Annals of Biology **41** (2) : 310-319, 2025 **http://agribioj.com** **Impact Score : 0.32 (Scopus)**

Harnessing Three Essential Oils against Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera:Noctuidae) and the Glassy Clover Snail, *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae): A Toxicological and Enzymatic Perspective

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(Received: May 12, 2025; Accepted: June 24, 2025)

ABSTRACT

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and the glassy clover snail, *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae) are among the major damaging pests affecting global agriculture. The present study assessed the insecticidal potential of three plant-derived volatile oils: French lavender (*Lavandula dentata*), citronella (*Cymbopogon nardus* L.) and lemongrass (*Cymbopogon citratus* L.) against the fourth instars larvae of *S. frugiperda* and *M. cartusiana* adults under controlled laboratory conditions. The composition of the examined volatile oils was determined through gas chromatography (GC) analysis. The findings indicated that mortality rates of *S. frugiperda* and *M. cartusiana* increased with increasing concentrations of the tested volatile oils (2.25, 4.5 and 9%) and exposure time. Among the tested oils, 9% citronella exhibited the highest larval mortality of *S. frugiperda* (65%), followed by lemongrass (60%) and French lavender (55%) by day 5. Similarly, 9% citronella oil caused the highest mortality in *M. cartusiana* (40%) by day 21, compared to lemongrass (33.33%) and French lavender (26.67%). Based on the strong bioactivity of citronella oil, it was further selected for enzymatic bioassays, which revealed significant reductions in AST and ALT activity levels in both pests over the tested periods. 9% citronella oil caused the highest significant decrease, indicating metabolic disruption and tissue damage. By G C analysis, the major components identified in the tested oils were 1,8-cineole (44.15%) and linalool (24.79%) in French lavender oil, geranial (41.51%) and neral (29.76%) in lemongrass oil, and citronellal (34.67%) and geranial (27.09%) in citronella oil. Overall, citronella oil demonstrated the most potent insecticidal activity and may serve as an eco-friendly, plant-based alternative within Integrated Pest Management (IPM) programs targeting *S. frugiperda* and *M. cartusiana*.

Key words: *Spodoptera frugiperda, Monacha cartusiana*, volatile oils, enzymes

INTRODUCTION

Insect-pests represent a major obstacle to agricultural productivity, causing notable yield losses and economic damage (Souto *et al*., 2021). Among these invasive pests, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a highly destructive polyphagous pest (Idrees *et al*., 2021). Interestingly, *S. frugiperda* feeds on over 80 plant species, including maize, sorghum, millet, sugarcane and various vegetables (Chimweta *et al*., 2020). In Egypt, the presence of *S. frugiperda* was initially documented in maize fields located near Kom Ombo in the Aswan Governorate by the Agricultural Pesticide Committee (APC) (Gamil, 2020). It is worth noting that by 2019, this pest was responsible for estimated annual economic losses ranging from US $ 2.5 to 6.2 billion across 44 countries in Africa (Bengyella *et al*., 2021).

Similarly, the land snail, *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae) was considered one of the major damaging molluscs in Egypt, causing severe injury to field and vegetable crops. This species damaged crops through direct feeding, slime secretion

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and contamination with feces, resulting in reduced quality and commercial value (Abdel-Azeem, 2025).

To mitigate losses caused by these pests, farmers traditionally relied on synthetic chemical insecticides. However, such chemicals posed environmental and health risks, including toxic residue accumulation in food and water, negative impacts on beneficial insects (Abraham *et al*., 2018; Abdel Hafez *et al*., 2024) and the development of insecticide resistance (Zhang *et al*., 2021). These challenges had prompted interest in safer, more sustainable alternatives such as biopesticides (Eldidamony *et al*., 2020; Idrees *et al*., 2021; Sakla *et al*., 2025), natural enemies (Cai *et al*., 2017) and plant-based molluscicides (Mahmood *et al*., 2021).

One of the promising control options is the use of volatile oils, due to their efficacy, affordability, low environmental impact, and not leaving any harmful residues on food (Radwan and Gad, 2021; Tewari *et al*., 2024). They are produced as secondary metabolites by aromatic plants and contain bioactive compounds such as alcohols, aldehydes, phenolics, esters and terpenoids (Stojanoviæ *et al*., 2018). Notably, volatile oils have demonstrated insecticidal and molluscicidal properties by inducing behavioural changes, physiological disruption, mortality, and reduced reproduction (Tewari *et al*., 2024). These advantages highlight the urgent need to explore plant-derived bioactive compounds for sustainable pest control.

