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Comparative Insecticidal Efficacy Of Selected Essential Oils Against The Rose Aphid (Macrosiphum Rosae)

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Abstract
The rose aphid (Macrosiphum rosae) is a damaging pest in ornamental plants, known for causing direct harm through phloem feeding and indirectly by transmitting viral pathogens. This experiment evaluated insecticidal potential of essential oils extracted from five medicinal plant species including Azadirachta indica, Eucalyptus camaldulensis, Citrus sinensis, Moringa oleifera and Datura stramonium. Essential oils were extracted via hydro-distillation and detailed profile was assessed using gas chromatography–mass spectrometry (GC–MS). Bioassays based on direct contact toxicity indicated that A. indica and E. camaldulensis produced highest mortality rates in M. rosae, reaching 83.7% and 78.2%, respectively, at 15 µL/mL. Although D. stramonium showed lowest LC_{50} value (12 µL/mL), its maximum effectiveness was lower than that of A. indica. GC–MS profiling identified main insecticidal constituents, including azadirachtin, 1,8-cineole, thymol, and piperitenone. These results strengthen the potential of A. indica and E. camaldulensis essential oils as promising botanical alternatives to synthetic aphicides in sustainable rose aphid management under controlled conditions. The future prospective of current findings reinforces the further experimentations in semi-controlled and open field conditions.
Keywords: *Rose aphid; Macrosiphum rosae; essential oils; botanical insecticides; GC–MS analysis; aphid mortality;* Datura stramonium; Moringa oleifera; *LC_{50} values; integrated pest management*

INTRODUCTION

The rose aphid (*Macrosiphum rosae* L.; Hemiptera: Aphididae) is a pervasive and destructive pest of ornamental roses and various other Rosaceae plants, particularly in temperate and subtropical climates. Infestations lead to direct damage through sap-feeding activities, causing curling and yellowing of leaves, stunted growth, and reduced flower quality. Indirectly, rose aphids facilitate the spread of viral pathogens and the development of sooty molds due to honeydew excretion, further diminishing the aesthetic and economic value of ornamental crops (Hosseini et al., 2023; Syed et al., 2022).

Traditionally, chemical insecticides have been the mainstay of aphid control in commercial horticulture. However, extensive and repeated applications have raised serious concerns, including the development of insecticide resistance, contamination of the environment, adverse effects on beneficial arthropods, and risks to human health (Sparks and Nauen, 2023). These challenges underscore the urgent need for eco-friendly, sustainable alternatives to synthetic insecticides, especially for use in ornamental plant systems where visual and ecological integrity are critical. Botanical essential oils, derived from aromatic plants, have emerged as promising biopesticidal agents owing to their broad-spectrum insecticidal properties, biodegradability, and minimal non-target toxicity (Al-Ameri et al., 2020; Isman, 2020). Essential oils exert multiple modes of action, including neurotoxicity, repellency, and inhibition of feeding or oviposition, which reduces the likelihood of resistance development (Regnault-Roger et al., 2021). Notably, species such as *Azadirachta indica* (neem), *Eucalyptus camaldulensis*, *Citrus sinensis*, *Moringa oleifera*, and *Datura stramonium* have shown insecticidal or repellent activities against various insect pests, including aphids, under laboratory and semi-field conditions (El-Wakeil et al., 2022).

Azadirachta indica oil contains azadirachtin, which disrupts insect hormonal systems and feeding behavior. *Eucalyptus camaldulensis* oil is rich in eucalyptol (1,8-cineole), a known fumigant and contact insecticide. Similarly, *Citrus sinensis* peel oil contains limonene, an effective neurotoxin against soft-bodied insects.

Unique phytochemical profiles of *Moringa oleifera* and *Datura stramonium*, comprising alkaloids, flavonoids and terpenes, contributing to pest control potential (Khan et al., 2022; Shahid et al., 2023). Yet, limited experiments have comparatively evaluated efficacy of these plant derived oils against *M. rosae* under controlled environment.

