

## BIOINSECTICIDAL ACTIVITY OF PLANT ESSENTIAL OILS AGAINST ROSE SAWFLY *Arge ochropus* (HYMENOPTERA: ARGIDAE)

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### ABSTRACT

The rose sawfly, *Arge ochropus* (Gmelin) (Hymenoptera: Argidae), is one of the most destructive insect pests attacking rose bushes. The increasing demand for eco-friendly control of insect pests has provoked a growing interest in biopesticides. In this study, the toxicity of three plant-based essential oils (PEOs); mint (*Mentha piperita*), eucalyptus (*Eucalyptus camaldulensis*), and lemongrass (*Cymbopogon citratus*) was tested against *A. ochropus* larvae using a leaf dip bioassay. The experiment was carried out using factorial with completely randomized design (CRD) in which concentration of each PEO was replicated six times. GC-MS analysis showed major compounds in plant essential oils: cubenol (8.4%) in *M. piperita*, 3,8-nonadien-2-one (23.6%) in *C. citratus*, and eucalyptol (42.3%) in *E. camaldulensis*. Results indicated a larval mortality rate of 70.4% after 48 hours of exposure to a 8.0% concentration of *M. piperita* oil. At 72 hours of exposure, the mortality rate was increased to 85.9%. *E. camaldulensis* at higher concentrations displayed a moderate efficacy, where 46.5% larval mortality was recorded at 48 hours and 70.9% mortality at 72 hours of application. The least effective PEO was *E. camaldulensis*, which showed only 50.2% larval mortality at 72 hours using higher concentrations. Probit analysis also confirmed the higher toxicity of *M. piperita* oil, with the lowest LC<sub>50</sub> values at both 48 hours (1.96%) and 72 hours (1.08%) compared to other PEOs. Our findings highlight that mint oil holds a significant promise as a potent biopesticide that could be integrated into sustainable management strategies against *A. ochropus*.

**Keywords:** *Arge ochropus*; botanical insecticides; eco-friendly pest control; lethal concentrations; bio-efficacy

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### INTRODUCTION

The sawfly, *Arge ochropus* (Gmelin) (Hymenoptera: Argidae) is a destructive insect pest of various crops and ornamental plants, and causes serious economic losses in the agricultural sector (Zhao and Hua, 2016). The larval stage of this pest voraciously feeds on foliage that leads to defoliation, stunted plant vigor, and low productivity (Sheykhnejad *et al.*, 2014). Rose plant is affected by several insects, such as thrips, aphids, whiteflies, leafhoppers, scales, and termites that attack different parts at every phenophase of growth of rose plants (Hegde *et al.*, 2020). *Arge ochropus* is widely distributed in Asia, including Pakistan (Perveen *et al.*, 2024). The larval feeding marks rose twigs to dry out with premature leaf drop and ultimately reduces both the quality and yield of the rose plants (Bolu *et al.*, 2021). The application of synthetic insecticides (such as spinetoram, flonicamid, chlorantraniliprole, gamma-cyhalothrin, spinosad, bifenthrin, carbofuran,

cypermethrin, and flubendiamide) is still a primary source of traditional insect pest control (Latif *et al.*, 2019; Khan *et al.*, 2021). Although these insecticides are effective, there are some serious implications, including toxicity to non-target organisms, environmental pollutants, and the development of resistance in insects (Wan *et al.*, 2025). These issues highlight a dire need to develop some alternate options for the sustainable management of pests. Plant-based biopesticides emerged as a valuable substitute for synthetic insecticides and are being regarded as a new and promising source of pest control (Wojciechowska *et al.*, 2016; Mawcha *et al.*, 2024).

The use of plant-based essential oils (PEOs) has become a prospective direction among biopesticides. Such PEOs and other secondary metabolites are being explored as environment-friendly (Souto *et al.*, 2021) and have several bioactivities, including repellent, ovicidal, and antifeedant effects against a wide number of insect pests (Kheloul *et al.*, 2019; Souto *et al.*, 2021; Zerkani *et al.*

*al.*, 2022). PEOs have several benefits, including less non-target effect, a large number of plant species, being safer for humans and the environment, and their natural biodegradability. Therefore, these PEOs may be applicable in the effective control of pests and minimizing the issue of resistance development in pests (Li *et al.*, 2022; Hu *et al.*, 2022).

