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RESEARCH ARTICLE

Insecticidal and repellent effects of essential oils from leaves of *Hyptis suaveolens* and *Ocimum canum* against *Tenebroides mauritanicus* (L.) isolated from peanut in post-harvest

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Abstract

The present work aims to evaluate the insecticidal and repellent effects of essential oils from fresh leaves of *Hyptis suaveolens* and *Ocimum canum* against *Tenebroides mauritanicus* (L.) (Coleoptera: Tenebrionidaea) isolated from peanut in post-harvest in Benin. Chemical analysis of essential oils by GC and GC-MS indicated that, in the volatile extracts, different groups of terpene and terpenoid were present. The results of contact toxicity tests indicated that at a concentration of 0.5 μl of essential oil/g of peanut the mortality rate of *Tenebroides mauritanicus* (L.) is 100% after 24 h for the essential oil of *Hyptis suaveolens*, while it is still at 20% for the essential oil of *Ocimum canum*. Results also indicated that *Hyptis suaveolens* oil has a high repellent activity, when compared to the essential oil of *Ocimum canum*. This essential oil with high repellent and insecticidal properties represents a novel approach in the protection of grains against *T. mauritanicus* and the reduction of post-harvest losses.

Keywords Peanut · *T. mauritanicus* (L.) · Essential oils · Insecticidal · Repellency · Benin

1 Introduction

Each year, large amounts of stored products in the world are destroyed or contaminated due to the presence of arthropods and beetles, forming the largest group of organisms that attack food (Campbell et al. 1989). It is estimated that 35% of crops all over the world are destroyed by insect pests (Shani 2000). For their survival, insects need nutrients, air and water. In most cases, stored foodstuffs provide a place of choice for the survival and growth of these insect pests because of the availability of food, air and water.

Peanuts are stored both as unshelled pods and as kernels for different uses. Both forms are vulnerable to attack by a plethora of insect pests after harvest. More than 100 insect species are known to live and feed on stored peanuts, some of which are of economic importance (Coskuncu and Kovanci 2005). The cadelle (*Tenebroides mauritanicus* L.), is one of the most commonly reported stored peanut pests (Coskuncu and Kovanci 2005). During periods of postharvest temperature, humidity and other factors play an important role in the growth of toxigenic fungi and insects in the storage ecosystem. The constant movement of insect populations within a granary ecosystem contributes to the dispersal of viable spores of fungi of various species, which are carried on the body surface or deposited in insect frass (Nesci et al. 2011).

In crop protection, chemicals largely used as pesticides could have undesirable effects such as ozone depletion, environmental pollution, and toxicity to non-target organisms and pest resistance (Isman 2006; Adjalian et al. 2015). Faced with the consequences of the use of synthetic insecticides, very few are nowadays allowed (Aktar et al. 2009).

The demand for pest-free products and reducing of pesticide use is large and the search for biorational pest control methods became necessary (Phillips 1997). Natural products are an excellent alternative to synthetic pesticides in order to reduce negative impacts to human health and the environment (Koul et al. 2008). Plants may provide a

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potential alternative to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Kim et al. 2002). Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts (Shaaya et al. 1997).

In Benin, several studies have focused on the protection of crops such as maize, pigeon pee and cowpea (Fandohan et al. 2004; Adjalian et al. 2015). Thus, the present study aims to investigate the insecticidal and repellent effects of essential oils of *Hyptis suaveolens* and *Ocimum canum* against adults of *Tenebroides mauritanicus* (L.), isolated from peanuts in post-harvest in Benin.

2 Materials and methods

2.1 Collection of plant leaves

Plant materials used for essential oil (EO) extraction were leaves from *Hyptis suaveolens* and *Ocimum canum*. Plants were collected at Abomey-calavi (south Benin) and identified at the Benin national herbarium, where voucher specimens are deposited.

2.2 Essential oil extraction

The collected plant materials were stored in the laboratory between 18 °C and 20 °C in the shade of the sunlight throughout the period of extraction. The essential oils were obtained by hydrodistillation of the leaves (450 g) for 5 h using a Clevenger-type apparatus. The oils recovered were dried over anhydrous sodium sulfate and stored at 4 °C (de Billerbeck et al. 2001).

