OPEN ACCESS Plant Protection, 10 (05) 2026. 863-876 DOI:10.33804/pp.010.05.6249

Check for updates

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

Research Article

Efficacy of Essential Oils in Suppressing Tomato Yellow Leaf Curl Virus Disease and Enhancing Growth and Yield of Tomato

Hussein Abdul Karim Qasim, Muhannad Abdul Ridha Khalaf Al-Waeli

Department of Plant Protection, College of Agriculture, University of Basrah, Iraq.

ARTICLE INFO

Article history

Received: 18th June, 2026

Revised: 23rd September, 2026

Accepted: 25th September, 2026

Keywords

Biological control

Essential oils

Rosemary oil

Thyme oil

Tomato, Tomato yellow leaf curl virus

TYLCV

ABSTRACT

Tomato yellow leaf curl virus (TYLCV) is a major constraint to tomato production, causing severe leaf curling, chlorosis, stunting, and yield reduction. This study evaluated the efficacy of thyme and rosemary essential oils at 1, 2, and 3% against TYLCV and their effects on tomato growth, biomass, chlorophyll content, and fruit yield. TYLCV infection was molecularly confirmed by PCR through amplification of the expected 777-bp coat protein gene fragment. Essential-oil treatments significantly improved vegetative growth and reduced disease severity compared with the untreated control. Rosemary oil at 3% produced the greatest plant height (158.2 cm), number of branches (10.2/plant), number of leaves (157.1/plant^{1}), leaf area (120.8 cm^{2}), and chlorophyll content (9.312). It also resulted in the highest shoot fresh and dry weights (1516.6 and 306.7 g, respectively) and root fresh and dry weights (49.2 and 24.0 g, respectively). Disease severity was reduced from 62.76% in the untreated control to 1.50% with 3% rosemary oil and 3.70% with 3% thyme oil. Fruit yield was also highest with 3% rosemary oil (25.081 kg per experimental sector), followed by 3% thyme oil (17.870 kg), compared with 6.990 kg in the untreated control. The responses generally increased with increasing essential-oil concentration. Overall, rosemary and thyme essential oils, particularly rosemary oil at 3%, were associated with reduced TYLCV severity and improved tomato growth, biomass accumulation, and yield, indicating their potential as botanical components of TYLCV management. Further studies are needed to elucidate the underlying antiviral and plant-defense mechanisms.

Corresponding Author: Hussein Abdul Karim Qasim

Email: hussain.abdulkarimm@uobasrah.edu.iq

Introduction

Tomato (*Solanum lycopersicum* L.), is one of the most important vegetable crops in the Solanaceae family, and is widely grown globally. It is native to Peru, South America. It has become an important economic and nutritional crop, as it is one of the most consumed in the world and is a part of the diet of many people due to its nutritional value (Wang et al., 2024).

Tomatoes rank second globally in terms of cultivated area and production, after potatoes, revealing their prominent position among vegetable crops. In Iraq, tomato cultivation has seen remarkable expansion over the past five decades, with a continuous increase in production. It contributes approximately 10% of total vegetable production in some growing seasons (Ministry of Planning, 2022). This is attributed to its high nutritional value and its role as an important source of vitamins and minerals essential for human health (FAOSTAT, 2021).

It grows in tropical, subtropical and temperate regions. Although naturally a perennial herbaceous plant, it is

cultivated as an annual for commercial purposes (Kumar et al., 2020). It exhibits both determinate and indeterminate growth patterns and is grown in fields or greenhouses and plastic covered tunnels, particularly in temperate regions, where they are a major crop for both local consumption and export (Parmar et al., 2020).

However, it is infected by numerous fungal, bacterial, and viral diseases that negatively affect both yield and quality. Among the most prevalent viral groups in the region are *Begomovirus* and *Potyvirus*, particularly those transmitted by insects. These viruses pose a significant challenge for farmers, especially due to the lack of effective and rapid diagnostic and control strategies (Al-Ani et al., 2011).

In the search for environmentally friendly methods to reduce the spread of plant viruses, many recent studies have shifted to the use of plant extracts and essential oils as bio-alternatives to chemical pesticides. Essential oils such as thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) are among the most important of these substances, due to their content of active compounds such as thymol, carvacrol and rosmarinic acid, which possess antiviral, antioxidant and antimicrobial properties (Vuko et al., 2022). Studies have shown that these oils contribute to reducing the severity of viral infection by inhibiting viral replication within plant cells as well as their role in stimulating systemic resistance mechanisms in plants. They also have an indirect effect on reducing the density of insect vectors such as the whitefly (*Bemisia tabaci*), thus limiting the spread of viruses such as *Begomoviruses* (Sen and Gencer, 2023). Therefore, these oils are considered promising strategies in integrated plant disease management programs (Bakkali et al., 2008; Isman, 2020).

Due to the limited local research on the use of essential oils in controlling tomato viral diseases, this study aimed to evaluate the efficacy of certain essential oils in reducing the severity of TYLCV infection and their effects on growth and yield under greenhouse conditions.

Materials and Methods

The experiment was conducted in a greenhouse at the Agricultural Research Station, College of Agriculture, University of Basrah, during the 2025–2026 growing season using a randomized complete block design (RCBD). Three blocks (replicates) of 8 treatments were set up. There were 9 plants per replicate, with a total of 27 plants per treatment and 216 plants for the entire experiment. The length of the greenhouse was 50 m and the width was 9 m. The tomatoes were planted in four rows with a plant to plant distance of 90 cm and row to row spacing of 100 cm. The treatments within each block were randomly assigned to reduce the environmental variation within the greenhouse (Al-Rawi and Khalafallah, 2000).

Soil and greenhouse preparation

The soil was twice plowed at right angles and then completely leveled in the greenhouse. Decomposed animal manure was then incorporated into the soil, and the soil was solarized for seven days prior to planting. The approved experimental design was implemented by dividing the greenhouse into four rows and three blocks.

Seedling cultivation

Tomato seeds of the Assel variety, susceptible to viral infection, were sown in Styrofam trays containing a specialized growing medium. After the seedlings reached an age of 45 days, they were transplanted to a greenhouse.

Source of infection

Tomato plants showing typical symptoms of tomato yellow leaf curl disease, including leaf curling and yellowing, were collected from tomato fields in Basrah Governorate and used as a source of infection. The presence of the virus in these plants was confirmed by PCR prior to their use in the inoculation experiment.

