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Trial of a control method using essential oil from *Ocimum canum* against *Diastocera trifasciata* (Fabricius, 1775), one of the main pests of the cashew tree in the central-western region (daloa; ivory coast)

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Abstract
With the aim of helping to improve cashew production by reducing the major pests of this crop, the profile of the essential oil of *Ocimum canum* was characterized, along with its insecticidal potential against adults of *Diastocera trifasciata*, one of the major pests of the cashew tree. The essential oils were extracted by hydrodistillation from fresh leafy stems and fruits, and chemical analyses were performed using GC (IR) and GC-MS. For the efficacy tests conducted at the Agricultural Production Improvement Laboratory of University Jean LOROUGNON GUEDE in Daloa, concentrations (0.04 g/mL, 0.05 g/mL, 0.07 g/mL, 0.12 g/mL, and 0.37 g/mL) were sprayed directly onto the insects in a controlled environment. Analysis of the chemical composition showed that the essential oil extracted from the leafy stems and fruits with a yield of 0.45% consists primarily of 1,8-cineole (49.7%) and camphor (15.4%). Test results revealed that the essential oil of *O. canum* possesses promising insecticidal properties. It caused 100% mortality of adult leaf miners at concentrations of 0.12 g/mL and 0.37 g/mL on the first day after spraying. The measured LC_{50} and LC_{90} values were 5.39×10^{-2} g/mL and 8.21×10^{-2} g/mL, respectively. Field trials will be necessary to confirm this preliminary result with a view to developing a biodegradable pesticide against this beetle.

Keywords: *Ocimum canum*, *Diastocera trifasciata*, essential oil, insecticidal activity, cashew pest, biodegradable pesticide

Introduction
Today, Africa accounts for more than 60% of global raw cashew nut production (FIRCA, 2022) [9]. Ivory Coast, the world's leading producer and exporter with more than 1,500,000 metric tons, accounts for 40% of global supply (FIRCA, 2022; CCAK, 2026) [9,7]. This crop is the second-largest agricultural export after cocoa and represents a vital source of income for several million rural producers (Ouali N'Goran *et al*., 2022) [22]. However, the sector is vulnerable to a diverse range of insects that cause considerable damage to orchards, particularly pest insects of the order Coleoptera. These pests alone account for nearly 39% of the total pest population (N'Guessan *et al*., 2025) [19].

Among these pests, D. trifasciata (Coleoptera: Cerambycidae), commonly known as the branch and young stem borer, is one of the major pests (N'Dépo *et al*., 2017) [17]. Adults of this species sever young branches and stems to feed on the bark and lay their eggs there after cutting the branches, causing the affected parts to dry out rapidly. They also cause nut yield losses that can exceed 55% in heavily infested plots (N'Dépo *et al*., 2017; Ouali N'Goran *et al*., 2022; N'Guessan *et al*., 2025) [17, 22, 19]. According to Akessé *et al*. (2020) [5], this pest is experiencing an alarming resurgence, fueled by the expansion of cashew plantations as well as ecological conditions that are increasingly conducive to its development. In response to this threat, several control strategies have been tested, ranging from mechanical control (sanitation) of plots to the use of pesticides (synthetic insecticides). However, the intensive use of these products leads to several drawbacks, including environmental contamination, risks to human health, the destruction of beneficial organisms, and the development of insect resistance (Yarou *et al*., 2017) [31]. Given these limitations, the search for effective, economically viable, and environmentally friendly alternatives is a priority in the context of sustainable agriculture.

Plant-based biopesticides, particularly essential oils extracted from aromatic plants, appear to be a promising approach to biological control of insect pests. These natural substances are recognized for their insecticidal, repellent, anti-feeding, and fumigant properties (Isman, 2020) [12].

