T.R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PROTECTION

BIOCONTROL OF *Trichoderma* spp. AND *Saccharomyces cerevisiae* AGAINST *Fusarium oxysporum* f.sp. *Lycopersici* IN TOMATO

M. Sc. THESIS

PREPARED BY: Shawen Zrar RASUL
SUPERVISOR: Assist. Prof. Dr. Emre DEMİRER DURAK

VAN-2019

T.R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PROTECTION

BIOCONTROL OF *Trichoderma* spp. AND *Saccharomyces cerevisiae* AGAINST *Fusarium oxysporum* f.sp. *Lycopersici* IN TOMATO

M. Sc. THESIS

PREPARED BY: Shawen Zrar RASUL

VAN-2019

ACCEPTANCE and APPROVAL PAGE

This thesis entitled **"BIOCONTROL OF *Trichoderma* spp. AND *Saccharomyces cerevisiae* AGAINST *Fusarium oxysporum* f.sp. *Lycopersici* IN TOMATO"** and prepared by Shawen Zrar RASUL under consultation of Assist. Prof. Dr. Emre DEMİRER DURAK in Department of Plant Protection, on date of 20/09/2019 it has been successful with a unanimous vote by the following jury and it has been recognized as a Master's Thesis, in accordance with Postgraduate Education and training regulation with the relevant provisions.

Chair: Assist. Prof. Dr. Mehmet Hadi AYDIN Signature:

Member: Assist. Prof. Dr. Mustafa USTA Signature:

Member: Assist. Prof. Dr. Emre DEMİRER DURAK Signature:

This thesis has been approved by the committee of The Institute of Natural and Applied Science on 27/09/2019 with decision number 2019-53-1

Signature
Prof. Dr. Nadir SENOY
Director of the Institute

THESIS STATEMENT

All information presented in the thesis obtained in the frame of ethical behavior and academic rules. In addition all kinds of information that does not belong to me have been cited appropriately in the thesis prepared by the thesis writing rules.

Signature
Shawen Zrar RASUL

ABSTRACT

BIOCONTROL OF *Trichoderma* spp. AND *Saccharomyces cerevisiae* AGAINST *Fusarium oxysporum* f.sp. *lycopersici* IN TOMATO

RASUL, Shawen Zrar
M. Sc. Thesis, Department of Plant Protection
Supervisor: Assist. Prof. Dr. Emre DEMİRER DURAK
September, 2019, 75 Pages

This study discussed the effectiveness of the *Trichoderma* species and dry yeast *Saccharomyces cerevisiae* in promoting tomato growth and managing Fusarium wilt disease, and evaluated conditions in vitro and in vivo, with two methods (seed coating and soil treatment). In vitro, the results indicated that *Trichoderma virens* significantly inhibited the pathogen's mycelial development. *Fusarium oxysporum* f. sp. *lycopersici* mycelium inhibition was more effective compared to *T. harzianum* (19.03 %), *T. asperellum* (21.77 %) and *T. virens* (44.60 %). In vivo, all species of *Trichoderma* and yeast also inhibited the development of Fusarium in tomato planting pots as indicated by a reduction in the severity of the disease. *T. virens* had the lowest disease severity in seed coating (10.22 %), but the highest was *T. asperellum* (53.33 %). However, the application of *S. cerevisiae* with *Trichoderma* mixture of the highest severity of the disease was found with *S. cerevisiae* and *T. harzianum* (58.33 %). According to the experimental results, soil treatment with the *Trichoderma* species together did not give the disease any severity (0), though; *T. asperellum* had the highest disease severity (25 %). Furthermore, the best result was the application of *S. cerevisiae* with *Trichoderma* species in soil treatment and had no disease. From the present in vitro and in vivo comparative studies, it is evident that *T. virens* was the most efficient antagonist of the species *Trichoderma* to *F. oxysporum*. Furthermore, *T. harzianum*, *T. asperellum*, and *T. virens* are capable of controlling pathogen attacks in tomatoes and can be considered an applicable disease control approach.

Keywords: Biological control, *Fusarium oxysporum* f. sp. *lycopersici*, *Saccharomyces cerevisiae*, Tomato, *Trichoderma* spp.

ÖZET

DOMATESTE *Fusarium oxysporum* f.sp. *lycopersici'* nin *Trichoderma* spp. VE *Saccharomyces cerevisiae* ILE BİYOKONTROLÜ

RASUL, Shawen Zrar
Yüksek Lisans Tezi, Bitki Koruma Anabilim Dalı
Tez Danışmanı: Dr. Emre DEMİRER DURAK
Eylül, 2019, 75 Sayfa

Bu çalışmada biyolojik mücadele elemanları *Trichoderma* spp. ve kuru maya *Saccharomyces cerevisiae'* nın tek ve kombinasyonlarının domates bitkisinin gelişim parametrelerine ve Fusarium solgunluğu hastalığına etkileri araştırılmıştır. In vivoda biyokontrol elemanları tohum kaplama ve toprak uygulaması olarak iki farklı şekilde denenmiştir. In vitro sonuçlarına göre *Trichoderma virens* patojenin misel gelişimini önemli oranda engellemiştir. *Fusarium oxysporum* f. sp. *lycopersici'* yi sırasıyla *T. virens* % 44.60, *T. asperellum* % 21.77 ve *T. harzianum* % 19.03 oranında engellemiştir. In vivoda bütün *Trichoderma* türleri ve maya patojenin domates bitkilerindeki hastalık şiddetinde azalmaya neden olmuşlardır. Tohum kaplama yönteminde *T. virens* uygulanan bitkilerde patojenin oluşturduğu hastalık şiddeti değeri en düşük (% 10.22) bulunurken *T. asperellum* (% 53.33) en yüksek değeri vermiştir. *Trichoderma* türlerinin birlikte kullanıldığı toprak uygulamasında ise patojenin engellendiği belirlenmiştir. Toprak uygulamasında diğerlerine göre *T. asperellum* en yüksek hastalık şiddetine (% 25) sebep olmuştur. Ayrıca, en iyi sonuç toprak muamelesinde *S. cerevisiae*' nin *Trichoderma* türleri ile birlikte uygulandığında görülmüştür. In vivo ve in vitro sonuçlara göre *T. virens F. oxysporum'* a en etkili antagonist olarak belirlenmiştir. Ayrıca bu çalışma ile *T. harzianum, T. virens* ve *T. asperellum'* un domateslerde patojeni kontrol edebildiği belirlenmiştir ve bu türler ile biyolojik mücadele uygulanabilir bir hastalık kontrol yaklaşımı olarak düşünülebilir.

Anahtar kelimeler: Biyolojik kontrol, *Fusarium oxysporum* f. sp. *lycopersici*, S*accharomyces cerevisiae*, Domates, *Trichoderma* spp.

ACKNOWLEDGMENT

First, I would like to thank God Almighty for providing me the power, knowledge, ability and chance to conduct this study of research and to persevere with it and to complete it satisfactorily.

I would like to thank my thesis advisor Dr. Emre DEMİRER DURAK of the Plant Protection Department at VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ. For her patience, encouragement, passion, and vast understanding, study and research. Her guidance helped me throughout this thesis ' research and writing. Without her assistance and dedicated involvement in every step throughout the process, this paper would have never been accomplished. I would like to thank you very much for your support and understanding over these past two years. I couldn't have thought I had a better MSc advisor. I am very grateful for what she presented me. I would also like to thank Assist. Gökhan BOYNO for his help and guidance during the laboratory work. He consistently allowed this paper to be my own work, but steered me in the right the direction whenever he thought I needed it. A very special thank you to Abdulrahman SMAIL and, Assist. Hilmi KARA for their invaluable advice and feedback on my research and for always being so supportive of my writing. Also My special thanks to Shreen Abdulkareem MUSA, my greatest friend and partner, for her love and support. I am also very grateful to all those at the phytopathology laboratory, especially Hasret GÜNEŞ and Necmettin TENİZ, and others who were always so helpful and provided me with their assistance throughout my dissertation.My acknowledgement would be incomplete without thanking the biggest source of my strength, my family. This would not have been possible without their unwavering and unselfish love and support is given to me at all times.

2019

Shawen Zrar RASUL

TABLE OF CONTENTS

Pages

Pages

LIST OF TABLES

Table **Pages**

LIST OF FIGURES

Figures **Pages**

Figures **Pages**

SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with Descriptions.

Symbols	Description
cm	Centimeter
pH	Power Hydrogen
^{o}C	Celsius degree
min	Minutes
G	Gram
L	Liter
mg	Milligrams
mL	Milliliters
hrs	hours
kg	kilogram

Abbreviations	Description
PDA	Potato dextrose agar
FOL	Fusarium oxysporum f. sp. lycopersici
FYM	Farmyard manure
DAS	Days after sowing
VAM	Vesicular-arbuscular mycorrhiza
BCA	Biological control agent
DNA	Deoxyribonucleic acid

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the world's most famous vegetables today (Rick, 1979). Its popularity as a source of vitamins A and C is due to its elevated dietary value, distinct use and dietary value. This crop is also very essential for dieting against prevalent cancers such as breast and prostate cancer (Barari, 2016). *Solanum lycopersicum* the cultivated tomato is grown widely for its output. Tomatoes originated in South America, but sometime in the 1500s were transferred to Europe, where they quickly became popular and exported all over the world. Tomatoes have been known by the name *Lycopersicon esculentum* for a long time, but the latest scientist's actions have shown that they really belong to the *Solanum* genus-as Linnaeus acknowledged when he first described the species. Today, for the cultivated tomato researchers and crop breeders all use the name *Solanum lycopersicum*. Tomato, *Lycopersicum esculentum* (syn. *Solanum lycopersicum* and *Lycopersicon lycopersicum*) is an annual herbaceous crop cultivated for its edible fruit in the *Solanaceae* family.

In many parts of the world, plant disease becomes the limiting factor in the production of tomatoes when cultivars are not planted with resistance to numerous diseases. Tomato diseases can be classified as parasitic caused by pathogenic microorganisms and non-parasitic diseases caused by other abiotic factors (physiological Disorders). Non-parasitic tomato diseases are triggered by extremes of light, heat, soil moisture, soil and dietary imbalances, unfavorable relationships between oxygen, atmospheric pollutants, herbicide, and other pesticides and lightning harm. Bacteria, fungi viruses, viroid nematodes, and insects cause parasitic diseases. A virulent pathogen and the proper environment must be present in order to develop a susceptible part of a susceptible host (Al-Shugairan, 2008).

1.1. Tomato Diseases Caused By Fungi

Among the most common tomato diseases caused by fungi according to (Jones et al., 1997) are stem canker or black mold (caused by *Alternaria alternaria*),

anthracnose (caused by *Colletotrichum* spp.), black root rot (caused by *Thielaviopsis basicola*), Fusarium wilt (caused by *F. oxysporum* f.sp. *Iycopersici*), powdery mildew (caused by *Leveillula taurica*), Septoria leaf spot (caused by *Septoria Iycopersici*), Verticillium wilt (caused by *Verticillium albo-atrum* and *V. dahliae*), white mold (caused by *Sclerotinia sclerotiorum*), *Rhizoctonia* diseases and damping-off, root rot basal stem rot (caused by *Rhizoctonia solani*). (Al-Shugairan, 2008).

1.1.1. Fusarium wilt disease of tomato

G.E. Massee was first described Fusarium wilt of tomato in England in 1895. It has been reported in at least 32 countries around the world. In southern states in the USA and Europe, the disease is particularly destructive in the field, but in northern parts of the United States, it is limited by temperature variation to greenhouse crops (Walker, 1971). In particular, when favorable soil conditions are available, Fusarium wilt can destroy tomato production under field conditions (Al-Shugairan and Mohammed, 2008).

The disease is caused by the vascular wilt fungus, *F. oxysporum* f. sp. *lycopersici* (FOL). The fungus penetrates directly into the vascular tissue and colonizes it (Inoue et al., 2002). After flowering and at the start of the fruit set, infestation often happens on mature crops. Yellowing starts to appear on one side of a leaf and then on the other half of the leaf all leaflets become yellow. Before maturation, an infected plant often dies. In seedling infection the older leaves fall and curve downwards, the vascular tissue darkens, and the plant fades and dies. The leaves yellow after blossoming in older diseased crops. The vascular tissue is dark brown in the stem, but the pith stays healthy. Infection of the fruit may occur, showing the same brown vascular discoloration (Mui-Yun, 2003).

Vascular system browning is a feature of the disease and can usually be used to identify it (Beckman, 1987). *Fusarium* is a type of genus producing macro-conidia, micro-conidia, and chlamydospores in hyphomycetes (subdivision Deutromycotina). *Fusarium* species are economically essential as pathogens on most plants cultivated worldwide in agriculture, horticulture, and forestry. Some species are more located in tropical or subtropical environments, temperate or cool, while others are cosmopolitan (Al-Shugairan, 2008).

In the culture of solid media like potato dextrose agar PDA, the various extraordinary shapes of *F. oxysporum* may appear to shift. Commonly, the airborne mycelium first appears white and then may change to a variety of shades-ranging from violet to dim purple-agreeing to the strain or uncommon shape of *F. oxysporum*.In the case of copious sporodochia, the culture may display color cream or orange (Smith et al., 1988).

F. oxysporum is mainly distributed through short separations of water and contaminated farm equipment from the water system. Furthermore, the fungus can be distributed in contaminated transplants or soil over lengthy separations. Even though in some cases the organism can contaminate the natural product and its seeds, it is exceptionally uncommon to spread the organism by seed (Agrios, 1988).

All types of *Fusarium oxysporum* are saprophytic and can grow and survive in the soil and rhizosphere of many plant species for lengthy periods (Garrett, 1970). Furthermore, a few strains of *F. Oxysporum* are pathogenic to separate plant species, when it invades the vascular system it penetrates the roots that induce either root-rots or trichomycosis. Many other strains may penetrate roots, but they do not attack the vascular frame or cause disease (Olivain and Alabouvette, 1997).

1.1.2. Control of *Fusarium oxysporum*

Diseases caused by *Fusarium* are difficult to control (Borrero et al., 2006; Elmer, 2006). Several chemical fungicides are used to suppress the disease by causing resistance, but they have a harmful effect on human health and are environmentally dangerous. The use of chemicals as the only control technique can lead to non-target impacts on populations of soil microorganisms. Soil microbes like *Trichoderma harzianum, T. asperellum, T. koningii, Penicillium spp.* and *Streptomyces griseoviridis* are a better option for chemicals residing in the plant rhizosphere and being able to suppress pathogens and boost plant growth through phytohormone manufacturing and complicated substrates degradation (Osuide et al., 2002; Syed et al., 2010; Borrero et al., 2011).

Various fungi and bacteria, including current bio-control strains with recognized activity against soil-borne fungal pathogens, as well as isolates from the roots and rhizosphere of field-growing tomato crops have been evaluated for their effectiveness in managing tomato wilt fusarium. Tomato seedlings have been treated within the nursery with potential bio-control specialists and transplanted into pathogen-infested field soil. Crop rotation is also used but is usually useless because of the pathogen's efficient survival strategies. There is the potential to develop new races that can overcome the resistance of cultivars (Anonymous, 2006).

1.2. Biological Control

Baker and Cook, (1983) defined the word biological control as reducing the inoculum density or disease that produces the activity of a pathogen or parasite in which one or more organisms are active or dormant, achieved naturally or by manipulating the host or antagonist environment, or by mass introducing one or more antagonists. Agrios, (2000) was described as the complete or partial destruction by other organisms of the pathogen population.More closely, biological control relates to the purposeful use of introduced or resident living organisms to suppress the operations and populations of one or more plant pathogens, other than disease-resistant host crops.

In biological control techniques involving the use of biocontrol agents, the potential for inoculum and host colonization by the pathogen could be impacted by the number and activity of a biocontrol agent on and around the host plant (Vinale et al., 2008). The methodology used during the soil-borne root pathogens biocontrol differs significantly from disease to disease. The chosen antagonistic microorganisms can generally be implemented in the following types: powder, transmitted straight to the soil surface (Elad and Chet, 1987), suspension to dip the roots of seedling (Brammall, 1986), mixing directly with soil (Whipps, 2001; El-Tarabily, 2006), seed dressing or coating of seed (Yuan and Carwford, 1995), some food substrates such as wheat bran have been discovered to be effective in introducing antagonists to the soil as a media (EI-Tarabily, 2006).

Tomato is affected by several diseases, negatively reflecting the improvement of plants and crops. Especially out of these pathogenic parasites, the wilt caused by *Fusarium* species remains a challenging administrative assignment. *Trichoderma* species are well known for their biocontrol action against many plant pathogens, which in the present rural situation causes significant problems. *Trichoderma* species are known to produce corrupting chemicals from cell dividers that can be used for business preparations. Some *Trichoderma* species genes can be used to resist biotic and abiotic stresses such as salt, warm and dry season (Kuc, 2001). *Trichoderma* species are widely used as bio-control experts as they have more advantages in plant development such as enhancing plant development, expanding the extraction from the soil and reducing the activity of soil-borne pathogens that have long-term effects on plant development (Harman, 2004). Among the different species of the *Trichoderma, T. Harzanum* is considered the leading effective specialist in bio-control (Gao et al., 2002). Since Fusarium wilt is soil-born, it is not practical to use fungicides to control these infections. Besides, chemicals pose serious health hazards to both an applicator and the consumer of the material being treated. Biocontrol arrangements of parasites, microscopic organisms, and yeast have been linked to seeds, seedlings and planting media to decrease greenhouse and field disease with different degrees of success (Sabuquillo et al., 2006).

1.3. *Trichoderma* spp.

Trichoderma species are well known for their bio-control action against various plant pathogens that in the present agrarian situation cause serious problems. *Trichoderma* species are known to generate enzymes that degrade cell walls that can be used for commercial production. Some genes of the species of *Trichoderma* can be used to resist biotic and abiotic stresses such as salt, heat, and drought (Kuc, 2001).

Trichoderma was introduced in the early 1930s as having the capacity to biocontrol (Weindling, 1934). *Trichoderma* is an opportunistic, avirulent plant symbionts fungus that acts as an antagonistic and parasitic fungus against many pathogenic plant fungi and provides protection from plant diseases. *Trichoderma* spp. has been proved in countless research. They are efficient biocontrol agents for plant

disease management and are presently available as biopesticides or soil improvements or as plant growth enhancers for *Trichoderma* commercial products (Papavizas, 1985; Chet 1987; Harman, 2004; Vinale et al., 2008). The observation and basis of fungal biocontrol today are based on *Trichoderma* spp. This fungus has attracted a great deal of attention as a model of biocontrol (Chet, 1993). Experiments performed on several crops such as peanut, tomato, cucumber, and durian show that chosen *Trichoderma* strains may decrease important diseases induced by fungal pathogens including *Phytophthora palmivora*, *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii* and *Pythium* spp. *Trichoderma* species have a greater efficacy on soil-borne fungal disease than fungicides and retain longer. The value produced through the growth, exploitation, and use of *Trichoderma* products is not only plant disease control but has also provided possibilities for local individuals to decrease health hazards, expenses and environmental harm owing to over-use of fungicides. Also, crops treated with *Trichoderma* grew better and yielded greater than without application.

1.3.1. *Trichoderma* spp. biocontrol mechanisms

Trichoderma spp. it is an efficient biocontrol agent against fungal pathogens. They can behave indirectly by competing for nutrients and space by changing environmental factors, or by promoting plant growth and plant defense and antibiotic mechanisms, or directly by mechanisms such as mycoparasitic (Papavizas, 1985; Howell, 2003; Vinale et al., 2008; Aydın, 2015).

1.3.1.1. Biocontrol by the nutrient and living space competition

Trichoderma spp., are fast-growing fungi with persistent conidia and a wide range of use of substrates. They compete very well for nutrition and living space (Hjeljord et al., 2000). *Trichoderma* spp. moreover, it is naturally resistant to many toxic compounds, including herbicides, fungicides, and phenolic compounds. They can, therefore, develop quickly and affect pathogens by generating metabolic compounds that impede germination of spores (fungistasis), destroy cells (antibiosis), or alter the

rhizosphere (E.g. acidifying the soil to prevent the growth of pathogens) (Benitez et al., 2004).

1.3.1.2. Biocontrol by mycoparasitism

The direct interaction between pathogen and *Trichoderma* is called mycoparasitism. Mycoparasitism is a complex mechanism generally involving the production of a lytic enzyme from the cell wall (Chet et al., 1998), defined four sequential steps in the process of mycoparasitism chemotropism and coiling, cell wall penetration, and host cell digestion. *Trichoderma* strains detect other fungi, grow directly towards them, and produce enzymes that degrade hydrolytic cell wall sequentially. *Trichoderma* attaches to the host and coils around the host, creates appressories on the host surface, penetrates the host cell and collapses the host hyphae (Steyaert et al., 2003; Aydın and Turhan, 2009).

