International Journal of Natural Sciences Research
2014 Vol. 2, No. 10, pp. 195-205
ISSN(e): 2311-4746
ISSN(p): 2311-7435

THE USE OF TWO NEW FORMULATIONS OF *OCIMUM CANUM* SIMS AND *CYMBOPOGON SCHOENANTHUS* L. IN THE CONTROL OF *AMITERMES EVUNCIFER* SILVESTRI (TERMITIDAE: TERMITINAE), IN TOGO

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ABSTRACT

Today, the main concern of farmers and scientists is finding an alternative to the use of chemical pesticides in pest management, environmental pollution prevention and poisoning of the population. For this purpose, more and more studies are being carried out on biopesticides from plants used in local pharmacopoeia for the management of insect pests of crops and food stocks. In this study, the effect of a mixture of essential oil of Ocimum canum Sims with starch and that of Cymbopogon schoenanthus with starch were separately evaluated against Amitermes evuncifer Silvestri, one of the main pestiferous termites in Togo. These formulations were prepared from each plant at six different concentrations with and without sawdust. They were effective and caused significant mortality of Amitermes evuncifer. The formulation of essential oil without sawdust was more effective than that with sawdust. For the formulation without sawdust, 75 and 38% mortality respectively were recorded with C. schoenantus and O. canum at a dose of 0.5 mg/cm^2, while 100% mortality was recorded at 2 mg/cm^2 for both plants in both formulations. However, between the two plants, the formulations made from S. schoenantus were more effective on A. evuncifer than those made from O. canum. These new formulations made from the essential oils and starch could be used as biopesticide to control pestiferous insects in the field.

Keywords: Mortality, *Amitermes evuncifer*, *Ocimum canum*, *Cymbopogon schoenanthus*, Essential oil, Starch.

Contribution/ Originality

This study is one of the very few studies which have investigated the efficiency of a new formulation made of a mixture of essential oils (Ocimum canum and Cymbopogon schoenantus) with starch, against pestiferous termite.

1. INTRODUCTION

Termites, Isoptera, are among the most pestiferous insects that cause serious damage to plants and woody materials [1, 2]. Approximately 5-8% of recorded species of termite attack human constructions in urban areas and also food crops, vegetables, industrial and forest resources [3]. Damage caused by termites and repair costs are estimated at more than € 22 billion each year [4].

In many parts of Africa, some species of termite are responsible for significant damage to cultivated plants, crops, forest trees and pastures [5, 6]. The damage by termites to crops and plantations are often greater than 15% and can sometimes reach 90% [3]. Furthermore, 50-100% losses have been reported in many tropical crops [7, 8]. In tropical areas of Africa, studies have shown that besides Macrotermitinae [3, 9] and Nasutitermitinae [6], species belonging to the Termitinae subfamily (including *Amitermes evuncifer* Silvestri) are also responsible for significant damage to crops, plantations, forest trees and fruit as well as horticultural plants [1, 10].

In Togo, studies have shown that *A. evuncifer* causes significant damage to sugar cane plantations [11], maize, cassava [12] and timber [13, 14].

Several decades ago, the control of these pestiferous termites as well as other insect pests was based on the use of non-specific chemicals. It consisted of repeated applications of the pesticide over a vast area killing many non-targeted species, destroying biodiversity and degrading the environment [15]. Most of these products belong to the group of organochlorines, which are very long-lasting and highly polluting. Since their banning and restriction, termite control becomes more difficult and scientists are seeking other alternatives. Thus, in recent years, many studies have been carried out to test the antimicrobial, insecticidal, insect repellent, fungicidal, and miticidal properties of some plants extracts.

Despite numerous studies on the biological properties of essential oils, very little has been consecrated to the confection of formulations from these oils. Therefore, the aim of this work is to evaluate the effect of two formulations made from the essential oils of *Ocimum canum* Sims and *Cymbopogon schoenantus* L., on *Amitermes evuncifer* Silvestri, one of the most redoubtable termite pests in Togo.

2. MATERIALS AND METHODS

2.1. Insects

Amitermes evuncifer Silvestri, (Termitinae) was used in this study. It was sampled from its nest at Kévé (50 km in the North East of Lomé) and brought to the laboratory where it was kept and acclimatized at 28 ± 2 °C and 80 ± 10% RH in a well ventilated room until the end of the tests.