Among the aromatic plants found in abundance in Ivory Coast, *Ocimum canum* (Lamiaceae) is attracting growing interest. It is widely used in traditional medicine and perfumery. Furthermore, this species produces an essential oil containing bioactive compounds that may affect the behavior, development, and survival of insect pests (Hassane *et al*., 2011) [11]. Despite numerous studies on the biological activities of these oils, to date, no study has evaluated the insecticidal activity of O. canum essential oil against adult D. trifasciata.

In this context, it appears necessary to evaluate the efficacy of O. canum essential oil with a view to improving the management of D. trifasciata. More specifically, the study will analyze the chemical composition of the essential oil extracted from O. canum, identify its major compounds, and evaluate its insecticidal activity against adult D. trifasciata.

Materials and Methods

Study site

The experiments were conducted in Daloa, a city located in the central-western part of Ivory Coast, 137 km from Yamoussoukro (the political capital). The climate is tropical, with a dry season and a rainy season. Precipitation ranges from 1,400 to 1,800 mm, with an annual average of 1,600 mm (Fondio *et al*., 2020) [10]. The average temperature is 27°C (Brou *et al*., 2005) [6]. Relative humidity ranges from 73% to 84%, with an average of 79.83 ± 4.12% (Coulibaly *et al*., 2021) [8].

Materials

The plant material used consisted of the leafy stems and flowers of *Ocimum canum* (Lamiaceae). The animal material consisted of adult *Diastocera trifasciata*. The equipment used included hand-held sprayers and 1,000 mL glass bottles for treatment. The extraction apparatus for extracting the essential oil from O. canum, as well as a binary synthetic insecticide (Sauver 62 EC) containing the active ingredients acetamiprid (32 g/L) and lambda-cyhalothrin (30 g/L).

Methods

Extraction of Essential Oil from O. canum

A total of 500 g of fresh material was extracted using the hydrodistillation method for two (2) hours with a Clevenger-type apparatus at an average temperature of 27.57 ± 1.28 °C and an average humidity of 76.00 ± 4.10%. The crude essential oil extracted was dried over anhydrous sodium sulfate and stored in pill bottles in the freezer (-8°C). The essential oil yield was calculated using the following formula :

$$\text{Yield (\%)} = \frac{mass\ (g)\ of\ essential\ oil}{mass\ (g)\ of\ plant\ material} \times 100$$

Essential Oil Analysis

The various samples of the essential oil were analyzed by gas chromatography combined with the gas chromatography retention index (GC-RI) and by gas chromatography coupled with mass spectrometry (GC-MS).

Analysis CPG (Ir)

Each sample was analyzed using gas chromatography with a PerkinElmer Clarus 500 gas chromatograph equipped with a retention index (GCP-RI). It is equipped with a flame ionization detector (FID) and two nonpolar columns BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol) each with the following specifications: 50 m × 0.22 mm; film thickness 0.25 μm.

The temperature is set as follows

- It begins at 60 °C and then gradually increases to 220 °C at a rate of 2 °C/min over 80 minutes, followed by a 20-minute isothermal hold at 220 °C:
- The injector and detector were maintained at 250 °C. Dihydrogen, which serves as the carrier gas, was delivered at a flow rate of 1.0 mL/min. A volume of 0.5 μL of each diluted sample was injected.

Analysis by CPG-SM

The analysis was performed using a PerkinElmer Clarus 580 Autosystem chromatograph coupled with a PerkinElmer Clarus SQ8S Turbo Mass electron impact mass spectrometer operating at 70 eV. The system consists of two fused silica capillary columns: BP-1 (polydimethylsiloxane) and BP-20. The sample injection volume and the GC-MS configuration are the same as previously described.

Identification of Constituents

Each compound in each sample was identified by its retention index (RI), calculated based on its retention time (RT) relative to a series of n-alkanes (C_8–C_{29}) using linear interpolation with Perkin-Elmer's "Target Compounds" software.