For instance, citronella oil, which is derived from citronella grasses (*Cymbopogon nardus* and *Cymbopogon winterianus*, Poaceae), exhibited a great effect against pests as it contains monoterpenes (citronellal, limonene and geraniol) which protect plants from pests (Caballero-Gallardo *et al*., 2023). Likewise, French lavender oil (*Lavandula dentate* L.) exhibits antimicrobial, antioxidant, antifungal and insecticidal properties (Duda *et al*., 2015). Another notable species, *Cymbopogon citratus* (lemongrass), the essential oil extracted from lemongrass, rich in terpenoid compounds, exhibiting strong toxic effects against a variety of pests (Khan *et al*., 2017).

Given these findings, volatile oils may offer a natural, non-toxic alternative for *S. frugiperda and M. cartusiana* control, presenting an innovative strategy that reduces the hazards associated with conventional chemical pesticides. In light of the urgent need for sustainable solutions, the present study evaluated the efficacy of three volatile oils: French lavender (*Lavandula dentata*), citronella (*Cymbopogon nardus* L.) and lemongrass (*Cymbopogon citratus* L.) on the mortality percentages of the 4th instar larvae of *S. frugiperda* and adults of the glassy clover snail, *M. cartusiana.* Also, the composition of the examined volatile oils was determined through gas chromatography (GC) analysis.

MATERIALS AND METHODS

Fall armyworm (*S. frugiperda*) (J. E. Smith) (Lepidoptera: Noctuidae) was cultured under laboratory conditions. The laboratory colony utilized in this study was derived from cultures maintained without insecticide exposure at the Plant Protection Research Institute, Egypt. During the experiment, the emergent butterflies were reared in glass jars (He *et al*., 2019), kept at (25±1°C, 60±5% RH and a 14L:10D photoperiod) and were fed on a 10% aqueous honey solution daily soaked in cotton (Kandil *et al*., 2020). Adult butterflies were permitted to mate and oviposit on white, clean paper. The egg masses were removed from the paper and placed in small plastic cups (7.0 cm in diameter and 3.5 cm in height) containing sawdust to reduce moisture. The larvae were then fed fresh and clean castor leaves (*Ricinus communis*) to prevent cannibalism.

Adult *M. cartusiana* snails were collected from infested cabbage fields in Sheeba Village, Zagazig District, Sharkia Governorate, Egypt. Healthy and morphologically similar individuals were selected and housed in a glass terrarium (50 × 30 × 30 cm) containing moist, sterilized loamy soil and maintained at 25±2°C and 75±5% soil moisture. Each terrarium was covered with a muslin cloth secured by a rubber band to prevent snails from escaping. The snails were fed fresh leaves of cabbage (*Brassica oleracea*) daily for two weeks to acclimate under laboratory conditions before conducting the experiments (Abd al-Rahman, 2020).

The hydro-distilled essential oils of French lavender (*Lavandula dentata*), citronella (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*) used in this study were obtained from the Medicinal and Aromatic

Plants Research and Production Branch, Horticulture Research Institute (HRI), Agricultural Research Centre (ARC), Egypt.

The chemical composition of the tested volatile oils was analyzed using gas chromatography (GC) at the Medicinal and Aromatic Plants Research Department, Horticulture Research Institute. Analyses were performed with a DsChrom 6200 gas chromatograph equipped with a flame ionization detector (FID) and a BPX-5 capillary column (30 m × 0.25 mm i.d., 0.25 ìm film thickness; 5% phenyl polysilphenylene-siloxane). A 1 µl aliquot of each sample was injected, and the oven temperature was programmed to increase from 70 to 200°C at a rate of 10°C/min. The detector temperature was set at 280°C. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min, with supplementary flow rates of 30 ml/min for hydrogen and 300 ml/min for air. Identification of the major constituents was based on comparison of their retention times with those of authentic standards analyzed under identical conditions. The relative percentage of each compound was determined from the corresponding peak area in the chromatogram.