2.2. Plant Materials

Plants used in this test are African Basil (*Ocimum canum* Sims) and Camel Grass (*Cymbopogon schoenantus* L.). The leaves and inflorescences of these plants were harvested at the flowering stage in Ouagadougou, Burkina Faso. These plant materials were brought to the Department of Natural Substances (Institute of Research in Applied Science and Technology) in Ouagadougou to be prepared for oil extraction.

2.3. Extraction and Analysis of Essential Oils

The essential oils of *O. canum* and *C. schoenanthus* were obtained by hydro-distillation for three hours using the Clevenger type apparatus. The essential oil was collected by decantation, dried with anhydrous sodium sulfate and stored in amber glass vials in a refrigerator at 4 °C.
Essential oils were identified by gas chromatography, coupled with mass spectrometry (GC and GC / MS) [16, 17].

2.3.1. Analysis by GC

The essential oils were analyzed by a Varian 3800 chromatograph equipped with two capillary columns: one polar and one apolar (Supelcowax 30 m, 0.25 mm and 30 m SPB1; 0.25 mm). In each case, the particle size of the column was 0.25 mm. The oven temperature was programmed as follows: 40 °C to 240 °C (2 °C / min) and stationary at 240 °C for 40 minutes. The injector and detector temperatures were respectively 230 °C and 250 °C, the vector gas used was helium [16, 17].

2.3.2. Analysis by GC/MS

Analysis by GC / MS was carried out using a Hewlett Packard type GC 5890 equipped with a SPB1 capillary column (30 mm, 0.25 mm, 0.25 mm) and a "Mass Selective Detector" Series 5972, column SPB-1. The temperature of the detector was 280 °C, and the injector 210 °C, the oven temperature was programmed as before and the transfer line temperature was 280 °C [16, 17].

2.3.3. Identification of the Components

The components were identified by comparing: (i) Kovats' indices with those of ESO bank data, (ii) mass spectra obtained with those in the literature [18, 19].

2.4. Preparation of the Formulations

The formulations were made by mixing 100 microliters of essential oil with a gram of starch. The mixture obtained was chilled for 24 hours, and placed in a vacuum desiccator for 12 hours. The flavored powder that constitutes the formulation was stored in non-transparent bottles in the refrigerator. The formulations produced were then transported to the Laboratory of Applied Entomology, (Faculty of Science, University of Lomé, Togo), where they were stored in a freezer at -20 °C until use.

2.5. Experimental Device

In vitro tests were carried out in the Laboratory of Applied Entomology.

Six different concentrations were prepared from the formulation: 0.5 mg/cm^2, 1 mg/cm^2, 2 mg/cm^2, 4 mg/cm^2, and 6 mg/cm^2. These doses were obtained from 32 mg, 64 mg, 127 mg, 254 mg and 382 mg respectively of the formulation depending on the surface area of the Petri dish (diameter = 9 cm).The tests were carried out either with the formulation without sawdust or mixed with 10 mg of teak sawdust per Petri dish. In the second case, the sawdust was used as attractant.The obtained formulate for each dose was used to impregnate filter paper (Whatman n°1) which was then placed at the bottom of the Petri dish and left for 24 hours before testing.Two positive controls were prepared: one with starch and the other with teak sawdust only. For the negative control, termites were simply exposed in an empty Petri dish without filter paper or sawdust.

For each dose, 30 workers of *A. evuncifer* were used and 6 replicates were done.

2.6. Bioassays

Before the tests, termites were kept under observation in laboratory conditions. Workers were selected and placed in Petri dishes containing moistened filter paper. These Petri dishes containing termites were kept in total darkness.For the bioassay tests, termites were introduced in Petri dishes containing different doses of each formulation. All the Petri dishes were well lidded and kept in total darkness at 28 ± 2 °C and a relative humidity of 80 ± 10%. The mortality and the behavior of termites were recorded each hour from the beginning to the sixth hour and after 24 hours (the end of the bioassay).At the end of the experiment, the mean of survival duration (D) of tested termite was calculated according to the formula:

$$D = \frac{\sum Nv_i * t_i}{\sum t_i}$$

Nvi = number of live termites at each observation time

ti = observation time (h)

2.7. Statistical Analysis

Data obtained from the calculation of survival duration mean for each dose, were subjected to an analysis of variance (ANOVA) at 5% and the averages were discriminated with the Student-Newman-Keuls (SNK) test using STATISTICA software, version 5.1 (1999).

