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Investigation of antiphytoviral effects of thymol, eugenol, and 1,8-cineol essential oils on cucumber mosaic virus in Turkey

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Abstract

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The exploration of natural antiviral agents, such as essential oils, is a critical area of research, offering promising alternatives for sustainable plant disease management and contributing to global agricultural resilience. In this regard, the present study investigated the antiviral efficacy of thymol, eugenol, and 1,8-cineol essential oils (EOs) against cucumber mosaic virus (CMV) *in vivo*. It focused on assessing the capability of these EOs, particularly at a concentration of 500 ppm, in both prophylactic and therapeutic contexts for plant health, particularly when applied prior to or concurrently with viral infection. The evaluation involved measuring a range of parameters, including local lesions of CMV on cucumber plants, symptom index, leaf width, plant height, and virus accumulation. Our findings demonstrated that thymol was significantly effective in mitigating CMV infection. Both eugenol and 1,8-cineole oils also showed efficacy in reducing local lesions when applied to host plants alongside the infecting virus. Based on our results, the essential oils exhibited substantial protective and curative effects against CMV, with the preventative (pre-inoculation) application proving more effective. In conclusion, this study highlights the potent antiviral activity of thymol, eugenol, and 1,8-cineole EOs against CMV, presenting them as viable alternative strategies for combating viral diseases in plants. In addition, our findings not only advance our understanding of plant-virus interactions, but also pave the way for more eco-friendly and effective agricultural practices, crucial in the face of escalating plant disease challenges worldwide.

Keywords: cucumber mosaic virus; antiphytoviral effect; plant virus; essential oils

Introduction

Viral infections in plants result in significant damage to a variety of vegetables and fruits globally, as highlighted by Lecoq and Katis (2014). The challenge of protecting crops from viral threats is considerable, particularly in the absence of effective direct control methods against viruses. Consequently, most strategies emphasize disease prevention, including the use of resistant plant varieties, controlling insect vectors, and employing hygienically certified reproductive materials (Paduch-Cichal et al., 2023). Although, techniques such as thermotherapy or meristem culture can eradicate plant pathogenic viruses, these methods are often costly, time-consuming, and not universally effective (Islam

et al., 2017; Taglienti et al., 2022). Therefore, research into alternative control methods for plant pathogenic viruses is of paramount importance.

Essential oils (EOs), as natural and biodegradable compounds, have emerged as a new trend in biological protection against plant pathogens (Chen et al., 2009). Their wide spectrum of biological activities, encompassing virucidal, bactericidal, insecticidal, fungicidal, and antiparasitic properties, have been recognized and utilized (Dunkic et al., 2011; Taglienti et al., 2022; Paduch-Cichal et al., 2023). Despite this, there is limited information available on the specific effects of EOs on plant viruses or viral infections (Dunkic et al., 2010; Arshad et al., 2014; Ruščić et al., 2017). Numerous studies have explored the use of essential oils in preventing crop losses due to plant diseases (Takikawa et al., 2002; Valero and Salmeron, 2003; Rao et al., 2010; Karpinski, 2020). However, research on the antiphytoviral activities of EOs, particularly against cucumber mosaic virus (CMV) and Tobacco Mosaic Virus (TMV), remains limited (Dunkic et al., 2010, 2011, 2013; Vuko et al., 2012; Ruščić et al., 2017; Taglienti et al., 2022; Paduch-Cichal et al., 2023). It is also not fully understood whether EOs directly inactivate viral particles or induce resistance/tolerance responses in host plants (Taglienti et al., 2022).

