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ANTI-OVIPOSITION AND REPELLENT ACTIVITY OF ESSENTIAL OIL FROM *MELALEUCA LEUCADENDRON* LEAF ACCLIMATED IN BÉNIN AGAINST THE ANGOUMOIS GRAIN MOTH.

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ABSTRACT

The fresh leaves of *Melaleuca leucadendron* Linn. (Myrtaceae) was hydrodistilled by Clevenger apparatus. Gas chromatography-mass spectrometry analyses revealed the presence of 25 components in essential oil obtained. 1,8-cineole (15.4%), (E)-nerolidol (37.3%) and ledol (19.5%) were found to be the major components. Cajeput oil has been historically reported for its antiseptic and anti-inflammatory actions. The evaluation of the activity of the anti-oviposition of the essential oil of *M. leucadendron* on female *S. cerealella* and repellent effect has been made in the laboratory by the method of fumigation in a closed glass vessel at a temperature 29 ± 2 ° C and natural photoperiod with a relative humidity of 70 ± 10%. The results show the toxicity of the essential oil was illustrated by the significant reduction or inhibition in egg laying compared to control groups whatever the form of rice storage. Repellent activity was evaluated by observing the behaviour of female beetles exposed to treated and untreated beans in a linear olfactometer. The extract of the leaves are repellents against *S. cerealella* with an average percentage of 61% repulsion. Our results indicate that botanical products may provide effective control of *S. cerealella* in rice storage.

Keywords: Essential oils, Anti-Oviposition Activity, Repellency, *Sitotroga cerealella*, *Melaleuca leucadendron*

INTRODUCTION

Insect pests cause heavy losses of stored grain quantity and quality worldwide (Madrid et al, 1990). Insect damage in stored grain can reach 10% - 40% in countries with modern storage technologies have been introduced **[1]**. The Angoumois grain moth, *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae), is a cosmopolitan insect Widespread All which is globally and a parasite of cereals. The infested grains smell and taste unpleasant **[2]**. Chemical control against insects in storage has been used for a long time, but has serious drawbacks **[3]**. Reckless and indiscriminate use of synthetic pesticides has led to a number of well-known problems. Plants are rich sources of natural substances that can be used in the development of environmentally friendly methods to control pests. The deleterious effects of certain phytochemicals purified or crude plant extracts on insects occur in several ways, including toxicity, inhibition of egg **[4, 5, 6]**, and the reduction of fertility and fertility **[7]**. The essential oils of Melaleuca have a strong repellent effect on ant *Wasmannia auropunctata* which is a pest of both the forest and plantations and fruit crops **[8]**. **[9]** showed that oil cajuput undiluted imposed a 100% mortality in *Andrector ruficornis* cowpea in Cuba and has distinct repellent effects. Cajeput oil is also known to prevent the infection of the plant of the herpes simplex type 1 on plant and inhibits the growth of fungi, bacteria and yeast **[10, 11, 12, 13, 14]**. *M. leucadendron* is a large tree, native to Asia Southeast, the Pacific Islands and Australia. It can be found in many countries such as Benin, Indonesia, Malaysia, Vietnam, the Philippines, Thailand, Cuba, Venezuela, Turkey, United States and Hawaii **[9, 10, 15]**. Moreover, there are few studies on the activities of the essential oil from the leaves of *M. leucadendron* against the insect pests. So the purpose of this study is to investigate the activities of *M. leucadendron* against *Sitotroga cerealella*, one of the major pests in rice stored in Benin.

MATERIALS AND METHODS

Plant material and extraction of essential oils

The leaves of *Melaleuca leucadendron* (Linn), Myrtaceae, were collected in Sèmè to the south of Benin in 2013. They were identified and certified at the National Herbarium of the University of Abomey-Calavi. In the laboratory, they are spread on the bench away from the light at 20 ° C. Essential oils are obtained by hydro distillation of the leaves (200 to 250g) for 3 hours using Clevenger-type extractor. The less dense than water species are collected by simple decantation and dried over anhydrous sodium sulfate. The extracted oils were stored at 4 ° C and protected from light in amber vials. Oils yields were calculated using the following formula:

$$\mathbf{Yield}\ (\%) = \frac{\mathbf{weight\ of\ oil}\ (\mathbf{g})}{\mathbf{Mass\ of\ plant\ materiel}\ (\mathbf{g})} \times \mathbf{100}$$

Insects

Strains *Sitotroga cerealella* used for mass rearing for this study come from the reserve WARDA (Bénin). They are reared in the laboratory, at the temperature of 29 ± 2 ° C relative humidity 70 ± 10% and natural photoperiod in glass jars or plastic on paddy rice as a substrate.

