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Efficacy Of Essential Oil From Algerian Rosemary (Rosmarinus Officinalis) As A Fumigant For Controlling Cydia Pomonella (Lepidoptera: Tortricidae) Larvae

Rahmouni Riyadh* [1], **Mezerdi Farid** [1]
[1]Department of Agronomic Science, Laboratory of Ecosystem Diversity and Agricultural Production System Dynamics in Arid Zones (DEDSPAZA), University of Mohamed Khider, BP 145 RP, 07000, Biskra, Algeria;
Email: riyadh.rahmouni@univ-biskra.dz[1*], f.mezerdi@univ-biskra.dz[1]

Abstract
The codling moth, Cydia pomonella, is a devastating pest for apple cultivation, and its increasing resistance to conventional controls necessitates the development of sustainable alternatives. This study investigates the potential of essential oil from Algerian wild rosemary (Rosmarinus officinalis) as a natural fumigant against C. pomonella larvae, determining its chemical composition and fumigant toxicity.The essential oil was extracted by hydrodistillation and analyzed by GC-MS. Fumigant toxicity was assessed by exposing fifth-instar larvae to various oil concentrations (2.08 to 16.16 µL L⁻¹ air). LC_{50} and LT_{50} values were calculated using probit analysis.The oil yield was 0.51% (v/w). The GC-MS analysis revealed a 1,8-Cineole chemotype, with this compound being the most abundant at 51.47%. The oil showed potent fumigant activity dependent on concentration and exposure time, achieving 100% mortality. The 24-hour lethal concentration (LC_{50}) was determined to be 08.72-µL L⁻¹ air, while the lethal time (LT_{50}) at the highest concentration was 14.13 hours. The essential oil of Algerian Rosmarinus officinalis, characterized by its high 1,8-Cineole content, exhibits strong fumigant toxicity against Cydia pomonella larvae. These findings position it as a promising candidate for developing a local botanical insecticide for post-harvest protection, offering an effective alternative to synthetic pesticides

Keywords: *Cydia pomonella, Rosmarinus officinalis, biopesticides, 1, 8-Cineole, chemotype.*

1. INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the world's most important fruit crops, representing a cornerstone of agriculture in many regions, including Algeria [1]. However, the sustainability of this industry faces continuous and multifaceted threats, including invasive pests, fungal and bacterial diseases, and adverse climatic conditions like spring frost [1, 2, 3]. The scale of the economic risk is highlighted by estimates suggesting that losses in a single major production region could exceed $500 million from the spread of a new quarantine pest, with costs directly linked to existing control programs [4]. Globally, apple cultivation spans 4.62 million hectares, yielding over 97 million tons annually, with Algeria's production reaching 539,852 tonnes in 2023, underscoring the importance of protecting this vital sector [5]

Invasive insect pests represent one of the most significant threats to this vital production, as they are capable of causing severe losses from the field through to the post-harvest stage. Among all arthropod pests, lepidopteran larvae, particularly from the Tortricidae family, are the primary cause of fruit damage in apple orchards across diverse climates [6]. The codling moth, *Cydia pomonella* L., stands out as a global invasive pest and has become an important quarantine object in many countries [7]. Genetic studies confirm its alien status in Algeria, suggesting an introduction from Europe followed by successful and widespread establishment [8]. The pest has an exceptional capacity for invasion, as a single mating pair can establish a new population [9], and its larvae cause severe economic damage by tunneling into the

fruit. Fresh fruits, along with packaging and padding materials, can act as primary vectors for spreading this moth by harboring its hidden larvae and pupae [7].

The threat posed by this pest is projected to intensify with climate change, as models indicate a potential northward expansion of its geographic range [10],with temperature being the most dominant climatic factor determining its suitability [10]. Furthermore, its resilience is compounded by complex biological strategies; it enters a winter diapause supported by remarkable physiological cold hardiness, lowering its supercooling point to approximately -20°C [11]. This biological complexity makes control timing critical, necessitating advanced tools like phenological models integrated with field monitoring data for precise decision-making [12 , 13].