Plants of the *Mentha* genus belong to the Lamiaceae family, commonly referred as mint, and are distributed globally. Most of the species from this genus are well-known for the production of economically significant secondary metabolites (Hajlaoui *et al.*, 2008). The antifeedant and repellent properties of *Mentha* spp. have been reported earlier against a wide range of insect pests, such as stored product pests including pulse beetles, *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae), rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Singh *et al.*, 2012), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Eliopoulos *et al.*, 2015) and field crop pests such as, *Aphis craccivora* Koch (Hemiptera: Aphididae) (Saifi *et al.*, 2023) and armyworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) (Kimbaris *et al.*, 2017). This underscores their potential to use as natural pest control agents (Sánchez-Borzone *et al.*, 2017). Similarly, lemongrass, *Cymbopogon citratus* (Poaceae), is also commercially produced worldwide (Tisserand and Young, 2013) and has been investigated against various insect pests such as granary weevil, *Sitophilus granarius*, and potato tuber moth *Phthorimaea operculella* (Plata-Rueda *et al.*, 2020; Jovanović *et al.*, 2020). Eucalyptus, *Eucalyptus camaldulensis* (Myrtaceae) is also a widespread plant in tropical and subtropical areas due to its easy cultivation, high adaptability, and rapid growth (Coppen, 2002). It has been reported that Eucalyptus oil has insecticidal qualities against a variety of insect pests, including coleopteran pests such as *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (Ebadollahi and Jalali Sendi, 2015; Parsia Aref *et al.*, 2016).

Several PEOs have been reported as promising agent against various species of insect pests, but their potential against *A. ochropus* remains undiscovered. No comprehensive studies are available on the effective management of *A. ochropus*, which is a serious defoliator of roses. Thus, there is a dire need for an eco-friendly management of this pest. To address this, our main objective was to evaluate the bioinsecticidal activity of selected plant essential oils against *A. ochropus* larvae. As far as we know, this study is novel in being the first attempt to evaluate PEOs in controlling this pest, thus contributing to more sustainable rose protection strategies.

## MATERIALS AND METHODS

**Insect culture:** Larvae of *A. ochropus* were collected from naturally infested rose plants nearby College of Agriculture, University of Sargodha during March, 2025. The culture was maintained in BioControl laboratory, Department of Entomology, University of Sargodha. Uniform-sized larvae (third instar) were selected and maintained at controlled conditions of  $25 \pm 2^\circ\text{C}$  temperature, and  $65 \pm 5\%$  relative humidity. Larvae were fed on fresh and soft leaves of rose plants. The pupae were kept in clean Petri plates and placed in adult rearing cages ( $30 \times 30 \times 30$  cm). As an adult feed, honey solution (10%) soaked in sterile cotton balls were placed in cages. The seedlings of rose plants were placed in cages to facilitate oviposition. The female laid eggs on soft young rose stems, and the newly emerged larvae were collected for further experiment.

**Plant oils:** Three plant essential oils, *E. camaldulensis*, *C. citratus*, and *M. piperita* were used for the bioassay. The extraction of oil was done as suggested by Paw *et al.* (2020). The fresh leaves of the plants were washed in running water to remove all dirt and insects. After cutting into small pieces, the extraction was done using hydrodistillation. 100 g of each plant was put in a 2-L flask that was filled with 800 mL of distilled water. Hydrodistillation was conducted using a Clevenger -type apparatus. The mixture was heated at  $50^\circ\text{C}$  in a heating mantle for 3 hours. At the end of the distillation process, we noticed two different phases: the aqueous phase, known as the aromatic water, and the organic phase containing the essential oil. The essential oil layer on top of the aqueous phase was gently decanted using a separator funnel. Samples were dehydrated with sodium sulphate and kept in a closed glass flask and stored at  $4^\circ\text{C}$  until further use (Altaf *et al.*, 2024). Five concentrations: 0.5%, 1.0%, 2.0%, 4.0%, and 8.0% per liter of water (v/v) of each EPO were prepared, and 0.1% Tween-80 was added as an emulsifier. In the control treatment, distilled water plus 0.1% Tween-80 was used.

