

Zucchini yellow mosaic virus (ZYMV) transmission by *Myzus persicae* vector in squash plants and inhibitory effects of some plant essential oils

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Abstract

Planting essential oils, or "plant-EOs," are increasingly interested in the global movement toward sustainable agriculture management and production. The current investigation aimed to assess the impact of peppermint, cinnamon, and lemon essential oils on the green peach aphid (*Myzus persicae*) settling and probing behaviour in The Cucurbita pepo cv. Eskandarani plants. The infected squash plant samples were employed as the viral-plant sources of inoculum and multiplied for normal stock cultures in a greenhouse with 26±1°C, relative humidity of 70–90%, and a photoperiod of 8–16 hours (light/dark). The current study reports on the anti-viral biological activity to increase the green peach aphid (*Myzus persicae*) mortality rate and decrease the ability of the Zucchini Yellow Mosaic Virus (ZYMV) to spread among squash plants by using three different plant-EOs. Plant essential oils concentrations ranging from 0.002 to 0.1 percentage across all investigated essential oils demonstrated exceptional efficiency. Research on essential oils (EOs) has shown that they exhibit promising insecticidal action against various problem insects.

Keywords: essential oils; *Myzus persicae*; anti-settling activity; Anti-viral biological activity; Zucchini Yellow Mosaic Virus

1. Introduction

Essential oils (EOs) are complex combinations of volatile and low molecular weight molecules that are generated and preserved in aromatic plant families that are especially rich in secondary metabolites, such as *Apiaceae*, *Asteraceae*, *Lamiaceae*, and *Myrtaceae* (Baser and Buchbauer, 2015). The most common methods for obtaining them are steam or hydro-distillation (Garzoli *et al.*, 2015; Božovic *et al.*, 2017). The components of these methods include aliphatic and aromatic chemicals,

hydrocarbons, alcohols, aldehydes, ketones, esters, phenols, and acids, among many other chemical classes. According to reports, several of these compounds have insecticidal, antimicrobial, antiviral, antimycotic, and antiparasitic effects on both plant and mammalian diseases (Garzoli *et al.*, 2018; Sabatino *et al.*, 2020). Plant virus infection poses a serious threat to agricultural products and results in significant financial losses on a global scale (Lecoq and Katis, 2014). Since there isn't a cure for controlling viruses in plants, like bacteria and fungi, that involves applying compounds of synthetic or natural origin (Rubio *et al.*, 2020), most defense tactics focus on prevention (using propagation material that has been sanitarily certified, controlling insect


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vectors, etc.) (Feres and Racciah, 2015; Golino *et al.*, 2017). Additional management strategies necessitate the utilization of resistant cultivars created through breeding (Paris and Brown, 2005; Martín-Hernández and Picó, 2020). On squash plants in northern Italy, the Zucchini Yellow Mosaic Virus (ZYMV), belonging to the Genus Potyvirus and Family Potyviridae, was found (Lisa *et al.*, 1981). The afflicted plants show severe stunting, yellowing and mosaic symptoms, and distortion of fruits and leaves. It is spread by numerous species of aphids in a non-persistent way (Radwan *et al.*, 2007; Al-Saleh *et al.*, 2014). According to Mansour and Al-Musa (1982), ZYMV is one of the most common viruses in squash plants (about 55%) and muskmelon (90%) (Cho *et al.*, 2019).

It's important to note that ZYMV can spread by seeds and aphids, while aphids are the primary vectors for non-persistent transmission. Although two species, *Myzus persicae* and *Aphis gossypii*, have the greatest recorded transmission efficiency (41% and 35%, respectively), a wide number of aphid species have been demonstrated to be capable of transmitting the virus (Katis *et al.*, 2006). The aphid stylet and two viral proteins, the coat protein (CP) and the helper component protein (HC-Pro), combine to cause vector transmission. ZYMV can spread vertically through seeds, which is less common than horizontal transmission through aphids. Simmons *et al.* (2011) have previously documented a transmission rate of only 1.6% from seed to seedling.

The study aimed to evaluate the effectiveness of ZYMV's insect vector transmission and the in vivo anti-viral biological activity of several essential oils. It might be a crucial technique for the agro-industrial goods' prototype of sustainable agricultural management.

