Antifungal Activities of Clove Oil Against Root Rot and Wilt Pathogens of Tomato Plants

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Abstract: The antifungal activities of clove essential oil at various concentrations (0, 0.5, 1, 2, 4% v/v) were evaluated in vitro and in vivo against tomato root rot and wilt pathogens. Several fungal pathogens were isolated from infected tomato plants collected from different locations of Giza, Monufia and Qalyubia governorates, Egypt. The purified isolates were identified as *Fusarium oxysporum*, *F. solani*, *F. semitectum* and *Rhizoctonia solani*. All fungal isolates were tested for their pathogenic ability on tomato plants under greenhouse conditions. In vitro, clove oil exhibited an inhibitory effect against the mycelial growth of all pathogens. All tested concentrations significantly reduced mycelial linear growth of the tested fungi compared to respective controls. Complete growth inhibition was observed in *Fusarium oxysporum* and *Rhizoctonia solani* when clove oil was applied at concentration of 4%. Effect of clove oil on fungi morphological features was studied by light microscope. Observations showed the disruption of the fungal growth and conidial malformation as well. In case of *Fusarium oxysporum*, clove oil decreased the conidial numbers, but increased the production of chlamydospores. Additionally, the alleviative effects of clove oil application on disease incidence and severity were also studied. Clove oil at concentration of 4% resulted a highly significant decrease in disease both incidence and severity. Therefore, it could be suggested that application of clove oil as potent antifungal agent could be used for controlling tomato root rot and wilt diseases.

Key words: Antifungal · Clove oil · *Fusarium oxysporum* · *Rhizoctonia solani* · *Fusarium solani* · Root rot · Wilt

INTRODUCTION

Tomato (Solanum lycopersicum Mill.) is one of the most important vegetable crops in the world, widely cultivated in tropical and sub-tropical regions. It has a very high nutritive value with antioxidant and curative properties [1]. Tomato plants are infected by several soil-borne fungal pathogens such as *Fusarium* spp and *Rhizoctonia solani* which cause serious diseases as root rots and wilt [2]. *Fusarium* wilt, caused by *Fusarium oxysporum* f.sp. *lycopersici*, is considered one of the limiting factors in tomato production areas. It has become one of the most prevalent and damaging diseases because, it can be soil-borne, air-borne or carried in plant residues and can be recovered from any part of the plant [3]. Tomato damping-off and root rot diseases caused by *Rhizoctonia solani* and *Fusarium* spp. are the most destructive diseases in tomato production areas which cause an annual loss of about 20% in the potential yield worldwide [4].

Control of root rot and wilt diseases depends mainly on fungicides [5]. Nowadays, the intensive application of chemical fungicides has been reported to cause adverse effects on plants, soil environment and human health [6]. Moreover, it leads to develop resistant strains of the pathogens against fungicides [7]. For these reasons, alternative methods with emphasis on the resistance inducers for controlling the diseases have been studied by several researchers to minimize fungicide application and decrease cost of plant production. Phytochemicals are considered to be environmentally safe as they are biodegradable and have little or nil toxicity to non-target animals [8, 9]. Recently, it has been reported that some plant essential oils have become a potent antimicrobial agents against foliar and soil-borne pathogens [10].
Plant essential oils (EO) are concentrated volatile hydrophobic liquids extracted from different parts of the aromatic plants [11]. They offer a variety of functions for the plants together with (i) protecting themselves of heat or cold, (ii) attracting or repelling insects and (iii) using chemical ingredients in the oil as defense equipment. As well, EO have shown promising results in vitro studies for their antifungal effects on mycelial growth and spore germination against many phyto-pathogenic fungi such as *Rhizoctonia solani*, *Fusarium moniliforme* and *F. oxysporum* [12]. The extent of inhibition depends on the concentration of essential oils [13]. The pathway of activity of these complexes against fungi is unidentified but may be related to their general capability to soften or otherwise dislocate the reliability of cell membranes and cell walls [14]. Clove oil has been known as a potential organic pesticide derived from different parts of the clove plant *Eugenia aromatic* [15]. It has been described to have useful antimicrobial effects on different types of plant pathogens such as fungi, bacteria and nematodes [16, 17]. It has been shown to inhibit the growth of several soil-borne fungi [18].