3. RESULTS

3.1. Chemical Composition of Essential Oils

Chemical analysis of essential oils revealed that both contain monoterpenes and sesquiterpenes. However they differ in their major components. *C. schoenanthus* is composed mainly of piperitone (63.43%), elemol (9.75%) and δ-2 carene (5.36%) (Table 1), while the major components of *O.canum* are 1,8-cineole (50.2%), camphor (13.5%) and beta-pinene (5.7%) (Table 2).

3.2. Bioassay Test with the Formulations Mixed with Teak Sawdust

The result of bioassay tests showed that the average rate of mortality of *A. evuncifer* workers was higher than that of the controls (Fig. 1). The termiticide activity of this formulation varies with concentration. The lowest concentration (0.5 mg/cm^2) of *C. schoenantus* caused 30% mortality while 100% mortality was reached at a dose of 2 mg/cm^2. For *O. canum*, the total mortality was effective at 4 mg/cm^2.

3.2. Effect of the Formulations without Sawdust

These formulations (for both plants) were very effective on the workers of *A. evuncifer* (Fig. 2). Their effect on this termite varies with concentration. At 0.5 mg/cm^2 and 1 mg/cm^2, the formulations from *C. schoenanthus* caused respectively 75% and 78% mortality while those of *O. canum* caused respectively 38% and 70% mortality for the same doses. Meanwhile 100% mortality was obtained at 2 mg/cm^2 for the two plants.

4. DISCUSSION

In this study, the formulations of *Ocimum canum* and *Cymbopogon schoenanthus* made from their essential oils have caused a significant increase in the mortality of *Amitermes evuncifer* workers, compared to the controls. These plants (belonging to aromatic plants) contain some compounds that cause the death of termites. Indeed many aromatic plants such as *O. canum* and *C. schoenanthus* of the Myrtaceae, Poaceae, Lauraceae, Myristicaceae, Lamiaceae families have insecticidal activity that could either kill insects or inhibit their reproduction [20]. Moreover, several authors have shown that many plants possess properties against termites such as antifeedant, repellency or toxicity [21]. In Togo, studies have shown that essential oils extracted from *Cymbopogon citratus* Stapf, *Cymbopogon nardus* L, *Cymbopogon schoenanthus* L, and *Cymbopogon giganteus* Chiov. are toxic to insect eggs, larvae and adults [22-26].

The effects on tested plants depend not only on the formulation but also on their concentration. Thus the formulation from *C. schoenanthus* was more effective on the mortality of *Amitermes evuncifer* than that of *O. canum*: the mortality rate obtained with the first plant was higher than that of the second. Therefore the formulation of *C. schoenantus* was the most toxic to *A. evuncifer*. The toxic products to adult insects are those causing high mortality at low concentrations [27]. Of the four Poaceae including: *C. citratus* Stapf, *C. nardus* L, *C. giganteus* and *C. schoenanthus* L, which had been studied in Togo, the essential oil from the last one was the most toxic to the adult *Callosobruchus maculatus* F, a pestiferous coleopteran beetle that causes serious damage to the stocks of *Vigna unguculata* (L.) Walp. On the other hand, Koba, et al. [28] recorded a total mortality (100%) of *Trinervitermes geminatus* Wasmann when exposed to the highest dose of essential oil of *O. canum* and *C. schoenantus* during 2 and 2 hours and a half respectively. The mortality rates observed here vary according to the concentrations, both formulations and the tested plants. This difference in sensitivity is due to the chemical constituents of the essential oils of the two tested plants. In fact, according to Djibo [16] and Nébié [17], the three major components of *O. canum* and *C. schoenanthus* were respectively, 1,8-cineole (50.2%), camphor (13.5%), alpha-terpineol (6.5%) and piperitone (63.43%), elemol (9.75%), δ-2 carene (5.36%). The different components of essential oils belong almost exclusively to two chemical groups including terpenoids (mono and sesquiterpenes, with low molecular weight) and phenylpropanoids [29]. However, the chemical composition of essential oils varies greatly depending on factors such as plant genetics, soil and climatic conditions of the environment of the plant. Indeed, in Togo, it was found that the major components of essential oils of *O. canum* include: terpineol-4 (36.40%), linalool (19.80%) and γ-terpinene (7.70%); and for *C. schoenantus*, these components are piperitone (68.51%) and 2-carene (16.48%) [28]. According to Delgarde [30], the monoterpenes in the essential oils such as citral, citronellal, eugenol and geranial are toxic to termites. These observations are similar to the results of Akantétou, et al. [31] who reported that the terpineol-4 in the essential oil of *O. canum* possessed some remarkable properties against aphids.

