EFFECT OF SOME PLANT EXTRACTS, PLANT OILS AND TRICHODERMA SPP. ON TOMATO FUSARIUM WILT DISEASE

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Received: Dec. 23, 2020 Accepted: Dec. 31, 2020

ABSTRACT: Plant extracts of *Aloe vera* and *Syzygium aromaticum* (clove) inhibited the growth and sporulation of *Fusarium oxysporum* f.sp. *lycopersici* (FOL) both under laboratory and green-house conditions. However, essential oils also reduced the growth and spore population of FOL significantly. The best results were obtained when clove oil was applied; followed by *Mentha arvensis* (mint) oil. *Trichoderma harzianum* and *T. asperellum* were the best tested *Trichoderma* spp. isolates in reducing the growth of FOL. Under green house and artificial soil infestation conditions, All the abovementioned treatments reduced the wilt disease incidence and improved the growth of tomato plants; significantly.

Key words: Fusarium oxysporum f.sp. lycopersici. Lycopersicon esculentum. Syzygium aromaticum. Mentha arvensis. Trichoderma harzianum. T. asperellum.

INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) belongs to the family Solanaceae, is a popular vegetable widely grown in the tropics and is the second most important vegetable crop next potato in Egypt and all over the world, Hafez et al., 2012. The vascular wilt of tomato caused by Fusarium oxysporum f.sp. lycopersici (FOL) (Sacc.) Snyder and Hansen, is one of the most destructive diseases, resulting significant yield losses Anam et al., 2017 and Aleaghaee et al., 2018. Aloe vera plant extract had inhibitory effect on Fusarium oxysporum in vitro Taiga et al., 2008 and Ali et al., 2013. However, Yeole et al., 2016 reported that clove extract exhibited 100% inhibition of FOL spores at 5 and 10 ml/L. They mentioned that antifungal potential of clove extract was confirmed as compared to the efficacy of chemical fungicides. Farrukh Aqil et al., detected that the maximum antifungal activity of essential oils due mainly to clove followed by peppermint and eucalyptus. They also mentioned that Fusarium chlamydosporum was

found to be most susceptible to essential oils in liquid as well as agar media. The antifungal activity of clove oil was also reported by many authors Abhishek Sharma et al., 2016 and Torre et al., 2016. Mohd Rajik et al., 2012 proved that Trichoderma harzianum and T. viride provided induced resistance in plant against Fusarium oxysporum lycopersici resulting declined disease incidence from 100 to 7.69% and the maximum inhibition was noted by T. harzianum. Trichoderma harzianum was observed to be good biocontrol agent against FOL by several authors (Akrami and Yousefi 2015; Lakshman Prasad et al., 2016; Andleeb Zehra et al., 2017; and Mwangi et al., 2019). Prachi Singh et al., 2019 reported that Trichoderma asperellum showed maximum inhibition of Fusarium oxysporum f.sp. lycopersici. Trichoderma asperellum strains significantly reduced wilt disease incidence and severity compared to FOL only infected plants. Moreover, the application of T. asperellum promoted tomato plant growth irrespective of the presence or absence of FOL.

The aim of this study was to find out some ecofriendly methods to control tomato fusarium wilt. Plant extracts, essential oils and *Trichoderma* spp. isolates were used for this purpose.

MATERIALS AND METHODS

Isolation, purification and identification of the tested fungi:

Both the pathogenic and antagonistic fungi were respectively isolated from diseased and healthy tomato plants grown in Sadat citv. Menoufia governorate. Disease tomato plants showing clear wilt symptoms were collected, roots and stem bases of such plants were gently washed by running tap water to remove the adhesive soil particles. The samples were surface sterilized by 70% ethanol, rinsed several times with sterilized distilled water, dried between sterilized filter papers, cut into small pieces and then planted on potato dextrose agar (PDA) medium contained (300 antibacterial antibiotic Streptomycin sulphate) to avoid the bacterial growth. Petri dishes were incubated at 25°C for and examined daily the abundant growth. meantime, healthy tomato plants showing were collected from the same fields and the rhizosphere soil was used to isolate the associated microorganisms on PDA medium. War cup soil plate Ammar, 2003 and dilution plate method achieve were followed to such microorganisms.

Streak and/or dilute/plate methods were carried out to obtain single propagule unit cultures. Pure cultures were kept at 5°C for the further studies.

All the obtained isolates were identified at Botany Department, Faculty of Agriculture, Menoufia University.

II- Pathogenicity test experiments:

These experiments were carried out under greenhouse and sterilization

condition at the Faculty of Agriculture, Menoufia University, during 2018 growing season. Clay loam soil was autoclaved twice at 121°C for an hour. Pots were sterilized by dipping in 5% formalin for 5 minutes and left for a week to allow formalin evaporation.

Inoculum were prepared by growing each isolate on sterilized Barley sand medium (75 g barley + 25 g sand + 100 ml water); using 500 ml flasks. Flasks which incubated 25°C for 2 weeks and shacked every second day to allow more fungal growth.

Soil infestation was conducted; separately; at the rate of 3% of soil weight. Potted soil was irrigated every second day for a week to allow the fungal distribution into the soil.

