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Application of some treatments to root zone for increases yield of tomato grown in field

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ABSTRACT:

The study was conducted to study the effects of different alternative, non-chemical strategies and its combinations on soil-disease control and fruit yield of tomato. Also, quantify the level of resistance of different tomato cultivars against root and vascular diseases. The results indicated ability of four tomato cultivars to infect with *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* as fungal pathogens. When tomato plants were treated with *Trichoderma* and cumin essential oil for overcome infection by *Fusarium oxysporum*, the results indicated that there were no significant differences in most studied characters except fruit total yield per plant when plants infected by *Fusarium oxysporum*. Also, the results showed that significant only in total yield per plant when tomato plant exposed to infect by *Fusarium solani*. The obtained results indicated the ability of *Trichoderma* and cumin essential oil to overcome the bad effect of fungal pathogens.

MATERIALS AND METHODS:

1-Field survey for the causal microorganisms of tomato root-rot and wilting diseases:

Survey of root-rot and wilting diseases were carried out on tomato plantations at different regions in greenhouses from Aswan, luxor, Qena, Sohag and Assiut Governorates. Samples of infected tomato plants and seedlings collected and used for isolating the causal microorganisms.

2- Isolation and identification of the causal pathogen:

Fungi were isolated from root sample of diseased tomato plants. The roots washed under tap water, chopped into 2 cm small pieces and surface sterilize in 0.5% NaOCl for two minutes then rinsed twice with triple distilled water and placed on Potato dextrose agar (PDA) and finally kept in an incubator at 27°C. All the procedure carried out into laminar hood under sterilizes condition. After five days of incubation, small colonies of fungus appeared, which were picked with a sterilize toothpick and transferred to fresh PDA plates. Identification of the fungal isolates carried out by using the morphological characteristics of mycelia and spores by Assiut University Mycological Center (AUMC), Assiut. Egypt.

3- Pathogenicity test:

Fungal isolates identified as *(F. oxysporum*, *F. solani*, *R. solani* and M. phaseolina). All isolates tested under greenhouse conditions for their pathogenicity using susceptible tomato cultivars (Super strain, Castle rock and Hyprid 448). Inocula of the tested isolates (taken from 5 days old culture grown in PDA medium at 27^{o}C) were prepared by growing in sterilized conical flasks (1000 ml) containing sorghum medium (100g sorghum seeds, 50g clean sand, 100 ml water and autoclaved on two consecutive days) and incubated at 27oC for 15 days. Sterilized plastic pots (30cm in diameter) filled with autoclaved sand clay soil and infested with each pathogen at the rate of 2% w/w of sand clay soil, mixed well, thoroughly irrigated and left 7 days to ensure establishment of the tested isolates in soil. None infested soil used as control. Five-week-old tomato seedlings grown in small pots filled with the cornmeal-sand soil mixture of an examined isolates. The experiments carried out in the greenhouse conditions maintained at 22 to 28°C. Development of symptoms on the plants inoculated by fungi and the controls monitored continuously at weekly intervals for eight weeks. Re-isolations from all isolate performed on PDA medium **(Nash and Snyder 1962)**. Disease severity percentage of root rot, wilt diseases and plant growth parameters recorded after 8 weeks from seedling date. The arbitrary (0-5) disease index scale as described by **Grunwald *et al.* (2003)** adopted. Where: 0= No visible symptoms, 1= slight hypocotyls lesions, 2= lesions coalescing around epicotyls and hypocotyls, 3= lesions starting to spread into the root system with root tips starting to be infected, 4= epicotyl, hypocotyls and root system almost completely infected and only slight amount of white, uninfected tissue was left, and 5= completely infected root. Disease severity percentage of root rot disease recorded using the following formula:

Disease severity (%) = $\sum$ [(n x V) /5xN)] x 100

Where, n= number of plants with in each infection category,

V= numerical values of infection categories.

N= total number of plants examined

5= constant, highest numerical value.

