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Evaluation of Effect of Ocimum Gratissimum (Lamiaceae) Extracts on Nasutitermes Termite, Cocoa Pests in Côte d'Ivoire

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ABSTRACT

The misuse of synthetic pesticides in agrosystems has led to many problems including insect resistance and environmental pollution. To solve this problem, scientific research is increasingly focused on plants with insecticidal properties. Thus, this study has the general objective of evaluating the effectiveness of the various extracts (crude extract, fraction of extract, and essential oil) of *Ocimum gratissimum* on termites of the genus *Nasutitermes*, pests of cocoa trees. The crude extracts were obtained by the maceration of the dried leaf powders; the extract fractions by fractionation of the crude hydro-ethanolic extract and the essential oil by hydrodistillation. The tests, repeated four times, were carried out at the Animal Biology Laboratory of Félix Houphouët-Boigny University. Thus, 50 workers deposited in Petri dishes of termites were exposed to different doses of extracts of this plant and the effect of the extracts on termites as compared to the negative control. The results have shown that all the tested concentrations have scored the highest rate of mortality (100%) after 3 hours of exposure. This toxicity would be linked to the monoterpenes which constitute 80% of the compounds of this extract. However, by fumigation, the essential oil is effective at high concentrations (5 and 10 mg/l). Regarding extracts and fractions of extracts, only the acetate extract fraction showed a toxicity for termites. Nevertheless, this extract acts after 48 hours of exposure and at high concentrations.

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

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INTRODUCTION

Some species of termites, due to their bio-ecology, can cause serious and irreversible damage to crops [1, 2]. Termites of the *Nasutitermes* genus of the Nasutitermitinae subfamily, by building arboreal nests, weaken the internal structure of these plants [3]. In addition, their diet (wood-feeders) is a brake on the development of the plants where they live. Several studies have shown that termites of this genus are the main pests of cocoa trees in Côte d'Ivoire [4, 5]. Pesticidal plants represent, to date, the oldest form of pest control in agriculture [6]. With the advent of synthetic pesticides in 1940 and the spectacular results that were obtained with their use, the use of plants for pest control was relegated to the background and defined as a traditional method [7, 8]. The excessive use of synthetic products has led to many problems in agriculture such as pest resistance, environmental pollution, and the occurrence of human diseases such as cancer, diabetes, and infertility [9, 10]. These disadvantages due to the use of synthetic insecticides have led to the search for insecticidal molecules derived from plants [11]. Indeed, plant extracts used as biopesticides have many advantages for the environment [12, 13]. These extracts are low pollutants, biodegradable, and not harmful to humans. Traditionally, farmers used plants to control insects in general and termite pests in particular [14]. Thus, the use of plant extract pesticides is increasingly common in the management of these pests [15, 16]. From numerous ethnobotanical studies, a non-exhaustive list of 2000 plant species has been identified as plants with insecticidal activities [17, 18]. They can act as insecticides, repellents, antifeedants, or inhibitors of termite development. Among these plants, the false basil *Ocimum gratissimum* occupies a prominent place. This shrub, native to India, has long been reported to have insecticidal properties. The fragrant leaves contain volatile compounds which in high doses can be toxic. In Côte d'Ivoire, various studies have highlighted the biological effectiveness of crude extracts of this plant on various harmful insects [19-21]. The objective of this present study is to evaluate the insecticidal effect of the different extracts (crude extract; fraction of extract and essential oil) of this plant on workers of termites of the genus *Nasutitermes*. It will also be a question of characterizing the chemical constituents which would make it possible to explain the insecticidal potential of the different extracts of this aromatic plant.

MATERIALS AND METHODS

Experimentation site

This study was carried out at the Zoology and Animal Biology Laboratory of the Félix Houphouët-Boigny University. The experiments were carried out under laboratory conditions (T°: 27 ± 2°C ; HR: 80%) and carried out in Petri dishes.

Biological material

The different extractions were carried out from the leaves of *Ocimum gratissimum* L (Lamiaceae) taken from the outskirts of Abidjan.

The workers of termites of the genus *Nasutitermes*, tested in this study, were collected at the Center National de Floristique (CNF) located within the University Félix Houphouët-Boigny.

