Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(4): 24-27
Received: 10-05-2021
Accepted: 12-06-2021

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Biological properties of essential oils from *Eucalyptus camaldulensis* and *Ocimum gratissimum* against *Sitophilus spp.* isolated from stored traditional yams chips

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Abstract
The present study aims to evaluate the efficacy of essential oils from *Ocimum gratissimum* and *Eucalyptus camaldulensis* leaves against *Sitophilus spp.*, isolated from stored traditional yam ships. Results indicated a very pronounced insecticidal effect by contact for the two essential oils tested on adult of *Sitophilus spp*, at a concentration of 2.5 $\mu l.g^{-1}$, with an exposure time of 24 hours. However, essential oil of *Ocimum gratissimum* appears to be more repellent ($p<5\%$) than those of *Eucalyptus camaldulensis* against the weevil. Based on the insecticidal and repellent effects of these essential oils, they could be incorporated in an integrated approach as alternative to synthetic pesticides, when used in reducing weevil infestation in traditional stored yam ships.

Keywords: essential oils, traditional yam ships, *Sitophilus*, biological activities, Benin

Introduction
According to Shani [1], it is estimated that 35% of crops over the world are contaminated by insect pests. In Benin, among the many food products harvested and often stored, starch products including yams have an important part. Yams are very popular food in West Africa countries. But its consumption in as fresh tubers still have strong constraints because of its seasonality and its perishability. In the northern part of Benin which is known to be one of the major yam growing areas, the natural drying of yam is rapid during the *harmattan* period. Thus, over time, commercial production of yam chips has developed to supply urban markets. Yam chip production technology, which includes operations such as precooking and solar drying of peeled tubers [2, 3], lead to limit post-harvest losses of the yams and thereby increase the availability of yams [4]. However, yam chips are sometimes severely attacked by boring insects which can reduce entire stocks to powder within a few months of storage [5, 6]. Among these pests, insects belonging to Coleoptera are the most abundant in infested stocks of yam chips [6]. To reduce pest infestation, farmers often used chemical synthetic insecticides. Unfortunately, this control method often results in numerous cases of food intoxication followed by the death of entire families, due to the consumption of yam chips contaminated with chemical insecticides [7, 8]. Several studies have also reported that faced with the increasingly intense attack by insect pests of yam chips and having no other appropriate solution, some populations resort to the use of insecticides based on *Endosulfan* which are intended for the management of cotton pests, despite the adverse effects of this product on human health [2]. It is therefore important to develop alternative methods of controlling insect pests that respect human health and the environment. Face with the demand for pest-free products as well as the reducing of the use of chemical pesticide, several studies have investigated the biorational pest control methods, and have shown that plant extracts, such as essential oils have insecticidal and repellent properties against pests [9, 10, 11, 12]. Thus, the present study aims to evaluate the effect of essential oils extracted from the fresh leaves of *Ocimum gratissimum* and *Eucalyptus camaldulensis* against *Sitophilus spp*. isolated from stored traditional yam chips in Benin.

Materials and methods

Collection of plant leaves

Fresh leaves of *Ocimum gratissimum* and *Eucalyptus camaldulensis* used for essential oil (EO) extraction were collected at Abomey-calavi (south Benin) and identified at the Benin national herbarium.

Essential oil extraction

Plant collected were stored in the laboratory between 18 °C and 20 °C in the shade of the sunlight throughout the period of extraction. Essential oils were extracted from leaves by hydrodistillation using a Clevenger-type apparatus. The oils recovered were dried over anhydrous sodium sulfate and stored at 4 °C [13].

Insects and rearing conditions

Insects were directly collected from infested yam chips samples. They were reared in laboratory at 25 ± 1 °C, with a photoperiod of 12 h/12 h (dark/light). The adults of *Sitophilus* obtained were put in separate insect cages.

Contact toxicity tests

Bioassays were performed using the method described by Adjalian *et al*. [14] and Adjou *et al*. [12]. The temperature of the test medium ranged from 25 to 31 °C and relative humidity was 80%. Four concentrations (2.5, 5, 10 and 15µl of EO/g of chips) of each EO were tested. Yam chips were treated with the essential oils and after 24 h, five adults of males and females of *Sitophilus* were deposited on the treated material. Overall, 4 doses x 3 repeats x 2 EO= 24 experimental units (containers) were implemented. Adult mortality was monitored each hour during the exposition in other to determine the exposure time necessary for 100% of mortality rate. Then the insects were separated from the treated yam chips. The emergence of new insects was then searched at intervals of 24 h in the experimental units to the 50th day after infestation.

Repellency tests

Repellency tests were performed as described by Zhang *et al*. [15]. Filter paper discs of 9 cm in diameter were cut into two equal parts of 31.8 cm^2 of surface. Four (04) concentrations of each EO (0.07, 0.14, 0.35, 0.7, 1.4 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 ten (10) of non-sexed adult insects. 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)] x 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%).

