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RECENT APPROACHES FOR MANAGEMENT OF TOMATO *FUSARIUM* WILT

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A B S T R A C T

Many fungal pathogens were isolated from tomato roots of plants appearing wilt symptoms. *Fusarium oxysporum* (FO) isolates were selected, purified, tested for their pathogenicity and identifying the formae speciales of the highest virulent one. Among the 17 isolates, 9 isolates caused root-rot, 5 resulted in wilt symptoms and 3 were un-pathogenic. The highest virulent isolate of FO, specialized in causing tomato wilt, was then named *Fusarium oxysporum* Schlect f. sp. *lycopersici* (FOL). Significant reduction to the linear growth and conidial germination of FOL was obtained by the culture filtrate of *Trichoderma bioagents*, compost tea, and the non-pathogenic isolates of FO in comparison with control treatment. The bi-combination of *Trichoderma asperellum* (TA)*, T.harzianum* (TH), cow dung compost (CDC), and the un-pathogenic isolate of FO lowered the infection by the disease and increased crop parameters significantly. However, fungicide Maxim was the superior treatment in lowering the severity of the disease and increasing crop parameters. Total phenolic compounds, photosynthetic pigments and vitamin-c considerably increased in tomato plants of *Trichoderma* treated plants, compost and un-pathogenic isolate of FO compared to the control.

Keywords: Tomato, Compost, *Fusarium* wilt, Disease control, *Trichoderma* bioagents, Fruit yield.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most sustainable Solanacous cash crops globally. In Egypt it´s grown for local consumption and exportation. Tomato fruit has high nutritional value of antioxidants, minerals, vitamins and some other compounds. Therefore, improving the bioproduction of vegetable and horticultural fruits is a singular urgent objective in modern agriculture. Tomato plants attack by many biotic occasions caused by *i.e.,* bacteria, fungi, virus and nematode in addition to abiotic physiological disorder. However, soil-borne fungal pathogens are considered the main devastative and destructive diseases affecting it´s plantation (Jones *et al*., 2013; Jha *et al*., 2018; López-Zapata *et al*., 2021).

Due to the planted area is limited in Egypt, so crop rotation is not applicable and this caused great deterioration to the grown plants, especially in case of specialized soil-borne plant pathogens such as the causal of tomato Fusarium wilt. The infection by the fungus *Fusarium oxysporum* f. sp. *lycopersici* can be occur in tropics temperate and desert zones, which reverse climatic conditions are prevail (Okungbowa *et al*., 2014). Therefore, many pathogenic *Fusarium* spp. are abundant in soil microbial community (Koyyappurath, 2015), and together with the another non-pathogenic strains make up a complex of endophyte strains that used to prohibit vascular wilt of tomato caused by *F.o.*f. sp. *lycopersici* (Wang *et al*., 2020).

Application chemical fungicides against such disease gives mostly adequate management. on the other hand, improper use of fungicides leads highly to resistance to the plant pathogens, environmental pollution and disasters all over the world. Hence, to overcome these disasters, it is urgent to disseminate another management trials as alternative safe applicable methods against such disease. Nowadays, using of compost and biological management are considered important

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approaches of modern agricultural biotechnology for managing many soil-borne plant pathogens (Jha *et al*., 2018; Wang *et al*., 2020; Abada *et al*., 2018).

Two formae speciales of the fungus *Fusarium oxysporum i.e., Fusarium oxysporum* Schlecht. f. sp. *radicis-lycopersici* Jarvis and Shoemaker (caused root-rot) and *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (caused wilt) are well known. In addition, *F.o.* f. sp. *lycopersici* is the major fungal pathogen causing economical yield losses in Egypt as well as several other tomato producing countries (Jones *et al*., 2016; Nogues *et al*., 2002; Nofal *et al*., 2021). Under optimally infection conditions, such as high soil moisture level, poor soil drainage, warm temperature, and soil compaction, the fungus can completely devastate the grown plants. The primary symptoms of tomato wilt begin as slight vein clearing on the outer leaflets, leaf chlorosis followed by yellowing and projection of the plant leaves, then xylem browning of the stem is happened on the basal stem and main root parts of the infected plants and finally dying the aboveground growth.

It is assumed that plant vindication response can be achieved by specific recognition of some biocontrol agents (Jacobsen *et al.,*2004). This, this action could be activated by their secreted bio-products and/or whole bio-agents under the influence of plant defense response (Abada *et al*., 2018; Albersheim and Valent, 1978; Akram and Yousefi, 2015).

A wide configuration of fungicides show efficiency against *Fusarium* wilt and they, generally, used frequently in alternative with crop rotation, resistant cultivars, sanitary methods, and other disease management strategies, especially in greenhouse plantations to manage this disease. Amini and Sidovich (2010) found that bromuconazole and prochloraz when used as soil drench (10 mg ai/ml) were more efficacious in lowering the severity of *Fusarium* wilt of tomato than another fungicides i.e., azoxystrobin, benomyl, carbendazim, and fludioxonil at the same dose. The caculated crop parameters and photosynthetic pigments were greatly reduced in tomato plants in restraint to *Fusarium* wilt (Akladious *et al*., 2014).

This work was performed to investigate the potential of compost tea, *Trichoderma bioagents* and non-pathogenic isolate of *F. oxysporum* on reducing the linear growth and germination of the conidiospores of *F.o.*f.sp. *lycopersici* . Also, the efficacy of both *T. asperellum* and *T.harzianum* in combination with compost and un-pathogenic isolate of *F. oxysporum* on management of tomato *Fusarium* wilt was assessed. In addition, total phenol compounds, photosynthetic pigments and vitamin-c were estimated in treated tomato plants with compost, *Trichoderma bioagents* and non-pathogenic isolate of *F. oxysporum* grown in soil infested with the pathogen.

MATERIALS AND METHODS

Associated fungi to tomato wilt: Tomato plants appearance characteristic symptoms of wilt were collected from Menofia, Kalubia, Giza and Fayoum governorates. The infected roots were thoroughly washed with tap water and cut into small segments (1.0-1.5 cm) with lesion having half healthy and half diseased root portions. The segments were surface sterilized with 2 % sodium hypochlorite for two min, washed thoroughly in three changes sterile water to eliminate the excess of sodium hypochlorite. The aseptic segments were dried on folds of sterile filter papers and transferred onto potato-dextrose-agar (PDA) medium in Petri-dishes. The dishes then were incubated at 25 ± 2°C and observed periodically for the fungal growth. Hyphal tip method and/ or single spore technique were achieved to get pure cultures of the isolated fungi. The purified fungi were then identified according to their morphological countenance and the specification of (Gilman, 1957; Booth, 1971; Domsch *et al*., 1980). The purified fungi were preserved on PDA slants throughout this work. The emerged *Fusarium* strains were selected for the current study.