4- Controlling of root rot and wilt diseases of tomato:

4.1 Biological control of root-rot and wilt diseases of tomato:

Source of biocontrol against:

T. harizianum was provided by Dr.: Kamal Abo El-Youser, Faculty of Agriculture, Assiut University. *T. hamatun* was purchased from Mycology center at Assiut University. Antagonistic bacteria were obtained from agricultural Botany Department, Faculty of Agriculture, South Valley University, Qena.

4.1.1. Preliminary tests for antagonistic capability of certain isolated microorganisms against tested isolates *in vitro*:

Antagonistic capability of fungal isolates studied in dual cultures according to **Hassan (1992)**. Sterilized Petri plates (9 mm in diameter), each containing 10 ml of PDA medium (pH 7) were used in this study. Disc from each antagonistic fungal isolates (6 mm in diameter) was inoculated on PDA medium in one site of Petri plate and opposite site was plated with the tested pathogenic isolates (6 mm in diameter taken from 5 days old culture grown in PDA medium at 27^{o}C). Three replicates used for each treatment. The inoculated plates incubated at 27°C until the fungal growth covered the plate surface compared with the control treatment. Diameter of fungal growth in all treatments was determined

The percentage of mycelial growth reduction calculated according to the formula proposed by **Fokkema (1973)** as follows:

Reduction % = C-T/C * 100

Where: C = growth of the pathogenic fungus in control plates, T = growth of the pathogenic fungus in dual organisms plates.

4.2 Effect of essential oil on incidence of root rot and wilt diseases of tomato

4.2.1. *In vitro* studies:

Pure-grade of the essential oils, *i.e.* Eucalyptus (*Eucalyptus obliqua* L.) and cumin (*Cuminum cyminum* L.) obtained from Cairo Company for oils and aromatic extractions, Cairo Industrial Development (CID), Egypt. The essential oils were stored in dark glass bottles.

The essential oils used to study their effect on mycelial growth of tested pathogenic fungi. Concentrations of each tested compound adjusted at 2, 4, 8, and 16 µl/gm, adding to conical flasks containing 100 ml Czapek's agar medium, mixed well, poured in Petri plates (9 cm in diameter) according to **El-Ganainy *et al.* (2002)**. The plates inoculated in the center with discs (6 mm in diameter) of tested pathogenic fungi (taken from 5 days old culture incubated at 27^{o}C). Inoculated Petri plates with pathogen without tested compounds used as control. Three replicates used for each treatment. Data were recorded by measuring the diameter of linear growth when the control plates completely covered by the fungal mycelium.

Percentage of mycelial growth reduction =(A-B/A) × 100

Where: A=Diameter of the hyphal growth in control.

B= Diameter of hyphal growth in the tested isolate.

5. Field experiment:

This study was carried out at the Experimental Farm of Faculty of Agriculture, South Valley University, Qena, Egypt during seasons of during seasons of 2015 and 2016 to evaluate the efficiency of the tested bio-control and essential oil treatments for controlling root rotting of tomato plants. The chosen field test area was naturally infested with *Fusarium oxysporum*. Tomato seeds of four tomato genotypes i.e., E448, Super red, Alshuruq and Super strain B were surface sterilized in 1% solution of sodium hypochlorite for 30 sec., rinsed thoroughly with several changes of distilled water, and then dried with sterile blotting paper. The seeds germinated in 15 x 20 x10 cm trays containing sterilized sand. Twenty-five days old, roots of tomato seedlings treated by immersion for 30 min in a solution of tested fungi **(EL-Mohamedy *et al*. 2014**). Roots of tomato seedlings treated with *F. oxysporum* by immersing them in a spore suspension of 1×10^8 cfu/ml. After that, the tomato seedlings transplanted in open field. For control treatment roots of tomato seedlings were dipped in pure water for 30 min. Disease incidence of post-rise damping-off and plant survival were recorded 45 days after sowing as shown in the following equations (**Khalifa 1987).** In our experiment, we are used two bio-control agents such as *Trichoderma* and Cumin oil. The experimental design was a complete randomized block with three replicates. Each experiment evaluated in a randomized complete block design (RCBD) with three replications. Each block contains 12 plots. The experimental unit area was $10.5m^2$ (3.5x3m). Each unit included four rows; each row was 3.5m in length and 75cm width. Tomato transplants were at a rate of 10 seedlings within each row. Planting and transplanting dates were 15th September in 2015 and 2016, respectively. The experimental field soil was sandy-clay in texture. The NPK mineral fertilizers applied at the recommended dose of Ministry of Agriculture and Land Reclamation. Normal agronomic practices of growing tomato carried out until harvest. Ten plants randomly selected from each plot in each replication for observing the data. The character included was total yield per plant (kg). Yield per plant recorded from randomly selected plants from each replication and average was calculated.