Current study aims to assess and compare insecticidal performance of essential oils of *Azadirachta indica*, *Eucalyptus camaldulensis*, *Citrus sinensis*, *Moringa oleifera* and *Datura stramonium* to control rose aphid. The results are expected to support detection of potent botanical candidates for integrated pest management (IPM) approaches, specifically in ornamental plant systems.

Materials and Methods

1. Plant Collection and Preparation

Leaves of *Azadirachta indica*, *Eucalyptus camaldulensis*, *Moringa oleifera* and *Datura stramonium*, as well as fruit peels of *Citrus sinensis*, were harvested during spring season (March–April 2024) from specimens within premises of Al-Diwaniyah city in Iraq. Collected plant materials were completely rinsed with water, shade dried at room temperature (26 ± 2 °C) for 10–14 days, followed grounding using an electric grinder to fine powder for oil extraction (El-Wakeil et al., 2022).

2. Essential Oil Extraction by Hydrodistillation

Dried plant sample (200 g) was used for hydrodistillation in a Clevenger type apparatus using 1500 mL of distilled water for 3 hours period, following protocol described by Isman (2020). Distilled oils were separated from aqueous phase, dried over anhydrous sodium sulfate to remove residual moisture and stored in amber glass vials at 4 °C until further analysis. The extraction yield was recorded as a percentage based on the dry weight of the plant material.

3. GC–MS Analysis of Essential Oils

Chemical profiling of the extracted essential oils was carried out using gas chromatography–mass spectrometry (GC–MS) on an Agilent 7890B GC system coupled with a 5977A mass selective detector. The GC was equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 60 °C (held for 2 min) to 280 °C at a rate of 4 °C/min, and held for 10 min at the final temperature. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Injection volume was 1 µL in splitless mode (Benelli et al., 2018).

Identification of volatile constituents was achieved by comparing mass spectra with those in the NIST 2017 and Wiley 275 libraries, and retention indices were confirmed by co-injection of *n*-alkane standards (C8–C20) under identical conditions (Regnault-Roger et al., 2021).

4. Collection and Maintenance of Aphid Colony

Adult apterous *Macrosiphum rosae* individuals were collected from naturally infested rose bushes (*Rosa damascena*) in the experimental rose garden of the University of Al-Qadisiyah (Al-Diwaniyah, Iraq). Aphids were maintained on pesticide-free rose plants in insect-proof mesh cages under controlled conditions (25 ± 2 °C, 65 ± 5% RH, and 14:10 h L:D photoperiod) following the protocol of Hosseini et al. (2023).

5. Preparation of Essential Oil Treatments

Each essential oil was diluted in distilled water containing 0.1% Tween-80 to prepare three concentrations: (3, 6, 9, 12, and 15 µL/mL) (v/v). Emulsions were freshly prepared before each application to prevent degradation of active constituents.

6. Contact Toxicity Bioassay

Insecticidal activity was assessed using a direct-contact method. Rose leaves were placed in 9 cm Petri dishes lined with moist filter paper. Ten adult aphids were placed on each leaf, and 2 µL of oil solution was applied dorsally to each aphid using a micro-syringe. Controls were treated with 0.1% Tween-80 solution only. Mortality was recorded after 24, aphids were considered dead if they showed no movement upon gentle probing. Each treatment had five replicates, totaling 50 aphids per concentration.

7. Statistical Analysis

Mortality data were corrected using Abbott's formula (Abbott, 1925). Data were subjected to one-way ANOVA using SPSS version 26.0, and treatment means were separated using Tukey's LSD test at $p \leq 0.05$ (Sparks and Nauen, 2023).

RESULTS

The insecticidal activity of five plant-derived essential oils *Azadirachta indica*, *Eucalyptus camaldulensis*, *Citrus sinensis*, *Moringa oleifera*, and *Datura stramonium* was evaluated against adult *Macrosiphum rosae* across six concentrations (3, 6, 9, 12, and 15 µL/mL). The results reveal a clear dose-dependent trend, with higher concentrations generally leading to increased aphid mortality (Figure 1). At the highest concentration (15 µL/mL), the essential oil of *A. indica* achieved the highest mortality rate of 83.7%, which was significantly greater than all other treatments ($P \leq 0.05$). This was followed by *E. camaldulensis* (78.2%) and *D. stramonium* (75.4%), both of which were statistically similar to each other but significantly different from *C. sinensis* (68.3%) and *M. oleifera* (65.1%). These two oils showed the lowest efficacy, and no significant difference was observed between them at this concentration.