Analysis of the volatile constituents

GC/MS: The essential oils were analyzed on a Hewlett- Packard gas chromatograph Model 7890, coupled to a Hewlett-Packad MS model 5875, equipped with a DB5 MS column (30m x 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Helium as carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperature: 250 and 280°C respectively. The MS working in electron impact mode at 70 eV; electronmultiplier: 2500eV; ion source temperature:180°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

GC/FID: The essential oils were analysed on a Hewlett- Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30m x 0.25mm; 0.25µm), programming from 50°C (5min) to 300°C at 5°C/min, 5min hold. Hydrogen was used as carrier gas (1.0 mL/min); injection in split mode (1:60); injector and detector temperature, 280 and 300°C respectively. The essential oil is diluted in hexane: 1/30. The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison Silverstein n of their mass spectra with those of reference substances **[16, 17, 18].**

Test

All tests were performed in the laboratory to a temperature of 29 ± 2 ° C and natural photoperiod a relative humidity of 70 ± 10%.

Anti-oviposition activity of the essential oil of *M. leucadendron* on female *Sitotroga cerealella*

The device used consists of glass jars containing 25g capacity of 1 liter of rice (*Oryza sativa* L.) variety of IR841, cotton mass was suspended in 0.3 g using a wire attached to the inner face of the lid of the jars. Concentrations (0, 0.2, 0.5, 1 and 3 $\mu l.ml^{-1}$) were selected after several preliminary tests of each essential oil dissolved in absolute ethanol were tested. The control (or control) was carried out with pure 96% ethanol. A 50µl volume of each solution thus prepared was taken and applied onto the cotton. Three replicates were performed for each dose. After 24 hours, a couple (1 male and 2 female) of *S. cerealella* from livestock, aged 0 to 24, are deposited on the treated plant material. In total, 5 x 3 x 3 = 45 experimental units are implemented. Three (3) days after the counting of eggs laid (hatched or unhatched) grain and on the wall of the glass jar is made with a stereoscopic microscope.

Repellency

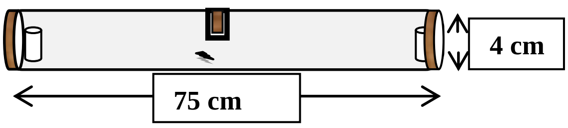

Figure 1: Olfactometer set-up

The repellent effect of the essential oil was tested on *S. cerealella* using an olfactometer (**Figure 1**) was done according to the protocol reported previously **[19]**. At each end of the tube a small jar was placed 10.0 g containing paddy treated with only ethanol and 10.0 g of paddy rice mixed with a solution of essential oil 0.1%, 0.5% and 1%. Ten (10) female *S. cerealella* aged 24h to 48h are released one after the other in the middle of the tube through the hole. Insect behavior is observed and the position was recorded every 15 min. All tests were replicated repellency 5 times for three or four days with new females for each repetition. Both volatile extracts were tested once before the second round. Insects that have a choice are those entered in one of the jars containing rice grains or those who have reached the last twenty-five centimeters of the tube. For those who chose the middle of the tube, more bugs have made a choice with the passage of time. The percentage of insects rejected is calculated using the following formula:

Percentage repellency (%) = (A-B) / (A+B) ×100
A = Average number of insects present in the untreated portion (insects repelled)
B = Average number of insects in the treated (not repelled insects) part

The average percentage of repulsion for the essential oil was calculated and assigned according previous ranking **[20]** 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%)

Statistical Analysis

The results from the observations were statistically processed by the method of analysis of variance (ANOVA) using SAS

(Statistical Analysis System) Version 9.1. Finally, he was made an average structure with the Newman-Keuls test. The results of statistical tests are considered significantly different if the probability of the hypothesis is less than or equal to 5%.