To evaluate the insecticidal activity of the tested volatile oils against the two target pests, three concentrations (2.25, 4.5 and 9%) were prepared. A stock solution (9%) was prepared by mixing 9 ml of each essential oil with 90.75 ml of distilled water and 0.25 ml of Tween 80 as an emulsifier. This stock solution was diluted to obtain the 4.5 and 2.25% concentrations. The control group was treated with distilled water supplemented with Tween 80. The insecticidal activity of the tested oils against 4th instar larvae of *S. frugiperda* was evaluated by applying 10 µl of each solution sprayed onto 1 cm-diameter castor leaf discs, which were placed in acrylic mini-plates lined with moistened filter paper to maintain humidity. Treatments and control groups were replicated three times, each with 100 larvae. Larval mortality was recorded daily for five days post-treatment.

For application against snails, the leaf spraying technique was employed. Approximately 15 snails were placed into rearing plastic boxes with a capacity of 3/4 kg. The snails were then fed fresh cabbage leaves that had been sprayed with the tested concentration and left to dry for one minute. The control group was fed fresh leaves sprayed with distilled water. Both treated and control groups were applied in three replicates; each replicate contained 15 snails. Notably, untreated cabbage leaves were provided daily after an initial two days of exposure, continuing for a total of 21 days. Snail mortality was assessed at 24-h intervals by gently tapping the snails with a stainless-steel needle (Abd al-Rahman, 2020). The mortality percentages were recorded at 1, 3, 7, 14 and 21 days.

The activities of the aminotransferase enzymes, alanine aminotransferase (ALT, U/L) and aspartate aminotransferase (AST, U/L) were assessed in 4th instar larvae of *S. frugiperda* and *M. cartusiana* adult soft tissues after 48 h of treatment with citronella oil at concentrations of 2.25, 4.5 and 9%. The pests were weighed, collected and homogenized as 1:10 (w/v) in distilled water, then centrifuged for 20 min at 5°C at 5000 rpm. The deposits were discarded and the supernatants were used for the determination of biochemical parameters. Colorimetric measurement of ALT (U/L) and AST (U/L) activity was estimated according to the method of Abdel-Azeem (2025). The statistical analysis was performed by using the Co-stat statistical software. The results were presented as mean values. Statistical variations, denoted by superscripts, were determined using one-way analysis of variance (ANOVA) followed by a student's t-test ($P<0.05$) (Mishra *et al*., 2019).

RESULTS AND DISCUSSION

The chemical composition of essential oils plays a crucial role in their biological activities, including anti-feedant and insecticidal effects (Shilaluke and Moteetee, 2022). In the present study, gas chromatography (GC) analysis was employed to identify and quantify the major constituents of French lavender (*Lavandula dentata*), lemongrass (*Cymbopogon citratus*) and citronella (*Cymbopogon nardus*) essential oils (Table 1).

Fifteen compounds were identified in French lavender oil, with 1,8-cineole (44.15%) and linalool (24.79%) as the major components (Fig. 1). On the other hand, 11 compounds were identified in lemongrass oil (Fig. 2), where the major components were geranial (41.51%) and neral (29.76%). In citronella oil, 13 compounds

Table 1. Gas chromatography for the French lavender, lemongrass and citronella oil

French lavender oil			Lemongrass oil			Citronella oil		
No.	Component	RC*	No.	Component	RC	No.	Component	RC
1.	β-pinene	4.38	1.	β-myrcene	14.94	1.	α-pinene	2.82
2.	Camphene	1.52	2.	Camphene	2.95	2.	Myrcene	1.49
3.	Sabinene	4.33	3.	β-pinene	0.49	3.	β-pinene	15.61
4.	P-Cymene	1.30	4.	β-ocimene	0.73	4.	P-Cymene	1.94
5.	Limonene	1.97	5.	Linalool	3.07	5.	Limonene	2.63
6.	1,8-Cineole	44.15	6.	α-terpineol	2.69	6.	Linalool	0.98
7.	Linalool oxide	1.05	7.	Isoneral	0.13	7.	Linalool oxide	3.44
8.	Linalool	24.79	8.	Neral	29.76	8.	Citronellol	2.48
9.	Camphor	1.83	9.	Geranial	41.51	9.	Geraniol	1.06
10.	Lavandulol	5.86	10.	Geranyl acetate	3.31	10.	Geranial	27.09
11.	Borneol	4.25	11.	β-Caryophyllene	0.42	11.	Citronellal	34.67
12.	Hexenyl butanoate	0.53		Total	100	12.	Geranyl acetate	5.06
13.	Linalool acetate	0.92				13.	β-Caryophyllene	0.75
14.	Lavandulyl acetate	1.42					Total	100
15.	Eugenol	1.70						
	Total	100						

*RC: Relative concentration (%).