2.3 Gas chromatography–mass spectrometry analysis

The EOs were analyzed by a gas chromatograph (Perkin Elmer Auto XL GC; Waltham, MA, USA) equipped with a flame ionisation detector, and the GC conditions were EQUITY-5 column (60 m × 0.32 mm × 0.25 µm); H_2 as the carrier gas; column head pressure 10 psi; oven temperature program isotherm 2 min at 70 °C, 3 °C/min gradient 250 °C, isotherm 10 min; injection temperature, 250 °C; detector temperature 280 °C. Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a Perkin Elmer Turbomass GC–MS. The GC column was EQUITY-5 (60 m × 0.32 mm × 0.25 µm); fused silica capillary column. The GC conditions were injection temperature, 250 °C; column temperature, isothermal at 70 °C for 2 min, then programmed to 250 °C at 37 °C/min and held at this temperature for 10 min; ion source temperature, 250 °C. Helium was the carrier gas. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the Electron ionization (EI) mode with 70 eV ionisation energy. The sector mass analyzer was set to scan from 40 to 500 amu (atomic mass unit) for 22 s. The identification of individual compounds is based on their retention times, retention indices relative to C_5–C_{18} n-alkanes, and matching spectral peaks available in the published data (Adams 2007).

2.4 Insects and rearing conditions

Insects were directly collected from infested peanut samples. They were reared in laboratory at 25 ± 1 °C, with a photoperiod of 12 h/12 h (dark/light). The adults obtained were put in separate insect cages and identified in the Department of Environmental Engineering of Polytechnic School of Abomey-Calavi University and the International Institute of Tropical Agriculture (IITA), (Benin).

2.5 Contact toxicity tests

Bioassays were performed using the method described by Adjalian et al. (2015). The temperature of the test medium ranged from 25 to 31 °C and relative humidity was 80%. Six concentrations (0.1, 0.2, 0.3, 0.4, 0.5 µl of EO/g of peanut) of each EO were tested. These low EO concentrations were chosen taking into account previous works on the use of essential oils in the preservation of post-harvest products available in the literature, and also the volatile nature of essential oils that could impact the organoleptic characteristics of treated peanuts.

Peanuts were treated with the essential oils and after 24 h, 5 adults of males and females of *T. mauritanicus* were deposited on the treated plant material. Overall, 6 doses × 3 repeats × 2 EO = 36 experimental units (containers) were implemented. Adult mortality was monitored 24 h after exposure to the essential oil. Then the insects were separated from the peanuts. The emergence of new insects was then observed at intervals of 24 h in the experimental units to the 50th day after infestation.

2.6 Repellency tests

Repellency tests were performed as described by Zhang et al. (2011). Filter paper discs of 9 cm in diameter were cut into two equal parts of 31.8 cm^2 of surface. Five concentrations of each EO (0.03, 0.06, 0.15, 0.23, 0.31 µl of EO/cm^2) were prepared by dilution with acetone. Then 0.5 ml of each concentration was spread on the half of the filter paper disc, while the other half received only 0.5 ml of acetone. After 10 min, the two half discs were tight and placed in a petri dish with 10 of non-sexed adult insects.

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The orientation of the tape was changed in each repetition to avoid the effect of any directional stimulus which could affect the orientation of insects. After half an hour, the number of insects present on the part of disc treated with acetone was determined and the percentage of repulsion (PR) is calculated by using the formula PR = [(A − B)/(A + B)] × 100 where A is the average number of insects present in the untreated portion (insects repelled) and B is the average number of insects in the treated (not repelled insects) part. The average percentage of repulsion for the essential oils was calculated and assigned to one of several repulsive classes ranging from 0 to V: class 0 (PR < 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%), and class V (PR = 80.1–100%).

2.7 Statistical analysis

The differences among the fumigant activities of essential oils tested were determined according to analysis of variance (ANOVA) test by using SPSS 13.0 software package. Differences between means were tested through Tukey's multiple comparison tests and values with $p < 0.05$ were considered significantly different.

3 Results and discussion

By hydrodistillation, fresh leaves of *Ocimum canum* yielded 1.1% of EO. Chemical analysis by GC and GC–MS analysis of EO enabled the identification of 32 components (Table 1), representing 95.2% of the EO. This EO has a chemical composition characterized by the presence of oxygenated monoterpenes (60.8%), hydrogenated monoterpenes (20.7%), hydrogenated sesquiterpenes (12.2%), oxygenated sesquiterpenes (0.5%) and aromatic compounds (95.2%). The major components of this essential oil were terpinen-4-ol (41.1%) and linalol (14.7%). The GC and GC–MS analysis of EO essential oil from *Hyptis suaveolens* (Table 2) indicated that this oil is characterized by the presence of oxygenated monoterpenes (31.2%), hydrogenated monoterpenes (33.7%), hydrogenated sesquiterpenes (25.6%) and oxygenated sesquiterpenes (3.5%). The major identified compounds were 1.8-cinéole (14.0%) and β-caryophyllene (9.8%).These results differ from those reported in early reports (Bassole et al. 2005), and indicated that essential oils have a heterogenic chemical composition depending on the geographic location of harvesting sites.