The retention index is then compared with those of reference compounds (König *et al*., 2001) [14]. Confirmation is performed by computer, based comparison of the mass spectrum with those of reference compounds compiled in digital databases, using AMDIS version 2.70 and NIST MS Search version 2.4 (König *et al*., 2001; Adams, 2007; NIST, 2020) [2, 14, 20].

The relative percentage of each constituent identified in the sample is obtained by peak area normalization.

Evaluation of the Efficacy of Essential Oil

For the tests, the average temperature was 27.32 ± 0.88 °C, with a humidity of 37.17 ± 3.76%.

Determination of Concentrations

The essential oil is extracted from O. canum. Diluting the essential oil (2 mL) in distilled water (45 mL; 35 mL; 15 mL; 10 mL and 5 mL) yielded five respective concentrations (0.04 g/mL; 0.05 g/mL; 0.07 g/mL; 0.12 g/mL and 0.37 g/mL).

The synthetic insecticide used was Sauver 62 EC. The recommended dose is 125 mL to be diluted in 15 liters of water. Diluting 1 ml of the product in distilled water (608 ml, 405 ml, 270 ml, 180 ml and 120 ml) yielded the following concentrations, respectively: 1.02×10^{-4} g/mL, 1.53×10^{-4} g/mL, 2.28×10^{-4} g/mL, 3.44×10^{-4} g/mL, and 5.12×10^{-4} g/mL.

Spraying the products on adult D. trifasciata

The prepared concentrations were sprayed onto each batch of (5) unsexed adult insects that is, 50 adults treated per concentration and 250 for the 5 concentrations tested and young cashew tree stems and thin branches were provided to them after spraying. Dead individuals were counted 24 hours, 48 hours, and 72 hours after spraying, and the average mortality rates were determined and then corrected as suggested by Abbott (1925) [1].

$$M = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ insects} \times 100$$

$$Mc = \frac{(Mo - Mt)}{(100 - Mt)} \times 100$$

Where Mc = adjusted mortality; Mo = observed mortality in the treatment group; and Mt = mortality in the control group.

The Abbott formula was not used because no dead D. trifasciata were found in the control group.

The lethal concentrations 50 (LC_{50}) and 90 (LC_{90}) are the concentrations that cause the death of 50% or 90% of a treated insect population after 24 hours. These values were determined using a regression model based on the log probit function.

Statistical analysis

Statistica version 7.1 was used for data analysis. An analysis of variance (ANOVA) was used to identify significant differences among the data. The Student-Newman-Keuls test at the 5% significance level was used to group the means into homogeneous clusters.

The LC_{50} and LC_{90} values were determined using RStudio version 4.3.2. The regression model used to determine the lethal doses is the log probit model, which is used to predict the values.

Results and discussion

Essential oil extraction yield

The essential oil is colorless, with a yield of 0.45%.

Chemical composition of O. canum essential oil

The results of the chemical analyses of the essential oil are presented in Table 1

Table 1: Chemical composition of O. canum (only compounds with a percentage of ≥ 0.1% are reported)

Identified Compounds	IKA	IKP	Contents
α-Pinene	930	1 016	5.3%
Camphene	943	1 067	2.4%
Oct-1-ene-3-ol	958	1 447	0.6%
Sabinen	965	1 124	0.8%
β-Pinene	970	1 113	4.0%
Myrcene	980	1 163	1.9%
α-Phellandrene	997	1 167	0.3%
p-Cymene	1 011	1 273	0.9%
1,8-Cineole	1 021	1 213	49.7%
β-Terpinene	1 048	1 247	0.5%
Terpinolene	1 078	1 285	0.3%
Linalool	1 083	1 547	0.9%
Camphor	1 121	1 515	15.4%
δ-Terpineol	1 147	1 682	0.5%
Borneol	1 149	1 698	0.2%
Terpineol-4	1 161	1 600	1.2%
α-Terpineol	1 172	1 695	1.4%
Myrtenol	1 177	1 787	0.3%
2-Phenylethyl acetate	1 242	1 768	0.3%
Bornyl acetate	1 269	1 579	1.7%
Myrtenyl acetate	1 304	1 686	0.5%
β-Caryophyllene	1 416	1 594	2.8%
α-Bergamotene	1 431	1 680	1.9%
(E)-β-Farnesene	1 445	1 670	0.4%
Germacrene D	1 474	1 705	0.2%
α-Farnesene	1 480	1 727	1.1%
Muurola-4(14),5-diene	1 513	1 643	0.3%
γ-Muurolene	1 513	1 682	0.9%
(Z)-β-Farnesene	1 546	1 667	0.6%
Caryophyllene oxide	1 568	1 974	0.3%
Total			97.6%
Oxygenated compounds			73.0%
Hydrocarbon compounds			24.6%
Oxygenated monoterpenes			71.8%
Hydrocarbon monoterpenes			16.4%
Oxygenated sesquiterpenes			0.3%
Hydrocarbon sesquiterpenes			8.2%