2. Materials and methods

2.1. Viral materials and inoculum

The *Cucurbita pepo* cv. Eskandarani plant samples infected with ZYMV were acquired from our earlier research (Radwan *et al.*, 2007). The infected squash plant samples were employed as the viral-plant sources of inoculum and multiplied for normal stock cultures in a greenhouse with $26\pm1^{\circ}\text{C}$, relative humidity of 70–90%, and a photoperiod of 8–16 hours (light/dark).

2.2. Aphid Culture and ZYMV transmission

The study's green peach aphid came from a stock culture housed in the biology department's greenhouse at the University of Hafr Al Batin in Saudi Arabia. In a carefully monitored environment, the stock culture was kept on winter squash seedlings at $22 \pm 1^{\circ}\text{C}$, 65% relative humidity (RH), and L16:D8 photoperiod. We employed adult apterous females for all following tests, 2–3 days after the last molt. In the biology department of the College of Science University of Hafr Al Batin, Saudi Arabia, test plants of zucchini squash (*Cucurbita pepo* L. cv. Eskandarani) cultivated in 1-litre pots in a greenhouse were used for the transmission experiments. *M. persicae*, virus-free, was cultivated on robust cv. Eskandarani zucchini squash plants. Apterous aphids were subjected to a 30-minute fasting period (FP) in plastic boxes before being placed on infected plants for a 20-minute acquisition feeding period (AFP). This was the method used to transmit aphids. For a one-hour inoculation access period (IAP), groups of five aphids were transplanted to test plants (Zucchini squash plants) that were virus-free. Inoculation of the zucchini squash plants occurred during the cotyledonary stage. Following inoculation, test plants were treated with insecticide to eradicate the aphids and allowed to undergo a 7-day latent period (LP) incubation. The effectiveness of *M. persicae* in spreading ZYMV to zucchini squash plants was assessed by contrasting the EO1, EO2, and EO3 treatments.




2.3. The anti-viral biological activity of plant-EOs

2.3.1. Plant Material and EO Extraction

Three plants were used to extract essential oils: citrus limon (*Citrus limon*), peppermint (*Mentha piperita*), and cinnamon (*Cinnamomum verum*) (Table 1). The plant pieces were taken from the local market. After being gathered, the material was allowed to dry in the shade at a temperature between 25 and 30 °C before being ground by hand. Using a Clevenger-style device, 50 g of plant material and 500 mL of water were hydro-distilled for three hours. After being dried over anhydrous sodium sulfate, the obtained oils were

filtered and kept in black vials at 4 °C until needed. The bioassay was conducted in a greenhouse environment. Tween-20 was added as an emulsifying agent to the diluted plant-EO solutions (0.002 to 0.2% v/v) obtained by diluting them with distilled water. The control solutions consisted of distilled water (DW) and a mixture of distilled water and Tween-20 (DWT), respectively. Under regulated settings, all bioassays were performed at $22 \pm 1^\circ\text{C}$, 65% relative humidity, and L16:D8 photoperiod. Stock solutions with a 40% (w/v) concentration were obtained by dissolving the evaluated EOs in ethanol.

Table 1. The lists of plant EOs for checking their antiviral activity

| No | Local name | Scientific name | Parts | Figure |
|----|------------|-------------------------|------------------|---|
| 1 | Cinnamon | <i>Cinnamomum verum</i> | Bark |  |
| 2 | Peppermint | <i>Mentha piperita</i> | Leaves and stems |  |
| 3 | Lemon | <i>Citrus limon</i> | Fruit peel |  |

2.3.2. Settling Inhibition Bioassays

Using a traditional settling choice test, the settling inhibition ability of the investigated EOs towards *M. persicae* was evaluated (Gutierrez *et al.*, 1997). Squash seedlings' trimmed leaves were air-dried for 30 minutes at room temperature after being dipped for 10 seconds in either a control solution or an essential oil solution (0.2%). To prevent drying out, treated and control leaves were then put in Petri dishes (20 cm in diameter) lined with moistened filter paper. The number of aphids that landed on each

leaf was counted at 1, 4, 8, and 24 hours after the apterous females (20 of them) were positioned in the middle of the dish between the two leaves. For every treatment, this experiment was repeated ten times. $\%SI = [1 - (\%T/\%C)] \times 100$ was the formula used to determine the settling inhibition index (%SI), where %T and %C represent the percentage of aphids that settled on the treated or control leaf, respectively (Gutierrez *et al.*, 1997).