Pathogenicity test of the seventeen *Fusarium oxysporum* isolates and identification of the formae speciales: The isolates of F. oxysporum were selected and their inoculum of was prepared by culturing on PDA medium for 7 days in Petri-plates. Conidial suspension was prepared by pouring 20 ml of sterile water in each Petri-dish and brushed by sterilizes camel brush. Conidial concentration was adjusted to $1x10^3$ conidia / ml water using haemocytom***eter.***

Three tomato transplants (Hybrid Super Strain-B) of 30 day-old, grown in foam trays contained disinfested soil (peat moss + clay+ sand +vermiculite), were transplanted in each plastic pot (30 cm.) contained infested clay + sand soil by spore suspension of any of the seventeen isolates of *F. oxysporum* at the rate of 250 ml inoculum / pot. Also, three transplants were transplanted in each plastic pot (30 cm.) contained disinfested clay soil and served as control. Five pots were used for each treatment. The plants were left to

grow under greenhouse conditions. The pots were irrigated when it was necessary and fertilized with recommended doses as recommended by the Egyptian Min. of Agric. and Land Recl.

The plants were examined for the infection by the tested *F. oxysporum* isolates two months after transplanting by gentle pull-off the plants, 2 hours after irrigation. The root system was carefully examined for root-rot and/or wilt, which longitudinally section was made in the main root and the basal stem (10-12 cm). The percentages of dead plant, type of infection (root-rot and/or wilt) caused by each isolate, plant height (cm), foliage fresh weight (g) of the foliage growth/ plant of the grown plants were estimated and recorded.

To identify the forma speciales of the most virulent isolate of *F. oxysporum* causing wilt (isolate Giza-4), eight tested plant species *i.e.*, bean (Bronco cv.), cucumber (Prince cv), eggplant (White Balady cv.), melon (Shad Edfina cv.), sweet pepper (California Wonder cv.), strawberry (Festival cv.), tomato (hybrid Super Strain cv.) and watermelon (Giza 1 cv.) were grown in plastic pots (20 cm) infested or not with *F. oxysporum* isolate causing wilt of Giza-4 governorate and left to grow for two months, then the symptoms of the infection by *F. oxysporum* were registered.

Isolation of *Trichoderma* strains: The rhizospheric soil of obviously healthy tomato plants grown in a field have severe infection by wilt at Kalubia governorate, was used to isolate the antagonists *Trichoderma* strains. Serial dilution by plate method was utilized to isolate the native *Trichoderma* strains using soil extract medium (Johoson *et al.,* 1959).

All cultures of the isolated *Trichoderma spp.* were selected, isolated and purified by hyphal tip procedure. The purified isolates of *Trichoderma spp.* were identified depending on the cultural and morphological specification (Rifai, 1969).

Characteristics of the three kinds of compost: Three kinds of compost prepared from cow dung (CDC), maize with rice straw (1:2) (MRSC) and poultry manure (PMC) were used. The three kinds of compost are produced by the Egyptian Company for Solid Waste Recycling (ECARU) at the rate of 10 m^3/ feddan and their characteristics are recorded in Table (1).

Table 1. Characteristics of the three kinds of compost

Compost characters	Kind of compost		
	CDC	MRSC	PMC
Weight kg./m3	620-665	560-590	510-555
Moisture content (%)	24-31	21-27	20-25
pH	7.2-7.4	7.0-7.2	6.8-7.1
EC (ds mG1)	2-4	2-4	2-4

***In vitro* impact of three kinds of compost tea on the linear growth and germinated conidiophores of *F.o.f.sp. lycopersici*:** Poultry manure compost extract (PMCE), cow dung compost extract (CDCE), maize and rice straw extract (MRCE) and tap water as control (TW) were used. The three composts were prepared to compost extract (compost tea) (Abada *et al*., 2018; Weltzien, 1991). The filtrate of each compost was sterilized by 0.25 µm syringe filter and added to the calculated amount of PDA medium after sterilization at the concentrations of 20, 40 and 80 %, shake well and poured into the Petri-dishes (20 ml/plate). Petri-dishes were inoculated after solidification with 5 mm. discs of *F. o.* f.sp*. lycopersici* cut from the seven-day old culture. PDA dishes inoculated with the tested pathogen, but not provided with compost tea were served as control. The plates were then incubated in an incubator at 30±2°C. Five replications were set up for each concentration of each kind of the composts. Linear growth of the pathogen was assessed when the dishes of the control treatment covered with the fungal growth. Inhibition percentage of the linear growth of the tested pathogen was assessed as mentioned before. The prepared concentrations of any of the three composted tea were, also, added to the fungal growth of *F. o.* f.sp*. lycopersici* in the Petri-dishes to make a conidial spore suspension. One ml. of conidial suspension was kept on each sterile glass slide, borne on two glass rods in a sterile Petri-dish (two slides in each Petri-dish) havening a piece of wetted cotton by sterilized water to maintain high relative humidity. The same procedure was made for a spore suspension of the tested pathogen kept in sterile water only as a control treatment. The prepared dishes were incubated in darkness at 30±2 °C for 24 hour. Five Petri-dishes for each treatment were set up as replicates. The

percentage of conidial germination was counted in a total of 100 conidiospores in each glass slide and the averages were assessed and recorded for each treatment.

In vitro* impact of the culture filtrate of five *Trichoderma* strains on the linear growth and germinated of conidiospores of *F. o.* f.sp*. lycopersici:

The restrained effect of the culture filtrate of five *Trichoderma* strains i.e., *Trichderma album, T. aperelleum, T. hamatum ,T. harzianum and T. viride* in vitro on the linear growth and germination of the conidiospores of *F. o.* f.sp. lycopersici was investigated. Steamer sterilized gliotoxin fermentation medium (GFM) was utilized to grow the tested *Trichoderma* strains in 250 ml bottles (Brain and Hemming, 1945). The medium in each bottle was inoculated with a disc of any of the tested *Trichoderma* strains (taken from five day-old culture). Inoculated bottles with *Trichoderma* strains were incubated on a rotary shaker at 200 rpm for two weeks at 30 ± 2°C. The obtained culture filtrate was filtered through Whatman No.1 filter paper, then the filtrate was antiseptic using 0.25 µm syringe filter. The culture filtrate of the *Trichoderma* strains was raised to the calculated offset of PDA medium just before solidification in different concentrations (20, 40 and 80 %), then shake well and poured into the Petri-dishes (20 ml/ dish). After solidification the Petri-dishes were inoculated with 5 mm. discs, possessed from the periphery of five-day old culture, of the tested pathogen. PDA plates inoculated with the tested pathogen, but not amended with the culture filtrate of the tested *Trichoderma* strains, were kept as control. The Petri-dishes were then incubated in an incubator at 30±2°C. Five replicates were set up for each treatment. The linear growth of the pathogen was measured when the plates of the control treatment were covered with the fungal growth. Inhibition percentage of the linear growth reduction over the control of the tested pathogen was calculated by using the following formula:

% Linear growth reduction over the control= The linear growth of the pathogen (mm) in the control - The linear growth of the pathogen (mm) in the treatment / The linear growth of the pathogen (mm) in the control x100.

Also, the prepared concentrations of any of the tested *Trichoderma* strains were added to the fungal growth in the Petri-dishes to prepare a conidial spore suspension. One ml. of conidial suspension was kept on each sterile glass slide, borne on two glass rods in each sterilized Petri-dish (two slides in each Petri-dish) having a piece of wetted cotton by sterile water to maintain high relative humidity. The same technique was made for a spore suspension kept in sterile water only as control treatment. The prepared dishes were incubated in darkness at 30±2 °C for 24 hours. Five Petri-dishes for each treatment were set up as replicates. The percentage of conidial germination was counted in a total of 100 conidiospores in each glass slide. The averages of the germinated conidia were calculated and registered for each treatment.