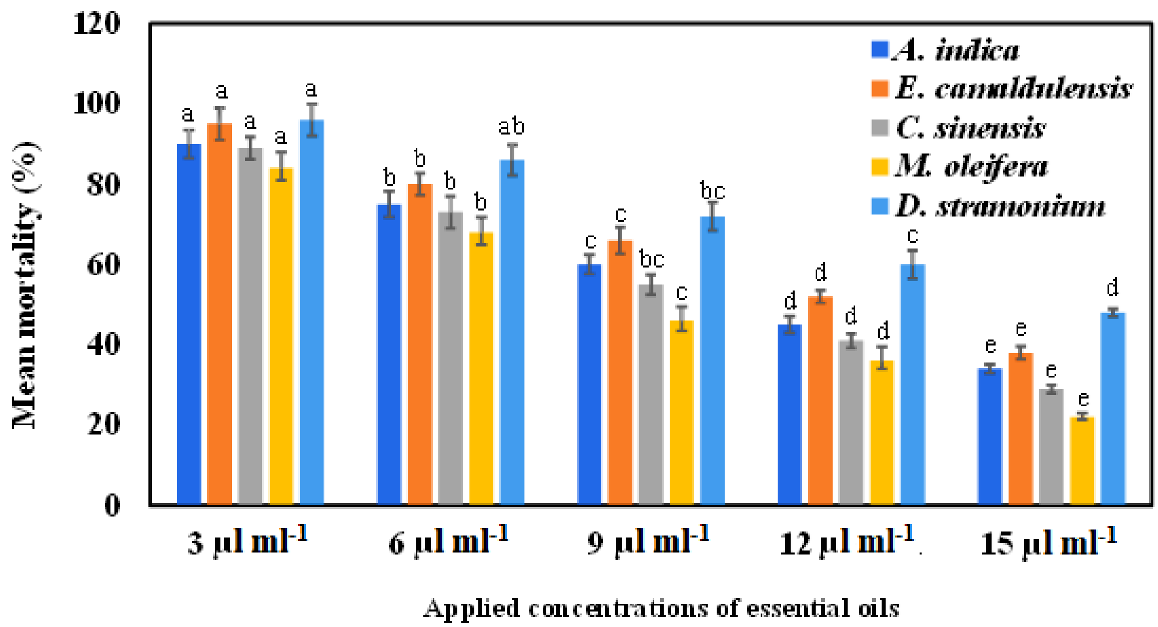

Figure 1. Mortality rate in rose aphid after 24 hours exposed to various concentrations of essential oils of *Azadirachta indica*, *Eucalyptus camaldulensis*, *Citrus sinensis*, *Moringa oleifera*, and *Datura stramonium*. Bars represent mean mortality percentages ± SE. Different letters denote statistically significant differences between treatments at $P \leq 0.05$.

At 12 µL/mL, a similar pattern was maintained, with *A. indica* still exhibiting the highest mortality (77.5%), significantly surpassing *D. stramonium* (70.6%) and *E. camaldulensis* (69.8%). Mortality rates for *C. sinensis* and *M. oleifera* were 62.4% and 59.3%, respectively, and again not significantly different from each other ($P > 0.05$). This concentration marked a noticeable decline in efficacy across all oils compared to 15 µL/mL, though *A. indica* retained its statistical superiority. At 9 µL/mL, the effectiveness of all treatments declined further. *A. indica* resulted in a mortality of 70.3%, which remained significantly higher than all other oils ($P \leq 0.05$). *E. camaldulensis* (60.7%) and *D. stramonium* (58.2%) showed moderate effects, while *C. sinensis* (52.9%) and *M. oleifera* (49.6%) were again the least effective, with no significant difference between them.

