Toxicity, Biochemical, and Genotoxic Effects of *Eucalyptus smithii* Essential Oil and Several Major Monoterpenes on the Land Snail *Massylaea vermiculata*

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ABSTRACT

***Massylaea vermiculata* is a significant pest in agriculture, prompting the search for safer, non-chemical methods of control. The present study assessed the lethal and sublethal effects of Eucalyptus essential oil (EEO) and the major components α-pinene and 1,8-cineole on adult *M. vermiculata*. The most potent compound, α-pinene (LD_{50}: 279.58 µg/g), followed by Eucalyptus EO (LD_{50}: 468.7 µg/g) and 1,8-cineole (LD_{50}: 834.60 µg/g), was assessed topically for acute toxicity. Physiological damage after exposure for 72 hours to sublethal doses (0.2 LD_{50}) was assessed via an integrated multi-biomarker approach. Neurotoxicity was further confirmed by the inhibition of acetylcholinesterase (AChE), with 1,8-cineole resulting in the greatest inhibition. Malondialdehyde (MDA) and glutathione S-transferase (GST) were found to increase, mainly due to the oxidative stress induced by α-pinene. Genotoxicity was reflected in decreased DNA content and an increase in micronuclei and binuclei in hemocytes (particularly with α-pinene). Moreover, genomic template stability (GTS) analysis using RAPD-PCR revealed that α-pinene induced the most severe DNA damage, followed by Eucalyptus essential oil, whereas 1,8-cineole exhibited the least genotoxic effect. These findings demonstrate that EEO and its components exert their molluscicidal effect through multiple modes of action, including neurotoxicity, oxidative stress, and genotoxicity. The best formulation and strategy for field application of compounds from Eucalyptus constitute potential candidates for ecofriendly molluscicides that need to be further explored.**

Keywords: Eucalyptus essential oil, α-pinene, 1,8-cineole, Molluscicide, Oxidative stress, Neurotoxicity, Genotoxicity, RAPD-PCR analysis, Biomarkers, Ecofriendly pest control.

INTRODUCTION

Terrestrial gastropods, particularly land snails such as the chocolate-band snail *Massylaea vermiculata*, pose a significant threat to global agriculture. These pests inflict substantial economic damage by consuming a wide variety of crops, reducing yield and marketability through feeding and contamination (Sabry and Ali, 2022). In regions such as Egypt, *M. vermiculata* causes considerable losses in diverse agricultural and horticultural settings (Mobarak, 2021). Owing to their high reproductive rate, adaptability, and hermaphroditic nature, effective management is crucial yet challenging for sustainable food production.

Historically, control strategies have heavily relied on synthetic chemical molluscicides, such as metaldehyde and carbamates (Mortada *et al.*, 2013 and De-Ley *et al.*, 2020). While effective initially, their persistent use raises serious environmental and health concerns. These include risks to non-target organisms (beneficial insects, wildlife), soil and water contamination, bioaccumulation, and the development of resistance in snail populations (Hamed *et al.*, 2007; Radwan *et al.*, 2008 and De-Roma *et al.*, 2018). These drawbacks necessitate the exploration of safer, ecologically sound alternatives.

Plant-derived essential oils (EOs) represent a promising alternative for eco-friendly pest management. Compared with synthetic mixtures, these complex volatile mixtures exhibit broad-spectrum activity, including molluscicidal effects, which are often coupled with lower environmental persistence and reduced non-target toxicity (Batish *et al.*, 2008 and Ayllón-Gutiérrez *et al.*, 2024). Their biodegradability and multiple modes of action offer potential advantages, possibly mitigating resistance development and providing efficacy against pests resistant to conventional chemicals (Gad *et al.*, 2023).

Eucalyptus essential oil (EEO) is derived from the leaves of eucalyptus trees, which are primarily native to Australia. It is known for its diverse biological activities, including pesticidal properties, largely attributed to major monoterpene constituents such as α-pinene and 1,8-cineole. (eucalyptol) (Fig. 1) (Danna *et al.*, 2023). These compounds are known to disrupt multiple physiological processes in pests, such as

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membrane integrity, neurotransmission, and oxidative balance. While EEO has potential as a natural molluscicide, a deeper understanding of the specific mechanisms underlying its effects is needed for optimized application (Ainane *et al.,* 2019).

Evaluating the mode of action for EOs requires looking beyond acute mortality to include sublethal effects. These effects, which occur at lower doses, can reveal crucial physiological and biochemical disruptions (Valavanidis *et al.,* 2006). Biomarkers are key tools in this assessment; neurotoxicity markers such as acetylcholinesterase (AChE) activity indicate nervous system impacts, whereas antioxidant markers such as malondialdehyde (MDA) and Glutathione-S-transferase (GST) reflect oxidative stress responses, providing insights into toxicity pathways (Al-Fanharawi *et al.,* 2018; Radwan *et al.,* 2019 and Baag *et al.,* 2021).

Cytogenetic and genotoxic effects are also critical considerations, as DNA damage can lead to long-term ecological consequences. Cytogenetic abnormalities such as micronuclei (Mn) and binucleated cells (Bn) indicate chromosomal damage or mitotic disruption (Fenech, 2007). Furthermore, assessing DNA structural integrity via methods such as the Random Amplified Polymorphic DNA (RAPD) assay reveals potential genotoxicity that could affect population viability (Atienzar *et al.,* 1999; Ali *et al.,* 2008 and Rocco *et al.,* 2014). Investigating these markers provides a comprehensive toxicity profile.