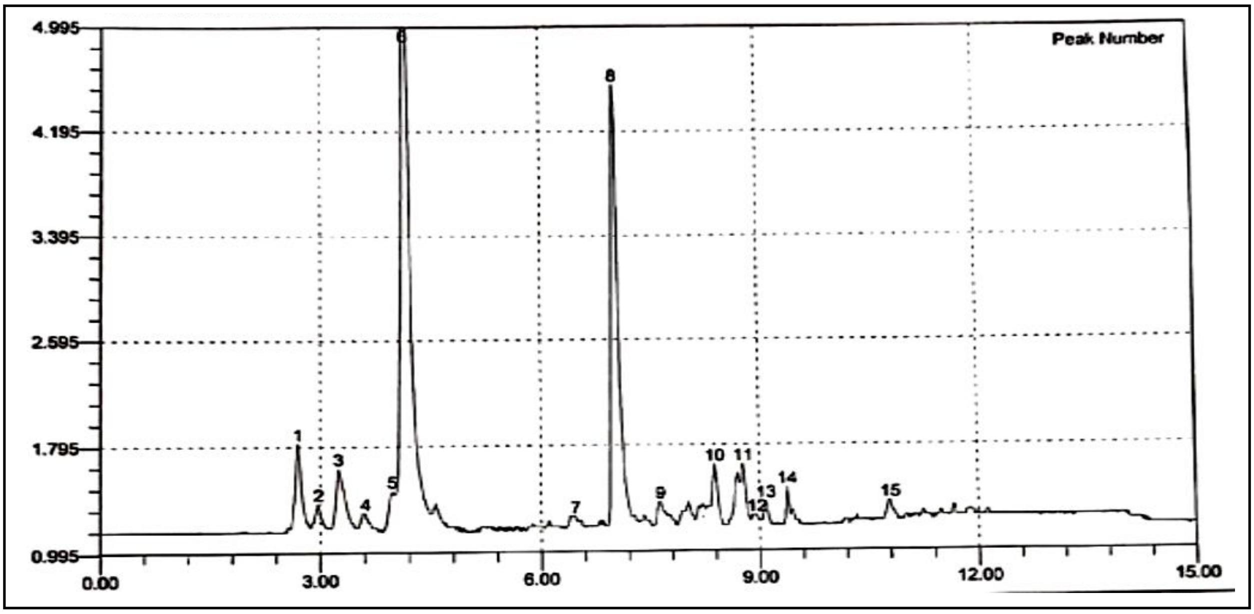

Fig. 1. The GC chromatogram of French lavender oil.

were identified, with citronellal (34.67%) and geranial (27.09%) as the major constituents (Fig. 3).

These results align with previous research findings. For instance, Kaur *et al*. (2021) identified citronellal as the dominant compound in citronella oil, typically ranging between 22.2 and 37.8%. As well, citronellal, along with geraniol and citronellol, were considered the primary bioactive constituents responsible for the insecticidal properties of *C. nardus* and *C. winterianus* (Hayati and Munawar, 2024).

Similarly, in lemongrass oil, the key active compounds were the citral isomers, geranial (citral A) and neral (citral B) which had been widely reported for their insecticidal and antimicrobial properties (Mansour *et al*., 2020; Moustafa *et al*., 2021; Hassan *et al*., 2023). Similar studies reported that neral, geranyl acetate, geranial and limonene and citral were identified as major components in *C. citratus* essential oil (Plata-Rueda *et al*., 2020).

