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Larvicidal activity of Neem oil and three plant essential oils from Senegal against *Chrysodeixis chalcites* (Esper, 1789)

Saliou Ngom[1], Raimundo Cabrera Perez[2], Ma Anta Mbow[1], Rokhaya Fall[3]✉, Saliou Niassy[5], Andreea Cosoveanu[2], Serigne Mbacké Diop[3], El Hadji Barka Ndiaye[3], Moussoukhoye Diop[3], Georges Lognay[4]

[1]*Institut Sénégalais de Recherches Agricoles, B.P. 3120 Dakar, Sénégal*

[2]*Unidad de Fitopatologia, Facultad de Biología, Universidad de la Laguna, 38206 San Cristobal de la Laguna, Tenerife, Islas Canarias, España*

[3]*Département de chimie, Faculté des Sciences et Techniques, Université Cheikh Anta Diop de Dakar, B.P. 5005 Fann, Sénégal*

[4]*Gembloux Agro-Bio Tech, Université de Liège, Chimie Analytique Department AgroBioTech, 2 Passage des Déportés-5030 Gembloux*

[5]*Development, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa*

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ABSTRACT

Objective: To evaluate the insecticide, larvicidal and repellent activity of the essential oils from *Callistemon viminalis*, *Melaleuca leucadendron*, and *Hyptis suaveolens* against *Chrysodeixis chalcites* and to compare it with neem oil (*Azadirachta indica*). **Methods:** The essential oils of the leaves of these aromatiques plants were extracted by steam distillation and contacts tests were carried out. **Results:** Essential oils in ethanol from *Callistemon viminalis* showed a higher biological activity than the neem with 100% larval mortality at the concentration of 2 µg/mL for 6 h, 100% and 90% in ethanol from *Melaleuca leucadendron* and *Hyptis suaveolens*, respectively at the concentration of 4 µg/mL for 24 h. By inhalation, the essential oils from *Melaleuca leucadendron* and of *Hyptis suaveolens* were more effective with mortality rates of larvae 100% and 50% respectively at 2 µg/L air applied after 24 h. Nevertheless, the neem has shown to be a repulsive plant and anti-nutritional plant. A significant difference in the percentages of consumption between leaves treated with neem oil and the control samples was observed (Newman-Keuls test) except for *Melaleuca leucadendron*. **Conclusions:** The results of the study highlight remarkable biocide, properties of tested extracts, which provides important opportunities for the development of biopesticides.

1. Introduction

In West Africa, horticulture is an important economic activity. It supplies major urban centres with fruits and vegetables and exports part of its production to Europe and Asia. However, the production is affected by high pest attacks. The use of chemical pesticides is the main means for pest control. Pesticides are used uncontrollably by the producers who ignore their consequences[1], which created

✉Corresponding author: Rokhaya Fall, Département de chimie, Faculté des Sciences et Techniques, Université Cheikh Anta Diop de Dakar, B.P. 5005 Fann, Sénégal.
E-mail: dabandia@yahoo.fr

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serious health and ecological problems. Soil and groundwater are strongly contaminated by pesticide residues[2], therefore, it is urgent to find an alternative to limit the damage.

Biocides plants are important sources of natural substances that could be used as alternatives[3,4]. In some countries of Asian, Eastern Europe and South America, extracts from plants are approved and currently used as insecticides for crop protection[5]. On the other hand, in Senegal and in the West African region, plant resources with insecticidal effects are enormous[6,7], but they are limitedly used by the populations. Farmers are aware of their potential, but lack of scientific knowledge has established their phytosanitary properties. Neem oil (*Azadirachta indica*) currently remains the only product used as a biopesticide by most producers. Yet the Senegalese flora is remarkably rich with diverse species of plants containing substances with interesting biocidal properties such as essential oils. Due to their specific and complex mechanisms of action, they can be used on their own repetitively without the potential risk of causing any form of resistance in pests[8]. In addition, it has been shown that essential oils generally have broad spectrum effectiveness. However, they were extensively studied in view of potential use in agriculture. Their use remains empirical and is not based on scientific basis.

Optimizing techniques for using these plants requires proven scientific knowledge on their physicochemical properties and biological effectiveness. The study was carried out to determine the chemical composition of the essential oils of three aromatic plants [*Callistemon viminalis* (*C. viminalis*), *Melaleuca leucadendron* (*M. leucadendron*) and *Hyptis suavolens* (*H. suavolens*)] and to compare their biological activity with that of neem oil against larvae *Chrysodeixis chalcites* (*C. chalcites*) (serious pest for several vegetable crops).