***In vitor* impact of the culture filtrate of three non-pathogenic isolates of *F. oxysporum* on the linear growth and germinated conidiospores of *F. o.* f.sp*. lycopersici*:** The three non-pathogenic isolates of *F. oxysporum* were grown in liquid medium (potato 20g +dextrose 20 g/ L water) for ten days then filtrate by Whatman filter paper No.1. The filtrate of each isolate was sterilized by 0.25 µm syringe filter and added to the calculated amount of PDA medium after sterilization at the concentrations of 20, 40 and 80 %, shake well and poured into the Petri-dishes (20 ml/plate). Petri-dishes were inoculated after solidification with 5 mm. discs of the causal pathogen cut from the seven-day old culture. PDA dishes inoculated with the tested pathogen, but not provided with culture filtrate of the non-pathogen fungi were served as control. The plates were then incubated in an incubator at 30±2°C. Five replications were set up for each concentration of each non-pathogenic isolate. Linear growth of the pathogen was assessed when the dishes of the control treatment covered with the fungal growth. Inhibition percentage of the linear growth of the tested pathogen was assessed as mentioned before.

The prepared concentrations of any of the three non-pathogenic *Fusarium* isolates, also, added to the fungal growth of the causal pathogen in the Petri-dishes to make a conidial spore suspension. One ml. of conidial suspension was kept on each sterile glass slide, borne on two glass rods in a sterile Petri-dish (two slides in each Petri-dish) havening a piece of wetted cotton by sterile water to maintain high relative humidity. The same procedure was made for a spore suspension of the tested pathogen kept in sterile water only as a control treatment. The

prepared dishes were incubated in darkness at 30±2 °C for 24 hours. Five Petri-dishes for each treatment were set up as replicates. The percentage of conidial germination was counted in a total of 100 conidiospores in each glass slide and the averages were assessed and recorded for each treatment.

Impact of the bi-combination among compost, non-pathogenic isolate of *F. oxysporum*, and the bioagents *T. asperellum and T. harzianum* in comparison of the Maxim fungicide on the severity of tomato wilt as well as some crop parameters, greenhouse experiment: Clay plots (25 x 90 x 30 cm) containing disinfested clay + sand soil by formalin was used in this experiment. The conidial spore suspension of *F. o.* f.sp. *lycopersici* (200 ml of $1x10^6$ spore / liter water) was added to each plot then irrigated and left for one week. Then after CDCE compost (200 g), *T.asperelleum* (200 ml. of $5x10^6$ spore/ ml water), *T.harzianum* (200 ml. of $5x10^6$ spore/ ml water) and the non-pathogenic isolate of *F.oxysporum* (200 ml. of $5x10^6$ spore/ ml water) were added to each plot , each alone or in combination, one week before transplanting tomato transplants. Also, the Maxim fungicide (200 ppm) (Fludioxonil; chemical class of phenylpyrazoles) was added as soil drench (200 ml/ plot), one week before transplanting tomato transplants (Hybrid Super Strain B). Plots without any adding any of the tested treatments were used as control treatment. Three transplants (hybrid Super Strain-B) of 30 day-old, grown in foam trays were transplanted in each plot. Three plots were set up for each treatment and kept in the greenhouse. The severity of wilt on the foliage growth and xylem vesicles was estimated (Amini and Sidovich, 2010; Ulloa *et al*., 2006). Also, the averages of plant height (cm), foliage growth weight (g), No. of fruits / plant and weight of fruits (g) / plant were estimated for the grown plants and recorded.

Disease assessment: The severity of Fusarium wilt was assessed 70 days after transplanting using the devised scale (0.0 to 5) by (Amini and Sidovich, 2010) on the foliage growth of tomato plants.

Also, the plants were examined for vascular discoloration by the causal fungus using the devised scale (0.0-5) by (Ulloa *et al*., 2006).

Impact of compost, *T.asperellum* , *T.harzianum*, non-pathogenic *F.oxysporum* isolate and the Maxim fungicide on biochemical constitute of tomato plants under the infection by *F. o.* f.sp. *lycopersici*:

Total phenols content: The treated tomato root samples (10 g) by the tested treatments were used to extract total phenol compounds using 10 ml of 80% methanol at 70 °C for 15 min. Total phenol compounds were expressed as mg gallic acid / 5g root segments (Zieslin and Ben-Zaken, 1993). 20, 40 and 60 days after inoculation gallic acid was determined.

Ascorbic acid and photosynthesis pigments: Free ascorbic acid (FAA) and total ascorbic acid (TAA) were assessed spectrophotometrically in the tomato fruits using a technique mentioned by (Hodges *et al*., 2001). Chlorophyll-a, chlorophyll-b and carotenoids were, also evaluated spectrophotometrically to tomato leaves of plants treated with the tested treatments, taken from five randomly plants resemble the categories of the disease (Metzner *et al*., 1965; Arnon, 1949).

STATISTICAL ANALYSIS

Obtained data were statistically analyzed by the standard methods for complete randomize block and split designs (Snedecor and Cochran, 1989). The obtained averages were then compared at 0.05 level using the least significant differences (L. S. D.) (Fisher, 1948).

RESULTS

Associated fungi to tomato wilt: Many fungal isolate were isolated from tomato plants showing symptoms of wilt, collected from four governorates *i.e.,* Menofia, Kalubia, Giza and Fauoum. The isolated fungi were purified and identified as: *Alternaria* spp., *Fusarium* spp., *F. oxysporum, F. solani, Macrophomina phaseolina, Phytophthora infestans, Pythium* spp., *Rhizontonia solani* and *Sclerotium rolfsii*.

The isolates of the fungus *F. oxysporum* were selected and tested for their pathogenicity and the most virulent isolate was used to identify its formae speciales.

Pathogenicity test of seventeen *F. oxysporum* isolates and identification of the formae speciales of the most virulent one: Among the seventeen isolates of *F. oxysporum*, nine isolates of Menofia-2, Menofia-3, Kalubia-1, Kalubia-3, Kaubia-5, Giza-1, Giza-2, Giza-5, Fayoum-1 and Fayoum-3 reveal that they were pathogenic to tomato plants showing typical root-rot symptoms and five isolates *i.e.,* Menofia-1, Kalubia-2, Giza-4- , Fayoum-2 and Fayoum-3 resulted in wilt symptoms (Table,2). Meanwhile, three *F. oxysporum* isolates *i.e.,* Kalubia-4, Giza-3 and Fauoum-4 failed to cause any infection by root-rot and wilt symptoms.

