



Evaluating the persistence and insecticidal effects of emulsifiable concentrate containing *Cananga odorata* essential oil on survival and enzymatic activity of *Spodoptera littoralis*

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Abstract The essential oil of *Cananga odorata* was prepared as an emulsifiable concentrate formulation and examined for its insecticidal activity against *Spodoptera littoralis* larvae. The effectiveness of *C. odorata* oil in both pure and EC formulations was investigated in terms of enzymatic activity and survival. In addition, their persistence effect was studied in semi-field conditions. Benzyl benzoate (18.86%), benzyl acetate (9.18%), β -Maaliene (15.53%), alloaromadendrene (6.41%), cis-Thujopsene (6.16%), cedrene (5.35%), and benzyl alcohol (5.98%) were the most abundant compounds found in *C. odorata* oil by gas chromatography-mass spectrometry analysis. The EC formulation exhibited the highest toxicity and produced LC values of 0.47, 1.02, and 3.78% for LC₄₀, LC₅₀, and LC₉₀, respectively, compared to 1.05, 1.70, and 4.96%, respectively, resulted from pure oil after 72 h of exposure. Following EC application, the mean survival time was only 3.60 ± 0.57 days, compared to pure oil treatment (7.70 ± 1.11 days) and control (14.0 ± 0.0 days). The LC₄₀ of EC formulation induced a more potent inhibitory effect for the detoxification enzyme carboxylesterases (CarE; α - and β -esterase) and the oxidative stress enzyme

superoxide dismutase (SOD). Under semi-field conditions, the cumulative mortality of *S. littoralis* larvae increased by increasing the exposure time to LC₉₀ of both formulations. The EC formulation had the highest mortality rates for the total exposure period (7 days). According to these results, *C. odorata* EC formulation demonstrated enhanced toxicity and persistence effects, and it can be utilized as a green bio-insecticide for the management of *S. littoralis* larvae.

Keywords *Cananga odorata* · Emulsifiable concentrate · Enzymes · Persistence · *Spodoptera littoralis* · Survival

Introduction

Throughout the world, a variety of economically significant crops are attacked by the seriously destructive polyphagous pest, Egyptian cotton leafworm *Spodoptera littoralis* (Boisd., 1833) (Hemmati et al., 2022). The host plants of *S. littoralis* are diverse and include ornamentals, orchards, castor oil trees, fruits, vegetables, wheat, maize, rice, sugarcane, soybeans, cotton, and numerous other crops (ElShahed et al., 2023). A 50% decrease in yield can occur when 20–70% of the leaf area of cotton is defoliated. This substantially negatively impacts the main cotton-producing territories and causes severe economic damage. Larvae have been observed to destroy leaves and pierce the fruits of different plants, causing them to

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be unsuitable for being eaten by humans (ElShahed et al., 2023). Resistance to several important pesticide classes has emerged due to the extensive use of conventional insecticides for controlling *S. littoralis* (Fouad et al., 2022). Furthermore, manufactured pesticides adversely impact non-target organisms like pollinators, mammals, and natural enemies, as well as the environment (Souza et al., 2014). Therefore, it is imperative to look for alternative pest control techniques that are less expensive and more environmentally friendly than traditional insecticides.

Essential oils are the main group of botanical pesticides; they are produced within different plant families as secondary metabolites (Ruttanaphan et al., 2019). Essential oil constituents have gained traction as eco-friendly substitutes for traditional insecticides over the last decade (Koul et al., 2013). Essential oils have low to moderate toxicity in mammals, degrade more quickly in the environment, and are frequently specific to natural enemies (Oliveira et al., 2024). Their application has been extensively studied for the control of different insect species. For instance, essential oils from *Lippia origanoides*, *Cymbopogon winterianus*, *Cymbopogon citratus*, *Salvia hispanica*, *Tanacetum vulgare*, and *Allium sativum* against *S. frugiperda*, *S. exigua*, *Acrobasis advenella*, and *Sesamia cretica* (Sombra et al., 2020; Chen et al., 2021; Magierowicz et al., 2020; Abdel-Hakim et al., 2021). Additionally, previous studies demonstrated the use of essential oils as a sustainable alternative to chemical insecticides for the control of *S. littoralis* (Awad et al., 2024; Moustafa et al., 2023).

Essential oils exhibit a range of insecticidal activities, including feeding deterrence, fumigant toxicity, and contact toxicity (Bincy et al., 2023; Moullamri et al., 2024). Furthermore, essential oils are known to disrupt the enzymatic activities of different insect species (Ruttanaphan et al., 2019; Abdelaal et al., 2021). Similar to vertebrates, insects have both enzymatic and non-enzymatic defence mechanisms (Ercan et al., 2022). Among the essential enzymes necessary for antioxidant and detoxification defence in insects are superoxide dismutase (SOD) (Awad et al., 2022) and carboxylesterase (CarE), which includes α - and β -esterase (Moustafa et al., 2021). Examining the enzyme activity in response to a possible insecticide is vital for knowing both the biological adaptability and the potential mechanism of resistance (Ercan et al., 2022).

The ylang-ylang tree [*Cananga odorata* (Lam.) Hook. f. & Thomson, F: Annonaceae] is native to Asian nations, and its essential oil is utilized for perfume production (Tan et al., 2015) and the food industry as a flavouring (Leung & Foster, 1996). From *C. odorata* oil, a variety of chemical compounds have been isolated, such as monoterpenes, sesquiterpenes, and phenylpropanoids. The essential oil of *C. odorata* has been found to exhibit a wide range of bioactivities in recent studies, including anti-microbial, anti-inflammatory, anti-vector, insect-repellent, and anti-diabetic properties (Tan et al., 2015).