Statistical analysis:

All data statistically analyzed and treatments means compared using Duncan's multiple range test according **Gomez and Gomez (1984).**

RESULTS

1- **Field survey for the causal microorganisms of tomato root-rot and wilt diseases:**

Data in **Table (1)** revealed that the highest percentage of disease severity recorded in Qena Governorate. While, the lowest percentage of disease severity was observed in Assiut Governorate. In addition, data indicated that 94 fungal isolates secured. These isolates included 39 isolates of *R. solani* followed by *F. oxysporum* including 32 isolates *F. solani* included 14 isolates and *M. phaseolina*. Included 9 isolates. Moreover, the highest isolate numbers of *R. solani* and *F. oxysporum* recorded in Qena followed by Aswan. While the lowest isolate numbers of *R. solani* and *F. oxysporum* recorded in Assiut. Data in **Table (1)** revealed that the highest infection percentage recorded in Qena Governorate While the lowest infection percentage observed in Sohag Governorate. On the other hand, the *M. phaseolina* was not isolated from Aswan and sohag but lowest isolate number were isolated from Qena and Assiut Governorates. The isolated fungi from different locations were identified as *F. oxysporurum F. solani R. solani* and *M. phaseolina.*

Table (1): Identity, occurrence and frequency of root-rot and wilting fungi isolated from diseased tomato plants collected from different Governorates.

Governorate	(%) of disease severity	No. of isolated Fungi				Total
		F. oxysporum	*F. solani*	*R. solani*	*M. phaseolina.*	
Aswan	14	9	5	12	0.0	26
Qena	16	11	2	14	6	33
Sohag	7	9	3	6	0.0	18
Assiut	11	3	4	7	3	17
		32	14	39	9	94

2. Pathogenicity test:

Data in **Table (2)** indicated that tested fungi differed in their virulence against tomato plants. In this respect, *F. oxysporum* seemed to be more aggressive which caused the highest percentage of disease severity.

Data **also**, indicated that *M. phaseolina* was less suppressive it recorded the lowest percentages of pre- and percentage of disease severity and gave the highly of healthy survival plants compared with the other fungi. On the other hand, data the hybrid 448 was more resistant to fungal pathogens compared with other tomato varieties (super strain and castle rock).

Table (2): Pathogenicity of pathogenic fungi under greenhouse conditions.

Tomato genotypes	% of disease severity				Total
	F. oxysporum	*F. solani*	*Rhizoctonia solani*	*M. phaseolina*	
Super strain	0/10 (0.0%)	0/10(0.0%)	0/10 (0.0%)	2/10 (20%)	2/40 (5%)
Castle rock	0/10(0.0%)	2/10 (20%)	2/10 (20%)	4/10 (40%)	8/40(40%)
Hyprid 448	1/10 (10%)	2/10 (20%)	1/10 (10%)	9/10 (90%)	13/40(32.5%)
Total	1/30 (3.3%)	4/30(13.3%)	3/30 (10%)	15/30 (50%)	23/120(19.2%)

3. Controlling of root-rot and wilt diseases of tomato:

3.1 Biological control of root rot and wilt diseases of tomato:

3.1.1. Preliminary tests for antagonistic capability of certain isolated microorganisms against tested isolates *in vitro*:

Antagonistic effect for biocontrol agents performed to select the more potent isolate for using in subsequent experiments in this research. Screening showed that two bacterial isolates identified as *Bacillus subtilis and B. megatherium.* Two bacterial strains do not have any antagonistic effect against the four-pathogen fungi *F. oxysporum F solani Rhizoctonia solani* and *M. phaseolina.*

Fungal isolates identified as *T.harzianum and T. hamatum* showed antifungal activity against the two pathogenic fungi *F. oxysporum* and *M.* but T. *harzianum* was less effective, which give antagonistic activity against *M. phaseolina*. only.