1.3.1.3. Production by *Trichoderma* spp. of antibiotics and secondary compounds

Trichoderma spp produces secondary compounds and antibiotics. Play an important position in the antagonistic activity of biocontrol (Vinale et al., 2008; Ajitha and Lakshmidevi, 2010). Antibiotics are often linked to the activity of biocontrol. The first secondary metabolite discovered in *Trichoderma* spp. was the peptide antibiotic paracelsin (Bruckner and Graf, 1983; Bruckner et al., 1984). Sivasithamparam and Ghisalberti (1991) proposed that *Trichoderma* spp. produced secondary metabolites could be grouped into three groups: (i) volatile compounds (e.g., 6-pentyl-alpha-pyrone), (ii) water-soluble compounds (e.g., heptelidic acid), and (iii) peptaibol compounds, These are linear oligopeptides made up of 12-22 amino acids that are rich in alpha-aminoisobutyrate, N-acetylated in the N-terminus, and have an amino alcohol group in the C-terminus.

1.3.1.4. Enhancement of plant growth by *Trichoderma* spp

Trichoderma spp. not only are pathogens controlled, they also boost plant growth and root development (biofertilizer) and enhance plant defense systems. It has been shown that some *Trichoderma* strains penetrate the epidermis and create stable and long-lasting root surface colonization (Harman, 2004). *Trichoderma* spp. the development of lettuce, tomato, and pepper crops has been shown to be improved (Vinale et al., 2006). *Trichoderma* spp. gluconic and citric acids were also produced, soil pH reduced and phosphate solubilization, micronutrients and mineral elements such as iron, magnesium, and manganese increased (Benitez et al., 2004; Harman et al., 2004; Vinale et al., 2008).

Trichoderma species in particular soil-borne fungal pathogens play a major role in controlling fungal plant pathogens. Using products based on *Trichoderma* is not only safe for farmers and consumers, it is also good for the environment. However, much more work must be done to develop formulations that are stable, cost-effective, easy to produce and easy to apply.

1.4. Yeasts

Saccharomyces cerevisiae is considered to promote yeast for various plants as new promising plant growth. It has become a positive alternative to chemical fertilizers used safely in humans, animals and the environment in the last century (Omran, 2000). Biological control of various plant diseases was mainly focused on bacteria or filamentous fungi (Whipps, 2001). Thus, the application of yeasts as agents of biocontrol acts as a new trend against various pathogens. The potential use of yeasts as biocontrol agents of soil-borne fungal pathogens and as promoters of plant growth has recently been explored (ElTarabily and Sivasithamparam, 2006). A wide range of yeasts has been extensively used for the biological control of fruit and vegetable diseases after harvest (Punja, 1997; Zheng et al., 2003), against moulds of stored grains (Petrsson et al., 1999) and to control powdery mildews (Urquhart and Punja, 1997).

Yeast describes a wide range of unicellular fungi (Moyad, 2008). *Saccharomyces cerevisiae* is one of the most widely used and studied yeast species and

is widely known as the yeast of the baker or brewer. According to Herskowitz (1988), to reproduce the *S. cerevisiae* are mostly asexual, with a budding period of approximately 1.5 hours. Notable features of *S. cerevisiae* include their capacity to grow in aerobic and anaerobic conditions and undergo metabolic processes (Ter Linde et al., 1999). Laboratory strains are also used as a model organism for all eukaryotic biology in different fields of study (Botstein and Fink, 2011), yeasts are best grown in a neutral or slightly acidic pH at optimal temperatures of 28-30 ° C under aerobic conditions with adequate nutrient supply (O'Kennedy et al., 2008).

Saccharomyces cerevisiae yeast specie has been used as a bio-control agent for soil-borne fungal plant pathogens *F. solani* and *Rhizoctonia solani* causing root-rot disease (Shalaby and El-Nady, 2008). The growth of the plant that promotes yeasts, *S. cerevisiae,* Candida sake and *Pichia membranifaciens* were used as bio-control agents for *Fusarium* wilt tomatoes under greenhouse conditions (Kamal et al., 2009).

The total number of soil yeasts is generally relatively low compared to the number of bacteria and filamentous fungi (Phaff et al., 1978). Yeasts are common in soils with a widely different texture, chemical composition, and moisture and pH values at different geographical locations and under different climatic conditions (Do Carmo Sousa, 1969; Alexander, 1977). Yeasts are especially numerous on the roots of certain plants such as cabbage, maize, sugar beet and oat (Bab'eva and Belyanin, 1966; Alexander, 1977; Phaff et al., 1978). Yeast populations are also affected by the depth in the soil and are most numerous in the upper layers, from about 2 to 10 cm in depth (Phaff et al., 1978). In summer, yeasts tend to be higher. The presence or absence of capsules on yeast species inhabiting soils, particularly arid and semiarid species, can affect the ability of yeast cells to survive under low humidity conditions (Spencer and Spencer, 1997). The number of yeasts in the soil is highly dependent on the number of nutrients available and is increased by adding metabolizable substances. Most of the yeasts found in the soil were not fermenting species (aerobic) (Phaff and Starmer, 1987).

The antagonism mechanisms of yeasts involved in the biological control of fungal plant pathogens were widely investigated in relation to leaves-related pathogens (Fokkema et al., 1979; Sundheim, 1986; Buck, 2002; Urquhart and Punja, 2002), and in relation to fruits (Wilson and Wisniewski, 1989; Droby and Chalutz, 1994; Filonow et

al., 1996; El Ghaouth et al., 2002). Understanding the modes of action of the antagonists among yeasts will assist not only to improve their efficiency as a result of enhancing their efficiency as biocontrol agents, but also to develop rapid screening requirements for superior biocontrol agents.

An increasing amount of research shows that yeasts in the rhizosphere can increase the development of plant roots directly or indirectly (Nassar et al., 2005; El-Tarabily and Sivasithamparam, 2006; Cloete et al., 2009).

1.5. Research Purpose

This thesis is aimed at developing a methodology for the biological control of tomato wilt disease induced by *F. oxysporum* f.sp. *lycopersici*. Laboratory experiments on the inhibitory effects of *Trichoderma* species on the growth of tomato Fusarium direct causal agent wilt (*Fusarium oxysporum* f.sp. *lycopersici*). Evaluation and comparison of *Trichoderma* and *Saccharomyces cerevisiae* applications techniques (soil and seed treatments) to determine the bio-agents effectiveness in decreasing the disease. Estimate the effectiveness of *Trichoderma* species native isolates in promoting tomato development parameters and managing *Fusarium* wilt infection under in vitro and in vivo conditions. Evaluate yeast to control tomato *Fusarium* wilt diseases.

2. LITERATURE REVIEW

Chaur- Tsuen Lo (1998), was studied the general mechanisms of action of microbial biocontrol agents. Understanding the mechanisms of biological control of plant diseases through the interactions between antagonists and pathogens may allow us to select and construct the more effective biocontrol agents and to manipulate the soil environment to create a conducive condition for successful biocontrol. The mechanisms of biocontrol may involve and be divided into (i) antibiosis, (ii) competition, (iii) mycoparasitism, (iv) cell wall degrading enzymes, and (v) induced resistance. However, these mechanisms of biological control are probably never mutually exclusive. They may include one and more processes.

In the broader sense, (Gardener and Fravel 2002), used the term biological control as they explained the current status of the study, business development, and implementation of plant pathogens biocontrol techniques. They conclude by defining prospects for the future for using biological control to reduce plant pathogen damage in both conventional and organic agriculture.

Sharma et al. (2011), studied the various types of, fungal genus *Trichoderma* plays a major role in controlling the plant diseases. The species of *Trichoderma* are known to produce different kinds of enzymes that have a significant role in biocontrol activity like cell wall degradation, biotic and abiotic stress tolerance, hyphal growth, antagonistic activity against plant pathogens.

Sundaramoorthy et al. (2013), was evaluated the efficacy of the native isolates of *Trichoderma* The species to promote the growth and yield parameters of tomato and to manage *Fusarium* wilt disease under in vitro and in vivo conditions. Also, tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62 cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolates and untreated control.

Efficacy of bio-control agents and organic amendments was evaluated by Theradimani et al. (2018), for their potential to manage the *Fusarium* wilt of tomato (*Lycopersicon escluentum* L.) caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Yeast, *Trichoderma viride, T. harzianum* and *Pseudomonas* spp. were collected from

tomato growing areas of Tamil Nadu, India, and tested for antagonistic activity against the pathogen using a dual culture technique in Petri dishes. The field experiment confirmed that Yeast 1 SA 2.5 kg ha-1 provided the best disease reduction over control and increased fruit yield.

This study investigated the ability of ten *Trichoderma* isolates to control the *fusarium* wilt pathogen of tomato, *Fusarium oxysporum* f. sp. *lycopersici*, as well as the effect of these isolates on tomato plant growth in the presence and absence of the pathogen. In the absence of the pathogen, none of the *Trichoderma* isolates consistently increased all plant growth parameters. The biocontrol mechanism of these *Trichoderma* isolates requires further investigation (Ghazalibiglar et al., 2016).

Demirer Durak (2018), studied the biological control of *Rhizoctonia* spp. from pepper. In vitro tests the most effective isolates were determined as *T. harzianum* (64,5%), *T. viride* (58,1%), *T. virens* (57,4%). According to in vivo test, while *T. harzianum* was found to be the most effective species, *T. viride and T. virens* were moderately, and *G. roseum* was the least effective genus.

Hesamadin (2010) studied the antagonistic effects of *Trichoderma* spp. against *F. oxysporum* f.sp. *lycopersici* under pot condition and found that when antagonist applied as a seed coating, could not reduce the disease significantly as wilt reduction was only 23.7 %.

Devi et al. (2013), evaluated the *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici* for biological control of tomato wilt and found that seed coating with different species of *Trichoderma* can significantly reduce the wilt incidence (34.47-56.36 %) and also increase germination percent (65.56-77.78 %). Soil treatment and seed + soil treatment proved better than seed treatment alone.

Sivan et al. (1987), found that when *T. harzianum* was applied as seed coating in tomato seeds the crown rot incidence of greenhouse-grown tomatoes reduced up to 80 % by 75 days after sowing. Furthermore, *S. cerevisiae* utilized as a biocontrol operator against soil-borne contagious plant pathogens causing root-spoil infection by *Fusarim solani* and *Rhizoctonia solani* of sugar beet and also plant development advertisers were ongoingly examined by (El-Tarabily, 2004; El-Tarabily and Sivasithamparam, 2006; Shalaby and El-Nady 2008).

Pandey and Upadhyay (1999), studied the chemical, biological and integrated approach for the management of *Fusarium* wilt of pigeon pea and found that minimum wilt incidence (10 %) and maximum disease control (81 %) has occurred when *T. viride* applied as seed coating 0.01 % biomass powder (w/w). This treatment was followed by seed coating with *T. harzianum* (c- isolate) with 13 % wilt incidence and 74 % disease control.

Demirer Durak (2016), studied with *T. harzianum, T. virens, T. asperellum,* and *T. viride* was isolated from the soil samples. At the in vitro tests, generally, it was determined that *Trichoderma* isolates have inhibited to *R. solani* and in vivo, they were reduced the effects of the disease and they were raised the development of the plant. In particular, it was determined that some isolates of the *T. harzianum* and *T. virens* have reduced the severity of the disease.

Singh et al. (2004), evaluated four antagonists viz., *T. viride, T. harzianum,* and *T. virens* and *Aspergillus nidulans* as seed, soil and seed+soil treatment for the control of tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici* under pot culture and reported that seed coating of tomato seeds with *T. viride, T. harzianum* and *T. virens* showed reduction in seedling mortality up to 85 % as compared to *A. nidulans* among different combinations.

Pandey and Pandey (2005), observed that coating the tomato seeds with bioagent *T. viride* was most effective against *F. solani* and *Sclerotium rolfsii* with 56.7 and 80.8 % seed germination, respectively. Whereas, *T. virens* was the best antagonist against *Rhizoctonia solani* (71.7 %) and Macrophomina phaseolina (75.8 %) under pot condition.

Verma and Dohroo (2005), tested six bioagents viz., *T. viride, T. harzianum, Pacellomyces lilacinus, G. virens, Laetisaria arvalis* and *P. fluorescens* against *F. oxysporum* f. sp. *pisi* under pot conditions on the pea. Among them, seed coating with *T. harzianum* and *T. viride* were found most effective with 93.83 % disease control.

Mehra (2006) tested five antagonist fungi viz., *T. viride, T. harzianum, Chaetomium globosum, Aspergillus* terreus and *Penicillium cyclopium* against *F. oxysporum* f. sp. *lycopersici* under pot conditions and reported that tomato seed coated with *T. viride, T. harzianum* and *C. globosum* showed higher seed germination 74, 72 and 68 % and disease control 71.5, 69.0 and 63 % over control.

Gangopadhyay et al. (2009), studied the effect of fungicides and antagonists (*T. harzianum* and *T. viride*) on *Fusarium* wilt of cumin and found that seed treatment with talc-based formulation of bioagents could control 41 to 60 % wilt incidence.

Demirer Durak (2011), studied with 52 *Trichoderma* isolates were obtained from strawberry plants. *Trichoderma harzianum* (28 isolates), *T. virens* (15 isolates), *T. asperellum* (5 isolates) and *T. rossicum* (4 isolates) were identifed from the groups. In the results of in vivo test, while *T. harzianum* was found to be the most effective species, *T. virens* and *T. asperellum* were moderately, and *T. rossicum* was the least effective species against *Rhizoctonia* spp.

Pan et al. (2009), reported that the application of *Trichoderma* as seedling dip + soil application 75g/kg reduces the mortality of cabbage to root rot up to 25.57 % as compared to 76.44 % mortality in control.

Kumar et al. (2010), studied the management of damping-off and powdery mildew disease of sweet pepper and growth promotion by *P. fluorescens* and *T. harzianum* and found that combined application of *T. harzianum* as seed biopriming + root dipping showed growth-inducing effect up to crop maturity in sweet pepper .

Akköprü and Demir (2005), were investigated the effects of the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* Schenck & Smith and four rhizobacteria (RB; 58/1 and D/2: *Pseudomonas fluorescens* biovar II; 17: *P. putida*; 21: *Enterobacter cloacae*), which are the important members of the rhizosphere microflora and biological control agents against plant diseases, were examined in the pathosystem of *F. oxysporum* f. sp. *lycopersici* [(Sacc) Syd. et Hans] (FOL) and tomato with respect to morphological parameters (fresh and dry root weight) and phosphorous (P) concentration in the roots.

Jamwal et al. (2011), studied the effect of biocontrol agents on wilt management and plant growth of tomato by using fungal and bacterial biocontrol agent *T. viride*, *T. harzianum* and *P. fluorescens* and found that dipping the tomato seedlings in bioagent suspenssion of *T. harzianum* recorded least wilt incidence (5.6 and 6.0 %) followed by *T. viride* (11.3 and 10.9 %) during two years.

Hadar et al. (1979), investigated that *T. harzianum* in the form of wheat bran culture as soil treatment effectively controlled damping-off of bean, tomato and eggplant seedlings caused by *R. solani*.

Dutta and Das (2002), studied the management of collar rot of tomato by *Trichoderma* spp. and chemicals and observed that soil application of FYM culture of *T. harzianum* was most effective in inhibiting the mycelial growth (61.5 %) as well as sclerotial production (90 %) of *S. rolfsii*.

Prasad et al. (2002), studied the effect of soil application of *T. harzianum* on pigeonpea wilt caused by *Fusarium oxysporum* f. *udum* under field conditions and found that soil application of *T. harzianum* at the rate 10 and 20 g/kg of FYM resulted in disease incidence of 32.7 and 15.2 5 %, respectively.

Mishra et al. (2004), reported that an isolate of *T. virens* was antagonistic to *F. oxysporum* f. sp. *gladioli*, a pathogen of gladiolus corm rot and wilt. The proliferation and population density of the pathogen in the soil was significantly reduced after the incorporation of *T. virens* along with FYM.

Rini and Sulochana (2006), studied the management of seedling rot of chilli using *Trichoderma* spp. and *P. fluorescens* and revealed that soil application of FYM based formulation of *T. harzianum* (TR20), and *T. pseudokoningii* (TR17) could significantly reduce disease incidence 20 and 17.5 %, respectively.

Singh (2007) reported that soil application of talc based formulation of *T. viride* and *T. harzianum* along with FYM significantly reduced severity of *Fusarium* wilt disease of chilli up to 60 to 70 % under field condition.

Singh et al. (2007), evaluated different strains of *Trichoderma* for the control of *Fusarium* wilt of tomato under greenhouse and field conditions and proved that soil application of WBB formulation of *T. viride* strain TvSV-II and TvSV-21 were most effective with 22 and 24 % wilt incidence, respectively.

Demirci et al. (2011), studied with Forty-five fungal isolates were obtained from sclerotia of *Rhizoctonia solani* on potato tubers. Some fungal isolates affected *R. solani* by antibiosis and/or parasitism. *Trichoderma harzianum* isolates were able to overgrow the mycelium of *R. solani*. Physical colony contact was observed between the remaining 21 fungal isolates and *R. solani.*

Kapoor (2008) tested efficacy of *Trichoderma* sp. against different soil borne pathogens and found that wheat bran alone or in combination with FYM in the ratio of 1:1 and 1:2 respectively supported maximum multiplication of *T. harzianum* and soil application of WBB formulation was found best delivery system in controlling the

tomato root rot with 89.12 percent control followed by talc based formulation with 72.32 % control.

Sharma et al. (2003), found that the combined effect of *Trichoderma* spp. + thiram was most effective in reducing the linseed wilt caused by *F. oxysporum* f. sp. *lini*. Jambhulkar et al. (2011), studied the comparative efficacy of fungicides and antagonists against *Fusarium* wilt of chickpea and reported that seed treatment with combination of *T. vride* and carbendazim could significantly reduce wilt incidence 54.20 %.

Bhatnagar et al. (2013), studied the management of cumin wilt caused by *F. oxysporum* f. sp. *cumini* through chemical and biological agents and found that combined seed treatment of cumin seeds with carbendazim and *T. vride* resulted in only 10.6 % disease incidence.

Barari (2016) evaluated biocontrol of tomato fusarium wilt by *Trichoderma* species under in vitro and in vivo conditions under in vitro conditions, the results revealed that *Trichoderma harzianum*, isolate N-8, was found to inhibit effectively the radial mycelial growth of the pathogen (by 68.22 %). Under greenhouse conditions, the application of *T. harzianum* (N-8) exhibited the least disease incidence (by 14.75 %). Also, tomato plants treated with *T. harzianum* (N-8) isolate showed a significant stimulatory effect on plant height (by 70.13 cm) and the dry weight (by 265.42 g) of tomato plants, in comparison to untreated control (54.6 cm and 195.5 g). Therefore, the antagonist *T. harzianum* (N-8) is chosen to be the most promising bio-control agent for *F. oxysporum* f. sp. lycopersici.

Ghazalibiglar et al. (2016), investigated the ability of ten *Trichoderma* isolates to control the Fusarium wilt pathogen of tomato, *Fusarium oxysporum* f. sp. *lycopersici*, as well as the effect of these isolates on tomato plant growth in the presence and absence of the pathogen. The isolates were obtained from the Lincoln Bio-Protection Research Centre Culture Collection and were inoculated into seed raising mix (0.5 % w/w) in two glasshouse studies. Two *Trichoderma* isolates significantly ($P<0.05$) reduced tomato Fusarium wilt incidence, as shown by 69 % fewer plants with vascular discoloration. In the presence of the pathogen, one isolate significantly increased tomato plant growth by 50 % or more. In the absence of the pathogen, none of the *Trichoderma*

isolates consistently increased all plant growth parameters. The biocontrol mechanism of these *Trichoderma* isolates requires further investigation.

Akrami and Yousefi (2015), were investigated biological control of *Fusarium* wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as antagonist fungi under greenhouse conditions. Tomato cultivar inoculated with *F. oxysporum* f. sp. *ciceri* and *R. solani*, showed greater wilt incidence, chlorosis of leaves and induced vascular discoloration in roots. Soil administration with biocontrol agents adjusted the severity of wilt in roots, substantially ($P<0.01$). The disease control was highest with a combination of *T. harzianum*, *T. asperellum*, and *T. virens* (80-87 %) followed by binary combination of *Trichoderma*. spp. (79-82 %), while the lowest control was done with *T. viride* (65 %). It is concluded that *T. harzianum*, *T. asperellum* and *T. viride* could control pathogen attacks in tomato and it can be considered as an applicable strategy in control measures against pathogens.

Patel and Saraf (2017), studied the biocontrol efficacy of *Trichoderma asperellum* MSST against tomato wilting by *Fusarium oxysporum* f. sp. *lycopersici* this study was carried out to evaluate the efficacy of *Trichoderma asperellum* MSST to promote the growth and yield parameters of tomato S-22, a susceptible variety. This study was also undertaken to manage Fusarium wilt disease under in vitro and in vivo conditions. A significant increase in vegetative parameters like root length, shoot length, plant weight and chlorophyll content 60 days after sowing (DAS) were observed. There was a reduction in the incidence of Fusarium wilt in tomato up to 85 %. An increase in the level of total phenol, peroxidase, polyphenol oxidase, and phenylalanine ammonium lyase activity on the 10th day of pathogen inoculation showed enhancement of plant defense mechanism by *T. asperellum* MSST against FOL. The Overall study revealed that isolate MSST was proven to be a potential biocontrol agent showing induced resistance against FOL.