The objectives of the present work were: (a) isolate and identify the causal agents of tomato root-rot and wilt disease from different Egyptian governorates and determination their pathogenicity. (b) to investigate the inhibitory effects of different concentrations of clove oil on the mycelial growth and spore germination of all isolated fungi. (c) to study the efficacy of clove oil as soil drench against the invasion of tomato plants by soil-borne fungi under greenhouse conditions.

**MATERIALS AND METHODS**

**Source of Plant Material and Clove Oil:** Tomato (*Solanum lycopersicum* L. cv. Super strain B) seeds were obtained from Unit of Vegetable Crops Seeds, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt. Clove oil (containing 75 - 80% eugenol as the major ingredient) was purchased from Sigma Aldrich, Germany.

**Isolation and Identification of the Causal Pathogens:** The fungal pathogens were isolated from naturally infected tomato plants showing typical root rot and wilt diseases symptoms according to Davet and Rouxel [19]. Samples were collected from Giza, Monufia and Qalyubia Governorates, Egypt. All emerged fungi were purified using the hyphal tip technique according to Barnett and Hunter [20]. The identification was based on their cultural properties, morphological and microscopical characteristics according to the identification keys of Leslie and Summerell [21] and Sneh et al. [22].

**Pathogenicity Test:** Pathogenicity tests were carried out using the method of Haggag and El-Gamal [23] to investigate the pathogenic potentiality of the isolated fungi as well as, to determine the most aggressive isolates that used in this study. Sterilized pots (10 cm diameter) were filled with disinfested soil. Inoculum of both *Fusarium* spp and *R. solani* isolates were prepared by growing each isolate in bottles containing sterilized sorghum grain media then incubated and kept for 15 days at 25±2°C. Soil infestation was achieved by mixing the inoculum of each isolate with the soil at the rate of 3% (w/w) potential inoculum. Four-weeks-old tomato seedlings were transplanted into the infested pots (2 seedlings per pot). The disease incidence was determined by recording the percentage of dead seedlings, 45 days after transplanting.

**In vitro Antifungal Activity Assay:** The influence of antifungal activity of clove oil on the radial growth of *Fusarium* spp and *Rhizoctonia solani* was performed by the agar medium assay according to Tatsadjieu et al. [24]. Potato Dextrose Agar (PDA) media with different concentrations of clove oil (0.5, 1, 2 and 4 % v/v) were prepared by adding appropriate quantity of clove oil to the melted media, followed by addition of Tween 20 (100 µl to 100 ml of media) for the dispersion. About 20 ml of the medium were poured into glass Petri-dishes (9 cm). Then inoculated at the center with a mycelial disc (0.5 cm diameter) taken from the margins of 4–6 days old *Fusarium* spp or *Rhizoctonia solani* cultures. Three replicates were conserved for each treatment. Additionally, positive control (without clove oil) was inoculated following the same procedure. Petri-dishes were incubated at 25°C and the colony diameter was daily measured until control petri dishes were fully covered with mycelia.

The percentage of Mycelial Growth Inhibition (MGI) was calculated according to the equation suggested by Djordjevic et al. [25] as follows: Mycelial Growth Inhibition (MGI) % = (Do – De) / Do x 100

where: Do = the diameter of the mycelia growth in the positive control – 0.5 cm
De = the diameter of the mycelia growth in the oil supplemented plates – 0.5 cm
Minimal Inhibitory Concentration (MIC) (defined as the lowest concentration of clove essential oil in which no growth occurred) was determined.

**Microscopical Observations:** The effects of different concentrations of clove oil on the structural and morphological characters of the isolated fungi were investigated using a light microscope (Leica – DM 2500). Representative samples were taken from the periphery of 8-day-old fungal colony grown on PDA at 28°C containing the essential oil. The samples were fixed in lacto-phenol–trypan blue stain and then microscopically investigated [26].

**Effects of Clove Oil on Disease Incidence and Severity under Greenhouse Conditions:** The most effective concentration of clove oil (4%) that showed great inhibition on the mycelial growth of the tested fungi *in vitro* was selected to evaluate its activity against *Fusarium* spp and *Rhizoctonia solani* under greenhouse conditions. Clove oil was emulsified with 0.05% Tween 20 at concentrations of 4% (v/v) before application [27]. It was used as seedling root dipping for 12 h before transplanting. Tomato seedlings (four-weeks-old) were transplanted into plastic pots (30 cm diameter) containing 3kg sandy loam soil (1:1 w/w). Pots were artificially infested with fresh inoculum of the tested fungus, *Fusarium* sp. and *Rhizoctonia solani* at the rate 3 % (w/w) of soil weight. There were 10 replicates per treatment, each pot contains 2 tomato seedlings. Similar numbers of pots only infected with pathogen were served as a control. Pots were arranged in a completely randomized block design then kept at 22–28°C, 70–80% relative humidity and 12 h photoperiod and watered as needed and fertilized as usual.