Data in **Table (3)** demonstrated that *T. harzianum* was the best strain since recorded 65.6% in growth reduction of *Macrophomina sp.* followed by *T humatum* (30.7 and 33.7 %) in growth reduction of *F. oxysporum* and *M. phaseolina* respectively. On the other hand *T. harzianum* and *T. hamtatum* were the best strains and showed the highest antagonistic effect against *Macrophomina sp.* These strains resulted the highest percentages of growth reduction of *Macrophomina sp.* being 65.6 and 33.7% respectively. On contrast, *T. harzianum* and *T. humitatum* do not lead to any effect against *Fusarium* spp.

Table (3): Effect of different bio-control agents on mycelial growth of different pathogenic fungi *in vitro*.

Biocontrol agent	*F. oxysporum*		*F. solani*		*R. solani*		*M. phaseolina*	
	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
T. harzianum	90.0	0.0	90.0	0.0	90.0	0.0	31.0	65.6
T. humitum	62.3	30.7	90.0	0.0	90.0	0.0	59.7	33.7
B. megatherium	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0
B. subtilis	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0
Control	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0

3.2 Effect of essential oil on incidence of root-rot and wilt diseases of tomato:

3.2.1. *In vitro* studies:

The essential oils were able to reduce the growth of tested pathogenic fungi **(Table 4)**. Eucalyptus oils at concentration of 16 µl/gm gave a high percentage of inhibition of mycelium growth of each tested pathogenic

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fungus. The highest inhibition found in cumin essential oils at 16 µl/gm tomato.

Table (4): Estimation of essential oils activity against disease severity induced by pathogenic fungi on tomato.

Fungal Pathogen	Eucalyptus oil concentration				Cumin oil concentration			
	2 µl/gm	4 µl/gm	8 µl/gm	16 µl/gm	2 µl/gm	4 µl/gm	8 µl/gm	16 µl/gm
Fusarium oxysporum	90.0	90.0	0.0	0.0	90.0	70.0	60.0	53.0
Fusarium solani	85.0	75.0	75.0	60.0	90.0	60.0	40.0	40.0
Rhizoctonia solani	90.0	66.0	56.0	41.0	90.0	80.0	50.0	30.0
Macrophomin sp.	90.0	75.0	63.0	60.0	0.0	0.0	0.0	0.0