Plant pathogenic viruses are recognized globally for their extensive host range and their potential to cause significant losses in crop yields (Fauquet, 2005; Strange and Scott, 2005). The CMV, belonging to the Cucumovirus genus within the *Bromoviridae* family, exemplifies this issue with its vast host spectrum among plant viruses (Negai et al., 2011). Characterized by a positive single-strand RNA genome of approximately 18×1300 nm, CMV infects over 770 plant species, leading to considerable economic detriment across various crops. Particularly acute are CMV infections in the *Cucurbitaceae* family, including pumpkins, melons, and cucumbers. In cucumbers, a globally cultivated vegetable, CMV induces productivity losses manifesting as stunted growth, yellow mosaic patterns, and curled leaves (Helal, 2019). The virus is transmitted non-persistently by aphids of the *Aphididae* order (Francki et al., 1979; Raccah et al., 1985). On the other hand, controlling plant viral diseases presents unique challenges, distinct from those posed by other plant pathogens (Islam et al., 2017). Conventional protection methods, such as eradication and sanitation, are often employed due to the impracticality of using chemical agents, which may be phytotoxic and economically burdensome. In recent years, the focus has shifted towards environmentally friendly and biologically based control methods to mitigate the impact on human health and the environment. A promising area of research in this context is the exploration of the antiviral properties of EOs derived from various plants. These studies have demonstrated the effectiveness of EOs in combating CMV. Thymol is a monoterpenoid phenol obtained from thyme oil, and is known for its antimicrobial and antiviral properties (Kowalczyk et al., 2020). Eugenol is one of the main components of clove oil and is known for its strong antioxidant and antiviral effects (Ulanowska and Olas, 2021). 1,8-cineole (also known as eucalyptol) is a monoterpene oxide found in various aromatic plants, especially eucalyptus oil. It has been shown in various studies to have anti-inflammatory and antiviral properties (Hoch et al., 2023). These compounds have attracted more attention in recent years, because they offer alternative solutions in sustainable plant protection approaches (Karakus, 2024; Kaya et al., 2025). To date, our understanding of the antiphytoviral activity of specific EOs such as thymol, eugenol, and 1,8-cineol, particularly *in vivo* contexts and economically crucial crops like cucumber, remains limited (Dunkic et al., 2010, 2013; Bezi´c et al., 2011, 2013; Vuko et al., 2012; Petrov et al., 2016; Helal, 2019; Taglienti et al., 2022; Paduch-Cichal et al., 2023).

This study represents a pioneering *in vivo* investigation into the antiphytoviral efficacy of thymol, eugenol, and 1,8-cineol EOs on cucumber plants infected with CMV. The research aims to elucidate both the protective and curative impacts of these EOs when applied before and after CMV infection via mechanical inoculation. The findings from this comprehensive study are anticipated to significantly contribute to the formulation of effective strategies for the management of plant viral infections, thereby enriching the field of plant virology with critical insights.

Materials and Methods

Essential oils, virus and plant host

The EOs utilized in this study, namely thymol (Aldrich C121452), eugenol (Fluka 45980), and 1,8-cineole (Aldrich 183164), were sourced from Sigma-Aldrich (Shanghai, China). These EOs were meticulously stored in dark conditions at 4°C to maintain their integrity. Initially, the EOs were prepared for experimental use by dissolving them in Tween-20. This process involved blending 10% EO with 88% sterile distilled water and 2% (v/v) Tween-20, ensuring thorough mixing for complete dissolution, as suggested by Lopez-Reyes et al. (2010). Subsequently, cucumber seeds (*Cucumis sativus* L. Beith alpha variety) were subjected to a sterilization process using a 5% sodium hypochlorite solution for 10 minutes, followed by rinsing with sterile distilled water. The sterilized seeds were then cultivated in plastic pots filled with fresh compost. The growth conditions in the greenhouse were care-

fully controlled at 23°C with a 16h/8h light/dark photoperiod, and the plants were watered as required. For the experimental phase, cucumber plants of the Beith Alpha cultivar, specifically those possessing five true leaves approximately four weeks after sowing, were selected for mechanical inoculation using phosphate buffer solution. Mechanical inoculation studies were conducted using tomato leaves previously identified as infected with CMV through a field survey. The presence of CMV was confirmed by the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). For inoculation, the CMV Cimenli 3-162 isolate, originally obtained from Hakkari, was selected. The viral inoculum was prepared from CMV-infected tomato leaves following the protocol described by Yardımcı and Eryiğit (2006).