During the tests, high rates of mortality of treated termites were recorded even at low concentrations (0.5 mg/cm² and 1 mg/cm²) especially with the formulations without sawdust. These mortality rates could be due to:

- the presence of starch in the formulations that considerably reduces the high volatility of the essential oils. This allows a good impregnation of termite cuticle by the tested formulation. In fact essential oils are different from other vegetable oils by their high volatility.

- the soft cuticle of termites which is very susceptible to the effect of essential oils. Chiason and Beloin [32] have shown that essential oils act directly on the cuticle of soft-bodied insects and mites including termites.

- the effect of major constituents and additive effect of other constituents of the essential oils of both plants. Essential oils have some insecticidal properties [33].

In this study, the formulations without sawdust were more effective on termites than those with sawdust. The sawdust used as attractant contains cellulose which is a food source for termites. The presence of this food source could influence the survival time of treated termites. These results are similar to those obtained by Togola [34] who showed that termites are found in large numbers in plots with teak sawdust compared to plots without sawdust.

5. CONCLUSION

The results of this study showed that the formulations from the essential oils of *C. schoenantus* and *O. canum* mixed with starch possessed some toxic properties on workers of *A. evuncifer* at low concentrations (0.5 mg/cm² and 1 mg/cm²). At 2 mg/cm² a total mortality of 100% was recorded. A survey of the persistence of the formulations needs to be carried out in order to determine how long the product remains effective after field application. Following this survey, new formulations of essential oils with starch could potentially be used as biopesticide against pestiferous insects.

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Table-1. Chemical Composition of the Essential Oil of *Cymbopogon Schoenanthus*

N°	Name	I.K.**	(%)
	Monoterpenes		
1	**□-2 carene**	990	**5.36**
2	limonene	1015	1.16
3	cis-p-menth-2-en-1-ol	1097	0.82
4	trans-p-menth-2-en-1-ol	1115	0.46
5	□-terpineol	1166	0.95
6	cis-piperitol	1187	0.17
7	trans-piperitol	1206	0.22
8	**piperitone**	1225	**63.43**
	sesquiterpenes		
9	□-elemene	1375	0.95
10	□-caryophyllene	1395	1.61
11	□-gurjunene	1408	0.08
12	□-humulene	1428	0.07
13	□-selinene	1455	0.05
14	□-selinene*	1459	0.08
15	epi-cubebol*	1459	
16	cuparene	1472	0.12
17	germacrene A	1487	0.93
18	□-cadinene	1489	0.07
19	□-cadinene	1497	0.10
20	**elemol**	1521	**9.75**
21	germacrene-d-4-ol*	1543	0.65
22	oxyde de caryophyllene*	1543	
23	□-eudesmol	1603	1.12
24	□-eudesmol	1608	2.25
25	□-eudesmol	1614	2.57
26	eremoligenol	1626	4.94
	Total	-	**97.91**

Both products are eluted at the same time

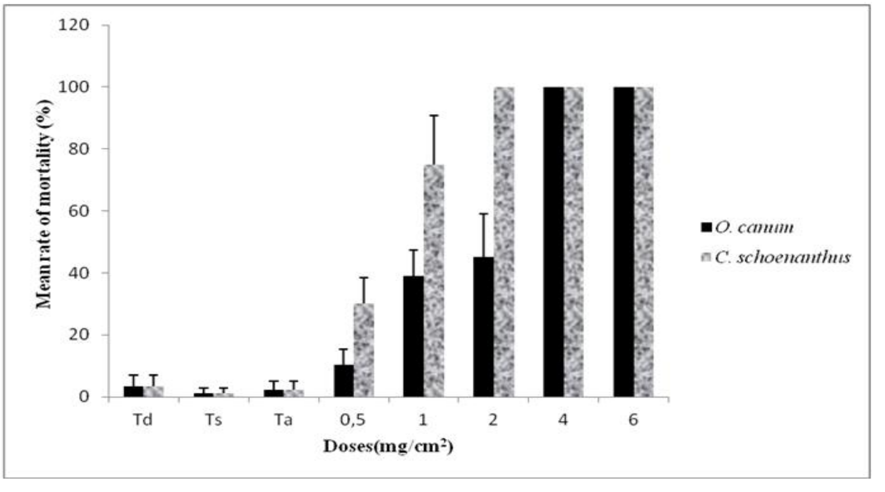
***Kovats' indices were calculated from the retention times determined on SPB1 column*

Table-2. Chemical composition of the essential oil of *Ocimum canum* Linn.

