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ANTIOXIDANT AND LARVICIDAL ACTIVITIES OF *Pistacia lentiscus* FRUIT ESSENTIAL OIL

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ABSTRACT

Pistacia lentiscus or mastic tree, a medicinal plant from Anacardiaceae family, is widely used in Algerian traditional medicine for its therapeutic properties. This study aimed to extract essential oil (EO) from its dried fruits by hydrodistillation using a Clevenger apparatus and determine its yield of extraction. The antioxidant activity of the EO was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, with ascorbic acid (AA) as a positive control, at concentrations of 0.25, 0.5, 1.0 and 2.0 µg/ml. In addition, its insecticidal activity was tested against third and fourth instar larvae of *Culex pipiens* at concentrations of 5, 10, 15, and 25 µl/ml. The EO yield was 0.4%, consistent with values reported in previous studies. Antioxidant tests showed significant radical scavenging activity, with inhibition ranging from 45.91 to 68.46% compared with 49.52 to 73.31% for AA, and Inhibition concentration IC_{50} values were 0.62 µg/ml and 0.12 µg/ml for the two samples respectively. Larvicidal assays revealed concentration-dependent mortality, meaning that higher concentrations caused higher mortality in both larval stages, with lethal concentrations LC_{50} and LC_{90} values estimated at 10 and 27 µl/ml for third instar larvae; 17 and 32 µl/ml for fourth instar larvae, respectively. These findings demonstrated that *P. lentiscus* fruit EO possesses promising antioxidant and larvicidal properties, suggesting its potential applications in natural pest control and health-related fields.

Keywords: *Pistacia lentiscus,* essential oil, hydrodistillation, antioxidant activity, larvicidal activity.

INTRODUCTION

The genus *Pistacia* belongs to the family Anacardiaceae, which comprises approximately 300–455 genera and 3000–3750 species. Members of this family are common and widely distributed in many regions of the world (Jayakodi *et al.,* 2024). Species of this genus grow in various soil types, particularly siliceous soils (Zaouali *et al.,* 2018).

Among these species, *Pistacia lentiscus* L. is a small evergreen tree widely distributed across the Mediterranean basin and the southwestern Black Sea region. Its use in traditional medicine dates back to antiquity, where it has been employed to treat ailments such as hypertension, gastralgia, dyspepsia, ulcers, and as a diuretic (Abdelwahed *et al.,* 2009). In addition, this plant exhibits a wide range of biological activities, including antioxidant, antimicrobial, anticoagulant, antileishmanial, and antidiabetic effects (Maaroufi *et al.,* 2021; Drioiche *et al.,* 2023; Bouakline *et al.,* 2024).

Synthetic antioxidants have been utilized in the food industry since the 1940s. However, recent trends in health-related industries have shifted preferences toward natural sources. As a result, the investigation of natural antioxidants has become a major research focus over the past two decades. Numerous research groups and institutions have explored plant-derived materials for their potential antioxidant properties. Plants are known to be rich in bioactive compounds that can promote human health and are widely used in the development of supplements and nutraceuticals (Patient *et al.,* 2022). The protective effects of plants are largely attributed to the presence of flavonoids, anthocyanins, and other phenolic compounds (Hemma *et al.,* 2018).

In addition to their role as natural antioxidants, plant extracts, including aqueous extracts and essential oils, have attracted considerable attention for their potential use as selective insecticides. These natural alternatives are being explored to reduce reliance on synthetic pesticides, which, despite their effectiveness, are associated with several negative impacts. The overuse of conventional pesticides has led to harmful side effects such as toxicity to non-target organisms, the development of pest resistance, secondary pest outbreaks, and the accumulation of persistent residues in water, soil, and the food chain (Pinheiro *et al.,* 2020).

By contrast, insecticides derived from plant extracts offer several advantages. They are generally biodegradable, selective towards target pests (especially mosquitoes) and present minimal toxicity to non-target organisms. As a result, these plant-based products represent promising eco-friendly substitutes for synthetic chemical pesticides (Djeghader *et al.,* 2018, 2019; Amira and Djeghader, 2024; Djeghader and Amira, 2024).

