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Evaluation of Ocimum Gratissimum L. (Lamiaceae) Extracts Against Nasutitermes Termites, Cocoa Pests in Côte d'Ivoire

Kenji J. Gonzalez[1], Elena Wilson[1]*

[1]Department of Agricultural Entomology, Faculty of Agriculture, University of Chile, Santiago, Chile.

***E-mail** ✉ ewilson@outlook.com

ABSTRACT

The overuse of synthetic pesticides in agricultural environments has caused numerous issues, including insect resistance and environmental contamination. As a result, research has increasingly turned toward plants with insect-repellent properties. This study aimed to evaluate the insecticidal potential of different extracts of *Ocimum gratissimum* L. (crude extract, extract fractions, and essential oil) against Nasutitermes termites that damage cocoa crops. The crude extracts were prepared by macerating dried leaf powder, the fractions were derived from the hydro-ethanolic extract, and the essential oil was extracted through hydrodistillation. All tests were performed in the Animal Biology Laboratory at Félix Houphouët-Boigny University and repeated four times. In each trial, 50 worker termites were placed in Petri dishes and exposed to different doses of the extracts, with the effects compared to a control group. The results showed that after three hours of exposure, all concentrations of the extracts caused 100% mortality in the termites. This high level of toxicity is likely due to the monoterpenes, which make up 80% of the extract. However, when used in fumigation, the essential oil was effective only at higher concentrations (5 and 10 mg/l). Among the various extracts, only the acetate fraction showed toxicity, although it required 48 hours to take effect at high concentrations.

Keywords: Termite pests, Crude extract, Insecticidal plants, Essential oil, Extract fraction, *Ocimum gratissimum*

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Introduction

Certain termite species can cause significant and often irreversible damage to crops due to their biological characteristics and feeding behavior [1, 2]. The Nasutitermes genus, a member of the Nasutitermitinae subfamily, is particularly destructive as it constructs arboreal nests that compromise the structural integrity of plants [3]. Additionally, their wood-feeding habits hinder the development of the plants they infest. Research has highlighted that termites of this genus are primary pests of cocoa trees in Côte d'Ivoire [4, 5]. Historically, plants have been one of the earliest forms of pest control in agriculture [6]. However, the introduction of synthetic pesticides in the 1940s, which showed dramatic results, led to the use of plant-based pest control methods being marginalized and seen as a traditional practice [7, 8]. The overreliance on synthetic chemicals has led to issues such as pest resistance, environmental pollution, and health problems, including diabetes, cancer, and infertility [9, 10]. These negative consequences have spurred the search for plant-based insecticidal compounds [11]. Plant extracts used as biopesticides are increasingly recognized for their environmental benefits, such as being biodegradable, low toxicity, and safe for humans [12, 13]. For generations, farmers have relied on plants to manage pests, particularly termites [14], and the use of plant-based pesticides continues to grow in importance [15, 16]. Ethnobotanical studies have revealed over 2,000 plant species with insecticidal properties, acting in various ways, such as insecticides, repellents, and growth inhibitors for termites [17, 18]. *Ocimum gratissimum* L., commonly known as false basil, is a noteworthy plant due to its insecticidal potential. Native to India, this shrub produces aromatic leaves containing volatile compounds that can be toxic in higher doses. In Côte d'Ivoire, several studies have confirmed the effectiveness of crude extracts from this plant in controlling various insect pests [19-21]. This research aims to evaluate the insecticidal properties of various extracts (crude extract, extract fractions, and essential oil) of *Ocimum gratissimum* against Nasutitermes termites. Additionally, the study aims to identify the chemical compounds responsible for the insecticidal effects of these extracts.

Materials and Methods

Experimentation site

The research was conducted at the Zoology and Animal Biology Laboratory of Félix Houphouët-Boigny University. All experiments were performed under controlled laboratory conditions (temperature: 27 ± 2 °C; humidity: 80%) using Petri dishes.

Biological material

The leaves of *Ocimum gratissimum* (Lamiaceae) were collected from the outskirts of Abidjan to prepare the different extracts used in the study. The Nasutitermes termite workers, tested in the experiments, were gathered from the Center National de Floristique (CNF) at the University Félix Houphouët-Boigny.