**GC/MS analysis of plant essential oils:** Gas chromatography–mass spectrometry (GC–MS) was conducted using an Agilent 5977B GC system equipped with a single quadrupole mass spectrometer (Agilent, USA), as described by Malik *et al.* (2022). Separation was done on a 0.25mm DB-5MS fused-silica capillary column (0.25mm in diameter, J&W Scientific, Folsom, CA). The chemical composition of PEOs was determined by mass-to-charge (m/z) ratio, and the results were compared with the MassHunter software library. Recording of mass spectra was done at 50–650 m/z range at a scan rate of 1.5 scans per second. The MS source temperature was  $230^\circ\text{C}$ , and the Quadrupole MS temperature was  $150^\circ\text{C}$ . The carrier was helium gas at a flow rate of 1 mL/min.

**Leaf dip bioassay:** The leaf dip bioassay method was used to check the insecticidal activity of PEOs against *A. ochropus* larvae (Saleem *et al.*, 2024). Healthy leaves from the rose plant were taken, washed, air-dried, and cut into uniform sections. Each leaf section was dipped into the oil solution for 10 seconds. Leaves were then air-dried for 30 minutes at room temperature to allow excess solution to evaporate. Dried samples of leaves were then kept in Petri plates lined with moist filter paper. Ten larvae of the 3rd instar were introduced into each Petri plate. Each concentration of each PEO was replicated six times, totaling 60 larvae per concentration. The experiment was performed in under controlled laboratory conditions of  $25 \pm 2^\circ\text{C}$  temperature and  $65 \pm 5\%$  relative humidity. Three plant oils (Factor A) with 5 concentrations, i.e., 0.5%, 1.0%, 2.0%, 4.0%, and 8.0% (Factor B) of each were designed in the experiment, and each concentration was replicated 6 times. The mortality data of larvae were recorded after 48 and 72 hours of post-treatment, and the data were corrected using Abbott's formula (Abbott, 1925).

**Data analysis:** The data normality was assessed using the Shapiro–Wilk normality test; however, original means are given in figure 2. Corrected mortality data were

analyzed by using a 2-factor factorial analysis of variance (ANOVA) under a completely randomized design by keeping plant oils and concentrations as main factors. Means were separated with the Tukey HSD all pairwise comparison test at 5% level of significance using SPSS 20.0 software. However, probit analyses were done using Minitab 17.0 software to calculate the lethal concentrations.

## RESULTS

**Volatile profile of plant essential oils:** Total yields of PEOs were 71.27%, 52.5% and 94.56% (w/w) for *C. citratus*, *M. piperita*, *E. camaldulensis*, respectively. Total compounds identified in the essential oils were 77 in *C. citratus*, 73 in *E. camaldulensis*, and 26 in *M. piperita*, respectively. Major compounds in *M. piperita* were cubenol (8.43%), trans-carveyl acetate (5.71%), and  $\alpha$ -cadinol (5.6%), while in *C. citratus*, 3,8-nonadien-2-one, 4,8-dimethyl- (23.66%), followed by neral (6.22%) and (Z)-2,3-epoxydecane (5.99%) were found. In *E. camaldulensis*, eucalyptol (42.21%),  $\alpha$ -pinene (12.09%), and pinocarveol (5.76%) were identified as the major compounds (Figure 1).

