CHEMICAL COMPOSITION AND ANTI-TERMITE ACTIVITY OF THREE TROPICAL ESSENTIAL OILS AGAINST TERMITE SPECIES *TRINERVITERMES GEMINATUS* (Wasmann)

COMPOSITION CHIMIQUE ET ACTIVITÉ ANTI-TERMITE DE TROIS HUILES ESSENTIELLES TROPICALES CONTRE L'ESPÈCE DE TERMITE: TRINERVITERMES GEMINATUS (Wasmann)

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Abstract

Aerial parts essential oils of *Ocimum basilicum*, *Ocimum canum* and *Cymbopogon schoenanthus* growing in Togo were extracted by steam distillation and analyzed by Gaz Chromatography (GC) and Gaz Chromatography –Mass spectrometry (GC-MS) for their percentage composition and in vitro anti-termite activity by inhalation method.

O. basilicum essential oil mainly contained estragole (85.50%) and 1,8 cineole (2.25%). That of *O. canum* consisted principally of terpineol-4 (36.40%) linalool (19.80%) γ-terpinene (7.70%) (E)-α-bergamotene (6.20%) and β-caryophyllene (5.30%). In the *C. schoenanthus* essential oil, the main constituents were piperitone (68.51%) and carene-2 (16.48%). In vitro anti-termite testing revealed an insecticidal activity of the test volatile oils on the termite species *Trinervitermes geminatus*. 100% mortality at a dose of 11.75x10^{-2}µl.ml^{-1} was obtained with all three tested volatile oils respectively after 2 hours for *O. canum* and 2.5 hours for that of *O. basilicum* and *C. schoenanthus*. In comparison, the commercial pesticide (Dursban) used as positive standard control required a 5 hours period of time to kill 100% of the tested termites.

These findings indicated that the tested essential oils have a potential as anti-termite alternatives to synthetic chemical pesticide in termite control.

Key words:
Ocimum basilicum, Ocimum canum, Cymbopogon schoenanthus, anti-termite, Trinervitermes geminatus.

Résumé

Les huiles essentielles des parties aériennes d'*Ocimum basilicum*, d'*Ocimum canum* et de *Cymbopogon schoenanthus* du Togo extraites par entraînement à la vapeur d'eau ont été analysées par chromatographie en phase gazeuse simple (CPG) et chromatographie en phase gazeuse couplée à la spectrométie de Masse (CPG-SM) pour la détermination de leur composition chimique et la recherche de leur activité anti-termite in vitro par la méthode d'inhalation. Les composés majoritaires des huiles essentielles sont pour *O. basilicum* l'estragole (85,50%) et le 1,8 cineole (2,25%) ; pour *O. canum*, le terpineol-4 (36,40%) le linalol (19,80%) γ- terpinène (7,70%) (E)-α-bergamotene (6,20%) et β– caryophyllene (5,30%) et pour *C. schoenanthus* la pipéritone (68,51%) et le carene-2 (16,48%).

Le test anti-termite in vitro a révélé une activité insecticide très marquée des huiles essentielles testées sur l'espèce de termite *Trinervitermes geminatus*. Une mortalité de 100% a été obtenue avec chacune des trois huiles essentielles à la dose de 11.75x10^{-2-}µl.ml^{-1} après 2 heures pour *O. canum*, 2,5 heures pour *O. basilicum* et *C. schoenanthus*. En comparaison, les huiles essentielles testées se sont révélées plus actives contre les termites plus que le pesticide commercial (Dursban) utilisé comme témoin positif standard qui n'a atteint ce taux de mortalité qu'après 5 heures.

Les huiles essentielles ont un réel potentiel et pourraient être utilisées comme matières actives alternatives aux pesticides chimiques de synthèse dans la lutte contre les termites.

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Mots clés:
Ocimum basilicum, Ocimum canum,, Cymbopogon schoenanthus huile essentielle, insecticide, Trinervitermes geminatus

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1. Introduction

Termites feed on plant material, such as wood, leaf litter, roots, dead herbs and grasses, dung and humus. Their food constitutes the most abundant organic material in terrestrial biosphere. Termites have the ability to digest cellulose and even some species assisted by symbiotic intestinal protozoa and bacteria can digest lignin.

About 200 termite species are known to attack trees and crops. Of tree and shrub crops, cocoa and tea are must seriously affected, but other cultures suffer as well. Of food and cash crops, sugarcane is the most susceptible to damage [1]. Maize is also seriously damaged by termites (maybe) particularly in Africa [2,3]. Collins [4] reports that loss in crops in Nigeria caused by termites could be range from 20 to 40% per year.