Mechanical inoculation of CMV

The procedure for infecting cucumber plants with CMV inocula began by blending CMV-infected leaves in a 0.01 M phosphate buffer (pH 7.2) at a chilled temperature of +4°C. After homogenization, a small amount of β-mercaptoethanol (1-2 drops) was added to the mixture. Next, the cotyledon leaves of the cucumber plants were prepared for inoculation. This procedure involved lightly coating the leaves with carborundum powder (400-500 mesh size) to create micro-abrasions. The prepared inoculum mixture was then applied to these cotyledon leaves. This was done by gently rubbing the virus-laden homogenate onto the surface of the leaves using a gloved finger dusted with carborundum powder, which mildly abrades the cuticle and facilitates virus entry. The leaves were then left undisturbed for five minutes to ensure adequate viral adsorption, after which excess inoculum was carefully rinsed off with distilled water. This approach follows standard procedures for mechanical inoculation, as described in previous studies. The inoculated cucumber plants were then utilized for further mechanical inoculation studies.

Antiviral effects of the EOs on the cucumber plants

The antiviral efficacy of EOs *in vivo* was evaluated through parameters such as the formation of local lesions, and the measurement of plant height and leaf width. For lesion induction, primary leaves were abraded with 500 mesh carborundum. Based on preliminary findings, a 10% stock solution of EOs was prepared, from which a concentration of 500 ppm was extracted for experimentation. Solutions comprising 500 ppm EOs were then mechanically inoculated with an equal volume of virus inoculum at a concentration of 20 g/mL, and subsequently applied to cucumber plants.

The experimental design included several control groups: Control group 1 comprised healthy plants without any treatment; Control group 2 included plants inoculated solely with the CMV Cimenli 3-162 isolate; and Control group 3 consisted of plants treated with both EOs and CMV. A total of 20 cucumber plants were used in the experiment and distributed equally among the three treatment groups, each with three leaves, which were cultivated in a greenhouse under controlled conditions (23°C, 16:8 h light/dark cycle). The occurrence of local lesions caused by CMV on inoculated cucumber leaves was monitored for ten days post-inoculation. The percentage inhibition of local lesions was calculated using the formula:

$$IP\ [\%] = [(CK - A)/CK] \times 100$$

Where: IP represents the inhibition of local lesions (%), CK the average number of local viral lesions in control group 3, and A the average number of local viral lesions in control group 2.

Plant height and leaf width were measured using a measuring tape at predetermined intervals (0, 10, 20, 30, and 40 days), and average values were calculated. The study aimed to investigate both the protective and curative effects of EOs against CMV infection, following methodologies established by Helal (2019) and Chen et al. (2009).

For assessing the protective effect, cucumber leaves were first treated with 500 ppm EO solutions, followed by mechanical inoculation with CMV after 24 hours. Conversely, for evaluating the curative effect, leaves were first mechanically inoculated with CMV Cimenli 3-162 isolate, and 500 ppm EO solutions were applied 24 hours post-inoculation.

The experimental groups were organized as follows:

Control (C): Leaves received no treatment.

Essential Oil (EO): Leaves were treated exclusively with essential oil.

CMV (CMV): Leaves were inoculated only with the virus.

Essential Oil + CMV (EO+CMV): Leaves were first treated with essential oil, followed by virus inoculation after one day.

Thymol + CMV: Leaves were treated with Thymol (500 ppm), followed by CMV inoculation.

Eugenol + CMV: Leaves were treated with Eugenol (500 ppm), followed by CMV inoculation.

1,8-cineol + CMV: Leaves were treated with 1,8-cineol (500 ppm), followed by CMV inoculation.

CMV + Essential Oil (CMV+EO): Simultaneous application of inoculum and essential oil to the leaves.

CMV + Thymol: Leaves were first inoculated with CMV inocula, followed by Thymol treatment (500 ppm).

CMV + Eugenol: Leaves were first inoculated with CMV inocula, followed by Eugenol treatment (500 ppm).

CMV + 1,8-cineol: Leaves were first inoculated with CMV inocula, followed by 1,8-cineol treatment (500 ppm).

The DAS-ELISA test

The DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay) test was conducted following the methodology outlined by Clark and Adams (1977), in accordance with the standard procedure provided by the antibody manufacturer, Bioreba AG, Switzerland. Absorbance readings of the samples were obtained at wave length (λ) 405 nm using a VersaMax microplate reader. For the determination of CMV presence, samples exhibiting absorbance values exceeding twice the mean absorbance of the negative controls were classified as positive.