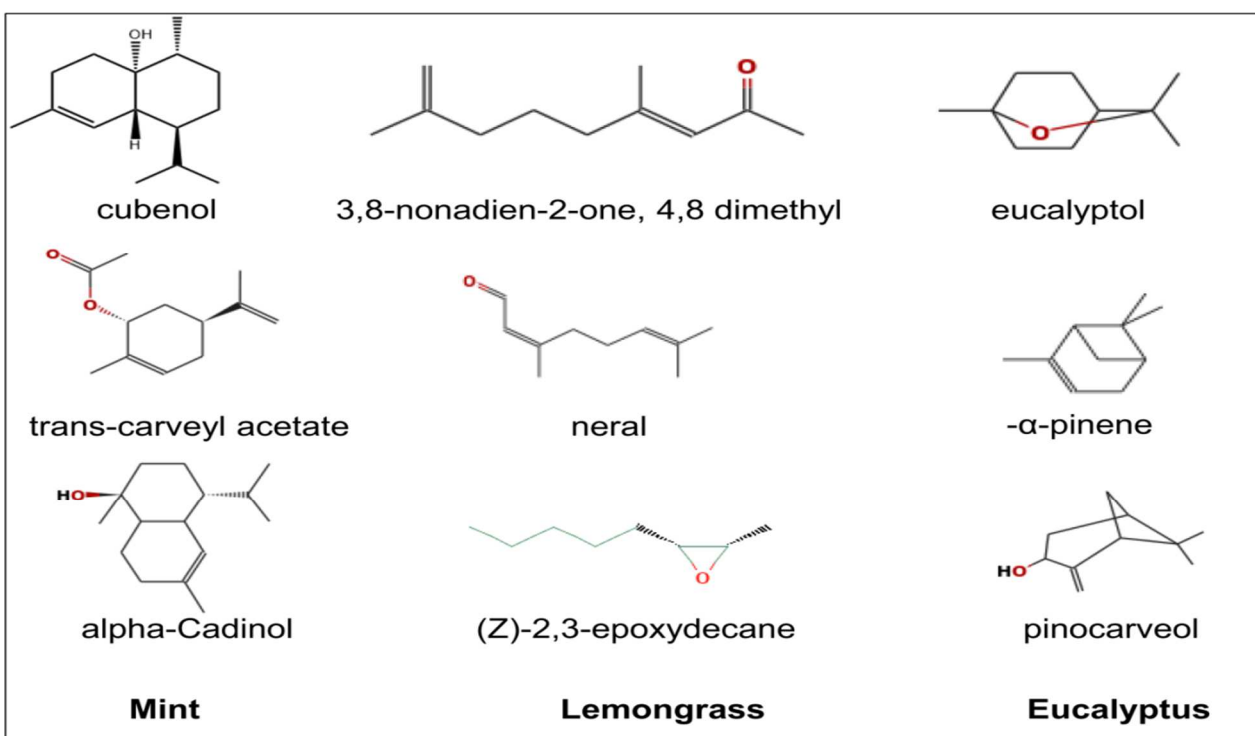


Figure 1. Molecular structures of major chemical compositions in the tested plant essential oils, source: <https://webbook.nist.gov/chemistry/name-ser/>

**Toxicity of PEOs against *Arge ochropus*:** A significant effect of PEOs ( $F_{2,89} = 74.4$ ,  $P < 0.001$ ) at different concentrations ( $F_{4,89} = 47.7$ ,  $P < 0.001$ ) was recorded on

*A. ochropus* larval mortality at 48 hours of exposure. However the interactive effect of PEOs and concentrations was non-significant ( $F_{8,89} = 0.85$ ,  $P > 0.05$ ). The highest

larval mortality (70.4%) was achieved using *M. piperita* oil at 8.0% concentration. However, *C. citratus* and *E. camaldulensis* oils, even at higher concentration were less effective with 46.5% and 37.9% larval mortality, respectively. Similarly, at 72 hours of exposure, PEOs ( $F_{2,89} = 69.6, P < 0.001$ ) and their concentrations ( $F_{4,89} = 78.5, P < 0.001$ ) showed a significant effect on the larval mortality. The interactive effect of PEOs and

concentrations was also significant ( $F_{8,89} = 2.18, P < 0.05$ ). The highest larval mortality was achieved by using *M. piperita* oil, and the mortality rate was 72.6% at 4.0% and 85.9% at 8.0% concentrations. At higher concentration, 70.9% larval mortality was attained by *C. citratus*. The lowest mortality of 50.2% was recorded when *E. camaldulensis* at 8.0% concentration was applied (Figure 2).