Methodology

Obtaining the essential oil and its chemical composition

Hydrodistillation is the method used to collect volatile substances from plants by steam distillation. These compounds can be more or less modified during the preparation [22]. The extraction of the essential oil was carried out with a Clevenger-type apparatus. 1300g of vegetable raw material are weighed and placed on a grid in the matrix (pressure cooker), serving as a boiler. The grid guarantees the separation between the plant organs and the water contained in the bottom of the matrix, whose steam will ensure the extraction of the essential oil. The condensation of the first drop of essential oil (EO), is counted three hours before ending the extraction by stopping the heating. The essential oil is collected and dried with anhydrous sodium sulfate (Na_2SO_4).

The compounds of the essential oil are analyzed using two techniques, Gas Chromatography (GC) and Nuclear Magnetic Resonance (^{13}C-NMR) under the same conditions as those described by [23].

Obtaining raw extracts

The powder of *Ocimum gratissimum* leaves is macerated for 24 hours in a hydro-ethanolic solvent in an 8-2 proportion and passed under a magnetic stirrer. After that, the mixture is filtered through Büchner with Whatman paper, then water cotton. The extract obtained is evaporated in an oven at 40°C until completely dry. The marc (residue obtained after maceration) from this first maceration, after drying, underwent a second maceration with hexane to extract the apolar compounds.

Obtaining extract fractions

The crude hydro-ethanolic extract obtained was then taken up successively in different solvents of increasing polarity to fractionate the starting extract and separate the molecules according to their polarity [20-22]. Liquid-liquid extraction was carried out with successive dichloromethane

(DCM), ethyl acetate, and distilled water in a separatory funnel. For a used solvent, the operation is repeated two to three times until exhaustion. The various fractions obtained are concentrated using a rotary evaporator, then dried in an oven at 40°C.

Characterization of chemical groups of extracts and fraction of extracts

The chemical groups were characterized by the usual reactions [24, 25]. These are the alkaloids (Dragendorff's reagent), the saponosides (foam test), the sterols and terpenes (Liebermann's reaction), the tannins (test with HCl concentrate), the quinones, the anthraquinones (test with chloroform extract) and polyphenols (reaction with ferric chloride).

Biological tests

Preparation of different concentrations tested

For the crude extract and the extract fractions, two stock solutions (S0) respectively from 100 mg or 200 mg of extract and 1 liter of distilled water (for water-insoluble extracts, a hydroalcoholic solvent is used) are prepared for a mass concentration of 100 mg/l or 200 mg/l. In the Petri dishes each containing 1 ml of distilled water, quantities of extracts (10, 20, 50, or 100 µl) are added using a micropipette for carrying out the tests. The different concentrations tested are given by 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; V1: Final volume.

For stock solutions with a concentration of 100 g/l, the concentrations tested are 1; 2 ; 5, and 10 mg/l. As for the stock solutions with a concentration of 200 g/l, the concentrations tested are 2; 4 ; 10, and 20 mg/l.

Touch test

In each Petri dish, 1 ml of distilled water was added. Depending on the inscription marked on the Petri dish, the corresponding quantity of extract (10 ; 20 ; 50, and 100 µl) is added using a micropipette. To this mixture is added 3.5 g of termite mound sand. A stick was used to spread the mixture in the Petri dish. After this manipulation, the Petri dish is left in the open air for 20 to 30 min. Next, fifty (50) termite workers were counted and placed in each Petri dish. Petri dishes are closed and stacked by concentration. Each test is repeated four times. For each test, 4 controls are carried out. The boxes are then stored in a cupboard in total darkness. The protocol is that of [26].

Fumigation test

Inside the lid of each Petri dish, absorbent cotton is stuck with tape. The preparations made in the Petri dishes and the concentrations tested are the same as those of the contact tests, except that the different concentrations of the products tested are placed on cotton wool. Thus, termites do not come into contact with the tested product. The fumigation test only concerned the essential oil to highlight its volatile nature.

Test follow-up

The tests are monitored every day, at 24-hour intervals, by observing the Petri dishes to verify the effectiveness of the extract tested. After the observations, the dead termites in each petri dish are counted and removed from the Petri dishes. The experiment stopped with the death of all the termites which were exposed to the extracts or the death of the control termites. The mortality rate is calculated and corrected according to the formula [27]:

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

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

Statistical analysis

The comparisons of the means are made with the one-factor ANOVA (analysis of variance) parametric test. For the parameters where the differences were significant, the means were separated using the Newman-Keuls test. The calculation of the different lethal concentrations of the extracts studied is carried out using the PROBIT analysis. These various tests were carried out at the 95% threshold using the XLSTAT 7.5 software.

