

Insecticidal and repellent activities of *Cananga odorata* leaf essential oil against *Sitotroga cerealella*, a post-harvest pest of rice

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Rice in storage is increasingly attacked by *Sitotroga cerealella*, a highly destructive and economically important pest. The use of plant extracts, especially essential oils, is a natural alternative to synthetic insecticides in crop pest control. This study determined the chemical composition of the essential oil of *Cananga odorata* leaves and evaluated its biological properties, the insecticidal and insect repellent effect of the oil against *Sitotroga cerealella*. The essential oil was obtained by hydro-distillation, and analysed by gas chromatography-mass spectrometry (GC-MS). Fumigation tests were carried out to evaluate the ovicidal, larvicidal, insecticidal and anti-oviposition properties of the oil on the insect and its insect repellent effect was determined using an olfactometer. The analysis showed that the oil contains 32 main compounds, the major ones being α -pinene (7.0 %), lavandulyl acetate (7.2 %), caryophyllene (30.3 %), β -humulene (13.4 %) and germacrene D (9.0 %). *C. odorata* essential oil showed high adulticidal toxicity with values CL_{50} of 0.094 μ l/mL/L air and CL_{90} of 0.46 μ l/mL. This oil has ovicidal and larvicidal or egg development retardant properties. It also caused strong oviposition inhibition activity with an insect repellency rate of 75.7 %. Its insecticidal effects preserved grain weight, with weight losses of no more than 0.38 % and a germination rate of paddy rice seeds of over 80 %. The essential oil from the leaves of the *C. odorata* can be used as an alternative to modern toxic synthetic chemicals, for protection against *Sitotroga cerealella*.

Keywords: *C. odorata*, essential oil, *S. cerealella*, fumigation, insecticidal, repellence, germination, rice

INTRODUCTION

Food losses during storage due to pest infestation are a major problem in our societies, both in developed and developing countries, causing significant financial losses (Gustavson et al., 2021). Stored cereals are, in fact, a food source for many insects, mites and fungi that degrade product quality and can be the source of 9 to 20% of net losses (Belouaer, 2020). Insect pests play a critical role in a country's food insecurity, especially in developing countries where losses of agricultural

products due to insects can reach more than 30%. Rice being one of the world's staple foods, it is infested by a wide range of storage pests including *Sitotroga cerealella*, *Sitophilus oryzae*, *S. granarius*, *Ryzopertha dominica*, as well as the *Scirpophaga incertulas* and the *Scirpophaga innotata* (Ashamo and Akinawonu, 2012). *Sitotroga cerealella* is quasi-cosmopolitan today, and includes almost the whole Europe, as well as areas as distant as Australia, Benin, Brazil, China, Indonesia, Japan, Samoa Islands and the United States. Under conditions of heavy infestation, stored produce can suffer losses of 100%. Attacks by the *S. cerealella* lead to a reduction in the weight of products, a reduction in the germination capacity of seeds and a loss of nutritional and commercial value. Currently, stored product insect control relies primarily on the use of synthetic insecticides and fumigants. There is therefore considerable interest in the development of natural products that are relatively less damaging to mammalian health and the environment than existing conventional pesticides, as important alternatives to non-selective synthetic pesticides for controlling pests medically and economically. The use of herbal insecticides has played an important role in the traditional method of stockpile pest control in Africa and Asia (Rajula et al., 2020). More specifically, essential oils also have interesting properties to replace synthetic insecticides (Huang et al., 2020; Oliveira et al., 2017). The growing interest in essential oils as an alternative to synthetic pesticides stems from their characteristics (Compolo et al., 2018). Traditionally, the leaves of *C. odorata* are used for their antimicrobial, anti-biofilm, antioxidant, antivectorial, insecticidal, insect repellent, anti-inflammatory, sedative, relaxing and harmonizing effects on mood and cognitive performance, spermatotoxic, antihyperglycemic and antidiabetic (Tan et al., 2015). The present study aims to evaluate under laboratory conditions some biological activities of the *Cananga odorata* leaf essential oil on the *Sitotroga cerealella*, an important pest of rice in storage.