Wolebo et al. (2015), were studied the evaluation of antagonistic activities of *Trıchoderma* isolates against Fusarium wilt (*Fusarium oxysporum*) of tomato (*lycopersıcon esculentum* mill.) isolates. The pathogenicity of *F. oxysporum* was determined on three different tomato varieties namely Cochoro, Miya and Fetane that were grown in 20 cm plastic pots containing 3 kg of autoclaved soil under the greenhouse. The antagonistic effect of *Trichoderma* isolates against the test pathogen

was tested both in vitro and in vivo conditions. From the three tomato varieties, Miya was more susceptible to *F. oxysporum* infection than both Cochoro and Fetane varieties. The antagonistic effects of *Trichoderma* isolates on the mycelial growth of the test pathogens, AUT9, AUT 8 and AUT10, showed 66 %, 61 %, and 58 % inhibition, respectively, on the mycelial growth of *F. oxysporum* isolate. All *Trichoderma* isolates achieved maximum mycelial growth at 25°C and minimum mycelial growth at 15°C. From the current comparative in vivo and in vitro (greenhouse) studies it is evident that the most effective antagonist of the *Trichoderma* isolates to *F. oxysporum* was AUT9 and the most resistant tomato variety was Fetane.

Prabha et al. (2017), were investigated the biocontrol effect of *Trichoderma viride* against *F. oxysporum* on tomato vegetable crops under nursery study. In this study, many types of fungal colonies were identified among the isolate the dominant fungal pathogen and antagonistic fungi were selected. The biocontrol agent resulted in an increase in soil fertility, control of minor pathogens and an increase in the general health of plants, ultimately leading to an increase in plant metabolism. If enhanced the growth producing stimulating factors.

Estifanos Tsegaye Redda et al. (2018), evaluated the antagonistic potential of different ısolates of *Trichoderma* against *Fusarium oxysporum, Rhizoctonia solani*, and *Botrytis cinerea.* The result indicated that the antagonistic potential of 380 isolates of *Trichoderma* strains against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* were varied which inhibited *Fusarium oxysporum* ranges 10.12-70.70 %, *Botrytis cinerea* (44.18-82.98 %) and *Rhizoctonia solani* (35.07-88.07 %). These potential isolates of *Trichoderma* may be further exploited as a biocontrol agent against *F. oxysporum*, *R. solani* and *B. cinerea* as well as other soilborne phytopathogenic fungi.

Zaidi Sara et al. (2014), studied the effect of in vitro and in vivo *Trichoderma* sp. (TR2) on the reduction of infection of the tomato variety (Elgon) contaminated with *Fusarium oxysporum* f. sp. *lycopersici*. It could inhibit 95.09 % from the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with test reference during seven days of the incubation at 25 ° C. Interesting results were also obtained in vivo: spraying tomato plants with a spore suspension of *Fusarium oxysporum* f.p. *lycopersici* and *Trichoderma* sp. reduced the incidence of root and neck fusariosis

compared to the untreated plants and inoculated ones by the pathogen. In addition, plants treated with *Trichoderma* sp had a greater vegetative development.

Babychan and Simon (2017), studied the efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici*. (FOL) infecting pre-and post-seedling of tomato. Under in vitro condition, the results revealed that *Trichoderma* isolate MiT-4 was found to be effectively inhibiting the radial mycelial growth of the pathogen by 58.4 %. Under the greenhouse condition application of *Trichoderma* isolates (MiT-1 and MiT-4) exhibits no wilt incidence in tomato seedlings. The germination of tomato seeds is greatly influenced by *Trichoderma* isolate (MiT-3) over *Fusarium oxysporium* f. sp *lycopersici* by 83.66 %. Application of *Trichoderma* isolates (MiT-3) on tomato seeds, seedlings showed a significant stimulatory effect on a shoot and root height by 7.53 cm and 7.1 cm respectively which is higher than the control.

Andera S. Mohammed et al. (2008), investigated Laboratory and pot experiments were conducted to evaluate the efficacy of *Trichoderma viride*, VA mycorrhiza and, dry yeast, separately and in combination, as an integrated strategy of *Rhizoctonia* disease management in potato crop. The challenge inoculation with *T. viride* caused a significant reduction in vitro in the linear growth of *Rhizoctonia solani*, particularly when it was performed closer to the time of pathogen inoculation. Except for the number of stems, yield and growth attributes of potato plants infected with *R. solani* were significantly affected. *T. viride* application significantly increased the growth components (i.e., plant height, shoot fresh and dry weights, root fresh and dry weights) and tuber yield (i.e., number and weight of tubers) compared to potato plants inoculated with *R. solani* alone. The dry yeast appeared to be the least efficacious biocontrol agent to *Rhizoctonia* compared to the other two organisms yet it also significantly improved the disease situation of the infected plants. The combined effect of *T. viride* and VA mycorrhiza with or without yeast excelled other treatments in the alleviation of almost all tested facets of the disease development.

Fravel et al. (2003), studied among rhizosphere microflora, *Fusarium oxysporum* is well known. Although all types are saprophytic, some are known to cause wilt or root rots on crops, while others are considered nonpathogenic. Several techniques have been created to characterize *Fusarium oxysporum* strains depending on phenotypic and genetic traits. The study showed the wide diversity that affects

Fusarium oxysporum's soil populations. Interactions between pathogenic and nonpathogenic strains result in disease control in suppressive soils. Non-pathogenic strains are therefore developed as agents for biocontrol.

Okungbowa and Shittu (2012), evaluated that the *Fusarium* species are vascular wilt soil-borne pathogens, one of the most significant phytopathogenic and toxigenic fungi. They are filamentous, belonging to the Ascomycetes class and Hypocreaceae family. Typically, Fusarium species produce macroconidia and microconidia, as well as mycelia and chlamydospores that act as propagules for host plants to be infected. It is possible to divide the life cycle into dormant, parasitic and saprophytic phases. Most species are harmless saprobes some species are parasitic, some of them on plants produce mycotoxins. The disease is generally hard to manage as physical, chemical and cultural control techniques are not only unsuccessful but also costly. Breeding for resistant cultivars is the best control method. Also fungi of the rhizosphere like *Trichoderma harzianum, T. Asperellum, T. Koningii*, and *Penicillium* spp. and the disease has been controlled by *Streptomyces griseoviridis*.

Sreenu et al. (2017), was studied *Fusarium oxysporum* f. sp. *lycopersici* is the causal organism that causes wilt disease in tomato all over the world. The least growth of pathogen was recorded in Carbendazim (treated control) (94.00) followed by Neem leaf (35.20) followed by Lantana camara (31.11), ginger bulb (26.31) and there was no growth in carbendazim treatment in poison food technique. All treatments were significantly decreased disease incidence.

Basco et al. (2017), studied the Biological management of *Fusarium* wilt of tomato using vermicompost biofortified with selected biological control agents (BCAs) i.e. *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* was the hypothesis of this study. In vitro test showed that all the selected microbes were antagonistic to *F. oxysporum* f. sp. *lycopersici*. The levels of different antioxidants, different plant growth parameters, and incidence of disease were recorded at different time intervals in designed treatments. According to the experimental results, significant variations in a reduction of disease incidence, enhancement in plant growth, yield and as well as higher stimulation of antioxidants were observed in tomato plants treated with biofortified vermicompost as compared to the control. Maximum values were recorded in plants treated with *T. harzianum* fortified vermicompost.

Hooykaas et al. (2006), studied the yeast (*Saccharomyces cerevisiae*). It is one of the best characterized eukaryotic organisms. This species has enabled a detailed study of the (genetic) requirements for Agrobacterium-mediated DNA transformation. For instance research with this yeast has led to the recognition that the transforming DNA molecules integrate into the eukaryotic chromosomes either by homologous recombination, which is the preferred pathway in *S.cerevisiae*, or by nonhomologous end-joining. Based on the protocol for Agrobacterium-mediated transformation of *S. cerevisiae* methodology has been developed for the transformation of many other yeast and fungal species.

Sarhan et al. (2011), evaluated the Effect of bread yeast application and seaweed extract on cucumber (*cucumis sativus* L.) plant growth, yield and fruit quality. The results showed that spraying bread yeast or seaweed extract resulted in a positive significant difference in shoot characteristics and in all yield traits as compared to untreated treatment. The interaction between yeast and seaweed extract was significantly enhanced by all detected traits. Since cucumber plant received 6 g.l-1 bread yeast and sprayed with a mixture of 0.33 ml.l-1 Alga 600 +2.5 ml.l-1 Sea force 2 were characterized by the highest values of all shoot and yield characteristics.

Applicability of *Saccharomyces cerevisiae* as a biocontrol agent of *Fusarium oxysporum* and as plant growth promoter was investigated by (Shalaby, 2008). Application of 5 g L-1 of yeast resulted in a reduction of the pre- and post-emergence damping-off 6.67 and 11.67 %, respectively. Survival of treated plants increased to 83.33 % in comparison with 30.00 % for the pots inoculated with the pathogen containing untreated seeds. Linear growth of *F. oxysporum* was inhibited with 39.52 % and 50 % by using 5 g L-1 and 6.35 g L-1 of the yeast, respectively.

3. MATERIALS and METHODS

3.1. Materials

3.1.1. Test plant

The study was conducted using the tomato seeds (*Lycopersicon esculentum*) Alsancak F1 hybrid which is the most widely grown tomato cultivar in the region.

3.1.2. Soil samples

Peat and perlite (1:1 w/w) were used.

3.1.3. Test pathogens

The *Fusarium oxysporum* f. sp. *lycopersici* and *Trichoderma* spp. (*Trichoderma harzianum*, *Trichoderma virens*, *Trichoderma asperellum*), fungi were originally isolated from tomato in Van, Turkey supplied by Yuzuncu Yil University in 2014. Yeast was purchased commercially (*Saccharomyces cerevisiae*).

3.1.4. Media and water

Media and water sterilized for 15 minutes in autoclave at 121 ° C.

3.1.4.1. The culture media were used in the present study

In the studies conducted within the scope of this thesis, PDA, water Agar and Yeast mannitol broth, Yeast extract media were used.

Table 3.1 Chemicals and amounts required for PDA medium used

1000 ml of PDA broth (Dhingra and Sinclair 1985)	
Chemical	Amount
Potatoes	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml
121 ° C for 15 min. autoclaved.	

Table 3.2 Chemicals and quantities for water agar medium used

1000 ml water agar media (Barry et al. 1970)	
Chemicals	Amount
Agar	15 g
121 ° C for 15 min. autoclaved.	

3.1.5. Working environments

The studies carried out within the scope of the master thesis were conducted in vitro and in vivo. The studies conducted in vitro were carried out at Van Yüzüncü Yıl University, Faculty of Agriculture, Plant Protection Department, Mycology Laboratories. The studies in the in vivo environment were also carried out in Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Plant Protection, Phytopathology climate room, The climate chambers were carried out at a temperature of 25 to 27° C and 12 hours of light and 12 hours of darkness. Tomato plant cultivation and pathogenicity test were carried out in such environments.

3.2. METHODS

3.2.1. In vitro

T. harzianum, T. virens and T. Asperellum were evaluated as antagonists in vitro against *F. oxysporum*. All pairings were replicated three times**.** Mycelial disks (5 mm diameter) were cut from 7 day old cultures of *F. oxysporum* and *Trichoderma* species disk were placed on the opposite side of a plate 48 hours after inoculation with *F.*

oxysporum, 15 mm from the Petri dish edge. PDA plates inoculated with *Fusarium* and *Trichoderma* only served as controls. The inoculated plates incubated in the dark at 25 °C for 5 days and then were observed for overgrowth or other interactions.

Paired cultures have been incubated on incubator at 24° C for 5 days, then they were scored for degree of antagonism on a scale of classes 1-5: (Bell et al., 1982).

1= *Trichoderma* completely overgrew the pathogen and covered the entire medium surface.

2= *Trichoderma* overgrew at least two-thirds of the medium surface.

3= *Trichoderma* and the pathogen each colonized approximately one-half of the medium surface (more than one-third and less than two thirds) and neither organism appeared to dominate the other.

4= the pathogen colonized at least two-thirds of the medium surface and appeared to withstand encroachment by Trichoderma.

5= the pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface.

Paired cultures were observed for a total of 9 days before being discarded. the researcher considered an isolate of *Trichoderma* to be antagonistic to the pathogen if the mean score for a given comparison (when rounded to the nearest whole class number) was ≤ 2, but not highly antagonistic if the number was ≥ 3. Selected organism was recorded. Inhibition of the pathogens development in dual culture was assessed by two parameters: the percentage inhibition of radial growth [100×(r1-r2) /r1] and the width of the zone of inhibition (Z1) measured at the smallest distance between both colonies (Figure 3.1). Zones of inhibition and inhibition of radial growth of the test organism by the potential antagonist haven been analyzed using a randomized complete block (split-plot) design with four replications.

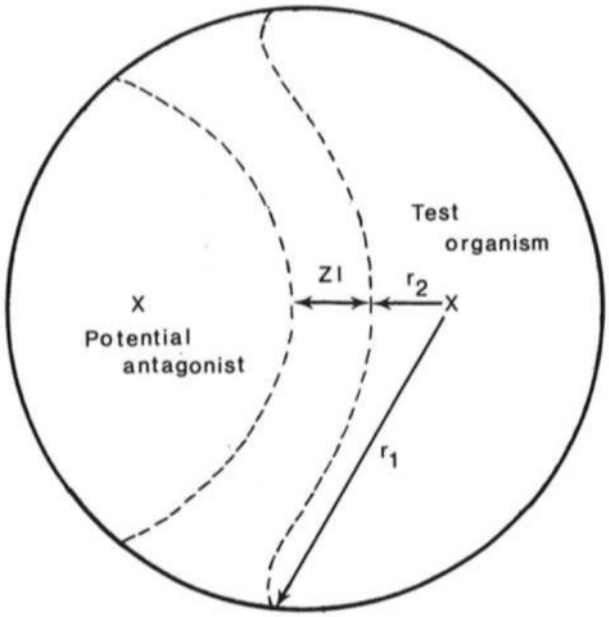

Figure 3.1. Diagram of the mode of inoculation of agar plates with the test organism and potential antagonist. Parameters for inhibition are the width of the zone of inhibition (Z1) and the percentage inhibition of radial growth [100×(r1-r2)/ r1]. (Royse and Ries, 1977).

3.2.2. In vivo evaluations

Completely randomize designs of seed coating and soil treatment methods have been accomplished in greenhouse and biocontrol effects of each *Trichoderma and* yeast isolates mixture of *Fusarium* isolates:

1- *F. oxysporum* (F) alone.

2- *Trichoderma harzianum* (T1) alone.

3- *Trichoderma virens* (T2) alone.

4- *Trichoderma asperellum (*T3*)* alone.

5- *Saccharomyces cerevisiae* (S) alone.

6- *T. harzianum + T. virens and T. asperellum.*

7- *T. harzianum + T. virens + T. asperellum and S. cerevisiae.*

8- *F. oxysporum + T. harzianum* (T1).

9- *F. oxysporum + T. virens* (T2).

10- *F. oxysporum* + *T. asperellum* (T3).

11- *F. oxysporum* + *S. cerevisiae* (S).

12- *F. oxysporum* + *S. cerevisiae* (S) + *T. harzianum* (T1).

13- *F. oxysporum* + *S. cerevisiae* (S) + *T. virens* (T2).

14- *F. oxysporum* + *S. cerevisiae* (S) + *T. asperellum* (T3).

15- *F. oxysporum* + *T. harzianum* + *T. virens* and *T. asperellum.*

16- *F. oxysporum* + *T. harzianum* + *T. virens* + *T. asperellum* and *S. cerevisiae.*

17- Control.

3.2.2.1. Seed coating method

Tomato seeds surfaced sterilized with 70% ethyl alcohol for 2 minutes followed by several washings with sterilized water. *Trichoderma* isolates have been grown on PDA at 24° C for 7 days. Spore concentration was determined by microscopic counting in a hemocytometer. *Trichoderma* species were prepared at rate of 10^7 conidium/ml with hemocytometer. Arabic gum has been added to suspensions at a rate of 2%. Tomato seeds were soaked with suspension for 10 minutes. Live commercial baker's yeast (*Saccharomyces cerevisiae*) and dead culture (autoclaved) have been used. The yeast grew in culture medium yeast extract and yeast mannitol broth for 5–7 days. The yeast concentration was prepared at a rate of 10^6 cells/ml with hemocytometer. Arabic gum was added to suspensions at a rate of 2%. Tomato seeds were soaked with suspension for 10 minutes. *Trichoderma* and yeast coating seeds were transferred to sterile paper. Seeds have been incubated one night at 25° C in an incubator.

Tomato seeds were sown in viols containing peat and perlite (1:1 w/w). After two or three weeks of germination, seedlings were transferred to pots. During the transfer, the pathogen has been inoculated to the seedlings. For this *Fusarium* isolate has been grown on PDA at 24° C for 10 days. The spores were prepared by adding 10 ml of distilled water to the growing medium in the petri dish and then gently releasing them with a sterile glass rod. The resulting suspension was transferred to a beaker, then the concentration of the spores was determined under a microscope. The concentration

of spores in the suspension was adjusted to approximately 10^7 conidia/ ml by adding distilled water. Seedlings were inoculated with the root dipping method. The plants have been removed from the pots and shaken gently, and the remaining soil particles on the roots were washed away under the tap water and put in a spore solution and then put aside for 10 minutes. The roots of the control plants were treated with distilled water for the same period.

Three replications were kept for each treatment. The plants were grown in a greenhouse, at a temperature range of 25 to 27°C, for up to 50-60 days. They received 10-12 hrs light and watered uniformly with tap water as required.

3.2.2.2. Soil treatment methods

Firstly peat and perlite (1:1 w/w) were contaminated with bio-control isolates for this method. *Trichoderma* isolates were grown on PDA at 24° C for 7 days. spore counting with hemocytometer of fungi, 20 ml of a *Trichoderma* suspension with 10^7 spores/ml was poured into each pot. From the dry yeast, a 100 ml suspension at a concentration of 2.5 gm/L was prepared and added to each pot. Sterile water was applied to the control pots (Arafat *et al*., 2012). pots were kept in a greenhouse at 25 - 27°C for a few days. Pots were watered every day.

Tomato seeds were sterilized, sown to viols and transferred to pots when they grew up. during the transfer process, the plants were inoculated with *Fusarium* isolate as described above. Treatments were replicated three times. Six-eight weeks after transplanting, all plant roots were cleaned and washed under running tap water. Disease incidence and severity, and seedling survival were recorded. The disease rating scale was as follows: 0 ¼ healthy roots; 1 ¼ slight brownings (usually at the tip of the root); 2 ¼ moderate brownings; and 3 ¼ severe browning. The experiment was repeated once. The experimental design was a complete randomized block design with three replicates. The parameters were measured plant height, fresh weights of shoot and root, which were determined immediately after harvest, whereas the shoot and root dry weights were determined after drying plant samples in an oven at 65°C for 48 hours.

3.2.3. Statistical analysis

The results of various parameters obtained from the experiments have been analyzed by statistical analysis using Duncan's Multiple Range Test. (Gomez, et al., 1984).

4. RESULTS

4.1. In Vitro Treatments

Cultural and characteristic of *Trichoderma* and *Fusarium oxysporum* isolates : *Fusarium* sp. was incubated at an incubation temperature of 25 ° C on PDA media. The colony appeared woolly to cotton, flat and spread. The color and pigmentation of the isolate on the PDA medium were creamy white to light pink Figure 4.1. *Fusarium* sp. is a slow-growing species that also agrees with this research in Figure 4.2. The *Fusarium* sp. in general characterized by creamy white to creamy, light pink to violet and light purple. Moreover, macro and micro-conidia characteristics that were thinly walled 3-5 septate, fusoid falcate macro-conidia with somewhat hooked apex and pedicellate base confirmed the isolate identification (Joshi et al., 2013).

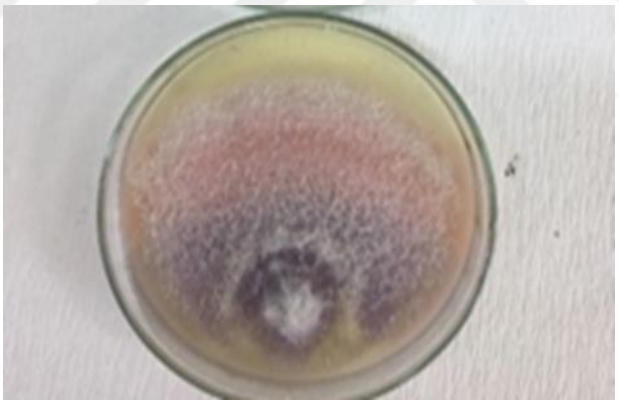

Figure 4.1. Morphology of *Fusarium oxysporum* isolate on PDA medium.

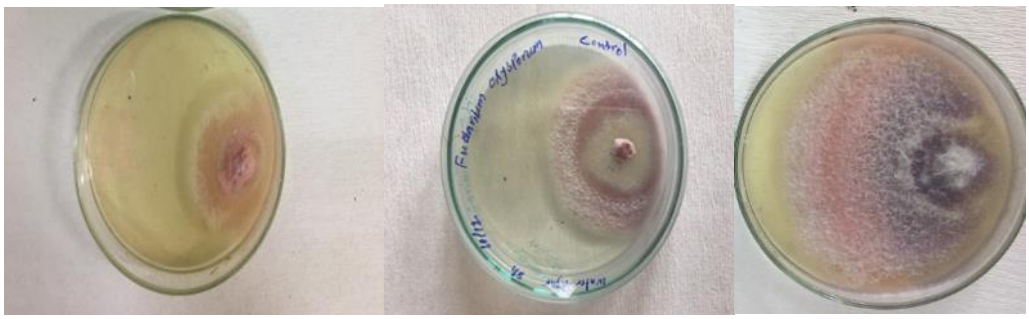

Figure 4.2. Growing of *Fusarium oxysporum* on PDA medium.