Annual economic damages to buildings caused by termites in urban areas are evaluated to about US$15-20 billion while a US$30 billion cost is estimated for damage to agriculture and forestry [5].

Persistent organic pesticides (aldrin, dieldrin, chlordane, endrin, heptachlor and mirex) have been the principal approach to termite management during the last 50 years. These synthetic chemical insecticides used in agroecosystems as seed dressing and furrow application have harmful effects (high toxicity, persistence in the environment, subject to long-range transport). They are subject to bioaccumulation in fatty tissues and are magnified through the food-chains, hence constituting a serious risk to human health and the environment. Some of their adverse effects are: cancer, reproductive and immune troubles, endocrine disruption as well as development troubles and other disorders [5].

In addition to their multiple applications in human nutrition, therapeutics, perfumery and cosmetics, aromatic plants could provide biologically active molecules with pesticide properties, capable of being effective, ecological and affordable chemical alternatives to synthetic molecules in fighting insect pests like termites. Research works report the insecticidal properties of tropical aromatic plants essential oils [6,7,8,9]. To our knowledge at least, there is very few literature reports on the anti-termite properties of plant essential oils [10]. Planting *Cymbopogon schoenanthus* in farms and house gardens is reported as an effective agricultural practice to control termites [11].

The Stockholm Convention on persistent organic pollutants (POPs) has been negotiated and adopted in May 2001 as a legally binding international treaty to promote a global action to ban the production and usage of POPs [12]. This treaty entered into force in May 2004 and many Parties are currently taking action to implement the Convention, which include, among others, provisions that encourage Parties to undertake research aimed at promoting alternatives to POPs. In line with this perspective, the United Nations Environmental Program (UNEP) has been encouraging Parties to develop alternatives for termite control in order to phase out the use of POPs [13].

In line with this global concern and in our attempt to contribute to the implementation of the Convention, our aim in this work was to assess the anti-termite properties of essential oils of three aromatic plants (*O. canum, O. basilicum* and *C. schoenanthus*) against termite species *Trinervitermes geminatus* (Wasmann) involved in the crops destruction and in man-modified habitats in West Africa [14].

2. Experimental

Plant material and volatile oils isolation

Aerial biomass (leaves and flowers) of the tested plants were harvested at the full flowering stage in August 2005, from the experimental field of the "Unité de

Recherche sur les Matériaux et les Agroressources" at the “Université de Lomé”, Togo.

Plant material was identified by Professor Akpagana at the “Université de Lomé", where Voucher specimens were stored in the Herbarium.

A sample (50 g) of each air-dried plants material was hydrodistilled for 4 hours in a Clevenger-type apparatus according to the standard procedure reported in the European Pharmacopoeia [15]. The extracted crude essential oil was stored in hermetically sealed dark glass flasks with rubber lids, covered with aluminium foil to protect the contents from light and kept under refrigeration at 4°C without any prior purification until biological test and analyses through GC and/or GC-MS.

2.1. Essential oils analyses

Gas chromatography (GC)

Essential oils were analyzed on a GC Varian 3300 type equipped with FID. Retention indices (Table 1) were measured on a polar (Supelcowax 10) and an apolar (DB-5) capillary column (30 m x 0.25 mm i.d.; film tickness 0.25 µm).

Temperature was programmed as follows:

The DB-5 column: 50°C (5min), 50°C to 250°C at the rate of 2°C/min. The Supelcowax 10 column: 50°C (5min), 50°C to 200°C at 2 °C/min. The temperature was held at 250°C and 300°C respectively for the injector and detector. The carrier gas was helium at a flow rate of 1.50 ml/min. Samples (0.2µl) of non diluted essential oils were injected manually.

2.2. Gas Chromatography - Mass Spectrometry (GC-MS)

The GC/MS analyses were carried out on a Hewlett Packard 5890 SERIES II type apparatus coupled to a mass spectrometer, a Hewlett Packard 5971 SERIES operating in the EI mode at 70 eV. The capillary column type was DB5-MS (30 m x 0.25 mm i.d.; film thickness 0.25µm). The amount of sample injected and GC/MS analyses parameters were the same as above mentioned.

The essential oils constituents were identified through their mass spectra comparison with Willet [16], NIST [17] databases references as well as matching up their retention indices with those obtained from authentic samples [18].

2.3. Pesticidal activity

Termites trapping

The termites are captured by trapping within the compound of "Université de Lomé". The traps were made of small clay pots, filled with dry cassava stems (*Manhiot esculenta*). The pots contents were slightly watered and their plant material content conveniently put in contact with a slightly wet ground. After a few days, termites invaded the pots for cassava stems cellulose consumption. The termites thus captured were isolated and taken in a clean vat and brought to the laboratory where they were determined before the test.

