

ORIGINAL RESEARCH

Repellency, contact toxicity, and anti-oviposition effects of three ethanol-extracted plant essential oils on *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Edited by D. Rothenberg

Abstract

There are more than 1200 species of whiteflies found across the globe. Due to the high level of resistance of whitefly against synthetic insecticides, alternate pest management measures have their significance. Plant essential oils (EOs) affect insect pests in many ways, such as via stimulatory, deterrent, toxic, and hormonal effects. This study was designed to determine the repellency of EOs, toxicity, and oviposition deterrent activities of *Allium ascalonicum*, *Cinnamomum camphora*, and *Mentha haplocalyx* against adult whiteflies. In repellency determination experiments, a single tomato plant was treated with 10 ml of ethanol-extracted EO with 1000 ppm concentration. Results showed that *C. camphora* EO was the most repellent for whitefly compared to *M. haplocalyx* and *A. ascalonicum*. The oviposition deterrent experiments revealed that *C. camphora* has the highest oviposition deterrent effect, followed by *M. haplocalyx* and *A. ascalonicum*. A single plant treatment method was used to assess the contact toxicity of three EOs against whitefly after 12, 24, 48, and 72 h of exposure. The results revealed that *C. camphora* is more toxic to whitefly than *M. haplocalyx* and *A. ascalonicum*. After determining the antagonistic effects of these EOs, the oils were analyzed using gas chromatography/mass spectrometry to identify the chemical components. It can be concluded that *C. camphora* is the most effective oil EO in terms of toxicity, repellence, and oviposition deterrence, followed by *M. haplocalyx* and *A. ascalonicum* under greenhouse conditions. Our results introduce some new eco-friendly plant EOs to control whiteflies.

1 | INTRODUCTION

Whitefly *Bemisia tabaci* Gennadius (1889) (Aleyrodidae: Hemiptera) is a small, 1–3 mm, sap-sucking pest. Whiteflies comprise more than 1200 species and can be found worldwide; they are pests causing tremendous economic damage to horticulture and field crops (Barro et al., 1998; Carlos & Kevin, 2016). The insect feeding habits damage plants directly, resulting in the destruction and spotting of fruit and vegetative parts and irregular ripening of fruits. The adults secrete honeydew during feeding, which encourages the production of sooty

mold growth, and restricts light absorption during photosynthesis (Anderson & Morales, 2005; Lapidot & Polston, 2006; Pappu et al., 2009). Indirectly, adults are responsible for transmitting around 150 plant viruses (Khan, Fan, et al., 2021; Lapidot & Polston, 2006; Polston & Anderson, 1997).

Once the whitefly population breaks out, dealing with them becomes challenging. Synthetic insecticides have been used to control this harmful pest. However, the excessive and unnecessary use of these poisons has led to several problems, including direct toxicity to users, adverse effects on non-target organisms, the resistance of

targeted species to pesticides, air and water pollution, and increasing expenses of application (Khan, Hafeez, et al., 2021; Okonkwo & Okoye, 1996; Shah et al., 2008). Using awareness about the harmful effects of chemical pesticides, scientists are now focusing on new, globally safe methods. Therefore, the approaches to handling whiteflies are rapidly evolving. Many aromatic plant essential oils have been introduced for their contact and fumigant toxicity, repellency, pathogenic properties, and growth regulation (Khan et al., 2020; Murray, 2000; Wagan, He, et al., 2016). In addition, they are responsible for protecting plants from many endogenous and exogenous infestations and infections. Essential oils are also responsible for defense against insect pests, herbivores, and microorganisms, and some are active in attracting pollinators (Bakkali et al., 2008; Pichersky & Gershenzon, 2002). The use of essential oils is as old as insect pest problems identified in the field and stored grains (Koul et al., 2008).

The use of essential oils from *Allium ascalonicum*, *Cinnamomum camphora*, and *Mentha haplocalyx* to kill, repel and deter oviposition of insects in greenhouses has not been well studied. Therefore, our study aimed to investigate repellency, toxicity, and oviposition deterrence activities against whitefly adults and to identify chemical components of *A. ascalonicum*, *C. camphora*, and *M. haplocalyx* essential oils through gas chromatography/mass spectrometry (GC-MS). We anticipated that our results would be helpful in the development of methods to control greenhouse whiteflies and promote a new eco-friendly whitefly management method.

2 | MATERIALS AND METHODS

2.1 | Insect rearing and plant material

A colony of whiteflies was reared on alternative hosts of sweet potato, cotton and tomato plants for 1 year without any application of essential oil or chemical pesticides at a greenhouse in the Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory at Huazhong Agricultural University. The environment in the greenhouse was $28 \pm 5^\circ\text{C}$ and $60 \pm 5\%$ RH.

The tomato and cottonseeds and sweet potato cuttings were sown/planted in round plastic pots 15 cm in diameter by 13 cm in height, filled with 1.5 kg of equal portions of organic and ground soil. After 40 days, the potted plants were used for rearing the whitefly. Only tomato plants were used for the greenhouse experiments.

2.2 | Essential oils preparation

Small leaves with inflorescence of *C. camphora*, the foliage of *M. haplocalyx* and whole plants of *A. ascalonicum* were purchased from a seed shop registered with Beijing Tongrentang Group, China. The method of extraction of essential oil was identical as mentioned by Su et al. (2009). The purchased materials were cleaned and then dried in an oven for 2–3 days. All the materials were ground, and the powder was sieved using 40 mm mesh. The powder was mixed with 95%

ethanol at a ratio of 5:1 (5 ml of ethanol with 1 g of powder) in a brown bottle and placed in a dark room at $20\text{--}25^\circ\text{C}$ for 7 days. The bottles were shaken twice a day for proper mixing. The solvent extract was filtered through a filter paper into a conical flask, and the residue was added with 2.5 ml ethanol per gram of powder and placed, shaken and filtered under the same condition as before. The first and second filtrates were combined in a flask and dried in a rotary evaporator. When the extracts were fully dried, samples were collected and weighed. The original 140.11 g of *A. ascalonicum* powder gave 7.44 g extract. Similarly, 25.21 g resulted from the original 155.85 g of *C. camphora* powder and 41.24 g from the original 561.00 g of *M. haplocalyx* powder.

