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Efficacy assessment of camphor (*Cinnamomum camphora*) methanol extract and oil against *Monacha cartusiana* (Gastropoda: Hygromiidae) land snail under laboratory and field conditions

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Abstract

Monacha cartusiana (Muller) (Gastropoda: Hygromiidae), a land snail, is considered one of the most destructive pests to various agricultural crops in Egypt. Due to the toxic impact of the chemical molluscicides, there is an interest in providing biological ecofriendly molluscicides. Camphor plants (*Cinnamomum camphora*) have been known to have insecticidal efficacy. So, this study aims to assess the molluscicidal activity of camphor extract and oil against *M. cartusian*. Different concentrations of camphor extract and oil were utilized to determine the LC₅₀ and mortality rates using the bait technique. Moreover, biochemical and histological investigations were assessed after exposure of snails to sublethal concentration (LC₂₅). Field trials were also evaluated, and results confirm the molluscicidal activity of camphor extract and oil on *M. cartusian*. The laboratory results showed that the LC₅₀ was 3227.202 and 3284.781 ppm for extract and oil, and the mortality rate is concentration dependent. Both tested compounds showed disturbance in antioxidants/oxidant biomarkers, where lipid peroxidation increased and reduced glutathione significantly decreased. As well as total lipid, cholesterol and protein levels decreased in treated snails. Both compounds at the tested doses caused a significant decline in acetylcholine esterase activity. Moreover, snail digestive and hermaphrodite glands showed pathological alterations after camphor extract and oil exposure. Regarding field application, both compounds caused a significant reduction in the *M. cartusiana* population by using the bait technique. Thus, camphor extract and oil can be applied as an eco-friendly alternative molluscicide for land snail protection programs.

Introduction

Land snails are believed to be harmful pests that are found in fruit, field crops, ornamental, orchards and medicinal plants (Sallam and El-Wakeil, 2012 and Gazzy *et al.*, 2019). Terrestrial gastropods have threatened sustainable agriculture and are also crucial in the transmission and spread of diseases to plants (Shahawy, 2019; De Ley *et al.*, 2020 and Kandil *et al.*, 2020). Chemical molluscicides are considered the primary method to control land snails (Ahmed *et al.*, 2023). Serious impact on environmental health caused by using synthetic molluscicides has prompted interest in looking for appropriate alternatives from other products, such as plants. Plant extracts and oil constituents, including alkaloids, terpenes, cardiac glycosides and flavonoids from certain plant species were proven to have potent molluscicidal efficacy (Nikoli *et al.*, 2014; Abd El-Atti *et al.*, 2019; Thakur *et al.*, 2019 and Ismail, 2024). Among these natural alternatives, camphor, derived from *Cinnamomum camphora*, has been shown to exhibit strong antimicrobial and insecticidal properties (He *et al.*, 2015 and Chandorkar *et al.*, 2021) making it a promising candidate for pest and mollusk control (Ali *et al.*, 2015). Its natural origin, biodegradability, and relatively low toxicity to humans enhance its suitability as a safer alternative to synthetic molluscicides. Therefore, evaluating the biological efficacy of camphor and its derivatives against agricultural pests such as *Monacha cartusiana* (Muller) (Gastropoda: Hygromiidae) is both scientifically and practically relevant. This study aims to assess the molluscicidal activity of camphor extract and camphor oil, using the bait technique. The findings may contribute to the development of eco-friendly pest

management strategies with minimal environmental impact.

Materials and methods

1. Experimental animals and materials:

Adults of *M. cartusiana* snails were obtained from fields of clover plants in Sids village, Beni-Suef Governorate, Egypt. Then they were transferred and kept under laboratory conditions in Sids Agricultural Research Station ($20 \pm 2^\circ\text{C}$) for 2 weeks before the beginning of the experiments.

2. Plant sampling and extraction:

The camphor tree (*C. camphora*) leaves were collected from Shandaweel Agricultural Research Station, Sohag, Egypt. The selected leaves were healthy and did not suffer from any morphological aberrations. Initially, the leaves were washed with tap water to remove any surface dust or contaminants and were left to shade-dried at room temperature for two weeks. The dried leaves were then ground into a fine powder. Thereafter, 50 g of the powder was dissolved in 500 mL of 80% methanol and shaken for 48 hrs. at room temperature. The resulting extract was filtrated through Whatman filter paper. To eliminate any remaining methanol, the extracts were concentrated with a rotary evaporator at 40°C and stored in a refrigerator at 4°C . While, camphor oil was purchased from El Hawag for Natural Oils located in Al Hay as Sabea, Nasr City, Cairo.