2. Materials and methods

2.1. Plant material

The aerial parts of the plants (*C. viminalis*, *M. leucadendron* and *H. suaveolens*) were harvested between October and December 2013 at the Botanical garden of the Faculty of Science and Technology of UCAD, at the Forestry Park of Hann and within the walls of the ISRA-LNERV institution. Representative copies were deposited in the Herbarium of the plant biology Department of FST/UCAD. In the Index Seminal (1985) of the botanical garden of the Faculty of Science and Technology, the plants are registered under the following numbers: *C. viminalis* 329; *M. leucadendron* 334 and *H. suaveolens* 250.

The collected samples were dried at room temperature on the benches, out of direct sunlight for 6 d.

2.2. Breeding of insects

Mass animal husbandry was conducted in the laboratory in plastic jars deposited at room temperature and the tests were performed on pepper (*Capsicum annuum*) plants grown in pots in the laboratory.

2.3. Extraction and analysis of essential oils

Essential oils were obtained by steam distillation using a Clevenger-type mounting tool for 2 h, then dried with anhydrous sodium sulfate. The extraction of essential oils was carried out in the laboratory of natural products at UCAD. Neemland-Senegal (Thies) provided us with neem oil samples.

The characterization of essential oils was performed using chromatograph gas coupled with a flame ionization detector and by gas chromatograph coupled with mass spectrometry.

The gas coupled with a flame ionization detector was equipped with a capillary column of type 5% phenyl-dimethylpolysiloxane (30 m×0.25 mm ID) with film thickness of 0.25 μm. The carrier gas used is helium (He) at a flow rate of 1.5 mL/min. The oven temperature was from 40 (5 min hold) to 280 ℃ at 8 ℃/min with a final hold at 280 ℃ for 5 min. The injector was in splitless mode at 290 ℃. The detector was set at 290 ℃ (air and hydrogen with respective flow rates of 350 mL/min and 35 mL/min); make up gas (N_2) at 30 mL/min.

The gas chromatograph coupled with mass spectrometry was equipped with a capillary column and was used under conditions identical to those of gas coupled with a flame ionization detector. The CPG was coupled to a mass spectrometer (FINNIGAN TRACE MS). Fragmentation was done by electron impact (70 eV) and the mass range was comprised between 35 and 300 amu.

The identification of the compounds was made using the spectral library (Wiley 275L) connected to the gas chromatograph coupled with mass spectrometry and by calculating the retention indices, they were compared to those in the literature[9,10].

2.4. Insect and bioassays

Bioassays were performed in the laboratory of plant pathology at the University of Luguna in Tenerife in the Canary Islands. Toxic, anti-repulsive and anti-nutritional effects of the extracts of plants were tested on *C. chalcites* (Lepidoptera, Noctuidae) larvae according to different applications: contact, inhalation and treatment on the entire plant and leaves cut into disks (disk-Leaf Bioassay) in a controlled environment. Only adult larvaes of the same age group were used for the tests after being starved for one hour. For this purpose, mass animal husbandry was conducted in the laboratory.

The tests were performed on pepper plants grown in pots in the laboratory. Concentrations at 0.4 μg/mL, 2 μg/mL and 4 μg/mL of essential oils dissolved in a solution of ethanol were tested in comparison with those of neem oil and the control sample.

To perform the contact efficacy tests, four larvae were placed in a Petri dish containing the food treated with various concentrations with a total of 5 repetitions (5 petri dishes/concentration). The number of dead larvae was counted during the time of exposure.

For the efficacy testing by inhalation, the larvae were placed in 250 mL glass jars with food and an open capsule containing cotton where essential oils were deposited with different doses (0.4 μg/L, 2 μg/L and 4 μg/L air). The number of dead larvae was counted during and after treatment.

For the repulsive and antinutritional tests, two experiments were conducted. For the first one, the plant leaves were cut into disc with a diameter of 1 cm. Four leaf discs were set equidistant on a plastic substrate in a petri dish. Then 2 μL of each of the prepared solutions was uniformly spread on the discs with choice (two discs treated with the extracts while the other two of the same box have only received the same volume of ethanol as control sample) and without selection (four discs in the same box treated with extracts, and as control sample four discs in the same box treated with ethanol). After the treatments, four larvae were locked in each device with five repetitions per device. For the second experiment, the leaves of a whole plant were marked and treated with the same concentrations. After 24 h of exposure the discs and the leaves of the plants were scanned and the area consumed by the larvae was calculated using the software (image J).