Statistical analysis

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

Results and Discussion

Essential oils from *Eucalyptus camaldulensis* and *Ocimum gratissimum* leaves were yellow in colour. Results obtained from the determination of exposure time corresponding to 100% of mortality rate of adult of *Sitophilus spp.* are present in Table 1. These results indicated a very pronounced insecticidal effect by contact for the two essential oils tested on adult of *Sitophilus spp.* Indeed, with a dose of essential oil of 2.5 $\mu l.g^{-1}$, a mortality of 100% of adult of *Sitophilus* is obtained after an exposure time of 24 hours, for all essential oils tested. However, with a dose of 5 $\mu l.g^{-1}$ of essential oil of *Eucalyptus camaldulensis*, the mortality rate of 100% is obtained after 12 hours of exposure, and after 24 hours of exposure for the essential oil of *Ocimum gratissimum*. Then the essential oil of *Eucalyptus camaldulensis* was found to be more active by contact than the essential oil of *Ocimum gratissimum* on *Sitophilus spp*. Table 2 presented the results of repellency tests of the essential oils tested against *Sitophilus spp*. These results indicated that the essential oils tested have also significant repellent activity ($p< 5\%$) against the adult of *Sitophilus*, depending on the essential oil concentrations used. Indeed, the dose of 0.14µl/cm^2 of the essential oil of *Ocimum gratissimum* leads to a total repellent (100%) of the adult of *Sitophilus* in contrary to the essential oil of *Eucalyptus camaldulensis* where the repellency rate is still 60%. The essential oil of *Ocimum gratissimum* therefore showed a very pronounced repellent activity compared to the essential oil of *Eucalyptus camaldulensis*. The high reproduction rate associated with the possibility of larvae and adults to damage food in postharvest, lead to insects belongs to *Sitophilus* species to be one of the major pests of stored products. The results of the present study indicated that the essential oils of *Ocimum gratissimum* and *Eucalyptus camaldulensis* have insecticidal and repellent activities and could therefore be used in replacement of chemical synthetic insecticides, which are not without consequences on the environment and the consumers of the preserved products. Indeed, it is generally accepted that pesticides play an important role in agricultural development because they can reduce the losses of agricultural products and improve the affordable yield and quality of food [16, 17, 18]. Then, according to Hayes *et al*. [19], three billion kilograms of pesticides are used worldwide every year. But, all of this quantity of pesticides are not effectively to control insect pests on target plants. The large amounts of remaining pesticides penetrate or reach non-target plants and environmental media. As a consequence, pesticide contamination has polluted the environment and caused negative impacts on human health [20, 21]. For this reason, there is a scope for new methods of protection or producing safe foods that have a natural or green image. One such possibility is the use of essential oils that have been known since antiquity to possess biological activity, such as larvicidal, repellents, and insecticidal effects [22].

Results from present research underlined the efficacy of essential oils of *Ocimum gratissimum* and *Eucalyptus camaldulensis* as control agent against *Sitophilus*. Other research has also reported the pronounced insecticidal effect by contact of essential oil and powders of Eucalyptus leaves on *Sitophilus zeamais* and *Tribolium castaneum* [23]. The insecticidal or repellent properties of essential oils are related to their chemical compositions. The constituents of essential oils are primarily lipophilic compounds that act as a deterrent to feeding and spawning a wide variety of pests. Some of them 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 [24].

Table 1: Exposure time (hours) corresponding for 100% of mortality

	Concentrations (µL of EO/g of chips)			
	2.5	**5**	**10**	**15**
Eucalyptus camaldulensis	24h^{a}	12h^{a}	6h^{a}	3h^{a}
Ocimum gratissimum	24h^{a}	24h^{b}	12h^{b}	6h^{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

Table 2: Results of repellency tests

Essential oils	Concentrations (µL of EO/cm^2)					Average percentage of repulsion	Repellence class	Property
	0.07	**0.14**	**0.35**	**0.7**	**1.4**			
Eucalyptus camaldulensis	25^{a}	60^{a}	100^{a}	100^{a}	100^{a}	77	class IV	Medium repellency
Ocimum gratissimum	65^{b}	100^{b}	100^{a}	100^{a}	100^{a}	93	class 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

Conclusion

There is necessary to control pesticide contamination and its negative influence on environmental and other non-target organisms by promote the use of biopesticides. Then the present research underlined the high insecticidal and repellent properties of essential oils of *Eucalyptus camaldulensis* and *Ocimum gratissimum* against *Sitophilus spp*. isolated from traditional yams chips in Benin. Based on the insecticidal and repellent effects of these essential oils, developing biocides from them would be one solution to the many problems posed by the use of synthetic insecticides. However, further investigations are necessary to identify the conditions that maximize their insecticidal and repellent activities without detrimental effects on the organoleptic and marketable properties of the traditional yam ships.

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