Historically, control strategies relied on chemical insecticides, but this approach is now hampered by widespread pest resistance [6]. The problem is particularly acute in the post-harvest stage, where larvae hidden inside harvested fruit can continue to cause damage, impeding international trade [4]. Conventional fumigants like methyl bromide and phosphine face increasing restrictions due to phytotoxicity, health and environmental risks, and the emergence of resistant strains [14]; [15]. Furthermore, alternative solutions have not proven to be panaceas; biological methods, including viruses (CpGV), the Sterile Insect Technique (SIT), and entomopathogens (EPNs & EPFs), face challenges of pest resistance or operational limitations [16 , 17 , 18 , 19]. Moreover, physical and agroecological approaches, such as exclusion netting, poultry integration, or soil cultivation, have shown complex results and unintended, negative side effects at the orchard-ecosystem level [20 , 21].

As pests develop resistance to both chemical and biological insecticides, it becomes imperative to urgently find novel control agents that operate via different modes of action [22], that are sustainable. In this context, essential oils from aromatic plants have emerged as a promising alternative. Their potential efficacy is attributed to their complex chemical nature as a mixture of volatile compounds that act via multiple mechanisms (e.g., respiratory and neural disruption), which reduces the likelihood of resistance compared to single-target pesticides. Studies on Algerian flora have demonstrated their effectiveness against various pests [15 , 23]. Rosemary (*Rosmarinus officinalis* L.) is a candidate of particular interest due to its known insecticidal properties, its chemistry rich in active compounds, and its availability as a sustainable local resource in Algeria [24].

Therefore, the following objectives were set for this study:

1. To evaluate the fumigant toxicity of essential oil from local Algerian rosemary against the larvae of the codling moth (C. pomonella) under laboratory conditions.
2. To fill a specific knowledge gap by investigating for the first time the efficacy of the 1,8-Cineole chemotype of Algerian rosemary against this pest.
3. To contribute to the scientific basis for developing a local, safe, and sustainable solution for the post-harvest control of apple pests.

2. MATERIALS AND METHODS

2.1. Material

2.1.1. Plant material

Two kilograms (2 kg) of wild rosemary (*Rosmarinus officinalis*) leaves were collected in April 2023 from the T'kout mountains in the Aurès region, Batna Province, northeastern Algeria (35°07′33″N, 6°16′08″E), where the plant grows spontaneously at an altitude of 923 meters above sea level. After harvesting, the plant material was air-dried at room temperature (20-25 °C) in darkness and then stored in cloth bags until oil extraction

2.1.2 Insect Material

Fifth-instar larvae of *Cydia pomonella* were used for this study, with species identification confirmed by Professor LAAMARI Malik. The larvae were collected using 10 cm-wide corrugated cardboard bands

wrapped around the trunks of selected apple trees in a neglected, non-treated family orchard. These traps, placed 40 cm above ground level and below the first branch, provided an artificial shelter for larvae seeking pupation and overwintering sites. The bands were installed in mid-July and retrieved in November **(Figure 1. a , b).** Following collection, the traps were stored in plastic containers covered with a fine mesh for ventilation. The larvae were subsequently maintained under standard laboratory conditions of 18 ± 2 °C, 60 ± 5% R.H., and a 16:8 (L:D) photoperiod to await experimentation.

(a) (b)

Figure 1. *Cydia pomonella* larval collection method. **(a)** Corrugated cardboard trap installed on an apple tree trunk; **(b)** Larvae found overwintering within the trap.

2.2. Methods

2.2.1. Extraction of essential oil and GC-MS

2.2.1.1. Extraction of Essential Oil

The essential oil was extracted using the following procedure:

1. Dried plant leaves (100 g) were subjected to hydrodistillation for 4 hours using a Clevenger-type apparatus with 500 mL of distilled water.
2. The collected essential oil was dried over anhydrous sodium sulphate to remove residual moisture.
3. The final purified oil was stored in sealed glass vials at 4°C until subsequent analysis by Gas Chromatography-Mass Spectrometry (GC-MS).