2.3.3. The biological tests for anti-viral

After the insect vector and squash plant were sprayed with active plant-EOs using the Direct Spray Method a modified method of Singh *et al.* (2014) differences in their durability periods were observed. Enzyme-linked immunosorbent Assay (ELISA) was used to gather and detect all tested treatments. The resulting data were then examined and contrasted with the control treatments.

2.3.4. The inhibitory efficiency of treated plants

Samples of diseased squash plants were gathered and treated with distilled water to prepare for cleaning. The squash plant samples were then air-dried for 1 hour before being sprayed with 50 mL of various plant-EO dilutions. After the drying phase, the non-viruliferous aphid samples (N=10) were released to feed on the sprayed squash plants (10/plant) for one day of AFP after being fasted for three hours of food. Next, the vectors were collected and detected by ELISA after being transplanted and raised on virus-free squash plant seedlings for 7 days to allow the LP and IP in the vectors, which operate as active viral transmitters.

2.3.5. The inhibitory efficiency of treated- *M. persicae* vector

The viruliferous populations of *M. persicae* (N=10) were gathered following a 5 mL spray of different plant-EO dilutions. After starving for three hours, the sprayed vector samples were then released and fed on the virus-free squash plant seedling for one day of the inoculation access period (IAP). After the inoculated squash plant samples were moved into the observation cage in a greenhouse for 14 days to allow the latent period (LP) and incubation period (IP) in the vectors, they were then collected and detected by ELISA.

2.3.6. The viral transmission inhibition rates

Plant-EOs' capacity to shield squash plants and vectors from ZYMV infection was assessed using the viral transmission inhibition rate (%), which was computed using the following formula: The formula for calculating the viral

transmission inhibition rate (%) is $[(C - T)/C] \times 100$, where C and T represent the number of control and treated samples, respectively.

2.4. ELISA assay

The indirect ELISA test was conducted using the methodology outlined by Koenig (1981) and refined by Fegla *et al.* (1997). Aphid or *C. pepo* samples were removed by hand-grinding them in a mortar using 0.01M pH 7.0 phosphate extraction buffer. There were both positive and negative controls. The ELISA test was performed in the biology lab of the College of Sciences at the University of Hafr Al Batin, following the manufacturer's instructions. An ELISA reader with a wavelength of 405 nm was utilized (Multiscan microplate reader model 357, Thermo Scientific). When A_{405} values were three times higher than those of the negative control, the samples were accepted as positive.

3. Results

3.1. The ZYMV-infected squash plant symptoms

After being injected by the viruliferous aphid vector, the susceptible squash variety Eskandarani began to exhibit symptoms. It displayed significant symptoms such as blistering, discoloration, yellow mosaic, deformity, and shoestri (Figure 1).

3.2. Settling Inhibition Activity

Aphid females settled on leaves treated with EOs far less frequently than on controls, according to the results of the leaf-choice bioassays (Table 2). For the whole twenty-four-hour period, the observed settling inhibitory effect persisted; however, with time, it diminished. With a range of 68.1 to 75.5%, peppermint oil had the highest settling inhibition activity of all the studied oils. Cinnamon essential oils exhibited a moderate level of activity against *M. persicae*, with specific activity values ranging from 61.2 to 67.3%. After applying lemon essential oil, the least

amount of settling inhibitory action was observed, with SI values ranging from 47.7 to 50.8%.

3.3. Effect of plant-EOs on ZYMV transmission by aphid vector

Depending on the dosage utilized, all EO treatments showed varied degrees of efficacy in the evaluation of EOs against putative ZYMV-transmission inhibitory effects. Table 3 summarizes the range of active plant-EO concentrations and shows that the inhibitory impact increased with increasing concentrations. According to the study's findings, all five of the tested EO concentrations inhibited ZYMV transmission from infected squash plants to non-viruliferous ZYMV status (Figure 2 and Table 2) and from viruliferous ZYMV status to virus-free

squash plants (Figure 2 and Table 2). When compared to lemon, the range of active EO concentrations for transmission inhibition was very efficient (55–65%) at 0.002 to 0.2%. The lemon was only somewhat efficient (27–35%). Conversely, when the squash plants were visually inspected, significant symptoms (malformation, blistering, and shoestri) were observed on ZYMV-infected plants that had not received EO treatment. On the other hand, squash plant seedlings injected with peppermint and cinnamon-sprayed viruliferous insects as vectors only showed vein clearing (Figure, 2A), but Figure, 2B illustrates that lemon-sprayed viruliferous aphids might produce more severe symptoms.



