Antiviral action of Lavender (lavendular vera) essential oil against tomato spotted wilt virus infected tomato plant.

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Abstract

An isolate of tomato spotted wilt virus (TSWV) was obtained from naturally infected tomato plants grown in Kafr El-Sheikh Governorate exhibiting a wide range of foliar disease symptoms including curling, stunting, bronzing and/or purpling, chlorosis and necrotic spot on the leaves. Dark brown streak also appear on leaf petioles and growing tips. The virus was biologically purified from single local lesion formed on Chenopodium amaranticolor Coste & Reyn. The isolated virus was identified on the basis of symptomatology, transmissibility and serological tests. The identification of the virus was confirmed serologically by indirect ELISA using authentic and induce antisera for TSWV. Five tomato cultivars were tested for pathogenicity to TSWV. Peto 86 tomato cv. was the highest infection under greenhouse conditions showing 80% infection. Two field experiments were conducted during tow successive seasons to study the effect of treatment with lavender essential oil with three concentrations (500, 1000 and 2000 µg/ml) to induce resistance against infection with TSWV in tomato plants (cv. Peto 86). The results showed that all treatments induced resistance to the virus infection when applied to plants as essential oil with TSWV in a mixed inoculum. Also all treatments gave a significant increase in photosynthetic pigments, total soluble sugars, total soluble phenols and flavonoids as well as the activity of catalase, peroxidase and polyphenol oxidase compared with infected plants. All treatments caused a significant role in reducing the incidence and severity of TSWV. The highest concentration of essential oil (2000 µg/ml) was the best treatment against TSWV infection.

Keywords:
Tomato (Lycopersicum esculentum Mill)
Tomato spotted wilt virus (TSWV)
Infection, lavender (lavendular vera)
Essential oil
Antiviral activity
Antioxidant enzymes

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1. Introduction

Tomato (Lycopersicum esculentum, Mill) is one of the most important vegetable crops in Egypt and worldwide grown for local human consumption and exportation. In Egypt, Tomatoes are cultivated on about 200.293 feddans which produced about 3623451 tons [3].

Tomato plant are widely infected by many virus disease in different areas of the world including, Tomato spotted wilt Tospovirus (TSWV) [8], Tobacco etch potyvirus (TEV)[29]; Tomato yellow leaf curl Geminivirus (TyLCV) [18]; Cucumber Mosaic Cucumovirus (CMV) [22]. Potato virus Y Potyvirus (PVY) [8]; Tomato chlorosis crinivirus (TOCV) [36] and Tomato infectious chlorosis crinivirus (TICV)[45].

TSWV is the type member of the genus Tospovirus in the family Bunyaviridae. The genus name is derived from the name of its first member, Tomato spotted wilt virus (TSWV). Initially observed in Australia in 1915[8].

TSWV has one of the widest host ranges of any plant virus, infecting over 1000 species of plants in more than 82 families including both monocots and dicots [38]. The Solanaceae and compositae families contain the largest numbers of susceptible plant species[42]. Symptoms vary with the host range, time of year, environmental conditions and include stunting, necrosis, chlorosis, ring spot and ring line patterns affecting leaves, stem and fruit[34,27]. In, Nature, TSWV is exclusively transmitted by several species of thrip (Thysanoptera: Thripidae[14], although it can be transmitted mechanically.

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TSWV is known to cause severe yield and quality losses of food and ornamental crops in temperate and subtropical regions of the world[30]. The loss of marketable tomato yield due to TSWV epidemics accounted for millions of dollars and reduced the tomato production by 50-90% in the Hawaiian islands[15].

It is known that the different essential oils from many plant extract contain volatile acetylene, monoterpenes and sesquiterpenes components has a variety of biological properties including antifeedant antimicrobial antioxidant, insecticidal activity and acaricidal [32,20].

In addition these compounds were easily biodegradable, non-phototoxic, more environment friendly and safe [13].

Lavender (Lavandula vera) is one of the most important aromatic plants in the world. Essential oils obtained from its flowers have long been used in perfumes and cosmetics. In addition it has anti-oxidant, antiviral, antibacterial activity and medicinal properties[37].