Percentage of disease incidence was calculated for each individual treatment by dividing the number of symptomatic plants over the total number of plants according to Song *et al.* [28].

Disease severity of both root rot and wilt was estimated at 10 days interval for 45 day after transplanting using a rating scale of (0 – 5) based on root discoloration or leaf yellowing grading, according to Abdou *et al.* [29] as follow.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 = neither root discoloration nor leaf yellowing.</td>
</tr>
<tr>
<td>1</td>
<td>1 = 1-25% root discoloration or one leaf yellowing.</td>
</tr>
<tr>
<td>2</td>
<td>2 = 25-50% root discoloration or more than one leaf yellowing.</td>
</tr>
<tr>
<td>3</td>
<td>3 ≥ 50-75% root discoloration with one wilted leaf.</td>
</tr>
<tr>
<td>4</td>
<td>4 = up to 75% root discoloration or more than one leaf wilted.</td>
</tr>
<tr>
<td>5</td>
<td>5 = dead seedlings.</td>
</tr>
</tbody>
</table>

The percentage of disease severity index (DSI) was estimated using the following formula according to Song *et al.* [28].

\[
\text{DSI} \% = \frac{\Sigma (d \times \text{number of plants in that grade})}{(d_{\text{max}} \times n) \times 100}
\]

where: 
\(d=\) is the disease rating of each plant 
\(d_{\text{max}}=\) the maximum disease rating 
\(n=\) the total number of plants/samples examined in each replicate.

**Plant Growth Parameters of Tomato Plants:** Six weeks after transplanting, five replicates of each treatment were destructively harvested and immediately separated into roots, leaves and stems. The plant height, number of branches/plant and fresh weight of the plant shoot were recorded. The root segments were then washed thoroughly with running water, blotted on tissue paper and their fresh weights were determined. Representative samples of roots and shoots were then oven dried at 70°C for 72 h for dry weights and water contents.

**Statistical Analysis:** All data sets were analyzed by variance analysis (ANOVA) using SAS software. The separation of means was done by using the Least Significant Difference (LSD) test at \(P \leq 0.05\) (30).

**RESULTS AND DISCUSSION**

**Isolation, Identification and Pathogenicity Test:** Four fungi were isolated from symptomatic tomato plants that were collected from different governorates in Egypt (Giza, Monufia and Qalyubia). All fungi were purified and identified based on their cultural properties, morphological and microscopical characteristics. Obtained results revealed that the most dominant fungi were identified as *Fusarium solani* from Giza, *Rhizoctonia solani* from Qalyubia and *Fusarium oxysporum* and *Fusarium semitectum* from Monufia. Each purified fungus was tested for its pathogenic ability on tomato plants under greenhouse conditions. The tested fungi were obviously varied in their ability to cause root rot or wilt infection of tomato plants. The most aggressive fungi
Table 1: Effect of clove oil on the radial growth of some isolated fungi of tomato plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fusarium oxysporum</th>
<th>Fusarium solani</th>
<th>Fusarium semitectum</th>
<th>Rhizoctonia solani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MG (cm)</td>
<td>MG%</td>
<td>MG (cm)</td>
<td>MG (cm)</td>
</tr>
<tr>
<td>Control</td>
<td>9a ±0.00</td>
<td>0e ±0.00</td>
<td>9a ±0.00</td>
<td>0e ±0.00</td>
</tr>
<tr>
<td>Clove oil 0.5%</td>
<td>7.5b ±1.00</td>
<td>16.6b ±11.00</td>
<td>7.6b ±0.52</td>
<td>15.5d ±5.88</td>
</tr>
<tr>
<td>Clove oil 1%</td>
<td>5.0c ±0.40</td>
<td>44.4e ±4.00</td>
<td>6.4c ±0.75</td>
<td>28.8e ±8.39</td>
</tr>
<tr>
<td>Clove oil 2%</td>
<td>1.66d ±0.76</td>
<td>81.3b ±8.00</td>
<td>3.7d ±0.50</td>
<td>58.8b ±5.59</td>
</tr>
<tr>
<td>Clove oil 4%</td>
<td>*0.5e ±0.00</td>
<td>100a ±0.00</td>
<td>0.9e ±0.36</td>
<td>91.8a ±19.80</td>
</tr>
</tbody>
</table>