IKA: Kováts Index on an Apolear Column; **IKP :** Kováts Index on the Polar Column

These results show that the essential oil of O. canum is predominantly monoterpenic (88.2%), with oxygenated compounds (73%) predominating, led by 1,8-cineole (49.7%) and camphor (15.4%). Among the hydrocarbon monoterpenes (16.4%), α-pinene is the most abundant compound, accounting for 5.3%. Sesquiterpenes (8.6%) are present in small amounts in this oil, with β-caryophyllene being the only identified compound, present at a low concentration (2.8%).

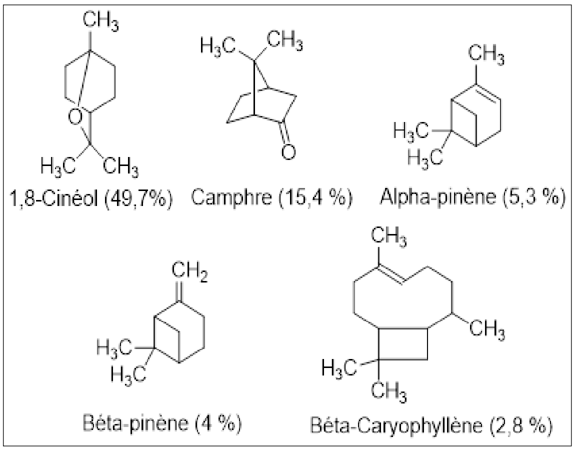

Fig 1: Some major compounds identified in the essential oil of O. canum

Effect of O. canum essential oil on adult D. trifasciata

Twenty-four hours (24 h) after treatment, the five concentrations 0.04 g/mL, 0.05 g/mL, 0.07 g/mL, 0.12 g/mL, and 0.37 g/mL recorded mortality rates of 14.28 ± 6.73%; 47.62 ± 17.82%; 85.71 ± 17.82%; 100% and 100%, respectively. Two days (48 h) after spraying, the lowest concentration, 0.07 g/mL, resulted in a maximum mortality rate of 100%. The lowest concentrations, 0.04 g/mL and 0.05 g/mL, resulted in mortality rates of 47.62 ± 17.82% and 90.48 ± 16.26% on day 3 (72 h), respectively (Table 2). Statistical analysis revealed significant differences in mortality rates among the various concentrations of O. canum ($F = 93.97$; ddf = 17; $p = 0.001$).

Table 2: Mortality rate (%) of adult D. trifasciata following treatment with O. canum in the laboratory

	Ocimum canum					
	00 g/mL	0.37 g/mL	0.12 g/mL	0.07 g/mL	0.05 g/mL	0.04 g/mL
24 h	0 ± 0 a	100 ± 0 a	100 ± 0 a	85.71 ± 17.82 b	47.62 ± 17.82 c	14.28 ± 6.73 c
48 h	0 ± 0 a	100 ± 0 a	100 ± 0 a	95.24 ± 12.60 a	57.14 ± 16.26 b	23.81 ± 8.26 b
72 h	0 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	90.48 ± 16.26 a	47.62 ± 17.82 a
F				93.97		
P				0.001		

H: time Means followed by the same letters in each column are not significantly different according to the Student-Newman-Keuls test at the 5% significance level.