The isolates *Trichoderma* were defined by the presence of rapidly growing colonies producing white, green or yellow lesions on PDA medium and sporulating stems with side-branched conidiophores with circular to oval-shaped green colored spores (Figure 4.3).

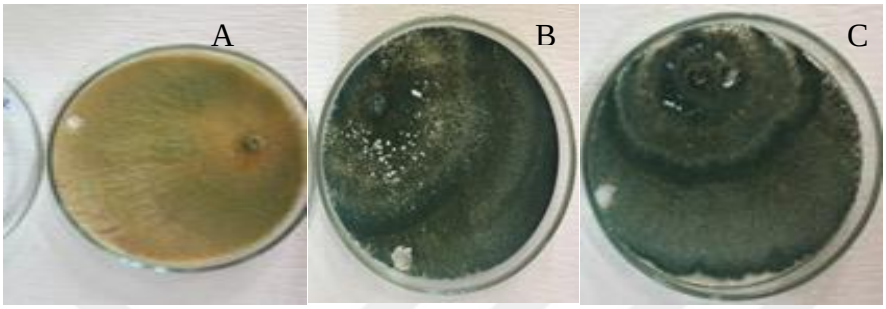

Figure 4.3. Morphology of *Trichoderma* spp. isolates on PDA medium, A. *Trichoderma harzianum*, B. *Trichoderma asperellum*, and C. *Trichoderma virens.*

4.2. In Vitro Biocontrol

All the *Trichoderma* species were tested inhibited mycelial growth of *Fusarium oxysporum* in dual culture. There have been significant differences among the species of *Trichoderma*. Growth inhibition of *F. oxysporum* was reduced by *T. harzianum* (T1) (by 19.03 %), *T. virens* (T2) (by 44.60 %), and *T. asperellum* (T3) (by 21.77 %), respectively. The antagonistic effect of *Trichoderma* species against the test pathogen was shown in Table 4.1. and (Figure 4.5). Accordingly, *Trichoderma virens* showed that the highest percentage inhibition of test pathogen growth with (44.60 %) compared *Trichoderma harzianum* with a percentage inhibition of (19.03 %) was lowest, and *Trichoderma asperellum* with (21.77 %), respectively.

Table 4.1. In vitro antagonistic activity of *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici*

Treatments*	**Inhibition Rate (%)**	**Antagonism (1-5 scale)**
	Mean ± SD**	Mean ± SD
F+T1	19.03 ± 7.86[a]***	2.16 ± 0.40[a]
F+T2	44.60 ± 5.37[b]	3.00 ± 0.00[b]
F+T3	21.77 ± 14.83[a]	2.83 ± 0.40[b]

*: F (*Fusarium oxysporum*),T1(*Trichoderma harzianum*),T2 (*Trichoderma virens*), T3 (*Trichoderma asperellum*).
**SD: Standard diviasion.
***:Data followed by the same letter are significantly different (P≤0.05), according to Duncan's multiple range test.

The difference between all applications and parameters is statistically significant (P≤0.05), as shown in Figure 4.4 and (Table 4.1). In the parameter, F+T2 and F+T3 have the highest values with (44.60 %), and scale (3.00), respectively. In general, the F+T2 application was found to be significantly effective.

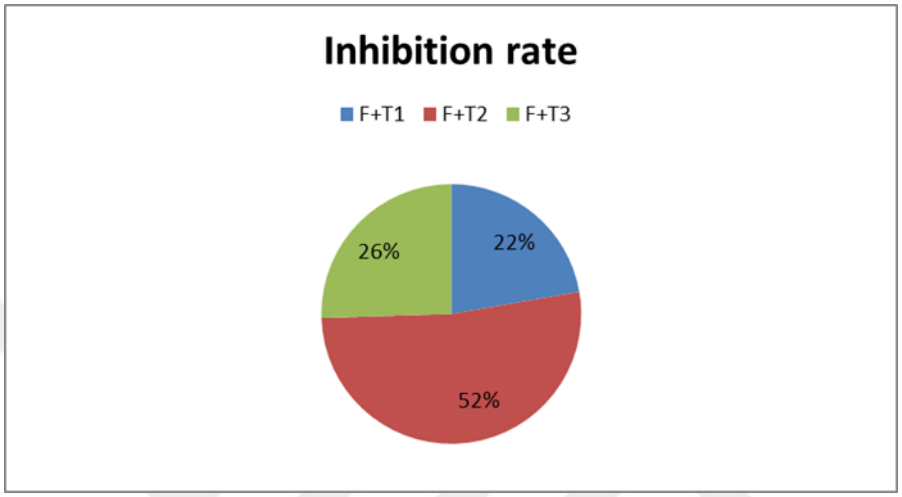

Figure 4.4. In vitro comparison of three species of *Trichoderma*'s antagonistic activity against *F. oxysporum* f. sp. *lycopersici.*

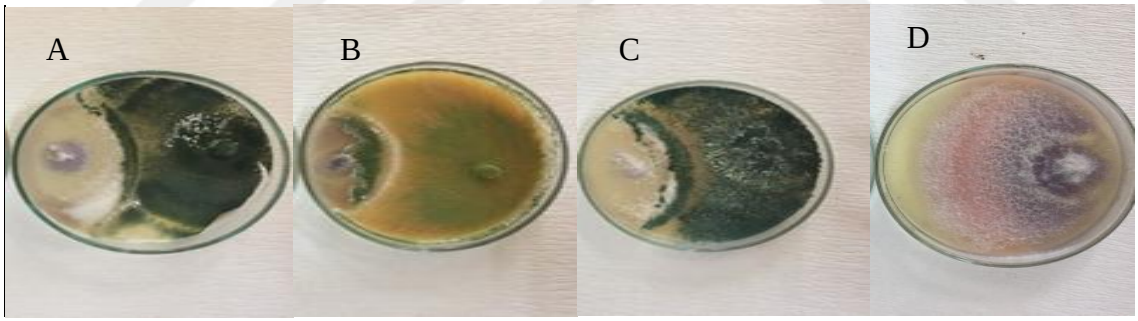

Figure 4.5. The antagonistic effect of *Trichoderma* species on the mycelial growth of *Fusarium oxysporum* isolate in the dual culture technique A. *T. virens*+ *F. oxysporum* B. *T. harzianum* + *F. oxysporum* C. *T. asperellum*+ *F. oxysporum* and D. Control.

4.3. In Vivo Treatments

4.3.1. Seed coating

Two techniques have evaluated the efficacy of the parameters used in this experiment: In the first technique, seed coating applications were investigated.

As early as 45 days after seed potting, the effects of various *Trichoderma* species and their application method on seedling growth promotion, and vigor were observed (Figure 4.6).

Harvest and parameter analysis three plants were harvested 80 days after transplantation from each treatment and the effect of each bioinoculant was registered on different development parameters. Change in height of plant (cm), fresh weight (g), and dry weight (g), Moreover, the results of this experiment revealed that *T. harzianum* significantly increased plant height (by 94.00 cm), *T. virens* (by 103.66 cm), *T. asperellum* (by 98.00 cm), *S. cereviasiae* (by 99.66 cm), T1+T2+T3 in conjunction (by 119.66 cm), S+T1+T2+T3 (by 94.50 cm), compared to untreated negative control (by 91.55 cm), and positive control (by 94.25 cm) (Figure 4.7) (Table 4.2).

Table 4.2. Effects of application groups on morphological development tomato plants

Treatment*	**Plant Height (Cm)**	**Plant Fresh Weight (G)**	**Plant Dry Weight (G)**
C-	91.55± 10.73^{a**}	68.18± 20.31^{cd}	9.03± 3.34^{ab}
C+	94.25± 6.70^{a}	34.98± 11.22^{a}	3.99± 1.19^{a}
T1	94.00± 7.54^{a}	78.15± 5.26^{d}	33.48± 5.55^{d}
T2	103.66± 9.07^{ab}	87.64± 8.13^{e}	35.72+1.42^{d}
T3	98.00± 9.53^{a}	67.40± 5.18^{cd}	25.05± 2.53^{c}
S	99.66± 17.61^{a}	39.59± 25.13^{ab}	13.03± 8.57^{b}
T1+T2+T3	119.66± 8.73^{b}	60.76± 9.89^{c}	25.26± 7.35^{c}
S+ T1+T2+T3	94.50± 17.44^{a}	48.34± 6.77^{b}	13.22± 3.31^{b}

*: **C(+):** positive control **C-:** negative control **T1:** *T. harzianum* **T2:** *T. virens* T3**:** *T. asperellum* **S**: *Saccharomyces cerevisiae.*

**: The difference between the means indicated by different letters in the same column is significant (P <0.05), according to Duncan's multiple range test.

Figure 4.6. Incubation in the climate chamber of tomato plant seedlings.

As a result, T1+T2+T3 together showed that the highest development of height plant (by 119.66 cm), but it did not have the same effect on the fresh and dry plant weight. *T. virens* significantly increased fresh plant weight (by 87.64 g), and dry plant weight (by 35.72 g) that the largest growth proportion, compared with the *T. harzianum*, *T. asperellum*, *S. cereviasiae* and untreated control (Figure 4.7). Application of dry yeast alone provided a significant height improvement over the effect of pathogen inoculation, but was still significantly shorter than other treatments to improve the weight of fresh and dry plants. On the other hand, plant fresh weight was significantly increased by yeast with *Trichoderma* spp. together (Figure 4.8) (Table 4.2).

Figure 4.7. Seed coating treatment effects of *Trichoderma* spp., and S. *cerevisiae*, on tomato plants, A. Positive control, B. Negative control, C. *S. cerevisiae*, D. *T. harzianum*, E. *T. virens*, F. *T. asperellum* .

Figure 4.8. Seed coating treatment effects of *S. cerevisiae*, on tomato root plants, A. negative control, B. positive control, and C. *Saccharomyces cerevisiae*.

The antagonistic activity of *Trichoderma* spp. was investigated against the soil-borne pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* and the results were summarized in (Table 4.3). The effect of *Trichoderma* species on tomato growth and the

ability to reduce the effect of FOL when subsequently applied to the pot experiment varied shown in (Figure 4.10). In the absence or presence of FOL inoculum, there have been statistically significant effects of *Trichoderma* treatments on plant growth parameters (Figure 4.9).

Table 4.3. The effect of *Trichoderma* species on the development of wilt disease in tomato plant morphological development parameters, scale value and disease severity

Treatment*	**Plant height (Cm)**	**Plant Fresh Weight (G)**	**Plant Dry Weight (G)**	**Scale Value (0_3)****	**Disease Severity (%)*****
C-	91.55±10.73^{a****}	68.18±20.31^{c}	9.03±3.34^{ab}	0	0
C+	94.25± 6.70^{a}	34.98± 11.22^{b}	3.99± 1.19^{a}	1.50	50
F+T1	106.00±23.45^{b}	23.29±7.74^{ab}	3.28±1.22^{a}	1.20	40
F+T2	100.20±4.43^{ab}	31.90±4.15^{b}	5.57±2.83^{a}	0.20	10.22
F+T3	82.40±8.17^{a}	21.51±5.48^{a}	3.14±1.14^{a}	1.60	53.33
F+T1+T2+T3	99.33±16.25^{ab}	76.65±6.02^{c}	28.17±4.01^{b}	1.0	33.33

*: **C (+):** positive control **C-:** negative control **F**: *Fusarium oxysporum* **T1:** *T. harzianum* **T2:** *T. virens* **T3:** *T. asperellum.*

**: Plants were evaluated using the 0-3 scale.

***: Disease severity index was calculated by using percentage on the 0-3 scale.

****: There are a difference between the means indicated by different letters (P≤0.05), according to Duncan's multiple range test.

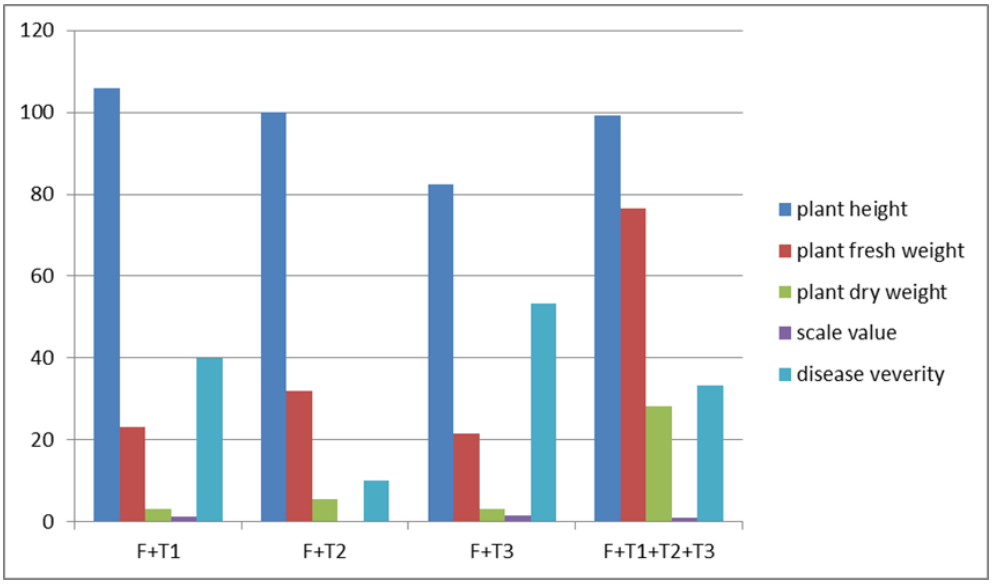

Figure 4.9. The effect of *Trichoderma* species on the development of wilt disease in tomato plant morphological development parameters, scale value and disease severity.

Trichoderma spp. *T. harzianum* increased plant height significantly (by 106.00 cm), compared to *T. virens* and *T. asperellum* the highest in plant growth parameters, but had no significant effects on plant fresh weight and plant dry weight compared to *T. virens* increased plant fresh weight (by 31.90 g), and plant dry weight (by 5.57 g), *T. asperellum* increased plant height (by 82.40 cm), plant fresh weight (by 21.5 g), and plant dry weight (by 3.14 g), it was least of all. Although all three species of *Trichoderma, T. harzianum, T. virens,* and *T. asperellum* were used together, plant fresh weight (by 76.65 g) was significantly increased and plant dry weight (by 28.17 g) was shown to be the highest in plant growth parameters (Figure 4.9).

Figure 4.10. The effect of *Trichoderma* species on the development of wilt disease in tomato plant, A. *F. oxysporum+T. harzianum*, B. *F. oxysporum+T* .virens, C. *F. oxysporum+T. asperellum*, D. *F. oxysporum+T. harzianum+T. virens+T. asperellum.*

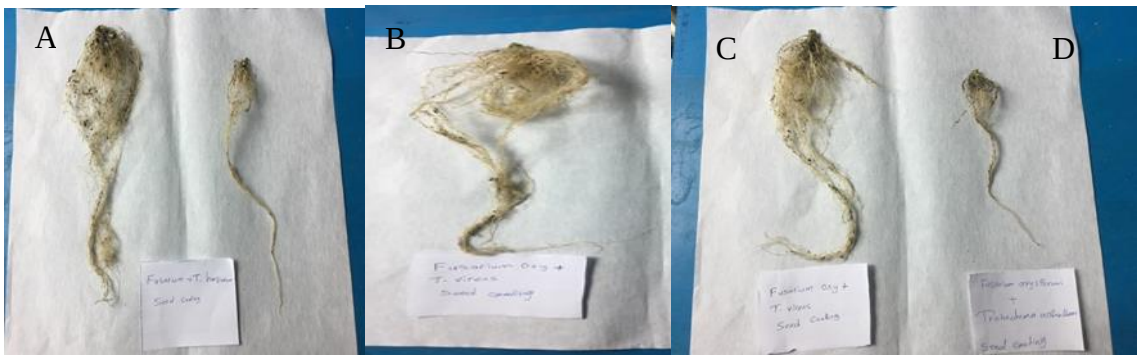

Figure 4.11. The effect of *Trichoderma* species on the development of wilt disease in tomato plant roots, A. *F.oxysporum+T. harzianum*, B. *F. oxysporum+T. virens*, C. *F. oxysporum+T. virens*, D. *F. oxysporum+T. asperellum.*

While F +T3 treatments had the highest results with 1.60 scales and 53.3%, disease severity, F+T2 showed the lowest values with 0.20 scales and 10.22%, disease

severity. In this group, the pathogenicity of the plant causing the most damage was observed by F+T3 (Figure 4.11).

The total height and weight per pot were not significantly affected by the different treatments, including inoculation with *F. oxysporum*, it was increased in response to an application of *T. asperellum* with yeast *S. cerevisiae* (Table 4.4). Treatment with yeast with *F. oxysporum* gave no improvement in the plant height (by 87.75 cm), was compared used with *Trichoderma* spp. F+S+T1 (by 98.75 cm), F+S+T2 (by 99.50 cm), F+S+T3 (by101,25 cm) F+S+T1+T2+T3 (by 105.66 g). Treatments F+S+T1+T2+T3 increased plant height (by 105.66 cm), and plant dry weight(by 17.12 g) that the largest growth proportion, but had no significant effect on plant fresh weight. F+S+T1 significantly reduced the plant dry weight (by 11.22 g). On the other hand, applications dry yeast with *F.oxysporum* produced the best plant fresh weight was significantly increased (by 54.21 g), but F+S+T1 were significantly the least effective treatment on plant fresh weight (by 37.40 g) respectively (Figure 4.12).

Table 4.4. The effect of *Trichoderma* and *Saccharomyces cerevisiae* against *Fusarium oxysporum* f. sp. *lycopersici* on plant disease morphological development parameters, scale value and disease severity

Treatment*	**Plant High (Cm)**	**Plant Fresh Weight (G)**	**Plant Dry Weight (G)**	**Scale Value (0_3)****	**Disease Severity (%)*****
C-	91.55±10.73[a]****	68.18±20.31[b]	9.03±3.34[a]	0	0
C+	94.25± 6.70[a]	34.98± 11.22[a]	3.99± 1.19[a]	1.50	50
F+S	87.75±8.53[a]	54.21±22.19[a]	14.36±4.75[a]	1.50	50
F+S+T1	98.75±16.45[a]	37.40±12.46[a]	11.22±8.33[a]	1.75	58.3
F+S+T2	99.50±5.80[a]	50.63±3.75[a]	15.70±1.72[a]	0.25	8.3
F+S+T3	101.25±10.30[a]	52.64±16.24[a]	15.81±10.38[a]	0.75	25
F+S+T1+T2+T3	105.66±8.38[a]	47.96±23.40[a]	17.12±3.48[a]	0.33	11.1

*: **C (+):** positive control **C-:** negative control **F**: *Fusarium oxysporum* **T1:** *T. harzianum* **T2:** *T. virens* **T3:** *T. asperellum.* **S**: *Saccharomyces cerevisiae.*

**: Plants were evaluated using the 0-3 scale.

***: Disease severity index was calculated using percentage on the 0-3 scale.

****: Data followed by the same letter are significantly no different (P≤0.05), according to Duncan's multiple range test.

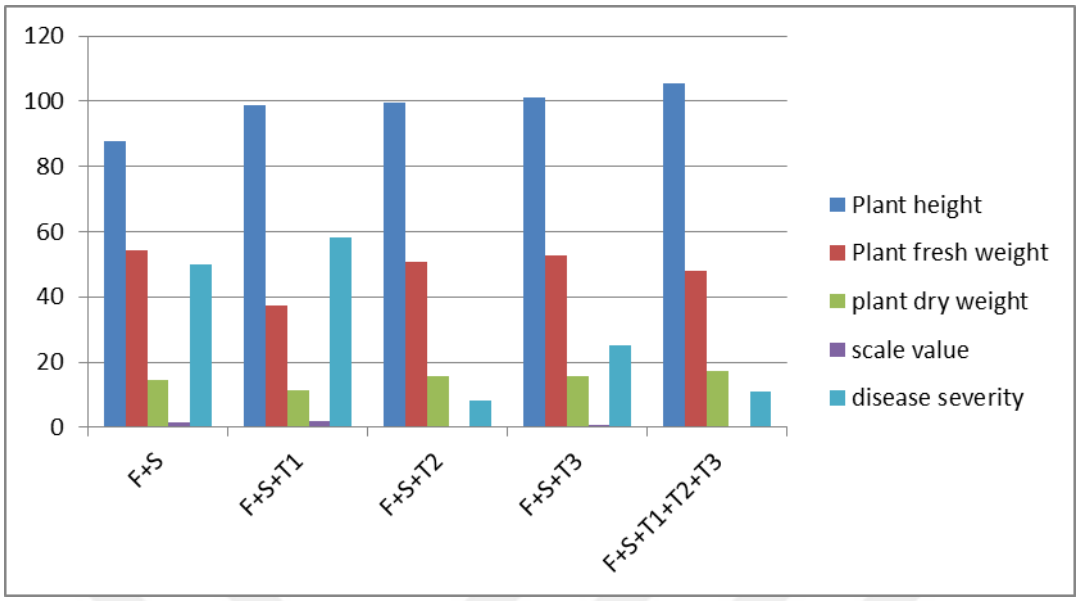

Figure 4.12. The effect of *Trichoderma* and *Saccharomyces cerevisiae* against *Fusarium oxysporum* f. sp. *lycopersici* on plant disease morphological development parameters, scale value and disease severity.