The crude oils (0.05 g) were dissolved in 0.3 ml dimethyl sulfoxide (DMSO) for dissolution and preparing the test solution, and 1% of Tween-20 was added. To make the final concentration of 10 mg/ml (10,000 ppm), double-distilled water was added until the volume reached 5 ml. To make concentrations of 5 mg/ml (5000 ppm) and 2.5 mg/ml (2500 ppm), we used 1 ml of the 10 mg/ml prepared solution and added it to 1 and 3 ml of double-distilled water, respectively. For the control solution, 0.3 ml of DMSO, 1% Tween-20, and double-distilled water were added to achieve the volume of 5 ml. This concentration was used as the working solution in the bioassay.

2.3 | Chemical constituents identification by GC-MS

The chemical constituents of the essential oils were identified by GS-MS on a Varian 450-GC/320-MS (Varian, Inc.), according to Wagan et al. (2017). An HP-5MS capillary column (film thickness: 30 m length \times 0.25 mm inner diameter) was used, and the compounds were detected with a flame ionization detector. For GS, the injector oven temperature was initially maintained at 60°C for 3 min, ramped at $10^\circ\text{C}/\text{min}$ to 180°C and maintained for 1 min, and ramped again at $20^\circ\text{C}/\text{min}$ to 280°C and maintained for 15 min. One microliter of the samples diluted with 1% hexane was injected with a split ratio of 1:10. The column pressure was maintained at 100 kPa. Helium gas passed at a 1.0 ml/min rate and was used as the sample carrier. The MS quadrupole, ion source, and transmission line temperatures were 150, 230, and 250°C , respectively. Chemical constituents were identified from the gas chromatogram with the online libraries of Wiley, REPLIB, MANLIB, and PMWTox3N (NIST, 2011).

2.4 | Repellency, contact toxicity, and oviposition test

Twenty (5 days old) potted tomato plants with 30–35 fully expanded leaves were sprayed with 10 ml of 10 mg/ml working solution with a hand sprayer; the same volume of the control solution was sprayed on the control plants. The plants were dried for 10 min after spray, then both pots (treatment and control plants) were placed in a thin and white cloth-made cage (60 cm in length, 80 cm in height, and

60 cm in width). Fifty (5 days old) adult female whiteflies were collected into a vial from the rearing colony and released between both pots into the cage. The behavioral choice of the whitefly adults was observed after 24, 48, and 72 h of bioassay in the cage. The observation was done in the early morning when the insects rested. The adult whitefly repellency percentage (PR) was calculated according to the formula of Liu et al. (2013):

$$\text{Percentage repellency}(\%) = \frac{C - T}{C + T} \times 100$$

where C represents the number of insects in control and T represents the number of insects in treatment.

In parallel, 10 leaves from each plant were collected after 24, 48, and 72 h and examined under a stereomicroscope for oviposition. The experiment was repeated with 5 and 2.5 mg/ml solutions, and eight replications were established for each solution.

A similar experiment was conducted where flies mortality was recorded at 12, 24, 48, and 72 h from the beginning of the bioassay.

2.5 | Contact toxicity

For contact toxicity, 20 (5 days old) potted tomato plants with 30–35 fully expanded leaves sprayed with 10 ml of 10 mg/ml working solution with a hand sprayer. The plant was dried for 10 min after spray and placed in a thin and white cloth-made cage (60 cm in length, 80 cm in height, and 60 cm in width). Fifty (5 days old) adult female whiteflies were collected into a vial from the rearing colony and released into the cage. The mortality was recorded at 12, 24, 48, and 72 h from the

beginning of the bioassay. The experiment was repeated with 5 and 2.5 mg/ml; the experiment was repeated eight times for each treatment.

2.6 | Data analysis

A paired t test was used to compare the number of insects and eggs between treatments with their corresponding controls. The percentage was subjected to an arcsine square-root transformation before analysis. Tukey test on the one-way ANOVA (SPSS 20.0 for Windows 2007) was used to analyze the mean repellency and oviposition differences. All the figures were drawn using Sigma plot version 10.0 for Windows 2007.

A chi-square test was used to compare the treatment and control repellencies. Paired t test was used to compare the number of eggs in the treated and control samples at $P < 0.05$.

3 | RESULTS

All the essential oils tested here exhibited repellency, contact toxicity and oviposition deterrence against whiteflies at 10 mg/ml concentrations in the greenhouse experiments. Meanwhile, the 5 and 2.5 mg/ml concentrations of essential oils showed nonsignificant differences in repellence and oviposition deterrence against whitefly (Figure 1).

3.1 | Whitefly repellency and anti-oviposition

The results showed that most whitefly adults were repelled from essential oil-treated plants. Results from ANOVA analysis revealed