Lavender essential oil contains monoterpenes and its derivatives alcohols, aldehydes, ethers, esters and ketones. Monoterpenoid alcohol isoborneol found in lavender essential oil showed viricidal action against viruses. The inactivation activity of isoborneol is significant only at high concentrations of the compound owing perhaps to inter lipids present on the virus envelope resulting loss of infective [25].

Therefore the present study was undertaken to investigate the nature of different constituents present in lavender essential oil from aerial parts, efficiency of these extracts on reducing the infection of TSWV in tomato plants under field conditions and investigate the proposed mechanism of action of these antiviral natural plant extracts throw the alteration of the chemical composition, secondary metabolites accumulation and activity of antioxidant defense enzymes compared with healthy plants.

2. Materials and methods

2.1 Virus source and symptoms:

Samples from naturally infected tomato (Lycopersicum esculentum, Mill) plants with suspected tomato spotted wilt symptoms showing curling, bronzing and / or purpling, chlorosis (yellowing) and necrotic spots on the leaves were collected from Kafr-El-Sheikh Governorate-Egypt . Infected plants were transferred and grown in 25 cm plastic pots filled with natural soil under greenhouse conditions. Upon recovery of plants, they were used for isolation, identification and serologic testing.

2.2 Virus isolation and propagation

Naturally infected tomato plants used as a source of TSWV were mechanically transferred onto tomato plants cv. Castle rock grown in the greenhouse. The virus was purified biologically through three consecutive passage onto the local lesion host Chenopodium amaranticolor Coste and Reyn plants[28], then transmitted mechanically to Gomphrena globosa L. which was used as a source and for virus propagation.

On the other hand, the isolate understudy was serologically identified using two authentic polyclonal antisera for TSWV obtained from Dr. N. Katis (Agristotelian University, Faculty of Agriculture, Plant Pathology Laboratory Greece) and an induced antiserum for TSWV [21]using indirect ELISA methods according to[16].

2.3 Cultivars susceptibility:

In greenhouse experiments, ten tomato plants from each of the five tested cultivars namely Castle rock, Peto 86, Super merman, UC 97 and Strain B were examined to study the sensitivity of different tomato cultivars to TSWV infection and to select the most sensitive cultivar as follow, sixty 25-cm pots were filled with soil and one plant was sown per pot. Upon plant emergence, 50 plants (in 50 pots) were inoculated mechanically by TSWV gently rubbing the primary leaves dusted with carborundum powder (600 mech). Ten healthy seedlings of the same age and cultivar were left as a control. Symptom manifestations were examined daily. Four days later, leaf samples from each pot were collected and examined for virus presence using indirect ELISA method.

2.4 Indirect ELISA method

Indirect ELISA method was done as described by[16] to study the reactions of different tomato cvs. to infection with TSWV under greenhouse condition by using induced antiserum of TSWV[21]. The most susceptible cultivar was used to design an integrated program to eliminate or reduce TSWV infection including natural alternatives as systemic resistance inducers.

2.5 Field experiment

Field experiments were conducted for two consecutive years at the experimental farm of the Faculty of Agriculture, Cairo University, Giza to study the effect of lavender essential oil with different concentrations on tomato plants resistance to TSWV.

2.5.1 Experimental design

Complete plot design for this experiment was used. Seeds of tomato plants CV peto 86 were seeded in plastic trays in the vegetable department, Fac. of Agric., Cairo Univ. Upon germination, tested cultivars were transferred under field condition and sown in eleven plots containing three lines / plot, five plants/ line and plot/ treatment. Treatments were used three concentrations from lavender essential oil (500, 1000, 2000 ug/ml). The plants in the tenth plot were inoculated only with TSWV as a positive control, whereas the plants in the eleventh plot were inoculated only with distilled water as a negative control.
2.5.2 Preparation of virus inocula
Inoculum was prepared by homogenizing fifty grams of freshly infected leaves of *gomphrena globosa* L. which used as a source plants for TSWV with distilled water (1:1 W/V) in a food grinder. Sap extract was squeezed through two layers of muslin cloth and the solution was centrifuged at 5000 rpm/10 min. The clear supernatant was used as virus inoculums.