RESULTS AND DISCUSSION

Effect of essential oil on termites

By touch

Whatever the concentration tested, the essential oil of the leaves of Ocimum gratisssimum concentrated at 100 mg/l, makes it possible to reach in 3 hours, the maximum mortality in all the termites tested.

By fumigation

The essential oil of *Ocimum gratissimum* concentrated at 100mg/l resulted in above-average mortality in 24 hours at concentrations of 5 and 10mg/l (respectively 42% dead termites and 64% dead termites). Maximum mortality (100%) was reached for these two concentrations after 72 hours **(Figure 1)**. For concentrations of 1 and 2mg/l, termite mortality was lower after 72 hours (respectively 22% and 45% of dead termites) although the results obtained were statistically different from those with the control (14%).

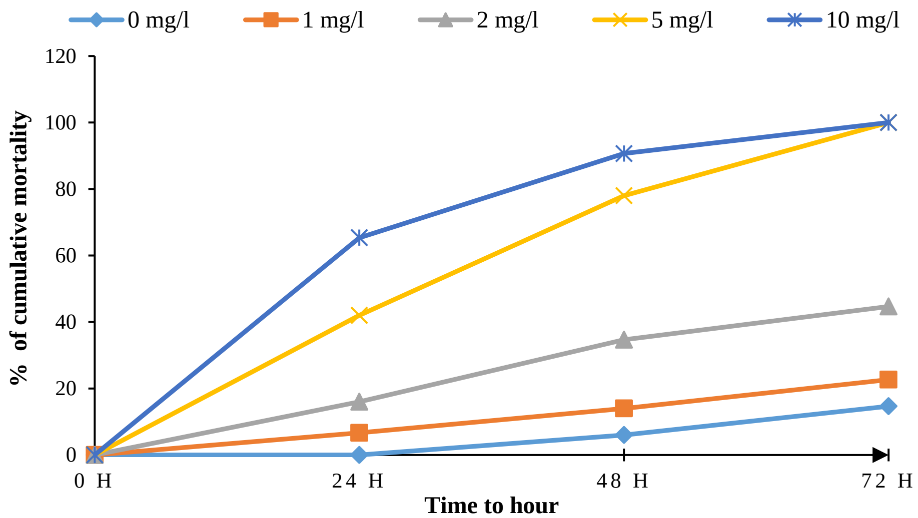


Figure 1. Cumulative mortality as a function of time of *Nasutitermes* workers treated by fumigation with the essential oil of *Ocimum gratissimum* concentrated at 100mg/l and tested at different concentrations

Effect of extracts and Fraction of extracts on termites

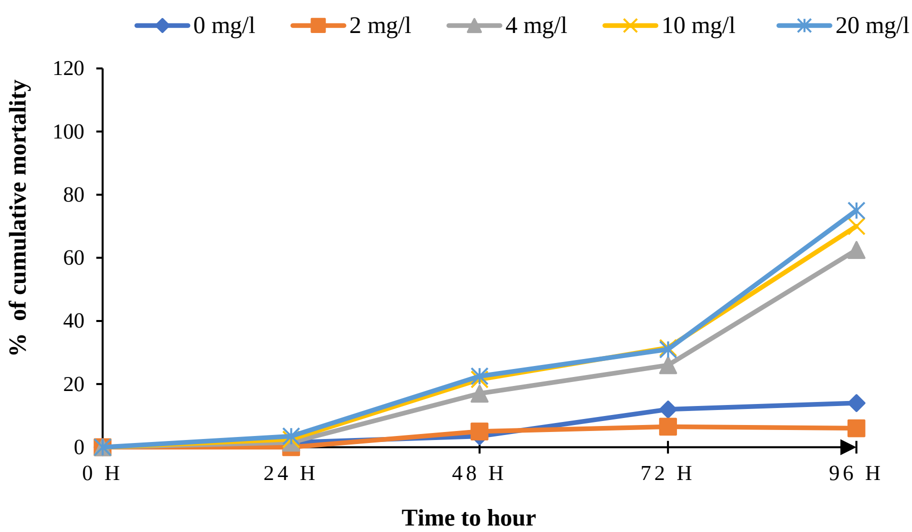
Except for the fraction with ethyl acetate which obtained statistically different results from the control at 48 hours of experiments, the extracts and fractions of extracts from the leaves of *Ocimum gratissimum* from stock solution concentrated at 100mg/l tested on termite workers showed no effectiveness against them. Concerning the ethyl acetate extract, the mortality rates recorded are low at 48 hours of exposure (respectively 24; 34 and 32% for the concentrations of 2; 4 and 10mg/l) compared to that of the control (6%). After 72 hours of exposure of the termites to the different products tested, no statistical difference was recorded between the concentrations tested and the control. As regards the aqueous extract and DCM fractions, the various mortalities recorded showed no significant difference between them and the control **(Table 1)**.