Tomato seedling (Cv. K-186) 24 days old were planted, after root sterilization in the infested soil. Control treatment had sterilized soil contained the same percentage of Barley medium (3%). The grown plants were examined every week for the wilt disease. After two months, the plants were picked up and longitudinal sections through stem bases and roots were caried out to measure the length of browning vesicles of each plant.

III- Laboratory experiments:

A complete randomized design (CRD) with three replicates was followed in these experiments.

III-1- Effect of some plant extracts on FOL growth and sporulation:

Two hundred grams of each tested plant (Aloe vera, clove, garlic, nigella and mint) were separately soaked in one-liter sterilized distilled water for 24h. The extracts were heated at 90°C for 30 m, filtered through filter paper, completed to be 1L and autoclaved at 90°C an hour. Extracts were added to PDA medium to obtain the concentrations of 5,10,15%

dilution method (Akaeze and Modupe, 2017).

III-2- Effect of some essential oils on FOL growth and sporulation:

Crude oils of clove, mint and garlic were obtained from El-Gomhouria company for oils, Cairo, Egypt. Each oil was emulsified with 3% (v: v) tween 20 and mixed with PDA medium to obtain 5, 10 and 15% concentrations (Fontes et al., 2018). Petri dishes contained Plant extracts or oils were inoculated with 5 mm disc of FOL (7days old PDA cultures and inoculated 25±2 °C for 7 days.

III-3- Effect of *Trichoderma* spp. on FOL fungal growth:

Five Trichoderma spp. isolates i.e., T. hamatum, T. harzianum, T. koningii, T. asperellum and T. viride were individually tested for their antagonistic effect(s) against FOL pathogen. Dual culture method was followed according to (Devi et al., 2015). Control plates had the pathogen disc 5mm in the middle. Petri dish were incubated at 25±2 °C for 7 days and examined daily.

III-4- Data recorded:

III-4-1- Growth diameter:

Three replicates were caried out per each treatment of the mentioned laboratory experiments. When a petri dish showed full growth; the average diameter of FOL fungal growth (mm) was recorded. Percent inhibition over control was calculated as per the formula (Sundaramoorthy and Balabaskar 2013):

PI % =
$$\frac{C-T}{C}$$
 x 100

Where,

PI= percent inhibition over control C: Mycelial radial growth in control

T: Mycelial radial growth in treatment

III-4-2- Spores population:

The tested isolate of FOL was grown on potato-dextrose agar (PDA) in

darkness at 22-25°C for one week. By the aid of camel hair brush; the formed spores were gently removed using 10 ml distilled sterilized water. **Spore** suspension (102 spore/ml) was then, filtered through a layer of Mira cloth and the suspension was diluted and counted using haemocytometer (Jahanshir and Dzhalilov 2010). Fifty random squares of the haemocytometer served as replicates and finally the average number of spores/ml was calculated as per the formula:

No. of spore /ml = No. of spores x dilution x factor

IV- Greenhouse experiments:

These experiments were carried out under greenhouse conditions at the Faculty of Agriculture, Menoufia University. Sterilization of the pots, the soil and soil infestation were conducted as mentioned in Pathogenicity test experiments. Three Pots (15 cm in diameter) were used as replicates for each treatment.

IV-1- Effect of plant extracts and oils on FOL spore population in the soil:

Tomato seedlings of the cv. K-186 (24 days old) were planted in the infested soil with FOL (3%). The pots were irrigated by different plant extracts and /or plant oils (75 ml/pot); after 3 days of planting. The concentrations of either extracts or oils were 5, 10 and 15%. Control pots were irrigated with sterilized distilled water. Irrigation of the different treatments was accomplished every week. Ten days after planting; one-gram soil from the middle of each pot was picked up separately and add to 99 ml sterilized distilled water. Spores of FOL were counted in this dilution using haemocytometer (1/400 m2); NEBAUER IMPROVED, Germany (Starovic et al., 2016). The abovementioned methods were carried out every 10 days (five times).

IV-2- Effect of plant extracts and oils on wilt disease incidence:

Both percentage and severity of infection with FOL were estimated after 55 days of sowing. Wilt disease percentage of infection (PI) was determined from according to this formula:

However, the severity of infection (SI) was estimated using 0-4 scale and the formula:

Sum of (disease grade x No. of plants in grade)
SI= x 100
No. of total plants x Max. grade infection

IV-3- Effect of plant extracts and oils on tomato growth parameters:

At the end of these experiments; average of plant height, number of branches and number of leaves/ plants were estimated.

IV-4- Effect of *Trichoderma* spp. on the disease incidence and plant growth:

Separate applications of five *Trichoderma* spp. isolates were tested for the disease control and tomato plants growth response. The biocontrol agents were individually added to the soil at 3% (w: w) and at the same time of soil infestation with FOL.

V- Statistical analysis:

All experiments were conducted in completely randomized design. Mean values were compared by the least significant difference (LSD) testing at p = 0.05. Duncan's multiple Range test at p = 0.05 was used to compare means. All statistical analyses were performed using Costate, Statistical Software.