Table 2. Pathogenicity test of seventeen isolates of *F. oxysporum* using tomato transplants (Hybrid Super Strain-B), greenhouse experiment, two months after transplanting

Isolates	% Dead plants	Type of infection	Average of plant height(cm)	Average of foliage fresh weight(g)/plant
Menofia (1)	15	Wilt	30.8	158.7
Menofia (2)	40	Root-rot	25.5	132.0
Menofia (3)	35	Root-rot	37.5	159.5
Kalubia (1)	30	Root-Rot	38.0	152.6
Kalubia (2)	35	wilt	28.5	135.8
Kalubia (3)	35	Root-rot	38.2	150.5
Kalubia (4)	0.0	No infection	38.5	154.5
Kalubia (5)	35	Root-rot	38.0	160.3
Giza (1)	30	Root-rot	27.3	153.0
Giza (2)	40	Root-rot	26.0	133.0
Giza (3)	0.0	No infection	37.6	148.4
Giza (4)	45	Wilt	21.0	125.1
Giza (5)	35	Root-rot	36.3	158.6
Fayoum (1)	40	Root-rot	25.4	132.5
Fayoum (2)	40	Wilt	23.0	128.2
Fayoum (3)	15	Wilt	30.8	148.0
Fayoum (4)	0.0	No infection	36.5	150.0
Control	0.0	No infection	37.0	152.0

Figure 1. Tomato plants showing symptoms of infection by *Fusarium* wilt due to the artificial inoculation with *F.o.*f.sp. *lycopersici..*

Figure 2. Discoloration of xylem vesicles of tomato plant artificially inoculated with *F.o* f.sp. *lycopersici*.

The isolates of Menofia-2, Giza-2 and Fayoum-1 were the highest virulent ones in causing root-rot and dead plants (40%), which resulted in the lowest values of plant height , being 25.5, 26.0 and 25.4 cm ; 432.0 , 430.0 and 425.5 g foliage fresh weight (g) / plant, respectively compared with the other isolates causing root-rot symptoms. Meanwhile, isolate Giza-4 resulted in the highest infection by (wilt) dead plants (45%) , plant height (31.0 cm) and foliage growth weight (345.1g). followed by isolate of Fayoum-2 (40% dead plant, 23.0 cm and 358.2 g)). Isolates of Menofia-1 and Fayoum-3 resulted in the lowest figures of wilt and dead plants (15%). The remained isolates caused intermediated infection by dead plants, root-rot and/ or wilt. No apparent infection by root-rot and wilt symptoms was observed due to the inoculation by isolates of Kalubia-1, Kalubia-4 and Fayoum-2 in addition to the control, which showed good growth. In addition, the virulent isolate of Giza-4 (caused wilt), were used to identify it´s formae sepeciales.

Testing eight different plant hosts, *i.e.* bean (Bronco cv.), cucumber (Prince cv.), eggplant (Balady White cv.), melon (Shad Edfina cv.), sweet pepper (California Wonder cv.), strawberry (Festival cv.), tomato (Hybrid Super Strain-B) and water melon (Giza 1 cv.) to the infection by the virulent isolate of *F. oxysporum* (Giza-4) indicated that the fungus caused high infection to tomato plants (Figure 1 and2) and trace or no apparent infection were occurred in case of the other plants. Therefore, the isolate of the fungus *F. oxysporum* named *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* and it used in the following experiments.

Isolation of *Trichoderma* strains: Isolation trials from the rhizospeheric soil of tomato roots yielded many fungal isolates. *Trichoderma* strains were selected, purified and identified as: *T.asperellum, T.album, T. hamatum, T.harzianum* and *T.viride*

***In vitor* impact of compost tea filtrate of three composts on the linear growth and the germinated conidiospores of *F. o.* f.sp*. lycopersici*:** Results shown in Table (3) show that the filtrate of the soaked three kinds of compost (compost tea) caused significant reduction to the linear growth of *F. o.* f. sp. *lycopersici* , seven days after incubation at 30±2°C compared with control treatment .This reduction was gradually increased by increasing the used concentration. The causal fungus failed to grow on the concentration of 40 % for CDCE and 80% for PMCE and MRCE. However, control treatment recorded 90 mm linear growth. In addition, CDCE was the most efficient one in reduction the fungal growth followed PMCE by MRCE, being 81.4, 67.9 and 66.6% reduction over the control.

Table 4 reveals that the filtrate of the soaked three kinds of compost (compost tea) resulted in significant reduction to the germinated conidiophores of the causal pathogen , 24 h after incubation at 30±2°C compared with control treatment. This reduction was gradually increased by increasing the used concentration. Also, the conidiospores of the causal fungus failed to germinate on the concentration of 40 % for CDCE and 80% for PMCE and MRCE. In the same time, control treatment recorded $4.2x10^2$ germinated conidiospores. In addition, CDCE was the most efficient one in reduction the fungal growth followed PMCE by MRCE, being 89.2, 69.2 and 78.0% reduction over the control.

Table 3. Impact of compost tea filtrate of different three composts on the linear growth of the causal pathogen, seven days after incubation at 30±2°C

Compost kind*	Average linear growth (mm) at conc. (%)			Mean	% Reduction over the control
	20	40	80		
PMCE	56.4	20.2	10.0	28.9	67.9
CDCE	50.0	0.0	0.0	16.7	81.4
MRCE	58.4	21.4	10.6	30.1	66.6
Control	90	90	90	90	-------
Mean	63.7	32.9	27.7	----	-------

* PDCE= Poultry manure compost extract, **= Cow dung compost extract and ***= Maize and rice straw extract. L. S. D. at 5 % for: Compost kind (CK) = 3.01, Concentration (C)= 2.27 and CKxC = 2.31.

Table 4. Impact of compost tea filtrate of different three composts on the germinated conidiospores of the causal pathogen ,24 hour after incubation at 30±2°C

Compost kind*	Average percentage of the germinated conidiospores (...x10^2) at conc.(%)				% Reduction over the control
	20	40	80	Mean	
PMCE	0.8	0.3	0.0	0.37	69.2
CDCE	0.4	0.0	0.0	0.13	89.2
MRCE	0.6	0.2	0.0	0.27	78.0
Control	1.2	1.2	1.2	1.20	------
Mean	0.75	0.43	0.30	-----	-----

* PDCE= Poultry manure compost extract, **= Cow dung compost extract and ***= Maize and rice straw extract. L. S. D. at 5 % for: Compost kind (CK) = 0.31, Concentration (C)= 0.22 and CKxC = 0.36.

In vitro* impact of culture filtrate of five *Trichoderma* strains on the linear growth and the germinated conidiospores of *F. o.* f.sp. *lycopersici: The effect of the five Trichoderma strains on the linear growth and the germinated conidiospores of the causal pathogen (isolate Giza-4) is shown in (Tables 5 and 6).

Data presented in Tables (5 and 6) show that all the tested five *Trichoderma* strains resulted in significant reduction to the linear growth and the germinated conidiospores of the causal pathogen compared with control treatment.

Table (5) indicates that *T.asperellum* followed by *T.harzianum* resulted in the highest reduction to the linear growth of the causal fungus, being 62.4 and 62.1% over the control, respectively. In addition, both bioagents caused complete inhibition to the linear growth of the pathogen at the concentration of 80%. Meanwhile, *T.album* followed by *T.hamatum* then *T.viride* resulted in the lowest reduction, being 58.6, 58.8 and 58.9 %, respectively. The reduction in the linear growth of the pathogen was gradually increased by arising the used concentration, being 75.9, 40.6 and 19.2 mm. at the concentration of 20, 40 and 80%, respectively.