Despite the recognized pharmacological potential of *P. lentiscus*, little is known about the biological activities of its fruit essential oil, particularly regarding its combined antioxidant and larvicidal effects. Existing studies have mainly focused on other plant parts or different biological activities (Floris *et al.,* 2024; Bouchfara *et al.,* 2025; Nouar *et al.,* 2025), leaving a gap in understanding the value of fruit-derived essential oil for vector control and oxidative stress mitigation. Furthermore, given the limitations of synthetic insecticides and the health risks associated with synthetic antioxidants, there is a pressing need to identify safe, natural alternatives. Addressing this gap, the present study investigates the antioxidant and larvicidal properties of *P. lentiscus* fruit essential oil. We hypothesize that this essential oil exhibits strong free radical scavenging activity and significant larvicidal potential, making it a promising candidate for natural pest management and health-related applications.

MATERIALS AND METHODS

Plant material: A large quantity of the aerial parts of *P. lentiscus* was collected in October 2022 from trees located in the commune of Beni belaid in the Wilaya of Jijel in northeastern Algeria (Geographical coordinates: 36.87° N latitude and 6.14° E longitude). In the laboratory, the fruits were separated from the other parts, cleaned, air-dried at ambient temperature away from direct sunlight, and then finely ground into powder, which was stored at room temperature in paper bags until extraction.

Extraction of essential oil: The essential oil extraction was conducted in the Laboratory of Physical, Chemistry and Biology of Materials- Higher Normal School of Technological Education of Skikda*,* from the fine powder obtained from the fruits of *P. lentiscus* using the hydrodistillation technique with a Clevenger-type apparatus (Clevenger, 1928). It is performed by boiling the whole or the ground plant. The vapors are cooled and condensed on a cold surface and the essential oil separates from the water as a floating liquid because it is denser (Bruneton, 1995). A total of 250 g of the plant powder was used. The oil began to appear after 25 minutes and was completely collected after 2 hours. The extracted essential oil was packaged and stored at 4°C till further use.

Yield of extraction: The extraction yield (Y) is defined as the ratio between the mass of extracted oil and the mass of plant material used. It is calculated using the following formula (Khajeh *et al.,* 2012):

$$\mathrm{Y\%} = (\mathrm{w1}/\mathrm{w2}) * 100$$

Where w_1 is the weight of the extracted oil and w_2 is the weight of the dried plant.

Antioxidant activity: The antiradical activity is measured by the degradation of DPPH: 2,2-diphenyl-1-picrylhydrazyl, which is a synthetic radical exhibiting an intense purple color. The electron layer of this radical becomes saturated upon contact with antioxidants, resulting in the disappearance of its coloration. This decolorization indicates the ability of the plant extract to scavenge this free radical. The estimation of antiradical activity is measured using different concentrations of the plant essential oil (0.25, 0.5, 1.0 and 2.0 µg/ml) dissolved in ethanol, nd mixed with 2 mL of a DPPH solution. After vigorous shaking, the mixture is left to stand in the dark for 90 minutes. The absorbance is measured at 517 nm using a UV-visible spectrophotometer, with a control sample without the extract. The lower the absorbance, the higher the antioxidant effect. Each concentration test is repeated three times. The same method was used for AA as a standard. The antiradical activity is estimated as a percentage of inhibition using the following formula (Amira *et al.,* 2024):

$$I(\%) = (\mathrm{A0} - \mathrm{As})/\mathrm{A0} \times 100$$

Where A_0: Absorbance of DPPH in solution (negative control or blank) and A_s: Absorbance of Samples

The IC_{50} (Inhibitory Concentration 50%), also known as EC_{50} (Efficient Concentration 50), is defined as the amount or concentration of antioxidants required to inhibit or eliminate 50% of free radicals. It is determined graphically by linear regression of the plot of inhibition percentages versus different antioxidant concentrations. The antioxidant capacity of a compound is higher when its IC50 is smaller (Rguez *et al.,* 2023a).