In the case of French lavender oil, linalool was identified as a principal component through GC-MS analysis in several studies (Martins *et*

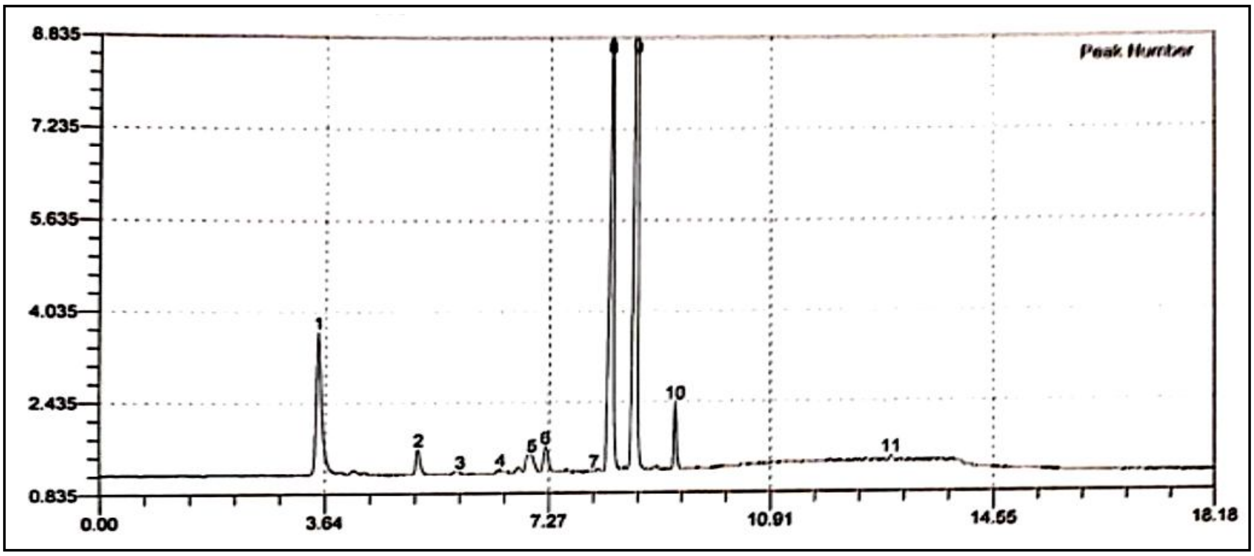

Fig. 2. The GC chromatogram of lemongrass oil.

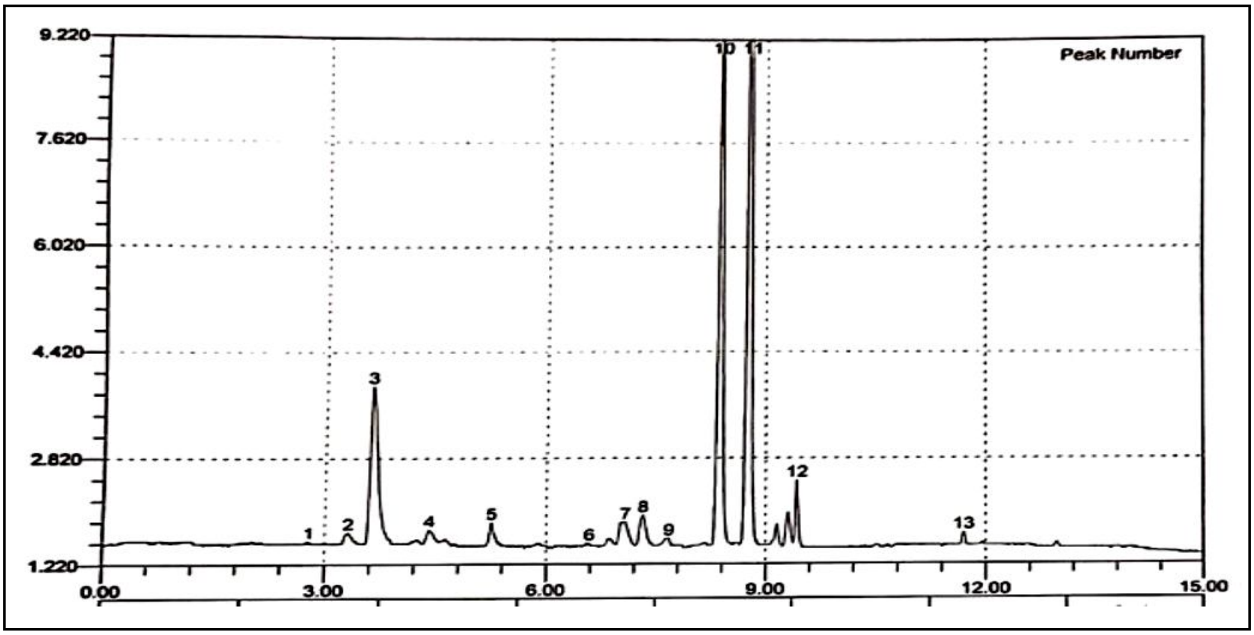

Fig. 3.The GC chromatogram of citronella oil.

al., 2019). Notably, lavender oil was rich in monoterpene compounds, including linalool, linalyl acetate, lavandulol, geraniol, borneol and eucalyptol, which contributed to its antimicrobial and antifungal activities (Bialoñ *et al*., 2019).