Data (Table, 6) reveal that the effect of the culture filtrate of the five *Trichoderma* stains on the germinated conidiospores of the causal pathogen showed similar trend to the effect of their filtrate on the linear growth of the pathogen. In addition, the conidiospores of the causal pathogen failed to geminate at the concentration of 40% of the culture filtrate of *T.asperellum and T.harzianum* and at the concentration of 80% for *T.album, T. hamatum* and *T.viride.*

Table 5. Impact of culture filtrate of five *Trichoderma* strains on the linear growth of the causal pathogen, seven days after incubation at 30±2°C

Trichoderma strains	Average linear growth (mm) at concentration (%)				% Reduction over the control
	20	40	80	Mean	
T.asperellum	68.8	32.6	0.0	33.8	62.4
T.album	70.4	35.2	8.2	37.3	58.6
T.hamatum	69.4	32.8	9.0	37.1	58.8
T.harzianum	69.0	33.2	0.0	34.1	62.1
T.viride	71.0	30.0	10.0	37.0	58.9
Control	90.0	90.0	90.0	90.0	------
Mean	75.9	40.6	19.2	-----	------

L. S. D. at 5 % for: *Trichoderma* strains (TS) = 2.7, Concentration (C)= 3.4 and TSxC = 4.6.

Table 6. Impact of culture filtrate of five *Trichoderma* strains on the average percentage of the germinated conidiospores of the causal pathogen , 24 hour after incubation at 30±2°C

Trichoderma strains	Average percentage of the germinated conidiospores (...x10²) at concentration (%)				% Reduction over the control
	20	40	80	Mean	
T.asperellum	3.8	1.2	0.0	1.7	61.4
T.album	4.2	1.8	0.4	2.1	52.3
T.hamatum	4.2	1.8	0.6	2.2	50.0
T.harzianum	3.8	1.4	0.0	1.7	61.4
T.viride	4.0	1.6	0.0	1.9	56.8
Control	4.4	4.4	4.4	4.4	-----
Mean	4.1	2.0	0.9	----	------

L. S. D. at 5 %: *Trichoderma* strains (TS) = 0.8, Concentration (C) = 0.7, TSxC = 1.2.

***In vitor* impact of the culture filtrate of three non-pathogenic isolates of *F.oxysporum* on the linear growth and the germinated conidiospores of *F. o.* f.sp. *lycopersici*:** The culture filtrate of three non-pathogenic isolates of *F.oxysporum* resulted in significant reduction to the linear growth of the pathogenic fungus, 7 after incubation at 30±°C (Table, 7).This reduction was gradually increased by increasing the used concentration. However, the causal fungus can grow on the three tested concentrations without complete inhibition at the concentration of 80 %. In addition, the culture filtrate of the non-pathogenic isolate of Giza-3 was more efficient in reduction of the linear growth of the pathogenic fungus followed by isolate of Kalubia-4 then isolate of Fayoum-4, being 34.2, 29.2 and 28.1 % reduction over the control.

Data presented in (Table, 8) indicate that the culture filtrate of three non-pathogenic isolates of *F.oxysporum* resulted in significant reduction to germinated conidiospores of the causal pathogen , 24 h after incubation at 30±2°C compared with control treatment. This reduction was gradually increased by increasing the used concentration. Also, the conidiospores of the causal fungus can germinate on the three tested concentration without complete inhibition at the high concentration (80%). In the same time, control treatment recorded 1.2x10² germinated conidiospores. In addition, the culture filtrate of the of isolate Giza-3 was the most efficient one in this regard followed by both isolates of Kalubia-4 and Fayoum-4, being 0.34, 0.28 and 0.28 % reduction over the control.

Table 7. Impact of the culture filtrate of three non-pathogenic isolates of *F. oxysporum* on the linear growth of the pathogenic fungus, 7 after incubation at 30±2°C

Fusarium isolates	Average linear growth (mm) at concentration (%)				% Reduction over the control
	20	40	80	Mean	
Kaubia-4	86.4	64.2	40.4	63.7	29.2
Giza-3	82.0	60.6	35.0	59.2	34.2
Fayoum-4	84.4	66.8	43.0	64.7	28.1
Control	90.0	90.0	90.0	90.0	-------
Mean	85.7	70.4	52.1	-----	

L. S. D. at 5 % for: Isolates (I) = 1.7, Concentration (C)= 3.1 and IxC = 3.5.

Table 8. Impact of the culture filtrate of three non-pathogenic isolates of *F.oxysporum* on the germinated conidiospores of *F .o.* f.sp. *lycopersici*, 24 hour after incubation at 30±2°C

Isolates of	(%) Average percentage of the germinated conidiospores (....x10²) at concentration (%)				% Reduction over the control
	20	40	80	Mean	
Kalubia-4	1.1	0.8	0.7	0.87	0.28
Giza-3	1.0	0.9	0.6	0.83	0.31
Fayoum-4	1.1	0.8	0.7	0.87	0.28
Control	1.2	1.2	1.2	1.20	-----
Mean	1.1	0.93	0.80	-----	-----

L. S. D. at 5 % for: Isolates (I) = 0.20 , Germinated spores(G)= 0.11 and IxG= 0.2.

***In vitro* impact of the bi-combination among compost, non-pathogenic isolate of F.oxysporum, and the bioagents *T.asperellum* and *T.harzianum* in comparison of the Maxim fungicide on the severity of tomato wilt as well as some crop parameters, greenhouse experiment:** Data presented in Tables (9 and 10) show that the bi-combination of compost, non-pathogenic isolate of *F.oxysporum, T.asperellum* and *T.harzianum* resulted in significant reduction to tomato wilt severity on the foliage growth and xylem vesicles with significant increase to plant height as well as the number of fruits and their weight/ plant compared with the control treatment (inoculated soil).

The severity of tomato wilt on the foliage growth after applying the tested treatments recorded 11.5 %, on the average on the foliage growth and 17.0%, on the average on xylem vesicles (Table, 9). The Table reveals, also, that the Maxim fungicide was the superior treatment for reducing the infection by the disease , being 4.7% disease severity, on the average followed by the bi-combinations among any of the tested treatments *i.e.,* compost (C) *F.oxysporum* (FO),*T.asperellum* (TA) and *T.harzianum* (TH). Applying the tested treatments, each alone, was of low efficiency in decreasing the disease, being 17.8, 17.9, 19.6 and 30.4 %,on the average, respectively compared with applying their bi-combination. In addition, the combination between C+FO and TA+TH were more efficient than the other combinations in this regard (10.1% disease severity, on the average) followed by the combination between C+TA (11.2%, on the average) then the combination between C+TH (11.6% disease severity, on the average). Inoculated treatment with the causal pathogen only recorded 39.3% disease severity, on the average. No symptoms were observed on tomato plants grown in un-inoculated soil with the causal pathogen.

Results shown in Table (10) show that the efficiency of the bi-combination among the tested treatments was more efficient in increasing the values of plant height, foliage fresh weight and fruit yield (number and weight / plant) compared with the treatment with each of them alone .In this regard, the Maxim fungicide was , also, the superior treatment in increased the estimated crop parameters, being 52.7 cm, 925 g, 25.3 fruit/plant and 885 g fruit /plant followed by the bi-combination of the tested items. In this regard, the bi-combination between CO and TA was more efficient than the other bi-combination, being 51.7 cm 850 g, 22.0 fruit fruit/plant and 765 kg fruit /plant. The inoculated plants with the pathogen recorded the shorted and low weight of the plants in addition to poor fruit yield (18.7 cm, 455 g, 11.7 fruit/plant and 342 g fruit /plant, respectively). Un inoculated plants recorded 54.3 cm, 985 g, 28.0 fruit/plant and 825 kg fruit /plant.

