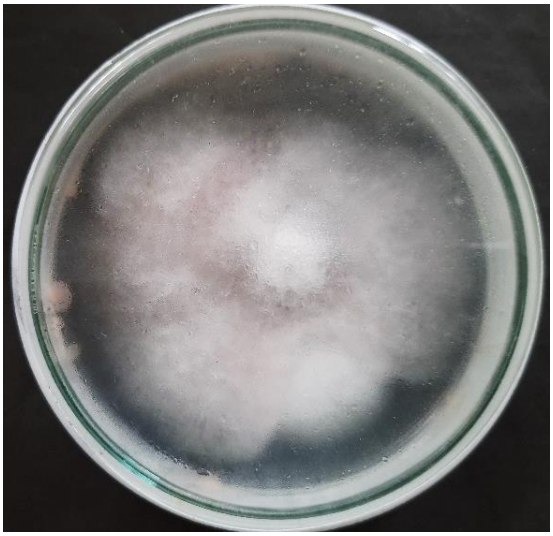


**Figure 10. Pure culture of *Fusarium***

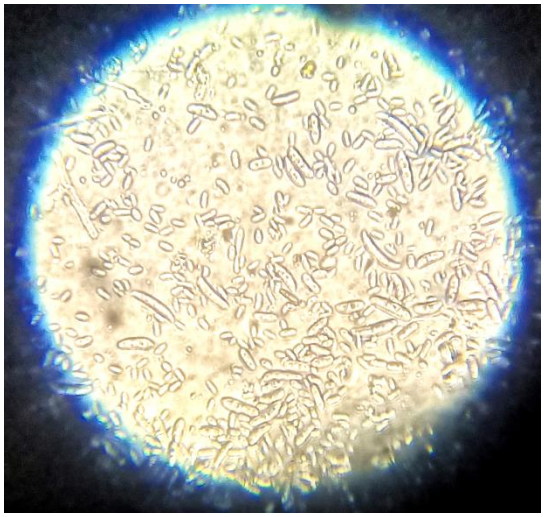


**Figure 11. Microscopic view of *Fusarium oxysporum***

### 4.5.2. Isolation, identification and characterization of *Ralstonia solanacearum* on the basis of different bio-chemical test (Gram staining, Motility, KOH solubility,Catalase, Simon's citrate test)

The results of gram staining exposed that the tested bacterium was gram negative, rod shaped and retained reddish pink colored cells. In the motility test, incubation few drops of triphenyl tetrazolium chloride (TTC) was dropped (10ml distilled water + 0.1gm TTC) into the test tubes, after 15 minutes red marks were visible. In the media where the bacterial growth was existing reaction happened and a red sign was formed. For performing the KOH test, a small

amount of 18 hrs. old bacterial isolate was macerated by a sterile loop. When the loop was put upside from the solution, a thin thread was seen that was confirmed the bacterium was gram negative and test was positive. Gas bubbles formation showed positive reaction for catalase test. Formation of Gas bubbles exposed the presence of aerobic and facultative anaerobic bacteria. For Simon's citrate test, after incubation it was seen that some slants turned into blue color from green. It was because the bacteria grew, absorbed the citrate of the media (Positive result).

From the above results (Plate 1) it was decided that the bacterium *R. solanacearum* was gram negative. The bacterium exposed positive reaction to all biochemical tests viz. motility test, KOH solubility test, catalase test and Simon's citrate test.

**Table 2. Bio-chemical characterization of *Ralstonia solanacearum***

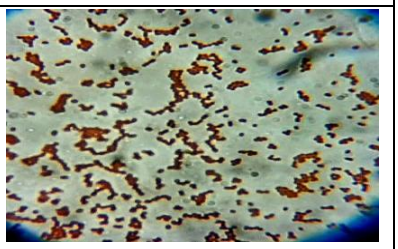
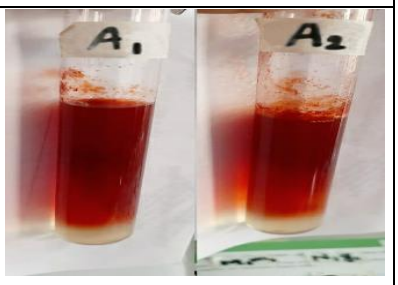
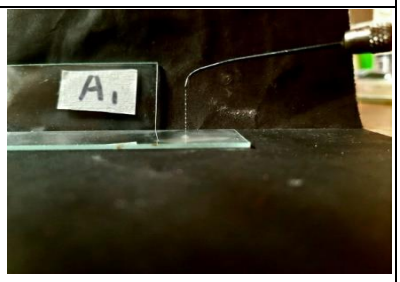
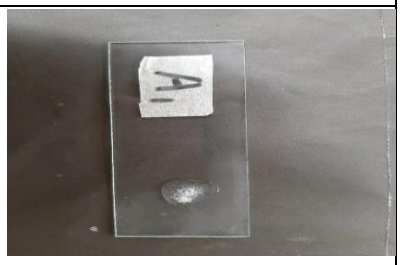
| S. No. | Biochemical test | Reaction | Pictorial view of the Bio-chemical reaction |
|---|---|---|---|
| 1. | Gram staining test: | Gram Negative |  |
| 2. | Motility test | Positive |   |
| 3. | Potassium hydroxide Test (KOH) | Positive |   |
| 4. | Catalase test | Positive |   |
| 5. | Simon's citrate | Positive |   |

**Plate 1. Biochemical characterization of *R. solanacearum;* Gram staining test (1), Motility test (2), KOH solubility test (3), Catalase test (4), Simon's citrate test (5).**

## 4.6. Effect of selected treatments on number of flower, fruits, fruits diameter, fruit weight and yield/plant

The treatments effect on number of flower, fruits, fruits diameter, fruit weight and yield/plant were varied significantly. The highest number (51.33) of flowers were counted in $T_9$ (Mustard oil cake) which was statistically similar with $T_6$ (Neem leaf extract, 46.67). The lowest number (18.33) of flowers were counted in $T_0$ (control). The moderate number of flowers were counted in remaining treatments which were statistically different from each and others.

The highest number (20.67) of fruits were counted in $T_9$ (Mustard oil cake) which was statistically different from each and others. The lowest number (13.33) of fruits were counted in $T_0$ (control) statistically similar with $T_4$ (Vat leaf extract, 14.00), $T_7$ (Tea Wastage, 13.33) and $T_8$ (Garlic powder, 14.00). The moderate number of fruits were counted in $T_6$ (Neem leaf extract) followed by $T_1$ (*Metarhizium anisopliae + Trichoderma harzianum,* 15.00), $T_2$ (*Metarhizium anisopliae + Verticillum lecanii,* 15.00), $T_3$ (*Metarhizium anisopliae + Beauveria bessiana*, 15.67), $T_5$ (Lantana leaf extract, 15.33) treatments which were statistically identical with each and others.

The highest number (14.17 cm) of fruits diameter were counted in $T_8$ (Garlic powder), which was statistically identical with $T_7$ (Tea Wastage, 13.80 cm). The lowest number (9.193 cm) of fruits were counted in $T_0$ (control). The moderate number of fruits diameter were counted in remaining treatments which were statistically similar each and others.

The highest number (24.67 gm) of fruit weight were counted in $T_9$ (Mustard oil cake) treatment. The lowest number (13.63 gm) of fruit weight were counted in $T_7$ (Tea Wastage) which was statistically similar with $T_0$ (control, 14.93 gm), $T_1$ (*Metarhizium anisopliae + Trichoderma harzianum,* 13.97 gm), $T_2$ (*Metarhizium anisopliae + Verticillum lecanii,* 14.07 gm), $T_4$ (Vat leaf extract, 14.17 gm), $T_5$ (Lantana leaf extract, 15.60 gm) and $T_8$ (Garlic powder, 14.07 gm) treatments. The moderate number of fruit weight were counted in $T_6$ (Neem leaf extract, 20.60 gm) and $T_3$ (*Metarhizium anisopliae + Beauveria bessiana*, 18.73 gm) treatments.