Even though plant-based products, like essential oils, have strong insecticidal properties against a variety of agricultural pests, the rapid biodegradability, volatility, and lack of water solubility of botanical pesticides present some difficulties and limitations for their widespread application in pest management (Pavela, 2014). This makes it essential to develop formulations that reduce the volatility of the bioactive ingredients to improve the persistence of these botanicals (Pavela et al., 2019). Thus, the present study aimed to develop an emulsifiable concentrate (EC) formulation of *C. odorata* essential oil and to assess its toxicity and persistence against *S. littoralis* larvae in both laboratory and semi-field experiments. In addition to determining how EC-formulated and non-formulated oil affected *S. littoralis*'s ability to survive and the activity of its enzymes such as the detoxification enzyme carboxylesterases (CarE) and the oxidative stress enzyme superoxide dismutase (SOD).

Materials and methods

Phytocompound screening

The *C. odorata* essential oil was obtained from El Nasr Pharmaceutical Chemicals Co. Egypt. Screening for the oil constituents was performed using gas chromatography-mass spectrometry analysis (GC-MS). At the Central Laboratories Network, National Research Centre, Cairo, Egypt, a mass spectrometer detector (5977 A) and gas chromatograph (7890B) were fitted with an Agilent Technologies GC-MS system. A DB-5MS column (30 m x 0.25 mm internal diameter and 0.25 μ m film thickness) was attached to the GC. Helium was used as the carrier gas in the assessments, with a splitless injection volume of 0.2

μL and a flow rate of 3.0 ml/min. The temperature program used was as follows: 40 °C for one minute; 10 °C/min to 200 °C and held for one minute; 20 °C/min to 220 °C and held for one minute; 30 °C/min to 320 °C and held for three minutes. At 250 °C and 320 °C, the injector and detector were operated. Using a spectral range of m/z 50–550 and a solvent delay of three minutes, mass spectra were obtained by electron ionization (EI) at 70 eV. Quad 150 °C and mass temperature were 230 °C. By comparing the spectrum fragmentation pattern with those found in Wiley and NIST Mass Spectral Library data, various constituents could be identified.

Preparation of *Cananga odorata* emulsifiable concentrate

The *C. odorata* essential oil was mixed separately with appropriate amounts of maxiver emulsifier (obtained from Egyptian starch yeast and detergents CO.) and natural solvents (vegetable and mineral oils) to formulate the emulsifiable concentrate (EC) of *C. odorata* EO. The proportions of oil, emulsifier, and solvent were 5:20:75 to produce a 5.0% concentration. Four concentrations of *C. odorata* oil were prepared as EC (5.0, 2.5, 1.25, and 0.625%).

EC emulsion stability and foam test

Stability and foam tests were performed to examine the emulsion properties. Five mL of EC was added with continuous stirring to 80 mL of distilled water, which was maintained at 30 ± 1 °C. The contents of the beaker were increased to 100 mL by the addition of distilled water. Next, the contents were swiftly poured into a graduated cylinder (100 mL). After that, the cylinder was kept at 30 ± 1 °C for 60 min. to observe the presence of any creaming or phase separation. According to WHO guidelines, the separation layer should not be larger than 5 mL (WHO, 1985). The produced foam, which should not bypass 5 mL, was also measured using the emulsion stability test (Dobrat & Martijn, 1995).

Insect

The stock culture of cotton leafworm *S. littoralis* was maintained for several generations without exposure

to insecticides in the Pests and Plant Protection Department, NRC, Egypt. The larvae were reared on leaves of castor-oil plant (*Ricinus communis* L.). The resulting pupae were kept in separate jars until the adult moths emerged. For oviposition, the freshly emerged adult moths were placed in jars with one male for every two females. Tafla leaves (*Nerium oleander* L.) were placed in each jar as a site for the moths to oviposit, and a cotton tuft saturated with honey solution (10%) was provided for the feeding of moths. Experiments and culture were held at $65 \pm 5\%$ RH, 28 ± 2 °C, and 16 L:8D h photoperiod.

Bioassay

Concentration-response test

Four concentrations (5.0, 2.5, 1.25, and 0.625%) of *C. odorata* were prepared as pure oil by dilution of the oil with water and 1 mL of Tween-80 mixture (v/v) (Sharaby, 1988). The third larval instar of *S. littoralis* was used to assess the toxicity of *C. odorata* pure and EC formulations. Applying the leaf-dipping method, the toxicity of both formulations was investigated. Castor oil leaf discs (2.5 cm diameter) were dipped for two min. in different concentrations and then allowed to air dry at room temperature. In glass Petri dishes provided with treated leaves, freshly moulted *S. littoralis* larvae were released. For every concentration, the experiment was replicated six times, releasing five larvae in each replicate. As a control, another set of larvae was given leaf discs treated with water (1.0% Tween-80 solution). The number of live and dead larvae was counted during the duration of the 72-hour observation period. To assess the lethal concentration values (LC_{40} , LC_{50} , and LC_{90}), larval mortality was recorded.