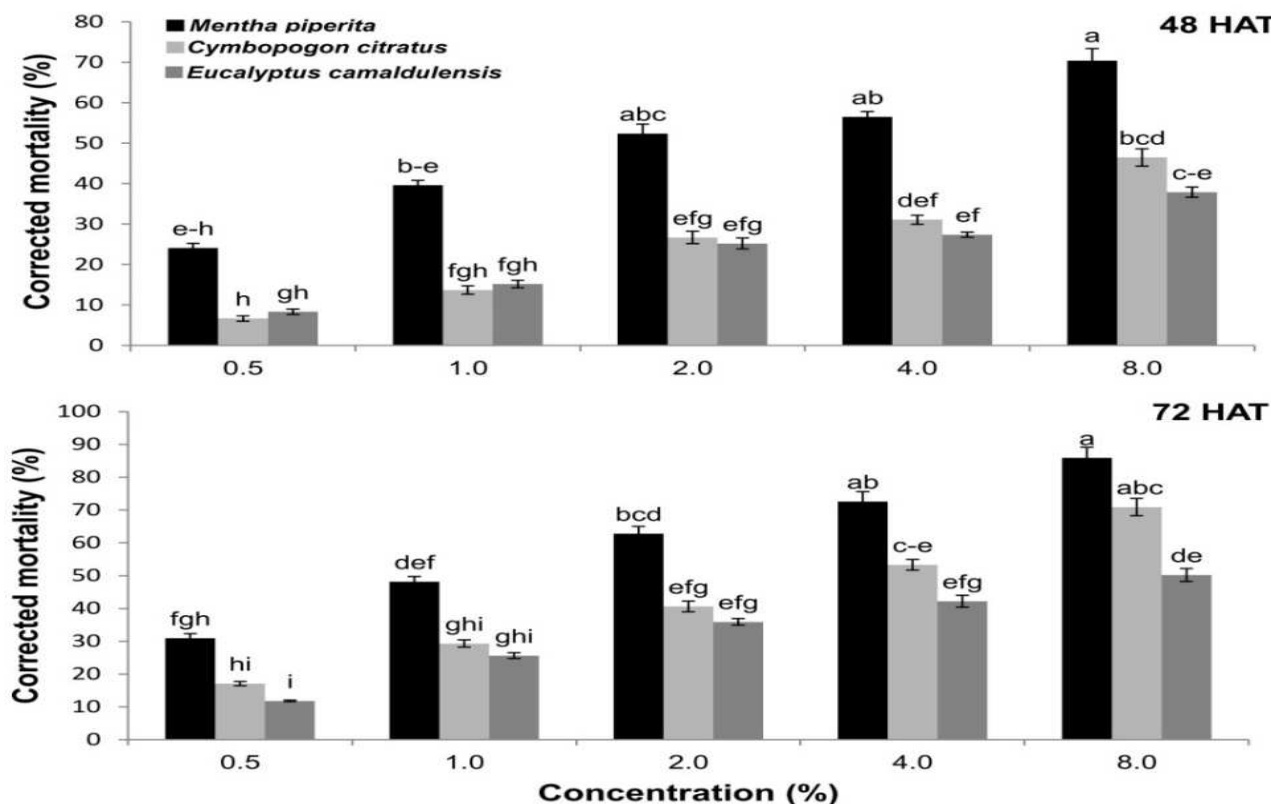


Figure 2. Percent (means ± SE) corrected mortality of *Arge ochropus* larvae exposed to plant essential oils at various concentrations at 48 and 72 hours of exposure, means sharing similar letters (plant oil x concentrations) are not significantly different at  $P > 0.05$

At 48 hours, the lowest  $LC_{50}$  value was recorded in the case of *M. piperita* (1.96%), compared to *C. citratus* (8.81%), and *E. camaldulensis* (17.6%). After 72 hours, *M. piperita*'s potency increased further as the  $LC_{50}$  value decreased to 1.08%.  $LC_{50}$  value for *C. citratus* was 2.81% and for *E. camaldulensis* was 8.24%, after 72 hours (Table 1).

Table 1. Probit analysis for plant essential oils against *Arge ochropus* larvae

Plant oils	$LC_{50}$ (%)	95% CL	$\chi^2$	P
<b>48 hours</b>				
<i>M. piperita</i>	1.96	1.32-2.88	7.64	< 0.001
<i>C. citratus</i>	8.81	5.56-21.1	12.87	< 0.001
<i>E. camaldulensis</i>	17.6	7.94-112.2	6.58	< 0.001
<b>72 hours</b>				
<i>M. piperita</i>	1.08	0.73-1.44	11.5	< 0.001
<i>C. citratus</i>	2.81	1.84-2.08	7.65	< 0.001
<i>E. camaldulensis</i>	8.24	3.79-15.7	6.11	< 0.001

Total number of insects (n) = 300 per plant oil, five concentrations were used to obtain  $LC_{50}$ , and six replicates were used for each concentration; CL = confidence limit,  $\chi^2$  = chi-square