While F+S+T1 treatments had the highest results with 1.75 scales and 58.3 % disease severity index, F+S+T2 showed the lowest values with 0.25 scales and an 8.33 % disease severity index. In this group, the pathogenicity of the plant causing the most damage was detected by F+S+T1. (Figure 4.13).

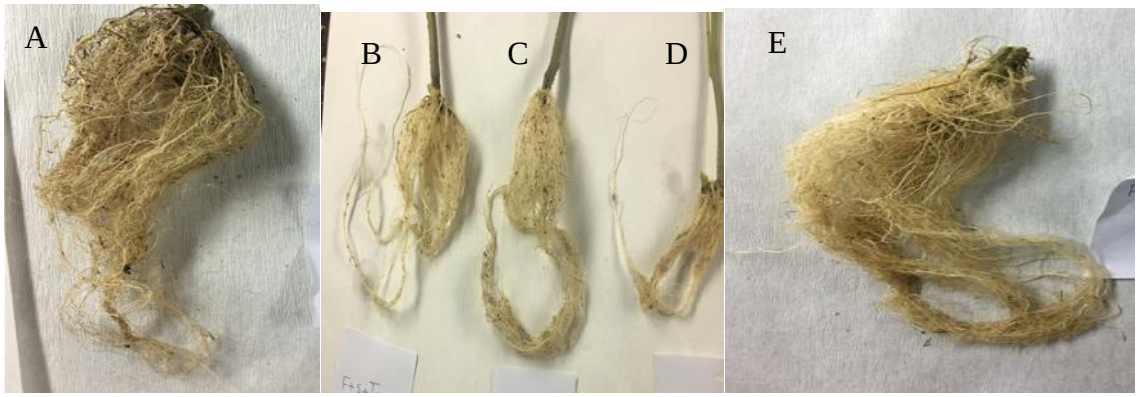

Figure 4.13. The effect of *Trichoderma* spp. and *Saccharomyces cerevisiae* against *Fusarium oxysporum* on tomato root plant disease, A. *F. oxysporum+S. cerevisiae*, B. *F. oxysporum+S. cerevisiae +T. asperellum*, C. *F. oxysporum+S. Cerevisiae +T. virens*, D. *F. oxysporum+S. Cerevisiae +T. harzianum*, E. *F. oxysporum+S. Cerevisiae +T. harzianum+T. virens+T. asperellum*.

4.3.2. Soil treatment

The efficacy of the parameters used in this experiment was tested by two methods: soil treatment applications were investigated in the second method. Individual and mixture of biocontrol organisms evaluated significantly affected the morphological development of tomato plants in greenhouse tests Figure 4.14. and results in Table 4.5 have been summarized.

Table 4.5. Effects of application groups on morphological development parameters of tomato plants

Treatment*	Plant Height (Cm)	Plant Fresh Weight (G)	Plant Dry Weight (G)
C-	91.55 ±10.73^{a**}	68.18±20.31^{bc}	9.03±3.34^{b}
C+	94.25±6.70^{a}	34.98±11.22^{a}	3.99±1.19^{a}
T1	99.66±10.59^{a}	87.96±10.80^{c}	43.50±7.29^{d}
T2	108.00±28.68^{a}	81.59±15.06^{bc}	40.13±16.68^{d}
T3	97.33±6.42^{a}	74.56±16.89^{bc}	21.33±10.78^{c}
S	110.66±20.98^{a}	71.61±28.06^{bc}	20.46±3.78^{c}
T1+T2+T3	111.66±10.50^{a}	52.52±10.76^{b}	8.35±4.10^{ab}
S+T1+T2+T3	98.33±5.50^{a}	52.38±6.21^{b}	18.18±3.40^{bc}

*: **C(+):** positive control **C-:** negative control **T1:** *T. harzianum* **T2:** *T. virens* T3**:** *T. asperellum* **S**: *Saccharomyces cerevisiae.*

**: There are a difference between the means indicated by different letters ($P \leq 0.05$), according to Duncan's multiple range test.

The result is shown in Table 4.5 gives the significant increase in height plants in treatment T1+T2+T3 (by 111.66 cm) as highest and least in T3 (by 97.33cm), and in a seed coating we obtained the same result with T1+T2+T3 as a highest (by 119.66 cm), but *T. harzianum* (by 94.00 cm) as a least.

Trichoderma spp. T1, T2, T3 and *S. cerevisiae* significantly increased plant height compared with non-inoculated plant control but had no significant effects on other plant growth parameters. In addition applications of T1+T2+T3 together were most effective on plant height and highest, but had no significant effects on plant dry weight and least (by 8.35 g), *T. harzianum* significantly increased plant fresh weight (by 87.96 g), and plant dry weight (by 43.50 g), as a highest respectively, but it had no the same effects on plant fresh and dry weight in a seed coating treatment. Moreover, *S. cerevisiae* was applied in a soil treatment given better results compare with the seed coating treatment.

Figure 4.14. Effects of application groups on morphological development parameters of tomato plants, **A.** Control and *T. harzianum*, B. Control and *T. virens*, C. Control and *T. asperellum*, D. Control and *S. cerevisiae.*

The antagonistic activity of *Trichoderma* species were screened against the soil borne plant pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* inoculated treatment *T. virens* the plant high was increased (by 99.75 cm) as a highest, in tomato plant *T. harzianum* (by 92.25 cm), *T. asperellum* (by 73.25 cm) was a least, and T1+T2+T3 together (by 99.00 cm), but *T. harzianum* was increased plant height (by 106.00 cm) as a highest and *T. asperellum* (by 82.40 cm) was a least in a seed coating treatment. However, treatments T1+T2+T3 together were increased plant fresh weight (by 68.67 g), and plant dry weight (by 25.04 g), was highest. The effects of *Trichoderma* species against *Fusarium oxysporum* on tomato plant root was shown in Figure 4.16. In addition species, T1+T2+T3 together gave the same result in seed coating treatments. *T. asperellum* was significantly the least effective treatment in both seed and soil treatments. *T. virens* was increased plant height but had no significant effects on plant fresh and dry weight. When the results of the scale and severity of the disease were investigated, it was seen that the highest isolate value *T. asperellum* (25 %), and T1+T2+T3 together was the lowest value (0), (Table 4.6) (Figure 4.15). Wilt disease symptoms on tomato plants caused by *Fusarium oxysporum* was seen in Figure 4.17.

Table 4.6. The effect of *Trichoderma* species on the development of wilt disease in tomato plant morphological development parameters, scale value and disease severity

Treatment*	Plant High (Cm)	Plant Fresh Weight (G)	Plant Dry Weight (G)	Scale (0-3)	Disease Severity (%)***
C-	91.55±10.73^{a****}	68.18±20.31^{b}	9.03±3.34^{a}	0	0
C+	94.25± 6.70^{b}	34.98± 11.22^{a}	3.99± 1.19^{a}	1.50	50
F+T1	92.25±8.09 **b**	35.21±9.36 **a**	9.26±1.27 **a**	0.50	16.66
F+T2	99.75±9.74 **b**	37.94±7.29 **a**	10.52±2.99 **a**	0.25	8.33
F+T3	73.25±10.50 **a**	27.86±5.31 **a**	8.43±1.36 **a**	0.75	25
F+T1+T2+T3	99.00±14.00 **b**	68.67±9.89 **b**	25.04±1.32 **b**	0	0

*: **C (+):** positive control **C-:** negative control F: *Fusarium oxysporum* T1: *T. harzianum* T2: *T. virens* T3: *T. asperellum* S: *Saccharomyces cerevisiae.*

**: Plants were evaluated using the 0-3 scale.

***: Disease severity index was calculated.

****: There are a difference between the means indicated by different letters (P≤0.05), according to Duncan's multiple range test.

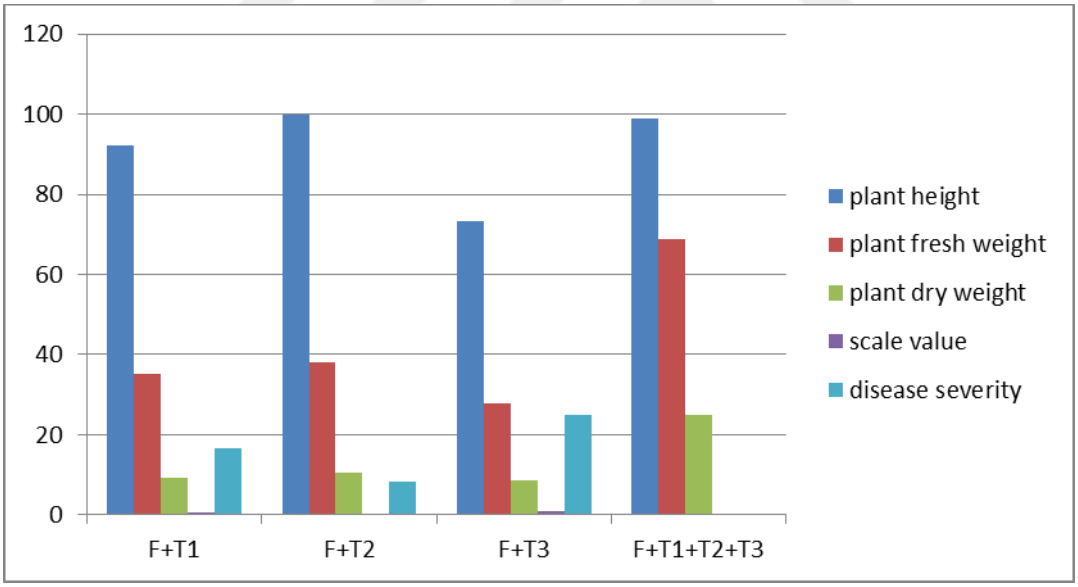

Figure 4.15. The effect of *Trichoderma* species on the development of wilt disease in tomato plant morphological development parameters, scale value and disease severity.

Figure 4.16. Effects of *Trichoderma* species against *Fusarium oxysporum* on tomato plant root *F. oxysporum*+ *T. harzianum* + *T. virens* + *T. asperellum*.

Figure 4.17. Wilt disease symptoms on tomato plants caused by *Fusarium oxysporum.*

On the other hand, infection with *Fusarium oxysporum* considerably decreased plant height, with the addition of *T.harzianum* negating the impact with yeast. The application of dry yeast alone resulted in a significantly lower improvement over the effect of pathogen inoculation but was still significantly higher with other treatments (Table 4.7) (Figure 4.18). The effect of *T. asperellum* and dry yeast *Sachharomyces cerevisiae* on the plant dry weight were highest (by 46.84 g), compare with the other treatments, but effects of *Sachharomyces cerevisiae* on fresh and dry plant weight alone

against *F.oxysporum* was least (by 35.21, 10.22 g), but in a seed coating treatment effects of *Sachharomyces cerevisiae* on fresh plant weight alone against *F.oxysporum* was a highest (by 54.21 g).

Increase in the plant height and plant fresh weight parameters was highest (by 125.66 cm), and (by 78.66 g), in the plants treated by S+T1+T2+T3 with respect to the untreated plant, but had no same effects on plant dry weight Figure 4.18, in the same time isolates S+T1+T2+T3 increased height plant (by 105.66 cm), as a highest in seed coating treatment.

Table 4.7. The effect of *Trichoderma* and *Saccharomyces cerevisiae* on plant disease morphological development parameters, scale value and disease severity

Treatment*	**Plant High (Cm)**	**Plant Fresh Weight (G)**	**Plant Dry Weight (G)**	**Scale (0_3)****	**Disease Severity (%)*****
C-	91.55±10.73[a****]	68.18±20.31[b]	9.03±3.34[ab]	0	0
C+	94.25± 6.70[a]	34.98± 11.22[a]	3.99± 1.19[a]	1.50	50
F+S	97.00±7.11[a]	35.21±9.36[a]	10.22±1.35[a]	1.00	33.33
F+S+T1	89.00±7.81[a]	62.88±14.77[ab]	20.73±6.58[b]	0	0
F+S+T2	97.66±10.01[a]	77.03±24.14[b]	22.63±6.77[b]	0	0
F+S+T3	98.00±11.78[a]	75.18. ±12.07[b]	46.84±23.03[c]	0	0
F+S+T1+T2+T3	125.66±24.82[b]	78.66±25.18[b]	33.02±11.86[bc]	0	0

*: **F:** *Fusarium oxysporum* **T1:** *T. harzianum* **T2:** *T. virens* T3**:** *T. asperellum* **S**: *Saccharomyces cerevisiae.*

**: Plants were evaluated using the 0-3 scale.

***: Disease severity index was calculated.

****: There are a difference between the means indicated by different letters (P≤0.05), according to Duncan's multiple range test.

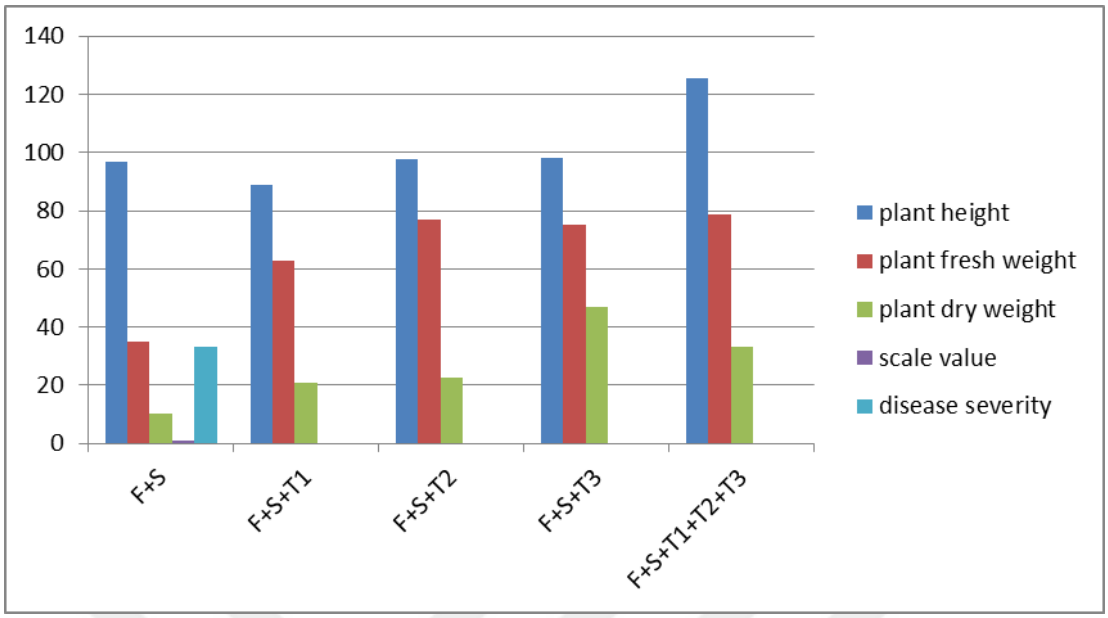

Figure 4.18. The effect of *Trichoderma* and *saccharomyces cerevisiae* on plant disease morphological development parameters, scale value and disease severity.

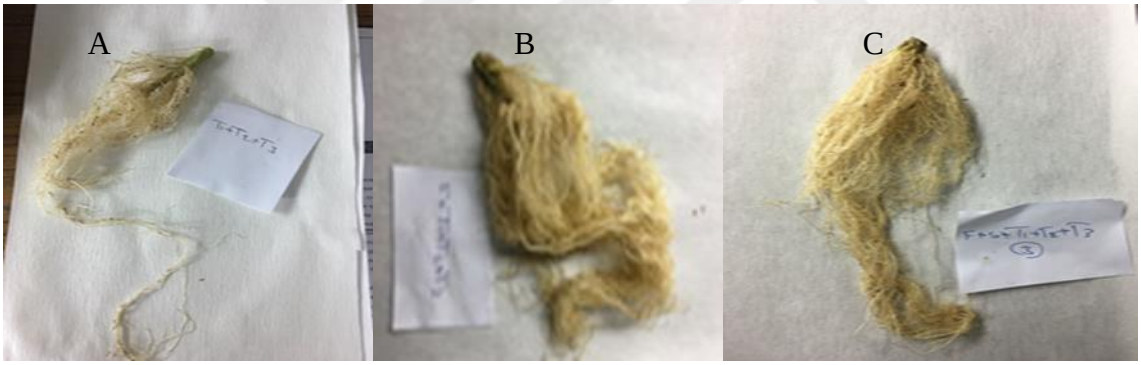

Figure 4.19. Applications effects against fusarium wilt disease on tomato root plants, A. T. *harzianum+T. virens+T. asperellum*, B. *F. oxysporum+ T. harzianum+T. virens+T. asperellum*, C. *F. oxysporum+S. cerevisiae+ T. harzianum+T. virens+T. asperellum.*

Scale values were generally between 0 and 1 and the highest scale value was found by F+S without *Trichoderma*, and disease severity was (33,33 %), but application the dry yeast *Saccharomyces cerevisiae* with *Trichoderma* against soil borne plant pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* in soil treatment method gave the highest significant development of tomato plant and suppressing the wilt incidence (0), (Figure 4.19).

The difference between all applications and parameters is statistically not significant (P≤0.05), as shown in (Table 4.8). The antagonistic activity of *Trichoderma*

spp. and yeast *Saccharomyces cerevisiae* was tested against soil borne pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* and the findings were summarized in Table 4.8. compared both of seed coating and soil treatment techniques that yeast *Saccharomyces cerevisiae* alone increased plant height (87.75 and 97.00 cm), plant fresh weight (54.21 and 35.21 gm), plant dry weight (14.36 and 10.22 gm), significantly increased plant height in soil treatment to compare with seed coating but was still significantly shorter than other treatments to improve the weight of fresh and dry plants. While *S. cerevisiae* with *T. harzianumt*, *T. virens,* and *T. asperellum* were most efficient in seed coating treatment at plant height to compare soil treatment, it did not have the same effect on fresh and dry plant weight.In addition, *Trichoderma* spp. T1, T2, T3 and *S. cerevisiae* together significantly increased plant height, fresh and dry plant weight in a soil treatment compared with seed coating and non-inoculated plant control. Scale values were normally between 0 and 1.50 and the highest scale value was found by F+S without *Trichoderma*, and the highest disease severity was found by F+S+T1 (58.33 %) in seed coating, but application the yeast *Saccharomyces cerevisiae* with *Trichoderma* against soil borne plant pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* in the soil treatment method gave the highest significant development of tomato plant and suppressing the wilt incidence (0).

Table 4.8. Comparison of seed coating and soil treatment against *F.oxysporum* with *Trichoderma* spp. and *S. cerevisiae*

Treatments	Plant height		Plant fresh weight		Plant dry weight		scale		Disease severity	
	Seed	soil	Seed	soil	Seed	soil	seed	soil	Seed	soil
C-	91.55± 10.73[a]		68.18± 20.31[a]		9.03± 3.34[a]					
C+	94.25± 6.70[a]		34.98± 11.22[a]		3.99± 1.19[a]		1.50		50	
F+S	87.75±8.53[a]	97.00±7.11[a]	54.21±22.19[a]	35.21±9.36[a]	14.36±4.75[a]	10.22±1.35[a]	1.50	1.00	50	33.33
F+S+T1	98.75±16.45[a]	89.00±7.81[a]	37.40±12.46[a]	62.88±14.77[a]	11.22±8.33[a]	20.73±6.58[a]	1.75	0	58.33	0
F+S+T2	99.50±5.80[a]	97.66±10.01[a]	50.63±3.75[a]	77.03±24.14[a]	15.70±1.72[a]	22.63±6.77[a]	0.25	0	8.33	0
F+S+T3	101.25±10.37[a]	98.00±11.78[a]	52.64±16.24[a]	75.18±12.07[a]	15.81±10.38[a]	46.84±23.03[a]	0.75	0	25	0
F+S+T1+T2+T3	105.66±8.38[a]	125.66±24.82[a]	47.96±23.40[a]	78.66±25.18[a]	17.12±3.48[a]	33.02±11.86[a]	0.33	0	11.11	0

[*]**: C (+):** positive control **C-:** negative control **F:** *Fusarium oxysporum* **T1:** *T. harzianum* **T2:** *T. virens* T3**:** *T. asperellum* **S**: *Saccharomyces cerevisiae.*
[**]**:** Plants were evaluated using the 0-3 scale.
[***]**:** Disease severity index was calculated.
[****]**:** There are a difference between the means indicated by different letters (P≤0.05), according to Duncan's multiple range test.