* The diameter of the initial disk (0.5 cm) was recorded in the case of full inhibition of mycelium. MG (cm) = Mycelia Growth (cm), MG% = Mycelia Growth Inhibition %. For each column, means followed by the same letter are not significantly different according to Least Significant Difference (LSD) test at ($P < 0.05$).

Fig. 1: Mycelial growth of different isolated fungi after 8 days incubation with different concentrations of clove oil (0, 0.5, 1, 2, 4 %)

were *F. semitectum* (M1) followed by *F. solani* (G2), as they recorded 95% and 86%, respectively. Meanwhile, *F. oxysporum* (M2) and *R. solani* (K1) had the least potentiality of infection to tomato plants (80% and 70%, respectively). These results are in harmony with those reported by Haggag and El-Gamal [23].

**Inhibition of Mycelial Growth of the Pathogenic Fungi in vitro:** Clove oil has antimicrobial activity against a variety of soil-borne fungi, plant pathogens and mycotoxigenic fungi [31- 33]. The main chemical components of clove oil are eugenol, acetyl eugenol, iso-eugenol and β-caryophyllene [34]. These phenolic compounds are responsible for the antibacterial and antifungal properties of essential oil [35]. The current work aimed to evaluate the antifungal activities of clove essential oil as a resistance inducer against *F. oxysporum*, *F. solani*, *F. semitectum* and *R. solani in vitro and in vivo*. The obtained data in Table (1) and Fig. (1) show that there was a significant inhibition in radial growth of all the tested fungi due to clove oil application. The inhibition was high at the lowest application rate (0.5%) and improved by increasing the tested concentration to 4%. The inhibition in linear growth of *F. semitectum* and *F. solani* was lower than that observed for *F. oxysporum* and *Rhizoctonia solani* in all tested concentrations. However, *Rhizoctonia solani* and *F. oxysporum* didn’t show any mycelium growth at concentration 4% (Fig. 1). Minimum inhibitory concentration (MIC) was tested for each pathogen and the results showed that MIC of clove oil was 4%, which completely inhibited the growth of *F. oxysporum* and *Rhizoctonia solani*. This concentration led, however, to 88.5 and 91.8% reductions in the growth of *F. semitectum* and *F. solani* respectively. Hence, it was selected for application in pot experiments under greenhouse conditions.
Our findings are in agreement with those obtained by Sharma, et al. [36] and Furdos [37], which revealed that clove essential oil, have the ability to inhibit radial growth, spore germination and to reduce the dry weight of Fusarium oxysporum f. sp. lycopersici. Similar type of study was conducted by Juniawan, et al. [38] who reported the sensitivity of Fusarium spp. against all the tested essential oils, but clove oil was the most effective among the tested oils. On the other hand, Abdulaziz and Younes [39] investigated the antifungal activity of four medicinal plants, (cinnamon, anise, black seed and clove) against pea root-rot fungus (Rhizoctonia solani). The highest antifungal activity was recorded for clove extract (4%) which cause complete growth inhibition for Rhizoctonia solani.

Effect of Clove Oil on Fungal Structures: There are several possibilities for antifungal mechanism of essential oils (EOS) described by researchers. For bioactivity, the EOS pass through the cell wall and cytoplasmic membrane [40]. Many authors emphasized that the antimicrobial effect of essential oil elements has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane [41, 42].