2.2.1.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Instrumentation: The analysis was performed on an Agilent Technologies gas chromatograph (Model GCMS2) coupled to a quadrupole mass spectrometer, operated by MassHunter GC/MS Acquisition software.

1. Injection Stage:

- A 2 µL sample is injected into the GC system.
- The injector port is heated to 250 °C to instantly vaporize the sample.
- The injection is performed in Split mode, meaning only a portion of the vaporized sample enters the column.

2. Separation Stage (in the Gas Chromatograph):

The vaporized compounds travel through an HP-5MS capillary column (30 m x 250 µm x 0.25 µm).

- Initial : Starts at 100 °C.
- Ramp 1 : Heats at 20 °C/min up to 190 °C and holds for 15 minutes.
- Ramp 2 : Heats at 4 °C/min up to 280 °C and holds for 5 minutes.

This process separates the compounds based on their different boiling points and chemical affinities to the column.

3. Detection & Identification Stage (in the Mass Spectrometer):

As each compound exits the GC column, it enters the MS detector.

- Ionization : The molecules are bombarded with electrons in the MS Source, which is heated to 230 **°C**, causing them to break into charged fragments (ions).
- Filtration : The ions pass through the MS Quadrupole (heated to **150 °C**), which acts as a mass filter, separating them by their mass-to-charge ratio.
- Identification : Components were identified by comparing their mass spectra with data from the Wiley 7n.l mass spectral library and by comparing their calculated Retention Indices (RI) to values found in scientific literature.

2.2.2. Laboratory Bioassays for Fumigant Toxicity

1. The fumigant toxicity of *Rosmarinus officinalis* essential oil was evaluated against fifth-instar larvae of *C. pomonella* in laboratory bioassays.
2. The experiments were conducted in sealed 60 mL transparent plastic containers.

3. For treatments, 2 cm strips of Whatman No.
4. 1 filter paper were impregnated with 0.1, 0.25, 0.5 and 1.0 µL of the neat essential oil, corresponding to final concentrations of 2.08,4.16, 8.33 and 16.66 µL L^{-1} air, respectively.
5. The treated paper was affixed to the inner surface of the screw cap.
6. For each replicate, ten larvae were placed at the bottom of a container before it was tightly sealed with Parafilm to prevent vapor loss.
7. Each experiment was replicated five times for each concentration (200 larvae in total) **(Figure 2 a; b).**
8. A control group was maintained under identical conditions, using filter paper strips without essential oil.

(a) **(b)**

Figure 2. Experimental setup for the fumigant toxicity bioassay. **(a)** Overview of the materials and preparation; **(b)** Vials containing *C. pomonella* larvae exposed to different concentrations.

Data analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 27.0, IBM Corp., Armonk, NY), with the significance level set at $p < 0.05$. Prior to analysis, percentage mortality data were transformed using an arcsine square root function to stabilize variance, and the normality of the data was confirmed with the Shapiro-Wilk test. A two-way Analysis of Variance (ANOVA) was employed to evaluate the effects of essential oil concentration and exposure time on larval mortality. Tukey's HSD test was used for post-hoc pairwise comparisons of means where significant differences were detected. Finally, lethal concentration (LC_{50}) and lethal time (LT_{50}) values, along with their respective 95% confidence limits (C.L.), were determined using probit analysis.