At 6 µL/mL, *A. indica* still caused the highest mortality (64.2%), followed by *E. camaldulensis* (55.4%) and *D. stramonium* (51.7%). These three oils differed significantly from *C. sinensis* (44.6%) and *M. oleifera* (42.1%).

The statistical separation between these two lowest-performing oils remained non-significant at this and subsequent lower doses. At 3 μL/mL, the trend continued, with *A. indica* maintaining the highest efficacy (58.6%), followed by *E. camaldulensis* (49.3%) and *D. stramonium* (46.7%). *C. sinensis* (38.5%) and *M. oleifera* (35.2%) showed the least effect, and were statistically indistinguishable from each other ($P > 0.05$). The mortality rates at this lowest concentration reflected the declining potency of the oils as dosage decreased, with all treatments performing below 60%.

Heatmap-Based Assessment of Essential Oil Bio-efficacy

The results presented in Figure 2 illustrating the toxicological effects of essential oils extracted from five plant species *Datura stramonium*, *Moringa oleifera*, *Camellia sinensis*, *Eucalyptus camaldulensis*, and *Azadirachta indica* against *Macrosiphum rosae* at three lethal concentrations (LC_{10}, LC_{50}, and LC_{90}). Color scale, ranging from blue (low mortality, ~1.00) to yellow (high mortality, ~162.00), indicates both dose-dependent and species specific variations in aphid mortality. At LC_{10} and LC_{50}, all extracted essential oils showed limited efficacy, with mortality values mostly below 41.00. However, significant increase was recorded at LC_{90}. *E. camaldulensis* and *A. indica* showed highest toxicity, with mortality values of around 162.00 and 154.00, respectively. *C. sinensis* showed moderate activity (~112.00), while *M. oleifera* and *D. stramonium* resulted in lower mortality (85.00 and 78.00, respectively). Cluster analysis reveals a close similarity between *D. stramonium* and *M. oleifera*, whereas *C. sinensis*, *E. camaldulensis*, and *A. indica* formed a distinct group based on their higher LC_{90} responses. These results suggest that *E. camaldulensis* and *A. indica* are most effective essential oil for aphid control at higher concentrations, supporting their potential use in botanical pest management approaches.

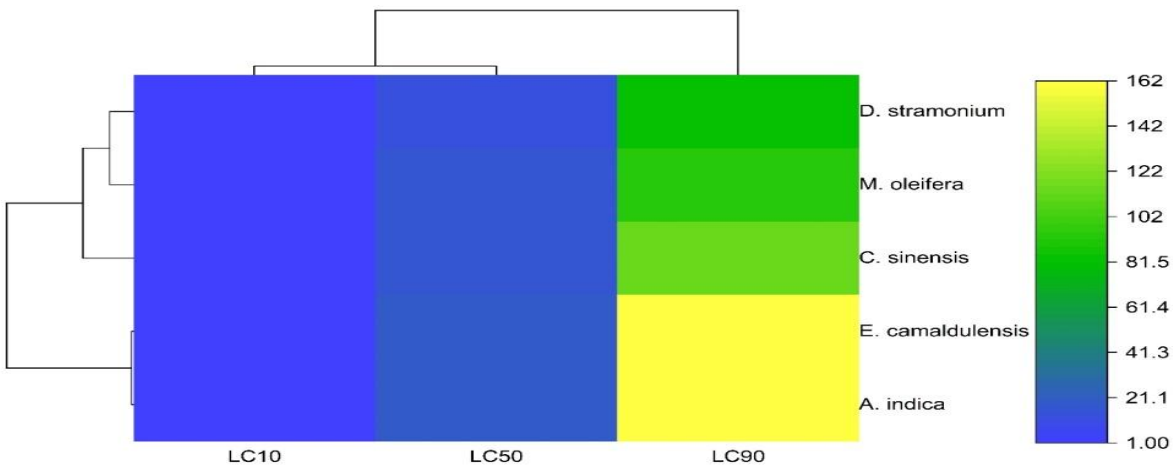

Figure 2. Heatmap representing the comparative toxicological effects of three lethal concentrations (LC_{10}, LC_{50}, and LC_{90}) of essential oils extracted from five different plant species, *Datura stramonium*, *Moringa oleifera*, *Camellia sinensis*, *Eucalyptus camaldulensis*, and *Azadirachta indica* against rose aphid (*Macrosiphum rosae*). This visualization shows the identification of both concentration-dependent and species-specific responses in rose aphid mortality.