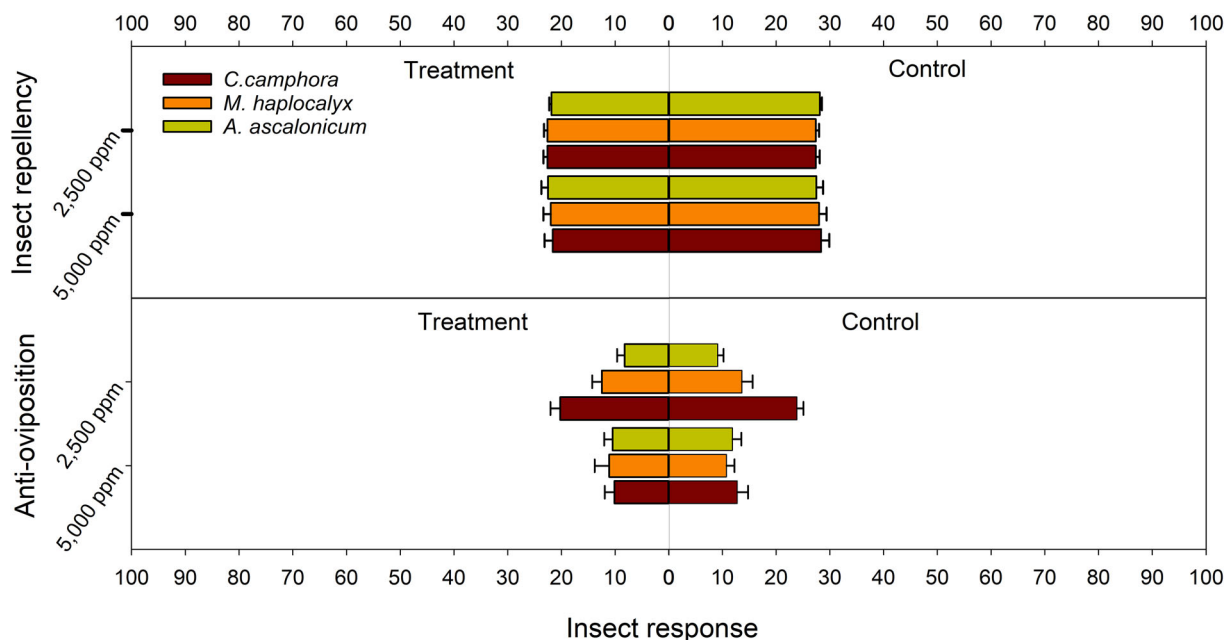


FIGURE 1 Whitefly repellency (mean \pm SE) at 24 h of exposure with different essential oils at a concentration of 5000 and 2500 ppm. The mean values of the treated and control were compared using the chi-square test at a significance level of $P < 0.05$ ($n = 8$ replications of each treatment and control).

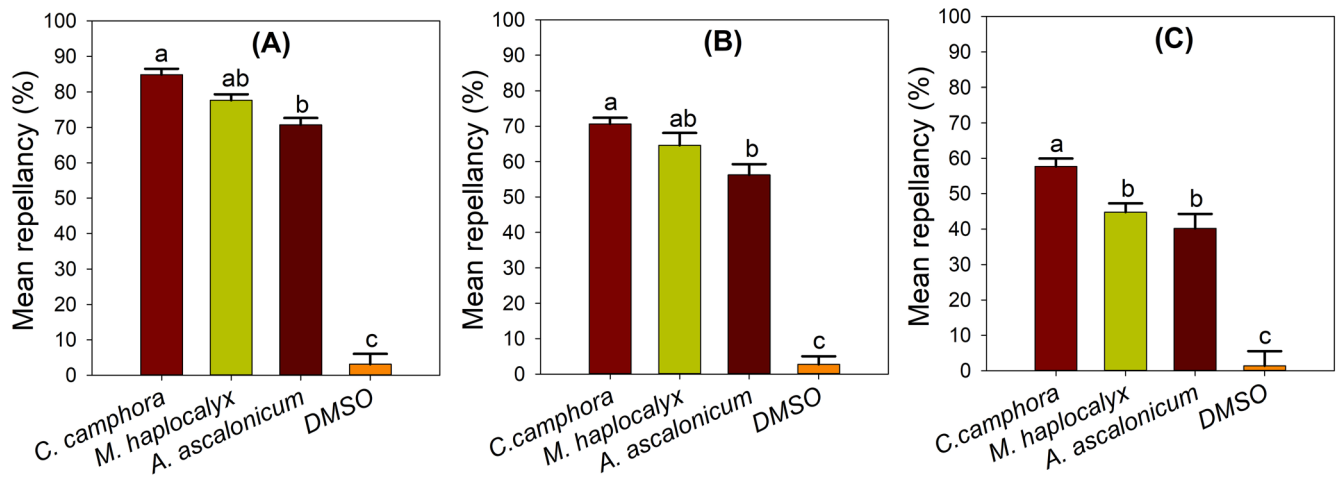


FIGURE 2 Repellency percentage (mean \pm se) of adult whiteflies on tomato plants sprayed with essential oils at a concentration of 10,000 ppm. Repellency at (A) 24 h, (B) 48 h, and (C) 72 h of exposure. The mean numbers of adults were analyzed by one-way ANOVA, using a Tukey HSD post hoc test at $P < 0.05$ ($n = 8$ replications of each treatment and control); means topped by the same letter are not significantly different.

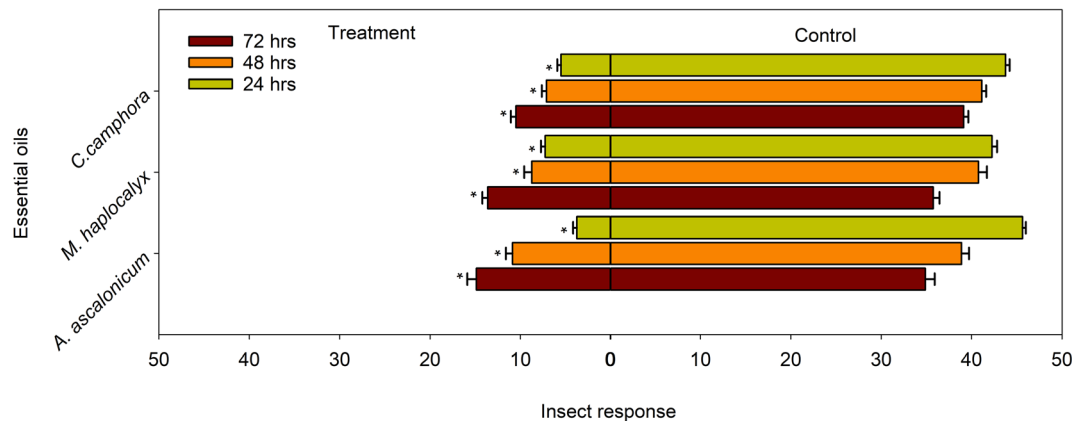


FIGURE 3 Whitefly repellency at 24, 48, and 72 h exposure with different essential oils at a concentration of 10,000 ppm (mean \pm se; $n = 8$ replications of each treatment and control). The bars are the mean values of eight replications of each treatment and control. Standard error bars represent the standard deviation of the mean. The mean numbers of adults in the treated and control were compared using the chi-square test at a significance level of $P < 0.05$. Asterisks indicate a significant difference between the treatment and control.