The effect of French lavender, lemongrass and citronella at three concentrations (2.25, 4.5 and 9%) against 4th instar larvae of *S. frugiperda over the period 2, 3, 4 and 5 days* showed an increase in the mortality percentage across time and concentration (Table 2). Citronella oil at 9% concentration exhibited the significantly highest larval mortality (65%) by the fifth day (P < 0.0001), followed by lemongrass oil (60%) and French lavender oil (55%). Meanwhile, lower concentrations resulted in modest mortality rates, with 2.25% French lavender oil showing the least efficacy (25% by day 5), in comparison with the control.

In contrast, the effect of the tested volatile oils against *M. cartusiana* was evaluated over 1, 3, 7, 14 and 21 days. After 21 days at a 9% concentration, citronella oil induced the highest mortality (40%), followed by lemongrass oil (33.33%) and French lavender oil (26.67%) (Table 3).

Table 2. Percentage mortality of the 4th instar larvae of *Spodoptera frugiperda* treated with three concentrations of French lavender, lemongrass and citronella oil

Tested oils	Conc. (%)	Days			
		2	3	4	5
French lavender oil	2.25	10^{ef}	15^{e}	20^{c}	25^{c}
	4.50	20^{de}	25^{de}	25^{c}	30^{c}
	9.00	45^{ab}	50^{ab}	55^{a}	55^{ab}
Lemongrass oil	2.25	15^{e}	20^{e}	25^{c}	30^{c}
	4.50	30^{cd}	35^{cd}	40^{b}	45^{b}
	9.00	50^{a}	55^{a}	60^{a}	60^{a}
Citronella oil	2.25	20^{de}	25^{de}	30^{bc}	30^{c}
	4.50	35^{bc}	40^{bc}	40^{b}	45^{b}
	9.00	55^{a}	60^{a}	65^{a}	65^{a}
Control	-	0.00^{f}	0.00^{f}	0.00^{d}	0.00^{d}
P		0.0001	0.0001	0.0001	0.0001
L. S. D. (P=0.05)		12.34	10.43	14.75	13.19

Different superscripts were significantly different at P≤0.05 level.

Table 3. Percentage mortality of *Monacha cartusiana* adults treated with three concentrations of French lavender, lemongrass and citronella oil

Tested oils	Conc. (%)	Days				
		1	3	7	14	21
French lavender oil	2.25	0.00^{b}	0.00^{c}	0.00^{e}	6.67^{de}	6.67^{de}
	4.50	0.00^{b}	6.67^{bc}	6.67^{de}	13.33^{cd}	13.33^{cd}
	9.00	6.67^{b}	13.33^{b}	26.67^{b}	26.67^{b}	26.67^{b}
Lemongrass oil	2.25	0.00^{b}	0.00^{c}	6.67^{de}	6.67^{de}	6.67^{de}
	4.50	0.00^{b}	6.67^{bc}	20.00^{bc}	20.00^{bc}	20.00^{bc}
	9.00	6.67^{b}	13.33^{b}	33.33^{ab}	33.33^{ab}	33.33^{ab}
Citronella oil	2.25	0.00^{b}	6.67^{bc}	13.33^{cd}	13.33^{cd}	13.33^{cd}
	4.50	6.67^{b}	13.33^{b}	20.00^{bc}	26.67^{b}	26.67^{b}
	9.00	20.00^{a}	26.67^{a}	40.00^{a}	40.00^{a}	40.00^{a}
Control	-	0.00^{b}	0.00^{c}	0.00^{e}	0.00^{e}	0.00^{e}
P		0.0002*	0.0002	0.0001	0.0001	0.0001
L. S. D. (P=0.05)		7.18	9.51	10.16	10.77	10.77

Different superscripts were significantly different at P≤0.05 level.