Methodology

Obtaining the essential oil and its chemical composition

The method of hydrodistillation is utilized to extract volatile compounds from plants through steam distillation. These compounds may experience varying degrees of modification during the extraction process [22]. To extract the essential oil, a Clevenger-type apparatus was employed. The process began with 1300 g of plant material, which was weighed and positioned on a grid inside the matrix (pressure cooker), functioning as the distillation chamber. The grid serves to separate the plant matter from the water below, with the steam generated from the water facilitating the essential oil extraction. The extraction is halted once the first drop of essential oil (EO) is observed, approximately three hours before completion, by stopping the heat. The collected oil is then dried using anhydrous sodium sulfate (Na2SO4). To analyze the compounds within the essential oil, gas chromatography (GC) and Nuclear Magnetic Resonance (13C-NMR) were employed, based on the protocols provided by Boti *et al*. [23].

Obtaining raw extracts

The powdered leaves of *Ocimum gratissimum* are immersed in a hydro-ethanolic solvent with an 8:2 ratio and left to macerate for 24 hours while being stirred magnetically. Once the maceration is complete, the mixture is filtered using Büchner with Whatman paper, followed by water cotton. The resulting extract is then dried in an oven at 40 °C until all moisture is evaporated. The remaining residue from the first maceration is dried and subjected to a second extraction using hexane to isolate the apolar compounds.

Obtaining extract fractions

The crude hydro-ethanolic extract was further processed by sequentially using solvents with increasing polarity to fractionate and isolate the molecules based on their polarity [20-22]. The liquid-liquid extraction was carried out in a separatory funnel with dichloromethane (DCM), ethyl acetate, and distilled water. Each solvent was applied 2 to 3 times until complete extraction was achieved. The fractions obtained were then concentrated with a rotary evaporator and dried in an oven at 40 °C.

Characterization of chemical groups of extracts and fraction of extracts

The identification of chemical groups was carried out using conventional reactions [24, 25]. Alkaloids were detected with Dragendorff's reagent, saponins through the foam test, sterols and terpenes with Liebermann's reaction, tannins via a concentrated HCl test, quinones and anthraquinones using a chloroform extract test, and polyphenols through a reaction with ferric chloride.

Biological tests

Preparation of different concentrations tested

For both the crude extract and its fractions, 2 stock solutions (S0) are prepared by dissolving one hundred mg or two hundred mg of extract in one liter of distilled water (for water-insoluble extracts, a hydroalcoholic solvent is used) to achieve concentrations of 100 mg/l or 200 mg/l. In each Petri dish containing one ml of distilled water, varying volumes of extracts (10, 20, 50, or 100 µl) are added using a micropipette for testing. The concentrations of the different test solutions are calculated using the following formula:

$$C0*V0=C1*V1 \quad (1)$$

$$C1= (C0*V0)/V1 \quad (2)$$

With C0: concentration of the stock solution; V0: volume has withdrawn; C1: concentration of the daughter solution; and V1: final volume.

For stock solutions prepared at a concentration of 100 grams per liter, the concentrations tested are 1, 2, 5, and 10 mg/l. For stock solutions with a concentration of two hundred grams per liter, the concentrations tested are 2, 4, 10, and 20 mg/l.

Touch test

In each Petri dish, one milliliter of distilled water is first added. According to the label on the dish, the required volume of extract (10, 20, 50, or 100 µl) is dispensed using a micropipette. Then, 3.5 grams of termite mound sand is incorporated into the mixture. A stick is used to evenly spread the mixture within the Petri dish. Afterward, the dish is left exposed to air for twenty to thirty minutes. Next, 50 termite workers are carefully counted and introduced into each dish. The Petri dishes are sealed and organized by concentration, with each experiment repeated four times. Additionally, four control groups are set up for each test. Finally, the dishes are stored in a cupboard in complete darkness. The procedure adheres to the method outlined in Tahiri *et al*. [26].

Fumigation test

Absorbent cotton is secured to the inside of the Petri dish lids using tape. The arrangement and concentrations in the Petri dishes are identical to those in the contact tests, with the key difference being that the various concentrations of the tested substances are placed on the cotton. This setup ensures that the termites do not directly interact with the tested product. The fumigation test was solely conducted with the essential oil to emphasize its volatile characteristics.