N°	Names	RI on DB-1	(%)
	Monoterpenes		
1	alpha-thujene	922	0.1
2	alpha-pinene	928	2.7
3	camphene	939	<0.1
4	sabinene	964	1.5
5	**beta-pinene**	966	**5.7**
6	myrcene	983	2.1
7	alpha-terpinene	1006	0.2
8	**1,8-cineole**	1019	**50.2**
9	limonene	1019	1.9
10	gamma-terpinene	1047	0.4

11	cis-p-menth-2-en-1-ol	1050	1.4
12	terpinolene	1075	0.2
13	linalol	1096	0.9
14	**camphre**	1127	**13.5**
16	terpinene-4-ol	1155	1.3
17	alpha-terpineol	1168	6.5
18	myrtenol	1172	0.2
19	ethyl phenylacetate	1220	0.3
	Sesquiterpenes		
22	beta-caryophyllene	1400	1.9
23	trans-alpha-bergamotene	1424	2.8
24	delta-cadinene	1481	2.5
25	nerolidol	1548	0.2
	Total		**96.6**

Figure-1. Termiticide activity of the formulations of *O. canum* and *C. schoenanthus* mixed with teak sawdust after 24 hours of exposure



O. canum F=300.20; ddf=7; p<0.000

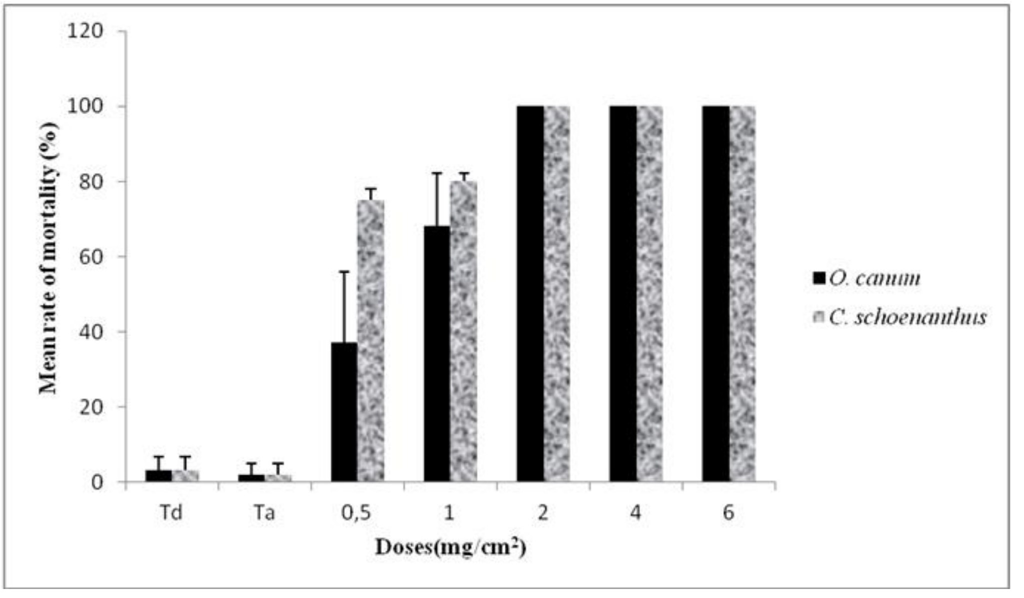
C. schoenanthus F=1204.07; ddf=7; p<0.000

Td: Negative control

Ts: positive control with sawdust

Ta: positive control with starch

Figure-2. Termiticide activity of the formulations of *O. canum* and *C. schoenanthus* without teak sawdust after 24 hours of exposure



O. canum F=180.89; ddf=7; $p<0.0000$

C. schoenanthus F=5186.42; ddf=7 ; $p<0.000$

Td: Negative control

Ta: positive control with starch