The highest number (494.33 gm) of yield were counted in $T_9$ (Mustard oil cake) treatment. The lowest number (180.30 gm) of yield were counted in $T_7$ (Tea Wastage) treatment. The moderate number of yield were counted in $T_6$ (Neem leaf extract, 356.47 gm) followed by $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*, 292.47 gm), $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*, 210.23 gm), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*, 211.13 gm), $T_5$ (Lantana leaf extract, 238.37 gm), $T_4$ (Vat leaf extract, 198.60 gm), $T_0$ (control, 198.60 gm) and $T_8$ (Garlic powder, 196.60 gm) treatments.

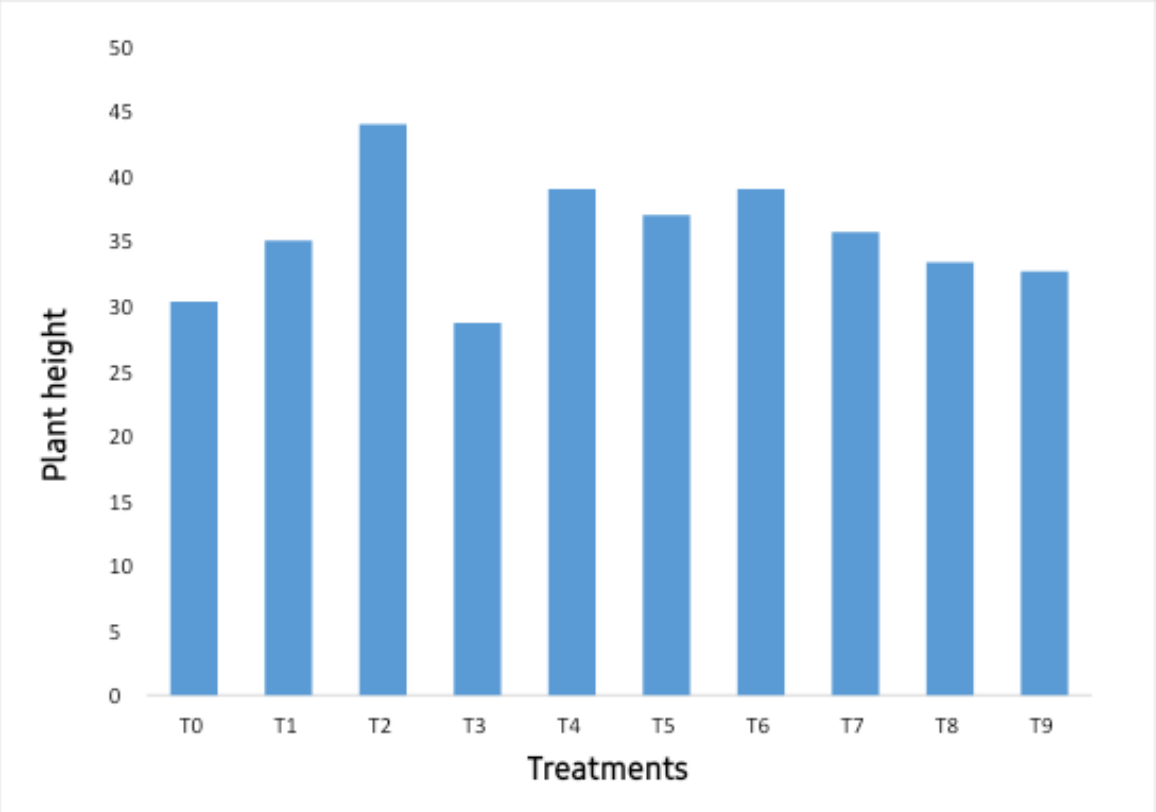
**Table 3. Effect of treatments on number of flower, fruits, fruits diameter, fruit weight and yield/plant**

| Treatment | Number of flowers | Number of fruits | Fruit diameter (cm) | Fruit weight (gm) | Yield/plant (gm) |
|---|---|---|---|---|---|
| $T_0$ | 18.33 c | 13.33 c | 9.19 f | 14.93 c | 198.67 e |
| $T_1$ | 28.00 b | 15.00 bc | 12.50 cd | 13.97 c | 210.23 de |
| $T_2$ | 26.67 bc | 15.00 bc | 12.44 cd | 14.07 c | 211.13 de |
| $T_3$ | 26.33 bc | 15.67 bc | 12.10 de | 18.73 b | 292.47 c |
| $T_4$ | 23.67 bc | 14.00 c | 13.17 bc | 14.17 c | 198.60 e |
| $T_5$ | 26.33 bc | 15.33 bc | 11.37 e | 15.60 c | 238.37 d |
| $T_6$ | 46.67 a | 17.33 b | 12.28 cde | 20.60 b | 356.47 b |
| $T_7$ | 25.00 bc | 13.33 c | 13.80 ab | 13.63 c | 180.30 e |
| $T_8$ | 29.33 b | 14.00 c | 14.17 a | 14.07 c | 196.60 e |
| $T_9$ | 51.33 a | 20.67 a | 11.79 de | 24.67 a | 494.33 a |
| **CV (%)** | 16.29 | 12.55 | 4.62 | 10.05 | 7.99 |

**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

### 4.7. Effect of selected treatments on morphological characters

The maximum plant height (44.00 cm) was measured in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*) treatment. The minimum number (28.67 cm) of Plant height was measured in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*) treatment. The moderate number of Plant height were measured in $T_0$ (control, 30.33 cm) followed by $T_9$ (Mustard oil cake, 32.67 cm), $T_8$ (Garlic powder, 33.33 cm), $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*, 35.00 cm), $T_7$ (Tea Wastage, 35.67 cm), $T_5$ (Lantana leaf extract, 37.00 cm), $T_4$ (Vat leaf extract, 39.00 cm) and $T_6$ (Neem leaf extract, 39.00 cm) treatments.




**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 12. Effect of selected treatments on plants height.**

The maximum number (7.25 inch) of Root Length were measured in $T_9$ (Mustard oil cake) which was identical with $T_0$ (control, 6.20 inch), $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*, 6.05 inch), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*, 6.53 inch), $T_5$ (Lantana leaf extract, 6.47 inch), $T_6$ (Neem leaf

extract, 6.83 inch) and $T_8$ (Garlic powder, 6.47 inch) treatments. The minimum number (4.43 inch) of Root Length was measured in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*) treatment. The moderate number of Root Length were measured in $T_4$ (Vat leaf extract) and $T_7$ (Tea Wastage) treatments.

The maximum number (28.67 gm) of Root weight were measured in $T_9$ (Mustard oil cake) treatment. The minimum number (6.80 gm) of Root weight were measured in $T_7$ (Tea Wastage) treatment. The moderate number of Root weight (14.76 gm) were measured in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*) which was identical with $T_4$ (Vat leaf extract, 13.57 gm), $T_6$ (Neem leaf extract, 13.03 gm) treatment and the remaining treatments which were statistically different from each and others.

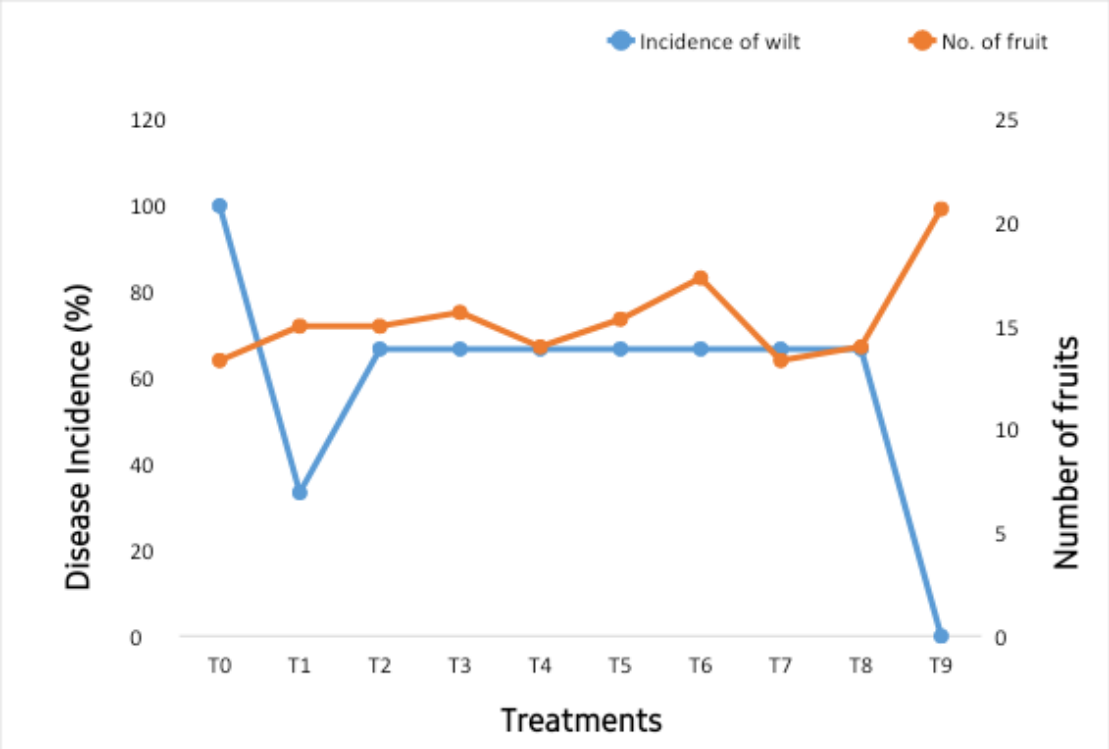
**Table 4. Effect of treatments on root length and weight**