Given the complexity of EOs, which contain numerous bioactive compounds that act via diverse pathways, an integrated multibiomarker approach is essential (Bakkali *et al.,* 2008 and Regnault-Roger *et al.,* 2012). Combining biochemical, cytogenetic, and genotoxic assessments allows for a holistic understanding of toxicity mechanisms (Ali *et al.,* 2015). This knowledge is crucial for predicting ecological impacts, enhancing target specificity, and developing effective, sustainable pest control strategies using plant-derived products (Campolo *et al.,* 2018).

Therefore, this study aimed to comprehensively evaluate both the lethal toxicity and the sublethal effects of Eucalyptus essential oil and its primary monoterpenes, α-pinene and 1,8-cineole, on the land snail *M. vermiculata*. The novelty lies in the use of an integrated multi-biomarker approach—simultaneously assessing neurotoxicity (AChE), oxidative stress (MDA, GST), cytogenetic alterations (DNA content, Mn, Bn frequency), and genotoxicity (RAPD analysis). This provides deeper insights into the biochemical and molecular modes of action, informing the development of targeted, environmentally safer molluscicidal formulations on the basis of these natural compounds.

MATERIAL AND METHODS

Chemicals and Essential Oil

Eucalyptus essential oil (EEO) was obtained from Imtenan (Obour City, Egypt). The pure monoterpene compounds α-pinene (98% purity) and 1,8-cineole (99% purity) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in the experiments were of analytical grade.

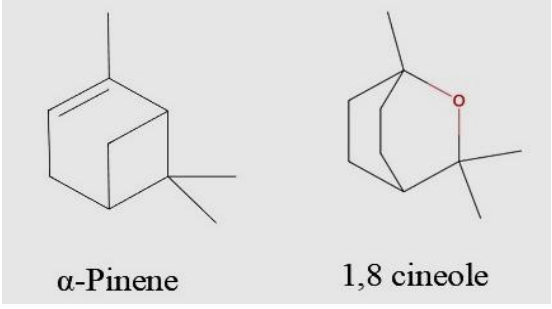

Fig.١. Chemical structure of the main constituent monoterpenes in Eucalyptus essential oil

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of the EEO was determined via an Agilent 7890A gas chromatograph interfaced with an Agilent 5975C mass spectrometer. Separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) with helium as the carrier gas (1 mL/min). The oven temperature program started at 60°C (2 min), increased 3°C/min to 240°C, and was held for 10 min. The injector temperature was 250°C, and the MS transfer line temperature was 280°C. A 1 μL diluted sample was injected (split ratio of 1:50). The mass spectrometer was operated at 70 eV (EI mode) and scanned at m/z 35–500. Components were identified by comparing mass spectra with NIST14 and Wiley 275 libraries, with relative percentages determined from peak areas against the total ion chromatogram (Gamal El-Din *et al.,* 2022).

Biological Material

Adult chocolate-band snails, *M. vermiculata* (Müller 1774), (Gastropoda: Helicidae), were collected during February–March 2023 from crop fields at Abis area, Alexandria Governorate. Snails were maintained in ventilated glass terraria (30 × 48 × 34.5 cm) and fed fresh lettuce leaves (Radwan *et al.,* 2008).

Toxicity Study

- **Topical application technique**

The acute toxicity of EEO, α-pinene, and 1,8-cineole to adult *M. vermiculata* was evaluated via topical application (Radwan *et al.,* 2008). Five concentrations of each test substance were prepared in DMSO. A 10 μL

volume was applied inside each snail's shell cavity, while control snails received only DMSO. For each concentration, 30 snails were used (3 replicates of 10 snails) and placed in ventilated plastic containers (10 cm diameter × 15 cm height) with moistened filter paper and lettuce leaves. Mortality was recorded at 8-hour intervals for 72 hours' post-treatment, with snails considered dead if unresponsive to foot stimulation with a needle (WHO, 1965).

- **Sublethal impact and tissue preparation.**

To assess the sublethal effects on physiological biomarkers, 80 snails were divided into four groups (20 each): a control group (DMSO only). Three treatment groups received 0.2 LD50 of EO, α-pinene, or 1,8-cineole. After 72 hours, hemolymph was collected from the visceral haemocoel and mixed 1:1 with anticoagulant buffer (0.1 M glucose, 15 mM EDTA, 13 mM citric acid, and 10 mM sodium citrate, pH 7.4). The hemocyte suspension was centrifuged (5000 rpm, 5 min, 4°C) and resuspended in PBS for cytogenic and genotoxic assessments. Soft body tissues were homogenized in 10 volumes (w/v) of ice-cold phosphate buffer (pH 7.4) and centrifuged (3000 rpm, 30 min, 4°C), and the supernatants were used for biochemical marker measurements.

Biochemical assessment

1. Oxidative stress markers

Lipid peroxidation was determined by measuring malondialdehyde (MDA) production via the thiobarbituric acid reaction method (Nair and Turner, 1984). The absorbance was measured at 532 nm and expressed as nmol MDA/g wet tissue. Glutathione-S-transferase (GST) activity was determined via the use of 1-chloro-2,4-dinitrobenzene according to Habig *et al.* (1974) Changes in absorbance at 340 nm were recorded for 3 minutes, with activity expressed as µmol/min/mg protein.