5. DISCUSSION AND CONCLUSION

In order to maintain the quality and abundance of food, feed, and fiber produced by farmers around the world, plant diseases must be controlled. Various approaches can be used to prevent, reduce or control diseases of plants. The biological control of soil-borne plant pathogens is a potential alternative to the use of chemical fungicides that have already been shown to be environmentally harmful. Several strains of the *Trichoderma* fungus under greenhouse and field conditions were isolated and found to be effective biocontrol agents of various soil-borne plant pathogenic fungi. An increase in plant growth was observed in all inoculated seedlings in the current investigation, although the extent of plant growth in all treatments was different. Because of the pathogen's soil-borne nature, it first attacks the host's root system and the root system of *Fusarium* alone plants has also been poorly developed in the present investigation compared with plants with bioagents. But perhaps due to *Trichoderma* spp. and dry yeast's antagonistic and beneficial activity, *Fusarium* growth was checked and root infection was reduced, producing healthier crops with a stronger root system.

Biological control includes the use of useful organisms, their genes and products, such as metabolites, which decrease the adverse impacts of pathogens on plants and promote positive plant reactions (Vinale et aI., 2008). Suppression of disease as mediated by agents of biocontrol is the result of interactions between plants, pathogens and the microbial community (Vinale et aI., 2008). Over the years, research has been carried out to screen and test potential biocontrol agents in vitro and under greenhouse and field conditions, as well as to develop improved delivery systems (Donumbou et al., 2001; EI-Tarabily and Sivasithamparam, 2006; Vinale et al., 2008). Biological control using microbial antagonists has appeared as one of the most successful options, either alone or as part of an integrated control approach to decrease dependency on chemical pesticides (Whipps, 2001; Donumbou et aI., 2001; Vinale et al., 2008). Some of the most common and severe plant diseases are caused by soil-borne fungal plant pathogens. Root diseases caused by these pathogens such as wilt, root rot, collar rot, foot rot, pre- and post-emergence seedling damping are the main problems that are becoming increasingly important in the world today and for which no direct

control measures have been developed to date (Whipps, 2001; Schumann and D'Arcy, 2006). *Fusarium* spp. Is a common soil-borne plant pathogen and is the causative agent of many economically valuable plants wilt disease (Jones et al., 1997). In most agricultural soils, *Fusarium* spp. is a limiting factor in plant productivity. Tomato wilt disease is a common problem in as well as almost every tomato field and greenhouse (Jones et al., 1997). *Fusarium oxysporum* f. Sp *lycopersici* is the causal organism that causes wilt disease in tomato all over the world (Sreenu et al., 2017).

(Crawford et aI., 1993; Yuan and Crawford, 1995), used the dual culture technique frequently to identify in vitro antagonisms between pathogens and antagonists. This method's concept is focused on the antagonist's production of effective metabolites, which diffuses into the agar medium and in tum inhibits pathogen development. This method allows observations to be made on the antagonist's effects on the agar culture's growth and survival of a pathogen.

Verma et al. (2017), researched that in the dual culture, the decrease in pathogen mycelial development (*Fusarium* sp.) was significantly higher ($p<0.05$) relative to pathogen control. It was probably due to the nutrient and space competition that was available.

In this study, all the *Trichoderma* species were tested (*T. harzianum, T. virens, T. asperellum*), inhibited mycelial growth of *Fusarium oxysporum* in dual culture. There have been significant differences among the species of *Trichoderma.*

Similarly, in another study, all *Trichoderma* species inhibited the development of *F. oxysporum* L-6 in dual culture. Zones of inhibition between pathogen colonies and *Trichoderma* species were detected. The inhibition zone could be due to the effect of diffusible inhibitory substances produced by the strains of *Trichoderma,* which suppressed growth *F. oxysporum* L-6. The presence and size of the inhibition zone were used as proof of antibiotic production by *Trichoderma* strains (Jackson et al., 1991; Crawford et al., 1993).

Growth inhibition in our study of *F. oxysporum* was reduced by *T. harzianum* (T1) (19.03 %), showed the lowest inhibition mycelial growth of *Fusarium oxysporum.* In contrast, Singh, J.et al., (2011), studied through in vitro screening of three bioagents, an extensive study was carried out to determine their potential as suitable bio-pesticides against *F. oxysporum* f. sp. *lycopersici*. Data analysis showed that *Trichoderma*

harzianum was very effective in controlling *F. oxysporum* f. sp. *lycopersici* where the inhibition zone development was highest (75.9 %) accompanied by *Trichoderma viride* and *Trichoderma koningii* with 67.7 and 55.6 % respectively inhibition zone.

In the present research Pathogenic radial mycelial growth was effectively inhibited by *Trichoderma harzianum* (19.03 %). In addition, Seed coating of tomato seeds with *T. harzianum* increased plant high (by 106.00 cm), fresh weight (by 23.29 g) and dry weight (by 3.28 g).

Barari (2016), researched *Trichoderma harzianum*, isolate N-8, was found to inhibit effectively the radial mycelial growth of the pathogen (by 68.22 %). Under greenhouse conditions, the application of *T. harzianum* (N-8) exhibited the least disease incidence (by 14.75 %). Also, tomato plants treated with *T. harzianum* (N-8) isolate showed a significant stimulatory effect on plant height (by 70.13 cm) and the dry weight (by 265.42 g) of tomato plants, in comparison to untreated control (54.6 cm and 195.5 g). Therefore, the antagonist *T. harzianum* (N-8) is chosen to be the most promising bio-control agent for *F. oxysporum* f. sp. *lycopersici*.

Babychan and Simon (2017), found the findings revealed under in vitro condition that *Trichoderma* isolate MiT-4 efficiently inhibited the pathogen's radial mycelial growth by 58.4 %. There were no wilt incidences in tomato seedlings under the greenhouse condition of *Trichoderma* isolates (MiT-1 and MiT-4).

The antagonistic effect of *Trichoderma asperellum* isolates against the test pathogen, *Fusarium oxysporum* was induced in this study at a ratio of 21.77 % . In another study the antagonistic activity of *T. asperellum* MSST against the pathogen FOL was carried out by dual culture method. It has been observed that isolate MSST inhibits the growth of FOL by (65 %), after 7 days of incubation (Patel and Saraf, 2017).

In the present research plant treated by *Trichoderma harzianum* isolate was increased plant height (94.00 %), and increased plant dry weight (33.48 %), in seed coating. However, in soil treatment *Trichoderma harzianum* isolate was increased plant high (99.66 %), dry weight (43.50 %), it was highest. Similarly, Sundaramoorthy et al. (2013), evaluated tomato plants also treated with *Trichoderma harzianum* (ANR-1) isolate had a significant stimulating effect on plant height (73.62 cm) and increased dry weight (288.38 g) of tomato plants compared with other isolates and untreated control.

In our research seed coating and soil treatment with three *Trichoderma* species (*T. harzianum, T. virens,* and *T. asperellum*) on the growth of the causal agent *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lycopersici*) caused disease decrease. In contrast, Ramezani (2010), researched that the seed treatment of five species of *Trichoderma* (*T. harzianum, T. koningi, T. longiconis, T. hamatum and T. viride*) on the growth of the causal agent *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lycopersici*) did not cause a decrease in disease but soil treatment caused a decrease of 92 % in disease.

In the research, *F. oxysporum* development was inhibited respectively by the use of dry yeast *saccharomyces cerevisiae*. Dry yeast increased plant height (87.75 %), fresh weight (54.21 %) and dry weight (14.36 %) in seed coating treatment, but soil treatment with dry yeast increased plant height (by 97.00 %), but reduced fresh and dry weight (35.21- 10.22 %). Shalaby (2008), studied application of 5 g L-1 of yeast resulted in a reduction of the pre- and post-emergence damping-off 6.67 % and 11.67 %, respectively. Survival of treated plants increased to 83.33 % in comparison with 30.00% for the pots inoculated with the pathogen containing untreated seeds. Linear growth of *F. oxysporum* was inhibited with 39.52 % and 50 % by using 5 g L-1 and 6.35 g L-1 of the yeast, respectively.

Ghazalibiglar et al. (2016), investigated in the absence of the pathogen, none of the *Trichoderma* isolates consistently increased all plant growth parameters. The biocontrol mechanism of these *Trichoderma* isolates requires further investigation.
In the study *Trichoderma* spp. antagonistic effects on *F. oxysporum* f.sp. *lycopersici* under pot condition and applied as seed coating could significantly reduce the disease. In contrast, Hesamadin (2010) evaluated the antagonistic effects of *Trichoderma* spp. against *F. oxysporum* f.sp. *lycopersici* under pot condition and applied as seed coating, could not reduce the disease significantly as wilt reduction was only 23.7 %.

In the present research exanimate *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici* for the biological control of tomato wilt and seed coating with different *Trichoderma* species can significantly reduce wilt incidence and increase plant weight, fresh and dry, increases germination as well as observed. Devi et al. (2013), evaluated the *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici* for biological control of tomato wilt and seed coating with different species of *Trichoderma* can significantly reduce the wilt incidence (34.47-56.36 %) and also increase germination per cent

(65.56-77.78 %). Soil treatment and seed + soil treatment proved better than seed treatment alone.Sivan et al., (1987), found when *T. harzianum* was applied as a seed coating in tomato seeds the crown rot incidence of greenhouse grown tomatoes reduced upto 80 % by 75 days after sowing.

(El-Tarabily and Sivasithamparam, 2006; El-Tarabily 2004; Shalaby and El-Nady 2008), examined the *Saccharomyces cerevisiae* utilized as biocontrol operator against of soil-borne contagious plant pathogens causing root-spoil infection by *Fusarim solani* and *Rhizoctonia solani* of sugar beet and also plant development advertisers.

Verma and Dohroo (2005), studied seed coating with *T. harzianum* and *T. viride* were found most effective with 93.83 % disease control under pot conditions on pea.
Mehra (2006), evaluated the under pot conditions tomato seed coated with *T. viride*, *T. harzianum* and *C. globosum* showed higher seed germination 74,72 and 68 % and disease control 71.5, 69.0 and 63 % over control.

Jamwal et al. (2011), studied *T. harzianum* was recorded least wilt incidence (5.6 and 6.0 %) followed by *T. viride* (11.3 and 10.9 %) during two years of dipping the tomato seedlings in bioagent suspenssion.

Mishra et al. (2004), investigated the proliferation and population density of the pathogen in the soil was significantly reduced after the incorporation of *T. virens* along with FYM.

In the present study least inhibition radial growth of *Fusarium oxysporum* was recorded by *Trichoderma harzianum* in vitro. In contrast, Basco et al. (2017), examinated maximum values were recorded in plants treated with *T. harzianum* fortified vermicompost.

Akrami and Yousefi (2015) evaluated the disease control was highest with a combination of *T. harzianum*, *T. asperellum*, and *T. virens* (80-87 %) followed by binary combination of *Trichoderma* spp. (79-82 %), while the lowest control was done with *T. viride* (65 %). It is concluded that *T. harzianum*, *T. asperellum* and *T. viride* could control pathogen attacks in tomato and it can be considered as an applicable strategy in control measures against pathogens.
Sara et al. (2014), studied that the interesting results were also obtained in vivo spraying tomato plants with a spore suspension of *Fusarium oxysporum* f.p. *lycopersici* and

Trichoderma sp. reduced the incidence of root and neck fusariosis compared to the untreated plants and inoculated ones by the pathogen. In addition, plants treated with *Trichoderma* sp. had a greater vegetative development.

The results agree with the idea that *T. virens* can promote plant growth by preventing the pathogen's harmful effects and decreasing the disease level. Comparison of applied *Trichoderma* spp. and yeast *S. cerevisiae* in soil and seed treatment has resulted in more efficient use of soil treatment and stronger management of tomato wilt disease. Moreover, further studies are required to confirm that *Trichoderma* induces systemic resistance as a mechanism for biological control of tomato Fusarium wilt. Also, applications of *Trichoderma* spp. were most effective against *Fusarium oxysporum* f.p. *lycopersici* in a soil treatment to compare with seed coating treatment.

The direct evidence from this research suggests that *Trichoderma* species can be used alone or with dry yeast *Saccharomyces cerevisiae,* would be an environmentally friendly strategy to plant disease management. It is found from the results of the current research that although all bio-control agents separately applied decreased incidence of disease. As well as, the most successful biocontrol agent for *Fusarium oxysporum* f. sp. *lycopersici* is the antagonist *T. virens.* Commonly known that environmental parameters such as abiotic and biotic factors as well as other factors such as application technique and timing may affect *Trichoderma* species biological control effectiveness. Based on this research, plant disease biocontrol agents could be utilized to save health danger for sustainable disease management programs. While several biocontrol agents were tried against Fusarium wilt disease, including botanicals, this deadly disease could not be completely controlled. In addition, a mixture of bioagents of different genera or a mixture of fungal and bacterial bioagents with or without fungicides or botanicals must be attempted to improve the level and extent of disease control under different environmental and soil conditions.

REFERENCES

Abo-Elyousr, K. A. M., Mohamed, H. M., 2009. Biological control of Fusarium wilts in tomato by plant growth-promoting yeasts and rhizobacteria. ***Plant Pathology Journal***, **25**(2): 199-204.

Agrios, G. N., 1988. ***Plant Pathology***, 3rd. edition. Academic press, Inc.: New York. 803.

Agrios, G. N., 2000. Significance of plant disease. ***Plant Pathology.*** Academic press London. 25-37.

Akköprü, A. and Demir, S., 2005. Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF Glomus intraradices and some rhizobacteria. ***Journal of Phytopathology***, **153**(9): 544-550.

Akrami, M., Yousefi, Z., 2015. Biological control of Fusarium wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as antagonist fungi. In ***Biological Forum-An International Journal,*** **7**(1): 887-892.

Alam, S. S., Sakamoto, K., Amemiya, Y., Inubushi, K., 2010. Biocontrol of soil-borne Fusarium wilts of tomato and cabbage with a root-colonizing fungus, ***Penicillium sp*. EU0013. In *19th World Congress of Soil Science, Soil Solutions for a Changing World***. August 2010.

Al-Shugairan, N. M. I., 2008. ***Biological Control of Fusarium oxysporum Wilt Disease of Tomato by Antagonistic and Plant Growth Promoting Actinomycetes.*** (MSc Thesis). United arab emirates university.

Andrews, J. H., 2002. Adhesion of yeasts to leaf surfaces. ***Phyllosphere Microbiology***. **47**(1): 25-35.

Armstrong, G. M., 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt disease. ***Fusarium: Disease, Biology, and Taxonomy***, **67**(3): 191-199.

Asghar, H., Diop, O. M., Weldegebriel, G., Malik, F., Shetty, S., El Bassioni, L., Akande, A. O., Al Maamoun, E., Zaidi, S., Adeniji, A. J., Burns, C. C., 2014. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. ***The Journal of Infectious Diseases***, **210**(1): 294-303.

AVRDC, Publication04-609, 2004. ***Tomato Mosaic Virus (ToMV).*** AVRDC. Available at: ***http://203.64.245.61/web_crops/tomato.*** Accessed 22 April 2015.

Aydın, M. H., Turhan, G., 2009. Studies of determination of fungal antagonists of *Rhizoctonia solani*. ***Anadolu***, **19**(2): 49-72.

Aydın, M. H., 2015. Biological control of fungul plant diseases with *Trichoderma*. ***Turkish Journal of Agricultural Research, 2***(2):135-148.

Babychan, M., Simon, S., 2017. Efficacy of *trichoderma spp*. against *Fusarium oxysporum* f.sp. *lycopersici*. (FOL) infecting pre-and post-seedling of tomato. ***Journal of Pharmacognosy and Phytochemistry***, **6**(4): 616-619.

Barari, H., 2016. Biocontrol of tomato Fusarium wilt by *Trichoderma* species under in vitro and in vivo conditions. ***Cercetari Agronomice in Moldova***, **49**(1): 91-98.

Basco, M. J., Bisen, K., Keswani, C., Singh, H. B., 2017. Biological management of Fusarium wilt of tomato using biofortified vermicompost. ***Mycosphere***, **8**(3): 467-483.

Beckman, C. H., 1987. ***The Nature of Wilt Diseases of Plants***. APS press.

Bell, D. K., Well, H. D., Markham, C. R., 1982. In vitro antagonism of *Ttrichoderma* species against six fungal plant pathogens. ***Phytopathology***, **72**: 379-382.

Benhamou, N., Chet, I., 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure and gold cytochemistry of the mycoparasitic process. ***Phytopathology-New York and Baltimore Then St Paul***, **83**: 1062-1062.

Benítez, T., Rincón, A. M., Limón, M. C., Codon, A. C., 2004. Biocontrol mechanisms of Trichoderma strains. ***International Microbiology***, **7**(4): 249-260.

Bhatnagar, K., Tak, S. K., Sharma, R. S., Majumdar, V. L., Meena, R. L., 2013. Management of cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini* through chemical and biological agents. ***Indian Phytopathology***, **66**(1): 101-102.

Borrero, C., Trillas, M. I., Delgado, A., Avilés, M., 2012. Effect of ammonium/nitrate ratio in nutrient solution on control of Fusarium wilt of tomato by *Trichoderma asperellum* T34. ***Plant Pathology***, **61**(1): 132-139.

Botstein, D., Fink, G. R., 2011. Yeast: An experimental organism for 21st century biology. ***Genetics***, **189**: 695-704.

Brammall, R. A., 1986. ***Host-Parasite Interactions in Fusarium Crown and Root Rot Disease of Tomato*** (Doctoral dissertation, Ph.D thesis, University of Toronto).

Brückner, H., Graf, H., 1983. Paracelsin, a peptide antibiotic containing α-aminoisobutyric acid, isolated from *Trichoderma ressei* simmons part A. ***Experientia***, **39**(5): 528-530.

Brückner, H., Graf, H., Bokel, M., 1984. Paracelsin; characterization by NMR spectroscopy and circular dichroism, and hemolytic properties of a peptaibol antibiotic from the cellulolytically active mnold *Trichoderma reesei*. Part B. ***Experientia***, **40**(11): 1189-1197.

Buck, J. W., 2002. In vitro antagonism of *Botrytis cinerea* by phylloplane yeasts. ***Canadian Journal of Botany***, **80**(8): 885-891.

CABI, Crop Protection Compendium, 2013. *Solanum lycopersicum* (tomato) datasheet. Available at: ***http://www.cabi.org/cpc/datasheet/31837***. Accessed 09.2.2014.

Cook R. J., Baker, K. F., 1983. The nature and practice of biological control of plant pathogens. ***American Phytopathological Society***. St.Paul. Minnesota. USA.

Crawford, D. L., Lynch, J. M., Whipps, J. M., Ousley, M. A., 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. ***Applied and Environmental Microbiology***, **59** (11): 3899-3905.

Demain, A. L., Fang, A., 2000. The natural functions of secondary metabolites. In ***History of Modern Biotechnology I***. Springer, Berlin, Heidelberg.1-39.

Dennis, C., Webster, J., 1971. Anntagonistic properties of species group of trichoderma, I, production of non_volatile antibiotics. ***Transactions of The British Mycological Society***, **57**: 25-39.

Devi, T. N., Linthoingambi, W., Singh, S. M., 2013. Evaluation of Trichoderma species against *Fusarium oxysporum* f. sp. *lycopersici* for biological control of tomato wilt. ***Indian Phytopathology,*** **66**: 81-87.

Demirci, E., Dane, E. and Eken, C., 2011. In vitro antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. ***Turkish Journal of Biology***, *35*(4): 457-462.

Durak, E.D., (2011). ***Anastomosis Groups, Pathogenetic And Biological Control Of Rhizoctonia Species Isolated From Strawberry Plants In Erzurum Province***.

(PhD Thesis). Ataturk University, Institute of Natural and Applied Sciences, Erzurum.

Durak, E.D., (2016), April. Biological control of Rhizoctonia solani on potato by using indigenous *Trichoderma* spp. **In *AIP Conference Proceedings***. **1726**(1): 020020.

Durak, E.D., (2018). Anastomosıs Groups, Pathogenıcıty And Bıologıcal Control Of Rhızoctonıa Specıes Isolated From Pepper (Capsıcum Annuum L.) Plants In Lake Van Basın. ***Fresenıus Envıronmental Bulletın***, ***27***(6): 4198-4205.

Dickinson, C. H., 1976. Fungi on the aerial surfaces of higher plants. ***Microbiology of Aerial Plant Surfaces***.

Dickinson, J. R., Schweizer, M., 2004. ***Life Cycle and Morphogenesis***. The metabolism and molecular physiology of *Sacharomyces cerevisiae*. London. 1-19.

Do Carmo-Sousa, L., 1969. Distribution of yeasts in nature. ***The Yeasts, Biology of Yeasts*, 1**: 79-105**.**

Doumbou, C. L., Hamby Salove, M. K., Crawford, D. L., Beaulieu, C., 2001. Actinomycetes, promising tools to control plant diseases and to promote plant growth. ***Phytoprotection***, **82**(3): 85-102.

Dutta, P., Das, B. C., 2002. Management of collar rot of tomato by *Trichoderma spp*. and chemicals. ***Indian Phytopathology***, **55** (2): 235-237.

Egel, D. S., Martyn, R. D., 2007. *Fusarium wilt of watermelon and other* cucurbits. ***The Plant Health Instructor*** *DOI: 10.1094*. PHI-I-2007-0122-01.

Elad, Y., Chet, I., 1987. Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. ***Phytopathology***, **77**(2): 190-195.

El-Tarabily, K. A., Sivasithamparam, K., 2006. Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. ***Soil Biology and Biochemistry***, **38**(7): 1505-1520.