2.5. Statistical analysis

For both tests, the mortality rate was calculated according to Abbott (1):

$M_c=(M_o-M_t)/(100-M_t)\times 100$ (M_o=mortality in the treated groups, M_t=mortality in the control group and M_c=calculated mortality).

Data was analyzed using SPSS IBM statistical software 21. The Newman-Keuls test was used to analyze non-parametric data. The difference is considered statistically significant when $P<0.05$.

3. Results

3.1. Chemical characteristics of essential oils of the plants used

Table 1 represents the main constituents of the essential oils of the three analyzed plants. Only compounds whose concentrations were above 1.86% (minor one) were registered.

Table 1
Main constituents in essential oils of plants.

Plants	Rt (mn)	RI	Major constituents	Content (%)
C. viminalis	8.86	926	α -pinene	2.49
	10.36	989	Myrcene	2.96
	11.42	1 030	Limonene	9.72
	11.46	1 034	1,8-cineol	58.12
	14.81	1 196	α -terpineol	9.56
	16.13	1 225	β -citral	6.02
	15.58	1 239	δ-elemene	3.53
M. leucadendron	8.90	926	α -pinene	12.22
	10.01	978	β -pinene	3.85
	11.35	1 030	Limonene	11.65
	14.78	1 196	α -terpineol	7.06
	21.57	1 605	Epiglobulol	23.06
	21.69	1 614	d-ledol	1.86
H. suaveolens	8.86	989	Myrcene	2.09
	9.94	973	Sabinene	31.49
	10.02	978	β -pinene	5.14
	11.69	1 031	E-ocimene	5.08
	12.51	1 086	Terpinolene	5.60
	17.27	1 339	δ-elemene	2.14
	18.79	1 426	β -caryophyllene	20.28
	18.96	1 435	α -bergamotene	3.51
	19.76	1 487	germacrene D	3.11
	20.15	1 502	β -bisabolene	5.46

Rt: retention times; RI: retention indices.

For *C. viminalis*, 34 compounds representing 99.07% of the essential oil were identified. The main compounds were 1.8 cineol (58.12%), limonene (9.72%), α -terpineol (9.56%) and β -citral (β -geranial) (6.02%).

In *M. leucadendron*, 44 compounds representing nearly 99.22% of the essential oil were identified. The main compounds were 1,8-cineol (28.87%), epiglobulol (23.06%), α -pinene (12.22%), limonene (11.65%), α -terpineol (7.06%), β -pinene (3.85%) and d-ledol (1.86%).

For the essential oil of *H. suaveolens*, 36 compounds representing 98.61% of the oil have been identified. It is essentially composed of: sabinene (31.49%), β -caryophyllene (20.28%), terpinolene, (5.60%), β -bisabolene (5.46%), β -pinene (5.14%), E-ocimene (5.08%), α bergamotène (3.51%) and germacrene d (3.11%).

3.2. Insecticidal properties of plant extracts

Table 2 represents the insecticidal effect of essential oils of *C. viminalis*, *M. leucadendron*, and *H. suaveolens* on the larvae of *C. chalcites*, compared to that of *Azadirachta indica,* neem oil.

The results showed that all the essential oils studied were more toxic than the neem formulation used in this study. At a concentration of 4 μg/mL ethanol, only 50% larval mortality was recorded on the neem oil after 24 h whereas, all essential oils have resulted in at least a 90% mortality using the same concentration after 24 h. However, oil of *C. viminalis* was more effective by contrast with a mortality

of 100% of the larvae at 4 μg/mL in an hour. The concentration of 2 μg/mL of this same oil has revealed a mortality of 90% after 2 h of application and 100% after 24 h. The essential oils of *M. leucadendron* and *H. suaveolens* have led to a mortality of 90% for larvae with the concentration of 4 μg/mL, in 2 h. However, essential oil of *M. leucadendron* at 4 μg/mL, killed all larvae after 24 h and 10% at 2 μg/mL.

Table 2

Effects larvicides extracts of plants in function of time and dose per contact (%).