The results of contact toxicity tests (Table 3) indicated that at a concentration of 0.5 µl/g of peanut the mortality rate is 100% after 24 h for the essential oil of *Hyptis suaveolens*, while it is still at 20% for the essential oil of *Ocimum canum*. The present study also indicated that the essential oil of *Hyptis suaveolens* has a high repellent activity, when compared to the essential oil of *Ocimum canum*. Indeed, the results of repellency tests (Table 4) indicated that from a concentration of 0.06 µl/cm^2, the EO of *Hyptis suaveolens* has a percentage of repellency of 100%, in opposite to the essential oil of *Ocimum canum*. The average percentage of repellency of the EO of *Hyptis suaveolens* in the concentrations range used indicated that this essential oil belongs to the high repellency class (V). These results underlined the strong insecticidal and repellent properties of the essential oil of *Hyptis suaveolens*.

Table 1 Chemical composition of tested essential oil of *Ocimum canum*

Compounds	RI	%
α-Thujene	928	1.3
α-Pinene	937	2.0
Camphene	952	0.3
Sabinene	968	0.2
β-Pinene	972	0.1
Acetate of (Z)-3-hexenyl	979	0.2
Myrcene	985	2.1
α-Phellandrene	1016	1.4
α-Terpinene	1020	1.7
Limonene	1030	3.4
γ-Terpinene	1058	6.9
Sabinene hydrate	1065	1.0
Terpinolene	1087	1.3
Linalol	1097	14.7
Acetate of octen-3-yl	1101	0.6
Camphre	1139	1.0
Borneol	1150	0.3
Terpinen-4-ol	1189	41.1
p-Cymen-8-ol	1192	0.6
α-Terpineol	1205	0.4
Fenchyl acetate	1219	0.9
Ethyl phenylacetate	1238	0.2
Bornyl acetate	1282	0.4
Myrtenyl acetate	1318	0.4
Butyrate of (Z)-3-hexenyle	1368	0.2
β-Caryophyllene	1439	4.1
trans-α-Bergamotene	1446	4.8
α-Humulene	1470	0.5
Germacrene D	1486	2.4
β-Bisabolene	1510	0.2
Nerolidol	1598	0.4
Caryophyllene oxide	1611	0.1
Total		95.2

RI retention index

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Table 2 Chemical composition of tested essential oil of *Hyptis suaveolens*

Compounds	RI	%
Tricyclene	923	1.0
α-Thujene	930	2.5
Sabinene	973	7.3
β-Pinene	979	5.4
Oct-1-en-3-ol	980	0.4
Myrcene	988	0.7
α-Phellandrene	1002	0.4
para-Mentha-1(7), 8-diene	1004	0.5
α-Terpinene	1014	2.4
para-Cymene	1023	1.8
1,8-cineole	1035	14.0
γ-terpinene	1057	3.8
cis-Sabinene hydrate	1068	2.8
para-Mentha-2(7), 8-diene	1083	7.9
Fenchone	1086	4.1
trans-Sabinene hydrate	1098	2.3
Exo-fenchone	1122	0.2
cis-para-Menth-2-en-1-ol	1124	0.2
trans-para-Menth-2-en-1-ol	1141	0.2
Camphre	1143	0.3
Terpinen-4-ol	1179	5.4
para-Cymen-8-ol	1183	0.2
α-Terpineol	1192	0.7
cis-Piperitol	1196	0.4
α-Cubebene	1340	0.2
α-Copaene	1370	0.9
β-Bourbonene	1377	0.5
β-Elemene	1383	0.3
Isocaryophyllene	1402	0.7
β-Caryophyllene	1417	9.8
cis-α-Bergamotene	1429	0.8
Aromadendrene	1437	1.0
α-Humulene	1450	0.8
Allo-aromadendrene	1458	0.8
9-epi-β-caryophyllene	1469	0.1
β-Acoradiene	1473	0.3
γ-Muurolene	1479	1.1
β-Selinene	1483	1.9
Viridiflorene	1494	3.0
δ-Amorphene	1510	0.5
Spathulenol	1570	2.9
Caryophyllene oxide	1583	1.2
Viridiflorol	1583	0.3
α-Cadinol	1652	0.3
Selin-11-en-4-α-ol	1656	0.4
Heliofolenol-C	1685	0.5
Manoyl oxide	1986	0.2
13-epi-Manoyl oxide	2004	0.6
Abietatriene	2044	0.3
Abietadiene	2077	0.4
Isopimarol	2311	0.3
Total		95.9