Time-response curves

The calculated LC_{50} was used to conduct insect survival assessments of *S. littoralis* third larval instar for both *C. odorata* pure and EC treatments. The experiment was conducted in thirty replicates, with one larva and either treated or untreated food given to each replicate. The larvae were fed on treated food for 72 h and then provided with untreated food till the end of the experiment. For 14 days, the same

conditions were used for the experiment, with dead insects collected and recorded every 24 h.

Biochemical analysis

Alteration in the enzymatic activity of *S. littoralis* was performed using sublethal concentration (LC_{40}) of EC and pure *C. odorata* oil. Newly moulted 3rd instar larvae were fed on treated and untreated castor oil leaf discs, as mentioned previously, for 72 h. To assess the activity of detoxifying and oxidative stress enzymes, surviving larvae were sampled in three replicates for each treatment and immediately preserved at $-20\text{ }^{\circ}\text{C}$ until further examination.

Preparation of tissue homogenates

Depending on the optimum enzyme source concentration required, a known weight of the insects was thoroughly crushed with liquid nitrogen to produce the crude homogenates, which were then suspended in cold sodium phosphate buffer (50 mM, pH 7). A manual homogenizer was used to homogenize the mixture in an Eppendorf tube at a temperature of about four $^{\circ}\text{C}$. To get rid of the residue, the extracts were centrifuged at $10,000 \times g$ for 10 min at four $^{\circ}\text{C}$. The enzymes' activities were measured in transparent supernatants.

Esterase activity assay

With slight modifications, the α - and β -esterase activity assays were conducted in a 96-well microplate using the clear supernatants, following the Penilla et al. (1998) method. To perform the α -esterase assay, ten μL of homogenate in a well was mixed with 200 μL of α -naphthyl acetate (α -NA) solution (100 μL of 30 mM α -NA in acetone in 10 mL of 0.02 M sodium phosphate buffer, pH 7.2). In the same way, ten μL of homogenate in another well was simultaneously added to 200 μL of β -NA solution (100 μL of 30 mM β -NA in acetone in 10 mL of 0.02 M sodium phosphate buffer, pH 7.2) for the β -esterase assay. After 15 min of incubation at room temperature, the reactions were terminated by adding 50 μL of o-dianisidine stain, which is a solution of 22.5 mg o-dianisidine in 2.25 mL of distilled water and 5.25 mL of 5% sodium lauryl sulphate in 0.1 mL of sodium phosphate buffer, pH 7.0. In the control wells, there

was 50 μL of o-dianisidine stain, 200 μL of α -NA or β -NA solution, and 10 μL of distilled water instead of homogenate. Using an ELISA reader, endpoint enzyme activity was determined at 570 nm.

Using bovine serum albumin (BSA) as a protein standard, the total protein was calculated using Bradford's (1976) recommended technique. The α - and β -naphthol standard curves were used to express the α - and β -esterase activities in each of the individual samples as mmoles of product formed/min/mg protein.

Superoxide dismutase (SOD) enzyme activity assay

SOD activity was measured utilizing the Sun et al. (1988) suggested method using a commercially available kit obtained from the Biodiagnostic Company for Research (Cairo, Egypt) by reacting each sample with nitro blue tetrazolium (NBT). The result was expressed as units per gram of tissue.

Foliar application of *Cananga odorata*

In a semi-field experiment, the persistence of *C. odorata* pure and EC using LC_{90} on *S. littoralis* larvae was assessed. The common bean plant, *Phaseolus vulgaris* (L.), was used as the host plant for *S. littoralis* in this bioassay. The plant was initially grown in pots with an 11 cm diameter, holding one plant in each pot. The plants were maintained in a natural photoperiod of 16 L:8D h, $28 \pm 2\text{ }^{\circ}\text{C}$, and $65 \pm 5\%$ RH. For each treatment, five pots were utilized, and five mL of the prepared formulations were sprayed onto the plant leaves (plant height: 10 to 15 cm) from all sides. As a control, five untreated plants were established. Following foliar application, ten freshly moulted third-instar *S. littoralis* larvae were utilized for each treatment to infest each pot using a fine brush. After treatments for 1, 3, 5, and 7 days, the insects were inspected, and dead insects were counted to calculate the corrected mortality.

Data analysis

After three days of exposure to *C. odorata* pure and EC, the effective concentrations that killed 40, 50, and 90% (LC_{40} , LC_{50} , LC_{90}) of treated insects, as well as the time needed to kill 50% of the individuals (The median lethal time; LT_{50}) were calculated using

Probit analysis (Finney, 1971). The survival distributions of *S. littoralis* insects treated with and without *C. odorata* pure and EC were compared using the Kaplan-Meier survival curve. The Log-rank (Mantel-Cox) test was used to compare the experimental groups' survival distributions. The one-way analysis of variance (ANOVA) by Duncan test ($P < 0.05$) was employed to examine the effect of *C. odorata* pure and EC on the enzyme activity of *S. littoralis* larvae. The persistent toxic activity of *C. odorata* pure and EC under semi-field conditions was investigated using an independent sample T-Test with varied concentrations at selected exposure intervals. All statistical analyses were conducted using SPSS version 20.0. The corrected mortality% was calculated using Abbott's formula (Abbott, 1925):

$$\%CM = 1 - \left(\frac{nT}{nC} \right) \times 100$$

%CM = Corrected mortality percentage

nT = Number of insects in treatment

nC = Number of insects in control

Results

GC-MS analysis

The phytochemical screening of *C. odorata* oil analyzed by GC-MS identified 57 components from various classes, including alcohols, esters, hydrocarbons, phenols, monoterpene, and sesquiterpene (Table 1; Fig. 1). The most abundant ester compounds were benzyl benzoate (18.86%) and benzyl acetate (9.18%). The major sesquiterpene compounds were β -Maaliene (15.53%), alloaromadendrene (6.41%), cis-Thujopsene (6.16%), and cedrene (5.35%). From the alcohol class, benzyl alcohol (5.98%) was the main identified compound. Monoterpenoid compounds like geraniol (1.13%), linalool (0.72%), alpha-Pinene (0.71%), citronellol (0.35%), and D-Limonene (0.08%) were also identified as well as a terpene alcohol alpha-Terpineol (9.99%).