RESULTS AND DISCUSSION

Six isolates of *Fusarium oxysporum* f.sp. *Lycopersici* (FOL) were observed and isolate No. 2 was chosen for this study where it produced more spores. All the obtained isolates were pathogenic to tomato plants. These results are in agreement with (Anam *et al.*, 2017 and Aleaghaee *et al.*, 2018).

In the meantime; five *Trichoderma* spp. isolates were obtained from the rhizosphere of healthy tomato plants. These isolates were identified as *T. harzianum*, *T. asperellum*, *T. viride*, *T. hamatum* and *T. koningii*.

I- Laboratory experiments:

I-1- Effect of plant extracts on the growth and sporulation of FOL:

Results present in Table (1) indicate that *Aloe vera* (cactus) and clove extracts completely inhibited the growth and sporulation of FOL even at the lowest tested concentration (5%). Such results were also obtained by Taiga *et al.*, 2008 and Ali *et al.*, 2013. However, Yeole *et al.*, 2016 mentioned that antifungal potential of clove extract was confirmed as compared to the efficacy of chemical fungicides.

I-2- Effect of plant oils on the growth and sporulation of FOL:

Results shown in Table (2) clear that all tested concentrations of plant oils reduced the linear growth of FOL significantly; in comparison with control. It is of logic that increasing oil gives more significant effect in reducing the fungal growth. Clove oil followed by mint oil had the best efficiency in reducing both growth and sporulation of FOL. Complete spore inhibition was observed when clove oil (at all concentration) and mint oil (15%) were individually applied. The antifungal activity of clove oil was also reported by Abhishek Sharma et al., 2016 and Torre et al., 2016.

Table (1): Effect of different concentrations of some plant water extracts on the growth and sporulation of *Fusarium oxysporum* f.sp. *lycopersici*:

| | <u> </u> | | | | | |
|---------------|-----------|--------------------|----------------------|------------------------------|---------------------------|--|
| Plant extract | Conc. (%) | Linear growth (mm) | Growth reduction (%) | No. of. spores/ml (x1000) | Sporulation reduction (%) | |
| | 5 | 00.00i | 100.00 | 00.00f | 100.00 | |
| Cactus | 10 | 00.00i | 100.00 | 00.00f | 100.00 | |
| | 15 | 00.00i | 100.00 | 00.00f | 100.00 | |
| | 5 | 00.00i | 100.00 | 00.00f | 100.00 | |
| Clove | 10 | 00.00i | 100.00 | 00.00f | 100.00 | |
| | 15 | 00.00i | 100.00 | 00.00f | 100.00 | |
| | 5 | 43.33f | 50.01 | 21.33e | 82.61 | |
| Garlic | 10 | 00.00i | 100.00 | 00.00f | 100.00 | |
| | 15 | 00.00i | 100.00 | 00.00f | 100.00 | |
| | 5 | 65.67b | 24.23 | 96.00b | 21.74 | |
| Mint | 10 | 53.33c | 38.47 | 53.33c | 56.52 | |
| | 15 | 47.67d | 45.00 | 32.00d | 73.37 | |
| | 5 | 45.00e | 48.08 | 32.00d | 73.37 | |
| Nigella | 10 | 35.33g | 59.24 | 21.33e | 82.61 | |
| | 15 | 25.67h | 70.38 | 16.00e | 86.96 | |
| Control | | 86.67a | 00.00 | 122.66a | 00.00 | |
| L.S.D 0,05 | | 1.52 | | 6.40 | | |

Table (2): Effect of different concentrations of some plant oils on the growth and sporulation of Fusarium oxysporum f.sp. lycopersici:

| Plant oil | Conc. (%) | Linear growth (mm) | Growth reduction (%) | No. of. spores/ml (x1000) | Sporulation reduction (%) |
|--------------------|--------------|--------------------------|----------------------|---------------------------------|---------------------------|
| | 5 | 00.00k | 100.00 | 00.00i | 100.00 |
| Clove | 10 | 00.00k | 100.00 | 00.00i | 100.00 |
| | 15 | 00.00k | 100.00 | 00.00i | 100.00 |
| | 5 | 58.67f | 32.31 | 64.00c | 45.45 |
| Garlic | 10 | 55.33g | 36.16 | 48.00d | 59.09 |
| | 15 | 49.00h | 43.46 | 26.66g | 77.27 |
| | 5 | 14.33i | 83.47 | 16.00h | 86.36 |
| Mint | 10 | 10.00j | 88.46 | 8.00i | 93.18 |
| | 15 | 00.00k | 100.00 | 00.00i | 100.00 |
| Control (tween) | | 86.33a | 00.39 | 114.66a | 2.27 |
| Control | | 86.67a | 00.00 | 117.33a | 0.00 |
| L.S.D 0,05 | | 00.10 | | | 6.56 |

I-3- Effect of different *Trichoderma* spp. isolates on the growth of FOL:

Results given in Table (3) clear that all Trichoderma spp. isolates inhibited the linear growth of FOL, significantly. In this request. harzianum and T. asperellum showed the best action while T. hamatum and T. koningii gave the least efficiency. Inhibition zones recorded 24.67, 24.33 and 20.67 mm between FOL in side and T. viride, T. koningii, and T. hamatum respectively in the other side. However, T. harzianum and T. asperellum overgrew on (FOL) colony. Such results were also observed by (Akrami and Yousefi 2015; Lakshman Prasad et al., 2016; Andleeb Zehra et al., 2017and Mwangi et al., 2019).