2. Neurotoxicity Marker

Acetylcholinesterase (AChE) activity was measured via the Ellman *et al.* (1961) method with acetylthiocholine iodide as the substrate and expressed as µmol/min/mg protein.

3. Cytogenetic Markers

The frequency of nuclear abnormalities was assessed according to Villela *et al.* (2006) by counting micronucleated and binucleated cells. Hemocytes were spread on glass slides, air-dried, fixed in methanol: acetic acid (3:1) for 15 minutes, and stained with 5% Giemsa for 20 minutes. A total of 1000 cells per animal were examined via light microscopy (1000× magnification). The DNA content was estimated via a NanoDrop, as outlined in section 2.5.4.1.

4. Genotoxicity markers

- **DNA extraction and quantification**

Genomic DNA was extracted from hemocytes via the modified CTAB method (Doyle and Doyle, 1987). Approximately 30 mg of hemocytes were homogenized in 600 µL of CTAB buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1% PVP, 0.2% β-mercaptoethanol) and incubated at 65°C for 45 minutes. After cooling, an equal volume of chloroform: isoamyl alcohol (24:1) was added, the mixture was centrifuged (10,000 × g, 10 min), and the aqueous phase was transferred. The DNA was precipitated with 0.6 volumes of isopropanol (-20°C, 1 hour), centrifuged (12,000 × g, 10 min), washed twice with 70% ethanol, and resuspended in 50 µL of TE buffer. RNA was removed via incubation with RNase A (10 mg/mL) at 37°C for 30 minutes. DNA purity and concentration were assessed spectrophotometrically (260/280 nm and 260/230 nm ratios), the DNA content was expressed as ng/µl, and integrity was confirmed via 1% agarose gel electrophoresis.

- **RAPD–PCR analysis**

The PCRs (25 µL) contained 1.5 ng of genomic DNA, 12.5 µL of EasyTaq master mix (Bio Helix, Taiwan), and 0.2 µM primer. The amplification conditions were as follows: initial denaturation at 94°C for 2 min; 45 cycles of 94°C for 60 sec, 36°C for 60 sec, and 72°C for 30 sec; and a final extension at 72°C for 10 min. Five primers were used (G10: 3'AGGGCCGTCT 5'; P5: 3'TGGACCGGTG 5'; P4: 3'GGTCCCTGAC5'; N05: 3'ACTGAACGCC5'; A09: 3'GGGTAACGCC5').

The PCR products were analyzed on 2% agarose gels (100 V, 0.5X TBE buffer), stained with ethidium bromide (10 µg/mL), and visualized via UV transillumination. Band sizes were compared against a 100 bp DNA ladder (bio helix, Korea). Clear, reproducible bands were scored as present (1) or absent (0) in a binary matrix.

- **Estimation of genomic template stability**

The genomic template stability (GTS, %) was calculated via the following formula:

$$GTS\ (\%) = \left(1 - \frac{a}{n}\right) X\ 100$$

Where (a) represents the average number of polymorphic bands and (n) represents the total number of control bands. Polymorphisms were identified as the disappearance of existing bands or the appearance of new bands compared with those in controls (Ibrahim *et al.*, 2022).

Statistical analysis

Mortality curves for all groups were determined using Kaplan–Meier survival analysis. Significant differences between curves were assessed using the log-rank test (Chi², $p < 0.05$). Probit analysis, as described by Finney (1971), was used to calculate the LD_{50} values, 95% confidence limits, and slope after 72 h of exposure. The results are presented as the means ± standard deviations (SDs). Statistical analysis was performed via one-way ANOVA with Tukey's HSD test ($p < 0.05$) to examine differences between groups (Graph Prism Software V. 10.2.0).

RESULTS AND DISCUSSION

Chemical composition of *Eucalyptus* essential oil

GC–MS/MS analysis revealed that the EEO predominantly contained four monoterpenes, with 1,8-cineole as the major constituent (78.68%), followed by α-pinene (11.62%), durene (5.99%), and γ-terpinene (3.71%) (Table 1, Fig. 2). 1,8-cineole is the predominant component reported in previous studies on EEOs, but exact proportions vary according to species, geographical origin, and extraction method (Batish *et al.*, 2008 and Sebei *et al.*, 2015).

Table 1. **Chemical composition of Eucalyptus essential oil**

Retention time (min)	Monoterpene	Molecular formula	Area %
4.78	α-Pinene	C10H16	11.62
6.65	durene	C10H14	5.99
6.81	1,8 cineole	C10H18O	78.68
7.56	γ-terpinene	C10H16	3.71

Comparative molluscicidal activity of EEO and its main constituent monoterpenes

The topical application of EEO and its major monoterpene constituents demonstrated significant molluscicidal activity against adult *M. vermiculata* snails. Probit analysis of the 72-hour mortality data revealed differential toxicity among the tested substances, with α-pinene exhibiting the highest potency (LD_{50} = 279.58 µg/g body weight), followed by EEO (LD_{50} = 468.7 µg/g body weight) and 1,8-cineole (LD_{50} = 834.60 µg/g body weight) (Table 2). Survival analysis via the Kaplan–Meier method and log-rank test further confirmed the differential efficacy of the tested substances, with highly significant differences in survival patterns among the treatment groups for all three tested substances ($p < 0.0001$) (Figs. 3A, B, C). These results provide robust evidence for the dose-dependent and time-dependent molluscicidal activity of EEO and its monoterpenes against *M. vermiculata*.