The effects of different dosages of clove oil on the morphological characterization of different pathogens were investigated. Microscopical investigations revealed that all concentrations of clove oil caused cytotoxicity to the tested fungi resulting in abnormal growth of mycelia, swollen of hypha and conidial malformation. Clove oil treatment decreased significantly the conidial numbers, but increased the production of chlamydospores relative to the control (untreated) and also mycelium that showed regular cell structures with homogenous cytoplasm (Figs. 2 & 3). Particularly, F. oxysporum and Rhizoctonia solani treated with clove oil at concentrations of 1% and 2% exhibited irregular branching of the terminal hyphae, severely collapsed mycelium and malformation in the morphology of the hyphae. In some cases, the hyphal cytoplasm was autolysis. While the production of Fusarium oxysporum conidia was severely impaired due to all clove oil concentrations. The presence of chlamydospores was increased in proportion to the increase of essential oil concentration (Figs. 2 &3). Such modifications may be related to the effect of the essential oil as enzymatic reactions regulating wall synthesis. Therefore, volatile phenolic compounds (carvacrol, eugenol, thymol) may interfere with cell wall enzymes (enzyme inhibition), possibly through reaction with sulphydryl groups or through interactions with proteins. They may change the permeability and fluidity of cell membrane and disintegrate fungal hyphae [43].

The microscopical observations are in agreement with the study conducted by each of LaTorre et al. [44] and Sharma et al. [36]. They found that clove oil exhibited considerable alterations in hyphal morphology and inhibited conidial germination of F. oxysporum f. sp. lycopersici in comparison to those of control. They confirmed that clove oil provided a complete inhibition after 24 and 48 h thus, probably killing the conidia. On the other hand, the microscopic observations of Soylu et al. [45] for Sclerotinia sclerotiorum showed changes in the hyphal morphology due to clove oil application. Shriveled hyphal aggregates reduced hyphal diameters and lyses of hyphal wall were commonly observed in oregano- or fennel oil-treated mycelium, compared with controls.

Effect of Clove Oil on Disease Incidence and Severity under Greenhouse Conditions: Many in vivo studies have reported the efficacy of essential oils against Fusarium spp and Rhizoctonia solani [46, 47]. The present study was planned to investigate the effect of clove oil (4%) on reduction the incidence and severity of wilt and root rot diseases on tomato plants under greenhouse conditions at 6 weeks after transplanting in soil infested with each of Fusarium spp and R. solani. Data illustrated in Figure (4) clearly indicate that clove oil (4%) reduced significantly (P < 0.05) the percentages of wilt and root rot disease incidence and severity on tomato plants compared with the untreated control plants. Actually, it decreased F. oxysporum and R. solani incidence by 66 and 75%, while F. solani and F. semitectum were reduced by 50 and 40%, respectively. However, clove oil treatments decreased the disease severity of F. oxysporum and R. solani by 72 and 81%, respectively, whereas, those of F. solani and F. semitectum were reduced by 54 and 46%, respectively. These results are in harmony with those conducted by LaTorre et al. [44]: Estrada et al. [18] and Sharma et al. [36] which revealed that increasing concentration of the clove oil can reduce the incidence and severity of Fusarium wilt of tomato under greenhouse conditions compared to control. Furthermore, Hamad et al. [48] mentioned that clove oil applied on guava seedlings roots completely inhibited F. oxysporum and R. solani.

Effect of Clove Oil on Plant Growth Parameters: Fungal infection with each of F. oxysporum, F. solani, F. semitectum and R. solani negatively affected the overall plant growth, the effect was less severe in plants
Fig. 2: Light micrographs showing the abnormality of Fusarium oxysporum mycelia due to clove oil application. (A) Hyphae growing on control medium. (B & C) effects of clove oil at concentration of (1%). Note: deformations of conidia and severely collapsed mycelium (large black arrows), chlamydomo- spores formation (small black arrows). (D & E) effects of clove oil at concentration of (2%). Note: irregular branching of the terminal hyphae (large black arrows), autolysis of the hyphal cytoplasm and necrosis. Bar = 40 µm.

Fig. 3: Light micrographs showing the abnormality of Rhizoctonia solani mycelia due to clove oil application. (A) Hyphae growing on control medium. (B & C) effects of clove oil at concentration (1%). (D & E) effects of clove oil at concentration of (2%). Note: deformations of hyphal shape (large black arrows), cytoplasmic coagulation, hyphal shrinkage and necrosis (small black arrows). Bar = 40 µm.
Fig. 4: Effect of clove essential oil (4 %) on root rot and wilt disease incidence and severity on tomato plants caused by *Fusarium spp* and *Rhizoctonia solani* under greenhouse conditions.