Larvicidal activity: The larvae of *Culex pipiens* were obtained from a laboratory stock colony and maintained at a temperature of 25-27°C with a photoperiod of 14L:10D. The larvae were fed a mixture of biscuit (75%) and dry yeast (25%), water changes and food additions were carried out every 2 to 3 days, with the quantity varying according to the different stages (Amira and Djeghader, 2024).

The larvicidal effect of *P. lentiscus* essential oil was evaluated on third and fourth-instar (L3, L4) larvae of *Culex pipiens.* A total of 45 freshly molted (L3, L4) larvae, divided into three replicates of 15 larvae each, were placed in containers with 150 ml of stored water. The larvae were exposed for 24 hours to different concentrations (5, 10, 15 and 25 µl) prepared in 1 ml of ethanol (Djeghader and Amira, 2024). Mortality in both control and treated series was recorded daily until the end of the treated stage.

Statistical analysis: Studies were conducted following completely randomized design with three replications and the results are shown as means±standard deviation. One-way analysis of variance was conducted for larvicidal activity whereas two-way ANOVA was utrilized for antioxidant activity tests. If treatments had significant differences, the Tukey test was employed to determine the existence of potential variations among the series themselves. Differences were considered statistically significant at $p < 0.05$ *, highly significant at $p < 0.01$**, and highly significant at $p<0.001$***. IC_{50}, LC_{50} and LC_{90} were determined by simple linear regression. All results (ANOVA tests, IC_{50}, LC_{50} and LC_{90}) were performed using R software (Version 4.2.2).

RESULTS

Yield of extraction: At the end of the extraction, the obtained essential oil was weighed (1.02 g), and the yield was calculated using the formula described above. The extraction yield of *P. lentiscus* essential oil was estimated at 0.40%.

Antioxidant activity: The scavenging activity was found to depend on the concentration of both the studied EO and AA. An increase in concentration resulted in a proportional decrease in absorbance. At the lowest tested concentration (0.25 µg/ml), the absorbance value was 0.530, which decreased to 0.309 at the highest concentration (2 µg/ml). Furthermore, the absorbance values of AA were lower than those recorded for *P. lentiscus* EO, the difference was statistically significant between samples ($p<0.001$), (Fig. 1).

The antioxidant activity, expressed as the percentage of inhibition, increased with concentration for both *P. lentiscus* essential oil and AA.

For EO, the inhibition was consistent at 0.25 and 0.5 µg/ml (45,91 ± 0,27 % and 48,90 ± 0,81 %, letter “ a ”). A marked elevation was detected at 1.0 µg/ml (52.71 ± 0.93%, letter “b”) and 2.0 µg/ml (68.46 ± 0.63%, letter “c”), suggesting concentration-dependency. A similar results were obtained for the ascorbic acid. The two lowest concentrations (0.25 and 0.5 µg/ml) were not significantly different (49.52 ± 1.34%) and 54.07 ± 4.06%) letter “a” but the inhibition became significantly higher at 1.0 µg/ml (67.07 ± 0.66%) letter “c” and at 2.0 µg/ml (73.31 ± 0.94%) letter “d”.

Two-ways ANOVA analysis indicated that both concentration and treatment had significant effect. These findings reveal that the inhibition was concentration dependent, with AA being more effective than EO at higher concentrations (different letters), while the two treatments are comparable at lower concentrations (letter “a”) (Table 1).