Figure 1. The symptoms of ZYMV-infected zucchini squash (*Cucurbita pepo* L. cv. Eskandarani) plants Notes: (A) discoloration, blistering, yellow mosaic; (B) shoestri.

Table 2. Effect of essential oils on the settling behavior of *M. persicae*.(n=10).

| EOs | Time (Hours) | Aphids/leaf Treated | Control | SI% |
|------------|--------------|---------------------|---------|------|
| Peppermint | 1 | 2.4 | 9.8 | 75.5 |
| | 4 | 3.7 | 12.7 | 70.8 |
| | 8 | 4.2 | 14.2 | 70.4 |
| | 24 | 4.5 | 14.1 | 68.1 |
| Cinnamon | 1 | 3.6 | 10.5 | 65.7 |
| | 4 | 4.3 | 12.4 | 65.3 |
| | 8 | 5.1 | 15.6 | 67.3 |
| | 24 | 5.7 | 14.7 | 61.2 |
| Lemon | 1 | 4.6 | 8.8 | 47.7 |

| | | | |
|----|-----|------|------|
| 4 | 5.8 | 11.8 | 50.8 |
| 8 | 7.3 | 14.5 | 49.6 |
| 24 | 7.6 | 14.8 | 48.6 |

Numbers represent the mean number of aphids that settled on the treated and control leaves; EOs- essential oils used in (0.2%) as a concentration; SI—settling inhibition activity in percentage.

Table 3. The summary of the ZYMV-transmission inhibitory effect of the EOs.

| EOs | Transmission from Infected Squash to Non-viruliferous Aphids | | Transmission from Viruliferous Aphids to Viral-free Squash Plants | |
|------------|--|----------------|---|----------------|
| | Active Concentration | Inhibition (%) | Active Concentration | Inhibition (%) |
| Peppermint | 0.002 | 55-65 | 0.002 | 71-74 |
| Cinnamon | 0.002 | 48-61 | 0.002 | 65-74 |
| Lemon | 0.02 | 27-35 | 0.02 | 42-58 |
| Control 1 | - | 0.0 | - | 0.0 |
| Control 2 | - | 0.0 | - | 0.0 |

Control 1: Distilled water; Control 2: Distilled water + Tween-20.

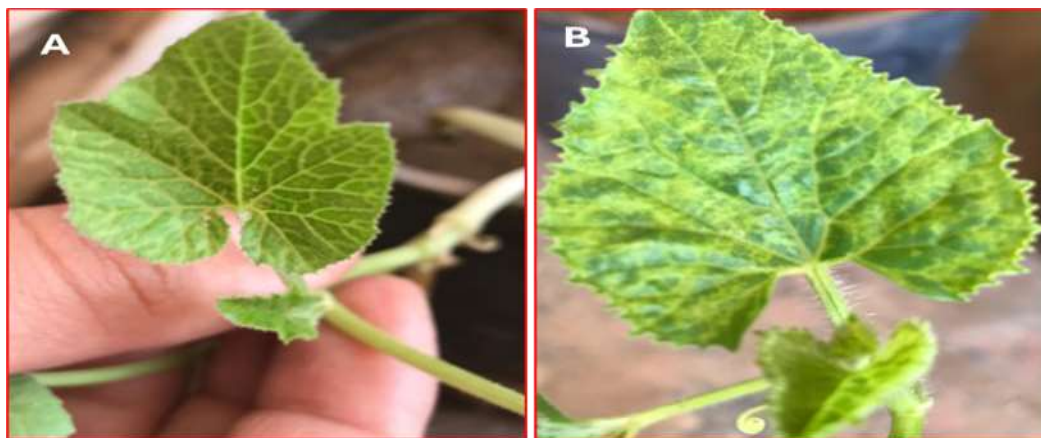


Figure 2. Visual examination of viruliferous-inoculated squash plants: Aphids treated with peppermint and cinnamon oils exhibit (A) vein-clearing symptoms, whereas those treated with lemon oil exhibit (B) yellow mosaic symptoms.

4. Discussion

Early-stage infected cucurbit crops suffer greatly from ZYMV. According to research by Blua and Perring (1989), a ZYMV infection at an early stage can reduce marketable cantaloupe by up to 94%. When zucchini squash was mechanically infected with a severe strain at varying dates following the seedling stage, a similar impact was seen; the earlier the inoculation, the fewer fruits overall per plant (Walkey *et al.*, 1992). In greenhouse-grown cucumbers inoculated with ZYMV, quantitative losses varied from 64 to

85%, and 95 percent of the infected fruits were unmarketable (Al-Shahwan *et al.*, 1995).