significant differences between all of the treatments at 24 ($F_{3,28} = 318.88$; $P < 0.01$), 48 ($F_{3,28} = 130.50$; $P < 0.01$) and 72 h ($F_{3,28} = 52.57$; $P < 0.01$) of the bioassay. Overall, *C. camphora* repelled the maximum adult whiteflies. It repelled $84.83 \pm 1.64\%$ of whiteflies at 24 h of the bioassay. At 48 and 72 h of the bioassay, the mean RP was $70.63 \pm 1.79\%$ and $57.70 \pm 2.22\%$, respectively (Figure 2), which corresponds to the first position on the repellency index. The chi-square test of repellency showed significant differences between the treatment and control at 24 h ($\chi^2 = 140.61$; $P < 0.01$), 48 h ($\chi^2 = 110.81$; $P < 0.01$) and 72 h ($\chi^2 = 72.26$; $P < 0.01$) of exposure (Figure 3).

The essential oils of *M. haplocalyx* also showed repellency ($77.67 \pm 1.69\%$) at 24 h of the bioassay, but its effect was lower than *C. camphora* oil. Its repellency decreased after 48 h ($64.61 \pm 3.48\%$) and 72 h ($44.78 \pm 2.50\%$) of the bioassay (Figure 2). A chi-square test of

repellency showed a significant difference between *M. haplocalyx* treated and controlled plants at 24 ($\chi^2 = 113.54$; $P < 0.01$), 48 ($\chi^2 = 92.76$; $P < 0.01$), and 72 ($\chi^2 = 41.99$; $P < 0.01$) hours of exposure (Figure 3).

A. ascalonicum showed the lowest RP ($70.70 \pm 1.97\%$). Its RP also decreased with increased time. The repellency was observed at 48 h with an RP of $56.25 \pm 3.03\%$ and $40.18 \pm 4.08\%$ at 72 h of the bioassay (Figure 2). A chi-square test of repellency showed a significant difference among *A. ascalonicum* treated and controlled plants at 24 h ($\chi^2 = 173.81$; $P < 0.01$), 48 h ($\chi^2 = 68.60$; $P < 0.01$), and 72 h ($\chi^2 = 33.59$; $P < 0.01$) of exposure (Figure 3).

The results of ANOVA of oviposition deterrent showed significant differences at all the concentrations at 24 ($F_{3,28} = 102.38$; $P < 0.00$), 48 ($F_{3,28} = 37.89$; $P < 0.00$), and 72 ($F_{3,28} = 26.01$; $P < 0.00$) of the bioassay. The maximum oviposition deterrent was observed from

C. camphora at 24, 48, and 72 h with mean values of 63.44 ± 3.25 , 52.40 ± 5.65 , and $49.68 \pm 5.78\%$, respectively (Figure 4). The *t* test showed that there was strong and significant difference in the *A. ascalonicum* and control at 24 ($t = 8.78$; $P < 0.00$), 48 ($t = 7.02$; $P < 0.00$), and 72 h ($t = 9.50$; $P < 0.00$). The *t* test showed that there was strong and significant difference in the *M. haplocalyx* and control at 24 ($t = 9.12$; $P < 0.00$), 48 ($t = 8.12$; $P < 0.00$), and 72 h ($t = 5.50$; $P < 0.00$). The *t* test showed that there was strong and significant difference in the *C. camphora* and control at 24 ($t = 11.35$; $P < 0.00$), 48 ($t = 8.75$; $P < 0.00$), and 72 h ($t = 6.14$; $P < 0.00$) (Figure 5).

3.2 | Whitefly contact toxicity

All essential oils showed contact toxicity against adult whiteflies. Toxicity started from 12 h of exposure and increased with time.