Toxicity test

Following 72 h of application, it was found that the EC formulation of *C. odorata* oil was more toxic than pure oil against the third larval instar of *S.*

littoralis (Table 2). The *C. odorata* EC presented values of 0.47, 1.02, and 3.78% for LC₄₀, LC₅₀, and LC₉₀, respectively. For *C. odorata* pure, the values of LC₄₀, LC₅₀, and LC₉₀ were 1.05, 1.70, and 4.96%, respectively. The *S. littoralis* treated with the non-formulated oil presented an LT₅₀ (9.21 days) more significant than the EC formulation (3.78 days) (Table 3).

Time-response curves

A significant relationship of survival time with both pure and EC formulations' treatments using LC₅₀ in *S. littoralis* larvae was observed ($\chi^2 = 64.410$, $df = 2$, $P < 0.0000$) (Fig. 2). Individuals in the control group survived the entire 14-day experiment. Compared to control groups, survival time was decreased after *C. odorata* application. The EC formulation had a more significant impact on *S. littoralis* larvae than pure oil. The mean survival time was only 3.60 ± 0.57 days following EC application. In contrast, the mortality was delayed following pure oil treatment and resulted in 7.70 ± 1.11 days of mean survival time compared to the control group (14.0 ± 0.0 day) (Table 4).

Biochemical activity

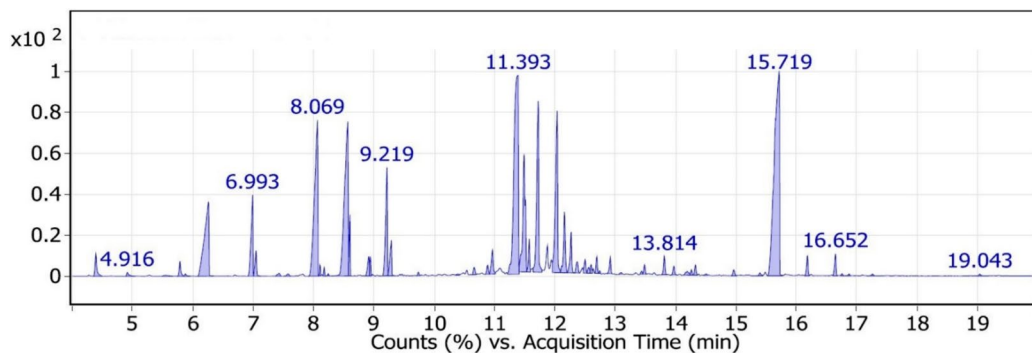
Figure 3 shows the activities of the detoxification enzyme CarE (α - and β -esterase) and the oxidative stress enzyme SOD following treatment of *S. littoralis* 3rd instar larvae with *C. odorata* pure and EC formulations. Both oil formulations exhibited a significant reduction effect in the activity of tested enzymes (F: 768.04, 792.84, and 224.46 for α -esterase, β -esterase, and SOD, respectively; $P < 0.000$). However, EC formulation significantly decreased the activity of α -esterase (0.71 ± 0.008 mmol/min/mg protein) and β -esterase (0.89 ± 0.009 mmol/min/mg protein) compared to their activities in pure oil (0.82 ± 0.005 and 1.02 ± 0.008 mmol/min/mg protein, respectively) and control (1.19 ± 0.01 and 1.48 ± 0.01 mmol/min/mg protein, respectively). Also, a remarkable decrease in the level of SOD enzyme was found in larvae treated with EC formulation being 1.12 ± 0.01 units/g tissue, followed by pure oil treatment (1.29 ± 0.02 units/g tissue) and compared to control (1.87 ± 0.03 units/g tissue).