Notably, mortality in *M. cartusiana* progressed more slowly than in *S. frugiperda*, with no significant effects observed in the first three days for most treatments. Lower concentrations of all treatments (2.25%) caused minimal mortality (≤13.33%) even after 21 days.

These findings were supported by previous studies reporting *Cymbopogon* essential oils exhibited high efficacy in controlling a wide range of agricultural pests (Hernandez-Lambraño *et al*., 2015). Notably, the toxicity of *Cymbopogon* oils can vary significantly depending on the plant species and the concentrations used (Bossou *et al*., 2015). Supporting this, lemongrass essential oil has shown strong insecticidal activity against various pest species (Hassan *et al*., 2023), especially *S. frugiperda* (Oliveira *et al*., 2018). Similarly, citronella oil has proven effective due to its high content of monoterpenes (citronellal, limonene and geraniol), which had a key role in protecting plants against insect-pests (Caballero-Gallardo *et al*., 2023).

Measuring AST and ALT enzyme activities is a key part of biochemical assessments, as these enzymes serve as biomarkers of physiological stress and tissue damage caused by botanical insecticides in insects and snails (Tony *et al*., 2023). The activity of AST and ALT enzymes was assessed following exposure to citronella oil (2.25, 4.5 and 9%) after 1 and 3 days, as it showed the highest mortality rates against both *S. frugiperda* larvae and *M. cartusiana* adults.

The data in Fig. 4 demonstrate that exposure to citronella volatile oil caused significant inhibition in ALT and AST activity of the 4th

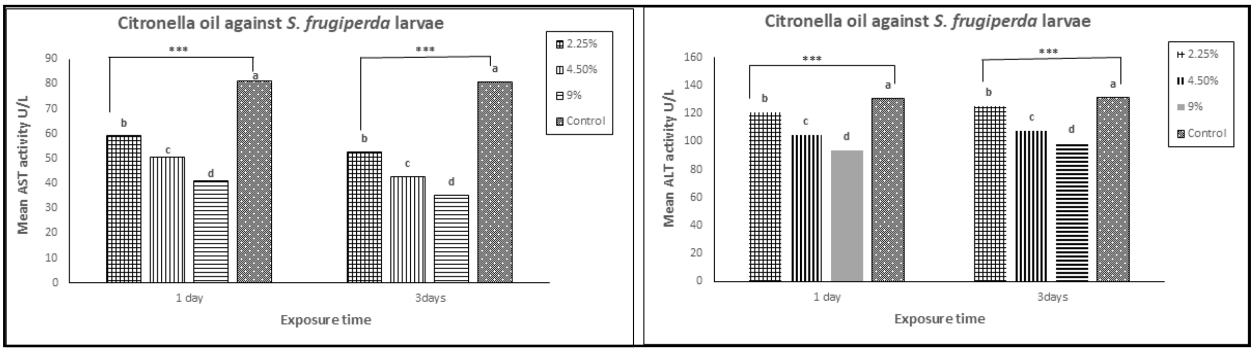

Fig. 4. Comparative analysis of alanine aminotransferase, ALT (U/L) and aspartate aminotransferase, AST (U/L), activity in 4th instar larvae of *S. frugiperda* following treatment with citronella volatile oil at concentrations of 2.25, 4.50 and 9%, along with a control group. Different superscripts between bars indicate a significant difference (P-value < 0.05). The stars on the top of each treatment group indicate the significance level between their means (***P < 0.001).