The greater toxicity of α-pinene than of 1,8-cineole despite its lower concentration in the essential oil (11.62% vs. 78.68%) indicates that the molluscicidal potency of EEO cannot be attributed solely to its most abundant constituents.

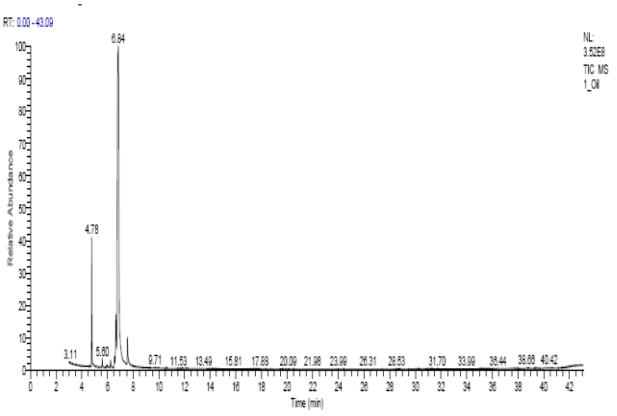

Fig. 1. GC–MSMS chromatograms of Eucalyptus essential oil

This finding aligns with those of Souza *et al.* (2020), who reported that the biological activities of plant EOs often result from complex interactions between their various constituents rather than from individual components. Similarly, Radwan *et al.* (2008) reported that α-pinene was more toxic than 1,8-cineole against the land snail *Theba pisana*, which is consistent with our observations in *M. vermiculata*. The greater toxicity of α-pinene also aligns with findings by Desouky *et al.* (2022), who demonstrated that certain monoterpenes in Eucalyptus globulus oil exert significant toxic effects on the land snail Theba pisana, affecting both survival and reproductive parameters. The different toxicity patterns observed can be attributed to the structural characteristics of these compounds. Compared with other monoterpenes, α-pinene, a bicyclic monoterpene with a reactive exocyclic double bond, has been reported to possess greater biological activity because of its lipophilic nature and structural features that facilitate interactions with biological membranes (Wojtunik-Kulesza, 2022). The intermediate toxicity of the whole essential oil to its individual constituents suggests complex interactions among its components. When applied as a mixture (Al-Sayed *et al.*, 2014).

Table 2. Acute toxicity of Eucalyptus essential oil and its main constituent monoterpenes to *M. vermiculata* after 72 h of topical exposure

EO/monoterpene	LD_{50} (µg/g bw)	95% Fudicial limit	Slope ± SE	X^2(df)	*R*
Eucalyptus EO	468.7	410.8 - 536.6	2.10 ± 0.20	2.97(3)	0.964
1,8 Cineole	834.60	743.62-947.70	2.36 ± 0.25	0.29(3)	0.996
α-Pinene	279.58	250.2 - 312.8	2.48 ± 0.26	1.42(3)	0.980

According to Tak *et al.* (2016), complex essential oils may exhibit modified penetration rates and biotransformation patterns compared with those of pure compounds, influencing their overall toxicity. Additionally, minor constituents not quantified in our analysis may contribute to or modulate the molluscicidal activity, as proposed by Raut and Karuppayil (2014) in their review of the biological activities of essential oils.

Physiological impact of sublethal exposure

1. Oxidative stress induction

Sublethal exposure to the tested substances induced significant oxidative stress in *M. vermiculata*, as evidenced by elevated MDA levels ($F = 68.34$, df = 3, $p < 0.0001$) and increased GST activity ($F = 194.84$, df = 3, $p < 0.0001$) (Figs. 4A, B). The most substantial increases in both markers were observed with α-pinene treatment (84.0% increase in MDA, 65.0% increase in GST), followed by Eucalyptus essential oil (55.1% increase in MDA, 53.4% increase in GST) and 1,8-cineole (42.1% increase in MDA, 40.6% increase in GST). MDA is a product of lipid peroxidation and serves as a biomarker of oxidative damage to cell membranes (Del-Rio *et al.*, 2005).