During the preparation of this manuscript, the author(s) used the generative artificial intelligence model Gemini (Version: August 2025, Google). The model's role was strictly limited to assisting with the comparative analysis of ideas, information, and results cited from existing literature, primarily within the introduction and discussion sections. The authors have reviewed and edited any output and take full responsibility for the content of this publication

3. RESULTS

3.1 Essential Oil Yield and Chemical Profile

Hydrodistillation of the aerial parts of *R. officinalis* yielded 0.51% (v/w) of essential oil. GC-MS analysis identified 11 compounds, representing 95.94% of the total oil composition. The chemical profile was dominated by oxygen-containing monoterpenes (75.25%). The main components were 1,8-Cineole (51.47%), followed by (-)-Camphor (11.63%) and α-Pinene (10.19%). The complete list of identified compounds, their retention indices, and relative percentages are presented in **Table 1**. **Figure 3.**

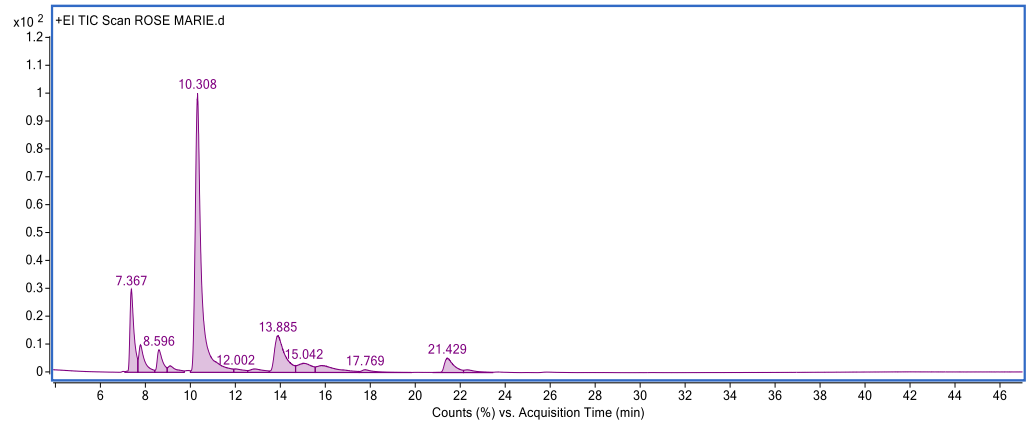

Figure 3. GC-MS chromatogram of the essential oil extracted from rosemary (*Rosmarinus officinalis*)

Table 1. Chemical components of the essential oil obtained from *Rosmarinus officinalis* aerial parts.

Compound Name	Area (%)	R.T.	Formula
α-Pinene	10.19	7.367	$C_{10}H_{16}$
Camphene	4.8	7.772	$C_{10}H_{16}$
β-Pinene	3.66	8.596	$C_{10}H_{16}$
5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	2.35	12.858	C10H18O
1,8-Cineole	51.47	10.308	$C_{10}H_{18}O$
(-)-Camphor	11.63	13.885	$C_{10}H_{16}O$
(-)-Borneol	3.89	15.042	C10H18O
L-α-Terpineol	4.25	15.835	C10H18O
Bornyl acetate	1.66	17.769	C10H20O2
Caryophyllene	1.05	21.429	C15H24
cis-α-Bisabolene	0.99	22.312	$C_{15}H_{24}$
Total Identified	**95.94**		
Monoterpene hydrocarbons	18.65		
Oxygen-containing monoterpenes	75.25		
Sesquiterpene hydrocarbons	2.04		