| Treatment | Root Length (inch) | Root weight (gm) |
|---|---|---|
| $T_0$ | 6.20 ab | 10.80 cd |
| $T_1$ | 6.05 ab | 12.24 bcd |
| $T_2$ | 6.53 ab | 14.76 b |
| $T_3$ | 4.43 c | 9.37 de |
| $T_4$ | 5.77 b | 13.57 bc |
| $T_5$ | 6.47 ab | 10.63 cd |
| $T_6$ | 6.83 ab | 13.03 bc |
| $T_7$ | 5.73 b | 6.80 e |
| $T_8$ | 6.47 ab | 10.92 cd |
| $T_9$ | 7.25 a | 28.67 a |
| **CV (%)** | 11.59 | 13.13 |

**T0 (Control), T1 (*Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

## 4.8. Relationship between number of fruit and disease incidence (%) of wilt complex

Different treatments that were used in the present study regarding number of fruit per pot and disease incidence (%), it was revealed that number of fruit was increased with the decreased of disease incidence (%). The highest number of fruit per pot (20.67) was found in $T_9$ (Mustard oil cake) where no disease incidence was found. Whereas the lowest number of fruit (13.33) was counted in $T_0$ (control) pot where the highest disease incidence was recorded as depicted in the figure 13.

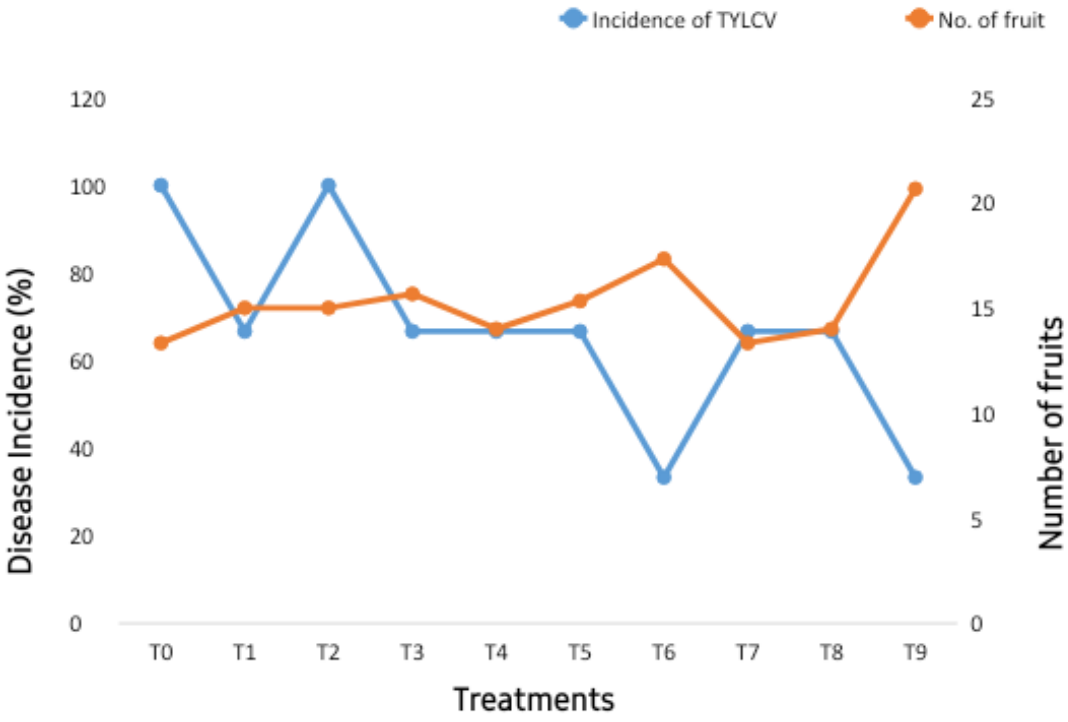



**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 13. Relationship between number of fruit and disease incidence (%) of wilt complex**

### 4.9. Relationship between number of fruit and Disease Incidence (%) of *TYLCV*

Different treatments that were used in the present study regarding number of fruit per pot and disease incidence (%), it was revealed that number of fruit become decreases with the increases of disease incidence (%). The highest number of fruit per pot (20.67) was found in $T_9$ (Mustard oil cake) with the lowest disease incidence (33.33%). In $T_0$ (control) pot the lowest number of fruit (13.33) was recorded where the highest disease incidence (100%) was estimated as clearly shown in the figure 14.

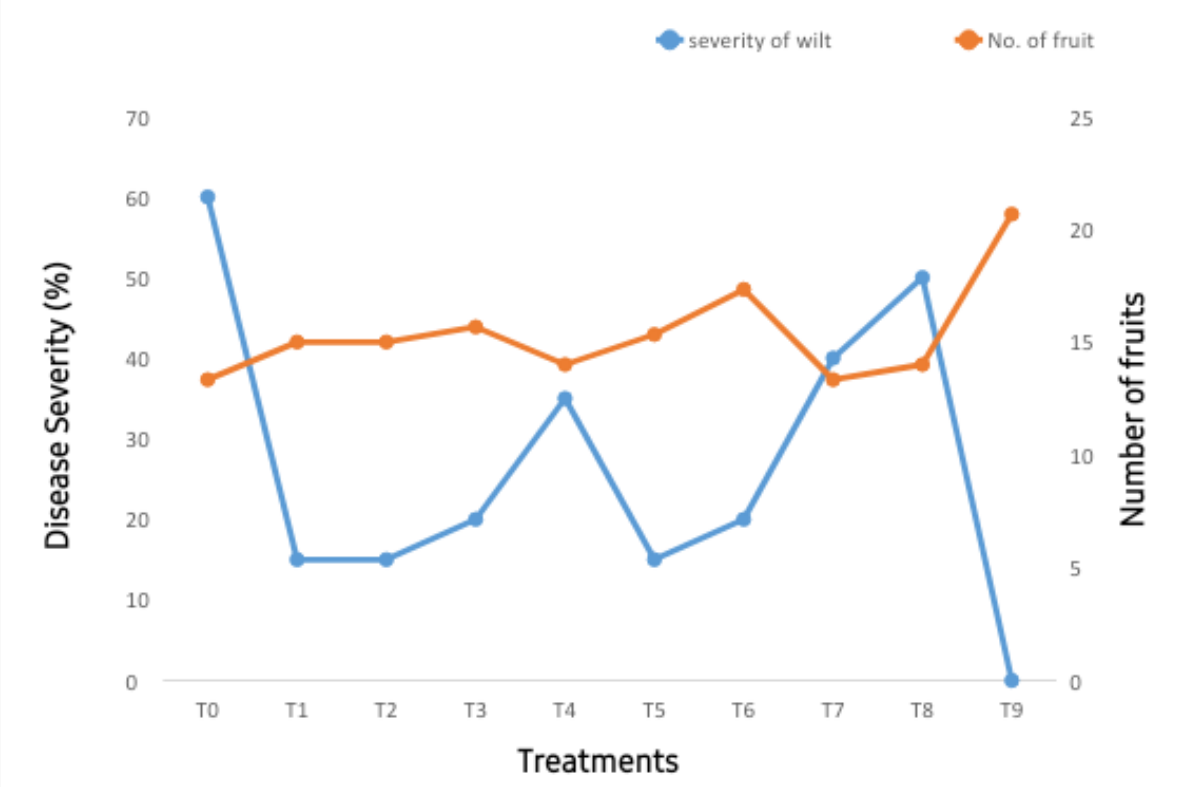



**T0 (Control), T1 (*Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 14. Relationship between number of fruit and disease incidence (%) of *TYLCV***

## 4.10. Relationship between number of fruit and Disease Severity (%) of wilt complex

Different treatments that were used in the present study regarding number of fruit per pot and disease severity (%), it was revealed that number of fruit was increased with the decreased of disease Severity (%). The highest number of fruit per pot (20.67) was found in $T_9$ (Mustard oil cake) and showed the lowest disease severity (0%). where $T_0$ (control) pot showed the lowest number of fruit (13.33) with highest disease severity (60%) as depicted in the figure 15.