Plants		Time (h)						
		1	2	3	4	5	6	24
Azadirachta indica	Control	0	0	0	0	0	0	0
	0.4 μg/mL	0	0	0	0	0	0	0
	2 μg/mL	0	0	0	0	0	0	0
	4 μg/mL	0	10	20	40	40	40	50
C. viminalis	Control	0	0	0	0	0	0	0
	0.4 μg/mL	0	0	0	0	0	20	100
	2 μg/mL	70	90	90	90	90	100	100
	4 μg/mL	100	100	100	100	100	100	100
M. leucadendron	Control	0	0	0	0	0	0	0
	0.4 μg/mL	0	0	0	0	0	0	0
	2 μg/mL	0	0	0	0	10	10	10
	4 μg/mL	80	90	90	90	90	90	100
H. suaveolens	Control	0	0	0	0	0	0	0
	0.4 μg/mL	0	0	0	0	0	10	20
	2 μg/mL	0	0	0	0	20	30	30
	4 μg/mL	80	90	90	90	90	90	90

By inhalation, the highest toxicity level was observed with the essential oil of *M. leucadendron*. After 24 h exposure, the essential oil of *M. leucadendron* showed a larval mortality rate of 40%, 60% and 80% for concentrations at 0.4, 2.0 and 4.0 μg/L air, respectively. For *H. suaveolens*, 50% larval mortality rate was observed with the concentration of 2.0 and 4.0 μg/L air, and 10% larval mortality rate was observed with the concentration of 0.4 μg/L airafter 24 h exposure. On the other hand, for *C. viminalis* the mortality rate was 10% at concentration of 2.0 and 4.0 μg/L air after 24 h of treatment.

Table 3 and Table 4 showed that all plant extracts had deterrent and repulsive activity against the larvae of *C. viminalis*. The percentage of consumption of treated leaf-disk with the extracts of the four plants (with choice) was lower than that observed for the control. Compared to the essential oils, neem oil (*Azadirachta indica*) revealed a significantly higher anti-feeding activity with a significant difference between the percentage of consumption of leaves treated with the neem oil and control (Mann-Whitney test): 4.4% for the treated leaves against 41.6% for the control on leaf-disk, with choice 3.8% against 54.3% on leaf-disk non-choice, and 1.8% against 19.9% for tests on whole plant.

Table 3

Leaf surface consumed (%) on leaf disk after 24 h of application of extracts of plants.

Plants	Choice		Non-choice		Control
	Treated	Untreated	Treated	Untreated	EtOH
Azadirachta indica	4.4±3.4	41.6±24.8	3.8±2.4	54.3±8.9	50.4±24.9
C. viminalis	13.7±6.7	15.9±8.4	13.7±9.6	20.6±8.9	31.0±9.1
M. leucadendron	32.5±16.5	38.8±19.6	18.2±12.7	30.4±18.6	23.5±11.6
H. suaveolens	6.5±5.8	24.1±10.77	4.8±3.6	36.5±13.8	27.5±9.8

Table 4

Leaf surface consumed on whole plant after 24 h of application of plant extracts (%).

Plants	Treatments	Leaf surface consumed (%)
Azadirachta indica	Untreated leaves	19.9±3.6^{a}
	Treated leaves	1.8±1.0^{c}
C. viminalis	Untreated leaves	16.3±2.8^{a}
	Treated leaves	8.7±2.6^{b}
M. leucadendron	Untreated leaves	4.9±1.9^{bc}
	Treated leaves	4.3±3.1^{bc}
H. suaveolens	Untreated leaves	14.7±3.8^{a}
	Treated leaves	2.8±1.7^{bc}

Means followed by different letters are significantly different according to the Newman-Keuls test (P<0.05).

4. Discussion

Several scientific studies have reported the chemical composition of the essential oils of *C. viminalis* from different areas[11,12]. This chemical profile of *C. viminalis* essential oil collected in Dakar is comparable to that described in Cameroon, Egypt and India[11-13]. Main compounds are very similar except the Brazilian species. 1,8-cineol (58.12%-71.77%) is the predominant constituent of the oil. However, the Indian and Egyptian species showed higher rates of α -pinene (20.43%-24.20%) as compared to the species in Cameroon and Senegal with 0.38% and 2.49% respectively. We also detected Δ3-carene and menthyl acetate only in Camerounian and Indian species in considerable rates (8.6% and 5.3%, respectively). For the Brazilian species, the principal compounds of the essential oils in the leaves were eucalyptol (84.60%) and α -pinene (10.28%)[14].