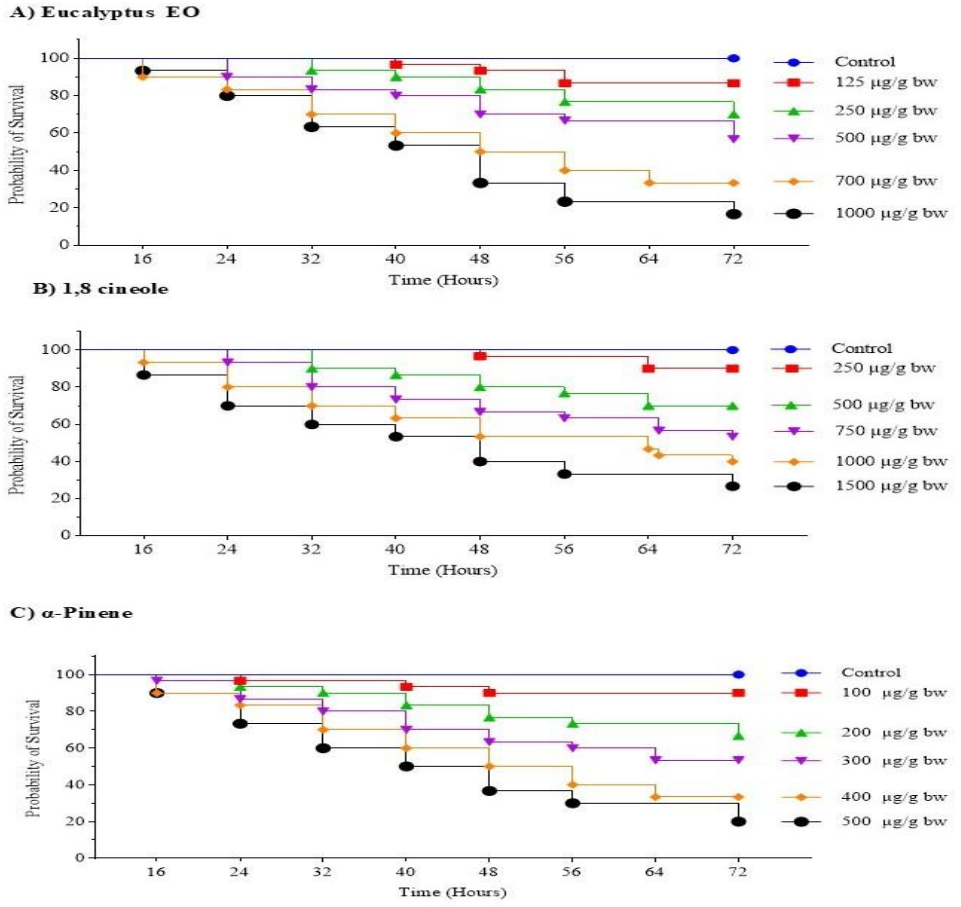

Fig. 2. K–M survival curves of Eucalyptus EO (A) and its main constituent monoterpenes 1,8 cineol (B) and α-pinene (C)

The observed increases in the MDA content indicate that Eucalyptus essential oil and its Monoterpenes induce lipid peroxidation in snail tissue, potentially compromising membrane integrity and cellular function. This finding is consistent with that of Bakkali *et al.* (2008), who reported that monoterpenes can accumulate in biological membranes due to their lipophilic nature, altering their fluidity and permeability, which may lead to mitochondrial dysfunction and increased production of reactive oxygen species (ROS).

GST plays a crucial role in cellular detoxification and antioxidant defense by catalyzing the conjugation of reduced glutathione with xenobiotics and oxidative stress products (Hayes *et al.*, 2005). The elevated GST activity observed in treated snails represents an adaptive response to oxidative challenge and xenobiotic exposure. Similar induction of GST activity by plant essential oils and monoterpenes has been reported in various invertebrates, including the land snail *Eobania vermiculata* (El-Gendy *et al.*, 2019) and the mosquito *Aedes aegypti* (Intirach *et al.*, 2018).

Neurotoxic impacts via acetylcholinesterase (AChE) inhibition

Sublethal exposure (0.2 LD50) to Eucalyptus essential oil and its major monoterpenes significantly reduced AChE activity in *M. vermiculata* tissue ($F = 284.66$, $df = 3$, $p < 0.0001$), with the most pronounced inhibition observed with 1,8-cineole (45.7% reduction), followed by Eucalyptus essential oil (38.6% reduction) and α-pinene (31.4% reduction), compared with the control (Fig. 4C). This inhibitory pattern is particularly noteworthy, as it contrasts with the acute toxicity ranking, suggesting that AChE inhibition represents a specific mechanism of action rather than a general toxic response.

Inhibition of acetylcholinesterase can lead to an accumulation of neurotransmitters, resulting in hyperexcitation, neuromuscular blockade, and eventually paralysis (Colovic *et al.*, 2013). The observed AChE inhibition therefore provides a mechanistic explanation for the molluscicidal activity through neurotoxic effects. Similar findings have been reported by Mills *et al.* (2004), who demonstrated that 1,8-cineole has a high ability to inhibit AChE in vitro, which is consistent with our in vivo observations in *M. vermiculata*.

The neurotoxic effects of monoterpenes through AChE inhibition have been documented in several studies. López *et al.* (2015) demonstrated that various monoterpenes inhibit AChE activity, with inhibitory potency related to their structural characteristics. The authors reported that bicyclic monoterpenes, including α-pinene, moderately inhibited AChE, whereas oxygen-containing monoterpenes such as 1,8-cineole exhibited stronger inhibitory effects, which is consistent with our observations. According to Miyazawa and Yamafuji (2005), the presence of an epoxide group in 1,8-cineole may enhance its binding affinity to the catalytic gorge of AChE, explaining its stronger inhibitory effect despite lower acute toxicity. This structure–activity relationship was further supported by Perry *et al.* (2000), who reported that oxygenated monoterpenes generally exhibited stronger AChE inhibitory activity than hydrocarbon monoterpenes did.

Cytogenetic impacts

Sublethal exposure to Eucalyptus essential oil and its monoterpenes induced significant cytogenetic effects in *M. vermiculata*, as evidenced by reduced DNA content ($F = 14.9$, $df = 3$, $p < 0.0001$) and increased frequencies of nuclear abnormalities (micronuclei: $F = 103.05$, $df = 3$, $p < 0.0001$; binucleated cells: $F = 87.81$, $df = 3$, $p < 0.0001$) (Figs. 4D, E). The most substantial genotoxic effects were observed with α-pinene treatment (30.5% reduction in DNA content, 172.6% increase in micronuclei, 43.6% increase in binucleated cells), which is consistent with its greater acute toxicity and stronger induction of oxidative stress. Compared with the control, the EEO and 1,8-cineole treatments led to intermediate DNA contents of 49.70 and 52.16 ng/µl, respectively, corresponding to 17.5% and 13.4% reductions.