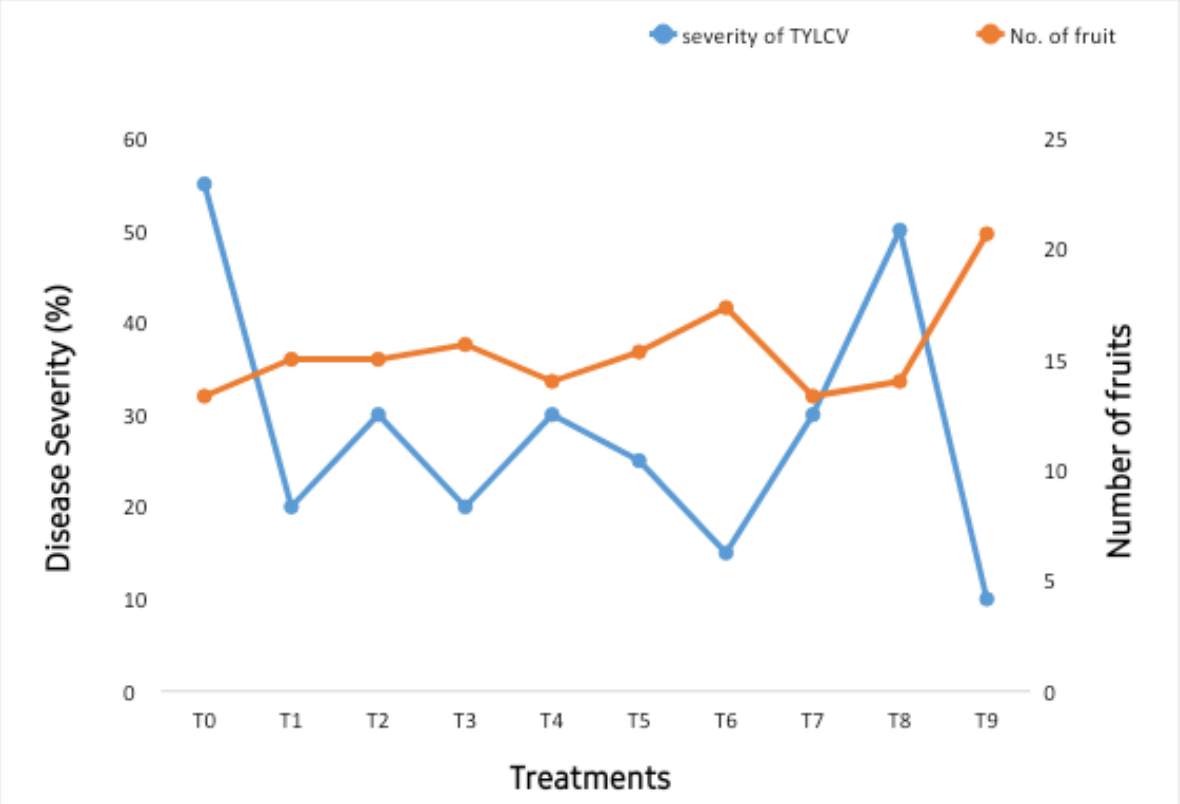



**T0 (Control), T1 (*Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 15. Relationship between number of fruit and disease severity (%) of wilt complex**

### 4.11. Relationship between number of fruit and Disease Severity (%) of *TYLCV*

Different treatments that were used in the present study regarding number of fruit per pot and disease severity (%), it was revealed that number of fruit was increased with the decreased of disease severity (%). The highest number of fruit per pot (20.67) was found in $T_9$ (Mustard oil cake) and showed the lowest disease severity (10%). where $T_0$ (control) pot showed the lowest number of fruit (13.33) with highest disease severity (55%) as depicted in the figure 16.

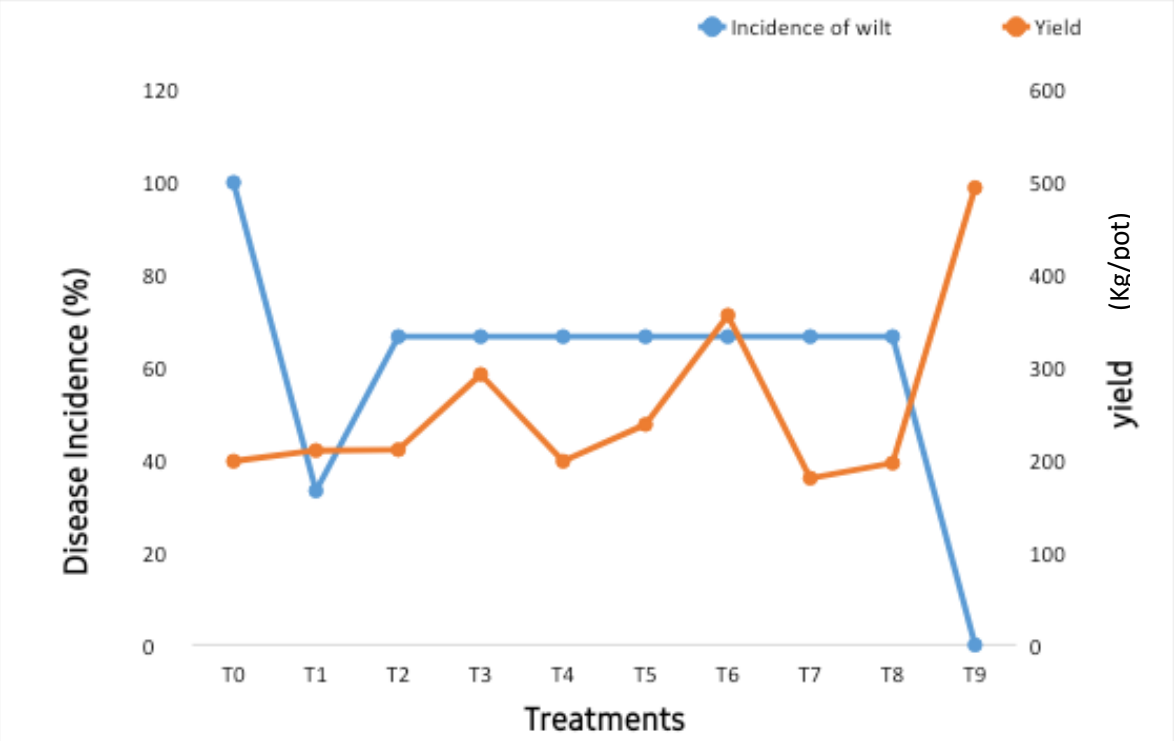



**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 16. Relationship between number of fruit and disease severity (%) of *TYLCV***

### 4.12. Relationship between yield per pot (kg) and Disease Incidence (%) of wilt complex

Different treatments that were used in the present study regarding yield per pot and disease incidence (%), it was revealed that yield was increased with the decreased of disease incidence (%). The highest yield per pot (494.33 gm) was found in $T_9$ (Mustard oil cake) and showed the lowest disease incidence (0%). where $T_7$ (Tea Wastage) pot showed the lowest yield (180.3 gm) with disease incidence (66.67%) as depicted in the figure 17.