Effect of Sauver 62 EC on adult D. trifasciata

Twenty-four hours (24 h) after spraying, the five concentrations 1.02×10^{-4} g/mL; 1.54 $\times10^{-4}$ g/mL; 2.28×10^{-4} g/mL;3.44×10^{-4}; 5.12×10^{-4} g/mL yielded mortality rates of 21.42 ± 9.45%; 50 ± 10.41%; 78.57 ± 9.45%; 92.86 ± 12.20% and 100%, respectively. The mortality rates induced by concentrations of 1.02×10^{-4} g/mL; 1.54 $\times10^{-4}$ g/mL and 2.28×10^{-4} g/mL reached 100% on day 3 (72 h) after application of the product (Table 3). Statistical analysis revealed significant differences in mortality rates among the different concentrations of Sauveur ($F = 129.96$; ddf = 17; $p = 0.001$).

Table 3: Mortality rate (%) of adult D. trifasciata after treatment with Sauveur in the laboratory

	Sauveur 62 EC					
	00 g/ml	5.12×10^{-4} g/ml	3.44×10^{-4} g/ml	2.28×10^{-4} g/ml	1.51×10^{-4} g/ml	1.02×10^{-4} g/ml
24 h	0 ± 0 a	100 ± 0 a	92.86 ± 12.20 ab	78.57 ± 9.45 b	50 ± 10.41 b	21.42 ± 9.45 c
48 h	0 ± 0 a	100 ± 0 a	96.43 ± 9.45 a	96.43 ± 9.45 a	75 ± 0 a	53.57 ± 9.45 b
72 h	0 ± 0 a	100 ± 0 a	100 ± 0 a	96.43 ± 9.45 a	78.57 ± 9.45 a	60.71 ± 9.45 a
F				129.96		
P				0.001		

H: time Means followed by the same letters in each column are not significantly different according to the Student-Newman-Keuls test at the 5% significance level.

Lethal concentrations (LC_{50} and LC_{90}) of the products tested on D. trifasciata

The lethal concentrations resulting in the death of 50% (LC_{50}) and 90% (LC_{90}) of adult D. trifasciata twenty-four hours (24 h) after treatment were calculated for each product. A comparison of the two products used shows that O. canum has a higher LC value than the reference chemical insecticide (Table 4).

Table 4: Lethal doses 50 and 90 (LD_{50} and LD_{90}) 24 hours after treatment

Lethal Doses		
Products	DL_{50}	DL_{90}
Ocimum canum	5.39×10^{-2} g/mL	8.21×10^{-2} g/mL
Sauveur 62 EC	1.45×10^{-4} g/mL	3.09×10^{-4} g/mL

Discussion

The essential oil yield of O. canum (0.45%) is similar to that reported by (Ntonga *et al*., 2012) [21], who found a yield of 0.44% from fresh leaves. This result also differs from those obtained in other studies conducted in Côte d'Ivoire (Oussou, 2009) [23] and in Burkina Faso (Coulibaly *et al*., 2023) [8]. This variability may be because the extraction was performed on fresh leaves, whereas our predecessors performed it on dry leaves. This difference in yield could also be related to the time of harvest, edaphic and climatic factors, or the plant's physiopathological condition (Tchoumbougnang *et al*., 2005; Tchoumbougnang *et al*., 2006) [29, 28].