II-Green house experiments:

II-1- Effect of some plant extracts on FOL spore population in the soil:

Results present in Table (4) indicate that the application of any tested plant extracts to the infested soil with FOL decreased the population of the pathogen's spore; significantly, in comparison with control. The best results

were obtained when *Aloe vera* plant extract was applied followed by clove one. Increasing the concentration of the plant extract resulted more reduction of FOL spore population. These results are in harmony with those obtained by Taiga et al., 2008; Ali et al., 2013 and Yeole et al., 2016.

II-2- Effect of some plant extracts on wilt disease incidence:

Results shown in Table (5) indicate that application of the plant extracts significantly decreased both percentage and severity of tomato wilt disease incidence. Aloe vera and clove plant extracts gave the best results where at the highest concentrations (15%), tomato plants were completely free of infection. However, at the lowest concentration (5%) of either Aloe vera or clove, severity of infection was 7.41 and 11.11% respectively. the nontreated control plants (FOL 92.6% only) resulted infection severity. It was noticed that more spore population in the soil resulted more disease incidence and vice versa such results are in logic and were also noticed by Yeole et al., 2016.

Table (3): Effect of different *Trichoderma* spp. isolates on the growth and sporulation of *Fusarium oxysporum* f.sp. *lycopersici*:

| | | Growth | Mode of action | | |
|------------------------|--------------------|---------------|----------------|-----------------|--|
| <i>Trichoderma</i> sp. | Linear growth (mm) | reduction (%) | O. G* (mm) | I. Z ** (mm) | |
| T. asperellum | 37.33d | 56.93 | + | _ | |
| T. hamatum | 51.33b | 40.78 | _ | 20.67 | |
| T. harzianum | 36.00d | 58.46 | + | _ | |
| T. koningii | 45.33c | 47.70 | _ | 24.33 | |
| T. viride | 42.67c | 50.77 | _ | 24.67 | |
| Control | 86.67a | 00.00 | | _ | |
| L.S.D 0,05 | 4.02 | | | | |

^{*} O.G: over growth (mm)

^{**} I.Z: Inhibition zone (mm)

Table (4): Effect of some plant extracts on spore population / I gm soil infested with FOL:

| Plant extract | Conc. (%) | 10* | R*% | 20* | R*% | 30* | R*% | 40* | R*% | 50* | R*% |
|---------------|--------------|-------|------|--------|------|--------|------|---------|------|--------|------|
| | 5 | 4.3hi | 58.1 | 3.3f | 71.5 | 3.0de | 73.0 | 2.7 de | 80.1 | 2.3 fg | 84.1 |
| Cactus | 10 | 3.7ij | 64.5 | 2.7fgh | 77.1 | 2.3efg | 81.1 | 2.0 efg | 85.7 | 1.3 hi | 90.1 |
| | 15 | 2.7k | 74.2 | 2.0h | 82.9 | 1.7g | 86.5 | 1.3 g | 90.5 | 1.0 i | 93.2 |
| | | | | | | | | - J | | | |
| | 5 | 6.0ef | 41.9 | 5.3de | 54.3 | 4.7c | 62.1 | 4.0c | 71.4 | 3.3de | 77.3 |
| Clove | 10 | 4.3hi | 58.1 | 3.0fg | 74.3 | 2.3efg | 81.1 | 2.3def | 83.3 | 1.7gh | 88.6 |
| | 15 | 3.0jk | 70.9 | 2.3gh | 77.5 | 2.0fg | 83.8 | 1.7fg | 88.1 | 1.3hi | 90.1 |
| | | , | |) | | , | | , | | | |
| | _ | | 40.4 | 0.01 | 04.5 | o =: | 45.0 | : | | 4 | |
| | 5 | 8.7b | 16.1 | 8.0b | 31.5 | 6.7b | 45.9 | 5.7b | 59.5 | 4.7bc | 68.2 |
| Garlic | 10 | 6.7de | 35.4 | 6.0cd | 48.6 | 4.7c | 62.1 | 4.0c | 71.4 | 3.0ef | 74.6 |
| | 15 | 5.3fg | 48.4 | 4.7e | 60.0 | 3.7d | 70.2 | 3.0d | 78.6 | 2.3fg | 84.1 |
| | | | | | | _ | | | | | |
| | 5 | 9.0b | 12.9 | 8.3b | 28.6 | 7.0b | 43.2 | 6.0b | 57.1 | 5.0b | 65.9 |
| Mint | 10 | 7.7c | 25.2 | 6.7c | 42.9 | 5.0c | 59.5 | 4.7c | 66.7 | 4.0cd | 72.7 |
| | 15 | 7.0cb | 32.2 | 6.0cd | 48.6 | 4.7c | 62.1 | 4.0c | 71.4 | 4.0cd | 72.7 |
| | _ | | | | | | | | | | -4.0 |
| l | 5 | 6.7de | 35.4 | 6.0cd | 48.6 | 5.0c | 59.5 | 4.3c | 69.1 | 3.7de | 74.9 |
| Nigella | 10 | 4.7gh | 54.8 | 3.3f | 71.5 | 2.7ef | 78.3 | 2.3def | 83.3 | 2.0gh | 86.4 |
| | 15 | 3.3jk | 67.8 | 2.3gh | 77.5 | 2.0fg | 83.8 | 1.7fg | 88.1 | 1.3hi | 90.1 |
| Control | | 10.3a | | 11.7e | | 12.3a | | 14.0a | | 14.7a | |
| L.S.D 0,05 | | 0.7 | | 0.8 | | 8.0 | | 0.7 | | 0.7 | |