Table 1. Average number of workers of Nasutitermes morts according to the concentrations and the extracts of the leaves of Ocimum gratissimum of concentration 100 mg/l according to the duration of observation

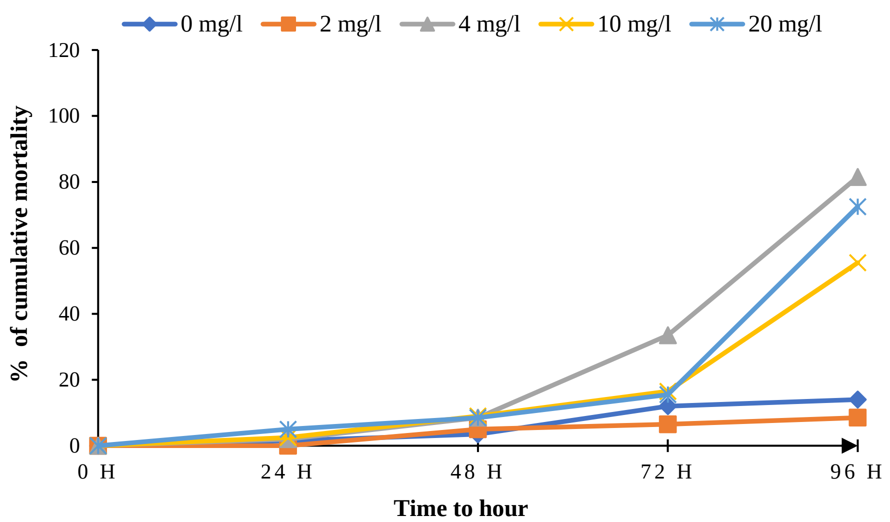
Temps	Extract studied and Concentration					
	Hexane extract crude					
	0 mg/l	1 mg/l	2 mg/l	5 mg/l	10 mg/l	P
24 h	0,0 ± 0	0,0 ± 0	1,0 ± 1	0,33 ± 0,57	0,67 ± 1,16	0,424
48 H	3,0 ± 1 a	1,67 ± 1,53 a	4,67 ± 2,08 a	3,33 ± 1,53 a	8,67 ± 2,08 b	0,005
72 H	34,0 ± 10,54	37,0 ± 10,97	42,33 ± 3,05	37,0 ± 4,58	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



a)



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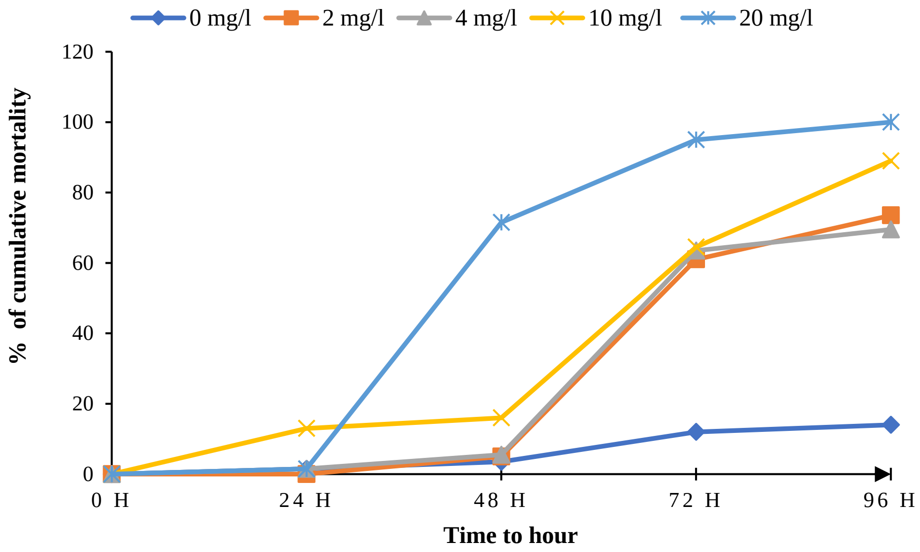