RESULTS

Anti-oviposition activity of *M. leucadendron* essential oil against the female of *S. cerealella*

The following table presents the results of the average egg laying by female *S. cerealella* stored in jars containing grains of paddy rice or parboiled paddy using different doses applied.

Repellent effect of essential oils tested

The percentages of the various doses of repulsion of the essential oil of the leaves of *Melaleuca leucadendron* are shown in **Figure 2** was found that after 15 min of exposure, the different doses of essential oils (0.1%, 0.5% and 1%) were caused 52.22% and 75.68% repellency, with an average rate of 61% repellency.

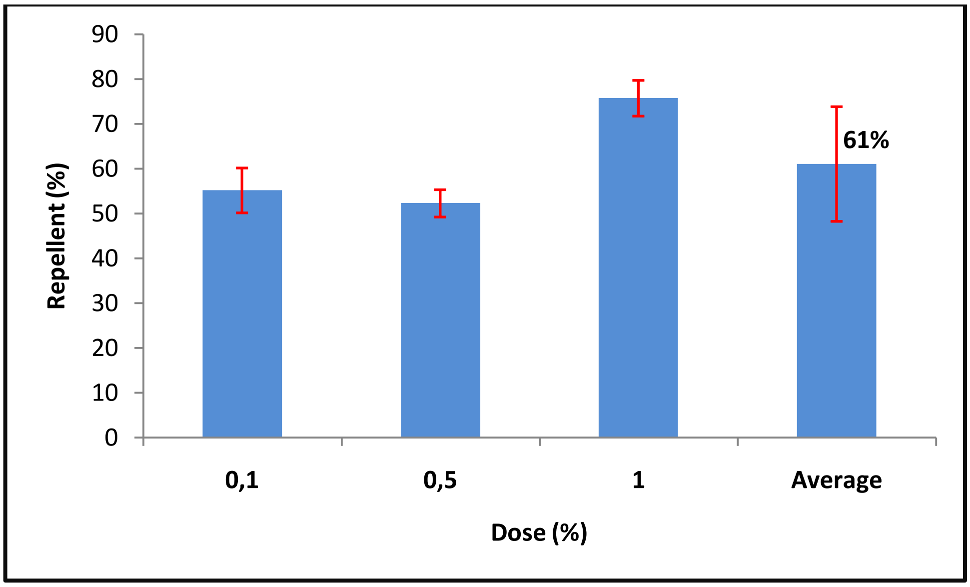

Figure 2: Percentage repellent effect of *Melaleuca leucadendron* essential oils against female *Sitotroga cerealella* adults in a olfactometer

Table 1: Yield and chemical composition of essential oil of *Melaleuca leucadendron* leaves

N°	Name of the compound	RI	(%)
1	α-pinene	931	4.4
2	benzaldehyde	956	0.5
3	β-pinene	975	1.5
4	myrcene	985	0.4
5	α-terpinene	1021	0.3
6	p-cymene	1026	4.3
7	1,8-cineole	1030	15.4
8	δ-terpinene	1054	0.5
9	terpinolene	1085	0.2
10	linalool	1094	0.2
11	terpinen-4-ol	1175	0.6
12	α-terpineol	1189	4.7

13	**citronellol**	**1226**	**t**
14	**(Z)-isoeugenol**	**1409**	**t**
15	**trans-β-caryophyllene**	**1421**	**2.0**
16	**α-humulene**	**1455**	**0.4**
17	**allo-aromadendrene**	**1462**	**0.2**
18	**δ-selinene**	**1496**	**0.8**
19	**ϒ-cadinene**	**1513**	**0.1**
20	**δ-cadinene**	**1521**	**0.2**
21	**(E)-nerolidol**	**1564**	**37.3**
22	**ledol**	**1600**	**19.5**
23	**ϒ-eudesmol**	**1633**	**0.4**
24	**epi-α-muurolol**	**1642**	**0.6**
25	**(2E, 6Z)-farnesol**	**1715**	**0.2**
Total (%)			**94.7%**
Essential oil yields (%)			**1.06%**
Monoterpenic hydrocarbons			**11.6%**
Oxygenated monoterpenes			**20.9%**
Sesquiterpenic hydrocarbons			**3.7%**
Oxygenated sesquiterpenes			**58%**
Others			**0.5%**
t (traces) ≤ 0.1% ; RI = Retention Index			