The observed reduction in DNA content may result from DNA fragmentation due to oxidative damage or apoptotic processes (Eastman and Barry, 1992). The ROS generated during oxidative stress can attack DNA, causing strand breaks, base modifications, and cross-linking, potentially leading to genomic instability and cell death. This correlation between oxidative stress markers and DNA.

This reduction in content suggests that genotoxicity may be secondary to oxidative damage, as proposed by Slameňová *et al.* (2009) for various plant essential oils. The cytogenetic potential of monoterpenes has been reported in previous studies. Türkez and Aydın (2016) demonstrated that certain monoterpenes, including α-pinene, can induce DNA damage through direct interactions with DNA molecules or indirectly through ROS generation. The authors suggested that the electrophilic nature of some monoterpenes enables them to form adducts with DNA, leading to structural alterations and potential mutagenic effects.

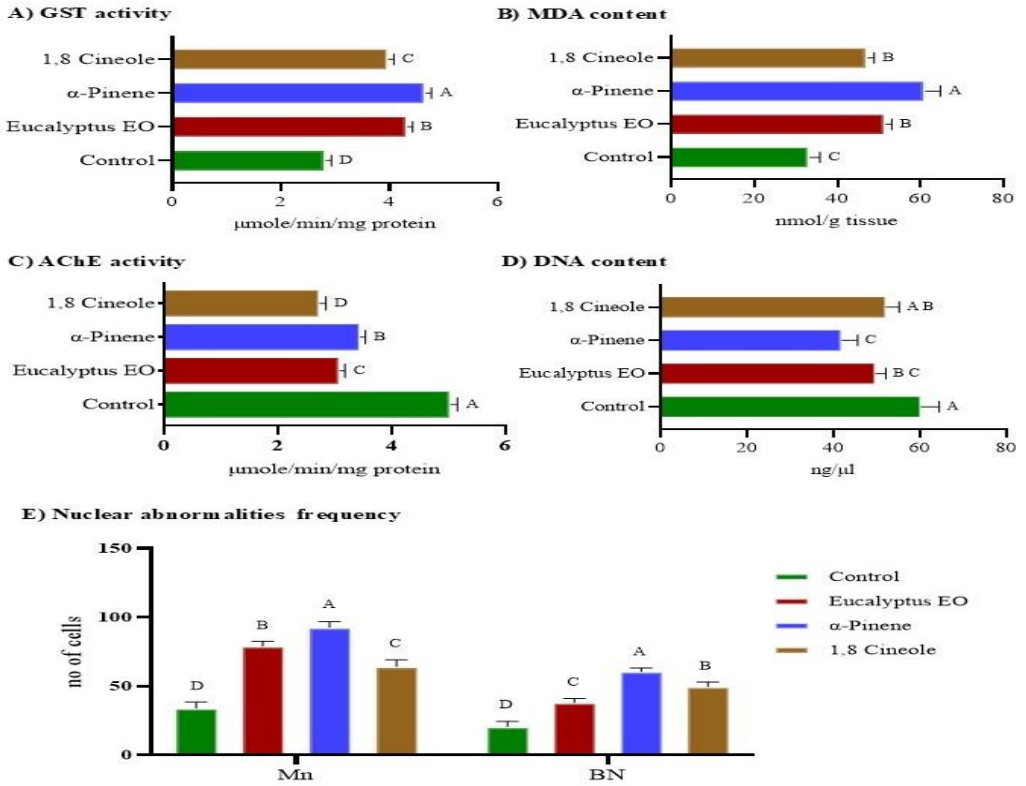

Fig. 3. Enzymatic oxidative stress (GST activity; A), nonenzymatic (MDA content, B), and neurotoxic biomarkers (AChE activity; C) cytogenetic toxicity parameters (DNA content; D) and frequency of nuclear abnormalities cells (micronuclei and binucleated cells; E)