Table 1 GC-MS analysis of *Cananga odorata* essential oil

Peak	RT	Compound	Area Sum %
1	4.395	.alpha.-Pinene	0.71
2	4.916	Benzaldehyde	0.14
3	5.551	2-Propanol, 1-(2-methoxypropoxy)-	0.08
4	5.791	Benzene, 1-methoxy-4-methyl-	0.42
5	5.877	D-Limonene	0.08
6	6.26	Benzyl alcohol	5.98
7	6.993	Benzoic acid, methyl ester	2.66
8	7.05	Linalool	0.72
9	7.433	5-Caranol, (1 S,3R,5 S,6R)-(-)-	0.12
10	7.582	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	0.09
11	8.069	Acetic acid, phenylmethyl ester	9.18
12	8.109	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	0.18
13	8.177	endo-Borneol	0.16
14	8.246	Terpinen-4-ol	0.06
15	8.572	.alpha.-Terpineol	9.99
16	8.606	Cyclohexanol, 1-methyl-4-(1-methylethylidene)-	1.04
17	8.915	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	0.61
18	8.944	Citronellol	0.35
19	9.219	1,6-Octadien-3-ol, 3,7-dimethyl-, formate	3.32
20	9.293	Geraniol	1.13
21	9.739	Estragole	0.08
22	10.397	13-Octadecenal, (Z)-	0.05
23	10.666	Phenol, 2-methoxy-3-(2-propenyl)-	0.22
24	10.89	Copaene	0.27
25	10.97	(E)-3,7-Dimethylocta-2,6-dienyl ethyl carbonate	0.81
26	11.393	1 H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	15.53
27	11.49	1 H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	5.35
28	11.576	1 H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	0.75
29	11.725	cis-Thujopsene	6.16
30	12.04	Alloaromadendrene	6.41
31	12.16	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	1.91
32	12.274	(1R,2 S,6 S,7 S,8 S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	1.2
33	12.372	.beta.-Longipinene	0.41
34	12.503	Di-epi-.alpha.-cedrene	0.39
35	12.56	Thujopsene-(I2)	0.19
36	12.6	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	0.21
37	12.698	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	0.4
38	12.744	trans-Calamenene	0.05
39	12.921	Isoamyl salicylate	0.4
40	13.093	Chloroacetic acid, tridecyl ester	0.06
41	13.436	1 H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	0.07
42	13.487	Caryophyllene oxide	0.24

Table 1 (continued)

Peak	RT	Compound	Area Sum %
43	13.814	Cedrol	0.48
44	13.974	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	0.24
45	14.197	.tau.-Cadinol	0.21
46	14.266	6-(2-Hydroxypropan-2-yl)-4,8a-dimethyl-2,3,4,6,7,8-hexahydro-1 H-naphthalen-1-ol, 1-acetate	0.13
47	14.329	.tau.-Muurolol	0.27
48	14.506	14-Hydroxycaryophyllene	0.05
49	14.964	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	0.18
50	15.399	(3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2 H-3a,7-methanoazulen-2-one	0.1
51	15.719	Benzyl Benzoate	18.86
52	16.188	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trienyl propionate	0.52
53	16.652	Benzoic acid, 2-hydroxy-, phenylmethyl ester	0.56
54	16.76	Benzenemethanol, 4-methyl-, benzoate	0.06
55	16.875	1,2-Dehydro-.alpha.-cyperone	0.06
56	17.264	2-(2-Benzyloxy-4-methyl-cyclohex-3-enyl)-propan-2-ol	0.06
57	19.043	2-Propenoic acid, 3-phenyl-, phenylmethyl ester, (E)-	0.07

**Fig. 1** GC–MS chromatogram of *Cananga odorata* oil**Table 2** Toxicity of *Cananga odorata* as pure oil and emulsifiable concentrate against *Spodoptera littoralis* 3rd larval instar after 72 h exposure

<i>C. odorata</i> formulation	LC ₄₀ (Confidence Limits µl/L air)	LC ₅₀ (Confidence Limits µl/L air)	LC ₉₀ (Confidence Limits µl/L air)	Slope ± SE	Chi ²
Pure	1.05 (0.008–1.69)	1.70 (0.92–2.33)	4.96 (3.96–7.19)	0.39 ± 0.08	3.21
EC	0.47 (-0.70–1.09)	1.02 (0.14–1.58)	3.78 (3.01–5.46)	0.46 ± 0.10	5.12

Foliar application

Data in Fig. 4 illustrates the persistence effect of *C. odorata* pure and EC formulations using LC₉₀

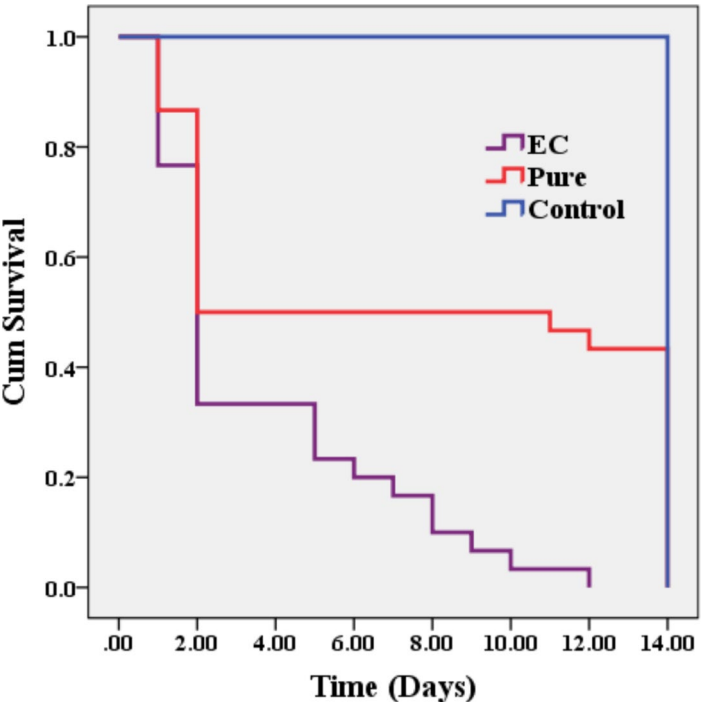
under semi-field conditions. It can be observed that the cumulative corrected mortality of *S. littoralis* larvae increased by increasing the exposure time. However, for the exposure period (7 days), the EC