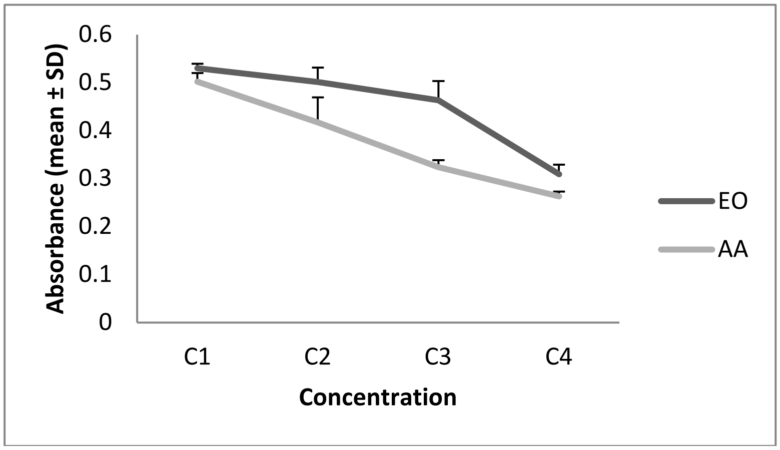


Fig. 1. Absorbance values of *P. lentiscus* essential oil (EO) and ascorbic acid (AA) at different concentrations: C1= 0.25, C2= 0.5, C3= 1.0 and C4= 2.0 µg/ml, (mean ± SD).

Table 1. Antioxidant activity at different concentrations of *P. lentiscus* EO and AA presented as percentage of inhibition.

Concentrations (µg/ml)	EO Inhibition (%)	AA inhibition (%)
0.25	45.91 ± 0.27a	49.52 ± 1.34a
0.5	48.90 ± 0.81a	54.07 ± 4.06a
1.0	52.71 ± 0.93b	67.07 ± 0.66c
2.0	68.46 ± 0.63c	73.31 ± 0.94d

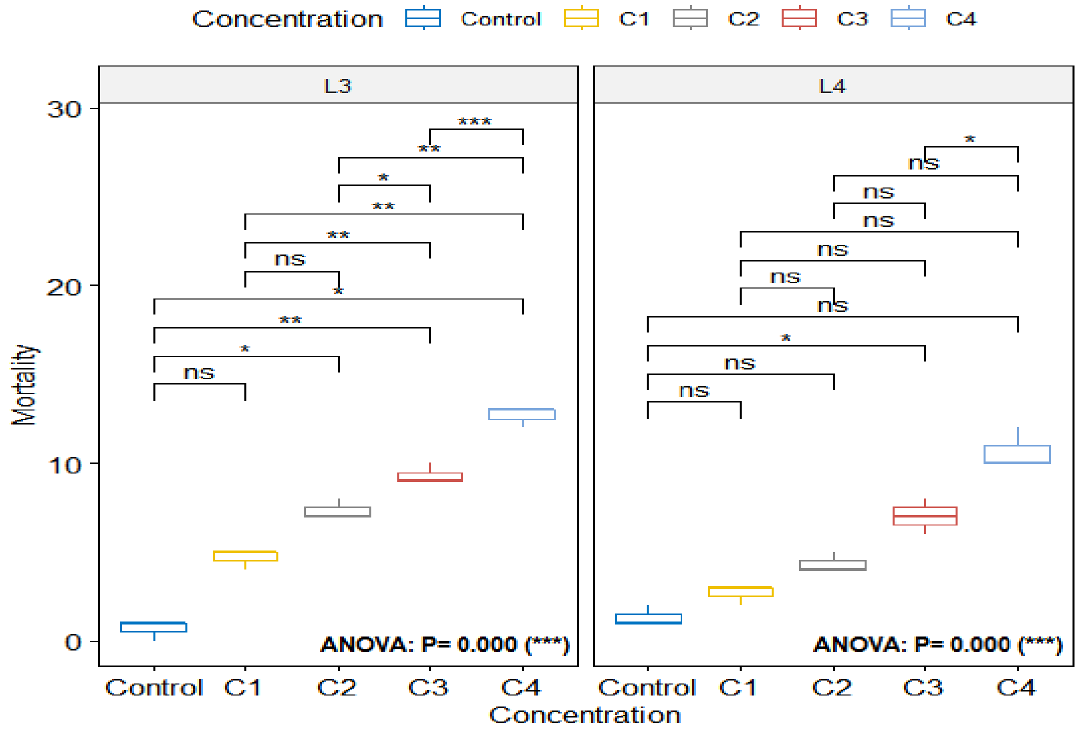
(mean ± SD). Different letters indicate significance differences between means.