Healthy tomato seedlings were maintained under protected conditions before exposure to the insect vector. Whiteflies were used as the virus vector. Twenty adult whiteflies were introduced per tomato plant during the transmission experiment. For virus acquisition, whiteflies were allowed to feed on PCR-confirmed infected tomato plants for an acquisition access period of 48 h. Subsequently, viruliferous whiteflies were transferred to healthy tomato plants, with 20 whiteflies used per plant, and were allowed to feed for an inoculation access period of 48 h. After completion of the inoculation period, plants were transferred to greenhouse conditions and maintained according to the experimental design. The same inoculation procedure was used for all treatments to ensure uniform virus exposure among experimental units (Li et al., 2017). The whitefly population used in the present study was identified morphologically as *Bemisia tabaci*; however, the specific biotype was not determined.

Molecular diagnosis

To diagnose TYLCV, leaf samples were randomly collected from tomato plants inside the greenhouse after the onset

of disease symptoms and throughout the experimental period. Total DNA was extracted from the plant samples using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Lohdi et al., 1994).

Applying polymerase chain reaction (PCR)

Tomato leaf samples were tested for the presence of the virus by PCR. Total DNA was extracted using the CTAB method according to Lodhi et al. (1994). PCR amplification was performed using the V1-CP ECORI-Forward primer (5′-GAATTCATGTCGAAGCGWCCMGGCGA-3′) and V1-CP ECORI-Reverse primer (5′-GAATTCTTAATTTKRTAYGAATCATAGAA-3′), as described by Kim et al. (2011). Each 25 µL reaction contained 12.5 µL of PCR Master Mix (Amplicon, Denmark), 1 µL of each primer, 2 µL of DNA template, and nuclease-free water to the final volume.

PCR amplification was carried out using an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The expected PCR product was approximately 777 bp and was visualized by agarose gel electrophoresis using a DNA molecular-weight marker. A confirmed virus-positive sample was included as the positive control, while a reaction containing all PCR components except template DNA was included as the negative control. Representative PCR products were not sequenced for molecular confirmation.

Effect of essential oils on plants

The greenhouse experiment took place at the Agricultural Research Station, College of Agriculture, University of Basrah, throughout the 2025–2026 growing season. The study was set up using RCBD featuring eight distinct treatments distributed across three blocks (Table 1).

Table 1. Treatments used in the experiment.

No.	Treatment	Concentration
1	Thyme oil	1%
2	Thyme oil	2%
3	Thyme oil	3%
4	Rosemary oil	1%
5	Rosemary oil	2%
6	Rosemary oil	3%
7	Mantra (pesticide)	1 g/L
8	Control	Distilled Water (DW)

Essential oil treatments and chemical control commercial thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) essential oils were used in the present study. The oils were obtained as commercial products from (SAC) S. Amden group of companies, Karachi, Pakistan. The essential oils were used as supplied by the manufacturer without further extraction or chemical modification in the laboratory. Thyme and rosemary oils were prepared at concentrations of 1%, 2%, and 3% (v/v) using distilled water. Tween 80 at a concentration of 0.1% was added as an emulsifying agent to facilitate the dispersion of the essential oils in water. The control treatment consisted of distilled water containing the same concentration of Tween 80 (0.1%) to ensure that the emulsifier was consistent across all treatments. The prepared essential-oil formulations were thoroughly mixed before application and sprayed uniformly onto the tomato plants. The treatments were applied as a foliar spray until run-off. A total of four applications were performed: the first application was conducted as a preventive treatment prior to virus inoculation, followed by three consecutive applications after inoculation, with a 14-day interval between successive sprayings. The treatments were applied according to the experimental schedule described above.

The commercial systemic insecticide Mantra® (active ingredient: Imidacloprid 17.8% SL; India) was included as a standard chemical control at a rate of 1 g/L. Imidacloprid, a neonicotinoid insecticide, was selected as the chemical control due to its high systemic efficacy against sucking insect vectors, particularly the whitefly, which serves as the primary vector for TYLCV.

Assessment of phytotoxicity

Phytotoxic effects on tomato leaves such as chlorotic spots, marginal burning, and necrosis were evaluated visually at 24, 48 and 72 h after spraying, with special attention to the highest concentration (3%) of essential oils.

Studied characteristics

Disease severity assessment

A descriptive scale of phenotypic symptoms was used to determine the severity of infection on the studied plants. Each plant was individually assessed and the level of infection was scored on a 0-4 scale with 0 rating a healthy plant (no symptoms) and 4 rating a highly infected plant with clear symptoms (Table 2, Figure 1) (Agrios, 2005).

The severity of the infection was then calculated using the disease severity index (DSI) equation as follows:

$$\text{Severity of Infection (\%)} = \frac{\text{Sum of (Infection Score} \times \text{Number of Infected Plants)}}{\text{High Infection Score} \times \text{Total number of plants}} \times 100$$

Table 2. The severity of the virus infection based on the apparent symptoms.

Score	Description of Symptoms
0	Healthy plants with free symptoms
1	Leaf mottling
2	Leaf yellowing
3	Leaf curling and yellowing
4	Leaf curling, yellowing and plant stunting



Figure 1. Symptoms of infection: A) Healthy plant, B) Leaf mottling, C) Leaf curling and yellowing, D) Stunting and severe yellowing.

Plant height

The height of the tomato plants was measured using a graduated measuring tape and the method of Jones et al. (2014). The height of the plant was measured from the soil surface at the base of the main stem to the growing tip of the plant. Measurements were taken on a number of plants randomly selected from each experimental unit. The average plant height for each treatment was then calculated and expressed in centimeters (cm). This indicator was used to evaluate the effect of different treatments on plant vegetative growth and their ability to improve plant growth or reduce the negative effects of viral infection.

Number of branches/plant

The number of branches in tomato plants was measured by counting all lateral and main branches formed on a randomly selected plant from each experimental unit 45 days after transplanting. The average number of branches per plant was then calculated for each treatment. The number of branches is an important indicator in evaluating plant vegetative growth, as it is associated with increased leaf area and improved plant utilization of available environmental resources, which positively influences growth and productivity (Khalil and Huthily, 2020).

Number of leaves/plant

The number of leaves in tomato plants was estimated by counting all leaves formed on randomly selected plants from each experimental unit. The average number of leaves per plant was then calculated for each treatment (Alam et al., 2021). The number of leaves is an important morphological characteristic that reveals the efficiency of plant vegetative growth, given its role in increasing the photosynthetic area, improving photosynthetic efficiency and accumulating dry material.