Table 3 LT₅₀ values of *Cananga odorata* as pure oil and emulsifiable concentrate against *Spodoptera littoralis* 3rd larval instar

<i>C. odorata</i> formulation	LT ₅₀ (Days)	95% confidence limits (Days)		Slope ± SE	Chi ²
		Lower	Upper		
Pure	9.21	6.30	15.07	0.07 ± 0.01	27.14
EC	3.78	2.57	4.68	0.27 ± 0.02	22.62

formulation had the highest mortality rates. The mortality rates for the EC significantly increased to 66.0 ± 6.0, 86.0 ± 2.45, 95.0 ± 2.59, and 100.0%, respectively, after one, three, five, and seven days of application (t: 6.17, 4.02, 3.07, and 6.18, respectively; df:8; $P < 0.000$). In contrast, pure formulation resulted in 26.0 ± 2.45, 68.0 ± 3.74, 81.56 ± 3.83, and 83.56 ± 2.66% mortality at the same intervals, respectively.

Fig. 2 Survival curve of *Spodoptera littoralis* larvae over exposure to LC₅₀ of *Cananga odorata* as pure oil and emulsifiable concentrate (EC) estimated by Kaplan-Meier method



Discussion

In this study, an emulsifiable concentrate containing *C. odorata* essential oil was prepared to evaluate its toxicity against *S. littoralis* larvae. The analysis of GC-MS in this study identified 57 components in *C. odorata* oil. The major components were benzyl benzoate (18.86%), β -Maaliene (15.53%), alpha-Terpineol (9.99%), benzyl acetate (9.18%), alloaromadendrene (6.41%), cis-Thujopsene (6.16%), benzyl alcohol (5.98%), and cedrene (5.35%). In addition to monoterpenoid compounds such as geraniol (1.13%), linalool (0.72%), alpha-Pinene (0.71%), citronellol (0.35%), and D-Limonene (0.08%) were also detected. These results agree with a prior study (Sedky et al.,

Table 4 Means for survival time after treatment with *Cananga odorata* as pure oil and emulsifiable concentrate against *Spodoptera littoralis* 3rd larval instar

<i>C. odorata</i> formulation	Mean ± SE	95% Confidence Interval	
		Lower Bound	Upper Bound
EC	3.60 ± 0.57	2.48	4.71
Pure	7.70 ± 1.11	5.51	9.88
Control	14.0 ± 0.0	14.0	14.0

Fig. 3 The levels of the detoxification enzyme carboxylesterases (α - and β -esterase) (mmol/min/mg protein) and the oxidative stress enzyme superoxide dismutase (SOD) (units/g tissue) in *Spodoptera littoralis* over exposure to LC₄₀ of *Cananga odorata* as pure oil and emulsifiable concentrate (EC). Means ($\pm$ SE) with similar letters within the same enzyme are not significantly different, Duncan test ($P < 0.05$)

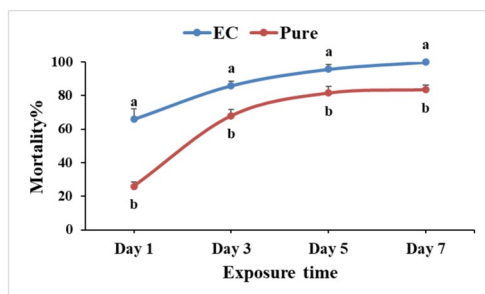
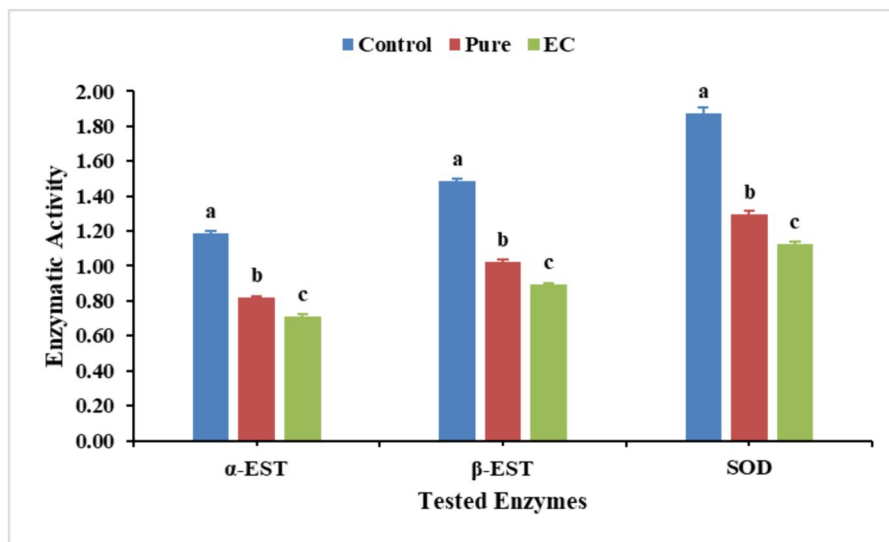


Fig. 4 Corrected mortality of *Spodoptera littoralis* 3rd instar larvae treated with LC₉₀ of *Cananga odorata* as a pure oil and emulsifiable concentrate (EC) under a semi-field experiment. Means ($\pm$ SE) with similar letters within the same time interval are not significantly different, Duncan test ($P < 0.05$)