Test follow-up

The tests are checked daily at 24-hour intervals by inspecting the Petri dishes to evaluate the effectiveness of the extract. After each observation, the deceased termites in the dishes are tallied and removed. The experiment ends once all termites exposed to the extracts have died or the control termites perish. The mortality rate is then determined and corrected using the formula outlined in Abbott [27].

$$Mc = (Me-Mt) / (100-Mt) \times 100 \quad (3)$$

With Mc: percentage-corrected mortality; Me: mortality of the sample tested; and Mt: mortality of the untreated control.

Statistical analysis

The differences in means were assessed using a one-way ANOVA (analysis of variance) parametric test. If significant variations were observed, the Newman-Keuls test was used to separate the means. The lethal concentrations of the studied extracts were calculated using PROBIT analysis. All statistical analyses were performed at a 95% confidence level with XLSTAT 7.5 software.

Results and Discussion

Effect of essential oil on termites

By touch

Regardless of the tested concentration, the essential oil of *Ocimum gratissimum* leaves at a concentration of 100 mg/l achieves the highest mortality rate in all the termites within 3 hours.

By fumigation

At a concentration of 100 mg/l, the essential oil of *Ocimum gratissimum* led to higher-than-average mortality within 24 hours at concentrations of 5 and 10 mg/l, with 42% and 64% of termites dying, respectively. These concentrations achieved complete mortality (100%) after 72 hours **(Figure 1)**. For the lower concentrations of 1 and 2 mg/l, termite mortality was lower after 72 hours, with 22% and 45% of termites dead, respectively, though these results were still significantly different from the control group, which had 14% mortality.

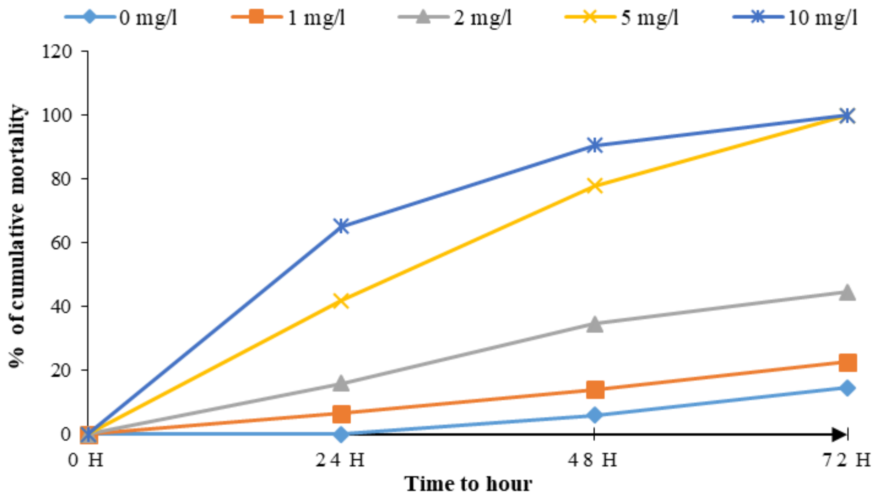


Figure 1. Cumulative mortality over time of *Nasutitermes* workers exposed to fumigation with the essential oil of *Ocimum gratissimum* at a concentration of 100 mg/l and evaluated at various concentrations.