El-Tarabily, K. A., Sivasithamparam, K., 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. ***Mycoscience***, **47**(1): 25-35.

El-Tarabily, K. A., 2006. Rhizosphere-competent isolates of streptomycete and non-streptomycete actinomycetes capable of producing cell-wall-degrading enzymes to control Pythium aphanidermatum damping-off disease of cucumber. ***Botany***, **84** (2): 211-222.

Fravel, D., Olivain, C., Alabouvette, C., 2003. Fusarium oxysporum and its biocontrol. ***New phytologist***, ***157***(3): 493-502.

Gangopadhyay, S., Gopal, R. and Godara, S. L., 2009. Effect of fungicides and antagonists on Fusarium wilt of cumin. ***Journal of Mycology and Plant Pathology***, **39**(2): 331.

Gao, K. X., Liu, X. G., Liu, Y. H., Zhu, T. B., Wang, S. L., 2002. Potential of *Trichoderma harzianum* and atroviride to control *botryosphaeria berengeriana* f.sp. piricola, the cause of apple ring rot. ***Journal of Phytopathology*, 150**: 271-276.

Gardener, B. B. M., Fravel, D. R., 2002. Biological control of plant pathogens research. commercialization, and application in the USA. ***Plant Health Progress***, **3**(1): 17.

Garrett, S. D., 1970. Pathogenic root-infecting fungi. ***Pathogenic Root-Infecting Fungi***. Ghazalibiglar, H., Kandula, D. R. W., Hampton, J. G., 2016. Biological control of Fusarium wilt of tomato by Trichoderma isolates. ***Microbial Control***, **69**: 57-63.

Ghisalberti, E. L., Narbey, M. J., Dewan, M. M., Sivasithamparam, K., 1990. Variability among strains of *Trichoderma harzianum* in their ability to reduce take-all and to produce pyrones. ***Plant and Soil***, **121**(2): 287-291.

Gomez, K. A., Gomez, A. A., 1984. Duncan's multiple range test. ***Statistical Procedures for Agricultural Research***, **2**: 540-544.

Hadar, Y., Chet, I., Henis, Y., 1979. ***Biological Control of Rhizoctonia Solani Damping-Off with Wheat Bran Culture of Trichoderma Harzianum [Soilborne Pathogens, Kidney Beans, Tomatoes, Eggplants]*** (No. RESEARCH).

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., Lorito, M., 2004. Trichoderma species opportunistic, avirulent plant symbionts. ***Nature Reviews Microbiology***, **1**: 43-56.

He, D., Zheng, X. D., Yin, Y. M., Sun, P., Zhang, H. Y., 2003. Yeast application for controlling apple postharvest diseases associated with Penicillium expansum1. ***Botanical Bulletin of Academia Sinica***, **44**.

Held, P., 2010. ***Monitoring Growth of Beer Brewing Strains of Saccharomyces Cerevisiae***. Monitoring of cell suspensions by kinetic absorbance measurements. Ph.D thesis. 2-6.

Herskowitz, I., 1988. Life cycle of the budding yeast *Saccharomyces cerevisiae*. ***Microbiological Reviews***, **52**(4): 536.

Hesamadin, R., 2010. Antagonistic effects of trichoderma spp. against *Fusarium oxysporum* f. sp. lycopersici causal agent of tomato wilt. ***Journal of Plant Protection Research***, **2**: 167-173.

Hjeljord, L. G., Stensvand, A., Tronsmo, A., 2000. Effect of temperature and nutrient stress on the capacity of commercial Trichoderma products to control Botrytis cinerea and Mucor piriformis in greenhouse strawberries. ***Biological Control***, **19**(2): 149-160.

Hooykaas, P. J., den Dulk-Ras, A., Bundock, P., Soltani, J., van Attikum, H., van Heusden, G. P. H., 2006. Yeast (*Saccharomyces cerevisiae*). In ***Agrobacterium Protocols Volume 2***, Humana Press, 465-473.

Houssien, A. A., Ahmed, S. M., Ismail, A. A., 2010. Activation of tomato plant defense response against Fusarium wilt disease using *Trichoderma harzianum* and salicylic acid under greenhouse conditions. ***Research Journal of Agriculture and Biological Sciences***, **6**(3): 328-338.

Howell, C. R., 2003. Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. ***Plant Disease***, **87**(1): 4-10.

Inoue, I., Namiki, F., Tsuge, T., 2002. Plant colonization by the vascular wilt fungus *Fusarium oxysporum* requires FOW1, a gene encoding a mitochondrial protein. ***The Plant Cell***, **14**(8): 1869-1883.

Jackson, A. M., Whipps, J. M., Lynch, J. M., 1991. In vitro screening for the identification of potential biocontrol agents of Allium white rot. ***Mycological Research***, **95**(4): 430-434.

Jambhulkar, P. P., Babu, S. R., Ameta, G. S., 2011. Comparative efficacy of fungicides and antagonists against Fusarium wilt of chickpea. ***Journal of Mycology and Plant Pathology***, **41**(3): 399.

Jamwal, S., Jamwal, A., Verma, V. S., 2011. Effect of bio control agents on wilt management and plant growth of tomato. ***Indian Phytopathology***, **64**(4): 381.

Jones, J. P., Jones, J. B., Miller, W., 1982. Fusarium wilt on tomato. ***Plant Pathology Circular***,**237**. ***https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=582733***

Jones, J. P., Stall, R. E., Zitter, T. A., 1991. Compendium of tomato diseases. ***American Phytopathological Society Press***.

Jones, J. P., Woltz, S. S., 1981. Fusarium incited-diseases of tomato and potato and their control. In '***Fusarium: Disease, Biology and Taxonomy***'. (Eds PE Nelson, TA Toussoun and RJ Cook.), 157–65.

Kopsell, D., 2000. Growing tomatoes. University of New Hampshire Cooperative Extension. Available at: ***http://extension.unh.edu/resources/fi***. Accessed: 09.02.2015.

Kuc, J., 2001. Concepts and direction of induced systemic resistance in plants and its application. ***European Journal of Plant Pathology,*** **107**: 7-12.

Lerner, B. R., Tomatoes. Purdue University Cooperative Extension. Available at: ***http://www.hort.purdue.edu/ext/HO-26.PDF***. Accessed: 09.02.2015.

Martini, A., 1993. Origin and domestication of the wine yeast saccharomyces cerevisiae. ***Journal of Wine Research,*** **4**: 165-176.

Massart, S., Jijakli, H. M., 2007. Use of molecular techniques elucidates the mechanisms of action of biocontrol agents: A review. ***Journal of Microbial Methods***, **69**: 229-241.

MAsse, E., 1895. GE: *Sclerotinia sclerotiorum* Mass. ***British Fungus Flora***, **4**: 280.

Mehra, R., 2006. Biocontrol of Fusarium wilt of tomato using antagonistic microorganisms. ***Plant Disease Research-Ludhiana-***, **21**(2): 146.

Mishra, P. K., Mukhopadhyay, A.N. and Singh, U.S., 2004. Suppression of *Fusarium oxysporum* f. sp. gladioli populations in soil by application of *Trichoderma virens* and in vitro approaches for understanding biological control mechanisms. ***Indian Phytopathology***, **57**(1): 44-47.

Morrell, J. J., Bloom, J. R., 1981. Influence of meloidogyne incognita on Fusarium wilt of tomato at or below the minimum temperature for wilt development. ***Journal of Nematology***, **13**(1): 57.

Moyad, M. A., 2008. Brewer's/baker's yeast (*Saccharomyces cerevisiae*) and preventive medicine: Part II. ***Urologic Nursing Journal***, **28**(1): 73-75.

Mwangi, M. W., Monda, E. O., Okoth, S. A., Jefwa, J. M., 2011. Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. ***Brazilian Journal of Microbiology***, **42**(2): 508-513.

Nagodawithana, W. T., 1991. ***Yeast Technology.*** Universal foods cooperation milwauke, Wisconsin.

Naher, L., Yusuf, U. K., Ismail, A., Hossain, K., 2014. *Trichoderma spp*.: a biocontrol agent for sustainable management of plant diseases. ***Pakistan Journal of Botany***, **46**(4): 1489-1493.

Naito, K., Nagumo, S., Furuya, K., Suzuki, H., 1981. Effect of benzyladenine on RNA and protein synthesis in intact bean leaves at various stages of ageing [kidney bean, chloroplast ribosome, polysome]. ***Physiologia Plantarum*** (Denmark).

O'Kennedy, K., Reid, G., 2008. Yeast nutrient management in winemaking. ***Yeast in Winemaking,*** **537**: 92-100.

Okungbowa, F. I., Shittu, H. O., 2012. Fusarium wilts: An overview. ***Environmental Research Journal***, **6**: 83-102.

Okungbowa, F. I., Shittu, H. O., 2012. Fusarium wilts: An overview. ***Environ. Res. J***, **6**, 83-102.

Olivain, C., Alabouvette, C., 1997. Colonization of tomato root by a non-pathogenic strain of Fusarium oxysporum. ***The New Phytologist***, **137**(3): 481-494.

Omran, Y. A., 2000. ***Studies on Histophysiological Effect of Hydrogen Cyanamide (Dormex) and Yeast Application on Bud Fertility, Vegetative Growth and Yield of, Roumi Red"Grape Cultivar***. Ph. D. thesis, Fac of Agric assiut univ Egypt.

Osuinde, M. I., Aluya, E. I., Emoghene, A. O., 2002. Control of Fusarium wilt of tomato (*Lycopersicon esculentum* Mill) by *Trichoderma species*. ***Acta Phytopathologica Et Entomologica Hungarica***, **37**(1-3): 47-55.

Pal, K. K., Gardener, B. M., 2006. ***Biological Control of Plant Pathogens***.

Pan, S., Bose, S., Jash, S., 2009. Management of root rot of cabbage (*Rhizoctonia solani*) and groundnut collar rot (*Sclerotium rolfsii*) with formulation of *Trichoderma harzianum*. ***Journal of Mycology and Plant Pathology***, **39**(2): 203.

Pandey, K. K., Upadhyay, J. P., 1999. Comparative study of chemical, biological and integrated approach for management of Fusarium wilt of pigeonpea. ***Journal of Mycology and Plant Pathology***, **29**(2): 214-216.

Papavizas, G. C., 1985. Trichoderma and Gliocladium: biology, ecology, and potential for biocontrol. ***Annual Review of Phytopathology***, **23**(1): 23-54.

Patel, S., Saraf, M., 2017. Biocontrol efficacy of *Trichoderma asperellum* MSST against tomato wilting by *Fusarium oxysporum* f. sp. lycopersici. ***Archives of Phytopathology and Plant Protection***, **50**(5-6): 228-238.

Pegg, G. F., Brady, B. L., 2002***. Verticillium Wilts***. CAB International. Oxford. Puhalla JE, Hummel H (1983). Vegetative compatibility. Pierson LS, Thomashow LS (1992) Cloning and heterologous expression of the phenazine biosynthesis locus from Pseudomonas aureofaciens. ***Molecular Plant-Microbe Interactions***, ***5***: 330-339.

Petersson, S., Jonsson, N., Schnürer, J., 1999. *Pichia anomala* as a biocontrol agent during storage of high-moisture feed grain under airtight conditions. ***Postharvest Biology and Technology***, **15**(2): 175-184.

Prasad, R. D., Rangeshwaran, R., Hegde, S. V., Anuroop, C. P., 2002. Effect of soil and seed application of *Trichoderma harzianum* on pigeonpea wilt caused by *Fusarium udum* under field conditions. ***Crop Protection***, **21**(4): 293-297.

Punja, Z. K., 1997. Comparative efficacy of bacteria, fungi, and yeasts as biological control agents for diseases of vegetable crops. ***Canadian Journal of Plant Pathology***, **19**(3): 315-323.

Ramezani, H., 2010. Antagonistic effects of *trichoderma spp*. against *fusarium oxysporum f.sp. lycopersici* causal agent of tomato wilt, ***Plant Protection Journal***, **2**(1): 167-173.

Redda, E. T., Ma, J., Mei, J., Li, M., Wu, B., Jiang, X., 2018. Biological control of soilborne pathogens (*Fusarium oxysporum F. Sp. Cucumerinum*) of cucumber (*Cucumis sativus*) by Trichoderma sp. ***Journal of Life Sciences***, **12**: 1-12.

Rick, C. M., 1976. ***Tomato. Evaluation of Crop Plant***, Simmonds, N.W (Ed) Longman. New York. 263-273.

Rick, C. M., 1979. The biology and taxonomy of the solanaceae. ***Biosystematics Studies in Lycopersicon and Closely Related Species of Solanum***. 667-677.

Rini, C. R., Sulochana, K. K., 2008. Substrate evaluation for multiplication of *Trichoderma spp*. ***Journal of Tropical Agriculture***, **45**(1): 55-57.

Royse, D. J., Ries, S. M., 1978. The influence of fungi isolated from peach twigs on the pathogenicity of Cytospora cincta. ***Phytopathology***, **68**(4): 603-607.

Sabuquillo, P., De Cal, A., Melgarejo, P., 2006. Biocontrol of tomato wilt by *Penicillium oxalicum* formulations in different crop conditions. ***Biological Control,*** **37**: 256-265.

Sarhan, T. Z., 2011. Effect of bread yeast application and seaweed extract on cucumber (*Cucumis sativus* L.) Plant growth, yield and fruit quality. ***Mesopotamia Journal of Agriculture***, **39**(2): 26-32.

Schumann, G. L., D'Arcy, C. J., 2006. ***Essential Plant Pathology***. American phytopathological society (APS Press).

Shalaby, M. E., El-Nady, M. F., 2008. Application of *saccharomyces cerevisiae* as a biocontrol agent against fusarium infection of sugar beet plants. ***Acta Biological Szegediensis,*** **52**(2): 271-275.

Sharma, p., Kumar, P. V., Ramesh, R., Saravanan, K., Deep, S., Sharma, M., Mahesh, S., Dinesh, S., 2011. Biocontrol genes from *Trichoderma* species. ***African Journal of Biotechnology***, **10** (86): 19898-19907.

Sherman, F., 2002. Getting started with yeast. ***Methods in Enzymology***, **350**: 3-41.

Shittu, H. O., Shakir, A. S., Nazar, R. N., Robb, J., 2009. Endophyte-induced Verticillium protection in tomato is range-restricted. ***Plant Signaling and Behavior***, **4**(2): 160-161.

Singh, F., Hooda, I., Sindhan, G. S., 2004. Biological control of tomato wilt caused by *Fusarium oxysporum* f. sp. lycopersici. ***Journal of Mycology and Plant Pathology***, **34**(2): 568-570.

Singh, J., kumar, V., Srivastava, S., Kumar, A., Singh, V. P., Hadian, S., Rahnama, K., Jamali, S., Eskandari, A., Dodson, M., Bachmann, J., 2011. Biocontrol mechanisms of Trichoderma strains. ***Plant Pathology Journal***, **17**(2): 2052-2057.

Singh, P. K., Mishra, M., Vyas, D., 2007. Efficacy of *Trichoderma spp*. strains in the control of Fusarium wilt of tomato. ***Journal of Mycology and Plant Pathology,*** **37**: 105-107.

Sivan, A., Ucko, O., Chet, I., 1987. Biological control of fusarium crown rot of tomato by trichoderma harzianum under field conditions. ***Plant Diseases***, **71**: 587-592.

Sivasithamparam, K., Ghisalberti, E. L., 1998. Secondary metabolism in Trichoderma and Gliocladium. ***Trichoderma and Gliocladium Basic Biology Taxonomy and Genetics***, **1**: 139-191.

Smith, I. M., Dunez, J., Phillips, D. H., Lelliott, R. A., Archer, S. A., 1988. ***European Handbook of Plant Diseases,*** Blackwell scientific publications: Oxford. 583.

Song, W., Zhou, L., Yang, C., Cao, X., Zhang, L., Liu, X., 2004. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. ***Crop protection***, **23**(3): 243-247.

Spencer, J. F. T., Spencer, D. M., 1997. Ecology: where yeasts live. In ***Yeasts in Natural and Artificial Habitats***, Springer, Berlin, Heidelberg. 33-58.

Sreenu, B., Zacharia, S., 2017. In vitro screening of plant extracts, *Trichoderma harzanium* and carbendazim against *fusarium oxysporum* f.sp. lycopersici on

tomato. ***International Journal of current Microbiology and Applied Sciences***, **6**(8): 2319-7706.

Srinon, W., Chuncheen, K., Jirattiwarutkul, K., Soytong, K., Kanokmedhakul, S., 2006. Efficacies of antagonistic fungi against fusarium wilt disease of cucumber and tomato and the assay of its enzyme activity. ***Journal of Agricultural Technology***, **2**(2):191-201.

Steyaert, J. M., Ridgway, H. J., Elad, Y., Stewart, A., 2003. Genetic basis of mycoparasitism: a mechanism of biological control by species of Trichoderma. ***New Zealand Journal of Crop and Horticultural Science***, **31**(4): 281-291.

Sundaramoorthy, S., Balabaskar, P., 2013. Biocontrol efficacy of *Trichoderma Spp.* agaenst wilts of tomato caused by *Fusarium oxysporum f.sp.* lycopersici. ***Journal of Applied Biology and Biotechnology***, **1**(03): 036-040.

Sunil, K., Arya, M. C., Ranjit, S., 2010. Management of sweet pepper diseases and growth promotion by *Pseudomonas fluorescens* and *Trichoderma harzianum* in mid Hills of central Himalayas, India. ***Indian Phytopathology***, **63**(2): 181-186.

Ter Linde, J. J. M., Liang, H., Davis, R. W., Steensma, H. Y., Van Dijken, J. P., Pronk, J. T., 1999. Genome-wide transcriptional analysis of aerobic and anaerobic chemostat cultures of *Saccharomyces cerevisiae*. ***Journal of Bacteriology***, **181**(24): 7409-7413.

Theradimani, M., Susitha, S., Amudha. C., 2018. Biocontrol of fusarium wilt in tomato caused by *Fusarium oxysporum f.sp. lycopersici*. ***International Journal of Current Microbiology and Applied Sciences***, **7**(09): 2319-7706.

Urquhart, E. J., Punja, Z. K., 1997. Epiphytic growth and survival of *Tilletiopsis pallescens*, a potential biological control agent of *Sphaerotheca fuliginea*, on cucumber leawes. ***Canadian Journal of Botany***, **75**(6): 892-901.

Verma, S., Dohroo, N. P., 2005. Novel approach for screening different antagonists against *Fusarium oxysporum f. sp. pisi* causing Fusarium wilt of autumn pea. ***Plant Disease Research-Ludhiana-***, **20**(1): 58.

Vinale, F., Marra, R., Scala, F., Ghisalberti, E. L., Lorito, M., Sivasithamparam, K., 2006. Major secondary metabolites produced by two commercial Trichoderma strains active against different phytopathogens. ***Letters in Applied Microbiology***, **43**(2): 143-148.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., Lorito, M., 2008. Trichoderma–plant–pathogen interactions. ***Soil Biology and Biochemistry***, **40** (1): 1-10.

Waksman, S. A., 1950. The Actinomycetes-their nature, occurrence, activities, and importance. ***The Actinomycetes-Their Nature, Occurrence, Activities, and Importance***.

Walker, J. C., 1971. Fusarium wilt of tomato: Monograph 6. ***The American Phytopathological Society, St. Paul, MN.***

Wareing, P. F., Phillips, I. D. J., 1970. The control of growth and differentiation in plants. ***The Control of Growth and Differentiation in Plants***.

Weindling, R., 1932. Trichoderma lignorum as a parasite of other soil fungi. ***Phytopathology***, **22**(8): 837-845.

Weindling, R., 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. ***Phytopathology***, **24**(1): 153-151.

Whipps, J. M., 2001. Microbial interactions and biocontrol in the rhizosphere. ***Journal of Experimental Botany***, **52**(1): 487-511.

Wilson, C. L., Wisniewski, M. E., Biles, C. L., McLaughlin, R., Chalutz, E., Droby, S., 1991. Biological control of post-harvest diseases of fruits and vegetables: alternatives to synthetic fungicides. ***Crop Protection***, **10**(3): 172-177.

Windels, C., 1992. Fusarium. ***Methods for Research on Soilborne Phytopathogenic Fungi***.

Wokoma, E. C. W., 2008. Preliminary report on diseases of tomato in choba, rivers state. ***Journal of Applied Sciences and Environmental Management***, **12**(3).

Wolebo, T., Alemu, T., Assefa, F., 2015. Evaluation of antagonistic activities of Trichoderma isolates against Fusarium wilt (*Fusarium oxysporum*) of tomato (*Lycopersicon esculentum* Mill.) isolates. ***Ethiopian Journal of Biological Sciences***, **14**(2): 129-145.

Yuan, W. M., Crawford, D. L., 1995. Characterization of streptomyces lydicus WYEC108 as a potential biocontrol agent against fungal root and seed rots. ***Applied Environmental Microbiology***, **61**(8): 3119-3128.