Table 9. Impact of the bi-combination among compost, non-pathogenic isolate of *F.oxysporum,* the bioagents *T.asperellum* and *T.harzianum* as well as the Maxim fungicide on the severity of tomato wilt (Hybrid Super Strain B), greenhouse experiment

Treatments	% Disease severity on		Mean
	Foliage growth	Xylem vesicles	
Compost (C)	12.5	23.0	17.8
F.oxysporum (FO)	13.0	22.8	17.9
T.asperellum (TA)	15.2	24.2	19.6
*T.harzianum (*TH)	16.0	24.8	20.4
C+FO	8.0	12.2	10.1
C+TA	9.1	13.3	11.1
C+TH	9.5	13.7	11.6
FO+TA	9.5	16.5	13.0
FO+TH	9.8	16.8	13.3
TA+TH	8.0	12.2	10.1
Maxim	3.4	6.0	4.7
Control (Inoculated)	38.1	40.5	39.3
Control (Un-inoculated)	0.0	0.0	0.0
Mean	11.5	17.0	-----

LSD at 5 % for: Treatments (T)= 2.0, Site of the disease (SD)=2.1 T x SD= 2.4 .

Table 10. Impact of the bi-combination among compost, non-pathogenic isolate of *F.oxysporum*, the bioagents *T.asperellum* and *T.harzianum* as well as the *Maxim* fungicide on some crop parameters of tomato plants (Hybrid Super Strain B), greenhouse experiment

Treatments	Av. of plant height (cm)	Av. of foliage growth weight (g)	Av. of the No. of fruits/ plant	Av. weight of fruits (g)/ plant
Compost (C)	47.3	885	20.3	695
F.oxysporum (FO	42.3	860	17.0	655
T.asperellum (TA)	44.3	845	18.0	660
*T.harzianum (*TH)	44.0	840	18.0	665
C+FO	50.0	845	20.3	755
C+TA	51.7	850	20.7	760
C+TH	51.0	845	21.0	785
FO+TA	45.3	865	20.7	690
FO+TH	45.0	862	20.3	680
TA+TH	46.3	870	21.0	685
Maxim	52.7	925	25.3	785
Control (Inoculated)	18.7	455	11.7	342
Control (Un-inoculated)	52.3	985	27.0	880
LSD at 5%	3.7	34	2.2	38

Impact of compost, non-pathogenic *F.oxysporum* isolate, the bioagents *T.asperellum* and *T.harzianum* as well as the Maxim fungicide on biochemical constitute of tomato plants under the infection by *F. o*. f.sp . *lycopersici* :

Total phenols content: Table (11) reveals that the tested compost, non-pathogenic isolate of *F.oxysporum* two *Trichoderma* bioagents, compost, the Maxim fungicide and pathogen-free treatment (un-inoculated) resulted in considerable increase in the phenol content in the roots of tomato plants with the inoculated plants. In addition, phenol content in the treated and untreated plants was gradually increased by increasing the age of the plants, being 0.66, 079 and 0.88 mg / 10 g fresh roots after 20, 40 and 60 days after inoculation by the pathogen. The highest increase was obtained by the treatment with compost (0.71 mg / 10 g fresh roots, on the average). Meanwhile, the treatment with the non-pathogenic *F.oxysporum* recorded the lowest value (0.66 mg / 10 g fresh roots, on the average). The other treatments recoded intermediate values. On the other hand, inoculated and un-inoculated plants recorded 0.59 and 0.72 mg / 10 g fresh roots, on the average, respectively.

Ascorbic acid and photosynthesis pigments: Data presented in Table (12) show the effect of the tested treatments compared with the inoculated andun-inoculated controls on free and total ascorbic acid of the fruits of tomato plants as well as photosynthesis pigments of tomato leaves. The treatment with Maxim resulted in the highest concentration of ascorbic acid, being 33.08 free ascorbic acid and 44.16 total ascorbic acid mg/100g fruit fresh weight and photosynthetic pigments, being 1.48 chlorophyll –a, 1.34 chlorophyll -b and 0.89 carotenoids mg/g fresh leaves weight). The treatment with compost ranked the second in this respect, being 1.46 chlorophyll –a, 1.32 chlorophyll -b and 0.87 carotenoids mg/g fresh leaves weight) and ascorbic acid, being 33.13 free ascorbic acid and 44.14 total ascorbic acid mg/100g fruit fresh weight. The other treatments recorded intermediate values.

Un-inoculated plants have the highest values of both photosynthetic pigments, being 1.63 chlorophyll –a, 1.42 chlorophyll -b and 0.97 carotenoids mg/g fresh leaves weight) and ascorbic acid , being 37.53 free ascorbic acid and 50.12 total ascorbic acid mg/100g fruit fresh weight. Meanwhile, the inoculated plants have a great reduction in both photosynthetic pigments (0.97 mg/g fresh leaves weight for chlorophyll –a, 1.05 mg/g fresh leaves weight for chlorophyll -b and 0.56 mg/g fresh leaves weight) and ascorbic acid (21.96 mg/g fresh leaves weight for free ascorbic acid and 28.82 mg/g fresh leaves weight for total ascorbic acid).

Table 11. Impact of compost, non-pathogenic isolate of *F.oxysporum*, the bioagents *T. aseperellum* and *T.harzianum* as well as the *Maxim* fungicide and pathogen-free and infected on the total phenols content of tomato roots, 20 and 40 and 60 days after inoculation with the causal pathogen

Treatments	Gallic acid in mg / 10 g fresh roots after (days)			
	20	40	60	Mean
Compost	0.60	0.72	0.82	0.71
F.oxysporum	0.55	0.68	0.75	0.66
T.asperellum	0.57	0.69	0.77	0.68
T.harzianum	0.58	0.69	0.77	0.68
Maxim	0.58	0.70	0.79	0.69
Control (inoculated)	0.40	0.50	0.58	0.59
Control (un-inoculated)	0.66	0.79	0.88	0.78

Table 12. Impact of compost, non-pathogenic isolate of *F.oxysporum*, the bioagents *T. aseperellum* and *T.harzianum* as well as the *Maxim* fungicide and pathogen-free and infected on ascorbic acid, and photosynthesis pigments of tomato leaves due to the inoculation with the causal pathogen

Treatments	Ascorbic acid *		Photosynthesis pigments **		
	Free	Total	Chloro-a***.	Chloro.-b	Carotenoids
Compost	33.12	44.14	1.46	1.32	0.87
F.oxysporum	30.89	41.81	1.43	1.22	0.84
T.asperellum	31.05	42.12	1.42	1.21	0.86
T.harzianum	30.98	42.09	1.42	1.21	0.86
Maxim	33.08	44.16	1.48	1.34	0.89
Control (inoculated)	21.96	29.82	0.97	1.05	0.56
Control (un-inoculated)	37.53	50.12	1.63	1.42	0.97

*mg/100g fruit fresh weight , **mg/g fresh leaves weight, and ***= Chlorophyll.