Furthermore, Mahmood *et al.* (2016) found that certain insecticide classes were hazardous to humans and could have long-term negative impacts as well as build up in the environment. As excellent, safe, and environmentally friendly substitutes for the eco-friendly management method, active plant-EOs were crucial. According to Svoboda and Greenaway (2003), essential oils (EOs) from over 17,500 plant species have the potential to be highly beneficial in preventing virus transmission via insect

vectors, particularly those that pierce and suck mouthparts. Complex combinations of organic chemicals, such as phenols, aldehydes, ketones, terpenoids, and esters, make up essential oils. Typically, these mixes have two or three primary components in rather high concentrations, with trace levels of minor components (Sadgrove *et al.*, 2022).

Aphid settlement and feeding numbers on a certain substrate are reliable predictors of that substrate's suitability (Dancewicz *et al.*, 2020). To ascertain the anti-settling action of the examined essential oils, a traditional settling choice test was conducted. The results obtained showed unequivocally that the green peach aphid was prevented from settling on the treated leaves by the essential oils derived from peppermint, cinnamon, and lemon. According to earlier research, aphids' ability to repel, discourage feeding, and stimulate movement is largely responsible for the settling inhibition of essential oils towards them (Hori, 1999). Furthermore, essential oil components might obscure the scents of the host plant, making it more challenging for insects to find a good host (Nottingham *et al.*, 1991). These effects lengthen the time required to locate suitable food sources, and particularly in outdoor settings, they may cause the insects to be exposed to biotic and abiotic environmental variables for substantially longer, increasing the insects' mortality (Hummelbrunner and Isman, 2001). The essential oils under investigation exhibited varying degrees of antisetling activity about *M. persicae*. The peppermint essential oil was the most active, followed by the oils from cinnamon and lemon. These plants' essential oils have previously been demonstrated to have biological activity against a variety of insect pests, including aphids. According to Belghasem and Araj (2017), peppermint essential oil is a potent aphid repellent against *M. persicae*.

In addition to depriving plants of vital nutrients, aphids are also carriers of many plant viruses. The acquisition and inoculation of different

virus types are exclusively linked to different stages of aphid feeding and probing. Aphids can spread non-permanent and semi-persistent viruses during short intracellular probes of epidermal and mesophyll cells; on the other hand, persistent viruses can spread when aphid stylets get to sieve elements (Moreno *et al.*, 2012; Jacobson and Kennedy, 2014). Therefore, reducing or eliminating aphids' ability to pierce plant tissues could lessen the spread of the virus. According to Gilling *et al.* (2014), active-EO inhibited the following mechanisms: (1) viral capsid or spike degradation, which reduced the activities of the virus infecting host cells; (2) viral nucleic acid denaturation, as reported by Helal (2019); (3) uncoating of viral particles by interfering with endosome-lysosome fusion, which resulted in non-specific binding to host cells (indiscriminate) and cell activity inhibition, as reported by Abonyi *et al.* (2009); and (4) blocking viral replication, entry into host cells, and cell-to-cell movement.

To consider bio-active compounds in plants as a promising agent for applying and creating new commercial anti-viral agents and bioinsecticides, which expanded the range of alternative methods to increase and promote the development of sustainable crop production systems, it was crucial to verify the transmission inhibition effects of plant viruses by active plant-EO. They safeguarded the environment, raised crop yields, and raised consumer and producer quality of life. The findings, however, pointed in several directions for future research, with an emphasis on the mechanisms by which plant-EOs from infected hosts are transmitted to understand the role that these compounds play in integrated pest management programs and the prevention of viral plant diseases in Thailand and around the world.

Overall, our research shows that the investigated essential oils (EOs) from the peppermint, cinnamon, and lemon plants have feeding deterrent and anti-settling properties against the green peach aphid. The most potent of them was

the peppermint essential oil, which was followed by oils from cinnamon and lemon.

5. Conclusion

According to our research, peppermint, cinnamon, and lemon plant-based essential oils exhibited potent antiviral properties that prevented ZYMV from spreading and killed aphids at every stage of their development. The outcomes showed that both plant-EOs might interfere with host infection and were possible candidates for additional research and development of antiviral drugs.

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All authors are contributed in this research

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest.

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