EXTENDED TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

DOMATESTE *Fusarium oxysporum* f.sp. *lycopersici'* nin *Trichoderma* spp. ve *Saccharomyces cerevisiae* ile BİYOKONTROLÜ

RASUL, Shawen Zrar
Yüksek Lisans Tezi, Bitki Koruma Anabilim Dalı
Tez Danışmanı: Dr. Emre DEMİRER DURAK
Eylül, 2019, 75 Sayfa

ÖZ

Bu çalışmada biyolojik mücadele elemanları *Trichoderma* spp. ve kuru maya *Saccharomyces cerevisiae'* nın tek ve kombinasyonlarının domates bitkisinin gelişim parametrelerine ve Fusarium solgunluğu hastalığına etkileri araştırılmıştır. In vivoda biyokontrol elemanları tohum kaplama ve toprak uygulaması olarak iki farklı şekilde denenmiştir. In vitro sonuçlarına göre *Trichoderma virens* patojenin misel gelişimini önemli oranda engellemiştir. *Fusarium oxysporum* f. sp. *lycopersici'* yi sırasıyla *T. virens* % 44.60, *T. asperellum* % 21.77 ve *T. harzianum* % 19.03 oranında engellemiştir. In vivoda bütün *Trichoderma* türleri ve maya patojenin domates bitkilerindeki hastalık şiddetinde azalmaya neden olmuşlardır. Tohum kaplama yönteminde *T. virens* uygulanan bitkilerde patojenin oluşturduğu hastalık şiddeti değeri en düşük (% 10.22) bulunurken *T. asperellum* (% 53.33) en yüksek değeri vermiştir. *Trichoderma* türlerinin birlikte kullanıldığı toprak uygulamasında ise patojenin engellendiği belirlenmiştir. Toprak uygulamasında diğerlerine göre *T. asperellum* en yüksek hastalık şiddetine (% 25) sebep olmuştur. Ayrıca, en iyi sonuç toprak muamelesinde *S. cerevisiae*' nin *Trichoderma* türleri ile birlikte uygulandığında görülmüştür. In vivo ve in vitro sonuçlara göre *T. virens F. oxysporum'* a en etkili antagonist olarak belirlenmiştir. Ayrıca bu çalışma ile *T. harzianum, T. virens* ve *T. asperellum'* un domateslerde patojeni kontrol edebildiği belirlenmiştir ve bu türler ile biyolojik mücadele uygulanabilir bir hastalık kontrol yaklaşımı olarak düşünülebilir.

Anahtar kelimeler: Biyolojik kontrol, Domates, *Fusarium oxysporum* f. sp. *lycopersici*, S*accharomyces cerevisiae, Trichoderma* spp.

1. GİRİŞ

Domates (*Lycopersicon esculentum*), dünyanın en çok üretilen ve tüketilen sebzelerin başında gelmektedir (Rick, 1979). Bu ürünün tazesi, konservesi ve kurutulmuşu tüketilebilmekte olup aynı zamanda son yıllarda kansere karşı koruyucu özelliği bulunduğu da bildirilmektedir (Barari, 2016).

Dünyanın birçok yerinde bitki hastalıkları domates üretiminde sınırlayıcı bir faktör haline gelmiştir. Domates hastalıkları patojenik mikroorganizmaların neden olduğu paraziter ve diğer abiyotik faktörlerin (fizyolojik hastalıklar) neden olduğu paraziter olmayan hastalıklar olarak sınıflandırılabilir.

Domates Fusarium Solgunluk Hastalığı

G.E. Massee ilk olarak 1895 yılında İngiltere'de domateslerde Fusarium solgunluğunu tanımlamıştır. Hastalığa sebep olan etmen vasküler solgunluk fungusu olan *F. oxysporum* f. sp. *lycopersici* (FOL)' dir. Fungus, doğrudan vasküler dokuya girer ve orada kolonize olur (Inoue ve ark. 2002). Vasküler sistemde koyulaşma hastalığın bir özelliğidir ve genellikle onu tanımlamak için kullanılabilir (Beckman, 1987).

Fusarium oxysporum'un kontrolü

Fusarium'un neden olduğu hastalıkları kontrol etmek zordur (Borrero ve ark. 2006; Elmer, 2006). Direnişe neden olarak hastalığı bastırmak için çeşitli kimyasal fungisitler kullanılır, ancak bunlar insan sağlığı üzerinde zararlı bir etkiye sahiptir ve çevre açısından tehlikelidir. Kimyasalların tek kontrol tekniği olarak kullanılması, toprak mikroorganizma popülasyonları üzerinde hedef olmayan etkilere yol açabilir. Toprak mikroorganizmaları olan *Trichoderma harzianum,* T. *asperellum, T. koningii, Penicillium* spp. ve *Streptomyces griseoviridis*, bitki rizosferinde kalıntı bırakan kimyasallara göre daha iyi bir seçenek olup patojenleri baskılayabilir ve fitohormon

üretimi ile komplike substratların bozunması yoluyla bitki büyümesini arttırabilir (Osuide et al., 2002; Syed vd., 2010; Borrero ve ark., 2011).

Biyolojik Kontrol

Biyolojik mücadele bazı araştırıcılara göre "bir organizmanın diğer organizmanın zarar verici aktivitelerini kontrol altına alması" olarak tanımlanmaktadır. Biyolojik mücadele mekanizmaları antibiyosis, yarışma, hiperparazitizm, hipovirülens, uyarılmış dayanıklılık ve çapraz koruma olmak üzere altı ana başlık altında toplanmaktadır. Ayrıca, biyokontrol organizmaların bitkilerin köklerindeki mikroflora kompozisyonunu değiştirmesi, bitkilerde dayanıklılığı uyarması, besin maddesi alımını arttırması ve kök gelişimini teşvik etmesi ve bunlara bağlı olarak bitki büyümesini ve gelişimini arttırması gibi etkilerinin de olduğu bildirilmektedir.

Trichoderma spp.

Trichoderma türlerinin, 1930'ların başında biyolojik kontrol kapasitesine sahip olduğu belirlenmiştir (Weindling, 1934). *Trichoderma*, pek çok patojenik bitki fungusuna karşı antagonistik ve paraziter bir organizma görevi gören ve bitki hastalıklarından korunma sağlayan, fırsatçı, avirulent bir bitki simbiyont fungusudur. *Trichoderma* türlerinin etkinliliği birçok araştırmada kanıtlanmıştır. Bitki hastalığı yönetimi için verimli biyolojik kontrol ajanlarıdır ve şu anda ticari biyopestisitler veya toprak iyileştirmeleri için bitki büyüme arttırıcıları olarak piyasada mevcuttur (Papavizas, 1985; Chet 1987; Harman, 2004; Vinale ve diğerleri, 2008).

Maya

Saccharomyces cerevisiae, eski çağlardan beri gıdalarda kullanılan bir maya türüdür ve son araştırmalarda bitki büyümesini teşvik edici özelliği de bulunduğu belirlenmiştir. Son yüzyılda insanlarda, hayvanlarda ve çevrede güvenle kullanılan kimyasal gübrelere olumlu bir alternatif haline gelmiştir (Omran, 2000).

Mayaların çeşitli patojenlere karşı biyokontrol ajanları olarak kullanılması yeni bir trend olmuştur. Mayaların, toprak kaynaklı fungal patojenlerinin biyolojik kontrol ajanları ve bitki büyümesinin destekleyicileri olarak potansiyel kullanımı son zamanlarda araştırılmıştır (ElTarabily ve Sivasithamparam, 2006).

Araştırma amacı

Bu tezde, *F. oxysporum* f.sp. *lycopersici*' nin neden olduğu solgunluk hastalığının biyolojik kontrol imkanları araştırılmıştır. *Trichoderma* türlerinin patojenin gelişimi üzerine etkisi in vitroda test edilmiştir. Bununla birlikte hem *Trichoderma* türlerinin hem de *Saccharomyces cerevisiae*' nın in vivo koşullar altında domates bitkisinin gelişim parametrelerine etkisi ve hastalığı baskılama durumu iki yöntemle (tohum kaplama ve toprak muameleleri) denenmiştir.

2. MATERYAL VE YÖNTEM

Materyal

Domates çeşidi olarak yörede en çok yetiştirilen Alsancak kullanılmıştır. Patojen *Fusarium oxysporum* f. sp. *lycopersici* ve biyokontrol *Trichoderma* spp. (*Trichoderma harzianum*, *Trichoderma virens*, *Trichoderma asperellum*) fungusları Van Yüzüncü Yıl Üniversitesi Bitki Koruma Bölümü Mikoloji kültür stoğundan temin edilmiştir. Ticari kuru maya (*Saccharomyces cerevisiae*) diğer biyokontrol organizma olarak kullanılmıştır. Çalışmada organizmaların geliştirilmesinde patates dektroz agar, su agarı, yeast mannitol broth ve yeast extract kullanılmıştır.

Yöntem

1. In vitro

T. harzianum, T. virens ve T. asperellum' un antagonistik etkisi in vitroda *F. oxysporum'* a karşı denenmiştir. Petri kaplarına karşılıklı ekim yöntemiyle yapılan denemede antagonizmin derecesini belirleyen 1-5 skalası kullanılmıştır.

Antagonizmin derecesini belirleyen 1-5 skalası:

1= *Trichoderma* tamamen patojenin üzerinde gelişmekte ve besiyerini tamamen kaplamaktadır.

2= *Trichoderma* ortam yüzeyinin üçte ikisini kaplamaktadır.

3= *Trichoderma* ve patojen her ikisi de hemen hemen ortam yüzeyinin yarısını kaplamakta ve hiçbiri bir diğerine baskın olamamaktadır.

4= Patojen ortamın üçte ikisini kaplamakta ve *Trichoderma*'nın baskısına dayanmaktadır.

5= Patojen tamamen *Trichoderma*'nın üstünde gelişmekte ve yüzeyi kaplamaktadır.

Bu skalaya göre eğer sonuç ≤2 olursa *Trichoderma* izolatı patojene karşı yüksek oranda antagonistik özellik göstermekte, ≥3 olursa antagonistik özellik yüksek olmamaktadır (Bell *et al*. 1982).

Fusarium izolatlarının gelişiminin antagonist tarafından engellenmesi aşağıdaki formül ile hesaplanmıştır (Royse and Ries 1978).

RI= (R1-R2)/R1x100

Burada;

RI (İnhibisyon oranı %): Büyümenin antagonist tarafından engellenmesi,

R1: Patojenin koloni yarı çapı

R2: Patojenin antagonist yönündeki büyüme yarı çapı.

2. In vivo

İklim odasında denemeler tohum kaplama ve toprak uygulaması olarak iki yöntemle yapılmıştır.

Tohum kaplama yöntemi

Tohumlar yüzeysel sterilizasyon işlemine tabi tutulduktan sonra *Trichoderma* türleri ile muamele edilmişlerdir. Bunun için izolatlar geliştirilmiş, hemositometre yardımıyla spor konsantrasyonları 10^7 konidi/ml olarak ayarlanmış ve %2 oranında arap zamkı eklenen süspansiyon hazırlanmıştır. Tohumlar 10 dakika süspansiyonda bekletilmiştir. Maya (*Saccharomyces cerevisiae*)' da besiyerinde geliştirildikten sonra hemositometre ile 10^6 cells/ml olarak ayarlanmış ve %2 oranında arap zamkı eklenen süspansiyonda 10 dakika bekletilmiştir. Tohumlar bir gece inkübatörde tutulmuştur. Viyollere ekilen tohumlar fide olunca saksılara şaşırtılmış ve patojen ile inokule edilmiştir. Bunun için geliştirilen Fusarium izolatı hemositometre ile 10^7 konidi/ ml olarak ayarlanmış ve şaşırtma işlemi sırasında bitkilere 10 dakika kök daldırma yöntemi uygulanmıştır. Üç tekerrürlü kurulan deneme iklim odası koşullarında yapılmıştır.

Toprak uygulaması yöntemi

Bu kısımda biyokontrol izolatlar hazırlanan ortama bulaştırılmıştır. Geliştirilen *Trichoderma izolatları* 10^7 spor/ml olarak hazırlanıp 20 ml süspansiyon her bir saksıya dökülmüştür. Maya ise 2.5 gm/L hazırlanıp 100 ml süspansiyon olarak saksılara dökülmüştür. kontrol saksılara ise steril su ilave edilmiştir. Bu şekilde hazırlanan saksılar iklim odasında birkaç gün tutulmuştur.

Domates tohumları steril edilmiş, viyollere ekilmiş, geliştiklerinde saksılara şaşırtılmıştır. Şaşırtma işlemi sırasında daha önce anlatılan şekilde Fusarium ile bulaştırılmışlardır. Altı- yedi hafta sonra bütün bitkiler sökülmüş, kökler yıkanmış hastalık şiddeti skalası kayıt edilmiştir. Kullanılan skala: 0, sağlıklı kökler, 1, genellikle kök uçlarında görülen hafif kahverengileşme, 2, orta derecede kahverengileşme ve 3, şiddetli kahverengileşme. Ayrıca bitki boyları ölçülmüş, yaş ağırlıkları tartılmış, 65°C' de 48 saat tutulduktan sonra kuru ağırlıkları da belirlenmiştir. Duncan çoklu karşılaştırma testi kullanılarak istatistiksel analizler yapılmıştır.

3. BULGULAR

In Vitro

Test edilen tüm *Trichoderma* türleri, ikili kültürde *Fusarium oxysporum*'un miselyel büyümesini inhibe etmiştir. Fakat Trichoderma türleri arasında önemli farklılıklar olmuştur. F. *oxysporum*'un büyüme inhibisyonu, sırasıyla *T. harzianum* (T1) (% 19.03), *T. virens* (T2) (% 44.60) ve *T. asperellum* (T3) (% 21.77) ile azaltılmıştır.

Buna göre, *Trichoderma virens*, test patojeninin gelişimini en yüksek yüzde (% 44.60) ile inhibe etmiştir.

In Vivo

1. Tohum kaplama

Bu denemede kullanılan organizmaların etkinliliği iki teknik ile değerlendirilmiştir. İlk teknikte tohum kaplama uygulamaları incelenmiştir. T1 + T2 + T3 birlikte uygulandığında bitki boyunun (119.66 cm) en yüksek olduğu, ancak taze ve kuru bitki ağırlığı üzerinde aynı etkiye sahip olmadığı belirlenmiştir. *T. virens,* diğer uygulamalar ile karşılaştırıldığında taze (87.64 g) ve kuru (35.72 g) bitki ağırlığını önemli ölçüde arttırmıştır. Patojen bulunan uygulamalarda *T. harzianum,* bitki boyunu, *T. virens ve T. asperellum*'a kıyasla önemli ölçüde arttırmıştır (106.00 cm), ancak bitkilerin taze ve kuru ağırlıkları üzerinde anlamlı bir etkisi olmamıştır. F +T3 denemesinde 1.60 skala ile % 53.3 en yüksek, F+T2 uygulamasında 0.20 skala ve % 10.22 hastalık şiddeti ile en düşük değerleri vermiştir. Maya ile beraber T. virens uygulandığında skala değeri (0.25) ve hastalık şiddetinin (% 8.3) en düşük oranda olduğu belirlenmiştir. Maya ile *F. oxysporum* muamele edildiğinde, bitki boyunda artış olmazken (87,75 cm), F + S + T1 (98.75 cm), F + S + T2 (99.50 cm), F + S + T3 (10, 25 cm) F + S + T1 + T2 + T3 (105.66 g) olarak belirlenmiştir. F + S + T1 + T2 + T3 bitki kuru ağırlığını (17,12 g) arttırmış, ancak bitki taze ağırlığı üzerinde önemli bir etkisi olmamıştır.

2. Toprak uygulaması

Tek olarak ve biyolojik kontrol organizmalarının karışımı, iklim odası denemesinde domates bitkilerinin morfolojik gelişimini önemli ölçüde etkilemiştir. Sonuç olarak, T1 + T2 + T3 muamelesinde boylarda belirgin bir artışın (111,66 cm) olduğu ve en az T3 (97,33 cm) uygulamasında olduğu belirlenmiştir. T1 + T2 + T3 uygulaması bitki boyu üzerinde etkili olmuş ancak bitki kuru ağırlığı üzerinde önemli bir etkisi olmamıştır. Patojen ile *Trichoderma virens'* in birlikte uygulandığında en yüksek bitki boyunun olduğunu (99,75 cm), F+T1+T2+T3 uygulamasında ise en yüksek yaş (68.67) ve kuru (25.04) ağırlık değerlerine sahip olduğu, ayrıca bu uygulamada hiçbir hastalık belirtisi göstermediği belirlenmiştir. Mayanın tek başına uygulandığında patojene etkisinin çok yüksek olmadığı, fakat *Trichoderma* türleri ile birlikte uygulandığında değerlerin yükseldiği ve hastalık şiddetinin 0 olduğu belirlenmiştir.

4. SONUÇ

Dünyadaki çiftçilerin ürettiği gıda ürünlerinin kalitesini ve bolluğunu korumak için bitki hastalıklarının kontrol edilmesi gerekir. Bitki hastalıklarının önlenmesi, azaltılması veya kontrol altına alınması için çeşitli yaklaşımlar kullanılabilir. Toprak kaynaklı bitki patojenlerinin biyolojik kontrolü, daha önce çevreye zararlı olduğu gösterilen kimyasal maddelerin kullanımına potansiyel bir alternatiftir.

Bu çalışmada, test edilen tüm *Trichoderma* türleri (*T. harzianum, T. virens, T. asperellu*m), *Fusarium oxysporum*' un miselyal büyümesini ikili kültürde inhibe etmiştir. Bunun yanısıra *Trichoderma* türleri arasında antagonizm açısından önemli farklılıklar oluşmuştur. *Fusarium oxysporum* f. sp. *lycopersici'* yi sırasıyla *Trichoderma virens* % 44.60, *Trichoderma asperellum* % 21.77 ve *Trichoderma harzianum* % 19.03 oranında engellemiştir.

In vivoda biyo organizmalar iki yöntem ile denenmiştir. Tohum kaplama yönteminde *T. virens* uygulanan bitkilerde patojenin oluşturduğu hastalık şiddeti değeri en düşük (% 10.22) bulunurken *T. asperellum* (% 53.33) en yüksek değeri vermiştir. *Trichoderma* türlerinin birlikte kullanıldığı toprak uygulamasında ise patojenin

engellendiği belirlenmiştir. Toprak uygulamasında diğerlerine göre *T. asperellum* en yüksek hastalık şiddetine (% 25) sebep olmuştur. Ayrıca, en iyi sonuç toprak muamelesinde *S. cerevisiae*' nin *Trichoderma* türleri ile birlikte uygulandığında görülmüştür. In vivo ve in vitro sonuçlara göre *T. virens F. oxysporum'* a en etkili antagonist olarak belirlenmiştir. Ayrıca bu çalışma ile *T. harzianum, T. virens* ve *T. asperellum'* un domateslerde patojeni kontrol edebildiği belirlenmiştir. Mayanın etkisi tek başına önemli olmazken diğer izolatlarla birlikte uygulandığında hem bitki gelişim parametrelerinde hem de hastalık üzerinde önemli oranda etkili bulunmuştur.

Bu araştırmadan elde edilen sonuçlar, *Trichoderma* türlerinin tek başına veya kuru maya ile kullanılabileceğini göstermektedir. *Saccharomyces cerevisiae*, bitki hastalıklarının yönetimi için çevre dostu bir strateji olacağını göstermektedir. Mevcut araştırma sonuçlarına göre, tüm biyo-kontrol ajanları ayrı ayrı uygulandığında da hastalık şiddetinin azaldığı tespit edilmiştir.

CURRICULUM VITAE

Shawen zrar RASUL was born in 1990 in Erbil province, Iraq. She completed primary, secondary and high school in Ranya district. She graduated from plant protection Department in Salahaddin University in 2012. She started her MSc. at Van Yuzuncu Yil University in Van -Turkey on September 2017.

UNIVERSITY OF VAN YUZUNCU YIL
THE ISTITUTE OF NATURAL AND APPLIED SCIENCES
THESIS ORIGINALITY REPORT

Date:27/09/2019

Thesis Title: **BIOCONTROL OF *Trichoderma* spp. AND *Saccharomyces cerevisiae* AGAINST *Fusariumoxysporum*f.sp. *Lycopersici* IN TOMATO**
The title of the mentioned thesis,above having total 75 pages with cover page, introduction, main parts and conclusion,has been checked for originality by Turnitingcomputer program on the date of 27/09/2019and its detected similar rate was 7% according to the following specified filtering

Originality report rules:

- Excluding the Cover page,
- Excluding the Thanks,
-Excluding the Contents,
- Excluding the Symbols and Abbreviations,
- Excluding the Materials and Methods
- Excluding the Bibliography,
- Excluding the Citations,
- Excluding the publications obtained from the thesis,
- Excluding the text parts less than 7 words (Limit match size to 7 words)

I read the Thesis Originality Report Guidelinesof VanYuzuncuYil University for Obtaining and Using Similarity Rate for the thesis, and I declarethe accuracy of the information I have given above and my thesisdoes not contain any plagiarism; otherwise I accept legal responsibility for any dispute arising in situations which are likely to be detected.

Sincerely yours

27/09/2019
Date and signature

Name and Surname: Shawen Zrar RASUL

Student ID#:17910001009

Science: Plant Protection

Program: Plant Protection

Statute: M. Sc.X Ph.D.☐

APPROVAL OF SUPERVISOR
SUITABLE

Dr. Öğr. Üyesi. Emre DEMİRER DURAK

(Title, Name-Surname, Signature)

APPROVAL OF THE INSTITUTE

(Title, Name-Surname, Signature)