Leaf area (cm^2)

Leaf area was measured using the digital method described by Aboukarima et al. (2017). To do this, the fourth leaf was taken from the top of three representative plants in each experimental replicate. The leaves were then laid flat against a white background, and clear digital photographs were taken next to a known scale (standard scale). The images were then transferred to a computer, and the precise leaf surface area was calculated using the standard digital image analysis software (Image J).

Fresh and dry weight of the vegetative and root system

Three plants were randomly removed from each experimental unit at the end of the experiment, and the vegetative system was separated from the root system at the crown. The root system was thoroughly washed with running water to remove the adhering soil and surface dried. Both systems were weighed on a sensitive

electronic scale and the weight readings were recorded in g/plant. The vegetative and root systems were separately placed in paper bags and the samples were dried at 65–70°C in the pneumatic oven for 72 h or until the dry weight became constant. The dry weight was subsequently determined by using a sensitive electronic scale after drying and the readings were expressed as g/plant (Taiz et al., 2015).

Total chlorophyll content in leaves

Total chlorophyll content of tomato leaf was estimated by the acetone extraction method (80%). A 0.5 g sample of the fresh leaves of tomato was extracted thoroughly after grinding with appropriate amount of 80% acetone for complete extraction. The extract was filtered and poured into a volumetric flask and the volume was made up to a known volume by adding the requisite amount of the same acetone. The absorbance of the extract was then determined at wavelengths of 645 and 663 nm by using the spectrophotometer. The chlorophyll a, chlorophyll b and total chlorophyll content were computed by applying the standard equations and the readings were expressed in mg/g fresh weight (Arnon, 1949).

Chl\ a = (12.7\times A_ {663})-(2.69\times A_ {645})

Chl\ b = (22.9\times A_ {645})-(4.68\times A_ {663})

Total\ Chlorophyll = (20.2\times A_ {645}) + (8.02\times A_ {663})

Fruit yield measurement

Fruit yield was measured for each experimental sector separately. During each harvesting period, all fruits produced within each sector were collected and weighed, and the total fruit weight obtained from each sector was recorded in kg. The mean fruit yield for each treatment was calculated from the three experimental sectors (replicates) (Dorais et al., 2001).

Statistical analysis

The data were analyzed using GenStat statistical software by analysis of variance (ANOVA) appropriate for the randomized complete block design (RCBD). Treatment means were compared using the least significant difference (LSD) test at 5% probability level ($P \leq 0.05$). The results were presented as treatment means, and different letters within the same column indicate significant differences among treatments at $P \leq 0.05$.

Results and Discussion

Effect of essential oils on plant height

The results shown in Table 3 indicate a significant effect of essential oils on the height of tomato plants infected with TYLCV. All treatments surpassed the control treatment, which recorded the lowest average plant height of 47.3 cm. This reveals the negative impact of viral infection in inhibiting the vegetative growth of infected plants. The results showed that the 3% rosemary oil treatment achieved the highest average plant height of 158.2 cm followed by the 3% thyme oil treatment at 148.0 cm, and the 2% thyme oil treatment at 138.3 cm. Lower concentrations recorded relatively lower values, indicating that increasing the concentration of essential oils led to a clear improvement in the growth of infected plants. The Mantra treatment also recorded an average height of 102.8 cm, indicating improved growth compared to the control treatment. Although, it was less effective than some essential oil treatments, particularly rosemary oil at a concentration of 3%.

Table 3. Effect of essential oils and chemical pesticide on vegetative growth characteristics of tomato plants.

Treatments	Vegetative growth				
	plant height	Number of branches	Number of leaves (plant^{-1})	Leaf area (cm^2)	Chlorophyll content
Control	47.3 g	2.5 g	47.5 g	22.0 g	3.731 f
Mantra	102.8 f	5.0 f	106.0 f	69.3 f	8.386 e
Thyme oil (1%)	115.2 e	4.8 ef	117.3 e	33.3 e	6.516 d
Thyme oil (2%)	138.3 cd	7.0 cd	139.8 cd	51.9 c	7.47 c
Thyme oil(3%)	148.0 ab	6.8 ab	142.6 ab	83.2 ab	8.91 a
Rosemary oil (1%)	90.4 de	5.2 de	109.0 de	64.4 d	7.043 c
Rosemary oil (2%)	120.0 bc	7.5 bc	130.1 bc	68.6 b	8.455 b
Rosemary oil (3%)	158.2 a	10.2 a	157.1 a	120.8 a	9.312 a
L.S.D 0.05	18.99	2.019	19.31	11.10	2.141

These results indicated the high efficiency of essential oils in reducing the damaging effects of viral infection and improving the vegetative growth of infected plants. TYLCV is one of the viruses that directly affect plant physiological processes, leading to reduced photosynthetic efficiency due to decreased chlorophyll content and disturbances in chloroplasts. This results in stunted plants and reduced vegetative growth and height. Therefore, the increase in height observed in plants treated with essential oils can be attributed to the ability of these natural compounds to decrease the physiological damage caused by viral infection and improve the efficiency of biological processes within the plant (Sofian et al., 2021). The improved plant height observed in the essential oil treatments can also be attributed to a reduction in severity of TYLCV infection compared to the control treatment. This reduced severity helped maintain the efficiency of plant physiological processes and promote better vegetative growth. Furthermore, the active compounds in thyme and rosemary oils contributed to dropping oxidative stress associated with viral infection, which positively impacted cell elongation and increased plant height (El-Serafy et al., 2023).On the other hand, increasing the concentration from 1% to 3% resulted in a significant improvement in plant height. This is likely due to the increased amount of active compounds entering plant tissues at higher concentrations, thus enhancing their effectiveness in stimulating resistance and mitigating the damaging effects of the virus. However, the response varies depending on the type and concentration of the essential oil and the nature of its active compounds. Rosemary oil showed higher efficacy compared to thyme oil at some concentrations, which can be attributed to differences in chemical composition and the percentage of active compounds between the two oils (Ibrahim et al., 2021).

These results are consistent with findings of Tzortzakis (2007) who reported that essential oils possess high biological activity in improving vegetative growth and reducing physiological stress caused by pathogens due to their content of active phenolic compounds. Abd El-Mageed et al. (2017) also pointed out that the use of plant extracts and oils improved vegetative growth characteristics and increased the efficiency of physiological processes in plants exposed to biological stress, because of their role in activating defense systems and improving metabolic activity within the plant.