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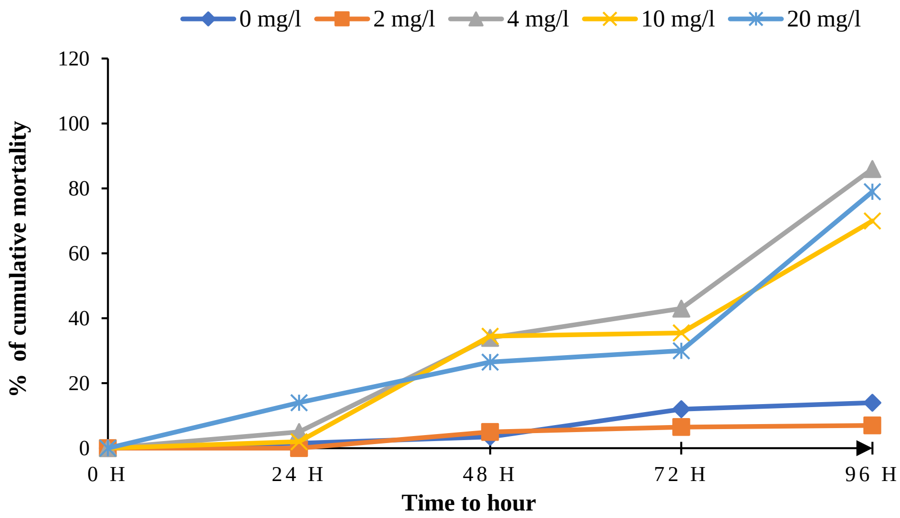

d)

Figure 2. Cumulative mortality as a function of time of *Nasutitermes* workers treated with extracts and fractions of extracts of *Ocimum gratissimum* at 200 mg/l at different concentrations
a) Aqueous fraction, b) hexane extract, c) Ethyl acetate, d) Dichloromethane fraction

However, the same extracts tested at a concentration of 200 mg/l at the different concentrations studied all showed efficacy **(Figure 2)**.

Although the maximum mortality was not reached for the termites exposed to the aqueous fraction, the results reveal the effectiveness of this extract at the concentration of 200 mg/l. The aqueous extract was effective after 48 hours. Nevertheless, it was after 96 hours that the average mortality was reached for the concentrations of 4, 10, and 20 mg/l (respectively 62%; 70% and 75% of dead termites) compared to the control (14% of dead termites).

Tests carried out with the DCM fraction showed late efficacy on termite workers. Although, after 24 hours of exposure, the results of the controls were statistically different from the tested, it was after 96 hours that 86; 70, and 78% of the termites tested died of the concentrations; of 10 and 20 mg/l respectively; compared to the control 14%. As for the ethyl acetate extract fraction, the mortality rates observed after 72 hours of the experiment were 60; 62; 64, and 94% for the concentrations tested (respectively 2; 4; 10, and 20 mg/l). However, the concentration of 20 mg/l reveals its effectiveness (71%) from 48 hours of experience compared to the control (3%).

Comparison of the effectiveness of different extracts

Regarding the LC50s, the smallest is recorded on the essential oil and is 7.33mg/l. The various LC50s calculated for the crude extracts and fractions of extracts from the leaves of Ocimum gratissimum are very high with interval limits very wide **(Table 2)**. Nevertheless, the smallest LC50 is obtained with the fraction of DCM extract concentrated at 200 mg/l (19.46 mg/l).

Table 2. Lethal concentrations (LC) to obtain 50 and 99% mortality of *Nasutitermes* workers and their 95% confidence limits for extracts of *Ocimum gratissimum* leaves at a concentration of 200 mg/l

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
Etlyl 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

The study of the chemical composition of the essential oil sample from the leaves of Ocimum gratissimum made it possible to identify 29 compounds representing 98.2% of the total composition **(Table 3)**. It is dominated by monoterpenes (80%). Sesquiterpenes have content of 18.6%. The major constituents of this oil are 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. Chemical composition of the essential oil of *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

Phytochemical screening performed on the hydroethanolic crude extract of leaves of false basil (*Ocimum gratissimum*) reveals the presence of five chemical compound families out of seven searched, of which the most abundant are flavonoids **(Table 4)**. The ethyl acetate and aqueous extract fractions contain the same families of chemical compounds (alkaloids, saponosides, catechic tannins, and flavonoids) while the DCM extract fraction contains three (saponosides and sterols, and terpenes) and the hexane extract, two chemical compound families (sterols and terpenes).