3. The molluscicidal activity of camphor extract and oil and the experimental design baiting technique:

Different concentrations of camphor extract and oil (500, 1000, 2000, 3000, 4000 ppm) were introduced to snails for 1 week the bait technique. Each of these concentrations is combined with 5% molasses + 93% bran. Three replicates of each concentration of the tested compounds are used. Each five grams of bait was given to each

replicate (Ten snails). Thirty other individuals (Three replicates of ten snails each) served as the control group. After 1 week, the mortality percent and the LC_{50} value were calculated according to Finney (1971). Dead snails were counted, and lethal concentrations (LC_{50} and LC_{90}) were computed by Probit analysis *via* statistical software of social sciences (SPSS) (IBM Corp. Armonk, NY, USA).

4. Biochemical and histological studies:

Sixty snails were divided into 3 groups. 1st group regarded as group (Control); 2nd group exposed to LC_{25} camphor extract; 3rd group exposed to LC_{25} camphor oil for a week. The animals were dissected, and the digestive tract and hermaphrodite gland were excised and fixed in Bouin's solution. Samples were dehydrated in ethanol, embedded in paraffin wax, cut into sections and then stained with hematoxylin and eosin. Slides were examined for any histological changes in comparison to control snails according to Mohamed and Saad (1990). For biochemical studies, 1 g of soft tissue of snails was homogenized in 10 mL of phosphate buffer saline. Then it was centrifuged for 15 min at 3000 rpm, and the supernatant was stored at -30°C until the biochemical markers were detected. Total protein, total lipids and cholesterol levels were assayed according to Kinsley and Frankel (1939), Zöllner and Kirsch (1962) and Tietz (2005). Glutathione content and malondialdehyde activity were estimated *via* the colorimetric method (Beutler *et al.*, 1963 and Ohkawa *et al.*, 1979) respectively, by using commercial kits purchased from Biodiagnostic Company (Egypt). Acetylcholinesterase (AChE) activity was determined according to Kovarik *et al.* (2003) method.

5. Field application:

Field efficacy of camphor extract and oil as bait against *M. cartusiana* snails was at Quftan Village, Sumsta District, Beni-Suef Governorate, nine clover-growing plots (Each 30 m²) were used that infested with *M. cartusiana*, three replicates were used for each tested compound and the control. Four meters between the plots are maintained. The most effective lab-tested concentrations of camphor extract and oil (4000 ppm) were used for evaluation against *M. cartusiana* in the field. After treatment, live snails were counted in each plot both before and after the application. The decline in snail population after 21 days of treatment was calculated using Henderson and Tilton equation (Henderson and Tilton, 1955).

6. Statistical analysis:

The present data was analyzed by using a one-way analysis of variance (ANOVA) (SPSS, version 20) statistical program (SPSS Inc., Chicago, IL, USA). Using Duncan's multiple range and two-tailed paired t-test, analysis of differences between the means of different groups was performed. Probit analysis (Finney, 1971) is used to calculate the lethal concentration values and their respective 95% confidence limit (CL).

Results and discussion

1. Molluscicidal activity of camphor extract and camphor oil against *Monacha cartusiana* snails:

The results indicated that the molluscicidal effect of camphor extract and camphor oil against the *M. cartusiana* snail was concentration dependent. It was shown that as concentration increased, the survival rate of *M. cartusiana* snails significantly declined. The highest mortality (60 % and 65 %) had occurred at the dose of 4000 ppm and the LC_{50} was reached at concentrations of (3227.202 and 3284.781 ppm) and LC_{90} was (6901.152 and 7216.517 ppm) for camphor extract and camphor oil respectively (Table 1).

Table (1): Molluscicidal activity of camphor extract and camphor oil against *Monacha cartusiana* snails.

Treatment	Conc. ppm	Mortality % Mean $\pm$ SEM	LC ₅₀ (95% CL)	LC ₉₀ (95% CL)	F (df = 5)	P
Camphor Extract	500	10.00 $\pm$ 1.58 ^b	3227.202 (2354.596-5904.609)	6901.152 (4881.096-17502.512)	130.667	0.001
	1000	30.00 $\pm$ 1.58 ^c				
	2000	35.00 $\pm$ 2.24 ^c				
	3000	45.00 $\pm$ 2.74 ^d				
	4000	60.00 $\pm$ 2.24 ^e				
Camphor oil	500	20.00 $\pm$ 4.18 ^b	3284.781 (2882.468-3870.166)	7216.517 (6074.647-9218.181)	36.96	0.001
	1000	25.00 $\pm$ 2.74 ^b				
	2000	30.00 $\pm$ 5.00 ^b				
	3000	40.00 $\pm$ 2.74 ^c				
	4000	65.00 $\pm$ 3.16 ^d				
Control		0.00 $\pm$ 0.00 ^a				

Data represented as means $\pm$ SEM, n = 5. Means are significantly different (P: $P \leq 0.05$). (f) frequency. (df) degree of freedom.