^{*}days after soil infestation

R*% Reduction of spore population %

Table (5): Effect of some plant extracts on the percentage and severity of infection with

| Plant extract | Conc. (%) | Percentage of infection (%) | Severity of infection (%) |
|---------------|-----------|-----------------------------|---------------------------|
| | 5 | 11.1fg | 7.4ijk |
| Cactus | 10 | 11.1fg | 3.7jk |
| | 15 | 00.0g | 00.0k |
| | 5 | 22.2efg | 11.1hij |
| Clove | 10 | 11.1fg | 4.9ijk |
| | 15 | 00.0g | 00.0k |
| | 5 | 55.6bcd | 33.3de |
| Garlic | 10 | 44.4cde | 25.9ef |
| | 15 | 33.3def | 20.4fgh |
| | 5 | 77.8ab | 55.5b |
| Mint | 10 | 66.7bc | 44.4c |
| | 15 | 55.7bcd | 34.0cd |
| | 5 | 44.4cde | 22.2fg |
| Nigella | 10 | 33.3def | 18.5fgh |
| | 15 | 22.2efg | 14.2ghi |
| Control | | 100.0a | 92.6a |
| L.S.D 0,05 | | 25.9 | 9.5 |

II-3- Effect of some plant extracts on tomato growth parameters:

Results shown in Table (6) clear that vegetative growth of tomato plants was positively improved by the application of variable plant extracts. The best results were observed when *Aloe vera* and clove plant extracts were individually applied to the infested soil with FOL. As example; plant height was more than two folds of control plants when the extracts of either *Aloe vera* or clove plants were applied. These results are in harmony with those obtained by Pattnaik *et al.*, 2012.

II-4- Effect of some plant oils on FOL spore population in the soil:

Results present in Table (7) indicate that all tested plant oils had significant effect in reducing the population of FOL spores in the artificially infested soil. Clove oil gave the best effects were noticed up to 55 days after soil infestation. Spore population in the soil was decreased in response to the oil application whereas it was increased by

time in control treatment (FOL only). Such results are confirmed by Farrukh Aqil et al., 2001 who detected that the maximum antifungal activity due mainly to clove oil followed by mint oil.

II-5- Effect of some plant oils on wilt disease incidence:

Results shown in Table (8) clear that all tested oils at all used concentration decreased both percentage and severity of infection with FOL significantly compared to control (-) treatment. The best results were obtained when clove oil and /or mint oil were applied to the infested soil at 15% concentration. As example; severity of infection with FOL recorded 5.6 and 7.4 % when clove oil and mint oil were individually applied to the soil at the concentration of 15%; respectively. The severity of infection with wilt disease recorded 94.4 and 0% in control (-) and control (+) treatments; respectively. The antifungal activity of clove and mint oils against FOL was also reported by Abhishek Sharma et al., 2018.

| Table (6): Effect of som | e plant extracts on tomato | growth parameters with FOL: |
|--------------------------|----------------------------|-----------------------------|
| | | |

| Plant extract | Conc. (%) | Plant height / (cm) | No. of branches (per plant) | No. of leaves (per plant) |
|---------------|--------------|------------------------|-----------------------------|------------------------------|
| | 5 | 36.4bc | 6.0bcd | 31.0d |
| Cactus | 10 | 38.0b | 6.7ab | 37.3b |
| | 15 | 40.6a | 7.3a | 43.0a |
| | 5 | 30.3e | 5.7cd | 28.3e |
| Clove | 10 | 32.2de | 6.3bc | 31.7d |
| | 15 | 34.3cd | 6.7ab | 37.3d |
| | 5 | 21.5i | 4.7ef | 23.7h |
| Garlic | 10 | 26.2gh | 5.3de | 24.3gh |
| | 15 | 29.8ef | 5.7cd | 28.7e |
| | 5 | 18.7J | 4.0fg | 21.7i |
| Mint | 10 | 21.0i | 4.3f | 23.0hi |
| | 15 | 25.5h | 4.7ef | 25.7fg |
| | 5 | 27.8fg | 5.3de | 26.7F |
| Nigella | 10 | 31.0e | 6.3bc | 30.7d |
| | 15 | 33.5d | 6.3bc | 35.7c |
| Control | | 15.7k | 3.3g | 13.3J |
| L.S.D 0,05 | | 2.2 | 0.8 | 1.4 |