Table 4. Some families of chemical compounds contained 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 tests carried out with the essential oil of the leaves of *Ocimum gratissimum* show its effectiveness at low concentrations (100mg/l). The most remarkable termiticidal effect was obtained by the contact test with the essential oil concentrated at 100 mg/l. Maximum mortality was reached for all doses tested (10 ; 20 ; 50 and 100 µl) after 3 hours of exposure. This could be explained by the presence in this oil of alcoholic and ketonic compounds which have the property of dissolving the protective integuments of insects [19]. Thanks to their composition, the majority of essential oils from plants of the genus *Ocimum* are effective against insects. Indeed, [28] revealed that the monoterpenes of essential oils are toxic to many insects. Furthermore, [29] has shown that the insecticidal efficacy of essential oil is due to the nature and chemical structure of its terpene constituents. Thus, the essential oil of *Ocimum sanctum* has shown its effectiveness on *Dysdercus voelkeri*, a cotton pest. The work of [30] revealed that the effectiveness of this essential oil would be due, on the one hand, to the action of these major compounds such as Germacrene-D (25%) and B-Caryophylene (21.28%). ; and, on the other hand, to the synergistic effect of these other minor constituents such as eugenol, elemol, B-elemene. According to the strong presence of monoterpene compounds in the essential oil of *Ocimum canum* would explain its efficacy against *Pectinophora gossypiella* adults. Similar results were obtained with the essential oil of *Senna Occidentalis* on the mortality of *Caryedon serratus*, a peanut pest in Senegal [22]. Indeed, the majority of aromatic plants are routinely tested on insects and their effects on insecticides no longer need to be demonstrated; [14, 31, 32]. The results obtained show that the effectiveness of the extracts from the powder of the leaves of *Ocimum gratissimm*, although late, depends on the concentration and the dosage. Termite mortality increased as the concentration of treatments increased. These results are similar to those of [33]. These authors who worked on the toxicity of ethanolic extracts of *Ocimum gratissimum* leaves showed that the average mortality of cockroaches depended on the concentration and dosage of the extract. They also demonstrated the late toxicity of ethanolic extracts of *Ocimum gratissimum* leaves on cockroaches. [21] have also obtained the same results in their experiments with extracts of powdered *Ocimum gratissimum* leaves on different insects. The most effective extracts proved to be the aqueous extract fractions and ethyl acetate fractions and other phenolic compounds.

These results are consistent with the work of [34] which leads to the same phytochemical screening on their chemotype of *Ocimum gratissimum*. However, these authors used 80% methanol as the solvent before doing the liquid-liquid extraction with hexane, chloroform, and ethyl acetate. [35] reveal that depending on their polarity, two solvents can extract the same molecules. The phytochemical screening carried out by [36] shows that apart from the presence of polyphenols, the leaves of *Ocimum gratissimum* also contain steroid compounds, terpenoids, and to a lesser extent alkaloids. According to [33], the Insecticidal activity of *Ocimum gratissimum* extracts can be attributed to eugenol, a terpene.

The presence of these compounds, indicative of active principles, gives the plant its antibacterial, antiparasitic, antifungal, and insecticidal characteristics which have been reported in the literature. The insecticidal activity was obtained with the different extracts studied. Indeed, for [37], they play an antinutritional role in affecting the growth, survival, behavior, or biology of living organisms that feed on plants or are associated with them.

CONCLUSION

The extracts obtained from the leaves of *Ocimum gratissimum* have made it possible to demonstrate its insecticidal effect on the workers of termites of the genus *Nasutitermes*. Taking into account the duration of the experiments and the concentrations tested, essential oil is the most toxic for termites. The smallest lethal concentrations were obtained with the essential oil. Although slower in action (from 48 hours to 72 hours), the ethyl acetate extract fraction shows interesting results at low doses. However, the other extracts have no effect on termite workers at low doses. All the extracts from the powder of the leaves of this plant have delayed effectiveness on termites.

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