MATERIAL AND METHODS

Plant material and distillation of volatile constituents

The *Cananga odorata* leaves commonly called ylang-ylang were collected on the campus of Abomey-Calavi, in southern Benin, Atlantic department. They were identified and certified at the National Herbarium of the University of Abomey-Calavi whose filing number is YH 738/HNB, and stored in the laboratory between 18 and 20°C away from direct sunlight throughout the extraction period. The essential oils were extracted by hydro-distillation of the leaves (450g) for 4 hours using a Clevenger-type extractor according to the method of the British Pharmacopoeia. The essential oils were then dried over anhydrous sodium sulphate and analysed by GC/MS.

The IR841 rice variety selected at the International Rice Research Institute (IRRI) and introduced to Benin in the 1970s is widely cultivated throughout the country. It is a variety suitable for both rainfed lowland cultivation and irrigated cultivation and well appreciated by producers and consumers for the fragrant aroma of its grain and for its good production yield. The IR841 rice grains used in our laboratory tests come from a rice field without the addition of chemical fertilizers or synthetic pesticides.

Volatile Constituents Analysis

GC/MS

The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 7890, coupled with a Hewlett-Packard MS model 5875, equipped with a DB5 MS column (30 m x 0.25 mm; 0.25 µm), 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; electron multiplier: 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 analyzed on a Hewlett-Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30 m x 0.25 mm; 0.25 μ m), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min 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 of their mass spectra with those of reference substances (Rösch et al., 1999; Adams, 1989; Swigar and Silverstein, 1981).

Animal material

Insect breeding

The strains of *Sitotroga cerealella* used in mass rearing for this study come from the WARDA reserve (Benin). At the Plant Production Laboratory of the Faculty of Agronomic Sciences/University of Abomey-Calavi (Benin), they were reared under optimal conditions for reproduction and obtaining new generations ($\square\square = 29 \pm 2^\circ\text{C}$, HR= $70 \pm 10\%$). Adults from 0 to 24 hours of age from mass rearing are used for the various tests. In addition, one-day-old *S. cerealella* eggs were also used in bioassays.

Obtaining *Sitotroga cerealella* eggs

The eggs of *S. cerealella* are obtained from a device whose main element is two black cardboard boxes with a triangular “V” shape (Ellington, 1930). This device allowed the female *S. cerealella* to insert her abdomen to deposit the eggs between the slits of the two contiguous triangles. The eggs are collected (counted progressively) with a fine spit so as not to crush them and placed on the rice grains.

Biological tests

The tests were all carried out in laboratory at a temperature of $29 \pm 2^\circ\text{C}$, and a relative humidity of $70 \pm 10\%$.

Essential oil fumigation toxicity test on adult *Sitotroga cerealella*

The method of Aiboud (2012) used for this test consisted in studying the fumigating effect of the essential oil of *Cananga odorata* on the mortality and emergence of young insects of *S. cerealella*. The device consists of glass jars with a volume of 1 litre containing 50 g of paddy rice (*Oryza sativa*) of the variety IR841, cotton with a mass of 0.3 g was suspended using a wire attached to the internal face of the lid of the jars. After cotton treatments, in each jar, 10 adult males and 20 adult females of *Sitotroga cerealella* aged between 0 and 24 hours, all hermetically sealed. The mortality of populations of *S. cerealella* exposed to the insecticidal activity by fumigation of the treatment is observed for 72 hours of exposure time for the five doses applied (0, 0.2, 0.5, 1, 5 $\mu\text{L/mL}$). The set is arranged in a complete random block device by application mode with three repetitions. Overall, $5 \times 3 \times 1 = 15$ experimental units are set up. The number of dead individuals was counted after exposure. If no wing or leg movement is observed, the insect is considered dead. There is, in fact, in any treated population a natural mortality which is added to the mortality caused by this toxicant, the mortality rates have been corrected by Abbott's formula. This monitoring of mortality is continued until the death of all the insects. Then all the insects were separated from the grains. The experimental units were then observed at regular time intervals (24 h) for the emergence rate of young insects recorded and counted up to the 50th day after infestation.