Table 2: Effect of clove oil on the growth parameters of tomato plants

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control</th>
<th><em>F. oxysporum</em></th>
<th><em>F. solani</em></th>
<th><em>F. semitectum</em></th>
<th><em>R. solani</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>NB/ per plant</td>
<td>8.00±1.00</td>
<td>8.30±0.57</td>
<td>4.60±0.57</td>
<td>8.30±0.57</td>
<td>4.00±1.00</td>
</tr>
<tr>
<td>Ph [cm]</td>
<td>34.60±1.52</td>
<td>35.00±4.00</td>
<td>19.60±3.21</td>
<td>36.30±5.50</td>
<td>17.60±2.51</td>
</tr>
<tr>
<td>RFW [g]</td>
<td>5.18±0.84</td>
<td>5.26±0.30</td>
<td>1.39±0.35</td>
<td>4.34±0.48</td>
<td>1.15±0.04</td>
</tr>
<tr>
<td>SFW [g]</td>
<td>8.96±0.56</td>
<td>9.00±0.50</td>
<td>4.15±0.17</td>
<td>8.33±0.51</td>
<td>4.50±0.36</td>
</tr>
<tr>
<td>RDW (%FW)</td>
<td>10.09±3.702</td>
<td>9.91±1.12</td>
<td>26.18±4.12</td>
<td>15.80±1.14</td>
<td>28.58±1.24</td>
</tr>
<tr>
<td>SDW (%FW)</td>
<td>13.70±2.32</td>
<td>14.43±1.97</td>
<td>14.22±3.67</td>
<td>10.57±1.24</td>
<td>11.33±1.60</td>
</tr>
</tbody>
</table>

For each column, means followed by the same letter are not significantly different according to Least Significant Difference (LSD) test at (P < 0.05). NP=number of branch/plant, Ph= plant height (cm), RFW= root fresh weight (g), SFW = shoot fresh weight, RDW= root dry weight (g), SDW= shoot dry weight (g), H.N.T= healthy plant un-treated, H.T= healthy treated, I.N.T= infected un-treated, I.T. = Infected treated

Infected with *R. solani*. The number of branches per plant was steadily decreased due to infection with *F. oxysporum*, *F. solani*, *F. semitectum* and *R. solani* by 42, 50, 58 and 37%, respectively, compared to the controls (Table 2). Heights of infected plants were also declined by 43, 50, 56 and 32%, respectively, compared to the respective controls. As a general trend, plant biomass was significantly suppressed upon fungal infection, with more severe effects on the root parts (Table 2). The infected plants exhibited about 73, 77, 80 and 66% reductions in their root fresh weight upon infection with *F. oxysporum*, *F. solani*, *F. semitectum* and *R. solani*, respectively. Shoot parts were less sensitive to fungal infection, as their fresh weights were reduced by 53, 50, 50 and 50% in plants infected with *F. oxysporum*, *F. solani*, *F. semitectum* and *R. solani*, respectively (Table 2). Fungal infections remarkably altered the dry weight (% fresh weight), the effect was varied between under- and aboveground plant parts. Root dry weights were increased by 159, 183, 89 and 85% in plants infected with *F. oxysporum*, *F. solani*, *F. semitectum* and *R. solani*, respectively. However, the shoot dry weights were slightly increased only in plants infected with *F. oxysporum* and *R. solani*, but decreased in those infected with *F. solani* and *F. semitectum* (Table 2). Whereas clove oil treatment (4%) did not significantly...
influence the morphological traits of non-infected tomato plants, it led distinctly to alleviate some of the detrimental effects of fungal infection (Table 2). Clove oil treatment significantly \( (P < 0.05) \) enhanced the number of branches per plant, plant height, root and shoot fresh weights of infected plants compared to non-treated infected ones (Table 2). These increases in growth may be attributed to elicitor’s effect on physiological processes in plant such as ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis \[49\]. These results are in agreement with those obtained by El-Mohamedy \textit{et al.} \[50\] and Hamad \textit{et al.} \[48\].

**CONCLUSION**

_Fusarium oxysporum, F. solani, F. semitectum and Rhizoctonia solani_ were isolated from infected tomato plants collected from different locations of Giza, Monufia and Qalyubia governorates, Egypt. In vitro, all concentrations of clove oil reduced mycelial growth of all tested fungi. Microscopical observations showed the disruption of the fungal growth and conidial malformation. Moreover, Clove oil at concentration 4% highly decreased disease incidence and disease severity. Therefore, it could be suggested that application of clove oil as a strong antifungal agent could be used for controlling tomato root rot and wilt diseases.

**ACKNOWLEDGEMENTS**

This research was financially supported by Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

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