DISCUSSION

The economic importance of tomato (*Lycopersicon esculentum* Mill.) plantation in the world could be explained by its high nutritional values from minerals, antioxidants, vitamins and other compounds in the fruits. Thereby, one of the objectives in the modern agriculture all over the world is improving the bioproduction of safe food.

Tomato plants are subjected to many fungal diseases, however the fungus *Fusarium oxysporum* f. sp. *lycopersici* is one of the most destructive causal pathogens causing tomato wilt (Biswal and Singh, 2020). The causal fungus invades tomato vascular vesicles, resulting in severe wilting and dying the foliage growth by blocking the xylem vesicle (s). In addition, it is mostly difficult to control most soil-borne plant pathogens by using conventional management methods.

Isolation procedures from the roots of wilted tomato plants collected from Dakahlia, Menofia, Kalubia, Giza and Fauoum governorates yielded many fungal isolates. The isolated fungi were purified and identified as: *Alternaria* spp., *Fusarium* spp., *F. oxysporum, F. solani, Macrophomina phaseolina, Phytophthora infestans, Pythium* spp., *Rhizontonia solani, Sclerotium rolfsii*. The isolated fungi were previously isolated from tomato roots (Agrios, 2005; Morsy *et al*., 2009; El-Mohamedy, 2014; Gilardi *et al*., 2014; Prasom *et al*., 2017; Srinivas *et al*., 2010; Hassanisaadi *et al*., 2018; El-Sheekh *et al*., 2022).

Pathogenicity test of seventeen isolates of *F. oxysporum*, nine isolates caused root-rot symptoms; five isolates caused wilt and three isolates proved to be non-pathogenic to the roots of tomato plants. In addition, isolate of Giza-4 revealed that that it was the most virulent one for causing wilt. The inoculated plants with the tested pathogenic isolates exerted different figures of root-rot and wilt infection. This infection was reflected on the plant height and the weight of the foliage growth, which exerted lower figures than the plants inoculated by the non-pathogenic three isolated and the control.

The obtained data showed that, testing eight plant species, *i.e.* bean (Bronco cv.), cucumber (Prince cv), egg-plant (Balady White cv.), melon (Shahd Edfina cv.), sweet pepper (California Wonder cv.), strawberry (Festival cv.), tomato (Hybrid Super Strain-B) and watermelon (Giza 1 cv.) to their infection by *F. oxysporum* proved that it infected tomato plants only causing wilt. Therefore, it named *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyder et Hansen. It has been reported that tomato infection process begins with the germination of the spores of *F.o.*f.sp. *lycopersici* due to the accumulation

of root exudates rich in amino acids, proteins, sugars, phenols and carbohydrates, and the branching of the germ tube of the spores leading to the formation of hyphae (García-Enciso *et al*., 2018). Then after, the fungus secretes enzymes such as polygaracturonidases (PGs), pectatoliases (PLs), cellulases, xylanases and proteases, which degrades the cell wall allowing penetration and subsequent cells colonization (Srinivas *et al*., 2010) then the roots hypodermis have been invaded by the pathogen, which it locates in the cortex.

The produced toxins by the fungus *F.o.*f.sp. *lycopersici* are fusaric acid, dehydrofusaric acid and lycomarasmin (Srinivas *et al*., 2010). The effect of fusaric acid is accompanied with reduction of photosynthesis, cell death, lipid peroxidation, leaf necrosis and finally death (Singh *et al*., 2017), decrease in cell viability and improvement in fungal strength (Srinivas *et al*., 2010). Also, the tomatinase, secreted by *F.o.* f. sp. *lycopersici* is an engaged enzyme recently identified as a virulence operator in the degradation of tomato defense component of tomatine (Murugan *et al*., 2020). In addition, xylem colonization by the fungus *Fusarium* intensions resistance to water circulation within the plant, so resulting in leaf water deficits that mostly lead to depression in leaf transpiration and longevity. Finally, the severe infection by the causal fungus leads to die and releases the chlamydospores to the soil, where they can preserve viable for long periods. The life cycle of *F.o.*f.sp. *lycopersici* duplicates many times when the optimal situations for spore germination and infection to the susceptible host are prevail (Agrios, 2005).

Amendment the biocontrol agents to the soil is become important process in managing many soil-borne plant pathogens, since they may raise beneficial microbial community, which lodging active for long periods._Five bioagents belonging to *Trichoderma* spp. *i.e.*, *T.asperellum. T.album, T.hamatum, T. harzianum* and *T.viride* were isolated from the rihzospheric soil of the apparently healthy tomato roots grown in a field of severe infection by Fusarium wilt.

It has been found that, both linear growth and the germinated conidia of the causal pathogen were completely inhibited by the culture filtrate of *T.asperellum* at the concentration of 40% and by the other bioagents at the concentration of 80%. Meanwhile, the linear growth as well as the germinated conidia of the causal pathogen were completely inhibited by compost tea of CDCE at the concentration of 40% and by the other two composts at the concentration of 80%. Similar results were obtained. In all cases, the conidiospores were more sensitive to the extracts of the tested *Trichoderma* bioagents and compost tea than the non-pathogenic fungus. In addition, both linear growth and the germinated conidia of the causal pathogen were not completely inhibited by the culture filtrate of the non-pathogenic *Fusarium oxysporum* even at the concentration of 80%. This may be due to the cultural filtrate of the non-pathogenic fungus have not enough toxic substances to suppress the causal fungus *in vitro*.

The tested compost, *Trichoderma* bioagents and the non-pathogenic fungus *F.oxysporum* resulted in significant reduction to tomato wilt with significant increase to the plant height, foliage fresh weight as well as the number of fruits and their weight / plant when added to soil inoculated with the causal pathogen compared with soil inoculation with the causal pathogen only. However, Maxim was the superior treatment in decreasing the severity of the disease and increasing plant height, foliage fresh weight and the produced fruit yield. In addition, the two tested *Trichoderma* bioagents were more efficient in reducing the severity of the disease and increasing values of plant height, foliage fresh weight and fruit yield (number and weight) / plant when the soil was amended with compost and the non-pathogenic isolate of *F.oxysporum* compared with control treatment. This may be due to compost comprises a substrate medium for the growth and reproduction of the two bioagents, hence increasing mode of action of both bioagents. Also, the non-pathogenic isolate of *F.oxysporum* can occupy the spaces between the sub-roots and the main root, which prevent the roots from the infection by the pathogenic fungus and/ or slight colonization of the xylem vesicles and this deprive the pathogen from occupation xylem vesicles well. In the present study, *Trichoderma* bioagents and the non-pathogenic isolate of *F.oxysporum* were protected tomato plants from the infection by the pathogenic isolate and also promoted plant growth (Nel *et al*., 2006). *F. oxysporum* species complex also includes numerous nonpathogenic strains, which have been shown to be effective in plant protection as biocontrol agents (Carmona *et al*., 2020). Depending on our results, we hypothesize that when the samples of wilted tomato plants collected to isolate pathogenic strains of *F. oxysporum*, there is a high proportion of isolating non-pathogenic *F. oxysporum* strains. In this regard, we obtained 3 non-pathogenic isolates during testing the

pathogenicity of seventeen strains of *F.oxysporum*. Depending on this data the non-pathogenic isolates collected in this investigation could be represent interesting candidates for further studies on the non-pathogenic strains of *F. oxysporum* as an alternative biocontrol approaches to control *Fusarium* wilt of many crops.