Data analysis

The results presented in this study were obtained from three samples for each treatment application, with each sample replicated thrice. Statistical analysis of the data was conducted using a one-way analysis of variance (one-way ANOVA) to compare the effects of different treatments. Post-hoc comparisons were made using the Tukey Test, with a significance threshold set at $P<0.05$. All statistical analyses were performed using the SPSS (version 20.0) software package.

Results

This research focused on evaluating the antiphytoviral properties of thymol, eugenol, and 1,8-cineol EOs in combating CMV infectivity in cucumber plants *in vivo*. The findings, as summarized in Table 1, indicate a significant impact of EOs on the virus's infectivity. Systemic symptoms of CMV were apparent on the tenth-day post-inoculation in plants exposed solely to the virus, while control plants exhibited no symptoms. The application of EOs resulted in a delay of systemic symptom onset by approximately two to three days, as shown in Table 1. A comparative analysis of lesion numbers on cucumber plants treated with EOs and control plants revealed that pre-inoculation treatment with EOs generally led to a reduction in lesion count. Among the tested EOs, thymol was the most effective, reducing the number of local lesions by 46.18-44.79% in both pre-inoculation and simultaneous application scenarios. Eugenol and 1,8-cineole also demonstrated effectiveness in reducing local lesions when applied concurrently with the virus, with respective activity rates of 39.21% and 37.26%, as detailed in Table 1. No significant distinction was observed between the antiviral activities of Eugenol and 1,8-cineol in either pre-inoculation or simultaneous application methods against the CMV isolate. The number of local lesions on leaves of plants treated exclusively with the CMV isolate displayed significant variation when compared to those treated with EO groups.

Table 1. Antiviral effect of essential oils on CMV infectivity on the cucumber plants

* GGroups	Number of local lesions	Inhibition of local lesions (%)
Control	0.0 ± 0.00	0
*CMV	10.76±0.35[a]	0
Thymol + CMV	5.79±0.20[b]	46.18
Eugenol + CMV	6.62±0.46[b]	38.47
1,8-cineol + CMV	6.74±0.50[b]	37.36
CMV + Thymol	5.94±0.05[b]	44.79
CMV + Eugenol	6.54±0.36[b]	39.21
CMV +1,8-cineol	6.75±0.43[b]	37.26

*Differences between solutions $p<0.05$ are significant. Angle transpiration was applied to the values.
Control (C): Leaves received no treatment.
CMV (CMV): Leaves were inoculated only with the virus.
Thymol + CMV: Leaves were treated with Thymol (500 ppm), followed by CMV inoculation.
Eugenol + CMV: Leaves were treated with Eugenol (500 ppm), followed by CMV inoculation.
1,8-cineol + CMV: Leaves were treated with 1,8-cineol (500 ppm), followed by CMV inoculation.
CMV + Essential Oil (CMV+EO): Simultaneous application of inoculum and essential oil to the leaves.
CMV + Thymol: Leaves were first inoculated with CMV inocula , followed by Thymol treatment (500 ppm).
CMV + Eugenol: Leaves were first inoculated with CMV inocula , followed by Eugenol treatment (500 ppm).
CMV + 1,8-cineol: Leaves were first inoculated with CMV inocula , followed by 1,8-cineol treatment (500 ppm).
Source: Authors' own elaboration

The impact of treatments, either simultaneous or before CMV inoculation, on leaf width and plant height in cucumber plants is detailed in Tables 2 and 3. Application of EOs alone, though not significantly altering plant height and leaf width compared to control plants, demonstrated a positive influence on plant growth. For instance, leaf width measured on the 40th day was 7.13 cm for the control, 6.40 cm for thymol-treated, 5.76 cm for eugenol-treated, and 5.83 cm for 1,8-cineol-treated plants. Plant heights were recorded as 15.30 cm for thymol, 15.36 cm for eugenol, and 14.80 cm for 1,8-cineol treatments, respectively. Statistical significance was observed in the results impacting plant height and leaf width when EOs were applied prior to mechanical inoculation with CMV ($p<0.05$). Applying EOs post-CMV inoculation also positively influenced plant growth compared to the group treated only with CMV. In this treatment, 500 ppm EO solutions were applied to the leaves 24 hours after mechanical inoculation with the CMV Cimenli 3-162 isolate, to assess the potential curative effect of the EOs. However, pre-inoculation EO application proved more effective than post-inoculation treatment, as indicated in Ta-

bles 2 and 3. Particularly notable was the efficacy of thymol, which emerged as the most potent essential oil against CMV, whether applied before or after inoculation.