RI retention index

Table 3 Results of contact toxicity tests (%)

Essential oils	Concentrations (μl of EO/g of peanut)				
	0.1	0.2	0.3	0.4	0.5
Ocimum canum	00[a]	10[a]	10[a]	10[a]	20[a]
Hyptis suaveolens	00[a]	10[a]	20[b]	80[b]	100[b]

Values are mean (n = 3). The means followed by different superscript letters (a or b) in the same column are significantly different according to ANOVA and Tukey's multiple comparison tests

Several studies have also underlined the insecticidal property of extracts from *Hyptis suaveolens* against lepidopteran pests (Prakash et al. 2008) and *Sesamia calamistis* on maize (Adda et al. 2011). According to Raja et al. (2005), extracts of *H. suaveolens* were also found to possess significant ovicidal and antifeedant activity against *Helicoverpa armigera* on cotton. Jayakumar et al. (2005) also reported the ovicidal and insecticidal effects of extracts from *H. suaveolens* against *Callosobruchus maculatus* on cowpea. This high insecticidal and repellent effects could be due to the presence in *H. suaveolens* plant of compounds with high insecticidal and repellent properties.

Several studies reported that the high insecticidal activity of essential oils may be related to its high content in monoterpenes such as 1.8-cineole present in EO of *H. suaveolens*. Indeed, Lee et al. (2004) reported that the majority of the oils showing potential fumigant toxicity were rich in 1,8-cineole. Rozman et al. (2006), on the basis of the results obtained by gas chromatography, came to the conclusion that 1,8-cineole was the principal active compound in essential oils of lavender, rosemary, thyme and laurel which have also a high insecticidal activity. Most monoterpenes are toxic to insects by penetrating the body through the respiratory system (fumigant effect), the cuticle (contact effect) or through the digestive system in case of ingestion (Gnankiné and Bassolé 2017). Rattan (2010) described three major targets of insect neurosystems (the cholinergic, octopamenergic and GABA systems).

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Table 4 Results of repellency tests

Essential oils	Concentrations (µl of EO /cm^2)					Average percentage of repulsion (%)	Repellence class	Propriety
	0.03	0.06	0.15	0.23	0.31			
Ocimum canum	00^a	60^a	40^a	80^a	80^a	52	III	Low repellency
Hyptis suaveolens	10^a	100^b	100^b	100^b	100^b	82	V	High repellency

Values are mean (n = 3). The means followed by different superscript letters (a or b) in the same column are significantly different according to ANOVA and Tukey's multiple comparison tests

Acetylcholinesterase plays a key role in cholinergic synapses that are essential for insects and higher animals (Fournier and Mutero 1994). It is known to be a class of enzymes that catalyzes the hydrolysis of the neurotransmitting agent acetylcholine. Inhibition of acetylcholinesterase causes accumulation of acetylcholine in the synapses, so that the post-synaptic membrane is in a state of permanent stimulation, which results in a lack of coordination in the neuromuscular system, and eventual death (Aygun et al. 2002).

Despite the fact that essential oils are volatile and could impact the organoleptic characteristics of treated food, many of individual oil components are approved food flavorings and also impart a certain flavor to foods. For example, carvacrol is known to produce a "warmly pungent" aroma, citral is "lemon-like" and geraniol is "rose-like" (Kim et al. 1995).

4 Conclusion

This research underlined the high insecticidal and repellent properties of essential oils of *Hyptis suaveolens* against *Tenebroides mauritanicus* (L.) (Coleoptera: Tenebrionidaea), isolated from peanuts in post-harvest in Benin. Developing biocides from this essential oil would be contributive for a lasting solution to the many problems posed by the use of synthetic insecticides. However, in relation to the composition of foods, further investigations are necessary to identify the conditions that maximize their insecticidal activity without detrimental effects on the organoleptic properties of the peanuts.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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