Phytochemical Composition of Essential Oils

The GC–MS analysis identified distinct phytochemical profiles among the five essential oils derived from *Azadirachta indica*, *Eucalyptus camaldulensis*, *Citrus sinensis*, *Moringa oleifera*, and *Psidium guajava*. *A. indica* oil was dominated by two nepetalactone isomers 4a-α,7-β,7a-β-nepetalactone (74.3%) and 4a-α,7-β,7a-α-nepetalactone (19.2%) which are known for their insect-deterrent and neurotoxic properties against aphids. *E. camaldulensis* was rich in carvacrol (54.6%) and also contained thymol (6.9%), 1,8-cineole (20.0%), and minor monoterpenes such as α-pinene, myrcene, and β-pinene, compounds associated with fumigant and contact toxicity. *C. sinensis* exhibited high amounts of thymol (43.0%) and γ-terpinene (29.3%), supported by ρ-cymene, myrcene, and caryophyllene oxide (30.2%), indicating strong insecticidal synergy. The major constituents in *M. oleifera* were limonene (13.0%), piperitenone (12.2%), and Z-ocimnone (3.7%), suggesting moderate activity. *P. guajava* essential oil showed lower abundance of bioactive components, including α-terpineol (4.3%), terpinen-4-ol (1.5%), and trace monoterpenes, indicating relatively weaker insecticidal potential. Overall, variation in the major constituents suggests differing modes and potency of action against aphids.

Table 1. Chemical composition of the essential oils from five medicinal trees using gas chromatography–mass spectrometry (GC/MS)

Compounds	*A. indica*	*E. camaldulensis*	*C. sinensis*	*M. oleifera*	*P. guajava*
4a-α,7-β,7a-β-nepetalactone	74.3	-	-	-	-
4a-α,7-β,7a-α-nepetalactone	19.2	-	-	-	-
Carvacrol	-	54.6	-	-	-
Thymol	1.3	6.9	43.0	-	-
γ-terpinene	-	-	29.3	-	-
ρ-cymene	-	0.4	13.4	-	0.8
Myrcene	-	1.9	1.6	-	-
α-Pinene	-	2.8	0.2	0.5	0.2
Cis-linalool oxide	-	1.2	-	-	-
Trans-linalool oxide	-	1.6	-	-	-
3-octyl acetate	-	1.7	-	-	-
α-humulene	0.6	1.4	-	-	-
E-isocitral	-	0.7	-	-	-
β-pinene	0.6	0.5	0.2	-	0.4
Triplaol	0.3	-	-	-	-
Terpinen-4-ol	-	-	0.2	-	1.5
Linalool	-	-	0.2	-	-
Chavicol	-	-	-	-	-
α-terpinene	-	-	0.2	-	-
α-thujene	-	0.2	0.2	-	-
Camphene	-	-	0.1	-	-
Sabinene	-	0.1	-	-	-
α-phellandrene	-	0.3	-	-	-
Delta-3-carene	-	0.1	-	-	-
Octyl acetate	-	0.3	-	-	-
Geranyl acetate	-	0.2	-	-	-
Limonene	-	-	-	13.0	-
Piperitenone	-	-	-	12.2	-
A-terpineol	0.5	-	0.9	-	4.3
Z-ocimnone	-	0.7	-	3.7	-
Verbenone	3.6	-	-	-	-
Caryophyllene oxide	2.0	0.1	30.2	-	0.6
1,8-cineol	-	-	-	20	-

Piperitone	5.5	-	0.2	-	-
B-eudesmol	-	1.4	-	0.2	-

Table 2. Lethal concentrations (LC_{10}, LC_{50}, and LC_{90} in µL ml^{-1}) of five essential oils (*Azadirachta indica, Eucalyptus camaldulensis, Citrus sinensis, Moringa oleifera,* and *Datura stramonium*) against Macrosiphum rosae, *estimated* 24 hours post-application using probit analysis. Values in parentheses represent 95% confidence intervals.