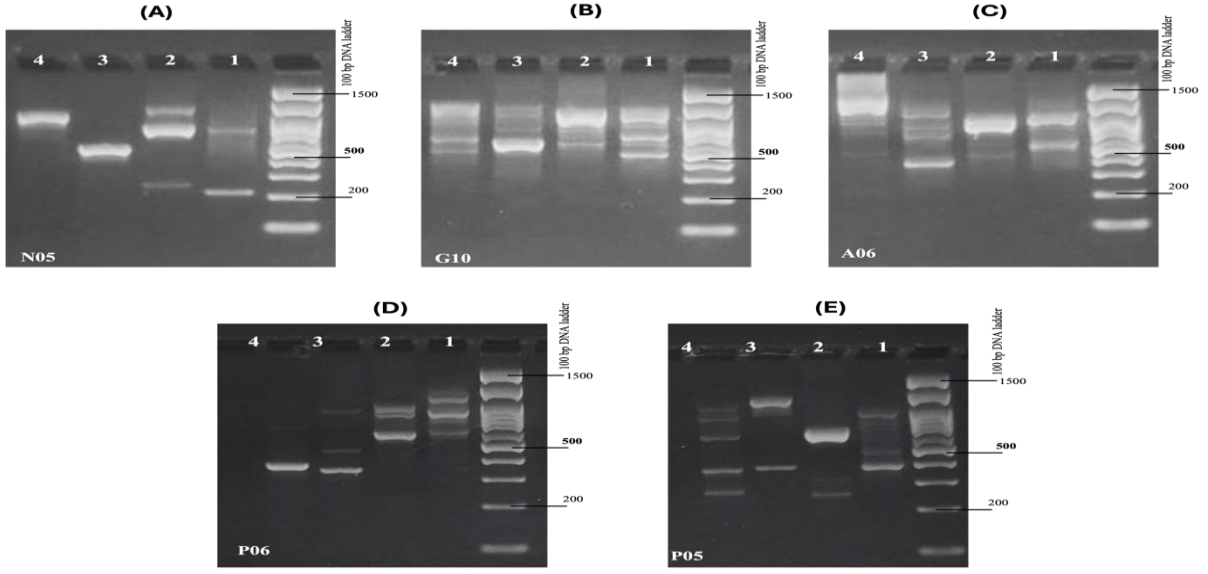

Fig. 5. Randomly amplified polymorphic DNA profiles of genomic DNA from M. vermiculata treated with Eucalyptus EO, 1,8-cineole, or α-pinene via five primers. (A) Profile generated by primer N05, (B) profile generated by primer G10, (C) profile generated by primer A06, (D) profile generated by primer P06, (E) profile generated by primer P05, samples run against a 100 bp plus DNA ladder; 1: Control, 2: α-Pinene 3: 1,8-Cineole 4: Eucalyptus EO

Table 1. RAPD primers used to evaluate genotoxicity in *M. vermiculata* snails after treatment with Eucalyptus EO, 1,8-cineole, or α-pinene

Primer	Length (bp)	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism ratio (%)
N05	1000-200	8	8	1	100.0
G10	1000-500	7	1	6	14.3
A06	1500-500	12	9	3	75.0
P06	1500-300	8	1	8	12.5
P05	1000-200	7	2	5	28.6

Micronuclei arise from chromosomal fragments or whole chromosomes that fail to incorporate into daughter nuclei during cell division, serving as biomarkers for clastogenic and aneugenic events (Fenech, 2007). The increased micronucleus frequency in treated snails indicates that Eucalyptus essential oil and its monoterpenes induce chromosomal damage, potentially through oxidative stress or direct interaction with DNA. Similar genotoxic effects have been reported by Di Sotto *et al.* (2013), who reported that β-caryophyllene oxide, a bicyclic sesquiterpene, exhibited clastogenic activity in mammalian cells, attributed to its pro-oxidant potential. Binucleated cells result from cytokinesis failure following nuclear division, reflecting disturbances in cell cycle progression and cytoskeletal organization (Fenech, 2007). The increased frequency of binucleated cells in treated snails, particularly those treated with α-pinene, suggests that these compounds interfere with mitotic processes or cytoskeletal dynamics. According to Pinheiro *et al.* (2015), monoterpenes can disrupt microtubule assembly and function, potentially explaining the observed effects on cytokinesis. Wang *et al.* (2012) demonstrated that *Rosmarinus officinalis* L. essential oil exhibited stronger cytotoxicity against human ovarian and liver cancer cell lines than did its individual constituents (α-pinene > β-pinene > 1,8-cineole), indicating synergistic interactions among components.

Genotoxic impact via RAPD–PCR polymorphisms

Random amplified polymorphic DNA (RAPD) analysis was used to evaluate the genomic impact of Eucalyptus EO and 1,8-cineole and α-pinene monoterpene treatment on the striped brown snail *M. vermiculata*. RAPD profiling was conducted on DNA extracted from both control and treated snails. Five primers were tested and produced clear and reproducible bands (Fig. 5). The DNA fragment length ranged from 200 to 1000 bp, with polymorphic changes, including the appearance of new bands and the disappearance of existing bands. Primer N05 presented the highest polymorphism ratio (100.0%), with 8 total bands, making it highly effective for detecting genetic variation. In contrast, primers G10 and P06 presented low polymorphism ratios (14.3% and 12.5%, respectively), indicating their limited ability to reveal genomic differences. Primer A06 was strongly balanced, producing 12 bands with a high polymorphism ratio of 75.0%, whereas primer P05 had a moderate polymorphism ratio (28.6%), with 7 bands. These results suggest that primers such as N05 and A06 are better suited for studies requiring high sensitivity to genetic diversity (Fig. 5 and Table 3).

RAPD fingerprints revealed significant differences between treated and control snails, reflected in altered DNA band patterns. These findings indicate a clear correlation between Eucalyptus EO, 1,8-cineole, and α-pinene treatments and genomic alterations in *M. vermiculata*.

Genomic template stability (GTS, %) is a measure reflecting changes in PCR amplification profiles caused by treatments relative to control profiles. In this study, GTS was calculated via RAPD analysis via five primers (Table 4), where decreases in GTS values indicated reduced genomic stability due to DNA damage. Compared with the control, exposure to α-pinene and Eucalyptus EO resulted in great decreases in GTS values of -7.69 and -3.85, respectively, which indicate a decrease in genomic stability, with α-pinene reflecting the highest level of genomic damage among the treatments. In contrast, the positive value (3.85) in samples treated with 1,8-cineole suggests slight maintenance or improvement of genomic stability, indicating minimal adverse effects. Overall, the results highlight that α-pinene is the most genotoxic of the three compounds, whereas 1,8-cineole is the least harmful and potentially even beneficial at sublethal levels.