The *in vitro* anti-termite properties of the essential oils were evaluated in the glass Petri dishes (80 mm diameter) using inhalation method described by Singh et al. [10].

The following quantities (µl.ml^{-1}) of the tested essential oil: 1.17x10^{-2}; 2.35x10^{-2}; 3.52x10^{-2}; 5.87x10^{-2} and 11.75x10^{-2}) were respectively applied on a piece of filter paper (15 mm diameter) and pasted in the inner surface of the cover of the Petri dish. A group of 20 tested termites, small parts of cassava stems (10 g) and (10 g) of soil were placed in each Petri dish in both treated and control sets. In this work, two types of control were used: Positive control (Dursban, 480g.l^{-1} commercial synthetic insecticide used against termites) and negative control (without any chemicals). Experiments were carried out in triplicate.

Table I: Chemical composition of three aromatic plants essential oils from Togo

Identified compounds	Retention indices	Peak Area [%]		
	RI	*O. basilium*	*O. canum*	*C. schoenanthus*
Monoterpene hydrocarbons		***2,88***	***20,05***	***18,63***
α-thujene	931		1.00	
α-pinene	941	0.16	2.30	
camphene	953		0.70	
sabinene	976	0.31		
myrcene	993	0.28	2.20	
α-phellandrene	1010		1.80	
carene-2	1017			**16.48**
α-terpinene	1023		2.80	
limonene	1036		0.15	2.15
(E)- β-ocimene	1058	1.77		
γ-terpinene	1078	0.20	**7.70**	
terpinolene	1095	0.16	1.40	
Oxygenated monoterpenes		***89.57***	***61,90***	***69,51***
1,8 cineole	1033	2.25		0.81
(Z)- sabinene hydrate	1076		4.20	
(E)-sabinene hydrate	1109		1.20	
linalool	1113	1.71	**19.80**	
camphor	1117	0.74		
estragole	1198	**85.50**		0.14
terpineol-4	1179	0.52	**36.40**	
α-terpineol	1190	0.75	0.30	0.56
piperitone		0.35		**68.00**
Sesquiterpene hydrocarbons		***3,72***	***16,80***	***2,39***
β-elemene	1387	0,43		0.82
β-caryophyllene	1420	0.40	5.30	1.10
(E)- α-bergamotene	1440	1.63	**6.20**	0.29
α-caryophyllène	1452		0.30	
germacrene D	1487	0.29	0.50	
β-selinene	1493		0.40	
bicyclogermacrene	1502	0.39	3.60	
germacrene A	1513	0.58		
δ-cadinene	1645		0.50	0.18
Oxygenated sesquiterpenes		***00.53***	***00.00***	***08,17***
elemol	1518	0.53		**5.76**
caryophyllene oxyde	1555			0.33
ß-eudesmol	1619			0.79
α-eudesmol	1643			1.19
Total identified		**96.70**	**98.60**	**98.43**

Peak area percentage is based on apolar DB-5 column, and values represent average of three determinations
Retention index on apolar DB-5 column

Sublethal toxicity of the tests essential oil was also investigated by observing the recovery of immobilized termites after transferring them in the fresh Petri dish without any chemicals.

Mortality rate was evaluated using the Abbott formula recommended by WHO and FAO for insecticides testing :

$$Pc = \frac{Po - Pt}{100 - Pt}$$

Where **Pc**: corrected mortality in %; **Pt**: mortality observed in the control and **Po:** mortality observed in the test.

3. Results and Discussion

The colourless essential oils of *O. basilicum*, *O. canum* and *C. schoenanthus* were obtained respectively in yield of 1.8%, 3.8% and 1.6% based on dried material. Chemical constituents of the studied essential oils are listed in Table I.

Twenty-two compounds were identified in the *O. basilicum* essential oil representing 96.70% of the detected constituents, with estragole (85.50%) and 1, 8 cineole (2.25%) as the major components. This *O. basilicum* essential oil was poor in monoterpene hydrocarbons (2.88%) sesquiterpene hydrocarbons (3.72%) and oxygenated sesquiterpenes (0.53%) and contained a high amount of oxygenated monoterpenes (89.57%). This sample was an estragole-rich one, very close to the Reunion type or Exotic type determined by a high estragole content [19,20,21].

The *O. canum* volatile oil contained twenty-one compounds representing 98.60% of the detected components. Terpineol-4 (36.40%) linalool (19.80%) γ-terpinene (7.70%) (E)-α-bergamotene (6.20%) and β-caryophyllene (5.30%) were the main constituents.