Chemical analysis of the essential oil of O. canum identified 1,8-cineole (49.7%) and camphor (15.4%) as the major constituents. These results are similar to those obtained by Coulibaly *et al*. (2023) [8], who reported the same major constituents in fresh leaves of O. canum. However, the essential oil from the same plant native to Cameroon, as reported by Ntonga *et al*. (2012) [21], is dominated by 1,8-cineole (29.04%) and linalool (19.07%). The Togolese species studied by Akantetou *et al*. (2012) [4], on the other hand, is rich in 4-terpineol (36.4%) and linalool (19.8%). These results show that the essential oils of O. canum exhibit distinct chemical variability. This variability in the volatile components could be explained by various factors, such as the plant's geographic origin, the plant part used, sunlight exposure, and the availability of mineral salts.

Adulticide tests showed that O. canum essential oil and the chemical insecticide (Sauver 62 EC) effectively eliminated adult D. trifasciata. Sauver 62 EC proved to be more effective against adult D. trifasciata than O. canum essential oil, depending on the duration of exposure. The efficacy of Sauver 62 EC was demonstrated by Akessé *et al*. (2020) [5] in their study of the same insect. Indeed, these authors reported that this product, at a concentration of 5.12×10^{-4} g/mL, eliminated 100% of adult D. trifasciata within a shorter observation period (1 day). This efficacy is believed to be due to the high concentration of the product's active ingredient. Sauveur consists of two active ingredients: acetamiprid and lambda-cyhalothrin. These two active ingredients act on the insect by penetrating its cuticle, disrupting its nervous system within a few minutes. They also cause cessation of feeding, loss of muscle control, and paralysis that can lead to death (Li-Ming *et al.,* 2008; Afrazeh & Jalali Sendi, 2025) [16, 3].

As for the O. canum essential oil, it also resulted in mortality rates that varied with time and concentration. At a concentration of 0.07 g/mL, the essential oil resulted in an 85% mortality rate within twenty-four hours after spraying the adults. The essential oil proved to be effective. This finding is consistent with those of Tano *et al*. (2019) [27], Yéboué *et al*. (2022) [32], and Parfait *et al*. (2025) [24], who reported that a product is effective when it induces a mortality rate of at least 70% in a pest insect within twenty-four hours (24 h) after spraying. Two concentrations (0.12 g/mL and 0.37 g/mL) induced 100% mortality. These findings confirm a study that reported 100% mortality rates in adults of Cylas puncticollis Boheman, the sweet potato weevil, at a dose of 50 µL/L after 72 hours of exposure, with an LC_{50} of 19.77 µL/L (Tia *et al*., 2019) [30]. These results are also consistent with other studies, which have demonstrated its effectiveness against adult Aphis gossypii (Akantetou *et al*., 2012) [4]. The insecticidal properties of essential oils from species of the genus Ocimum in the control of various orders of insect pests have already been reported by several authors (Kobenan *et al*., 2018; Tano *et al*., 2019; Yoboué *et al*., 2022) [13, 27, 32]. The efficacy of this essential oil against insects may be due to synergistic effects of the insecticidal action of 1,8-cineole, camphor, and (α/β)-pinene active compounds in the essential oil of O. canum (Langsi *et al*., 2020; Sun *et al*., 2020; Nebie, 2023) [15, 25].

Conclusion

This study determined the extraction yield and chemical composition of O. canum essential oil and evaluated the oil's efficacy against adult D. trifasciata. The physicochemical parameter determined in this study was the extraction yield, which was 0.45%. Analysis of the chemical composition of O. canum oil revealed that it consists primarily of 1,8-cineole (49.7%), camphor (15.4%), α-pinene (5.3%), β-pinene (4%), β-caryophyllene (2.8%), and camphene (2.4%). This oil also exhibited significant insecticidal activity against adult D. trifasciata. The mortality rate reached 100% on the 1st and 3rd days of treatment at the various concentrations of 0.07 g/mL, 0.12 g/mL, and 0.37 g/mL. The results obtained from the elimination rates show that O. canum oil is effective. However, field trials would be needed to confirm its insecticidal activity against adult D. trifasciata. The essential oil could thus serve as an alternative to synthetic chemicals in the management of D. trifasciata in cashew nut crops.

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