2. Biochemical investigations:

Total protein, total lipids and cholesterol levels of *M. cartusiana* significantly decreased ($P \leq 0.001$, $P \leq 0.01$) following the exposure to the LC₂₅ of camphor extract. Meanwhile, camphor oil induced significant decrease in total protein and cholesterol ($P \leq 0.001$, $P \leq 0.01$) while the decline in the total lipids content was non-significant ($P > 0.05$) (Figures 1, 2 and 3). Regarding the GSH content *M. cartusiana* significantly decreased ($P \leq 0.05$) following the exposure to the LC₂₅ of camphor oil while the decrease in the camphor extract treated snails was non-significant. Moreover, camphor oil showed significant elevation in MDA level ($P \leq 0.05$) but camphor extracts increased MDA activity insignificantly (Figures 4 and 5). In addition, both tested compounds significantly decreased the acetylcholinesterase activity ($P \leq 0.001$, $P \leq 0.05$) (Figure 6).

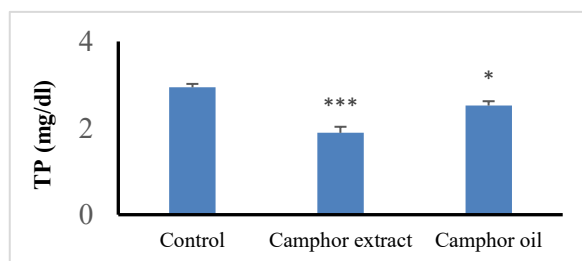


Figure (1): Total protein (TP) of treated *M. cartusiana* snails treated with LC₂₅ of camphor extract and camphor oil. *, * Significantly different at $P < 0.05$, $P < 0.001$ over their respective controls.**

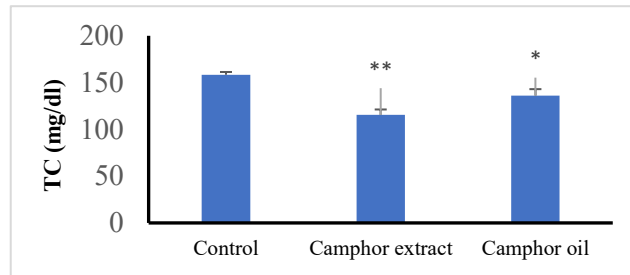


Figure (2): Total cholesterol (TC) of treated *M. cartusiana* snails treated with LC₂₅ of camphor extract and camphor oil. *,** Significantly different at $P < 0.05$, $P < 0.01$ over their respective controls.

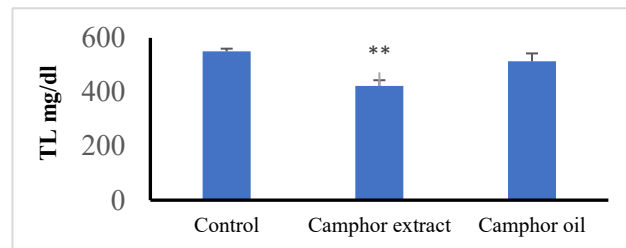


Figure (3): Total lipid (TL) of treated *M. cartusiana* snails treated with LC₂₅ of camphor extract and camphor oil. ** Significantly different at $P < 0.01$ over their respective controls.

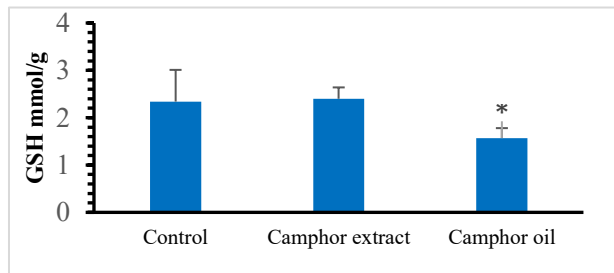


Figure (4): Effect of treatments of LC₂₅ of camphor extract and camphor oil on GSH content in *M. cartusiana* along with their respective controls. * Significantly different at $P < 0.05$ over their respective controls.