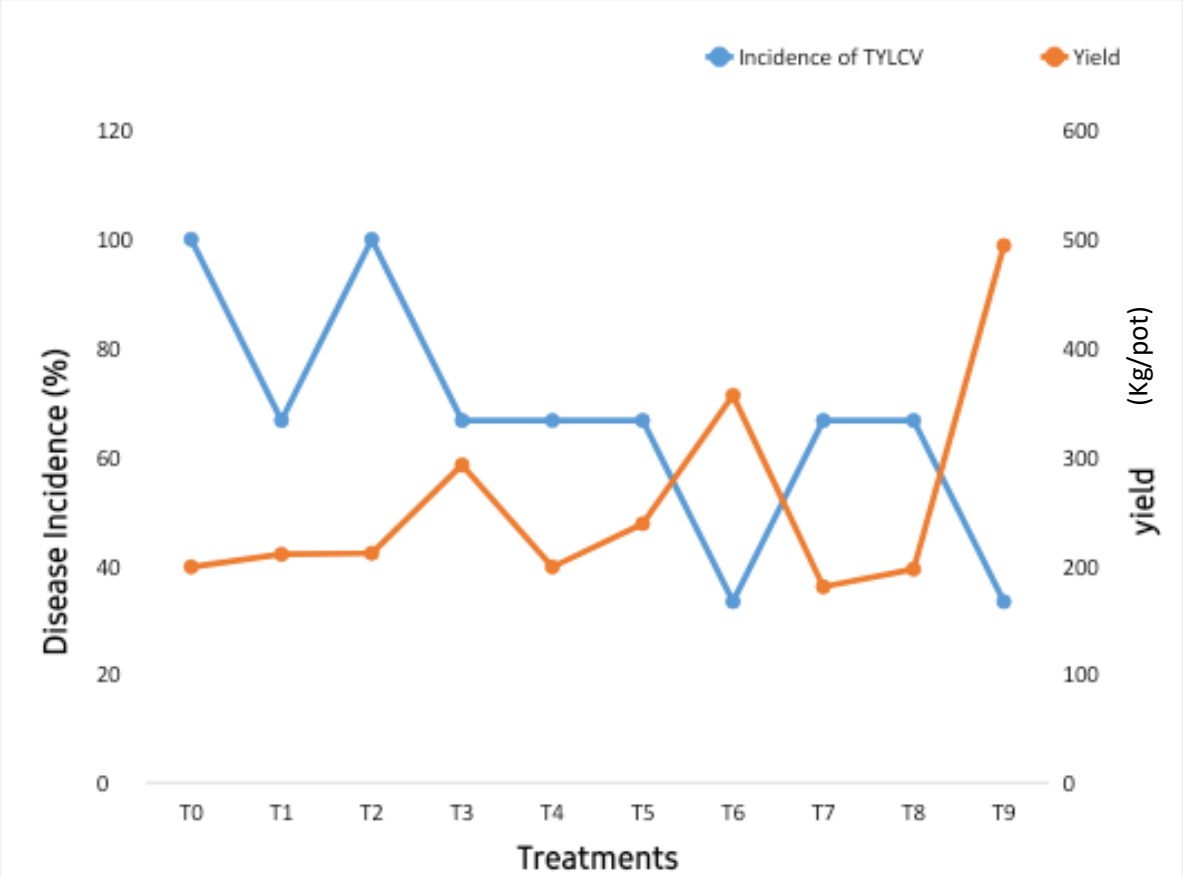



**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 17. Relationship between yield per pot (kg) and disease incidence (%) of wilt complex**

### 4.13. Relationship between yield per pot (kg) and Disease Incidence (%) of *TYLCV*

Different treatments that were used in the present study regarding yield per pot and disease incidence (%), it was revealed that yield was increased with the decreased of disease incidence (%). The highest yield per pot (494.33) was found in $T_9$ (Mustard oil cake) and showed the lowest disease incidence (33.33%). where $T_7$ (Tea Wastage) pot showed the lowest yield (180.3) with disease incidence (66.67%) as depicted in the figure 18.

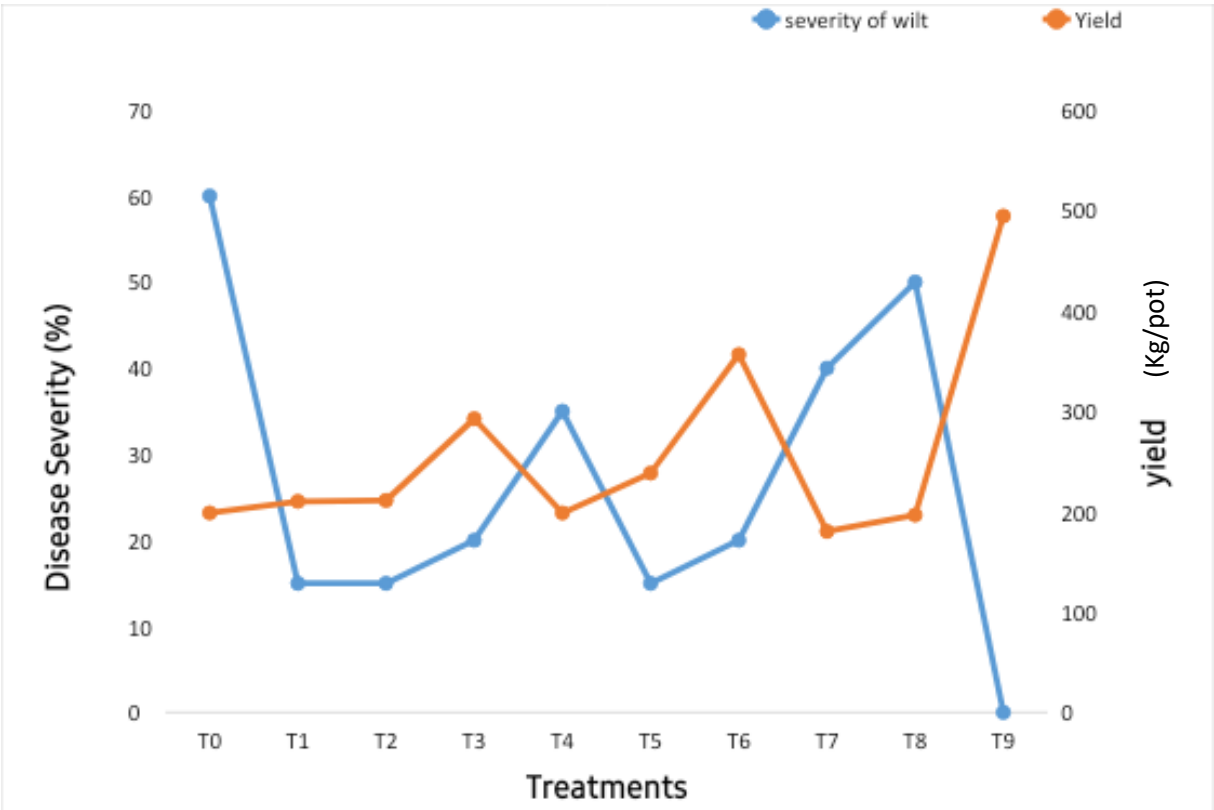



**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 18. Relationship between yield per pot (kg) and disease incidence (%) of *TYLCV***

## 4.14. Relationship between yield per pot (kg) and Disease Severity (%) of wilt complex

Different treatments that were used in the present study regarding yield per pot and disease severity (%), it was revealed that yield was increased with the decreased of disease severity (%). The highest yield per pot (494.33 gm) was found in $T_9$ (Mustard oil cake) and showed the lowest disease severity (0%). where $T_7$ (Tea Wastage) pot showed the lowest yield (180.3 gm) with disease severity (40%) as depicted in the figure 19.