Anti-oviposition activity of essential oil of *C. odorata* on female *Sitotroga cerealella*

The device used consists of glass jars of 1 litre capacity containing paddy rice (*Oryza sativa*) variety of IR841, the 0.3 g cotton mass was suspended using a wire fixed to the internal face of the lid of the jars with the concentrations of (0; 0.2; 0.5; 1 and 3 $\mu\text{L}/\text{mL}$). The control (or control) was carried out with 96% pure ethanol. Three replicates were performed for each dose. After 24 hours, 1 male and 2 females of *S. cerealella* O. from the farm, aged 0 to 24 hours, is placed on the plant material. In total, $5 \times 3 \times 1 = 15$ experimental units are implemented for the test. Three days after the counting of the eggs laid (hatched or not) on the grains and on the wall of the glass jar is carried out using a stereoscopic microscope.

Effect of treatments on egg development

The method of Aiboud (2012) used for this test consisted in studying the fumigant effect of essential oil on the evolution of *S. cerealella* eggs. 10 *S. cerealella* eggs aged between 0 and 24 hours were deposited on 50 g of paddy rice. Each of the doses (0, 0.2, 0.5, 1 and 3 $\mu\text{L}/\text{mL}$) applied to cotton and then the behaviour of the eggs was observed after 72 h. The rice grains removed from the pots were placed in a Petri dish so that unhatched eggs could be easily counted under a binocular magnifying glass at 40x magnification and then reintroduced into their respective pots and counted as the larvae emerged from the treated grains up to day 45 after treatment. The following variables were measured: egg hatching rate after 72h of exposure for each dose of essential oil; emergence rate of young *Sitotroga cerealella*; calculation of the viability rate of the larvae; average development time of the insect. The egg hatching rate after 72 hours of exposure for each dose of essential oil:

The emergence rate of *Sitotroga cerealella* up to the 45th day after treatment:

The viability rate of *Sitotroga cerealella* larvae:

Repellence tests

The repellent action of plant was tested on *S. cerealella* using an olfactometer (Figure 1) described by Boeke et al. (2002).

The repellent action of the plants was tested in an olfactometer (Figure 1), consisting of a 75 cm glass tube of 4 cm in diameter, with a 29 mm hole in the middle. At each end of the tube, a small jar was placed containing either 10.0 g of paddy rice treated only with ethanol, and on the other side the rice was treated with an essential oil solution diluted with ethanol at the dose of 0.1%, 0.5% and 1%. Ten *S. cerealella* females aged 24-48 hours were released one after the other through the hole in the middle of the tube. The females were chosen for this test because of their need to lay their eggs on the rice. The behaviour of the insects was observed and their position was recorded every 15 minutes. All repulsion tests were repeated 5 times over three or four days with new females for each repetition. The insects that made a choice were those that entered one of the pots containing the rice grains or those that reached the last 25 cm of the tube. For those that chose the middle of the tube, the number of insects that made a choice increases as time passes. The percentage of insects repelled is calculated according to the following formula:

A = Average number of insects present in the untreated part (insects repelled)

B = Average number of insects present in the treated part (insects not repelled)

The average repellence rate of the essential oil was calculated and assigned, according to the previous classification (Mc Donald et al. 1970), to one of the repellence classes ranging from 0 to V: class 0 (repellence rate (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%).

Estimation of grain losses after treatment

To assess the damage caused by the insect *S. cerealella* on rice seeds, the grains of the substrate resulting from the treatments were collected, counted and weighed. The rate of grain dry matter weight loss was determined according to MCP (Method of Counting and Weighing) and MSVW (Method of Standard Volumetric Weight). Two damage evaluation criteria are commonly used: the grain attack rate (A%) and the weight loss rate (B%) calculated respectively by the following formulas:

where N_a is number of attacked grains, N_s is number of healthy grains, P_a is weight of damaged grains, and P_s is weight of healthy grains.