Table (7): Effect of some plant oils on spore population / I gm soil of FOL:

| Plant oil | Conc. (%) | 10* | R*% | 20* | R*% | 30* | R*% | 40* | R*% | 50* | R*% |
|-------------|---------------|--------------------------|----------------------|------------------------|----------------------|-----------------------|----------------------|------------------------|----------------------|-----------------------|----------------------|
| Clove | 5 10 15 | 14.3d 12.3f 10.3h | 21.8 32.7 43.6 | 8.0f 7.3fg 6.7g | 57.9 61.4 64.9 | 6.3g 5.0h 4.0i | 66.7 73.7 78.9 | 4.7f 4.0f 2.7g | 77.4 80.6 87.1 | 4.3de 3.0f 2.0g | 80.6 86.6 91.0 |
| Garlic | 5 10 15 | 18.3a 15.3c 13.7e | 00.0 16.4 25.4 | 14.3b 12.0c 9.3e | 24.6 36.8 50.9 | 11.0b 9.7c 9.0d | 42.1 49.1 52.6 | 9.3b 7.7c 7.0cd | 54.9 62.9 66.1 | 8.0b 6.3c 6.0c | 64.2 71.7 73.1 |
| Mint | 5 10 15 | 16.3b 14.0de 11.3g | 10.9 23.6 38.2 | 10.3d 8.0f 7.0g | 45.6 57.9 63.2 | 8.0e 7.3f 3.0j | 57.9 61.4 84.2 | 6.0de 5.0ef 2.3g | 71.0 75.8 88.7 | 5.0d 4.0e 1.7g | 77.6 82.1 92.5 |
| Control (+) | | 8.0i | 56.4 | 3.7h | 80.7 | 2.0k | 89.5 | 1.0h | 95.2 | 0.0h | 100.0 |
| Control (-) | | 18.3a | | 19.0a | | 19.0a | | 20.7a | | 22.3a | |
| L.S.D 0,05 | | 0.6 | | 0.7 | | 0.6 | | 1.1 | | 8.0 | |

Table (8): Effect of some plant oils on the percentage and severity of infection with FOL:

| Plant oil | Conc. (%) | Percentage of infection (%) | severity of infection (%) |
|-------------|--------------|-----------------------------|---------------------------|
| | 5 | 33.3cde | 16.7cde |
| Clove | 10 | 16.7de | 9.3e |
| | 15 | 16.7de | 5.6e |
| | 5 | 83.3ab | 42.6b |
| Garlic | 10 | 66.7abc | 35.2bc |
| | 15 | 50.0bcd | 29.6bcd |
| | 5 | 50.0bcd | 20.4cde |
| Mint | 10 | 33.3de | 13.0de |
| | 15 | 16.7de | 7.4e |
| Control (+) | | 00.0e | 00.0f |
| Control (-) | | 100.0a | 94.4a |
| L.S.D 0,05 | | 43.1 | 18.0 |

II-6- Effect of some plant oils on tomato growth parameters:

Results given in Table (9) indicate that the average Plant height, No. of branches and No. of leaves per plant were significantly increased in response to the application of different oils to the infested soil. The best results were obtained when clove oil was applied and this was followed by mint oil. Such results were recommended by the abovementioned authors.

II-7- Effect of different *Trichoderma* spp. isolates on wilt disease incidence:

Results present in Table (10) clear that all tested *Trichoderma* spp. tested isolates had significant effects in reducing both percentage and severity of infection with FOL when applied to the infested soil; in comparison with control (-) treatment. *Trichoderma harzianum* and *T. asperellum* were the best tested biocontrol agents in reducing the disease

incidence. On the other hand, T. hamatum was the least effective one in disease incidence. reducing wilt However, Mohd Rajik et al., 2012 proved that Trichoderma harzianum and T. viride provided induced resistance in plant against Fusarium oxysporum lycopersici resulting declined disease incidence from 100 to 7.69% Trichoderma harzianum was observed to be good biocontrol agent against FOL by several authors (Akrami and Yousefi 2015; Lakshman Prasad et al., 2016; Andleeb Zehra et al., 2017 and Mwangi et al., 2019).

II-8- Effect of different *Trichoderma* spp. isolates on tomato growth parameters:

Results present in Table (11) clear that *T.harzianum* and *T.asperellum* were the best biocontrol agents which improved tomato plant height, the average number of branches and number of leaves per plant. In comparison with control; all five tested *Trichoderma* species improved tomato growth parameters; significantly. This couldbe due to the resistance induction by *Trichoderma* spp. as mentioned by Andleeb Zehra *et al.*, 2017 and / or the antifungal activity of *Trichoderma* spp. isolates as reported by many authors; mentioned before.