Effect of extracts and Fraction of extracts on termites

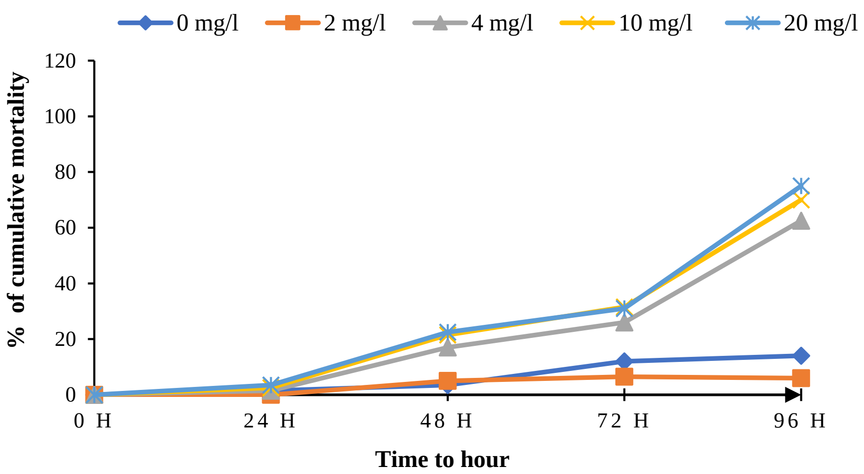
Apart from the ethyl acetate fraction, which produced statistically different results from the control at 48 hours, none of the extracts or fractions from *Ocimum gratissimum* leaves, prepared from a 100 mg/l stock solution, demonstrated any effectiveness against termite workers. For the ethyl acetate extract, mortality rates were relatively low at 48 hours (24%, 34%, and 32% for concentrations of 2, 4, and 10 mg/l, respectively), in comparison to the control group, which had 6% mortality. After 72 hours of exposure to the various tested products, no significant differences were observed between the concentrations and the control. The mortality observed in both the aqueous extract and DCM fractions did not differ significantly from that of the control **(Table 1)**.

Table 1. A mean number of *Nasutitermes* workers killed at different concentrations of *Ocimum gratissimum* leaf extracts at 100 mg/l, based on the observation period.

Temps	Extract studied and concentration					
	Hexane extract crude					
	0 mg/l	1 mg/l	2 mg/l	5 mg/l	10 mg/l	P
24 h	0.0 ± 0	0.0 ± 0	1.0 ± 1	0.33 ± 0.57	0.67 ± 1.16	0.424
48 H	3.0 ± 1 a	1.67 ± 1.53 a	4.67 ± 2.08 a	3.33 ± 1.53 a	8.67 ± 2.08 b	0.005
72 H	34.0 ± 10.54	37.0 ± 10.97	42.33 ± 3.05	37.0 ± 4.58	43.67 ± 8.51	0.601
	Dichloromethane (DCM) extract fraction					
	0 mg/l	1 mg/l	2 mg/l	5 mg/l	10 mg/l	P
24 h	0.0 ± 0	0.0 ± 0	0.67 ± 1.16	1.33 ± 0.58	1.33 ± 0.58	0.056
48 H	3.0 ± 1	6.0 ± 5.29	8.0 ± 7.55	9.0 ± 2.65	7.0 ± 4	0.592
72 H	34.0 ± 10.54	38.33 ± 11.06	35.33 ± 10.02	38.33 ± 5.51	35.67 ± 13.2	0.979

Ethyl acetate extract fraction						
	0 mg/l	1 mg/l	2 mg/l	5 mg/l	10 mg/l	P
24 h	0.0 ± 0	0.0 ± 0	1.67 ± 1.53	0.67 ± 1.16	4.33 ± 4.04	0.113
48 H	3.0 ± 1 a	5.0 ± 3.60 ab	12.0 ± 3 bc	17.67 ± 3.06 c	16.67 ± 2.52 c	0.001
72 H	34.0 ± 10.54	34.67 ± 6.81	34.67 ± 6.66	42.33 ± 7.09	30.0 ± 9.54	0.513
Aqueous extract fraction						
	0 mg/l	1 mg/l	2 mg/l	5 mg/l	10 mg/l	P
24 h	0.0 ± 0	0.33 ± 0.57	0.0 ± 0	0.33 ± 0.57	0.67 ± 1.16	0.682
48 H	3.0 ± 1	1.67 ± 2.08	8.0 ± 6.25	12.0 ± 7.94	12.67 ± 7.77	0.134
72 H	34.0 ± 10.54	34.0 ± 7	38.67 ± 5.51	35.67 ± 9.87	39.0 ± 11	0.922

ANOVA test at the 95% threshold; Newman-Keuls pairwise multiple comparison test lowercase letters show differences in a row