The antioxidant activity was expressed as IC$_{50}$, which is defined as the concentration of antioxidant required to reduce 50% of the initial DPPH concentration. The results showed that the positive control (AA) exhibited the strongest antioxidant activity, with an IC$_{50}$ value of 0.12 µg/ml, compared to *P. lentiscus* EO, which showed an IC$_{50}$ value of 0.62 µg/ml. The determination coefficients (R^2) of the two regression equations indicated a very strong positive relationship between concentration and percentage of inhibition (Table 2).

Table 2. Regression equations and IC$_{50}$ values (µg/ml) of *P. lentiscus* EO and AA.

Samples	Equation of regression	R^2	IC$_{50}$ (µg/ml)
P. lentiscus EO	$Y= 12.842x + 41.955$	0.98	0.62
Ascorbic Acid	$Y= 13.57x + 48.271$	0.89	0.12

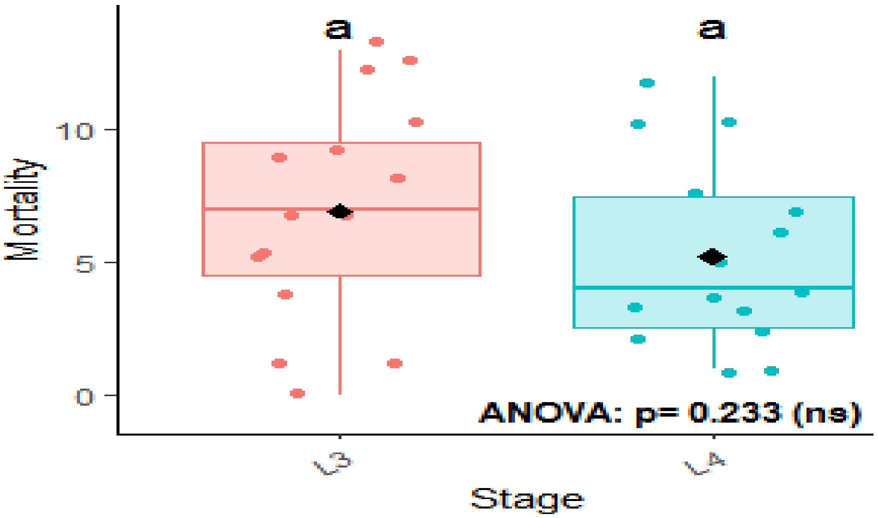
Larvicidal activity: The toxicological study evaluated the effects of different concentrations (5, 10, 15, and 25 µl/ml) of *P. lentiscus* fruit EO on newly molted third and fourth instar larvae (L3 and L4) of *Cx. pipiens*. The results obtained, demonstrated a concentration-dependent increase in larval mortality compared to the control. The one-way ANOVA (concentration factor) revealed a highly significant effect of the tested concentration on larval mortality (p = 0.000). For the L3 stage, mortality increased significantly with increasing concentrations, with a particularly marked effect starting at concentration C3 ($p < 0.05$) and a highly significant effect at concentration C4 ($p < 0.001$). A similar trend was observed for the L4 stage, although significant differences were only detected at the higher concentrations. These findings confirm the concentration-dependent larvicidal activity of *P. lentiscus* EO (Fig. 2).



ns: non significant difference, $p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***.

Fig. 2. Mortality of third and fourth instar larvae of *Cx. pipiens* treated with *P. lentiscus* EO, analyzed using one-way ANOVA. (C1= 5, C2= 10, C3= 15 and C4= 25 µl/ml).

The statistical analysis revealed that the larval stage factor, assessed by one-way ANOVA, had no significant effect on larval mortality (p = 0.233), indicating that third- (L3) and fourth-instar (L4) larvae responded similarly to treatment with *Pistacia lentiscus* essential oil. The absence of a significant difference between the two stages suggests that the larvicidal effectiveness of the essential oil depends primarily on the applied concentration rather than the larval stage (Fig. 3).



Same letter (a) means no significant differences between stages.

Fig. 3. Larval mortality of *Cx. pipiens* treated with *P. lentiscus* essential oil: one-way ANOVA test (stage factor: third and fourth instar larvae).