This sample of *O. canum* oil consisted principally of monoterpene hydrocarbons (20.05%) oxygenated monoterpenes (61.90%) and sesquiterpene hydrocarbons (16.80%). This sample of *O. canum* composition was quite identical to those previously described by Yayi et al. [21] and Sanda et al. [22].

In the *C. schoenanthus* essential oil, sixteen constituents were identified and piperitone (68.51%) and carene-2 (16.48%) were the major constituents. Our sample contained mainly monoterpene hydrocarbons (18.63%) and oxygenated monoterpenes (69.51%) and very few sesquiterpene hydrocarbons (3.39%) and oxygenated sesquiterpenes (08.17%).

This composition was quite similar to one previously described by Koumaglo et al. [23] and Koba et al. [24], but it differed sharply from that reported by Shahi and Tava [25] for an Indian *C. schoenanthus* oil type 2-undecanone (14.68%) limonene (19.54%) and camphene (7.98%) as major constituents.

Bioassays (Table II) of the volatile oils against termite (*T. geminatus*) showed that efficacy depended on both the dose of the essential oils and the termites exposure duration. Indeed, a dose of 3.52x10^{-2} µl.ml^{-1} essential oils of *O. basilicum C schoenanthus* caused 100% mortality after a 5-hour exposure while that of *O. canum* gave the same mortality only after 2.5 hours of exposure. Increasing the dose to 11.75x10^{-2} µl.ml^{-1} 100% mortality was

Table II: Insecticidal properties of three essential oils against termites (*T. geminatus*)

Essential oils/ synthetic insecticide	Concentrations µl.ml^{-1}	Mortality* at different exposure time (%)								
		0.5 h	1h	1.5h	2h	2.5h	3h	5	12	24
O. basilicum	1.17x10^{-2}	0	5	5	10	15	15	22.22	35.29	50
	2.35x10^{-2}	0	5	15	20	30	45	44.44	58.82	68.75
	3.52x10^{-2}	10	15	35	45	75	95	100	100	100
	5.87x10^{-2}	10	15	35	75	90	100	100	100	100
	11.75x10^{-2}	15	30	65	90	**100**	100	100	100	100
O. canum	1.17x10^{-2}	0	15	20	80	85	90	94.44	100	100
	2.35x10^{-2}	0	15	35	85	85	95	100	100	100
	3.52x10^{-2}	10	30	50	95	100	100	100	100	100
	5.87x10^{-2}	15	30	70	100	100	100	100	100	100
	11.75x10^{-2}	20	40	85	**100**	100	100	100	100	100
C. schoenanthus	1.17x10^{-2}	0	5	15	15	20	30	38.88	47.05	68.75
	2.35x10^{-2}	0	5	20	45	55	65	80	95	100
	3.52x10^{-2}	10	20	50	60	85	95	100	100	100
	5.87x10^{-2}	10	20	60	75	100	100	100	100	100
	11.75x10^{-2}	15	25	60	90	**100**	100	100	100	100
Dursban	11.75x10^{-2}	25	30	30	40	80	90	**100**	100	100
Control		0	0	0	0	0	0	10	15	20

* Average of three replications

observed only after a 2.5-hour exposure for *O. basilicum* and *C. schoenanthus* essential oils and 2 hours for the *O. canum* oil. In comparison, the commercial synthetic pesticide (Dursban) used as positive control in this work required a 5-hour exposure at the dose of $11.75\text{x}10^{-2}$ µl.ml^{-1} to cause 100% of mortality of the termites.

Termites immobilized after exposure to the essential oils did not recover when transferred in untreated Petri dishes.

The major components of the tested essential oils: estragole (*O. basilicum*), terpineol-4 (*O. canum*) and piperitone (*C. schoenanthus*) have been reported as insecticidal molecules [8**,**7]. Here we assume that they more specifically have anti-termite properties at least against the tested *T. geminatus* species.

The main finding of this work was that the naturally occurring plant essential oils tested as ecological anti-termites products proved to be more effective than the commercial synthetic pesticide used as positive control. More particularly, the *O. canum* essential oil chemotype seems to have an outstanding potential to serve as a natural product for termites control.

This finding is of a great import as the tested termite species is a major pest for food crops like cassava (*Manhiot esculenta*) potato (*Ipomea batata*) and rice (*Oryza* sp.) in West Africa. Using *O. canum* volatile oil to control termites, not only in agriculture but also in building, is technically and economically a viable alternative that needs further investigation (toxicity to humans, cost, availability, etc.) for development and extension. The environmental benefits of this alternative approach relate to using a chemically safer product and preserving plant biodiversity through the promotion of useful locally available plant resource.

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