Essential oils	N	LC_{10}[1]	LC_{50}[1]	LC_{90}[1]	Slope ± S.E	χ^2
A. indica	250	2 (0.00–3)	21 (13–31)	260 (90–610)	1.240 ± 0.206	1.750
E. camaldulensis	250	2 (0.00–4)	20 (14–29)	260 (110–570)	1.275 ± 0.209	1.230
C. sinensis	250	1 (0.00–2)	17 (10–24)	215 (70–502)	1.195 ± 0.214	1.220
M. oleifera	250	1 (0.00–2)	15 (8–21)	174 (60–420)	1.255 ± 0.223	0.889
D. stramonium	250	1 (0.00–2)	12 (6–16)	125 (50–320)	1.295 ± 0.207	0.876

[1]Values of LC_{10}, LC_{50}, and LC_{90} were estimated 24 hours after application using probit analysis.

Toxicological Bioassay against Rose Aphid (Table 3)

The lethal concentration data (LC_{10}, LC_{50}, LC_{90}) derived from 24-hour probit analysis of essential oils against *Macrosiphum rosae* demonstrated variable insecticidal efficacy. *Datura stramonium* showed the highest potency with the lowest LC_{50} (12 µL L^{-1}), followed closely by *M. oleifera* (15 µL L^{-1}) and *C. sinensis* (17 µL L^{-1}), indicating strong aphidicidal effects at relatively low doses. These oils also exhibited steeper slopes in dose-response curves, suggesting higher mortality acceleration with increased concentration. In contrast, *A. indica* (LC_{50} = 21 µL L^{-1}) and *E. camaldulensis* (LC_{50} = 20 µL L^{-1}) were comparatively less toxic within the same exposure period, though their systemic or residual effects may contribute to delayed mortality. All essential oils showed acceptable goodness-of-fit in the probit models ($\chi^2 < 2$), supporting the reliability of the concentration–response estimates. These results confirm the high potential of *D. stramonium*, *M. oleifera*, and *C. sinensis* as promising botanical alternatives for aphid control due to their acute toxicity and rapid action at low concentrations.

DISCUSSION

Present study demonstrated that five essential oils tested against *Macrosiphum rosae*, at different degrees of efficacy that were clearly dose dependent. Among treatments, essential oil extracted from *Azadirachta indica* constantly induced the highest mortality across all concentrations, showing its potential as a botanical insecticide. Superior control of *A. indica* is mainly attributable to presence of azadirachtin, nimbin and salannin bioactive constituents known to interfere with insect hormonal systems, feeding behavior and molting processes (Singh et al., 2023). Its performance even at lower concentrations (e.g., 58.6% mortality at 3 µL/mL) confirms previous findings by Kaushik and Guleria (2022), who found notable aphidicidal activity of neem based formulations against several aphid species. Moderate performance observed in *Eucalyptus camaldulensis* and *Datura stramonium* essential oils, particularly at higher concentration, is likely due to their distinct volatile constituents. *E. camaldulensis* essential oil is rich in 1,8-cineole and α-pinene which have been linked with neurotoxic effects in insects (Abd El-Ghany et al., 2021; He et al., 2024). *D. stramonium* contains alkaloids such as atropine and scopolamine, which inhibit insect nervous system, although its activity looks less consistent than neem.

On other hand, *Citrus sinensis* and *Moringa oleifera* essential oils displayed comparatively low aphid mortality, especially at concentrations below 9 µL/mL. While *C. sinensis* essential oil contains well known fumigant limonene and contact poison it may not be present in adequate concentration to cause high mortality in current aphid species (Benelli and Pavela, 2023). Similarly, M. *oleifera* EO, although rich in fatty acids and flavonoids, seems to lack potent insecticidal constituents, explaining its limited efficacy (Ogunyemi et al., 2022). Significantly, plant based insecticides such as essential oils provide a safer, environment friendly alternative to synthetic pesticides, which are increasingly associated with pest resistance, environmental pollution and non-target toxicity (Regnault-Roger et al., 2021). Efficacy of A. *indica* EO in particular supports with integrated pest management (IPM) approaches.