3.2. Fumigant Toxicity against *Cydia pomonella* Larvae

The fumigant toxicity of *R. officinalis* essential oil against *C. pomonella* larvae was found to be dependent on both concentration and exposure time (Two-way ANOVA: $F = 555.32$; $p < 0.001$), As illustrated in Larval mortality reached 100% after 48 h at the highest concentration (16.66 µL L^{-1} air) and after 144 h at the lowest concentration (2.08 µL L^{-1} air), while no mortality was observed in the control group **Figure 4.** The lethal concentration required to kill 50% of the larvae (LC_{50}) decreased over time, with a 24-hour LC_{50} value of 8.72 µL L^{-1} air. The full probit analysis for LC_{50} values is presented in **Table 2.** Correspondingly, the lethal time required to kill 50% of the larvae (LT_{50}) was shorter at higher concentrations; the LT_{50} values ranged from 14.13 h (at 16.66 µL L^{-1} air) to 77.36 h (at 2.08 µL L^{-1} air). The full analysis for LT_{50} values is detailed in **Table 3.** The probit analysis indicated a good fit for the statistical models in all cases, as the Chi-square (χ^2) values were not statistically significant ($P > 0.05$), confirming the accuracy and reliability of the calculated **LC_{50} and LT_{50} values**.

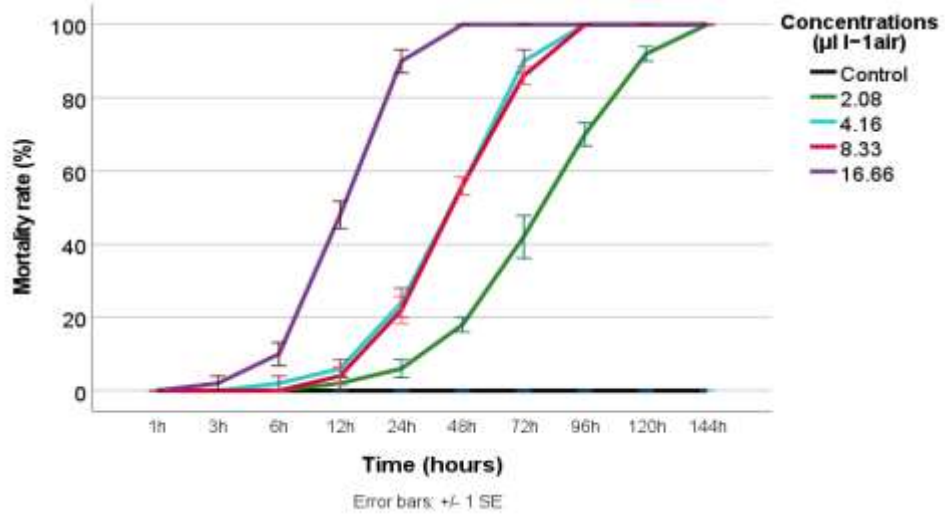

Figure 4. Mortality rate (%) of fifth-instar *Cydia pomonella* larvae after fumigant exposure to different concentrations of ***Rosmarinus officinalis*** essential oil over 144 hours. Error bars represent the standard error (± SE).

Table 2. Toxicity (LC_{50}) of ***Rosmarinus officinalis*** essential oil against the fifth instar of *Cydia pomonella*

Time (hours)	LC50 (95% C.L.) (µl l-1air)	Regression equation	R^2	χ^2	Pvalue

1	-	-	-	-	-
3	25.98 (-)	-	-	0.003	1.00ns
6	31.51 (22.27 ; 73.66)	Y= -2.29 + 0.06X	0.996	1.235	0.74ns
12	16.30 (14.21 ; 19.48)	Y= -2.15 + 0.13X	0.948	3.327	0.344ns
24	8.72 (6.13 ; 12.84)	Y= -1.64 + 0.18X	0.954	7.290	0.063ns
48	4.66 (3.02 ; 6.97)	Y= -1.46 + 0.32X	0.938	7.607	0.055ns
72	2.48 (2.14 ; 2.82)	Y= -1.69 + 0.71X	1	1.87	0.600ns
96	1.81 (-)	-	-	0.009	1.000ns
120	1.39 (0.62 ; 1.68)	-	-	0.141	0.986ns
144	1.06 (0.20 ; 1.73)	-	-	0.125	0.989ns

x^2 : Pearson χ^2 goodness-of-fit test on the probit model.
P_{value} : Significance of Chi-square test
^{ns} : P > 0.05 indicates that the model provides a good fit to the data.
$^{**, ***}$ P < 0.01 or 0.001 indicates a significant deviation from the model.