The lethal concentrations (LC_{50} and LC_{90}) were estimated from the regression equations of the two stages. The LC_{50} values were 10 µl/ml for third-instar larvae and 17 µl/ml for fourth-instar larvae, while the LC_{90} values were 27 µl/ml and 32 µl/ml for L3 and L4, respectively. The coefficient of determination (R^2) indicates a moderate to strong positive relation between mortality and the concentration used (R^2= 0.54 for L3 and R^2= 0.95 for L4) (Fig. 4).

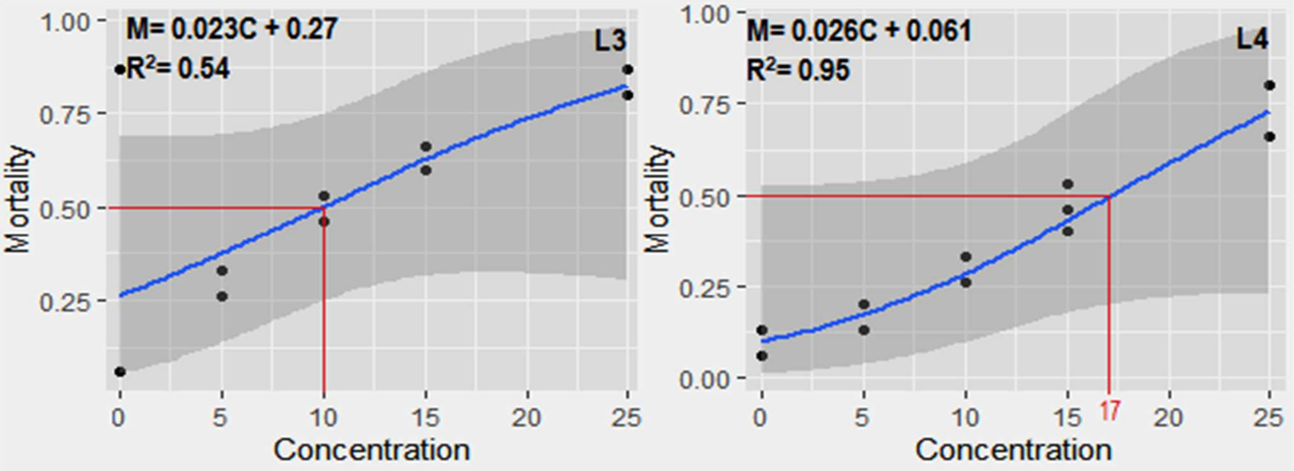


Fig. 4. Regression equations and LC_{50} (µl/ml) values of *P. lentiscus* EO on third and fourth instar larvae of *Cx. pipiens*.

DISCUSSION

Oxidative stress is known to play a key role in the development and progression of numerous diseases, including diabetes, cardiovascular diseases, inflammatory disorders, cancer, and aging. Antioxidants can help mitigate oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and acting through various other protective mechanisms, thereby potentially reducing the risk of these diseases.