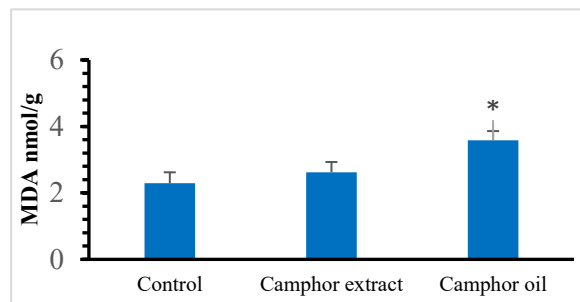


Figure (5): Effect of treatments of LC₂₅ of camphor extract and camphor oil on MDA activity in *M. cartusiana* along with their respective controls. * Significantly different at $P < 0.05$ over their respective controls.

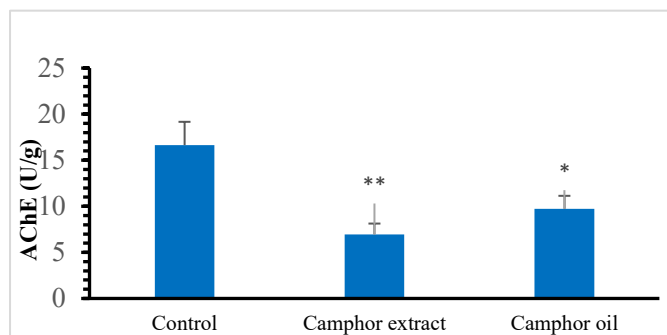


Figure (6): Achetylcholine activity (AChE (U/g)) of treated *M. cartusiana* snails treated with c of camphor extract and camphor oil. *,** Significantly different at $P < 0.05$, $P < 0.01$ over their respective controls.

3. Histological studies:

Both camphor extract and oil induced remarkable impact in the digestive gland, where treatment with camphor extract caused destruction of the tubules and degeneration of the digestive cells (Figure 7b). Camphor oil treatment instigated severe atrophy of tubules, destruction of digestive tubule and also, rupture of the basement membrane (Figure 7c). On the other hand, normal structure of the digestive gland with normal branched tubules, basement membrane, digestive cell and excretory cell found in untreated group (Figure 7a). Regarding the

hermaphrodite gland, camphor extracts induced destruction of the follicular epithelium, deformation of the follicle, degeneration of the oocytes as well as lysis of gonad cells (Figure 8b). In the same way, camphor oil caused deformation of the follicle, the oocytes, mature ovum and lysis of gonad cells (Figure 8c). Untreated snails displayed normal developing oocytes, different stages of spermatocytes, mature ovum and follicular epithelium (Figure 8a).

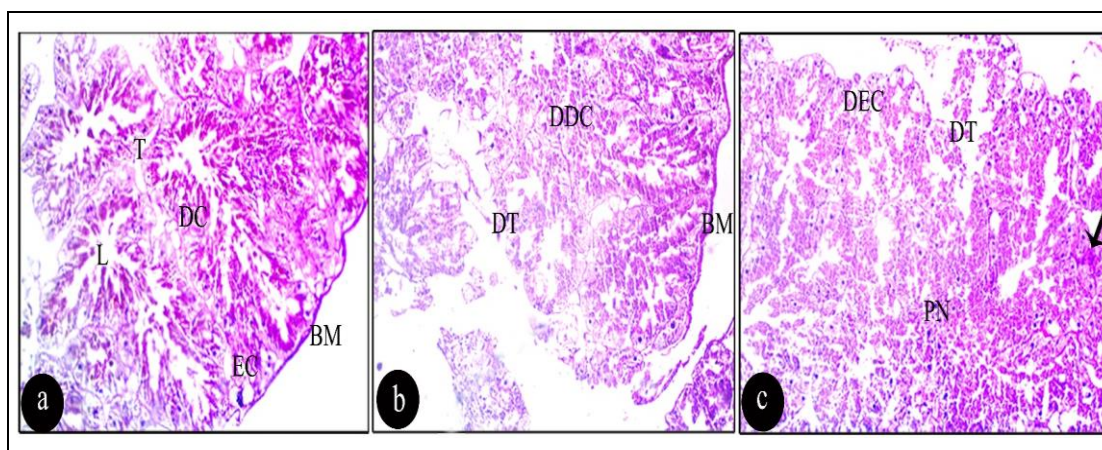


Figure (7): Photomicrographs of sections of the digestive gland of *Monacha* snail, stained with H&E. (a): control group demonstrating branched tubules (T), Basement membrane (BM), Digestive cell (DC) and Excretory cell (EC). (b): Camphor extracted treated (LC₂₅) group showing Destructed tubules (DT) and Degenerative digestive cell. (c): Camphor oil treated (LC₂₅) group showing severe atrophy of tubules, Destructed digestive tubule (DDT) and Ruptured basement membrane (BM). (x 200).