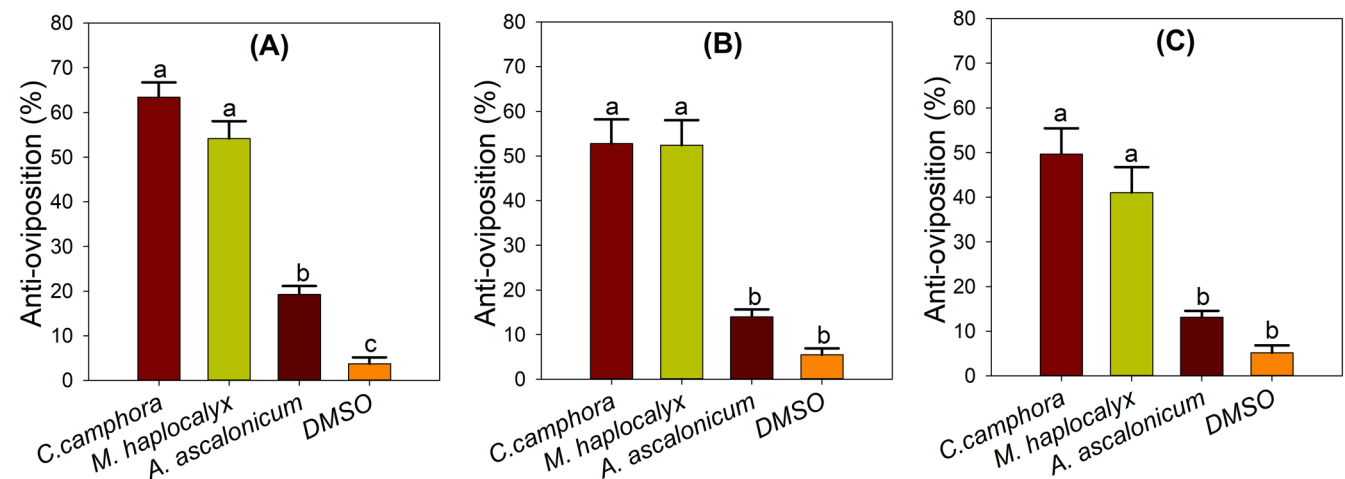


FIGURE 4 Oviposition deterrence percentage (mean \pm SE; $n = 8$ replications of each treatment and control) on tomatoes sprayed with essential oils at a concentration of 10,000 ppm. Oviposition deterrence at (A) 24, (B) 48, and (C) 72 h of exposure. The mean numbers of eggs laid were analyzed by one-way ANOVA, using a Tukey HSD post hoc test at $P < 0.05$; means topped by the same letter are not significantly different.

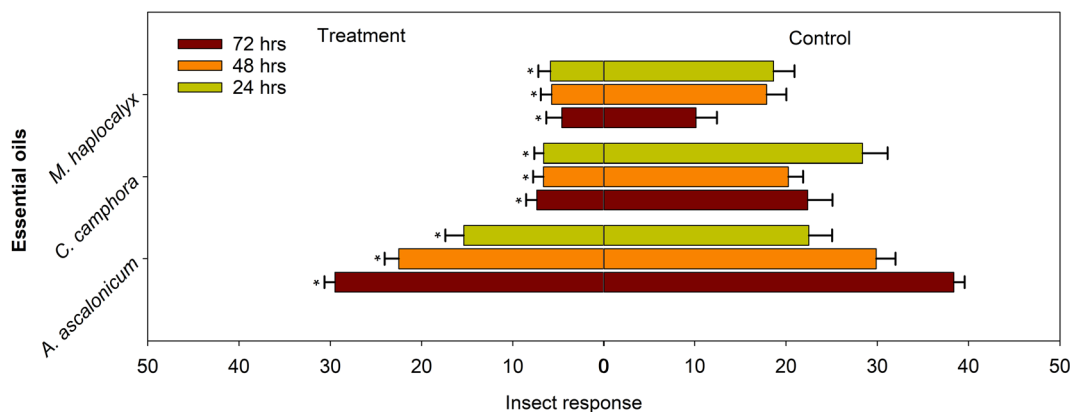


FIGURE 5 Whitefly oviposition at 24, 48, and 72 h exposure with different essential oils at a concentration of 10,000 ppm (mean \pm SE; $n = 8$ replications of each treatment and control). The mean numbers of eggs in the treated and control were compared using the chi-square test at a significance level of $P < 0.05$. Asterisks indicate a significant difference between the treatment and control.

The highest contact toxicity was observed with the essential oil of *C. camphora* ($64.25 \pm 2.12\%$), followed by *M. haplocalyx* (58.25 ± 2.22) and *A. ascalonicum* (44.75 ± 2.83) at 72 h of exposure. The ANOVA analysis revealed significant differences among all of the treatments at 12 h ($F_{2,21} = 22.51$; $P < 0.01$), 24 h ($F_{2,21} = 6.11$; $P < 0.01$), 48 h ($F_{2,21} = 4.02$; $P < 0.03$), and 72 h ($F_{2,21} = 17.20$; $P < 0.01$) of exposure (Figure 6).

3.3 | Chemical composition of the essential oil

All the essential oils showed positive effects of repellency and toxicity against whiteflies; therefore, these three essential oils were analyzed with GC-MS to identify their chemical components. The results showed four major chemical components in *A. ascalonicum*, eight major chemical components in *C. camphora* and nine major chemical

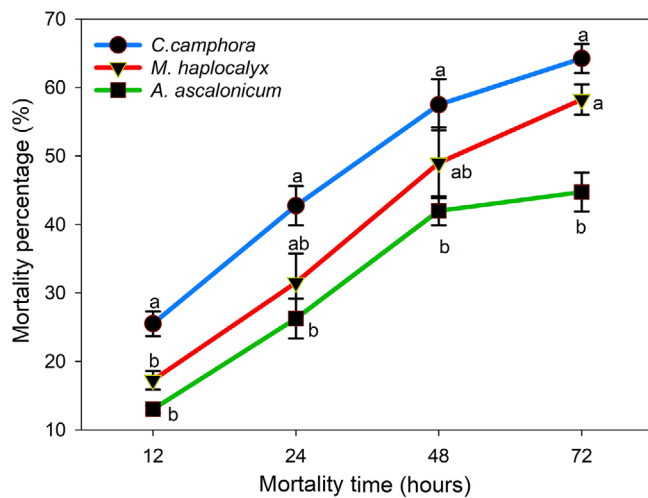


FIGURE 6 Whitefly contact toxicity at 12, 24, 48, and 72 h during the bioassay. The points are the mean values of eight replications of each treatment and control. Standard error bars represent the standard deviation of the mean. The mean numbers of adults were analyzed using one-way ANOVA and Tukey HSD post hoc tests at $P < 0.05$. Small lettering on each point represents the mean difference in each treatment at a specific time.

components in *M. haplocalyx* essential oils. Essential oil chemical components, component retention times on GC-MS, and component percentages of the totals are presented in Table 1.