Effect of essential oils on the number of branches and leaves in tomato plants

The results shown in the Table 3 pointed out that there were obvious and significant differences between the essential oil treatments in the vegetative growth characteristics of tomato plants infected with TYLCV. Regarding the number of branches, the control treatment recorded the lowest average of 2.5 branches/plant, while the 3% rosemary oil treatment significantly outperformed it, yielding the highest average of 10.2 branches/plant. This was followed by the 2% rosemary oil treatment at 7.5 branches/plant and the 2% thyme oil treatment at 7.0 branches/plant, while the Mantra treatment recorded an average of 5.0 branches/plant (LSD = 2.019).

As for the feature of number of leaves, the control treatment recorded the lowest average at 47.5 leaves/plant, while the 3% rosemary oil treatment yielded the highest average at 157.1 leaves/plant. This was followed by the 3% thyme oil treatment at 142.6 leaves/plant, and the thyme oil treatment at a concentration of 2% resulted in 139.8 leaves/plant, while the Mantra treatment resulted in 106.0 leaves/plant (LSD = 19.31).

The superiority of essential oils in increasing the number of branches and leaves compared to the control treatment was attributed to their effective role in reducing the negative effects of TYLCV. The virus typically causes physiological disturbances affecting cell division, elongation and vegetative growth as well as reducing photosynthetic efficiency and chlorophyll production, leading to a decrease in the number of leaves and branches and stunting of infected plants. However, treatment with essential oils assisted in decreasing the severity of the infection and improved the efficiency of vital processes, which positively influenced the increase in vegetative branching and the number of leaves. Furthermore, increasing the concentration from 1% to 3% resulted in a significant improvement due to the increased concentration of active compounds within plant tissues, which enhances metabolic activity and reduces oxidative stress within the plant. These results are consistent with those reported by Pradhanang et al. (2003), Najar et al. (2020) and Moustafa et al. (2021) who discussed the role of essential oils in improving vegetative growth characteristics and reducing pathological effects by stimulating plant defense systems.

Effect of essential oils on leaf area in tomato plants

As shown in Table 3, there were significant differences between the essential oil treatments in leaf area of tomato plants infected with TYLCV. All treatments surpassed the control treatment, which recorded the lowest average leaf area at 22.0 cm^2. This reveals the negative impact of viral infection in reducing vegetative growth and limiting leaf expansion in infected plants. In contrast, the 3% rosemary oil treatment recorded the highest average leaf area of 120.8 cm^2, followed by the 3% thyme oil treatment at 83.2 cm^2, and the Mantra treatment at 69.3 cm^2. Treatments with lower concentrations recorded relatively lower values. The value (L.S.D = 11.10) indicates a significant difference among different treatments at a probability level of 0.05.

The results show that increasing the concentration of essential oils from 1% to 3% led to a gradual increase in leaf area (Ali et al., 2021a), indicating the efficacy of essential oils in improving vegetative growth and mitigating the negative effects of viral infection. This can be attributed to the role of these oils in enhancing the efficiency of physiological processes within the plant. Infection with TYLCV disrupts photosynthesis and reduces chlorophyll production, in addition to affecting cell division and elongation, which directly results in reduced leaf area and stunted growth in infected plants. However, treating the plants with essential oils assisted in decreasing the severity of infection and improving plant vitality, leading to increased leaf expansion and overall improvement in vegetative growth (Helmy et al., 2020).

The increase in leaf area in treated plants might be a result of improved plant health and reduced severity of viral symptoms, as tomato yellow leaf curl disease leads to decrease leaf expansion, leaf deformation and a reduction in effective green area. Therefore, the reduced severity of infection was displayed in an increase in leaf area and the plant's ability to capture light and perform photosynthesis more efficiently.

The increase in leaf area is also likely due to improved nutrient uptake and elevated metabolic activity within the plant, which is reflected in increased cell division and elongation of leaf cells. This, in turn, leads to increased photosynthetic efficiency and enhances the plant,s ability to produce food, thus improving vegetative growth and following productivity of infected plants.

Pradhanang et al. (2003) confirmed that essential oils improved vegetative growth characteristics and reduced pathogenic effects in tomato plants. Najar (2020) also indicated that essential oils possess antiviral activity in plants by stimulating defense systems and reducing oxidative stress within the plant. Al-Qahtani et al. (2020) also explained that essential oils contribute to promoting plant growth and increasing the efficiency of physiological processes due to their content of bioactive compounds with anti-pathogenic activity.

The effect of essential oils on the chlorophyll content in tomato leaves

The results in Table 3 also indicate significant differences between the essential oil treatments in the chlorophyll content of tomato leaves infected with TYLCV. All treatments showed a marked increase in chlorophyll content compared to the control treatment, which recorded the lowest value at 3.731. This demonstrates the negative impact of viral infection in reducing the formation of photosynthetic pigments and weakening the physiological efficiency of infected plants.

A 3% rosemary oil treatment significantly surpassed all others, yielding the highest chlorophyll content of 9.312. This was followed by the 3% thyme oil treatment at 8.91, and the 2% rosemary oil treatment at 8.455, while the Mantra treatment recorded a value of 8.386. Treatments with lower concentrations yielded relatively lower values, but they still outperformed the control treatment. The LSD value (2.141) indicates a significant difference between the different treatments at a probability level of 0.05.

The reduced chlorophyll content in infected and untreated plants can be attributed to the effect of the TYLCV on chloroplasts, inhibiting chlorophyll production and affecting the activity of enzymes involved in photosynthesis. This leads to yellowing of the leaves, reduced photosynthetic efficiency and complete stunted plant growth. Moreover, viral infection increases free radical formation and oxidative stress within plant cells, causing chlorophyll degradation and damage to cell membranes and chloroplasts.

The higher chlorophyll content in treated plants can be due to a lower viral infection severity compared to the control group. TYLCV infection disrupts chloroplast activity and reduces photosynthetic efficiency, which in turn affects chlorophyll content. Therefore, the improvement in chlorophyll levels across different treatments indicates an improvement in the physiological state of the treated plants.