2.6 Preparation of Plant materials

2.6.1 Preparation of lavender essential oil
Essential oil was extracted from dried aerial parts of lavender which obtained from the experimental station for medicinal plant researches ,Faculty of pharmacy , Cairo University using steam distillation apparatus for 3 hrs then yield oil collected over sodium sulphate anhydrous for drying.

2.6.2 GC-MS analysis of essential oil.
Gas Chromatography-Mass Spectroscopy was used for identification of components of essential oil according to[1]. Analytical GC-MS was carried out on a HP chemstation version A02.12 data system. A carbowax capillary column, 50m*0.53 mm 1.D. 1.5 m thickness (HP Company, U.S.A) was used with helium as carrier gas (flow rate 1.5 ml/min).

2.6.3 Treatments with antiviral compounds
Different concentrations of lavender essential oil was mixed with 0.3% tween20 as emulsifying agent then mixed (1:1 V/V) with virus inoculum in sterilized mortar and pestle concentration according to[4]. Leaves of seedlings previously dusted with carborundum (600 mesh) were inoculated with the virus inoculum in sterilized mortar and pestle then mixed (1:1 V/V) insoluble polyvynilpyrolidione. The homogenate was centrifuged at 10,000 xg for 30 min and supernatant kept stored in separate aliquots at - 40°C, to determine catalase, peroxidase and polyphenol oxidase activity according to the method reported by[43].

2.7 Chemical analysis of tomato leaves
The ethanol extracts of tomato leaves after four days from the virus infection were used to determine total soluble, reducing and non-reducing sugars, total soluble phenols, and total flavonoids.

2.7.1. Determination of total flavonoids
The total flavonoids content were determined according to the aluminum chloride colorimetric method described by [12]

2.7.2 Determination of Phenolic compounds:
Total phenolic contents were determined by the Folin-Ciocalteu method [31].

2.7.3 Total soluble, reducing and non-reducing sugars
Total soluble sugars were determined using the phenol-sulfuric method according to[19], reducing sugars were determined using dinitrosalicylic acid method[33] and non-reducing sugars were calculated by the difference.

2.7.4 Determination of total pigments:
Chlorophyll a, chlorophyll b and carotenoids were extracted from tomato leaves after four days of inoculation according to the method of [24]. The concentration of chlorophyll a, b, total chlorophyll and carotenoids were calculated by means of wettstein's formula [44].

2.8 Antioxidant enzymes determination:
2.8.1 Enzymes extraction:-
The leaves collected after 1,2,3, and 4 days from infection (3 :1 buffer volume: fresh weight) were homogenized in a pastel and mortar with 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 3mM DL-dithiothreitol and 5% (W/V) insoluble polyvynilpyrolidione. The homogenate was centrifuged at 10,000 xg for 30 min and supernatant kept stored in separate aliquots at - 40°C, to determine catalase, peroxidase and polyphenol oxidase activity according to the method reported by[43].

2.8.2 Soluble Protein Determination
Soluble protein was estimated by using the Coomassie Brilliant Blue G-250 according to [7]method with bovine serum albumin as standard .

2.8.3 Determination of Catalase specific activity
Catalase was assayed in leaves extracts by measuring the decrease in absorbance due to disappearance of H2O2 at 240 nm according to[11]

2.8.4 Determination of peroxidase activity
Peroxidase activity was assayed in leaves extracts by photochemical method as described by [2].

2.8.5 Determination of polyphenol oxidase activity:
Polyphenol oxidase activity was assayed by using photochemical method as described by [17].

2.9 Statistical analysis:
Statistical analyses have done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

3. Results and discussion
3.1. Cultivars susceptibility
The obtained results from table (1) showed that, the most susceptible cultivar to TSWV infection compared with the other cultivars (castle rock, super merman, UC97 and strain B) was peto 86 cultivar when tested by indirect ELISA using polyclonal antiserum, which realized the highest O.D.(0.80) compared with the negative control (0.32). so that, the most adequate cultivar for continuing the antiviral investigation studies was the Peto 86.
Table 1 Reactions of different tomato plant cultivars to infection with TSWV.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>O.D.(405)nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.32± 0.007e</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.84± 0.020d</td>
</tr>
<tr>
<td>Castle rock</td>
<td>0.51± 0.019d</td>
</tr>
<tr>
<td>Peto 86</td>
<td>0.80± 0.045a</td>
</tr>
<tr>
<td>Super merman</td>
<td>0.61± 0.019c</td>
</tr>
<tr>
<td>UC97</td>
<td>0.68± 0.003b</td>
</tr>
<tr>
<td>Strain B</td>
<td>0.53± 0.023d</td>
</tr>
<tr>
<td>LSD</td>
<td>0.056</td>
</tr>
</tbody>
</table>

O.D = optical density determined by indirect ELISA at 405 nm.