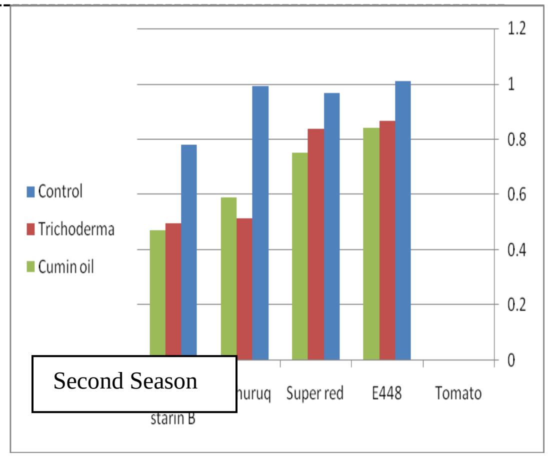
Field experiments

Evaluated four local tomato genotypes exposed to infect with *F. oxysporum* and planted in open field are presented in Table 5 and Figure 1. The results indicated that there were significant differences among the tomato genotypes in this trait. Both E488 and Super red genotypes were significantly higher than other two genotypes (Alshuruq and Super strain B) in both studied seasons 2015 and 2016. The highest value was recorded from E488 tomato genotype while the lowest value was obtained from Super stain B the same trend was clear in the second season 2016. Compared among the control plants and treated tomato plants with essential oil are also presented in Table 5 and Figure 1. The results showed that control plant gave the highest value and significantly higher than other treatments. Interaction between tomato plants and treatments in **Table (5)** presented that there were significant differences among these interactions. The range of total yield per plants was (1.01 to 0.471 kg) in the first season 2015 and was (1.100 to 0,480 kg) in the second season 2016. The highest value in this trait was recorded from interaction (E488 x control) while the lowest value of total yield per plant was recorded from interaction (Super strain B x Cumin oil) in both studied seasons.

Table (5): The effect of both *Trichoderma harzianum* and Cumin oil on total yield per plants character of four tomato genotypes infected with *Fusarium oxysporum* in open field during seasons 2015 and 2016 in Qena governorate.

Total yield per plants(kg)				
First season 2015				
Treatments / Tomato	**Control**	***Trichoderma*** *harzianum*	**Cumin oil**	**Mean**
E448	1.010 a	0.8653 a	0.8413 a	0.9056 A
Super red	0.9667 a	0.8387 b	0.7513 b	0.8522 A
Alshuruq	0.9900 a	0.5140 c	0.5910 c	0.6983 B
super starin B	0.7800 b	0.4960 c	0.4710 c	0.5823 B
Mean	0.9367 A	0.6785 B	0.6637 B	
Second season 2016				
Treatments / Tomato	**Control**	***Trichoderma harzianum***	**Cumin oil**	**Mean**
E448	1.100 a	0.8700 a	0.8600 a	0.9433 A
Super red	0.9800a	0.8400b	0.7600b	0.8600 A
Alshuruq	0.9900 a	0.5250c	0.5910 c	0.7020B
super starin B	0.7900 b	0.5000c	0.4820c	0.5906B
Mean	0.9650 A	0.6820B	0.6720B	

Values followed by the same letter or letters are not significantly different at 5% level.



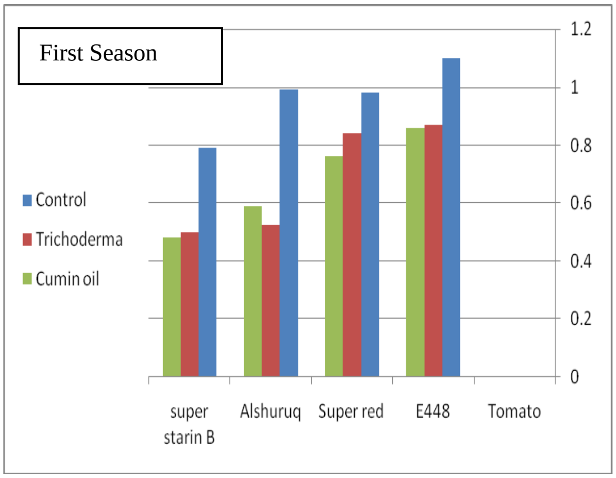


Figure (1): The effect of both *Trichoderma harzianum* and Cumin oil on total yield per plants character of four tomato genotypes infected with *Fusarium oxysporum* in open field during seasons 2015 and 2016 in Qena governorate.

DISCUSSION:

Tomato is one of the most important vegetable crops. Soil borne diseases including wilt and root rot cause important considerable losses in yield. In the present investigation, extensive survey conducted throughout three Egyptian governorates (Assuit, Sohag, Qena and Aswan) to determine the main causal pathogens of these diseases. The obtained fungal isolates belonging to three genera were isolated from diseased plants viz. *F. oxysporum F. solani R. solani* and *Machrophomina* sp. Pathogenicity test proved all the obtained isolates were pathogenic and virulent for tomato plants. The most aggressive fungi are *F. oxysporum R. solani* and *Machrophomina* sp. These results are in harmony with those reported by other researchers **(Saad 2006 Morsy et al. 2009 Abd El-Monaim 2010)**. Controlling such, diseases mainly depend on fungicides treatments. However, fungicidal applications cause hazards to human health and increase environmental pollution. The antagonistic effect of *Trichoderma* spp might be attributed to producing cell wall degrading enzymes ghicanase and chitinase, which lyse the cell wall of the pathogenic fungi inhibition of the host mycelium and hyphal penetration, by *Trichoderma*. It found that *T*