Table (9): Effect of some plant oils on tomato growth parameters grown in infested soil with FOL:

| Plant oil | Conc. (%) | Plant height / (cm) | No. of branches (per plant) | No. of leaves (per plant) |
|-------------|--------------|------------------------|-----------------------------|------------------------------|
| | 5 | 25.8d | 5.0bcd | 24.3de |
| Clove | 10 | 28.2c | 5.7bc | 32.0bc |
| | 15 | 37.3b | 6.7ab | 33.3b |
| | 5 | 17.7g | 4.0cd | 15.7f |
| Garlic | 10 | 19.5fg | 4.3cd | 19.7ef |
| | 15 | 20.5f | 4.7bcd | 21.7e |
| | 5 | 23e | 5.0bcd | 23.0e |
| Mint | 10 | 27.2cd | 5.3bc | 28.3cd |
| | 15 | 35.6b | 6.0bc | 31.0bc |
| Control (+) | | 46.2a | 8.0a | 48.3a |
| Control (-) | | 12.2h | 3.0d | 9.3g |
| L.S.D 0,05 | | 2.1 | 1.4 | 4.4 |

Table (10): Effect of different *Trichoderma* spp. isolates on the percentage and severity of infection with FOL:

| Trichoderma sp. | Percentage of infection (%) | severity of infection (%) |
|-----------------|-----------------------------|---------------------------|
| T. asperellum | 22.2d | 6.2cd |
| T. hamatum | 77.7ab | 41.9b |
| T. harzianum | 11.1d | 1.0d |
| T. koningii | 55.5bc | 20.1c |
| T. viride | 33.3cd | 12.3cd |
| Control | 100.0a | 93.8a |
| LSD 0.05 | 29.3 | 17.2 |

| 9.0 | g. • · · · · · · · · · · · · · · · · · · | | | | | | | |
|-----------------|--|--------------------------------|------------------------------|--|--|--|--|--|
| Trichoderma sp. | Plant height / (cm) | No. of branches (per plant) | No. of leaves (per plant) | | | | | |
| T. asperellum | 40.0b | 7.0ab | 41.3b | | | | | |
| T. hamatum | 29.3e | 5.0c | 25.7e | | | | | |
| T. harzianum | 43.0a | 7.7a | 46.0a | | | | | |
| T. koningii | 33.1d | 6.0bc | 30.0d | | | | | |
| T. viride | 35.3c | 6.3b | 36.7c | | | | | |
| Control | 13.2f | 3.0d | 12.0f | | | | | |
| LSD 0.05 | 1.8 | 1.2 | 3.0 | | | | | |

Table (11): Effect of different *Trichoderma* spp. isolates on tomato growth parameters grown in artificially infested soil with FOL:

REFERENCES

Abhishek Sharma, Gunjan Sehra, Priti Sabnis, Anju Kamra and Sharma Satyawati (2016). Development of clove oil based nanoformulation against *Fusarium oxysporum*. Indian Phytopathology; 69 (4s):313-315.

Abhishek Sharma, Sharma, N. K., Ankit Arti Srivastava, Kataria, Saurabh Dubey, Satyawati Sharma and Kundu (2018). **Bishwajit** Clove and lemongrass oil based non-ionic nanoemulsion for suppressing the growth of plant pathogenic Fusarium oxysporum f.sp. lycopersici. Industrial Crops and Products; 123: 353-362.

Akaeze, O. O. and A. O. Aduramigba-Modupe (2017). Fusarium wilt disease of tomato: screening for resistance and *in-vitro* evaluation of botanicals for control; the Nigeria case. Journal of Microbiology, Biotechnology and Food Sciences.

Akrami, M. and Z. Yousefi. (2015). Biological control of fusarium wilt of tomato (Solanum lycopersicum) by Trichoderma spp. as antagonist fungi. Biological Forum; 7(1): 887-892.

Aleaghaee, S., S. Rezaee, M. Ebadi and H. R. Zamanizadeh (2018). The efficacy of some native Trichoderma isolates in induction of resistance in tomato against Fusarium oxysporum f.sp. lycopersici, the causal agent of fusarium wilt disease. [Persian] Applied Entomology and Phytopathology; 85(2): 219-233.

Ali, M. O.H. D., M. E. H. I. Lal, A. N. I. S. Khan, V. I. V. E. K. Singh and P. K. Singh (2013). Evaluation of leaf extracts and essential oils against *Fusarium oxysporum* f.sp. *pisi*, the causal agent of pea wilt disease. Indian Phytopathology; 66(3): 316-318.

Ammar, M. M. (2003). Fungi, second part, physiology, reproduction and relations with human and environment. Arabic book (597pp.). El-Dar El-Arabia for Press and Distribution.

Anam Moosa, Sahi, S. T., Imran-ul-Haq, Ayaz Farzand, S. A. Khan and Javaid Khushboo (2017). Antagonistic potential of Trichoderma isolates and manures against fusarium wilt of tomato. International Journal of Vegetable Science; 23(3): 207-218.

Andleeb Zehra, Mukesh Meena, M. K. Dubey, M. Aamir and R. S. Upadhyay (2017). Synergistic effects of plant defense elicitors and *Trichoderma harzianum* on enhanced induction of antioxidant defense system in tomato against fusarium wilt disease. Botanical Studies; 58(44) :(2 November 2017).

Devi, S. S., Y. Sreenivasulu and K. V. B. Rao (2015). *In vitro* antagonistic activity of Trichoderma isolates against phytopathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.). Journal of Pure and Applied Microbiology; 2015. 9(3): 2673-2680.