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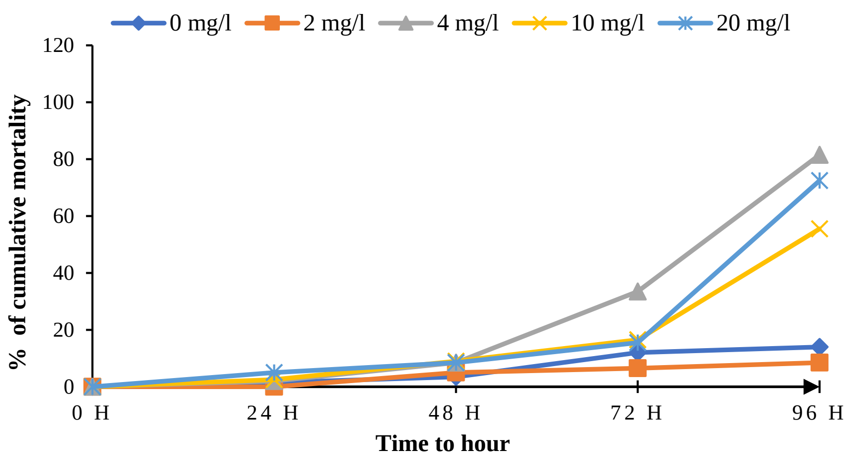

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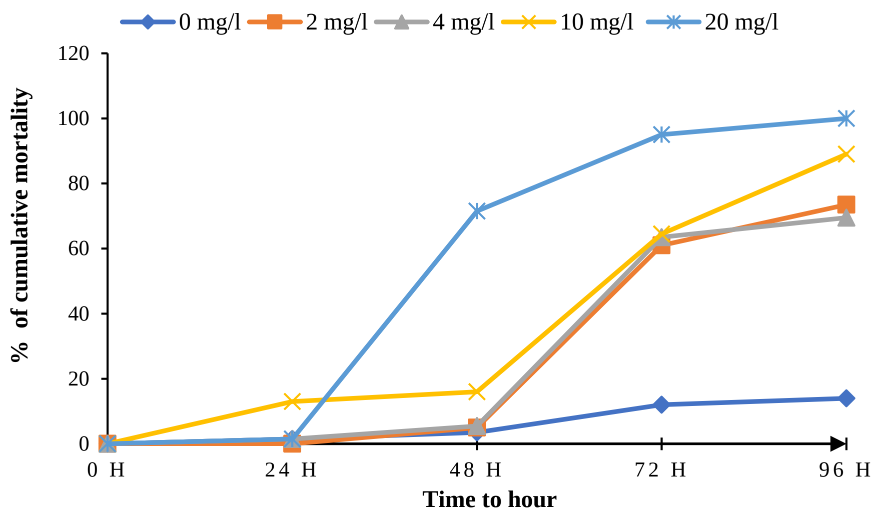

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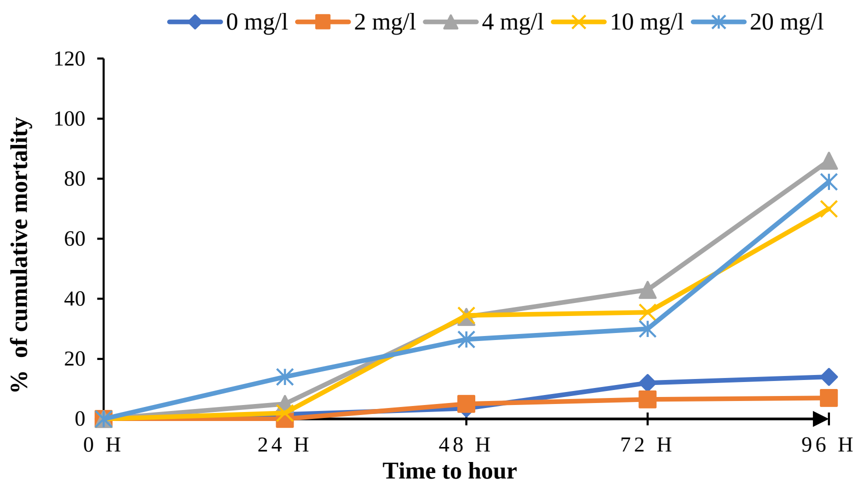

d)

Figure 2. Cumulative mortality over time of *Nasutitermes* workers exposed to extracts and extract fractions of *Ocimum gratissimum* at 200 mg/l across various concentrations; a) aqueous fraction, b) hexane extract, c) ethyl acetate, and d) dichloromethane fraction.

At a concentration of two hundred milligrams per liter, all the extracts tested at various concentrations demonstrated effectiveness **(Figure 2)**. Although the aqueous fraction did not reach maximum mortality, it showed notable effectiveness at the 200 mg/l concentration. The aqueous extract began to show results after 48 hours, with average mortality reaching 62%, 70%, and 75% at 4, 10, and 20 milligrams per liter, respectively, after 96 hours, compared to the control group (14% mortality).