Effect of treatment doses on the preservation of the germination capacity of paddy rice seeds

At the end of the tests, one hundred seeds from each treatment based on the essential oil of *Cananga odorata* including the controls, are mixed and divided into two batches of 100 seeds, were placed in Petri dishes the bottom of which contains a hydrophilic paper soaked in water. This device made it possible to subject the seeds to germination and to evaluate their germination power. Thus, all the germinated seeds were counted and the germination rates in the treatments were determined. Germination tests were carried out in the laboratory at a temperature of $29 \pm 2^\circ\text{C}$, relative humidity of $70 \pm 10\%$ and lasted 7 to 11 days

Statistical analysis

The means of the various parameters studied (with the exception of those recorded at the level of the olfactometer) were subjected to analysis of variance (ANOVA) using the statistical analysis software SAS (Version 9.1). The Student-Newman-Keuls test was used to separate the means. The results of the statistical tests are considered to be significantly different when the probability of the null hypothesis is less than or equal to 5%.

To more accurately assess the efficacy of oil toxicity by fumigation, we calculated the CL50 and CL90. They were deduced from the regression plot by Finney's method. For this, the corrected mortality rates are converted into probits.

RESULTS

Yield and chemical composition of *Cananga odorata* essential oil

The composition and yield of the volatile compounds is listed in table 1. The essential oil yield of *Cananga odorata* leaves, harvested at Abomey-Calavi Campus, Atlantique department, Benin is 0.41%. The main compounds of the essential oil are α -pinene (7.01%), lavandulyl acetate (7.19%), caryophyllene (30.3%), α -humulene (13.4%) and germacrene D (8.99%). Monoterpenoids represent 11 of the 32 compounds, which corresponds to 23.9% of the whole oil, while 17 of the 32 constituents are sesquiterpenoids (72.1% of the crude essential oil).

The essential oil from the leaves of *C. odorata* has fumigant toxicity against adults of *Sitotroga cerealella* O. with 100% mortality recorded at the lowest dose of 0.2 $\mu\text{L}/\text{mL}$ with a complete absence of insect emergence from of the dose of 0.5 $\mu\text{L}/\text{mL}$ compared to the control group with 404 young *Sitotroga cerealella* counted (Table 2).

To further assess the toxicity efficacy of this oil, we calculated its CL50 and CL90. It is clear from this table 3 that the essential oil of *Cananga odorata* seems to have significant efficacy. These results are confirmed by the CL50 and CL90 values obtained from a function of the regression line and which correspond to 0.094 $\mu\text{L}/\text{mL}$ and 0.46 $\mu\text{L}/\text{mL}$ (Table 3). Indeed, the CL50 is lower than the first dose (0.2 $\mu\text{L}/\text{mL}$), so that the CL90 is between the first and the second dose.

Evaluation of the anti-oviposition activity of the essential oil of *C. odorata* on the female of *S. cerealella*

The table 4 presents the effects of fumigation with *Cananga odorata* essential oil on the oviposition capacity of *S. cerealella* females on its preferred host, paddy rice.

A drastic decrease of 98.7 % in the oviposition rate of females was noted for the fumigation treatments for the lowest dose 0.2 $\mu\text{L}/\text{mL}$ compared to the control which recorded an average oviposition rate of 53.3 eggs. Egg-laying activity was totally inhibited in females from a dose of 0.5 $\mu\text{L}/\text{mL}$. There is a highly significant difference between the effects of the treatment doses on lay and are statistically different from the control. We therefore observed a significant reduction in the number of eggs laid by the females during and/or after their exposure to the various essential oils compared to the control at the various doses. *Cananga odorata* essential oil remains active on females, inhibiting their egg-laying by more than 90%.