The DPPH radical scavenging assay measures the ability of antioxidants to reduce DPPH radicals, which is observed as a color change and a corresponding decrease in absorbance with increasing extract concentration. In the present study, *P. lentiscus* essential oil exhibited high inhibition activity, consistent with the findings of Haddi *et al.* (2024), who reported strong free radical scavenging activity in methanolic extracts of the leaves and fruits of the same plant, with IC_{50} values of 0.121 mg/ml and 0.26 mg/ml, respectively. The difference in concentrations between the two studies is attributed to the nature of the extract used (Essential oils often exhibit higher antioxidant activity than other extracts, such as alcoholic extracts). In general, differences in the intensity of free radical scavenging activity among essential oils are attributed to variations in their chemical composition, particularly the presence of phenolic compounds and flavonoids (Agati *et al.,* 2020; Barbouchi *et al.,* 2020; Drioiche *et al.,* 2023). Based on these results, a relationship can be established between the high free radical scavenging activity of *P. lentiscus* extract and the elevated content of phenolic compounds in the fruits of this plant. Furthermore, the present study demonstrated that the essential oil of *P. lentiscus* exhibits significant toxicity against the larvae of the mosquito *Cx. pipiens*. Statistical analysis revealed a clear concentration-dependent response, with larval mortality increasing as the concentration of the essential oil increased. This trend was further supported by the calculated lethal concentrations (LC_{50} and LC_{90}), which confirmed the effectiveness of the essential oil in eliminating mosquito larvae. These findings are consistent with previous studies reporting the insecticidal activity of *P. lentiscus* essential oils against mosquito larvae and other insect species (Traboulsi *et al.,* 2002; Bachrouch *et al.,* 2010; Behi *et al.,* 2019; Tabti *et al.,* 2020). The toxic effect of *Pistacia lentiscus* essential oil against *Cx. pipiens* larvae is mainly attributed to its complex chemical composition, which is rich in bioactive molecules with insecticidal properties. Among the principal identified constituents are monoterpenes and sesquiterpenes (Zaouali *et al.,* 2018; Rguez *et al.,* 2023b; Belkessam *et al.,* 2025). Monoterpenes, the main constituents of essential oils, are lipophilic compounds capable of penetrating the insect cuticle and exhibiting high vapor pressure, which contributes to their fumigant toxicity (Tarelli *et al.,* 2009). These compounds primarily exert their insecticidal effects through neurotoxic mechanisms, including modulation of ion channels, inhibition of acetylcholinesterase (AChE) activity, and interference with gamma-aminobutyric acid (GABA) receptors, ultimately leading to paralysis or death (Peixoto *et al.,* 2015). Moreover, monoterpenes can induce cytotoxic effects by disrupting cell membranes, interfering with metabolic processes, and generating reactive oxygen species (ROS), which in turn cause oxidative stress and damage to essential cellular components such as DNA and proteins. Some monoterpenes may also impair mitochondrial function, leading to energy depletion and ultimately resulting in cell death (Qasim *et al.,* 2024). In addition to their neurotoxic effects, essential oils and their monoterpenes may also target other enzymes involved in insect detoxification, such as cytochrome P450 monooxygenases and carboxylesterases (Xie *et al.,* 2020).

Furthermore, the multi-target mode of action of these compounds may explain the effectiveness of *Pistacia lentiscus* essential oil, even at relatively low concentrations. Unlike conventional chemical insecticides, which often act on a single target site, *P. lentiscus* essential oil affects multiple biological pathways simultaneously, thereby reducing the likelihood of resistance development in mosquito populations.

The antioxidant and larvicidal activities observed in this study can be justified by the chemical composition of *P. lentiscus* essential oil, which is typically rich in monoterpenes such as α-pinene, limonene, β-myrcene, and terpinen-4-ol (Bouchfara *et al.,* 2025). These compounds act as effective hydrogen donors, contributing to radical scavenging activity, while also exhibiting neurotoxic and respiratory inhibitory effects on mosquito larvae. Additionally, the variation in biological activities reported in the literature is often linked to differences in chemical composition due to geographic and environmental factors, which influence the proportion of key compounds (Sehaki *et al.,* 2022; Benterrouche *et al.,* 2023; Bouchfara *et al.,* 2025). Thus, the combined presence of these bioactive molecules and the influence of geographic origin likely account for both the strong antioxidant effect and the significant larvicidal activity recorded for *P. lentiscus* essential oil.

These properties highlight *Pistacia lentiscus* essential oil as a promising natural alternative to synthetic insecticides for the control of *Cx. pipiens*, offering a potentially more environmentally friendly and safer option for human health.

Conclusion: The essential oil extracted from *P. lentiscus* fruits exhibited significant antioxidant and larvicidal activities. These effects are likely attributed to the high content of secondary metabolites, particularly terpenes present in the plant. The results obtained in this study support the traditional use of *P. lentiscus* in folk medicine in Algeria. However, this study has certain limitations. The chemical composition of the essential oil was not analyzed, which limits the ability to correlate specific compounds with biological activities. Future research should include GC-MS profiling of the oil to identify its major constituents and establish clear relationships between chemical composition and biological effects.

Conflict of interest: The authors declare no conflicts of interest.

Author's contribution: KA and ND performed experiments and Data analysis. The manuscript was written by KA. All authors revised and approved the final version.

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