These results are consistent with the findings of Burt (2004) who stated that essential oils possess significant

biological effects in protecting plant cells and reducing damage caused by biological stress, which positively influences photosynthetic efficiency and increases chlorophyll content in treated plants. Sangwan et al. (2001) also stated that the active compounds in essential oils play a crucial role in improving physiological processes within the plant, maintaining the integrity of chloroplasts, and increasing photosynthetic activity. Kordali et al. (2008) further explained that essential oils contribute to improved plant growth and reduce the damaging effects of pathogens due to their antioxidant and anti-pathogenic properties. Soliman and Badeaa (2002) indicated that the use of plant and essential oils led to improved physiological characteristics in plants and increased chlorophyll content as a result of their role in reducing oxidative damage and maintaining the integrity of plant tissues.

Effect of essential oils on the fresh and dry weight of the shoots and roots of tomato plants

The results shown in Table 4 indicate significant differences between the essential oil treatments in both the fresh and dry weights of the vegetative and roots of tomato plants infected with TYLCV. All treatments showed a significant advantage over the control treatment, which recorded the lowest rates for both traits. This reveals the negative impact of viral infection in weakening physiological activity and inhibiting the vegetative and root growth of infected plants.

Regarding vegetative characteristics, the control treatment recorded the lowest average fresh weight of 353.6 g, due to the direct effect of the virus in reducing photosynthetic efficiency and decreasing dry material formation within the plant. While the 3% rosemary oil treatment significantly outperformed the others, yielding the highest average weight of 1516.6 g. It was followed by the 3% thyme oil treatment at 1371.8 g, and then the 2% rosemary oil treatment at 1048.7 g. Lower concentrations recorded relatively lower values, while the Mantra treatment showed a clear improvement compared to the control, reaching 902.4 g. The LSD value (475.29) indicates a significant difference between the treatments at a probability level of 0.05.

Regarding the dry weight of the vegetative system, the control treatment recorded the lowest average at 75.2 g, while the 3% rosemary oil treatment yielded the highest average at 306.7 g, followed by the 3% thyme oil treatment at 222.0 g, and then the 2% rosemary oil treatment at 211.3 g. This indicates the positive role of essential oils in improving dry matter accumulation within infected plants. In contrast, the Mantra treatment recorded an average dry weight of 150.7 g, confirming its effectiveness in improving growth compared to the control, although less effective than some of the essential oil treatments, with a minimum significant difference (LSD = 79.99).

As for the characteristics of the root system, the results indicated obvious significant differences in both fresh weight and dry weight. The control treatment recorded the lowest fresh weight at 16.5 g, resulting from the direct effect of the virus in reducing the efficiency of vital processes and weakening the plant's ability to absorb water and nutrients. In contrast, the 3% rosemary oil treatment significantly surpassed the control, achieving the highest weight at 49.2 g, followed by the 3% thyme oil treatment at 44.8 g, and then the 2% thyme oil treatment at approximately 41.2 g. Treatments with lower concentrations recorded relatively lower values. The Mantra treatment showed improvement compared to the control, yielding 35.6 g, with the lowest significant difference (LSD = 12.93) at a probability level of 0.05.

Table 4. Effect of essential oils on the fresh and dry weight of the shoot and root systems of the tomato plant.

Treatments	Vegetative system		Root system	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Control	353.6 f	75.2 e	16.5 f	7.5 f
Mantra	902.4 e	150.7 cd	35.6 e	10.8 e
Thyme oil (1%)	903.7 d	142.6 c	32.5 d	14.0 a
Thyme oil (2%)	1041.1 bc	207. 7 ab	41.2 bc	13.6 bc
Thyme oil(3%)	1371.8 a	222.0 a	44.8 a	17.1 a
Rosemary oil (1%)	756.6 c	159.5 b	30.3 c	12.6 c
Rosemary oil (2%)	1048.7 ab	211.3 a	37.7 ab	14.7 b
Rosemary oil (3%)	1516.6 a	306.7 a	49.2 a	24.0 a
L.S.D 0.05	475.29	79.99	12.93	6.299

Concerning the dry weight of the root system, the control treatment recorded the lowest average of 7.5 g, while the 3% rosemary oil treatment yielded the highest average of 24.0 g, followed by the 3% thyme oil treatment at 17.1 g, and then the 2% rosemary oil treatment at 14.7 g. This confirms the positive role of essential oils in improving root growth and increasing dry matter accumulation within the roots. Finally, the Mantra treatment recorded an average dry weight of 10.8 g, demonstrating its ability to improve growth compared to the control, although it was less efficient than some of the essential oil treatments, with the lowest significant difference value (LSD = 6.299). The significant decrease in the fresh and dry weights of the vegetative and root systems in the control treatment is attributed to the direct and negative impact of infection with the TYLCV. The virus reduces the efficiency of photosynthesis, inhibits metabolism, disrupts the activity of vital enzymes and protein and carbohydrate synthesis, and decreases nutrient uptake. It also impedes the flow and transport of photosynthetic products from the shoots to the roots, thus hindering root expansion and reducing dry matter and total plant biomass accumulation.

In contrast, the significant improvement observed with essential oil treatments (rosemary and thyme) is attributed to their effectiveness in reducing viral infection severity and preserving leaf area and chlorophyll content. This contributes to improved physiological efficiency and biomass accumulation, consistent with the findings of Pradhanang et al. (2003). Furthermore, increasing the concentrations used (from 1% to 3%) led to greater penetration of active phenolic and terpene compounds and natural antioxidants into the tissues. This provided high protection for cell membranes against oxidative stress resulting from infection of disease, a mechanism elucidated by Bakkali et al. (2008) in explaining the role of aromatic compounds in reducing oxidative damage and stimulating growth.

Moreover, the physiological improvement and restoration of photosynthetic efficiency in the vegetative parts led to increased nutrient flow to the roots. This positively influenced the weight of both fresh and dry roots and enhanced their ability to absorb water and nutrients, thus promoting vegetative growth. These results are in agreement with the scientific observations of Pistelli et al. (2020), Mahmoud et al. (2021), and Abdul-Hussein et al. (2022) on the role of plant extracts and oils in protecting the root system, enhancing its physiological efficiency and increasing the plant's resistance to biotic and pathological stresses. The superiority of rosemary oil, in particular, in terms of biomass weight can be attributed to the nature and concentration of its main active compounds compared to thyme oil. Furthermore, visual inspection revealed no signs of phytotoxicity (e.g., leaf scorching, chlorosis, or necrosis) on tomato foliage treated with thyme or rosemary essential oils across all evaluated concentrations, including the 3% level, confirming their safety for crop application (Sertkaya et al., 2010).