Nowadays, compost is considered one of many obtainable sustainable approaches that may be used to mitigate, prevent and/ or to manage many soil-borne plant diseases. Thereby, organic amendments such as compost play an important role as sustainable alternative process to protect plants against soil-borne pathogens and eco-friendly. Therefore, soil amendment using composted agricultural wastes fortified with bioagents could be acceptable approach in this regard. The use of organic agricultural wastes in this respect can be an advantageous in recycling of agricultural wastes, causes soil fertility and could provide a potent tool for management of soil-borne plant diseases. It has been reported that several composts and/or composts fortified with bioagents utilized as soil amendments reduced the density of pathogens propagules and prevented the grown plants from many soil-borne plant pathogens (García-Enciso *et al*., 2018; Khalil and El-Mghrabia, 2010). However, although the effectiveness of disease management by compost can be changeable (Termorshuizen *et al*., 2007), the economic significance and environmental benefits obtaining from utilizing compost can prevail any form of distrust that could hover on operators. In addition, it is supposed that compost may be labors as a reach nutrient source and refuge for the bioagents that compete antagonist with plant pathogens.

Recently, there has been a growing interest in using *Trichoderma* bioagents due to their efficacy as bioagents for managing many soil-borne plant diseases in many crops. Application of the two *Tichoderma* strains to tomato transplants has been found to be effective for suppressing wilt disease and has successfully induced systemic resistance in the treated plants. Protection of plants from the infection by many plant pathogens is may be due to induction of systemic resistance by many elicitors, where this is of low harmful and co-friendly to the environment in comparison with many agrochemicals that applied to manage plant diseases. The ability of *Trichoderma* spp. to suppress plant diseases is fundamentally due to their direct antagonistic effect on the pathogen by their capability to produce many lytic enzymes e.g. chitinases and β-1, 3-glucanases. The secreted enzymes by *Trichoderma* spp. hydrolyze and degradate the pathogen's cell wall occurs, thereby preventing the growth and reproduction of fungal pathogens (Hassan *et al*., 2020). Also, the reduction in the infection by plant pathogens and the raise in the plant height and foliage fresh weight of the treated plants might be due to both *T.asperellum* and *T.harzianum* produce plant growth regulators.

The obtained dated revealed that compost, the non-pathogenic isolate of *F.oxypsorum, T. asperellum, T.harzianum* and the Maxim fungicide resulted in considerable higher production of phenol compounds without valuable variation in their values compared with soil inoculated with *F.o.* f.sp*. lycopersici*. A cosiderable increase was observed in the total phenol compounds of plants treated with compost followed by the two tested bioagents. It has been found that phenolic compounds have been in the converge of many demonstrations of plant-defense mechanisms to plant pathogens *i.e.*, bacteria, fungi, and viruses, and many abiotic stresses like drought, salinity and UV (Kumar *et al*., 2020). Also, phenolic compounds exhibit antioxidant possessions and antimicrobial, which help the plants to prevent their major tissues from toxic action of reactive oxygen species as well as hedge pathogenic infections. Similar results were obtained (Nicholson and Hammerschmidt, 1992; Panina *et al*., 2007; Shalaby and Howitz, 2015; Islam *et al*., 2019).

To understanding of plant signaling pathways due to implementation of bioagents is elicitors and/or activators that encourage the synthesis of specialized pathogenesis related proteins, where it is one of the most remarkable and effective plant defense mechanisms versus many pathogens. So, elicitors induce similar defense restraints in plants as induced by the successful implementation of bioagents and /or the infection by the pathogen (Vilasinee *et al*., 2019).

Plant phenols are known as secondary metabolites and coprise several classes structurally diverse from natural biogenetically products driving from the shikimate-phenylpropanoids-flavonoids pathways. The plants want phenolic compounds for many purpose *i.e.,* growth, reproduction, pigmentation, resistance to plant pathogens and for other functions. In addition, some antibiotic phenols are found in plant cells as inoperative bound structures but by hydrolyzing enzymes of the plant (glycosidases) they are readily converted into biologically

active antibiotics in echo to pathogens invasion. Moreover, these phenolic compounds can be considered as preformed antibiotics, which the plant enzymes are perhaps found in the plants to animate them but are insular from their outriggers by compartmentalization (Osbourn, 1996).

The excessed amount of phenols provides sufficient substrate to oxidative reactions, which catalyzed by POD and/or by PPO that, exhaustion oxygen and producing fungitoxic quinones. This makes the medium unfavorable to the suitable development of the causal pathogens. So, if pre-existing antifungal phenolics are not sufficient to stop the development of procedure of the infection, plant cells usually comply by increasing the scale of pre-existing antifungal phenols in the infection situ, after the elicited raised activity of the enzymes (chaconne synthase and PAL) by the biosynthetic pathway (Christensen *et al*., 1998).

The phytoalexin α-tomatin in tomato plants is famed to induce programmed cell dying by activating signaling pathways out of protein G and tyrosine kinase resulting in intracellular accumulation of reactive oxygen species (ROS) and Ca++ viz. hydrogen peroxide (H_2O_2) (Gonzalez *et al*., 2012).

The estimated free and total ascorbic acid and photosynthesis pigments *i.e.,* chlorophyll a, chlorophyll and carotenoids were considerably increased due to the tested treatments *i.e.*, compost, both bioagents and the non-pathogenic isolate of *F.oxypsorum* in tomato plants grown under infection by *F.o.* f.sp*. lycopersici*. Both bioagents followed by compost were the best treatments for reduction the impact of the disease on free and total ascorbic acid (vitamin-C) in the fruits as well as photosynthesis pigments in the tomato leaves. Plants free from the infection by the causal fungus and without any another treatment signalized the highest values of photosynthesis pigments and total ascorbic acid. The negative impact of the tested treatments on the photosynthetic pigments and ascorbic acid may be due to the retardation of the activity of green plastids and vitamin-C metabolism. When a plant is subjected to physical or physiological effect, the free ascorbic acid can be oxidized into dehydro-ascorbic acid. Moreover, several workers have shown that the application of *Trichoderma* spp. caused an increment to chlorophyll content. The combined inoculation of the five isolates of *T. asperellum, T. harzianum* and *T. virens* (three isolates) impress to a considerable increase in chlorophyll-a, chlorophyll-b in tomato plants and personage isolates was less effective in case of combined inoculation(Elshahawy and El-Mohamedy, 2019). In addition, the treatment of onion plants with *T. viride* resulted in increasing chlorophyll- a by 7.7%, chlorophyll-b by 17.7%, and carotenoid by 18% content (Metwally and Al-Amri, 2020). Analogues results were obtained (Hunter *et al*., 2011).

CONCLUSION

In recent years, biological control is considered an important approach of agricultural biotechnology for managing many plant pathogens, which considers an environmentally friendly and sustainable alternative approach for disease management. The obtained results give a potential of the combination of compost, non-pathogenic isolate of *F.oxypsorum,* the bioagents *T.asperellum* and *T.harzianum* for efficient protection treatments against tomato Fusarium wilt. These treatments resulted in considerable increase in phenol compounds, photosynthesis pigments and vitamin-C.

CONFLICTS OF INTEREST

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