Table 4 illustrates the results of the DAS-ELISA test, which measures absorbance at 405 nm, for cucumber plants treated with various EO applications in conjunction with the CMV isolate. The test aimed to quantify the viral load in the plant samples based on their absorbance levels. The average optical density (OD) at 405 nm in the group inoculated solely with CMV was observed to be higher than that in the other experimental groups. Across the experimental duration (from day 0 to day 40), a general decrease in absorbance values was noted in the experimental groups treated with EOs. This trend suggested a reduction in the viral titer due to the EO treatments. In contrast, the negative control group displayed an increase in absorbance values over time. The application of EOs in conjunction with the CMV isolate resulted in reduced mean absorbance (OD405 nm) compared to the CMV group, in which leaves were inoculated only with the virus. There was no marked difference in the absorbance values when comparing the different EOs tested against each other. The results also note no significant difference in absorbance values between the groups where EOs were applied to cucumber leaves either before or simultaneously with CMV inoculation.

Discussion

The application of EOs as agents for controlling plant viral diseases has garnered significant attention in contemporary phytopathological research. The robust biological activities exhibited by EOs, as described by Wink (2003), have been demonstrated to be of substantial importance in diverse scientific domains. Notably, previous research has provided evidence of the antiphytoviral properties of EOs extracted from various plant species. Nevertheless, the specific antiphytoviral activity of thymol is not extensively documented in the literature, with only a handful of studies touching upon this subject (Dunkic et al., 2010; Helal, 2019; Paduch-Cichal et al., 2023). To our knowledge, the antiphytoviral activities

Table 2. Effects of EOs and different application times on leaf width (cm)

Groups	0. Day	10. Day	20. Day	30. Day	40. Day
Control	5.70±0.10^{a}	6.26±0.15^{a}	6.46±0.15^{a}	6.76±0.05^{a}	7.13±0.15^{a}
CMV	5.73±0.05^{a}	5.66±0.05^{b}	5.20±0.20^{c}	4.73±0.11^{e}	4.56±0.05^{f}
Thymol	4.83±0.05^{bc}	5.20±0.10^{d}	5.46±0.05^{bc}	5.83±0.11^{b}	6.40±0.09^{b}
Eugenol	4.76±0.05^{bcd}	5.46±0.05^{bc}	5.63±0.05^{b}	5.36±0.35^{cd}	5.76±0.05^{cd}
1,8-cineol	4.43±0.37^{d}	5.10±0.09^{de}	5.40±0.11^{bc}	5.56±0.05^{bc}	5.83±0.05^{c}
Thymol + CMV	5.06±0.05^{b}	5.26±0.05^{cd}	5.40±0.09^{bc}	5.46±0.05^{bc}	5.63±0.05^{cd}
Eugenol + CMV	4.70±0.09^{bcd}	4.80±0.09^{f}	5.16±0.05^{cd}	5.60±0.09^{bc}	5.73±0.05^{cd}
1,8-cineol + CMV	4.66±0.05^{cd}	5.10±0.09^{de}	5.40±0.09^{bc}	5.56±0.05^{bc}	5.60±0.00^{d}
CMV + Thymol	5.06±0.05^{b}	5.20±0.10^{d}	5.23±0.15^{c}	5.03±0.15de	4.86±0.05^{e}
CMV + Eugenol	4.76±0.05^{bcd}	4.93±0.05^{ef}	4.73±0.05^{e}	4.63±0.05^{e}	4.63±0.05^{f}
CMV +1,8-cineol	4.73±0.05^{bcd}	4.86±0.05^{ef}	4.80±0.09^{de}	4.70±0.09^{e}	4.60±0.09^{f}

Source: Authors' own elaboration

Table 3. Effects of EOs and different application times on plant height (cm)