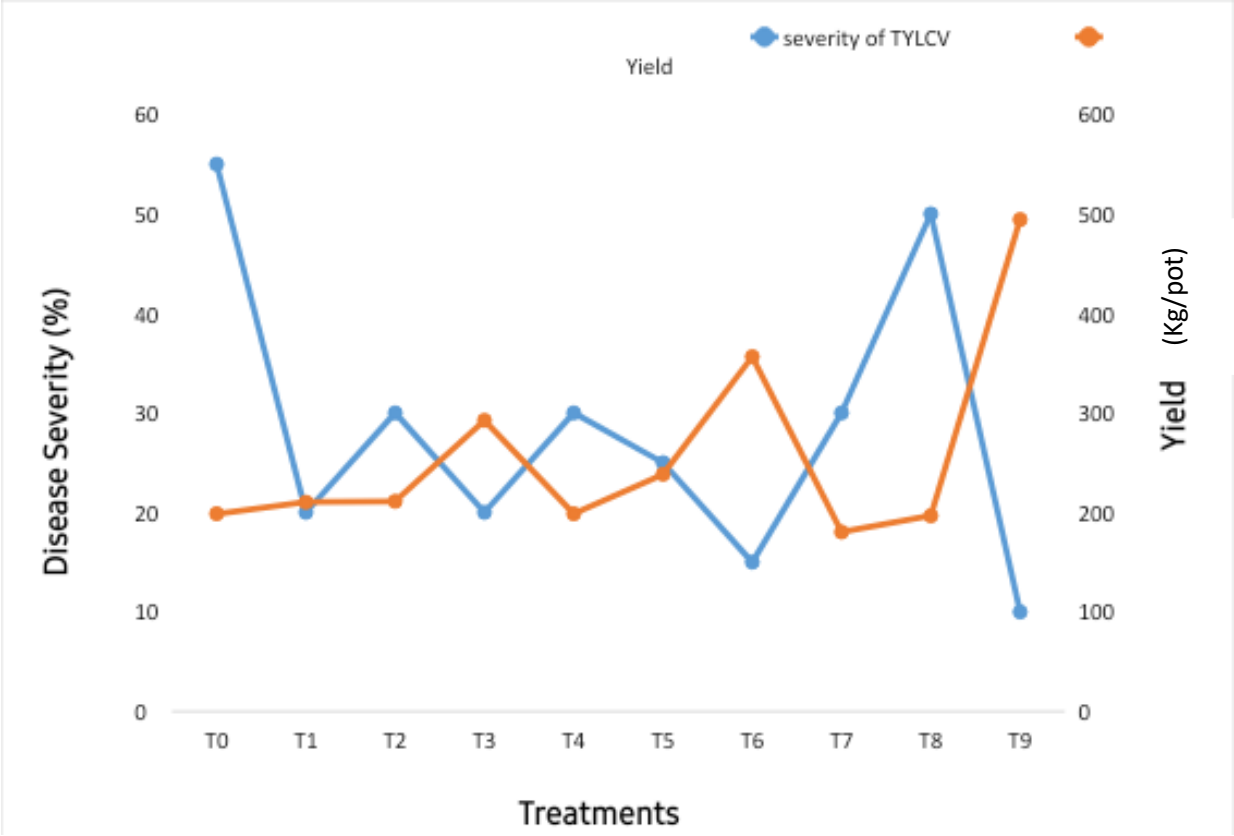



**T0 (Control), T1 (*Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 19. Relationship between yield per pot (kg) and disease severity (%) of wilt complex**

### 4.15. Relationship between yield per pot (kg) and Disease Severity (%) of *TYLCV*

Different treatments that were used in the present study regarding yield per pot and disease severity (%), it was revealed that yield was increased with the decreased of disease severity (%). The highest yield per pot (494.33 gm) was found in $T_9$ (Mustard oil cake) and showed the lowest disease severity (10%). where $T_7$ (Tea Wastage) pot showed the lowest yield (180.3 gm) with disease severity (30%) as depicted in the figure 20.

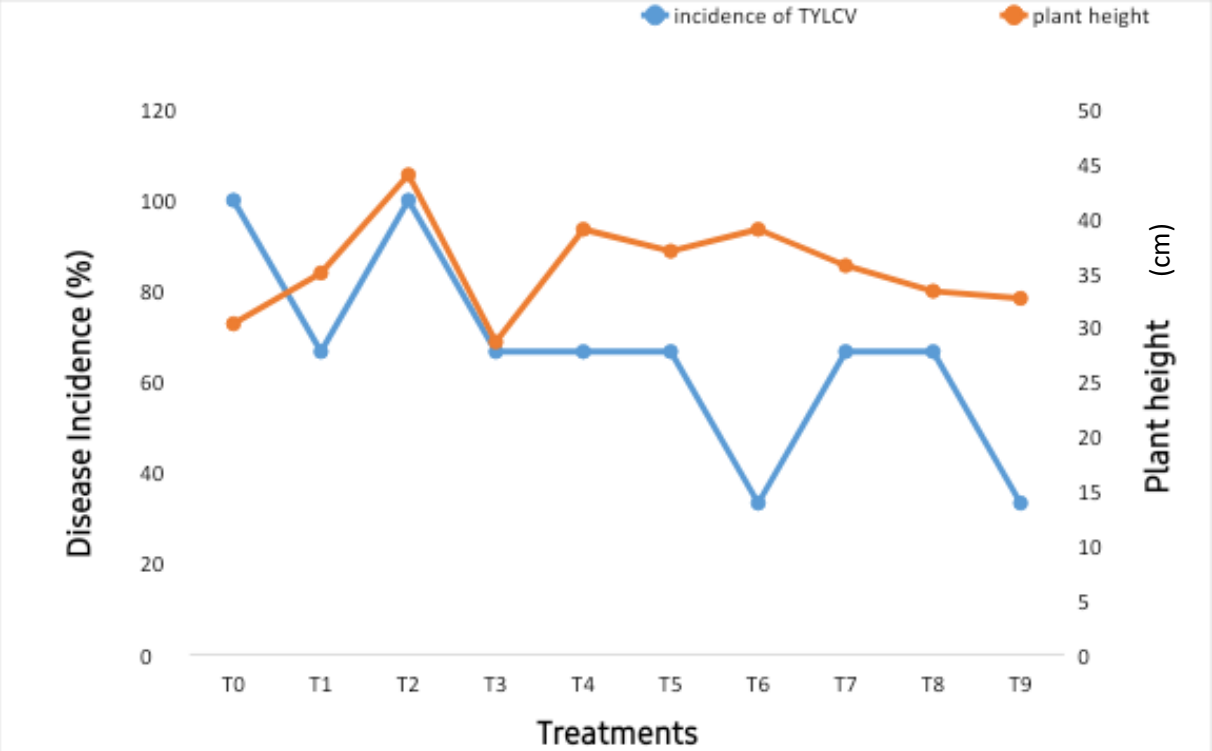



**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 20. Relationship between yield per pot (kg) and disease severity (%) of *TYLCV***

### 4.16. Relationship between plant height and Disease Incidence (%) of *TYLCV*

Different treatments that were used in the present study regarding plant height and disease incidence (%), it was revealed that plant height was increased with the decreased of disease incidence (%). The highest plant height (39 cm) was found in $T_6$ (Neem leaf extract) and showed the lowest disease incidence (33.33%). where $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*) pot showed the lowest plant height (28.67 cm) with disease incidence (66.67%) as depicted in the figure 21.