Molecular detection of TYLCV

The PCR results revealed amplification of the expected size DNA fragment using the V1-CP ECORI-Forward and V1-CP ECORI-Reverse primers, indicating the presence of TYLCV in the tested plants (Figure 2).

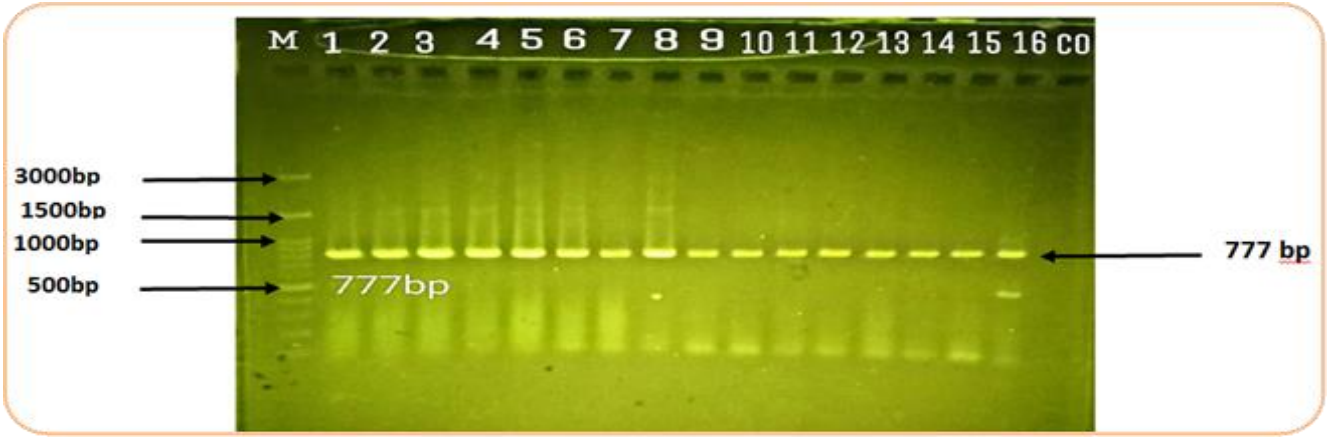


Figure 2. PCR amplification of a 777 bp fragment of TYLCV coat protein gene using primers V1-CP ECORI-Forward and V1-CP ECORI-Reverse. Lane M: DNA ladder (500–3000 bp); Lanes 1–16: Positive tomato leaf samples collected from the field showing TYLCV infection; Lane C0: Negative control (nuclease-free water template).

Effect of essential oils on TYLCV severity

The results in Table 5 show significant differences among various treatments in their effect on the severity of infection by TYLCV, the causal agents of leaf curl and yellowing symptoms. The control treatment recorded the highest infection severity at 62.76%, indicating the strong impact of the virus on untreated plants due to continued viral replication within plant tissues and the plant's reduced ability to resist infection. In contrast, infection severity decreased significantly when plants were treated with the preparations and essential oils used in the experiment.

Table 5. Effect of essential oils on the severity of Begomovirus infection in tomato plants.

Treatments	Infection Severity
Control	62.76 a
Mantra	25.93 b
Thyme oil (1%)	21.30 bc
Thyme oil (2%)	14.81 cd
Thyme oil (3%)	3.7 f
Rosemary oil(1%)	13.89 d
Rosemary oil(2%)	3.70 e
Rosemary oil(3%)	1.5 f
L.S.D 0.05	7.50

Treatments of thyme oil at a concentration of 3% and rosemary oil at a concentration of 3% achieved the best results, reducing infection severity to 3.7% and 1.5%, respectively, compared to the control treatment. This demonstrates the effectiveness of these oils in reducing the severity of disease symptoms associated with Begomoviruses infection compared to the control treatment. This can be attributed to the role of the active compounds in these oils in enhancing the plant's physiological state and increasing its resistance to infection, which was reflected in the reduced severity of the observed symptoms during the experiment. It was also noted that increasing the concentration from 1% to 3% led to a gradual improvement in treatment efficacy, indicating a positive relationship between oil concentration and the degree of infection severity reduction. Thyme oil at a concentration of 2% resulted in a significant decrease in infection severity of 14.81%, while the infection severity reached 21.30% when using thyme oil at a concentration of 1%. Rosemary oil treatments yielded infection severity of 13.89% and 3.70% at concentrations of 1% and 2%, respectively, demonstrating increased oil efficacy with increasing concentration. The Mantra treatment also showed a significant decrease in infection severity of 25.93% compared to the control treatment.

The 3% essential oil concentration showed the highest efficacy in reducing the severity of the disease which might be due to the higher concentration of bioactive phenolic and terpene compounds that could increase their antiviral activity in plant tissues. Furthermore, these essential oils can induce antioxidant defense responses thus enhancing the plant resistance to viral attack. This superiority can also be attributed to the fact that increased concentration of volatile compounds directly contributes to a repellent and anti-feeding effect against the whitefly, the primary vector of the virus, thus limiting the efficiency of virus transmission and spread among plants (Smith and Johnson, 2020; Ali et al., 2021b; Zhang et al., 2021).

These results are consistent with what Ibrahim et al. (2021) indicated that essential oils extracted from medicinal plants possess high efficacy in reducing the severity of plant virus infections due to their content of potent phenolic compounds with antiviral effects. Taglienti et al. (2022) demonstrated that thyme oil and rosemary oil contributed to reducing the severity of tomato virus infection and improving the physiological characteristics of infected plants due to their role in activating systemic resistance and reducing oxidative stress within plant tissues. Similarly, Abd-Elkader et al. (2019) showed that the use of aromatic plant oils inhibited the replication of plant viruses and reduced the appearance of disease symptoms in infected tomato plants.

Effect of essential oils on total yield in tomato fruits

The results presented in Table 6 showed significant differences among the treatments in tomato fruit yield. The rosemary oil treatment at 3% recorded the highest mean fruit yield per experimental sector, reaching 25.081 kg, followed by the thyme oil treatment at 3%, which recorded 17.870 kg per sector. Rosemary oil at 2% and thyme oil at 2% recorded mean fruit yields of 15.751 and 16.389 kg per sector, respectively. The chemical control treatment (Mantra) recorded a mean fruit yield of 9.955 kg per sector, whereas the untreated control recorded the lowest mean fruit yield, reaching 6.990 kg per sector. The LSD at the 5% probability level was 5.281 kg.