Groups	0. Day	10. Day	20. Day	30. Day	40. Day
Control	9.63±0.15^{cd}	11.43±0.32^{b}	13.60±0.20^{c}	14.63±0.15^{a}	15.43±0.30^{e}
CMV	9.70±0.10^{d}	11.60±0.17^{b}	12.80±0.09^{b}	13.60±0.10^{a}	14.90±0.51^{cde}
Thymol	9.73±0.15^{d}	12.26±0.15^{c}	13.40±0.26^{c}	14.30±0.20^{a}	15.30±0.10^{de}
Eugenol	9.40±0.09^{bc}	12.16±0.05^{c}	13.43±0.05^{c}	14.50±0.10^{a}	15.36±0.15^{e}
1,8-cineol	8.80±0.09^{a}	10.73±0.11^{a}	12.33±0.05^{a}	13.76±0.05^{a}	14.80±0.09^{bcd}
Thymol + CMV	9.46±0.05^{bcd}	11.60±0.09^{b}	12.76±0.05^{b}	13.80±0.10^{a}	14.86±0.05^{cde}
Eugenol + CMV	9.66±0.05^{cd}	11.30±0.10^{b}	12.56±0.05^{ab}	13.60±0.10^{a}	14.56±0.05^{abc}
1,8-cineol + CMV	9.30±0.09^{b}	10.83±0.05^{a}	12.40±0.09^{a}	13.40±0.10^{a}	14.20±0.09^{a}
CMV + Thymol	9.56±0.05^{bcd}	10.80±0.09^{a}	12.50±0.10^{ab}	13.30±0.10^{a}	14.60±0.09^{abc}
CMV + Eugenol	9.53±0.05^{bcd}	11.53±0.05^{b}	12.66±0.05^{ab}	13.05±0.10^{a}	14.60±0.09^{abc}
CMV +1,8-cineol	9.46±0.15^{bcd}	11.53±0.30^{b}	12.46±0.15^{ab}	13.50±0.10^{a}	14.30±0.17^{ab}

Source: Authors' own elaboration

Table 4. DAS-ELISA results for plants inoculated with EO treatments with CMV isolate*

Groups	0. Day	10. Day	20. Day	30. Day	40. Day
CMV	0.3022	0.4325	0.3844	0.3477	0.3029
Thymol + CMV	0.2085	0.2987	0.2458	0.2122	0.2074
1,8-cineol +CMV	0.1796	0.2487	0.2141	0.2139	0.1896
Eugenol + CMV	0.2270	0.2078	0.2607	0.2466	0.2181
CMV + Thymol	0.2156	0.2673	0.2087	0.1960	0.1902
CMV +1,8-cineol	0.2159	0.1992	0.2315	0.2048	0.2067
CMV + Eugenol	0.2194	0.2377	0.2102	0.2018	0.1841

*Negative control absorbance value was evaluated as 0.1366-0.1149, and values greater than 0.24 were considered positive (Aktaş and Akdura, 2023). (Absorbance = 405 nm)

Source: Authors' own elaboration

of eugenol and 1,8-cineol have not been previously reported, which underscores the innovative aspect of our research. Our study extends the existing body of knowledge by revealing that thymol, eugenol, and 1,8-cineol possess potent antifungal and antibacterial activities, suggesting their potential efficacy against a variety of fungal and bacterial pathogens (Batish et al., 2008; Pitarokili et al., 2003; Karpinski, 2020; Salehi et al., 2018). This broad-spectrum bioactivity prompted an examination of their effects on CMV, a virus with the most extensive host range among plant viruses (Negai et al., 2011), thereby accentuating the need for effective antiphytoviral agents. In addition, our study aimed to was to ascertain the antiphytoviral activity of pure EOs against CMV, contributing novel insights into the previously unexplored antiphytoviral potential of thymol, eugenol, and 1,8-cineol. Our results indicated that the EOs in question inhibited CMV, aligning with previous reports that EOs extracted from plants such as *Satureja montana* L. ssp. variegate, *Teucrium arduini* L., *Micromeria croatica* L., *Eryngium amethystinum* L., *E. alpinum* L., *Micromeria fruticulosa* L., and *Greek oregano* L. are effective against TMV and CMV (Dunkic et al., 2010, 2011, 2013; Ruščić et al., 2017; Vuko et al., 2012; Paduch-Cichal et al., 2023). On the other hand, through a comprehensive examination of parameters such as local lesions, symptom index, leaf width, plant height, and virus accumulation in cucumber plants, our study demonstrated that the protective application of EOs before mechanical inoculation was more efficacious than the curative application at the time of inoculation. Thymol, in particular, exhibited the most substantial inhibitory effect on CMV infection, followed by eugenol and 1,8-cineol, which also contributed to a reduction in the number of local lesions (Paduch-Cichal et al., 2023). These findings resonate with Helal (2019), who reported a 91.5% inhibition rate and maximum protection against CMV in cucumber plants with the pre-application of thyme EO. Given that the antiphytoviral activities of eugenol and 1,8-cineole have not been previously examined, our results present a pioneering contribution to the field. The delay in systemic symptoms and the observed positive effects of EO applications on leaf width and plant height (Othman and Shoman, 2004; Helal, 2019) suggest the potential of EOs as both protective and curative agents against CMV. The antiphytoviral activity of EOs may be achieved through direct or indirect inhibition of virus replication or induction of systemic resistance in the host plant, a hypothesis that aligns with the findings of Othman and Shoman (2004), Helal (2019), and Paduch-Cichal et al. (2023).