2023), which reported the detection of 52 volatiles in *C. odorata* oil, which was classified into six categories: sesquiterpene hydrocarbons, monoterpene hydrocarbons, esters, phenols, phthalate esters, and alcohols. A previous study reported that benzyl benzoate, linalool, benzyl salicylate, benzyl alcohol, benzyl acetate, geraniol, and methyl benzoate (20.25, 11.00, 9.53, 9.10, 7.48, 6.79, and 6.08%, respectively) were identified as main compounds in *C. odorata* oil (Zhang et al., 2016). Also, it was reported that p-methyl anisole, benzyl salicylate, methyl benzoate, geranyl acetate, germacrene-D, (E)-caryophyllene, benzyl benzoate, benzyl acetate, and linalool were found as major components in *C. odorata* oil (Mal-lavarapu et al., 2016). The phytoconstituents makeup

of aromatic plant essential oils may be influenced by a number of variables, including the extraction process, the condition of the flowers, where they grow, climate, and seasonal changes (Chakira et al., 2022).

Significant toxicity of *C. odorata* oil on *S. littoralis* larvae was demonstrated in this work, and the oil's composition may have contributed to this toxic effect. Some of the phytoconstituents detected in *C. odorata* essential oil were reported to have insecticidal toxic effects. For instance, benzoates are widely recognized for having insecticidal properties. Methyl benzoate, for example, is effective against *Manduca sexta*, *Drosophila sukuzii*, *Plutella xylostella*, *S. frugiperda*, and *Aphis gossypii* (Zhao et al., 2022; Feng & Zhang, 2017; Mostafiz et al., 2019). Furthermore, benzyl benzoate exhibited contact toxicity against stored product insects (Qi et al., 2023). Terpenoids are among the groups identified in *C. odorata* oil that have been demonstrated to promote insecticidal properties (Khaleel et al., 2018). Alpha-Terpineol, for example, showed 100% mortality 96 h post-treatment with the maximum dosage (30 $\mu\text{L}/\mu\text{g}$) against *Sitophilus zeamais* adults (Yildirim et al., 2013). Moreover, monoterpenes such as D-limonene, citronellol, geraniol, linalool, and α -pinene were effective against stored product pests, *Rhyzopertha dominica*, *Callosobruchus analis*, *S. oryzae*, *Stegobium paniceum*, and *Tribolium castaneum* (Brari & Thakur, 2015; Tripathi et al., 2003).

The desire to use essential oils in integrated pest management (IPM) programmes has grown

enormously in recent decades because of their minimal harm to humans and low adverse environmental effects (Passos et al., 2022). However, plant metabolites need to be formulated in order to prevent degradation and to be used practically as biocides while handling and applying (Abo-El Seoud et al., 2005). Therefore, the EC formulation of *C. odorata* oil was prepared in this work as an oil-in-water emulsion by adding an emulsifier along with natural vegetable and mineral oils as solvents that help prevent the emulsion from separating. The findings show that the EC formulation successfully passed the foam formation and emulsion stability tests. It was reported that the EC formulations are relatively easy to use and could be diluted with water to produce the proper concentrations for various purposes (Abo-El Seoud et al., 2005).

The toxicity test in the present study revealed that *C. odorata* oil, both non-formulated and EC, had a significant toxic effect against *S. littoralis* after 72 h of exposure. However, the EC formulation was more potent than pure oil and resulted in lower LC values. Similarly, *Sophora alopecuroides* extract and the *Derris* EC formulations were more efficient than extract solutions against *Diaphorina citri* and *S. litura* (Wiwattanapatapee et al., 2009; Rizvi et al., 2019). In a previous study, non-formulated essential oils from *Proserpinaca palustris* and *Terminalia chebula* were less toxic than their EC formulations against *Aphis fabae* (Metwally et al., 2022). Abdelmaksoud et al. (2023) reported that among different tested essential oils, the EC formulation of *Rheum palmatum* ($LC_{50}=1003.60 \text{ mg L}^{-1}$) was slightly more toxic than the corresponding nanoemulsion formulation against *Aphis fabae* ($LC_{50}=1105.34 \text{ mg L}^{-1}$). Essential oils disrupt the physiological, metabolic, and behavioural processes of insects, impairing their ability to grow, develop, reproduce, or survive (Passos et al., 2022). Consistent with this, our findings demonstrated that the tested *C. odorata* oil severely affected the survival and enzymatic activity of *S. littoralis*. According to our findings, *S. littoralis*'s survival period was shortened following the application of *C. odorata*. In particular, the EC formulation at LC_{50} resulted in a lower survival time (3.60 ± 0.57 days) than that observed after non-formulated oil treatment (7.70 ± 1.11 days). This is the first study of the effects of EC formulation containing *C. odorata* oil on the enzymatic activity and survival of *S.*

littoralis. However, with a CL_{50} ($0.094 \mu\text{L/mL}$) and a CL_{90} ($0.46 \mu\text{L/mL}$) against *Sitotroga cerealella*, the insecticidal activity of non-formulated *C. odorata* oil demonstrated high efficacy (Elvis et al., 2022). Furthermore, after being exposed for 24 h, Thai *C. odorata* had an LC_{50} value of 0.042 ppm, while Indian *C. odorata* had an LC_{50} value of 0.064 ppm against *Aedes aegypti* (Chaiphongpachara & Laojun, 2020).