The DCM fraction exhibited delayed effectiveness. While significant differences were observed after 24 hours, it was only after 96 hours that mortality rates of 86%, 70%, and 78% were recorded for the 10 and 20 milligrams per liter concentrations, respectively, compared to the control (14%). The ethyl acetate fraction showed mortality rates of 60%, 62%, 64%, and 94% after 72 hours for the respective concentrations of 2, 4, 10, and 20 milligrams per liter. However, the 20 mg/l concentration demonstrated its effectiveness (71%) as early as 48 hours, in contrast to the control (3%).

Comparison of the effectiveness of different extracts

The lowest LC50 value was observed for the essential oil, which was 7.33 milligrams per liter. The LC50 values for the crude extracts and their fractions from *Ocimum gratissimum* leaves were significantly higher, with broad confidence intervals **(Table 2)**. However, the lowest LC50 value among the fractions was found for the DCM extract, concentrated at 200 milligrams per liter, with a value of 19.46 milligrams per liter.

Table 2. Lethal concentrations (LC) are required to achieve 50% and 99% mortality of *Nasutitermes* workers, along with their 95% confidence intervals, for *Ocimum gratissimum* leaf extracts at a concentration of 200 milligrams per liter.

Extract	LC50 (mg/l)	LC50 Terminals at 95 %	LC99 (mg/l)	LC99 terminals at 95%.
HE (100 mg/l)	7.33	6.68-8.12	19.56	17.38-22.65
Hexane	30.31	20.51-78.37	60.19	38.55-161.72
Aqueous	34.67	21.68-159.95	67.33	39.78-335.76
Ethyl acetate	66.83	-	149.26	-
DCM	19.46	15.88-26.85	39.16	30.49-57.43

Probit model of lethal concentration calculation

Chemical composition of extracts

Essential oil

Analysis of the chemical composition of the essential oil extracted from *Ocimum gratissimum* leaves revealed 29 compounds, accounting for 98.2% of the total composition **(Table 3)**. The oil is primarily composed of monoterpenes, which make up 80% of the total, followed by sesquiterpenes at 18.6%. The key components include thymol (34.6%), p-cymene (25.2%), α-selinene (6.8%), myrcene (5.4%), β-caryophyllene (4.9%), and α-thujene (4.5%).

Table 3. Constituents of the essential oil extracted from *Ocimum gratissimum* leaves.

N°	Compounds	Content (%)
1	α-Thujene	**4.5**
2	α-Pinene	1.5
3	Camphene	0.1
4	Verbenene	0.2
5	Sabinene	0.2
6	β -Pinene	1.7
7	β -Myrcene	**5.4**
8	α-Terpinene	0.4
9	p –Cymene	**25.2**
10	γ-Terpinene	1.6
11	Trans Hydrate de Sabinene	1.6
12	Trans Thujone	0.6
13	Terpinene 4 ol	0.7
14	α –Terpineol	0.2
15	Thymol methyl ester	1.4
16	Thymol	**34.6**
17	Carvacrol	0.1
18	α-Copaene	1.6
19	α-Cubebene	0.2
20	Isoledene	0.6
21	β-Caryphyllene	**4.9**
22	α-trans Bergamotene	0.4
23	β -cis Guaiene	0.3
24	β -Humulene	0.8
25	α -selinene	**6.8**

26	δ -Cadinene	0.7
27	Humulene 1,2 epoxyde	0.1
28	Cubenol epi	0.7
29	Benzoate de benzyle	1.5
	Total	**98.6**
	Hydrocarbon monoterpenes	40.8
	Oxygenated monoterpenes	39.2
	Hydrocarbon sesquiterpenes	16.3
	Oxygenated sesquiterpenes	2.3

Extracts and extract fractions

The phytochemical analysis of the hydroethanolic crude extract from *Ocimum gratissimum* leaves identified five of the seven chemical compound families tested, with flavonoids being the most prevalent **(Table 4)**. Both the ethyl acetate and aqueous fractions exhibited the presence of alkaloids, catechic tannins, saponins, and flavonoids. The DCM extract fraction contained three families: saponins, sterols, and terpenes, while the hexane extract showed only two families, namely sterols and terpenes.