The obtained data was in accordance with [34] they found that, various variables affecting symptom expression include the cultivar, age, nutritional and environmental conditions of the plant, and differences between different isolates of TSWV on the same hosts.

3.2. Chemical composition of lavender essential oil

Chemical composition of lavender essential oil was analyzed by GC-MS and the results are summarized in Table (2). Thirty-five components were identified (83.06%) and (16.94%) components were unknown. The main constituents in the oil were 1,8-cineole (13.21%), isocaryophyllen (7.56%), camphor (5.40%), eseroline (4.83%), linalyl hexanoate (4.68%), α-pinene (4.21%), and longifolene (4.07%). In addition to fifteen compounds were recorded as minor constituents (less than 4%) camphene (3.85%), tricyclene (3.56%), supinidine (2.93%), ledene (2.88%), pulegone (2.75%), 1-borneol (2.57%), linalool (2.51%), santolina alcohol (2.49%), camphor (5.40), 1-4-Terpineol (1.71), linalyl hexanoate (4.68), Cyclopentanone (0.71), Cuminum (1.01), Isobornyl acetate (2.35), Delta-4-carene (1.37), Isocaryophyllen (7.56), Ledene (2.88), Supinidine (2.93), Curcumene (0.52), β – Funerebne (0.54), Delta- cadinene (0.48), Eseroline (4.83), Longifolene (4.07), β – Guaiene (1.56), 1-Benzopyran-4-one (0.84), β – selinene (1.55), Cyclopentaneethanil (1.35), Trace-β.farnesene (0.65), Methyloctadecanoyl pyrrolidine (0.48), Unknown (11.55).

Table 2 Chemical constituents of essential oil from Lavandula vera.

<table>
<thead>
<tr>
<th>Name</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propenylester</td>
<td>0.75</td>
</tr>
<tr>
<td>Pentane-1-chloro</td>
<td>2.26</td>
</tr>
<tr>
<td>Tricyclene</td>
<td>3.56</td>
</tr>
<tr>
<td>Camphene</td>
<td>3.85</td>
</tr>
<tr>
<td>α-pinene</td>
<td>4.21</td>
</tr>
<tr>
<td>α-terpinene</td>
<td>0.51</td>
</tr>
</tbody>
</table>

3.3 Effect of treatments with lavender essential oil on elimination of TSWV in tomato plants:

lavender essential oil at three concentrations (500, 1000 and 2000 µg/ml) were tested for their ability to inhibit TSWV multiplication and spread of virus infection in systemically infected tomato plants. Results demonstrated in Table (3) reveal that all these concentrations induced resistance to virus infection. 2000 µg/ml was the most effective from all the three concentrations as the percentage of TSWV infection which was decreased by 40.97%.

Table 3 Effect of various concentrations of lavender essential oil on TSWV elimination in tomato plants Peto 86 cultivar.