For the essential oil of *M. leucadendron*, the chemical composition of the species and the species studied by Farag *et al* in Cairo (Egypt) [15] showed slight differences with the absence of epiglobulol. The egyptian species contains more 1,8-cineol (64.3% against 28.87%) and α -terpineol (11.02% against 7.06%). Inversely, the oil essential collected in Senegal on *M. leucadendron* in Dakar showed higher rates of α -pinene, limonene and β -pinene (12.22%, 11.65% and 3.85%, as compared to the specie in Egypt with 4.24%, 6.70% and 1.67% respectively)[16]. 1,8-cineol (0.1%) and epiglobulol were

found in traces in Pakistani variety of *M. leucadendron*. According to Saima *et al*[17], eugenol methyl ether (95.4%) was the major component in the essential oil of *M. leucadendron* species grown in Pakistan.

For *H. suaveolens*, results obtained on the chemical profile of the essential oil were compared with those reported in the literature. The biochemical profile obtained for the essential oil of *H. suaveolens* is different from that found in Benin[18]. In addition to the main compounds identified, the latter contains other relevant compounds at higher rates such as 1.8-cineol (14.0%-24.6%), β-phéllandrène (10.2%) and fenchone (4.1%-8.1%). However, the extract of *H. suaveolens* studied in Burkina Faso by Djibo[19] presents a similar chemical profile to our study with dominant compounds sabinene (36%), β-caryophyllene (17%) and terpinolene (7.3%) in the Burkinabe species.

Insecticidal activity of the essential oil against *C. chalcites* were determined by the bioassays in the laboratory conditions and compared with neem oil *Azadirachta indica*. All the essential oils showed a larvicidal activity. However, only the essential oil of *C. chalcites* showed a larvicidal activity at the lowest concentration (0.4 μg/mL). The high larvicidal activities of *C. chalcites* and *M. leucadendron* could be explained by their important compounds such as the 1-8-cineole for *C. chalcites* and the epiglobulol for *M. leucadendron*. Indeed, a strong biological activity of essential oils rich in compounds with alcohol function was reported by several authors[20,21]. 1-8-cineole has been reported to possess insecticidal activity against several insects including *Tribolium castaneum*, *Callosobruchiis maculatus*, *Rhyzopertha dominica*, and *Sitopliilus oryzae* L.[22,23]. In addition, these two plants also contain other compounds such as α- and β-pinene, limonene, α-terpineol at considerable amounts and their insecticidal properties have been demonstrated. The larvicidal activity of essential oils with similar compounds has been proved by several authors[24–26].

For the essential oils, *H. suavolen*s seems to show a stronger anti-feeding and repulsive activity with a consumption of 6.45% on treated leaves against 24.08% for the control leaf-disk with choice, 4.80% against 36.47% on leaf-disk non-choice and 4.80% against 14.65% for tests on whole plant. Although the essential oils of *C. chalcites* and *M. leucadendron* were considered repulsive according to Lachance[27], our results have revealed that this property is low. However, this low repulsive and anti-feeding activity observed with the essential oils could be explained by the concentration and the mode of treatment used for the tests as this did not allow a strong adherence of the product on the leaves.

To overcome this limitation on the mode of application we notify that in the established formulas of biopesticides, essential oils are diluted in the neem oil. In addition, the essential oils will induce a toxic effect associated with the activity anti-feeding repulsive effect of neem.

In Senegal and other countries of the West African sub-region, the use of insecticides against pests of cultures is not based on any scientific evidence. This study is therefore an important contribution to the knowledge of the chemical profile of the essential oils of the plants (*C. viminalis*, *M. leucadendron*, and *H. suaveolens*) as well as their insecticidal potential. The chemical characterization showed that the essential oils of *C. viminalis* and *M. leucadendron* are rich in oxygenated compounds (monoterpene oxides, monoterpenic alcohols sesquiterpene). *H. suaveolens* is essentially composed of monoterpenes and hydrocarbon sesquiterpenes. By contrast, the essential oils of the three plants showed a remarkable biological activity on larvae of *Chrysodeixis*. Neem treatment showed a repulsive and anti-feeding activity. On the basis of these results, it suggests that the association of essential oils with the neem oil is efficient in the management of lepidopterans particularly *C. chalcites*.

Conflict of interest statement

We declare that we have no conflict of interest.

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