4 | DISCUSSION

The essential oils tested in our experiment showed positive and significant effects of repellency against adult whiteflies in a greenhouse environment. Essential oils from *C. camphora* showed maximum repellency and anti-oviposition effects against adult whiteflies up to 72 h after exposure. Previous studies have reported on the bioactivity of the essential oil of *C. camphora*. For example, the aromatic seed oil of *C. camphora* was found to repel 89.86% of cotton aphids up to 24 h after the bioassay at a concentration of 20 $\mu\text{l/ml}$ (Jiang et al., 2016). Ethanol extraction of *C. camphora* essential oil can repel $83.75 \pm 1.83\%$ of *O. surinamensis* adults in a laboratory environment (Wagan, Hu, et al., 2016). In a lab experiment, *C. camphora* essential oil repelled 54.35% of *Tribolium castaneum* at a 0.75% concentration and 39.55% of *O. surinamensis* at a 1% concentration (Al-Jabr, 2006). Our results also showed that *C. camphora* has a significant repellent impact on whiteflies (Figures 1–4). In the present study, after *C. camphora*, the essential oil of *M. haplocalyx* was found to have the most effective repellent and anti-oviposition activity; similar results were reported by Ren et al. (2007), where *M. haplocalyx* essential oil showed repellency, toxicity, reproduction regulatory and oviposition activities as well as growth inhibitor activities against several insect species. The essential oils of *M. haplocalyx* can repel up to 83% of red flour beetle adults for up to 72 h after exposure in the area preference test in Petri dishes (Wagan, He, et al., 2016). These studies confirm

TABLE 1 Chemical components identified in essential oils based on GC-MS analysis

| Chemical components | Retention time | Percentage of total |
|--|----------------|---------------------|
| <i>Allium ascalonicum</i> | | |
| Methane, sulfinylbis | 5.09 | 6.56 |
| Isolongifolene | 10.11 | 0.04 |
| Diethyl phthalate | 11.22 | 1.47 |
| Ethylene brassylate | 13.63 | 1.06 |
| <i>Cinnamomum camphora</i> | | |
| Linalool | 7.65 | 0.72 |
| trans-Caryophyllene | 10.23 | 2.82 |
| alpha-Humulene | 10.46 | 0.98 |
| Nerolidol | 10.98 | 12.18 |
| 1,5-Heptadien-4-one, 3,3,6-trimethyl acetic acid | 12.00 | 59.94 |
| 2-Propanol, 1-chloro, phosphate | 12.33 | 1.27 |
| Phytol | 13.88 | 1.02 |
| <i>Mentha haplocalyx</i> | | |
| L-(–)-menthol | 7.85 | 8.26 |
| Cyclohexanol, 5-methyl-2-(1-methylethyl) | 7.89 | 2.03 |
| 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl | 11.94 | 1.22 |
| Hexadecanoic acid | 12.64 | 4.40 |
| Hexadecanoic acid, ethyl ester | 12.74 | 1.16 |
| Phytol | 13.33 | 1.27 |
| 9,12-Octadecadienoic acid (Z,Z) | 13.49 | 1.57 |
| 9,12,15-Octadecatrienoic acid, (Z,Z,Z) | 13.52 | 4.48 |
| 24(Z)-methyl-25-homocholesterol | 23.88 | 1.36 |

Abbreviations: GC, gas chromatography; MS, mass spectrometry.

the results of the current study (Figures 1–4). Our results showed that *M. haplocalyx* was the second most effective essential oil to manage whitefly with repellence and oviposition deterrent activity. In our results, the essential oil of *A. ascalonicum* was active in repelling the whiteflies, and it had oviposition deterrence effects against those insects. Ethanol-extracted essential oils from *A. ascalonicum* were reported to have a repellency of 66.25 and 63.75% after 6 h of exposure against *Sitophilus zeamais* adults and larval stages, respectively (Wagan et al., 2018). Freshly extracted sap from *A. ascalonicum* at a concentration of 0.25% was demonstrated to have antifungal activity when applied to 11 species of dermatophytes, saprophytic, and *Candida* fungi (Mahmoudabadi & Nasery, 2009). Its essential oil also showed bioactivity against some bacterial species, such as *Escherichia coli*, *Lactobacillus* sp., and *Salmonella* sp. (Moazin et al., 2015). These studies follow the results of the current study, which showed that *A. ascalonicum* significantly repels pests. However, in our study, *A. ascalonicum* was the least effective essential oil than other oils (Figures 1–4).

In the present study, all the tested essential oils exhibited the highest mortality rate against the whiteflies, regardless of the type of oil used. Our results correspond with the findings reported in previous studies investigating the toxicity of these essential oils against some insect species. Purified *C. camphora* oil showed LC₅₀ to *Helicoverpa armigera* at 1839 ppm; LC₅₀ to *Culex pipiens pallens* at 168 ppm (Zhou et al., 2000). The steamed distillation of the essential oil of *C. camphora* was found to have strong fumigant and contact toxicity against *Lasioderma serricorne* and *T. castaneum* adults (Guo et al., 2016). Ethanol-extracted essential oils from *M. haplocalyx* and *A. ascalonicum* showed toxicity against larval and adult stages of maize weevil *S. zeamais* in a laboratory test (Wagan et al., 2018). The maximum larval toxicity of 50.29 and 46.36% and adult toxicity of 26.35 and 37.34% were recorded for *A. ascalonicum* and *M. haplocalyx*, respectively, at 16 h of exposure (Wagan et al., 2018). No other study has investigated these essential oils against greenhouse insect species. However, many studies have described the bioactivity of these essential oils in a laboratory, in stored grain pests, and using some pathogens. The present study is the first to show the repellency of these essential oils, toxicity, and oviposition deterrence against adult whiteflies (Figure 6).