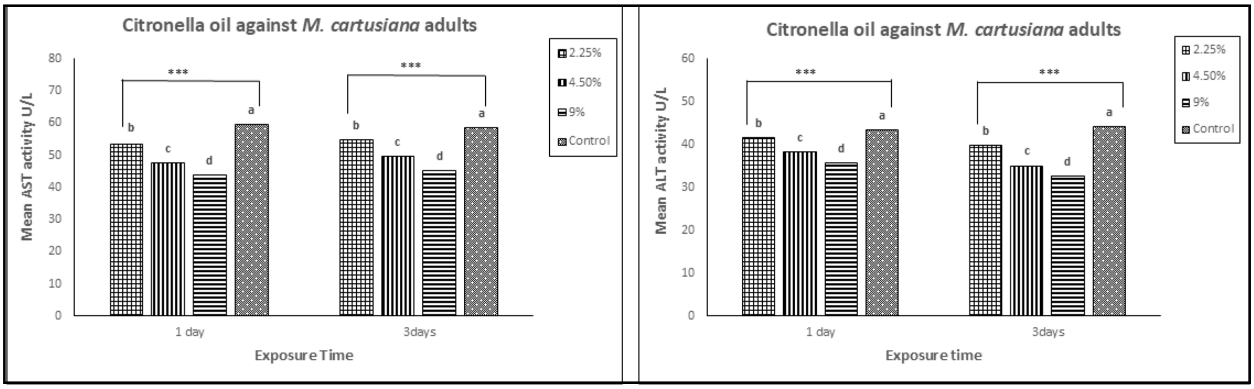

Fig. 5. Comparative analysis of alanine aminotransferase, ALT (U/L) and aspartate aminotransferase, AST (U/L), activity in *M. cartusiana* adults following treatment with citronella volatile oil at concentrations of 2.25, 4.50 and 9%, along with a control group. Different superscripts between bars indicate a significant difference (P-value < 0.05). The stars on the top of each treatment group indicate the significance level between their means (***P < 0.001).

instar larvae of *S. frugiperda* at all experimental periods. A dose-dependent decrease in enzyme activity was observed. For instance, the highest concentration (9%) resulted in a significant reduction in AST levels, reaching 41.09 and 35.22% after 1 and 3 days, respectively, compared to the control. Similarly, ALT levels decreased to 93.65 and 98.78% after 1 and 3 days, respectively, compared to the control group.

In the same manner, citronella oil caused significant inhibition in the ALT and AST activity of *M. cartusiana* adults (Fig. 5). Whereas, the 9% citronella oil decreased AST activity with values of 43.91 and 45.25% after 1 and 3 days, respectively. Likewise, 9% citronella oil decreased ALT activity, with values of 35.76 and 32.58% after 1 and 3 days, respectively, compared to the control group. These findings suggested that *C. nardus* oil had a potent biochemical impact on both the pests, potentially contributing to its insecticidal mode of action by impairing metabolic enzyme functions.

The present study findings were consistent with previous research indicating that exposure to botanical pesticides can significantly reduce the activity of key detoxifying enzymes, such as AST and ALT, in various insect and mollusk species. For example, El-Maghraby *et al*. (2023) demonstrated that natural oils, including clove, neemazal T/S, garlic, ginger and orange, markedly decreased AST and ALT levels in some pests. Concerning *S. frugiperda*, Abdullah and Sukar (2025) noted that most tested pesticides inhibited AST and ALT activity, aligning with the present study findings. On the contrary, Abdullah *et al*. (2024) reported elevated AST and ALT enzyme activity in *S. frugiperda* larvae treated with extracts of *Cladosporium cladosporioides*, *Verticillium* *lecanii* and *Ricinus communis*. On the same manner, Mahmoud *et al*. (2024) documented increased AST and ALT levels in *S. frugiperda* treated with chlorpyrifos, methomyl, and spinosad. Similarly, a comparable decline in ALT was observed in the small sand snail *Helicellavestalis* following exposure to chemical insecticides (Al-Akraa and Mohammed, 2015). In contrast, some studies have indicated increased ALT and AST activities in land snails after pesticide exposure (Gaber *et al*., 2022). Overall, the reduction in AST and ALT activity observed in this study may be attributed to metabolic disruption caused by the chemicals tested (Khalil, 2016).

CONCLUSION

The plant-derived volatile oils citronella, lemongrass and French lavender oil showed insecticidal and molluscicidal efficacy against *S. frugiperda* larvae and *M. cartusiana* adults. Citronella oil exhibited strong bioactivity among the three tested oils. Gas chromatography analysis confirmed the presence of major bioactive constituents such as citronellal, citral isomers and linalool, which are known for their pesticidal properties. In addition, a significant reduction in the aminotransferase enzymes (AST and ALT) in treated pests further indicated the physiological toxicity of citronella oil. These findings support the potential use of these volatile oils, especially citronella oil, as natural, safe and environmentally-friendly alternatives to conventional synthetic pesticides. Their application in IPM strategies could contribute to reducing chemical pesticide reliance, mitigating resistance development and preserving ecological balance. However, further research under field conditions and in combination with other biocontrol agents is recommended to validate their practical applicability and enhance their efficacy in large-scale agricultural systems.

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