<table>
<thead>
<tr>
<th>Treatments with lavender essential oil</th>
<th>% Reduction</th>
<th>% Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 µg/ml</td>
<td>20.26± 1.15c</td>
<td>79.74± 2.99a</td>
</tr>
<tr>
<td>1000 µg/ml</td>
<td>31.12± 1.49b</td>
<td>68.88± 2.08b</td>
</tr>
<tr>
<td>2000 µg/ml</td>
<td>40.97± 2.37a</td>
<td>59.03± 2.33c</td>
</tr>
<tr>
<td>Infected (positive control)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>LSD</td>
<td>0.90</td>
<td>0.96</td>
</tr>
</tbody>
</table>
The reduction of infection with TSWV may be due to the treatments with lavender essential oil contain natural compounds which already exist in these extracts which separately or together act as antiviral against viral infection with TSWV throw its inhibition effect against reverse transcriptase or interfering with virus coat protein attachment with target cells. In this respect [20], discussed the antiviral activity of monoterpenes which found in the essential oils from some plants, such as methyleugenol, 1,8-cineole, terpinen-4-ol and caryophyllene oxide, they have effect as virucidal. Also essential oils and their compounds have been shown to exhibit antiviral, antimycotic, antioxygenic, antiparasitic and insecticidal properties [32]. At the same time the essential oil directly inactivates the virus particles, thus preventing adsorption of virion to host cells. Its also capable of preventing cell to cell virus spread in infected cells [20].

3.4. Effect of treatments with lavender essential oil on photosynthetic pigments of tomato leaves: -

Data in Table (4) showed that healthy plants (negative control) recorded the highest content of chlorophyll a, b and carotenoids (0.748, 0.531 and 0.396 mg/g F.W, respectively) while the lowest values were found in plants infected with virus (positive control) (0.535, 0.331 and 0.252 mg/g F.W, respectively).

Table 4 Effect of various concentrations of lavender essential oil on chlorophylls and carotenoids contents as (mg/g FW) in experimental tomato leaves.

<table>
<thead>
<tr>
<th>Treatments with essential oil</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Total chlorophyll</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>(500 µg/ml)</td>
<td>0.539±0.034b</td>
<td>0.347±0.012b</td>
<td>0.886±0.037g</td>
<td>0.352±0.026a</td>
</tr>
<tr>
<td>(1000 µg/ml)</td>
<td>0.557±0.021b</td>
<td>0.351±0.014b</td>
<td>0.908±0.048f</td>
<td>0.334±0.019a</td>
</tr>
<tr>
<td>(2000 µg/ml)</td>
<td>0.614±0.030ab</td>
<td>0.405±0.021b</td>
<td>1.019±0.082b</td>
<td>0.358±0.011a</td>
</tr>
<tr>
<td>Healthy plants</td>
<td>0.748±0.054a</td>
<td>0.531±0.023a</td>
<td>1.279±0.069a</td>
<td>0.396±0.018a</td>
</tr>
<tr>
<td>Infected</td>
<td>0.535±0.012b</td>
<td>0.331±0.019b</td>
<td>0.866±0.036ge</td>
<td>0.252±0.017c</td>
</tr>
<tr>
<td>LSD</td>
<td>0.1229</td>
<td>0.0869</td>
<td>0.0165</td>
<td>0.033</td>
</tr>
</tbody>
</table>

- Each value represents the mean ± (SE).

A significant increasing was noticed in chlorophylls and carotenoids contents with different treatments from essential oil. The levels of chlorophyll a, b and carotenoids at the concentration 2000 µg/ml reached (0.614, 0.405 and 0.358 mg/g F.W) respectively compared to positive control. These data showed that chlorophylls and carotenoids gradually increased according to the increase of antiviral compound concentration. These changes in chlorophylls and carotenoids content may be due to virus infection frequently involves yellow mosaic mottling or generalized yellowing of the leaves. Such changes are obviously due to loss of the chlorophylls giving the yellowish coloration due to carotene and xanthophylls, but the latter pigments are also decreased in some diseases [35,9,10]. These changes are occur because many viruses appear to multiply and accumulate in other parts of the cell, generally, the increase of chlorophylls and carotenoids content after treatments with lavender essential oil may be due to high content from monoterpenes (isoborneol) which make at high concentrations owing perhaps to interaction between alcoholic moiety and the lipids present on the virus envelope resulting in loss of infective [20].

3.5 Influence of treatments with lavender essential oil on total soluble, reducing and non-reducing sugars percentage of tomato leaves: -

From data in Table (5), it could be noticed that total soluble, reducing sugars percentage were increased after infection with virus and reached 5.56 mg/g F.W compared to healthy plants (5.36 mg/g F.W). While the non-reducing sugar percentage was decreased in infected plants and reached 3.08 mg/g F.W compared to control (3.10 mg/g F.W).