Our results expand upon the scope of existing literature concerning the application of EOs as antiphytoviral agents by examining not just the EOs in their entirety, but specifically isolating and testing the individual components known for their potent biological activity. Prior research has primarily focused on the antiphytoviral effects of whole essential oils extracted from various plants against CMV (Dunkic et al., 2010, 2011, 2013; Vuko et al., 2012; Ruščić et al., 2017; Paduch-Cichal et al., 2023). Our study innovates in this area by specifically targeting the individual constituents-thymol, eugenol, and 1,8-cineol, known for their high biological activity, thus pioneering the investigation into their direct antiphytoviral properties. In addition, the findings of our study are significant as they elucidate, for the first time, the antiphytoviral potential of these selected EO components against CMV. The demonstration of delayed systemic symptoms, the quantifiable reduction in the formation of local lesions, and the observable promotion of plant growth in infected cucumber plants suggest a robust inhibitory effect of these EO components on the virus. Such effects signify a breakthrough in the understanding of EO component-specific activities and provide a foundation for the development of targeted antiphytoviral strategies in the agricultural sector. Moreover, our research contributes to a deeper comprehension of the mechanism, by which these EOs exert their antiviral effects. The notable delay in symptom onset and mitigation of lesion development could indicate an interference with the virus repli-

cation cycle or an enhancement of the plant's innate defense mechanisms, a phenomenon that warrants further molecular investigation. The observed positive effects on plant growth parameters, such as leaf width and height, in the presence of these EO components, further the argument for their use not only as antiviral agents, but also as potential growth-promoting substances. This dual functionality emphasizes the versatility and utility of EO components in integrated pest and disease management programs, which aim to reduce reliance on synthetic chemicals.

Conclusions

The current investigation offers initial evidence suggesting that the EOs thymol, eugenol, and 1,8-cineol may provide a viable protective strategy against CMV in cucumbers. This research substantiates the potential application of these EO constituents as antiphytoviral agents. A statistically significant reduction in the number of local lesions was observed in plants treated with these oils, indicating their efficacy in managing viral infections. Upon analysis, thymol emerged as the superior EO in mitigating CMV infection, surpassing the other tested EOs in effectiveness. Notably, the application of EOs prior to the inoculation with the virus yielded more favorable outcomes compared to their simultaneous application with the virus. This study, to the best of our knowledge, represents the inaugural foray into the utilization of EO components for plant viral disease management in cucumbers within Turkey, laying the groundwork for the development of innovative plant protection methodologies. The implications of these findings are profound, as they not only establish a precedent for the use of EOs in the fight against local and systemic plant viral diseases, but also signal the inception of this line of inquiry in the region. Future research endeavors, encompassing comprehensive molecular studies and expansive field trials, are imperative to ascertain the efficacy and feasibility of integrating these EO components into established crop protection protocols on a larger scale.

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Author contributions

S.K., N.A. and H.C-K. were accountable for conducting the research and gathering data. S.K. oversaw, interpreting the results, analyzing the data, and authoring the manuscript. All authors have reviewed and consented to the final version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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