Table 3. Letal time values LT_{50} of ***Rosmarinus officinalis*** essential oil against the fifth instar of *Cydia pomonella*

Concentration (l-1air)	n	LT50 (95% C.L.) (hours)	Regression equation	R^2	χ^2	Pvalue
2.08	50	77.36 (72.08 ; 82.93)	Y= -2.39 + 0.03X	0.997	3.003	0.934ns
4.16	50	43.97 (40.03 ; 48.36)	Y= -2.14 + 0.05X	0.983	5.186	0.738ns
8.33	50	27.11 (22.52 ; 33.15)	Y= -1.73 + 0.06X	0.914	15.120	0.057ns
16.66	50	14.13 (12.67 ; 15.86)	Y= -2.26 + 0.15X	0.965	5.504	0.703ns

x^2 : Pearson χ^2 goodness-of-fit test on the probit model.
P_{value} : Significance of Chi-square test
^{ns} : P > 0.05 indicates that the model provides a good fit to the data.
$^{**, ***}$ P < 0.01 or 0.001 indicates a significant deviation from the model.
Note : Confidence limits (-) could not be estimated in some cases due to very high or low mortality rates.

DISCUSSION

The global imperative to find sustainable alternatives to synthetic pesticides has intensified research into plant-based biopesticides like essential oils. The present study contributes to this field by characterizing a specific *Rosmarinus officinalis* essential oil that is promising not only for its high-efficacy chemical profile but also for its viable, regionally-consistent extraction yield, positioning it as a strong candidate for local biopesticide development.

Toxicity and Speed of Action in a Comparative Context

The 24-hour LC_{50} value of 8.72 µL/L positions ***Cydia pomonella*** among pests highly sensitive to rosemary essential oil, particularly when compared to other Lepidopteran species. For instance, the potato tuber moth, ***Phthorimaea operculella***, demonstrated even greater sensitivity, with 48-hour LC_{50} values as low as 4.55 µL/L [25]. In contrast, the Mediterranean flour moth, ***Ephestia kuehniella***, was significantly more tolerant, requiring a much higher 48-hour LC_{50} of 198.25 µL/L [24]This wide range of toxicity is consistent with findings for other pests, where LC_{50} values have varied from 14.02 to 171.11 µL/L depending on the oil's geographical origin [26]. When compared to pests from other insect orders, the sensitivity of C. *pomonella* is comparable to the highly susceptible cowpea weevil, ***Callosobruchus maculatus*** (Coleoptera) (LC_{50} of 5.51 µL/L), but other coleopteran pests like ***Tribolium confusum*** (LC_{50} of 22.14 µL/L) and the highly tolerant red flour beetle, ***Tribolium castaneum*** (LC_{50} of 140.78 µL/L), exhibited greater resistance [27 , 28 , 29]. It should be noted that while the probit model showed a significant deviation in some cases, which can be attributed to natural biological variability, the calculated LC_{50} and LT_{50} values still provide a robust and valuable estimate of the oil's toxicity.

Regarding the speed of action, the LT_{50} values for C. *pomonella* (ranging from 14.13 h to 77.36 h) indicate a relatively rapid effect. While ***T. confusum*** showed a similar LT_{50} of 10.77 hours, it required a substantially higher concentration to achieve this. Other pests, such as the broad bean weevil and the potato tuber moth, were more tolerant, with LT_{50} values measured in days rather than hours [28 , 25 , 30].

It is worth noting a potential limitation of this study concerning the insect material. The use of fifth-instar larvae collected directly from a field environment, rather than a standardized laboratory-reared colony, may introduce a degree of biological variability. Factors such as minor differences in larval age, nutritional status, or physiological condition could contribute to variations in susceptibility. However, this approach also presents a significant strength, as testing on a wild population provides a more realistic assessment of the essential oil's efficacy against the natural genetic and physiological diversity of the target pest, thereby enhancing the practical relevance of the findings.