Table 5 Effect of various concentrations of lavender essential oil on reducing, non-reducing and total soluble sugars contents (mg/g FW).

<table>
<thead>
<tr>
<th>Treatments with essential oil</th>
<th>Reducing sugars</th>
<th>Non reducing sugars</th>
<th>Total soluble sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>(500 µg/ml)</td>
<td>2.25±0.172b</td>
<td>3.40±0.197a</td>
<td>5.65±0.240a</td>
</tr>
<tr>
<td>(1000 µg/ml)</td>
<td>2.18±0.129b</td>
<td>3.39±0.201a</td>
<td>5.57±0.268ab</td>
</tr>
<tr>
<td>(2000 µg/ml)</td>
<td>2.15±0.103b</td>
<td>3.19±0.169a</td>
<td>5.34±0.216bc</td>
</tr>
<tr>
<td>Healthy plants</td>
<td>2.26±0.102b</td>
<td>3.10±0.132a</td>
<td>5.36±0.127bc</td>
</tr>
<tr>
<td>Infected</td>
<td>2.48±0.098a</td>
<td>3.08±0.167a</td>
<td>5.56±0.142ab</td>
</tr>
<tr>
<td>LSD</td>
<td>0.173</td>
<td>0.173</td>
<td>0.173</td>
</tr>
</tbody>
</table>

- Each value represents the mean ± (SE).

Values in Table 5 also indicated that the treatments with essential oil decreased total soluble and reducing sugars percentage by increasing antiviral compound concentration. The lowest decrease of total soluble and reducing sugars found at the concentration 2000 µg/ml from oil. These may be due to virus infection which can decrease the rate of accumulation of starch in leaves and increase the total soluble sugars content. These results are in harmony with those obtained by [41] they reported that antiviral compounds inhibit virus infection and activate the photosynthesis processes while in virus infected leaves a rise in glucose, fructose and sucrose were noticed.

3.6. Effect of treatments with lavender essential oil on total soluble phenols and flavonides content in tomato leaves: -

From the results in Table 6 it could be noticed that infection with virus and treatments with antiviral compounds increased the content of phenols and flavonides compared with healthy plants. This increase may be due to high content from flavonoids which already exist in these extracts which separately or together act as antiviral against viral infection with TSWV throw its inhibition effect against reverse transcriptase while in virus infected leaves a rise in glucose, fructose and sucrose were noticed.

<table>
<thead>
<tr>
<th>Treatments with essential oil</th>
<th>Reducing sugars</th>
<th>Non reducing sugars</th>
<th>Total soluble sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>(500 µg/ml)</td>
<td>2.25±0.172b</td>
<td>3.40±0.197a</td>
<td>5.65±0.240a</td>
</tr>
<tr>
<td>(1000 µg/ml)</td>
<td>2.18±0.129b</td>
<td>3.39±0.201a</td>
<td>5.57±0.268ab</td>
</tr>
<tr>
<td>(2000 µg/ml)</td>
<td>2.15±0.103b</td>
<td>3.19±0.169a</td>
<td>5.34±0.216bc</td>
</tr>
<tr>
<td>Healthy plants</td>
<td>2.26±0.102b</td>
<td>3.10±0.132a</td>
<td>5.36±0.127bc</td>
</tr>
<tr>
<td>Infected</td>
<td>2.48±0.098a</td>
<td>3.08±0.167a</td>
<td>5.56±0.142ab</td>
</tr>
<tr>
<td>LSD</td>
<td>0.173</td>
<td>0.173</td>
<td>0.173</td>
</tr>
</tbody>
</table>

- Each value represents the mean ± (SE).
Table 6 Effect of various concentrations of lavender essential oil on total soluble phenols, and flavonoids content (mg /g FW) in tomato leaves.