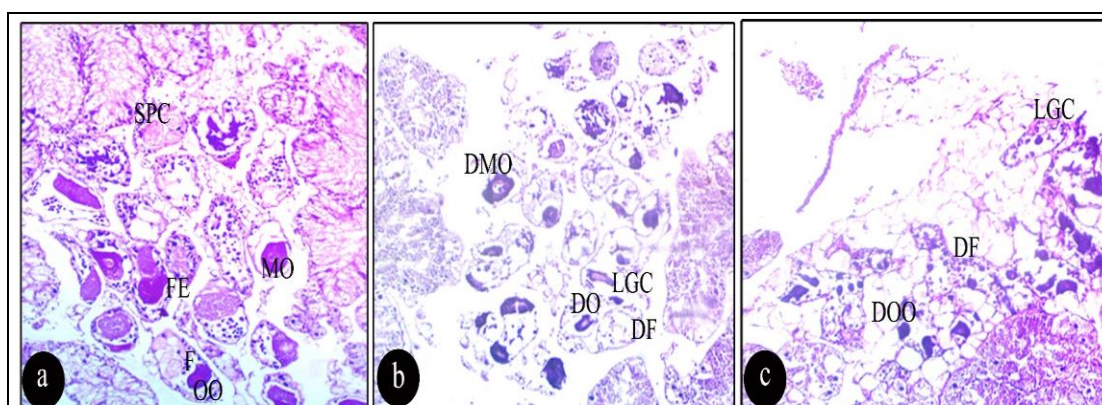


Figure (8): Photomicrographs of sections of the hermaphrodite gland of *Moncha*, stained with H&E. (a): control group demonstrating hermaphroditic follicles containing the developing oocyte (OO), different stages of spermatocytes (SPC), mature ovum (MO) and follicular epithelium (FE). (b): section of the hermaphrodite gland of Camphor extracted treated (LC₂₅) group showing destruction follicular epithelium (FE), deformed follicle (DF), degenerated oocytes (OO) and lysis of gonad cells (LGC). (c): The hermaphrodite gland treated with LC₂₅ of Camphor oil showing deformed follicle (DF), lysis of gonad cells (LGC) deformed oocyte (DO) and deformed mature ovum (DMO) (x 200).

4. Field efficacy:

Field studies showed significant reductions in the *M. cartusiana* population after treatment with camphor extract and camphor oil achieving reductions of 74.54 and 77.88 %, respectively (Table 2).

Camphor plant has potent insecticidal activity against a broad range of insects (Fu *et al.*, 2015 and Wang *et al.*, 2024). Camphor extract and oil induce mortality to land snails in our study. This may be due to the presence of alkaloids and terpenes like cineole, which contribute to camphor toxicity (Fu *et al.*, 2015 and Adawy *et al.*, 2024). Treatments with sub-lethal doses of camphor extract and oil reduced protein and lipid content as well as cholesterol levels and this may be related to induction of catabolism and impairment of the metabolic functions. This is in line with (Sobhi *et*

al., 2020) who found a decrease in total protein and lipid in cotton leaf worm after treatment with camphor oil. Moreover, treatments enhanced oxidative stress *via* increasing MDA and decreasing GSH levels. Our results are in parallel with those of Du *et al.* (2021). Meanwhile, AChE activity decreases after treatments, especially extracts, suggesting neurotoxicity. This disturbance in its level can trigger the nervous system overstimulation promoting death. Histopathological changes that have appeared in the digestive and hermaphrodite glands promote biochemical disturbances. The field results support the potential efficacy of camphor extract and oil as eco-friendly molluscicidal products. So, camphor extract and oil could be involved in the integrated pest management strategies.

Table (2): Field performance of camphor extract and camphor oil against land snails, *Monacha cartusiana* , comparing with control after 21 days of treatment.

Treatment	No. pretreatment	No. post treatment	t	P	Percent of change
Camphor Extract	34.2± 1.77	8.8± 0.37	15.02	0.001	74.54
Camphor oil	34.00±1.92	7.6±1.08	9.29	0.001	77.88
Control	37.2 ± 1.66	37.6± 1.63	1.0	0.374	

Data represented as means ± SEM, (P ≤ 0.05)

In conclusion, both camphor extract and camphor oil demonstrated molluscicidal effects on *M. cartusiana*. These compounds caused biochemical disturbances in enzyme activities, notably affecting levels of malondialdehyde (MDA) and acetylcholinesterase (AChE). Additionally, they resulted in reduced concentrations of glutathione (GSH), cholesterol, total protein, and total lipids. Furthermore, the treatments led to the degeneration and destruction of digestive and hermaphroditic cells.

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