Table 4. Various families of chemical compounds are found in the leaves of false basil (*Ocimum gratissimum*).

Chemical compound	hydroethanolic crude	DCM	Aqueous	Ethyl acetate	Hexane
Alkaloids	+	-	+	+	-
Saponosides	++	±	++	+	-
Sterols and terpenes	+	+	-	-	++
Catechic tannins	++	-	++	++	-
Quinones	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Flavonoids	+++	-	+++	++	-

The essential oil derived from *Ocimum gratissimum* leaves has proven effective at low concentrations, such as 100 mg/l. The highest termiticidal activity was observed during the contact test using the oil at this concentration, where maximum mortality was achieved across all tested doses (10, 20, 50, and 100 μl) after three hours of exposure. This can be attributed to the presence of alcoholic and ketonic compounds in the oil, which are known to dissolve the protective coverings of insects [19]. Essential oils from Ocimum species are typically effective against insects due to their composition. Kanda *et al*. [28] found that the monoterpenes in these oils are toxic to various insect species. Moreover, Ghasemi *et al*. [29] established that the insecticidal action of essential oils is closely tied to the chemical structure of their terpene components. For example, the essential oil of *Ocimum sanctum* has shown effectiveness against the cotton pest *Dysdercus voelkeri*. According to Nadio *et al*. [30], the efficacy of this essential oil is partly due to major compounds like Germacrene-D (25%) and B-Caryophyllene (21.28%), as well as the synergistic effect of minor components such as eugenol, elemol, and B-elemene. The high concentration of monoterpenes in *Ocimum canum* essential oil likely accounts for its success in targeting *Pectinophora gossypiella* adults. Similar findings were noted for the essential oil of *Senna occidentalis*, which was effective against the peanut pest *Caryedon serratus* in Senegal [22]. The insecticidal properties of aromatic plants have been well-documented [14, 31, 32]. The findings from this study suggest that while the extracts from powdered *Ocimum gratissimum* leaves exhibit delayed effects, their effectiveness is concentration- and dosage-dependent, with termite mortality increasing as the concentration rises. These results are consistent with those reported by Ukoroije and Bobmanuel [33], who found that ethanolic extracts of *Ocimum gratissimum* leaves caused cockroach mortality that was dependent on extract concentration and dosage. They also confirmed the delayed toxicity of these ethanolic extracts. Likewise, Ukoroije *et al*. [21] observed similar outcomes with powdered *Ocimum gratissimum* leaf extracts, showing that aqueous and ethyl acetate fractions, along with other phenolic compounds, were the most effective.

The results align with the study by N'guessan *et al*. [34], which performed a similar phytochemical screening on their *Ocimum gratissimum* chemotype. However, in their approach, they used 80% methanol as the solvent and followed up with liquid-liquid extraction using hexane, chloroform, and ethyl acetate. As stated by Kaderides *et al*. [35], different solvents can extract the same compounds depending on their polarity. The phytochemical investigation conducted by Kpètèhoto *et al*. [36] revealed that besides polyphenols, *Ocimum gratissimum* leaves also contain steroids, terpenoids, and, to a lesser extent, alkaloids. According to Ukoroije and Bobmanuel [33], eugenol, a terpene, is the main component responsible for the insecticidal effects of *Ocimum gratissimum* extracts. These active compounds contribute to the plant's diverse biological activities, including antibacterial, antifungal, antiparasitic, and insecticidal effects, which have been well-documented. The insecticidal activity was observed in various extracts studied, and according to Ndiaye *et al*. [37], these compounds may play an antinutritional role, affecting the growth, survival, and behavior of organisms that consume or interact with the plant.

Conclusion

The leaf extracts of *Ocimum gratissimum* have been shown to have insecticidal effects on Nasutitermes termite workers. Among the treatments, the essential oil proved to be the most toxic, with the lowest lethal concentrations observed. Although it took longer to show results (48-72 hours), the ethyl acetate extract fraction demonstrated notable effectiveness even at low doses. However, the other extracts had no noticeable impact on termite workers at lower doses. All extracts from the powdered leaves exhibited a delayed action against termites.

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Ethics Statement: None

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