Yield and Chemical Profile in a Regional Context

The essential oil yield of **0.51**% obtained in this study is a promising and competitive result. While this value is lower than some yields reported in Tunisia (0.82% to 1.69%) and other North African countries like Egypt and Morocco, it is comparable to other findings in Algeria and falls within the range of yields from regions outside the Mediterranean, such as Kenya and Iran [26 , 24 , 27 , 28 , 31]. From a chemical perspective, the oil is characterized as a **1,8-cineole chemotype**, with its main components being 1,8-Cineole (51.47%), (-)-Camphor (11.63%), and α-Pinene (10.19%). This composition strongly aligns with the predominant 1,8-cineole/camphor/α-pinene chemotype documented in Tunisia and fits within the known chemical diversity across North Africa, Europe, and globally, where "cineoliferum," "camphoriferum," and "pineniferum" chemotypes are prevalent [26 , 24 , 27 , 32].

Explaining Efficacy and Variability

The potent insecticidal efficacy of this oil stems from a complex synergy among its constituents, which exert a multi-pronged attack on pests [33]. The primary mechanism is **neurotoxicity**, where monoterpenoids interfere with key neurotransmitter systems like GABA-gated chloride channels and the acetylcholinesterase (AChE) enzyme [34 , 35 , 36]. In addition, the oil exhibits strong **behavioral effects** (repellency and antifeedant action) and can cause asphyxiation [37 , 28]. This comprehensive efficacy extends across multiple pest life stages, with studies confirming clear **ovicidal, larvicidal, and adulticidal** effects, although some stages, like the pupa, may show tolerance [26 , 25].

The observed variability in both yield and chemical profile is not random but is determined by a complex interplay of factors. These include endogenous factors like the plant part used and its age, environmental conditions, and post-harvest processes such as the drying and extraction techniques [38 , 39 , 40 , 25]. Therefore, a robust chemotaxonomic framework requires a holistic approach combining physical, chemical, and molecular analysis [41].

Practical Challenges and Future Directions

While essential oils are effective, their direct application as biopesticides is often limited by their high volatility and poor water solubility [42]; [43]. This highlights the disconnect where the chemical profile alone is not always a perfect predictor of field efficacy [44 , 33]. To overcome these limitations, research into innovative formulations like **nanoencapsulation** is emerging as a highly effective solution [45]. This technology provides controlled-release properties, enhances economic viability, and can even help preserve the quality of the protected product [46 , 47 , 48]. The classification of oils into chemotypes is therefore an essential step, paving the way for the development of a new generation of more stable and potent biopesticides.

CONCLUSION

In response to the growing challenge of resistance in the codling moth,
Cydia pomonella, to conventional pesticides, this study provided a scientific validation for the essential oil of local Algerian rosemary as a sustainable biopesticide. The findings conclusively demonstrate that the essential oil from the T'kout region, characterized by its 1,8-Cineole dominant chemotype, possesses potent and rapid fumigant toxicity against the fifth-instar larvae of this major pest. The lethal concentration and time values (LC_{50} and LT_{50}) not only quantify this high efficacy but also position this specific oil as a powerful natural alternative for post-harvest protection.

To translate these promising laboratory results into a practical application, future research should focus on several key areas. Future work should therefore focus on developing stable nano-formulations and evaluating them under field conditions, not only for their efficacy against *C. pomonella*, but also for their potential impacts on non-target organisms and overall environmental safety to validate them as a truly sustainable solution. Further investigation into the oil's efficacy against other life stages of *C. pomonella*, such as eggs and adults, would also provide a more comprehensive pest management strategy.

In conclusion, this research not only presents an effective solution to a pressing agricultural problem but also opens new avenues for the valorization of local biodiversity. The essential oil of Algerian *Rosmarinus*

officinalis stands as a strong candidate for the development of a new generation of safe, effective, and sustainable biopesticides.

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