- Farrukh Aqil, Beg, A. Z. and Ahmad Iqbal (2001). *In vitro* toxicity of plant essential oils against soil fungi. Journal of Medicinal and Aromatic Plant Sciences; 22/23(4A/1A):177-181.
- Fontes, M. G., R. R. Costa-Carvalho, I. L. Coelho, E. R. Araujo, J. L. S. Carvalho Filho, D. Laranjeira, A. F. Blank, J. O. Melo and P. B. Alves (2018). Effect of essential oils from plants of the genus Lippia on *Fusarium oxysporum* f. sp. *lycopersici*. Acta Horticulture; (1198): 35-39.
- Hafez, E. E., M. M. Balbaa, S. S. A. Kabeil, M. A. El-Saadani and S. A. Ahmed (2012). Molecular studies on the biocontrol effect of *Trichoderma viride* and *Bacillus subtilis* on *Fusarium oxysporum* and *Rhizoctonia solani* infected tomato plants. World Applied Sciences Journal; 19(1): 89-99.
- Jahanshir Amini and Dzhalilov Fevzi Sidovich (2010). The effects of fungicides on *Fusarium oxysporum* f.sp. *lycopersici* associated with fusarium wilt of tomato. Journal of plant protection research. Vol. 50, No.2.
- Lakshman Prasad, Sorabh Chaudhary, Sushma Sagar and Tomar Akash (2016). Mycoparasitic capabilities of diverse native strain of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *Iycopersici*. Journal of Applied and Natural Science; 8(2): 769-776.
- Mohd Rajik, S. K. Biswas and Shiv Shakti (2012). Biochemical basis of defense response in plant against fusarium wilt through bio-agents as an inducer. African Journal of Agricultural Research; 7(43): 5849-5857.
- Mwangi, M. W., W. M. Muiru, R. D. Narla, J. W. Kimenju and G. M. Kariuki (2019). Management of *Fusarium oxysporum* f.sp. *Iycopersici* and rootknot nematode disease complex in tomato by use of antagonistic fungi, plant resistance and neem. Biocontrol

- Science and Technology; 29(3): 229-238.
- Pattnaik, M. M., Manoranjan Kar and R. K. Sahu (2012). Biopesticidal effects of some medicinal plant extracts on growth parameters and control of diseases in Solanum melongena L. International Journal of Biosciences, Agriculture and Technology (IJBSAT); 4(3): 14-22.
- Prachi Singh, Jyoti Singh, R. S. Rajput, Anukool Vaishnav, Shatrupa Ray, R. K. Singh and H. B. Singh. (2019). Exploration of multitrait antagonistic microbes against *Fusarium oxysporum* f.sp. *lycopersici*. Journal of Applied and Natural Science; 11(2): 503-510.
- Starovic, M., D. Ristic, S. Pavlovic, M. Ristic, M. Stevanovic, F. AlJuhaimi, N. Svetlana and M. M. Ozcan (2016). Antifungal activities of different essential oils against anise seeds mycopopulations. Archives fur Lebensmittelhygiene; 67(3): 72-78.
- Sundaramoorthy, S. and P. Balabaskar (2013). Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*. Journal of Applied Biology and Biotechnology; 1(3): 036-040.
- Taiga, A., M. N. Suleiman, W. Sule and D. B. Olufolaji (2008). Comparative in vitro inhibitory effects of cold extracts of some fungicidal plants on Fusarium oxysporum mycelium. African Journal of Biotechnology; 7(18).
- Torre, A. Ia, F. Caradonia, A. Matere and V. Battaglia (2016). Using plant essential oils to control fusarium wilt in tomato plants. European Journal of Plant Pathology; 144(3): 487-496.
- Yeole, G. J., H. M. Kotkar, N. P. Teli and P. S. Mendki (2016). Herbal fungicide to control fusarium wilt in tomato plants. Biopesticides International; 12(1): 25-35.

تأثير بعض المستخلصات النباتية، الزبوت العطرية وأنواع التريكوديرما على مرض الذبول الفيوزارمي في الطماطم

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يهدف هذا البحث إلى مكافحة مرض الذبول الفيوزارمى فى الطماطم بإستخدام مركبات صديقة للبيئة بديلة عن المبيدات الفطرية. وأظهرت النتائج أن المستخلصات المائية لنبات الصبار والقرنفل تؤدى إلى إختزال معنوى لنمو وتجرثم الفطر (FOL) Fusarium oxysporum f.sp. lycopersici (FOL) وذلك سواء تحت ظروف المعمل أو الصوبة فى التربة المعداه صناعيا بالفطر. كما أدت المعاملة بالزيوت النباتية إلى إختزال نمو الفطر FOL وتجرثمه بصورة معنوية ، وسجلت أقل نسبة إصابة أو شدة إصابة عند إستخدام زيت القرنفل يليه زيت النعناع. وكان الفطرين Trichoderma وسجلت أقل نسبة إصابة أو شدة إصابة عند إستخدام أنواع جنس Trichoderma الخمسة المختبرة فى إختزال معدل نمو الفطر FOL . وتحت ظروف الصوبة والعدوى الصناعية للتربة بالفطر الممرض أدت المعاملة بأى من المعاملات المذكورة سابقاً إلى نقص معنوى فى حدوث مرض الذبول الفيوزارمى وتحسن ملحوظ فى مواصفات النمو لنباتات الطماطم.

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