This experiment confirms that different EOs significantly in their insecticidal efficacy against M*acrosiphum rosae*, with *Azadirachta indica* oil being most effective. Its high efficacy is characterized to azadirachtin and related limonoids, which are known to interrupt insect growth, feeding and reproduction (Isman, 2020; Raveendran et al., 2022). At highest concentration (15 µL/mL), *A. indica* achieved over 82% mortality, significantly higher than other EOs applied. *Eucalyptus camaldulensis* also showed strong insecticidal efficacy, due to eucalyptol (1,8-cineole) and α-pinene, which have been linked with neurotoxic characteristics in insects (Oliveira et al., 2021). *Datura stramonium* showed moderate mortality, potentially due to slower action of its alkaloids. In contrast, *Citrus sinensis* and *Moringa oleifera* were nominal, which may associated with lower doses of quick acting bioactives or higher volatility of their main compounds (Al-Tamimi et al., 2022).

Highest efficacy of *Datura stramonium*, *Moringa oleifera*, and *Citrus sinensis* against *Macrosiphum rosae* may be characterized to their high content of compounds such as piperitenone, limonene and thymol. These constituents have been reported to contain neurotoxic characteristics on aphids by interrupting their respiratory and nervous systems (Mossa et al., 2023; Benelli and Pavela, 2022). *Azadirachta indica*, although rich in nepetalactone isomers, displayed moderate performance, possibly due to slower action or volatile compounds. Nepetalactones perform as feeding deterrents and interfere with aphid reproduction, which may require longer exposure periods to exert full effects (Roh et al., 2022). Moderate toxicity of *Eucalyptus camaldulensis* is consistent with previously reported studies carvacrol and 1,8-cineole as contact and fumigant agents against soft bodied insects (Ali et al., 2022). Diversity in LC_{50} values across essential oils reflects differences in both active compounds and their synergistic relations. For instance, combination of thymol and γ-terpinene in *C. sinensis* has been shown to improve membrane permeability in insects, leading to rapid mortality (Knaak and da Silva, 2023). Similarly, piperitenone and limonene in M. *oleifera* may perform synergistically to impair aphid neuromuscular coordination (Abbas et al., 2024).

Current experiment clearly depicted that all five EOs had insecticidal effects on *Macrosiphum rosae*, with significantly variability in efficacy. Significant, *Azadirachta indica* and *Eucalyptus camaldulensis* produced highest mortality, mainly at LC_{90} levels, where mortality values reached 154.00 and 162.00 respectively. These results agree with presence of azadirachtin and 1,8-cineole, compounds known for their neurotoxic and growth disrupting characters (Ghosh et al., 2019; Isman, 2020). In contrast, although *Datura stramonium* showed lowest LC_{50} (12 µL/mL), its aphidicidal effect plateaued at higher concentration, limiting its practical effectiveness. Similarly, *Moringa oleifera* and *Citrus sinensis* showed lower aphid mortality across all doses, due to less potent or lower concentrations of active volatiles compounds like limonene and piperitenone (Benelli and Pavela, 2023). Heatmap analysis further authenticate that *A. indica* and *E. camaldulensis* grouped together as most toxic oils at higher concentrations, strengthening their suitability as plant based insecticides in integrated pest management strategy. Their highest efficacy under controlled conditions permits further investigation under semi field and field conditions to confirm persistence and non-target properties.

CONCLUSION

Present experiment exhibited that EOs extracted from *Datura stramonium*, *Moringa oleifera* and *Citrus sinensis* exhibit strong insecticidal property against M. *rosae*, with significantly low LC_{50} values. Performance of these EOs is closely linked with their rich content of bioactive compounds like piperitenone, limonene and thymol. Although *Azadirachta indica* and *Eucalyptus camaldulensis* also indicated insecticidal potential, their effects were comparatively moderate. Overall, findings support use of selected plant based EOs as promising botanical alternatives to synthetic insecticides for sustainable aphid management in horticultural systems. Further studies on formulation stability and field validation are recommended to improve practical application.

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