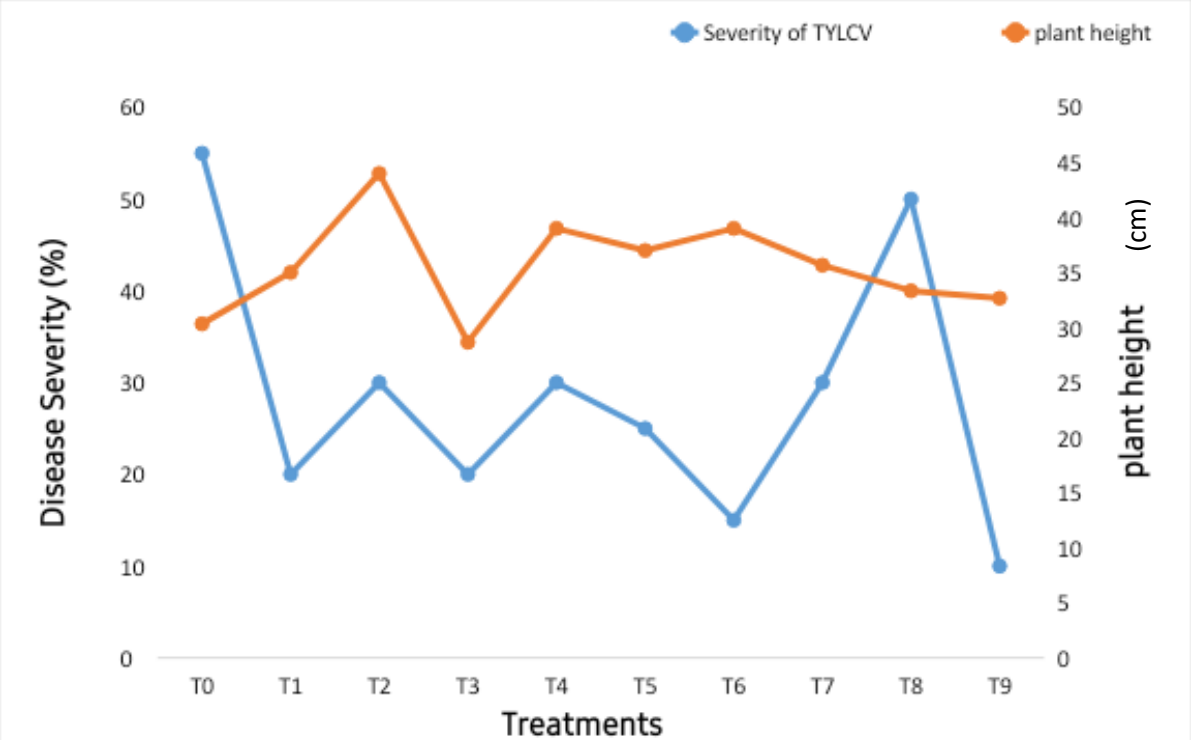



**T0 (Control), T1 *(Metarhizium anisopliae + Trichoderma harzianum*), T2 (*Metarhizium anisopliae + Verticillium lecanii*), T3 (*Metarhiaium anisopliae + Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 21. Relationship between plant height (cm) and disease incidence (% of *TYLCV***

### 4.17. Relationship between plant height and Disease Severity (%) of *TYLCV*

Different treatments that were used in the present study regarding plant height and disease severity (%), it was revealed that plant height was increased with the decreased of disease severity (%). The highest plant height (39 cm) was found in $T_6$ (Neem leaf extract) and showed the lowest disease severity (15%). where $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*) pot showed the lowest plant height (28.67 cm) with disease severity (20%) as depicted in the figure 22.




**T0 (Control), T1 (*Metarhizium anisopliae* + *Trichoderma harzianum*), T2 (*Metarhizium anisopliae* + *Verticillium lecanii*), T3 (*Metarhiaium anisopliae* + *Beauveria bessiana*), T4 (Vat leaf extract), T5 (Lantana leaf extract), T6 (Neem leaf extract), T7 (Tea wastage), T8 (Garlic powder) and T9 (Mastard oil cake)**

**Figure 22. Relationship between plant height (cm) and disease severity (%) of *TYLCV***

# DISCUSSION

Tomato (*Solanum lycopersicum* L.) is an important and widely grown vegetable crop good sources of minerals vitamins (A, C, E) and antioxidants. It is also the good source of tomatine that reduce the risk of cancer and very good for blood purifier. In Bangladesh, tomato mainly grow in winter season but presently it also can grow in the summer season . In summer the crop suffer from many biotic and abiotic stresses viz. disease pest infestation hit injury etc. It causes the reduction in yield and quality of tomato fruits a among the diseases, wilt complex and *TYLCV* are the most importance which limit the tomato production in summer season. This disease cause serious loss in tomato production in sometime fail to the production. Therefore the present study carried out to evaluate the combined effect of selected bio-agents, botanicals and agro bio-products against the wilt complex and *Tomato yellow leaf curl virus (TYLCV)* under pot condition. The results generated during the study is discuss here.

In this study, the disease incidence and severity of wilt complex and *Tomato yellow leaf curl virus (TYLCV)* on the basis of visible symptoms in response to different treatments were recorded at 30, 45 and 60 days after transplanting (DAT). All treatments reduced the disease incidence and severity of wilt complex and *TYLCV* over control untreated. Based on the disease incidence of wilt complex recorded at 60 DAT, the highest disease incidence (100%) was recorded in untreated control. Among the selected treatments used for bio-fortification of the pot soil, the lowest disease incidence (33%) was recorded in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*) and no disease symptoms was found in $T_9$ (mustard oil cake) treatment. Remaining treatments also gave satisfactory result over control that was statistically similar each other. Based on the disease incidence of *TLCV* recorded at 60 DAT, the highest disease incidence (100%) was recorded in $T_0$ (untreated control) and $T_2$ (*Metarhizium anisopliae + Verticillum lecanii*). Among the selected treatments used for bio-fortification of the pot soil, the lowest disease incidence (33%) was recorded in $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake). Rest of the treatments also gave satisfactory result over control that was statistically similar each other.

Based on the disease severity of wilt complex recorded at 60 DAT, the highest disease severity (60%) was recorded in untreated control. Among the selected treatments used for bio-fortification of the pot soil, the lowest disease severity (15%) was recorded in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*) which is statistically similar with $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*) and $T_5$ (Lantana leaf extract). No disease symptoms was found in $T_9$ (mustard oil cake) treatment. Other treatments also gave satisfactory result over control in terms of disease severity recorded at 60 DAT. Based on the disease severity of *TYLCV* recorded at 60 DAT, the highest disease severity (55%) was recorded in $T_1$ (untreated control). Among the selected treatments used for bio-fortification of the pot soil, the lowest disease severity (10%) was recorded in $T_9$ (mustard oil cake). Rest of the treatments also gave satisfactory result over control at last observation. From the present study, it is revealed that the selected bio-agents, botanicals and agro bio-products are showed significant antifungal and antibacterial activities as well as also can prevent the viral activities. Results from the present study is closed to some recent studies. Neem oil, derived from the seeds of *Azadirecta indica*, offers a wide range of therapeutic qualities, including antifertility, antibacterial, antifungal, antipyretic, and immune stimulant properties (Biswas *et al.,* 2002). Da-Costa *et al.* (2010) discovered that neem extract at concentrations ranging from 0.5–5.0% suppressed the growth of *Aspergillus flavus*. Adepoju *et al.* (2014) found that *A. indica* seed oil totally inhibited *Curvularia* sp. Growth and significantly slowed the growth of *Aspergillus* and *Fusarium* species. However, the treatment had no notable effect on *Rhizopus stolonifera.* Different formulations of *A. indica* leaf extracts viz., crude form, ethanolic form, boil form, and powder form were utilized to evaluate antifungal potential which reported that boil extract showed significant results against *Rhizoctonia bataticola* and *Alternaria* sp., and crude extracts were found most effective against *Fusarium* sp (Kaurav, 2019). However significant antibacterial activity was also reported against *Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus,* and Proteus *mirabilis*. Umaiyal *et al.* (2016) reported considerable antimicrobial properties against several gram-positive and gram-negative *bacilli* strains. Al-Ghamdi *et al.* (2019) reported the significant

antibacterial activity of *S. chinensis* against *B. subtilis, P. vulgaris, S. aureus,* and *P.mirabilis*. The results closely matched with the report of Ambia (2006) where the lowest disease incidence and disease severity of rhizome rot of ginger was found in case of application of *Trichoderma harzianum* and neem leaves extract at different days after planting and those treatments resulted maximum yield of rhizome. The results also closely matched with the previous reports, where soil application of bio-control agents like *Trichoderma harzianun* and *Pseudomonas fluorescens* during planting time at 2-5% gave effective control of the diseases.

From the present study, it was also found that the wilt complex of tomato caused by fungal pathogen *Fusarium* (*Fusarium oxysporum* f.sp. *lycopersici )* and Bacterium (*R. solanacearum ). Fusarium* isolates was confirmed and characterized on the basis of cultural and morphological characteristics as well as on the basis of macro and micro conidial structure. The results are matched with the reported findings of Booth (1971), where microscopic study of *Fusarium* isolates were identified as *F. oxysporum* on the basis of macro conidia characteristics which were thin walled generally 3-5 septate, fusoid falcate macro conidia with somewhat hooked apex and pedicillate base. From the results of bacteria isolation, it was decided that the identified bacterium is *R. solanacearum* that was confirmed by the biochemical tests viz. gram staining, motility, KOH solubility, catalase and Simon's citrate test. Those biochemical test results are stated with some previous biochemical reports (Singh *et al.,* 2015; Bannihatti, R.K., 2015; Rahman *et al.*, 2010).