<table>
<thead>
<tr>
<th>Treatments with essential oil</th>
<th>Total flavonoids</th>
<th>Total soluble phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>(500 µg/ml)</td>
<td>0.262± 0.019b</td>
<td>0.82± 0.047c</td>
</tr>
<tr>
<td>(1000 µg/ml)</td>
<td>0.314± 0.016b</td>
<td>0.99± 0.042b</td>
</tr>
<tr>
<td>(2000 µg/ml)</td>
<td>0.324± 0.021b</td>
<td>1.02± 0.097b</td>
</tr>
<tr>
<td>Healthy plants</td>
<td>0.081±0.006b</td>
<td>0.25± 0.016d</td>
</tr>
<tr>
<td>Infected</td>
<td>0.162±0.012b</td>
<td>0.74± 0.025c</td>
</tr>
<tr>
<td>LSD</td>
<td>0.162</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Each value represents the mean ± (SE).

3.7. Induced systemic resistance of tomato (cv. Peto 86) and specific enzymes activity

The results showed in Figs. 1, 2 and 3 indicated that, TSWV infection significantly stimulated the activity of catalase, peroxidase (POD) and polyphenol oxidase (PPO) enzymes compared with healthy plants. On the other hand, all treatments with lavender essential oil realized the highest stimulation of their activity compared with the infected plants. These may be due to increased specific defense gene expression or one possibility is that the initial signal from the inoculated leaf leads to the activation of an enzyme or group of defense enzymes downstream[26].

3.7.1. Specific activities of catalase in tomato leaves treated with lavender essential oil

The obtained data (illustrated in Fig.1) revealed that the catalase activity significantly was stimulated under viral infection stress. On the other hand, all treatments positively affected catalase activity against viral infection. The catalase activity was stimulated to reach the highest activity by the third day of all treatments then inhibited to get its lowest activity levels by the forth day. The highest catalase activity (4.46 unite) have been recorded with 2000µg/ml of oil concentration compared with healthy plants (1.49 unit).

The stimulated catalase activity in response to antiviral natural products reflect the induction effect of these extracts on the transcription gene levels of antioxidant defense catalase enzymes against induced highly levels of hydrogen peroxide signaling molecules during viral infection. These data are in accordance with [26] who noted that H₂O₂ have been implicated in plant responses to stress. Catalases and peroxidases are the primary enzymatic detoxifiers of H₂O₂ in most plant tissues under stress conditions.

3.7.2. Specific activities of peroxidase in tomato leaves which treated with lavender essential oil.

The obtained data illustrated in Fig. 2 revealed that, the peroxidase activity significantly was stimulated under viral infection stress compared with healthy plants, on the other hand, all treatments significantly increased the peroxidase activity against viral infection of this study. The peroxidase activity stimulated to reach the highest activity by the third day of all treatments, then inhibited by the fourth day. The highest peroxidase activity have been realized with oil (2000 µg/ml) to realize 2.97 unite activity compared with healthy plants (1.29).

The induced peroxidase activity in response to various essential oil concentrations reflect the induction effect of these extracts on the transcription gene levels of antioxidant defense
Data illustrated in Fig. 3 revealed that, the polyphenol oxidase anion, hydrogen peroxide (H$_2$O$_2$), hydroxyl radical and single sequence ROS are predominantly represented by superoxide of the interaction between the plant and the pathogen [6]. In be an early event that can fundamentally influence the balance accumulation of reactive oxygen species (ROS) is thought to defence mechanisms, including the rapid production and attacked by pathogens they respond by activating a variety of enzymes of H$_2$O$_2$ in most plant tissues under stress conditions.

3.7.3 Specific activities of polyphenol oxidase in tomato leaves which treated with lavender essential oil.

The obtained data was in accordance with [39] they noted that, antiviral principals against TSWV infection revealed that they induced defense mechanisms in plants challenged with TSWV and accumulation of peroxidase was observed from frist day after challenge inoculation with TSWV on cowpea. Also, [26] found that H$_2$O$_2$ have been implicated in plant responses to stress. Catalases (CAT) and peroxidases are the primary enzymatic detoxifiers of H$_2$O$_2$ in most plant tissues under stress conditions.

4. Conclusion

In this study we have investigated the effect of treatments with lavender essential oil to induced resistance against TSWV infection in tomato plants under field conditions when applied to plants as essential oil with TSWV in a mixed inoculum. This work shows that all tested treatments gave a significant increase in photosynthetic pigments, total soluble sugars, total soluble phenols and flavonoids as well as the activity of catalase, peroxidase and polyphenol oxidase compared with infected plants.

References