Essential oils are chemically characterized as natural mixtures of chemical components, and they can show bioactivities against several species of insects. Previous research has reported on the effects of the major compounds of the essential oils of *C. camphora*, *M. haplocalyx*, and *A. ascalonicum* against insect and noninsect species. For example, the insecticidal, nematicidal, and antioxidant characteristics were probably due to hexadecenoic acid ethyl ester (Jananie et al., 2011). Linalool, found in some plant species, has been applied as an insecticide against insect species, such as fleas, fruit flies, and cockroaches (Yang et al., 2004). Phytol is a naturally occurring plant compound used as a plant defense system (Krishnaiah et al., 2009). It has also been found to have an anticonvulsant effect in mice (Costa et al., 2012). Toxic properties were observed from menthol; it showed LD50 values of 7.91 µg/adult against *L. serricorne* (Zhang et al., 2015). It is extensively used as a topical antibacterial agent in dental care, affecting several types of bacteria, such as streptococci and lactobacilli (Freires et al., 2015). Nerolidol can cross the plasma membrane and interfere with proteins in a cell. It has been found to have antioxidant, antifungal, anticancer, and antimicrobial bioactivities (Chan et al., 2016). Humulene naturally occurs in aromatic plant species, exhibiting anti-inflammatory activity in *in vitro* studies (Fernandes et al., 2007). Some studies tested chemicals using rat and mouse controls; caryophyllene was selectively effective in rodents, showing cannabinimetic anti-inflammatory effects in rats (Javed et al., 2016) and mice (Gertsch et al., 2008). Diethyl phthalate was reported to cause skeletal malformation, especially in rats (Singh et al., 1972) (Table 1).

5 | CONCLUSION

In the present study, essential oils from *C. camphora*, *M. haplocalyx*, and *A. ascalonicum* protected the plants and kept the adult whiteflies away, decreasing their infestation and oviposition activity up to 72 h

after the bioassay in a greenhouse environment. *C. camphora* essential oil was the most effective in repellency, contact toxicity and oviposition deterrence, followed by *M. haplocalyx* and *A. ascalonicum*. Further studies are needed to assess the bioactivity of the chemical components of these essential oils on other insect species in the greenhouse and open field settings.

AUTHOR CONTRIBUTIONS

Hongxia Hua designed research. Tufail Ahmed Wagan reared the insects and conducted the experiments. Tufail Ahmed Wagan, Hakan Bozdoğan, and Muhammad Musa Khan analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENT

Thanks to Dr. Pankaj Bhatt for reviewing the manuscript and for his helpful suggestions.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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REFERENCES

- Al-Jabr, A.M. (2006) Toxicity and repellency of seven plant essential oils to *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Scientific Journal of King Faisal University*, 7, 49–60.
- Anderson, P.K. & Morales, F.J. (2005) *Whitefly and whitefly-borne viruses in the tropics: building a knowledge base for global action*. Cali, Colombia: Centro Internacional de Agricultura Tropical, p. 345.
- Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008) Biological effects of essential oils—A review. *Food and chemical toxicology*, 46, 446–475.
- Barro, P.J.D., Liebrechts, W. & Carver, M. (1998) Distribution and identity of biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in member countries of the Secretariat of the Pacific Community. *Australian Journal of Entomology*, 37, 214–218.
- Carlos, E.B. & Kevin, M.H. (2016) *Whiteflies*. The Texas A&M University System. <https://extensionentomology.tamu.edu/publications/whiteflies/>
- Chan, W.K., Tan, L.T., Chan, K.G., Lee, L.H. & Goh, B.H. (2016) Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules*, 21, 529.
- Costa, J.P., Ferreira, P.B., DE-Sousa, D.P., Jordan, J. & Freitas, R.M. (2012) Anticonvulsant effect of phytol in a pilocarpine model in mice. *Neuroscience Letters*, 523(2), 115–118.
- Fernandes, E.S., Passos, G.F., Medeiros, R., DA-Cunha, F.M., Ferreira, J., Campos, M.M. et al. (2007) Anti-inflammatory effects of compounds alpha-humulene and (–)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *European Journal of Pharmacology*, 569(3), 228–236.
- Freires, I.A., Denny, C., Benso, B., de Alencar, S.M. & Rosalen, P.L. (2015) Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review. *Molecules*, 20(4), 7329–7358.
- Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J.Z., Xie, X.Q. et al. (2008) Beta-caryophyllene is a dietary cannabinoid. *Proceeding of the National Academy of Science of the United States of America*, 105(26), 9099–9104.