These results indicate that the application of thyme and rosemary essential oils, particularly rosemary oil at 3%,

was associated with increased tomato fruit yield compared with the untreated control. The higher fruit yield observed in the treated plants might be related to the improved vegetative growth and lower disease severity recorded under these treatments. However, the present study did not directly investigate the physiological or molecular mechanisms responsible for the increase in fruit yield; therefore, possible mechanisms should be considered as hypotheses rather than definitive explanations.

Table 6. Effect of thyme and rosemary essential oils on tomato fruit yield.(Mean fruit yield per experimental sector (kg))

Treatments	Mean fruit yield per experimental sector (kg)
Control	6.990 f
Mantra	9.955 e
Thyme oil (1%)	8.416 d
Thyme oil (2%)	16.389 bc
Thyme oil (3%)	17.870 a
Rosemary oil(1%)	12.630 c
Rosemary oil(2%)	15.751 b
Rosemary oil(3%)	25.081 a
L.S.D 0.05	5.281

Conclusions

The study results showed significant differences among the studied treatments in most vegetative, physiological and productive characteristics, indicating the effectiveness of essential oils in improving tomato plant growth and reducing the negative effects of viral infection. LSD values also showed obvious differences between the means, especially when using rosemary oil at a concentration of 3%.

This superiority can point to the vital role of phenolic and terpene compounds present in essential oils, which inhibit pathogen activity and stimulate the plant's defense response, as well as reduce oxidative stress resulting from infection. This dominance is also attributed to the high concentration, as the increased concentration of volatile compounds directly contributes to a repellent and anti-feeding effect against the whitefly, the primary vector of the virus, thus limiting the efficiency of virus transmission and spread among plants.

Based on the results obtained, foliar spraying of rosemary and thyme essential oils can be used as a safe and sustainable alternative to reduce the severity of TYLCV infection and may interfere with virus transmission mediated by its insect vector, *B. tabaci*. Future studies are also recommended to evaluate the synergistic effects when this oil is combined with other essential oils, and to investigate the molecular mechanisms and gene-induced responses within the plant.

Author Contributions

HAKQ and MARKA conceptualized and designed the study. Both authors contributed to the investigation, methodology, and formal analysis. HAKQ and MARKA were involved in the preparation of the initial manuscript draft, including presentation of the results and interpretation of the findings. Subsequently, both authors critically reviewed and edited the manuscript, contributed to its intellectual refinement, cross-checked the data and analyses, and approved the final version for submission.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 3: Good Health and Well-being
SDG 12: Responsible Consumption and Production

Policy Addressed

The findings support botanical and integrated approaches for sustainable TYLCV management. Policies should promote research, standardization, registration, and field validation of essential-oil-based products, with emphasis on IPM integration, environmental safety, phytotoxicity, residues, non-target effects, formulation stability, and cost-effectiveness.

References

Abd El-Mageed, T.A., Semida, W.M., Rady, M.M., 2017. Foliar-applied effective microorganisms or compost tea enhanced growth, yield and defense system in tomato plants under stress conditions. Scientia Horticulturae 224, 1-10.

Abd-Elkader, H.S., Elsharkawy, M.M., Kamel, S.M., 2019.

Induction of systemic resistance in tomato plants against Tomato yellow leaf curl virus using natural plant extracts and essential oils. Journal of Plant Protection and Pathology 10(7), 349-356.

Abdul-Hussein, A.K., Abdul-Amir, M.J., Hussein, S.A., 2022. Effect of some plant extracts and essential oils on improving tomato plant growth and reducing disease severity. Basrah Journal of Agricultural Sciences 35(2), 55-68.

Aboukarima, A.M., Al-Hamed, S.A., Sayed, S.M., 2017. Assessment of leaf area for some crops using ImageJ software. Journal of Agricultural Sciences, Belgrade 62(1), 77-89.

Agrios, G.N., 2005. Plant Pathology (5th ed.). Elsevier Academic Press, Burlington, MA, USA.

Alam, M.A., Islam, M.S., Rahman, M.M., Islam, M.R., 2021. Growth and yield performance of tomato as influenced by different nutrient management practices. International Journal of Agricultural Research, Innovation and Technology 11(1), 55-62.

Al-Ani, R.A., Adhab, M.A., Hamad, S.H., 2011. Tomato yellow leaf curl virus (TYLCV), identification, virus-vector relationship, strains characterization in Iraq. Academic Journals of Agricultural Research 6(21), 4983-4991.

Ali, A.M., Al-Zubaidi, H.A., Mohammed, K.A., 2021a. Effect of using some volatile vegetable oils as repellent and feeding inhibitors for whiteflies (*Bemisia tabaci*) on vegetable crops. Journal of Agricultural and Environmental Sciences 15(2), 45-58.

Ali, A.M., Ibrahim, M.A., Sharaf, A.E., 2021b. Botanical essential oils as eco-friendly alternatives for controlling plant viral diseases and improving growth performance of tomato. Journal of Applied Phytopathology 10(2), 112-125.

Al-Qahtani, S.M., Al-Ghamdi, A.A., Al-Qurainy, F., 2020. Essential oils and bioactive compounds in improving plant growth and physiological processes under stress conditions. Saudi Journal of Biological Sciences 27(11), 3120-3128.

Al-Rawi, K.M., Khalaf Allah, A.A.M., 2000. Design and Analysis of Agricultural Experiments, 2nd ed. Dar Al-Kutub for Printing and Publishing, University of Mosul, 488 pp.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology 24(1), 1-15.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils - A review. Food and Chemical Toxicology 46(2), 446-475.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods - A review. International Journal of Food Microbiology 94(3), 223-253.

Dorais, M., Papadopoulos, A.P., Gosselin, A., 2001. Greenhouse tomato fruit quality. Horticultural Reviews 26, 239-319.

El-Serafy, R.S., El-Sawy, M.M., Rezk, A.A., 2023. Exogenous application of rosemary and thyme essential oils mitigates oxidative stress and improves growth and physiological traits of virus-infected tomato plants. Acta Physiologiae Plantarum 45(3), 42.

FAOSTAT, 2021. FAO Statistical Database. Food and Agriculture Organization of the United Nations, Rome, Italy.

Helmy, M.S., El-Sawy, A.M., Abd El-Gawad, H.G., 2020. Ameliorative effects of some essential oils on growth, yield, and physiological aspects of tomato plants (*Solanum lycopersicum* L.) infected with Tomato yellow leaf curl virus. Egyptian Journal of Virology 17(1), 45-59.