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harzianum suppressed the growth of *F. oxysporum* and a direct contact observed between the two fungi. Thereafter *T harzianum* grew over mycelium of *F. oxysporum.* Within the large reservoir of natural fungicides that exist in plants examples exist that would serve as safe and effective alternatives to synthetic fungicides. Such compounds (volatile components essential oils) if properly formulated and applied could be used directly or could serve as templates for synthetic analogs. In the present study preliminary data suggested, that the evaluated essential oils are capable of fungal growth inhibition *in vitro* when tested in direct contact. Application of Eucaliptus and cumin essential oils as seed coating revealed their efficacy against seed or plant invasion under *in vivo* conditions, which result in a significant reduction in root rot and wilt incidence of tomato under greenhouse conditions. The suppression of wilt and root rot development under greenhouse seem to corresponds with the ability of these essential oils to reduce disease incidence. Eucaliptus oil had a superior effect in this concern against *F. oxysporium* out of other fungi. On the other hand, the cumin oil was highly active against root rot caused by *Machrophomin* sp. The used essential oils are reported to contain many volatile compounds **(Singh *et al.*,1980)** so it seems that the antifungal effects are the result of compounds acting synergistically. This means that the individual components by themselves are not sufficiently effective. Similar results were also report by many researchers indicating the efficacy of essential oils as antifungal inhibitors for a large number of soilborne pathogens **(Akgul and Kivanc 1988; Kumar and Tripathi 1991; Singh 1992 and 1994)**. The mode by which microorganisms are inhibiting by essential oils and their chemical compounds seems to involve different mechanisms. It has been hypothesize that the inhibition involves phenolic compounds because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents **(Juven 1994)**. Many authors empha sized that the antimicrobial effect of essential oil constituents has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane. In the light of these results, it could be conclude that the application of biological control and essential oils is applicable safe and cost-effective method for controlling soilborne diseases. In addition, the use of essential oils in agriculture as fungicides has the advantages as they disintegrate in nature and do not leave a toxic residue of the product. The yield of four tomato genotypes screened in our study under infected with four fungi i.e., *Fusarium oxysporum* *Fusarium solani* *Rhizoctonia solani* and *Macrophomina phaseolina*. The results indicated that there were significant differences among these genotypes in all experiments except when infected tomato plants with *Rhizoctonia solani*. The highest values obtained from Alshuruq while the lowest value obtained from super strain B genotype. The variation in yield among these genotypes are in the line with those reported by **Todorov and Pevicharova (2003)** they reported that foreign hybrid tomato cultivars showed higher variation of fruit yield, good adaptability, high yield potential and good quality compared to common (nonhybrid) cultivars. **Singh *et al*., (2002)** they studied variability of 92 genotypes of tomato with regards to number of fruit clusters plant in India during winter seasons 2000-2001. They reported that the high genotypic and phenotypic variation found for number of fruit clusters plant. The phenotypic and genotypic variation observed among 92 genotypes of tomato concerning number of fruit plant. **Singh *et al*., (2002)** reported that highly significant variation observed in case of number of fruits plant-1 among these evaluating under agro-climatic conditions of the area and to compare performance of local variety **(Rehman *et al*., 2000)**. **Islam *et al*., (1999)** studied 10 yield components in 26 diverse genotypes of tomato and observed highest genetic variability for number of fruits plant. A significant range of variation for number of fruits plant among 11 varieties of tomato reported by **Fatunla (1969)**. **Kabir (2004)** set up an experiment with 14 tomato genotypes reported that the highest fruit yield (1.35 kg) was recorded in CLN2443A and the lowest fruit yield (0.52 kg) was obtained from Momotaru.

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