Table 2: Average egg laying by *Sitotroga cerealella* as essential oil doses and forms of conservation of rice

Doses µl.ml^{-1}	**Mean of egg laying (±SD)**		
	Paddy rice	**Milled rice**	**Parboiled rice**
0	**1.73±0.04a**	**1.67±0.06a**	**1.64±0.03a**
0.2	**0.00±0.00b**	**0.26±0.14b**	**0.46± 0.08b**
0.5	**0.30±0.17b**	**0.33± 0.20b**	**0.00±0.00c**
1	**0.36±0.05b**	**0.10±0.10b**	**0.10±0.10c**
3	**0.10±0.10b**	**0.10±0.10b**	**0.00±0.00c**
Probability	**<0.001*****	**<0.001*****	**<0.001*****
CV (%)	**23.08**	**31.35**	**17.13**

NOTE: 0: ethanol treatment corrected with the control without treatment * = very highly significant difference (0.1%); The averages followed by the same letter were not significantly different at the beginning of 5% (Newman and Keuls test)**

DISCUSSION

The leaf oil of *M. leucadendron* derived by steam distillation is green in color with a camphor odor and is medicinally farming such as antiseptic, stomachic, stimulant, analgesic, antirhematic, expectotant, and for treatment of intestinal worms **[21, 15]**. The yield of essential oil from the leaves of *Melaleuca leucadendron* is 1.06%. These results are similar to those obtained by **[22]** in Benin. Twenty-five (25) compounds have been identified in the essential oil of the leaves, which represents 94.7% of the oil. This volatile extract is rich in 1,8-cineole (15.4%), (E) -nerolidol (37.3%) and ledol (19.5%) (**Table 1**). Indeed, it is rich in oxygenated monoterpenes (20%) and oxygenated sesquiterpenes (58%). The major constituents of the samples studied in the current work differs truly in proportion to those works published by **[14]** in Egypt and **[23]** in India. It has been reported a high rate respectively 1,8-cineole (64.30%) and the β-eudesmol presence (15.8%) α-eudesmol (11.3%), viridifloral (8.9%) and guaiol (9.0%). The content variability recorded for the essential oil of *M. leucadendron* could be related to the magnitude of the secretory cells in the leaves of each sample, physiology or harvest period. This essential oil contains 1, 8 - cineole, nerolidol, alloromadendrene, viridiflorol and α - terpineol as the major components **[10, 24]**. The evaluation of insecticidal oil essential leaf shows a very significant reduction in the potential for egg laying ($p < 0.001$) after 3 days of exposure to different doses of vapors of some essential oil of the form of rice storage (**table 2**). The anti-oviposition character shown by fumigation of adult females of *S. cerealella*, may be linked to the main volatile compounds extracted reportedly acting alone or in synergy with other minor constituents. Indeed, among insecticides herbal essential oils as fumigants have received much attention from scientific groups. Many essential oils have been reported for their larvicidal and antifeedant activity **[25, 26, 27, 28]** the ability to delay development, adult emergence and fertility **[27, 28, 29]**; and disincentives laying. In this study, the repulsive activities of the oil against *M. leucadendron* the Angoumois grain moth, *Sitotroga cerealella* were studied. The essential oil of *M. leucadendron* showed significant repellent activity against adults of Angoumois grain moth. In effect as the dose increases, the percentage of repulsion increases ranging from 52.22% to 75.68% with an average of 61% (**figure 2**) which gives the repulsive class IV according to the classification of **[20]**. The oil repellent activity of *M. leucadendron* against other insects was also demonstrated **[30]**. **[31]** found that the essential oil from the leaves of *Melaleuca quinquenervia* has a repulsive potential of *T. castaneum*. Plants can therefore provide safer alternatives for synthetic lethal toxic modern chemicals.

CONCLUSION

The essential oil of *Melaleuca leucadendron* is potentially rich in oxygenated compounds. In this study, anti-insect repellent against oviposition and *Sitotroga cerealella* activities were evaluated in the presence of paddy rice, parboiled and milled. Outside the antifungal and antimicrobial activities, volatile extract from the leaves of *M. leucadendron* significantly reduced or inhibited the potential of spawning females of the pest by fumigation to the treatment dose and is an excellent repellent against the Angoumois grain moth.

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