## DISCUSSION

*Arge ochropus* is an important defoliator pest on rose bushes, and its larvae are external leaf feeders that cause substantial damage and defoliation in the ornamental plants (Sahragard and Heidari, 2001). The bioactivity of essential oils derived from *E. camaldulensis*, *C. citratus*, and *M. piperita* against *A. ochropus* larvae. Overall, all PEOs showed time and dose-dependent toxicity. According to our findings, *M. piperita* showed the highest insecticidal activity against *A. ochropus* compared to other oils; therefore, it can be considered an effective botanical agent for pest management. The higher mortality rate with the application of *M. piperita* oil can be ascribed to its rich composition of bioactive secondary metabolites, which are well-known for their antifeedant and repellent effects against various insect pests (Khani and Asghari, 2012; Tourabi *et al.*, 2023).

Some chemical constituents in PEOs have potential of insecticidal activities against insect pests (Kumar *et al.*, 2011). The presence of more than one compound in the *M. piperita* oil may be considered an advantage in pest control. Previously, researchers have reported different compounds in the essential oil of *M. piperita*, including menthone, 1,8-cineole, menthyl acetate,  $\alpha$ -terpinene, isomenthone, caryophyllene,  $\beta$ -pinene, trans-carveol, pipertitinone oxide, D-limonene,  $\alpha$ -pinene, and  $\beta$ -caryophyllene (Djenane *et al.*, 2012). However, in our study, higher content of cubenol, trans-carveyl acetate, and alpha-Cadinol was found in the oil of *M. piperita*. However, the variation in the chemical composition of PEOs may be influenced by climatic conditions and other factors such as the plant part utilized, plant maturity, the stage of the vegetative cycle, genetic differences among plants, and time of harvest (Tourabi *et al.*, 2023). There could be another possibility of contribution of certain minor constituents or a synergistic effect of various constituents in controlling insect pests. As reported by Isman (2020), monoterpenes, phenylpropanoids, and sesquiterpenes are among the main constituents of various PEOs that are important in their effectiveness against insect pests. Our findings related to the higher effectiveness of *Mentha* oil are supported by previous studies such as Mackled *et al.* (2019) and Tourabi *et al.* (2023), who reported the insecticidal activity and chemical characterization of different wild populations of *Mentha longifolia* oil in controlling insect pests. Gospodarek *et al.* (2023) reported the impact of *M. piperita* oil against aphids. Prasannakumar *et al.* (2023), reported that *M. piperita* oils showed greater control of *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelichiidae).

The higher activity of *M. piperita* oil was also confirmed with probit analysis, with a lower LC<sub>50</sub> value as compared to the other two oils. The observed toxicity

of *M. piperita* oil can be attributed to the ability to cause an injury to the normal functioning of the nervous system of insects, as described by Chaubey (2017) and Djemaa and Hayette (2023). The presence of major compounds in the plant oils can also lead to the malfunctioning of the insect respiratory and digestive systems, leading to death (Kumar *et al.*, 2011). The efficacy of PEOs against insect pests is largely influenced by the plant type where EO is collected (Abdelgaleil *et al.*, 2016; Al-Harbi *et al.*, 2021), the insect life-stage (El Kasimi *et al.*, 2023; Kavallieratos *et al.*, 2024), and environmental factors (Mehalaine and Chenchouni, 2021), which influence composition, volatility of oils, and insect behavior and physiology.

The usage of *M. piperita* in the field may be beneficial to protect rose plantations and could be an attractive possibility to be utilized in an integrated pest management (IPM) strategy. However, PEOs' insecticidal performance under field conditions still needs to be studied in the context of environmental limitations. As these oils are biodegradable, eco-friendly, and safer for humans, they might be integrated into a sustainable rose protection program. Their integration into pest management programs may be helpful in reducing pesticide use, resistance development in insects, and support environment friendly horticultural practices. Future studies should focus on developing stable formulations of these oils and evaluating their compatibility with other biological control agents.

**Conclusion:** *M. piperita* oil showed strong insecticidal activity against *A. ochropus* larvae, which supports its potential role as a natural alternative to chemical insecticides. Its efficacy and eco-friendly control make it a promising candidate in integrated pest control strategies for future use.

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