Ibrahim, H.M., El-Banna, A.A., Al-Ameri, M.S., 2021. Efficacy of rosemary and thyme essential oils in controlling viral diseases and improving growth of plant crops. Journal of Agricultural Sciences, 15(2), 112–124.

Isman, M.B., 2020. Botanical insecticides in the twenty-first century. Annual Review of Entomology 65, 233-249.

Jones, J.B., Zitter, T.A., Momol, T.M., Miller, S.A., 2014. Compendium of Tomato Diseases and Pests, 2nd ed. APS Press, St. Paul, Minnesota, USA.

Khalil, S.O., Huthily, K.H., 2020. Effect of organic and bio-fertilization on growth and yield of tomato (*Solanum lycopersicum* L.). Plant Archives 20(1), 1231-1236.

Kim, S.H., Oh, S., Oh, T.K., Park, J.S., Kim, S.C., Kim, S.H., Kim, Y.S., Hong, J.K., Sim, S.Y., Park, K.S., Lee, H.G., 2011. Genetic diversity of tomato-infecting Tomato yellow leaf curl virus (TYLCV) isolates in Korea. Virus Genes 42, 117-127.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E., 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated

from Turkish oregano. Bioresource Technology 99(18), 8788-8795.

Kumar, R., Singh, M., Sharma, S., 2020. Advances in tomato production and management under protected cultivation. Journal of Horticultural Sciences 15(2), 120-135.

Li, M., Li, X.J., Su, Y.L., 2017. Flue-cured tobacco confirmed as a reservoir host plant for Tomato yellow leaf curl virus by agro-inoculation and *Bemisia tabaci* MED-mediated transmission. PLoS One 12(12), e0190013.

Lodhi, M.A., Ye, G.N., Weeden, N.F., Reisch, B.I., 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Molecular Biology Reporter 12(1), 6-13.

Mahmoud, N.A., Al-Qahtani, W.H., El-Wakeil, N.E., 2021. Potential role of essential oils in plant disease management and growth promotion. Environmental Science and Pollution Research 28, 48428-48435.

Ministry of Planning, 2022. Estimation Report on Secondary Crop and Vegetable Production in Iraq for 2021. Central Statistical Organization, Directorate of Agricultural Statistics, Baghdad, Iraq.

Moustafa, S.M.N., Mahmoud, N.A., Al-Qahtani, W.H., El-Wakeil, N.E., 2021. Potential role of essential oils in plant disease management and growth promotion. Environmental Science and Pollution Research 28, 48421-48438.

Najar, B., Pistelli, L., Ferri, B., Angelini, L.G., 2020. Essential oils as antiviral agents against plant viruses and their role in plant protection: A review. Plants 9(11), 1573.

Parmar, D.K., Singh, A.K., Maurya, R.A., 2020. Genetic variability, character association and path coefficient studies in tomato (*Solanum lycopersicum* L.) grown under terai region. International Journal of Chemical Studies 8(2), 569-573.

Pistelli, L., Ferri, B., Angelini, L.G., 2020. Essential oils as antiviral agents against plant viruses and their role in plant protection: A review. Plants 9(11), 1576.

Pradhanang, P.M., Momol, M.T., Olson, S.M., Jones, J.B., 2003. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. Plant Disease 87(4), 423-427.

Sangwan, N.S., Farooqi, A.H.A., Shabih, F., Sangwan, R.S., 2001. Regulation of essential oil production in plants. Plant Growth Regulation 34, 3-21.

Şen, E., Gençer, N.S., 2023. Toxic effects of essential oils including *Thymus vulgaris* against *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) and its certain important natural enemies. Pakistan Journal of Agricultural Sciences 60(1), 33-41.

Sertkaya, E., Kaya, K., Soylu, S., 2010. Chemical compositions and insecticidal activities of the essential oils from several medicinal plants against the cotton whitefly, *Bemisia tabaci*. Asian Journal of Chemistry 22(4), 2982-2990.

Smith, J., Johnson, R., 2020. Essential oils as repellent and deterrent agents against *Bemisia tabaci* in tomato crops. Journal of Pest Science 93(2), 521-534.

Sofian, M., El-Sawy, A.M., Al-Amri, M.S., 2021. Physiological and biochemical alterations in tomato plants infected with Tomato yellow leaf curl virus and the ameliorative role of some botanical extracts. Physiological and Molecular Plant Pathology 115, 101673.

Soliman, K.M., Badeaa, R.I., 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology 40(11), 1669-1675.

Taglienti, A., Donati, L., Ferretti, L., Tomassoli, L., Sapienza, F., Sabatino, M., Di Massimo, G., Fiorentino, S., Vecchiarelli, V., Nota, P., Ragno, R., 2022. In vivo antiphytoviral activity of essential oils and hydrosols from Origanum vulgare, Thymus vulgaris, and Rosmarinus officinalis to control zucchini yellow mosaic virus and tomato leaf curl New Delhi virus in Cucurbita pepo L. Frontiers in Microbiology, 13, 840893.

Taiz, L., Zeiger, E., Møller, I. M., Murphy, A., 2015. Plant Physiology and Development, 6th ed. Sinauer Associates, Sunderland, Massachusetts, USA.

Tzortzakis, N.G., 2007. Maintaining postharvest quality of fresh produce with volatile compounds. Innovative Food Science and Emerging Technologies 8(1), 111-116.

Vuko, E., Dunkić, V., Maravić, A., Ruščić, M., Soldo, B., Nazlić, M., 2022. In vivo antiphytoviral activity of essential oils and hydrosols from Origanum vulgare, Thymus vulgaris, and Rosmarinus officinalis to control Zucchini yellow mosaic virus and Tomato leaf curl New Delhi virus (Begomovirus) in Cucurbita pepo L. Frontiers in

Plant Science 13, 881031.

Wang, H., Wang, L., Qureshi, A., 2024. Enhancing tomato (*Solanum lycopersicum* Mill.) plant growth and rhizosphere microbiome with carbon nanodots and mycorrhizal fungi. International Agrophysics 38(2), 115-127.

Zhang, L., Wang, X., Liu, Y., 2021. Antifeedant and repellent activities of essential oil components against the whitefly *Bemisia tabaci*. Crop Protection 139, 105380.

Publisher's note: EScience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.