Our findings demonstrate a significant reduction in the activity of the detoxification enzyme CarE (α - and β -esterase) and the oxidative stress enzyme SOD, particularly after *C. odorata* EC treatment. These enzymes play a significant role in the defence mechanism of insects. Herbivorous insects are commonly known to metabolize potentially harmful secondary plant metabolites using detoxification enzymes like CarE (Ramsey et al., 2010). Esterases are the main enzymes responsible for the formation of insecticide resistance mechanisms. They break phosphodiester and carboxyl ester bonds (Abdelaal et al., 2021). In the present study, the activity of both α - and β -esterase enzymes was decreased significantly in *S. littoralis* following *C. odorata* pure and EC treatments. Although their roles have only occasionally been verified at the biochemical level, detoxification enzymes usually play a significant role in allelochemical metabolism and tolerance (Li et al., 2006). One mechanism of insecticide resistance is the increased activity of detoxification enzymes (Karuppaiah et al., 2017). Our findings indicate that *C. odorata* EC formulation has the ability to cause acute toxicity in *S. littoralis* larvae through the inhibition of α - and β -esterase enzyme activity. Previous studies reported the inhibition activity of essential oils and their constituents on detoxification enzymes (Tak et al., 2016; Huang et al., 2020). For instance, *C. winterianus* was found to significantly inhibit the activity of detoxifying enzymes, specifically glutathione s-transferase, cytochrome P450, and carboxylesterase (α - and β -esterase) in *S. littoralis* (Awad et al., 2024). Essential oils showed variable effects on *Agrotis ipsilon* 2nd instar larvae. When tested at LC_{10} and LC_{30} , *Origanum majorana* essential oil significantly inhibited the α -esterase activity at 96 h and 72 h post-treatment, respectively (Ahmed et al., 2024). In their study, only LC_{30} of *Rosmarinus officinalis* inhibited the activity of the same enzyme at 48, 72, and 96 h post-treatment. It has been reported that the primary constituents of *Ocimum basilicum* and *Alpinia galanga*

essential oils, linalool and 1,8-cineole, inhibited the activity of the detoxification enzymes carboxylesterase and cytochromes P450 (Ruttanaphan et al., 2019). Numerous defence systems protect cells from oxidative damage. For instance, antioxidant enzymes like SOD eliminate reactive oxygen species (ROS) (Ercan et al., 2022). These enzymes mitigate the oxidative stress that free radical production causes in tissues (Bas & Pandir, 2016). Consequently, an increase in ROS levels happens if the cell's antioxidant enzyme activity is insufficient. Determining the activity of these enzymes is, therefore, crucial to identifying oxidative stress. Reducing the activity of SOD in this study demonstrates that the oil EC formulation produces more oxidative stress than pure oil, which in turn leads to shorter survival rates in the treated *S. littoralis*. Similarly, *S. littoralis* treated with *C. winterianus* and *O. majorana* essential oils exhibited reduced activity of the SOD enzyme (Awad et al., 2024). It is important to examine insects' tolerance to insecticides in order to develop a proper formulation. Our results demonstrate that the EC formulation increased the susceptibility of *S. littoralis* larvae compared to pure oil and control. This toxicity might be attributable to the inhibition of these enzymes. However, additional research could lead to a more accurate understanding.

Most botanical pesticides rapidly lose their persistence because of their volatility and fast degradation (Ahmadi et al., 2018). In this study, the persistence effect of *C. odorata* oil at LC₉₀ was evaluated against *S. littoralis* under semi-field conditions. The EC formulation was found to have higher mortality rates than pure oil during the duration of the 7-day experiment. Consistent with this result, under semi-field conditions, the aqueous methanolic solution of *Sophora alopecuroides* lost its persistence 48 h after application against Asian citrus psyllid, while the EC formulation at 15, 30, and 50 mg/mL concentrations offered complete protection for a duration of 168 h (Rizvi et al., 2019). In a greenhouse assessment, the essential oil of 20% EC *Cephalotaxus sinensis* showed a notable control effect against *Megoura japonica*. The effects of EC at a concentration of 0.25% were 68.55%, 78.57%, and 85.67% on the first, third-, and seventh days following treatment, respectively (Ma et al., 2020). According to our results, using *C. odorata* oil in an EC formulation increased its control efficacy against *S. littoralis*. It

can be assumed that the EC formulation improved the oil's insecticidal activity by reducing evaporation and increasing stability. Large-scale field trials are still necessary to evaluate the viability and long-term advantages of utilizing EC formulations as a fundamental component of the integrated pest management program for the control of *S. littoralis*.

Conclusions

Plant essential oils could provide eco-friendly substitutes for traditional insecticides. In the present study, the potential insecticidal activity of *C. odorata* essential oil was investigated against *S. littoralis* larvae. The emulsifiable concentrate containing *C. odorata* was formulated to increase the toxicity and persistence effects of the oil. Our results indicate that the EC formulation exhibited higher toxicity than pure oil. The survival time of *S. littoralis* larvae was shortened significantly after the EC treatment. Furthermore, a remarkable inhibition effect was observed in the detoxification and antioxidant-tested enzymes, particularly after EC treatment. It can be observed that EC formulation resulted in a more persistent impact and increased larval mortality when tested under semi-field conditions. Therefore, producing *C. odorata* in EC formulation can be effectively used in the management of *S. littoralis* larvae. However, more research is needed to determine how EC formulation performs in field trials, as well as how it affects non-target organisms and whether phytotoxicity is a possibility.

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Data availability The data set of the study is available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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