- Guo, S.S., Geng, Z.F., Zhang, W.J., Liang, J.Y., Wang, C.F., Deng, Z.W. et al. (2016) The chemical composition of essential oils from *Cinnamomum camphora* and their insecticidal activity against the stored product pests. *International Journal of Molecular Sciences*, 17(11), 1836.
- Jananie, R.K., Priya, V. & Vijayalakshmi, K. (2011) Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. *New York Science Journal*, 4, 16–20.
- Javed, H., Azimullah, S., Haque, M.E. & Ojha, S.K. (2016) Cannabinoid type 2 (cb2) receptors activation protects against oxidative stress and neuroinflammation associated dopaminergic neurodegeneration in rotenone model of Parkinson's disease. *Frontiers in Neuroscience*, 10, 26–39.
- Jiang, H., Wang, J., Song, L., Cao, X., Yao, X., Tang, F. et al. (2016) GC-TOFMS analysis of essential oils composition from leaves, twigs and seeds of *Cinnamomum camphora* L. Presl and their insecticidal and repellent activities. *Molecules*, 21, 1–12.
- Khan, M.M., Fan, Z., Rothenberg, D.O.N., Peng, J., Hafeez, M., Chen, X. et al. (2021) Phototoxicity of ultraviolet-A against the whitefly *Bemisia tabaci* and its compatibility with an entomopathogenic fungus and whitefly parasitoid. *Oxidative Medicine and Cellular Longevity*, 2021, 1–13.
- Khan, M.M., Hafeez, M., Elgizawy, K., Wang, H., Zhao, J., Cai, W. et al. (2021) Sublethal effects of chlorantraniliprole on *Paederus fuscipes* (Staphylinidae: Coleoptera), a general predator in paddle field. *Environmental Pollution*, 291, 118171.
- Khan, M.M., Huang, Q., Wagan, T.A., Hua, H., Cai, W., Zhao, J. et al. (2020) Behavioral response of *Nilaparvata lugens* (Stål), *Cyrtorhinus lividipennis* Reuter and *Paederus fuscipes* Curtis to three synthetic volatile chemical compounds. *Journal of Asia-Pacific Entomology*, 23, 2020–2276.
- Koul, O., Walia, S. & Dhaliwal, G.S. (2008) Essential oils as green pesticides: potential and constraints. *Biopesticides International*, 4, 63–84.
- Krishnaiah, D., Devi, T., Bono, A. & Sarbatly, R. (2009) Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plant Research*, 3, 67–72.
- Lapidot, M. & Polston, J.E. (2006) Resistance to tomato yellow leaf curl virus in tomato. In: Loebenstein, G. & Carr, J.P. (Eds.) *Natural resistance mechanisms of plants to viruses*. New York, NY: Springer Verlag, pp. 503–540.
- Liu, X.C., Li, Y.P., Li, H.Q., Deng, Z.W., Zhou, L., Liu, Z.L. et al. (2013) Identification of repellent and insecticidal constituents of the essential oil of *Artemisia rupestris* L. aerial parts against *Liposcelis bostrychophila* Badonnel. *Molecules*, 18, 10733–10746.
- Mahmoudabadi, A.Z. & Nasery, M.K.G. (2009) Anti-fungal activity of shallot *Allium ascalonicum* Linn. (Liliaceae) in vitro. *Journal of Medicinal Plants Research*, 3, 450–453.
- Mozin, S., Rosyidi, D., Sjoftan, O. & Widodo, E. (2015) The effect of shallot (*Allium ascalonicum* L.) by-product as an antibacterial and alternative phytobiotic on characteristics of small intestine of broiler. *Livestock Research for Rural Development*, 27, 78.
- Murray, M. (2000) Mechanisms of inhibitory and regulatory effects of methylenedioxyphenyl compounds on cytochrome P450-dependent drug oxidation. *Current Drug Metabolism*, 1, 67–84.
- NIST. (2011) *NIST/EPA/NIH mass spectral database (NIST 11) and NIST mass spectral search program (version 2.0g)*. Gaithersburg, MD: US Department of Commerce National Institute of Standards and Technology Standard Reference Data Program, 20899.
- Okonkwo, E.U. & Okoye, W.I. (1996) The efficacy of four seed powders and the essential oils as protectants of cow-pea and maize grain against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. *International Journal of Pest Management*, 42, 143–146.
- Pappu, H., Jones, R. & Jain, R. (2009) Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. *Virus Research*, 141, 219–236.
- Pichersky, E. & Gershenzon, J. (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current opinion in plant biology*, 5, 237–243.
- Polston, J.E. & Anderson, P.K. (1997) The emergence of whitefly transmitted geminiviruses in tomato in the western hemisphere. *Plant Disease*, 81, 1358–1369.
- Ren, W., Liao, H. & Du, L.F. (2007) Inhibitory effect of trypsin inhibitor from *Cassia obtusifolia* seeds against *Pieris rapae* larvae. *Sichuan Journal of Zoology*, 26, 635–637.
- Shah, M.M.R., Prodhon, M.D.H., Siddique, M.N.A., Mamun, M.A.A. & Shahjahan, M. (2008) Repellent effect of some indigenous plant extracts against saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.). *International Journal of Sustainable Crop Production*, 3, 51–54.
- Singh, A.R., Lawrence, W.H. & Autian, J. (1972) Teratogenicity of phthalate esters in rats. *Journal of Pharmaceutical Sciences*, 61, 51–55.
- Su, Y.P., Yang, C.J., Hua, H.X., Cai, W.L. & Lin, Y.J. (2009) Bioactivities of ethanol extracts from thirteen plants against *Nilaparvata lugens* (Stal). *Chinese Agricultural Science Bulletin*, 25(1), 198–202.
- Wagan, T.A., He, Y.P., Cai, W.L., Zhao, J. & Hua, H.X. (2016) Fumigant and contact toxicity and oviposition deterrent effects of plant essential oils on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Florida Entomologist*, 99, 673–677.
- Wagan, T.A., Hu, D.B., He, Y.P., Nawaz, M., Nazir, T., Mabubu, J.I. et al. (2016) Repellency of three plant-derived essential oils against red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Turkish Journal of Entomology*, 40, 347–354.
- Wagan, T.A., Wang, W.J., Hua, H.X. & Cai, W.L. (2017) Chemical constituents and toxicity, repellency, and oviposition deterrence effects of ethanol-extracted *Myristica fragrans* oil against *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). *Florida Entomologist*, 100(3), 594–601.
- Wagan, T.A., Wang, W.J., Khan, M.M., Hua, H.X. & Cai, W.L. (2018) Repellency, fumigant toxicity and chemical constituents of ethanol-extracted essential oils from *Mentha haplocalyx* and *Allium ascalonicum* on *Sitophilus zeamais* (Coleoptera: Curculionidae). *African Entomology*, 26(2), 369–378.
- Yang, Z., Bengtsson, M. & Witzgall, P. (2004) Host plant volatiles synergize response to sex pheromone in codling moth, *Cydia pomonella*. *Journal of Chemical Ecology*, 30(3), 619–629.
- Zhang, W.J., Yang, K., You, C.X., Wang, C.F., Geng, Z.F., Su, Y. et al. (2015) Contact toxicity and repellency of the essential oil from *Mentha haplocalyx* BRIQ against *Lasioderma serricorne*. *Chemistry & Biodiversity*, 12, 832–839. <https://doi.org/10.1002/cbdv.201400245>
- Zhou, X., Li, X.D., Yuan, J.Z., Tang, Z.H. & Liu, W.Y. (2000) Toxicity of cinnamon—a new type II ribosome-inactivating protein to bollworm and mosquito. *Insect Biochemistry and Molecular Biology*, 30, 259–264.

How to cite this article: Wagan, T.A., Hua, H., Bozdoğan, H. & Khan, M.M. (2022) Repellency, contact toxicity, and anti-oviposition effects of three ethanol-extracted plant essential oils on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Physiologia Plantarum*, 174(6), e13799. Available from: <https://doi.org/10.1111/ppl.13799>