Table 4. Summary of the changes detected in the RAPD profiles of each sample, including the total bands in the control, variations in band patterns by primer sets, and % GTS in *Massylaea vermiculata* snails exposed to EEO, 1,8-cineole, and α-pinene

Primer No.	Primer name	Control (1)	α-Pinene		1,8-Cineole		Eucalyptus EO	
			p	d	p	d	p	d
1	N05	2	1	1	0	0	0	0
2	G10	6	4	2	3	2	1	5
3	A06	2	1	1	1	1	0	0
4	P06	5	2	3	1	4	0	4
5	P05	4	0	4	1	3	2	2
Total bands		19	8	11	6	10	3	11
A			28.00		25.00		27.00	
a/n			1.08		0.96		1.04	
1-(a/n)			-0.08		0.04		-0.04	
%GTS			-7.69		3.85		-3.85	

CONCLUSION

The significant molluscicidal effect of the EEO and its main monoterpene constituents, α-pinene and 1,8-cineole, was demonstrated against the agricultural pest *Massylaea vermiculata*. The determination of acute toxicity revealed that the LD_{50} dose-dependently increased, with α-pinene being the most effective, followed by whole EO and then 1,8-cineole. Notably, investigations into sublethal effects revealed major physiological disturbances via an integrated multibiomarker approach after exposure to a 0.2 LD_{50}. Neurotoxicity was revealed by significant inhibition of acetylcholinesterase (AChE) activity, indicating neurotoxic effects, which included increased malondialdehyde (MDA) levels and changes in glutathione S-transferase (GST) activity as a result of oxidative stress induction. Genetic effects were corroborated by decreased DNA content and increased numbers of nuclear wounds (micronuclei, binucleated cells) in the hemocytes, in addition to modifications in the RAPD profiles related to preparation with α-pinene. This evidence indicates that Eucalyptus EO and its constituents exploit multiple actions to exert molluscicidal effects at the level of the nervous system, causing oxidatively induced damage and compromising genomic integrity. The complex influences of these EOs and their components point to the complexity of the bioactivity associated with essential oils. Overall, EEO, α-pinene, and 1,8-cineole are promising candidates for the development of environmentally safe molluscicides. Future research will aim to optimize compositions to improve effectiveness and stability while conducting field trials to establish practical applications and further investigate non-target effects to support ecological safety.

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الملخص العربي

السمية والتأثيرات البيوكيميائية والجينية لزيت الأوكالبتوس سميثي العطري و المونوتربينات الرئيسية على الحلزون الأرضي *Massylaea vermiculata*

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تعتبر حلزون *Massylaea vermiculata* آفة خطيرة في الزراعة، مما دفع إلى البحث عن طرق أكثر أمانًا وغير كيميائية لمكافحتها. قامت الدراسة الحالية بتقييم التأثيرات المميتة وشبه المميتة لزيت الأوكالبتوس العطري (EEO) والمكونات الرئيسية α-pinene و١،٨ cineole - (الفا-بينين و١،٨-سينئول) على *M. vermiculata* الكاملة. تم تقييم المركب الأكثر فعالية، الفا بينين (LD_{50}: 279.58 α-pineneميكروجرام/جرام)، يليه زيت الأوكالبتوس EO (LD_{50}: 468.7ميكروغرام/غرام) و(١،٨-سينيول: LD_{50} 834.60ميكروغرام/غرام)، موضعياً للتأكد من سميته الحادة. تم تقييم الضرر الفسيولوجي بعد المعاملة ب ٧٢ ساعة لجرعات شبه مميتة (0.2 LD50) من خلال تقدير العديد من العلامات الحيوية. تم تأكيد السمية العصبية أيضًا عن طريق تثبيط إنزيم الأسيتيل كولينستراز (AChE)، حيث أدى ١،٨-سينيول إلى أكبر تثبيط. وُجِد أن المالونديالدهيد (MDA)والجلوتاثيون-S ترانسفيراز (GST) يزدادان، ويرجع ذلك أساسًا إلى الإجهاد التأكسدي الناجم عن ألفا بينين. انعكست السمية الجينية في انخفاض محتوى من الحمض النووي وزيادة النوى الصغرية (micronuclei) والنوى المزدوجة (binuclei) في خلايا الدم اللمفاوي (خاصة مع α-pinene). علاوة على ذلك، كشف تحليل استقرار القالب الجينومي (GTS) باستخدامRAPD-PCR أن α-pinene تسبب في أشد تلف في الحمض النووي، يليه زيت الأوكالبتوس العطري، في حين أظهر ١،٨-سينيول أقل تأثير سام للجينات. توضح هذه النتائج أن تكافؤ الفرص في التوظيف ومكوناته و تأثيرها القاتل للحلزون الأرضي من خلال طرق عمل متعددة، بما في ذلك السمية العصبية، والإجهاد التأكسدي، والسمية الجينية. تشكل أفضل تركيبة واستراتيجية للتطبيق الميداني للمركبات من الأوكالبتوس مرشحين محتملين لمبيدات الحلزون الصديقة للبيئة والتي تحتاج إلى مزيد من الاستكشاف.

الكلمات المفتاحية: زيت الأوكالبتوس العطري، ألفا بينين، ١،٨-سينول، مبيد الرخويات، الإجهاد التأكسدي، السمية العصبية، العلامات الحيوية، مبيدات الآفات الصديقة للبيئة.RAPD-PCR analysis السمية الجينية.