The performance of the treatments in respect of yield and yield contributing characters against wilt complex and *TYLCV* of summer tomato varied significantly. All the treatments effect were found effective in terms of number of fruits per plant, individual fruit weight and fruit diameter. The highest number of fruits/plant was found in $T_9$ (Mustard oil cake, 20.67). Among the bio-fortified treatments, the highest individual fruit weight was obtained in $T_9$ (Mustard oil cake, 24.67) treatment. Individual fruit weight in others bio-fortified treatments were found almost same and both was also statistically similar. Among the treatments, the maximum fruit diameter was obtained in $T_8$ (Garlic powder, 14.16 cm) treatment

followed by $T_7$ (Tea Wastage, 13.80 cm) and $T_4$ (Vat leaf extract, 13.17 cm) which was statistically similar. The minimum fruit diameter was obtained in untreated control which was statistically different from all the treatments. In respect of fruit/plant, all the selected treatments varied significantly. Among the bio-fortified treatments, the highest number of fruits/plant was counted in $T_9$ (Mustard oil cake, 20.67) while the lowest was counted in $T_7$ (Tea Wastage, 13.33). The present findings of the study regarding the reduction of disease incidence and severity of wilt complex and *TYLCV* of summer tomato and improving the yield attributing characters and yield were supported by the previous report. Meena and Mathur (2005) worked on both bio-control agent and fungicides and showed that rhizomes were treated with fungicides followed by the soil application of bio-agents resulted suppression of the disease and increasing the yield.

This finding will help to utilize these oilseed cakes for agricultural disease management. The standardized and recognized formulations of these oilseed cakes will be further applied under field environments to find out the efficiency of oilseed cakes in natural conditions. These are industrial by products, further we can combine these with other bio-management strategies to cope up with disease resistance of pathogens and devastating crop losses by these phyto-pathogenic fungi, bacteria and viruses. From the environmental point of view, this would reduce the adverse effect of chemical fungicides on the environment by transforming oilseed cake, which is currently a waste product left in large quantities, into an effective and inexpensive antifungal and antibacterial agent as well as for preclusion the plant viruses.

# CHAPTER V

# SUMMARY AND CONCLUSION

# SUMMARY AND CONCLUSION

A pot experiment was conducted in net house at the central farm of Sher-e-Bangla Agricultural University, Dhaka, to evaluate the selected bio-agents (*Metarhizium anisopliae*, *Trichoderma harzianum*, *Verticillum lecanii*, *Beauveria bessiana*) botanicals (Vat leaf extract, Lantana leaf extract and Neem leaf extract) and agro bio-products (Tea Wastage, Garlic powder, Mustard oil cake) against the wilt complex and *Tomato yellow leaf curl virus (TYLCV)* in summer tomato cultivation. For conducting the pot experiment, the experimental pots were prepared with bio-agents, botanicals and agro bio-products for bio-fortification of soil fifteen days before of seedling transplantation. In the present study, in response to different selected treatments, percent disease incidence and severity on the basis of visible symptoms, yield and yield attributes were recorded. The data obtained for different characters were statistically analyzed to find out the significance of the difference among the treatments. An encouraging performances of the treatments used in the experiment was observed in reducing the disease incidence and severity in terms of plant infection and appearance the visible symptoms in comparison to manage at 30, 45, 60 days after transplanting (DAT).

In case of wilt complex of tomato, At 30 DAT, the disease incidence was found in $T_0$ (Control), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage) and $T_8$ (Garlic powder). No disease symptom was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake). At 45 DAT, the highest disease incidence (66.67%) was found in $T_0$ (Control) similar results was recorded in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), and $T_8$ (Garlic powder) treatments. The lowest disease incidence (33.33%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage) treatments. No disease symptom was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease incidence (100%) was found in $T_0$ (Control). The moderate

disease incidence (66.67%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage) and $T_8$ (Garlic powder). The lowest disease incidence (33.33%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*). No disease symptoms were found in $T_9$ (Mustard oil cake).

The effect of different treatments on disease severity (%) at 30 DAT, the highest disease Severity (10%) was found in T0 (Control) .The lowest disease Severity (5%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage), $T_8$ (Garlic powder). No disease symptom was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_6$ (Neem leaf extract), $T_9$ (Mustard oil cake). At 45 DAT, the highest disease Severity (35%) was found in $T_0$ (Control) followed by $T_8$ (Garlic powder), $T_4$ (Vat leaf extract), treatments. The lowest disease Severity was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage) treatments. No disease severity was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease severity (60%) was found in $T_0$ (Control) followed by $T_8$ (Garlic powder). The moderate disease severity was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_6$ (Neem leaf extract), $T_7$ (Tea Wastage). The lowest disease severity was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_5$ (Lantana leaf extract). No disease severity was found in $T_9$ (Mustard oil cake).

At 30 DAT, the viral disease symptoms was appeared in $T_0$ (Control), $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_7$ (Tea Wastage) and $T_8$ (Garlic powder). No disease symptoms were appeared in $T_3$ (Metarhizium + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake).

At 45 DAT, the highest disease incidence (66.67%) was found in $T_0$ (Control), similar results was recorded in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_7$ (Tea Wastage), $T_8$ (Garlic powder) treatments. The lowest disease incidence (33.33%) was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract) treatments. Again no disease symptom was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease incidence (100%) was found in $T_0$ (Control) and $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*). The moderate disease incidence (66.67%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage), $T_8$ (Garlic powder).The lowest disease incidence (33.33%) was found in $T_6$ (Neem leaf extract) and $T_9$ (Mustard oil cake).

The effect of different treatments on disease severity (%) at 30 DAT, the highest disease Severity (10%) was found in $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_8$ (Garlic powder). The lowest disease Severity (5%) was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_7$ (Tea Wastage) treatments. No disease symptom was found in $T_0$ (Control), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_6$ (Neem leaf extract), $T_9$ (Mustard oil cake). At 45 DAT, the highest disease Severity (40%) was found in $T_8$ (Garlic powder) treatments. The moderate disease Severity was found in $T_0$ (Control), $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract), $T_7$ (Tea Wastage) treatments. The lowest disease Severity was found in $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_6$ (Neem leaf extract). No disease symptom was found in $T_9$ (Mustard oil cake). At 60 DAT, the highest disease Severity was found in $T_0$ (Control) followed by $T_8$ (Garlic powder). The moderate disease Severity was found in $T_1$ (*Metarhizium anisopliae* + *Trichoderma harzianum*), $T_2$ (*Metarhizium anisopliae* + *Verticillum lecanii*), $T_3$ (*Metarhizium anisopliae* + *Beauveria bessiana*), $T_4$ (Vat leaf extract), $T_5$ (Lantana leaf extract),

$T_6$ (Neem leaf extract), $T_7$ (Tea Wastage). The lowest disease Severity was found in $T_9$ (Mustard oil cake).

The performance of the treatments in respect of yield and yield contributing characters against wilt complex and *TYLCV* of summer tomato varied significantly. All the treatments effect were found effective in terms of number of fruits per plant, individual fruit weight and fruit diameter. The highest number of fruits/plant was found in $T_9$ (Mustard oil cake, 20.67). Among the bio-fortified treatments, the highest individual fruit weight was obtained in $T_9$ (Mustard oil cake, 24.67) treatment. Individual fruit weight in others bio-fortified treatments were found almost same and both was also statistically similar. Among the treatments, the maximum fruit diameter was obtained in $T_8$ (Garlic powder, 14.16 cm) treatment followed by $T_7$ (Tea Wastage, 13.80 cm) and $T_4$ (Vat leaf extract, 13.17 cm) which was statistically similar. The minimum fruit diameter was obtained in untreated control which was statistically different from all the treatments. In respect of fruit/plant, all the selected treatments varied significantly. Among the bio-fortified treatments, the highest number of fruits/plant was counted in $T_9$ (Mustard oil cake, 20.67) treatment while the lowest was counted in $T_7$ (Tea Wastage, 13.33) treatment

# CHAPTER VI

# REFERENCES

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