Ovicidal and larvicidal properties of *C. odorata* essential oil

Table 5 presents the results of the effect of fumigation treatments according to dose on egg development on paddy rice.

Egg hatching and adult emergence rates were low to zero as the application rate of *Cananga odorata* leaf oil increased. More than 80% of the eggs failed to hatch and more than 70% of the emergences were inhibited by fumigation with *Cananga odorata* essential oil. The fumigation delayed the insect's development cycle by 10 to 15 days at the low doses applied or prevented the development of the insect's cycle at the high doses of essential oil application.

Repellence effect of the essential oil tested

The repellence rates for the different doses of *Cananga odorata* leaf essential oil are summarized in (Table 6).

The different doses of essential oils (0.1%, 0.5% and 1.0%) caused a rate of $59.5\% \pm 9.46\%$ repellence; $75.5\% \pm 8.74\%$; $92\% \pm 10.9\%$. This clearly shows that the repellence rate increases with dose.

Effects of essential oil doses on paddy rice grains weight preservation

Table 7 shows the rates of attack and weight loss caused by the *S. cerealella* insect populations on rice seeds from essential oil treatments. At the end of the biological tests, the attack rates and the weight losses recorded at the level of the treatments are at most, respectively 0.45% and 0.3% compared to the control where the insects caused up to 25% of the attacks on the grains for an estimated 20% weight loss. No losses were recorded for the highest doses of this fumigation treatment

Effect of essential oil on preservation of germination power of paddy rice seeds

Figure 2 illustrates the percentages of germinated paddy rice grains according to the different doses of essential oil. For the grains resulting from the fumigation treatments, it is observed that the essential oil of *Cananga odorata* preserved the germination power of the infested paddy rice seeds. The germination rate recorded at the lowest dose was greater than 80% and reached 99% at the highest dose of 3 $\mu\text{L}/\text{mL}$. In all cases, the germination rates recorded with the treatments are much higher than the germination rates recorded with the control, which was 41.7 % and the germination rates of 80% recommended by the National Agricultural Research Institute of Benin.

DISCUSSION

The essential oil yield of *Cananga odorata* leaves ($R=0.41\% \pm 0.03\%$) harvested at Abomey Calavi Campus, Atlantique department, Benin is higher than those obtained by Cheng et al. (2012) ($R=0.12\%$) in China, and in Indonesia ($R=0.04\%$). The chromatographic analysis of the essential oil extracted from the leaves of *C. odorata* contains 32 chemical compounds representing 100% of the weight of the essential oil.

Regarding the adulticidal effects of the essential oil tested against *Sitotroga cerealella*, a highly significant difference was noted between treatments. The slight reduction in emergence rates at low concentrations would result from the manifestation of the ovicidal or larvicidal effect of the active volatile extract, which would have destroyed egg or larval development. However, the low emergences observed in the tests could well be from eggs probably laid inside the walls of the pots. Our results corroborate the work of Thiam et al, (2021) and Demeter et al, (2021). The essential oil of *C. odorata* has a fumigant toxicity against *Sitophilus zeamais* with an LC50 value ($LC50 = 14.8$ mg/L) and overall shows a potential for contact and fumigant toxicity against cereal storage insects. The presence of linalool in the essential oil, which is a competitive inhibitor of acetylcholinesterase, could be the active component that explains the insecticidal activity of the essential oil of *C. odorata*. On the other hand, the insecticidal activity of ylang-ylang flower essential oil was also studied by Phasomkusolsil and Soonwera (2011) against the three types of mosquito species with LC50 values ($LC50 = 9.77\%$, 8.82% and 4.99%) respectively.

The reduction in oviposition observed in *Sitotroga cerealella* females in the presence of *Cananga odorata* essential oil would probably also be related to the early death of some females or the morbidity observed in some. Furthermore, the evaluation of the anti-oviposition effect of *Cananga odorata* essential oil informs us that the inhibition of oviposition of *S. cerealella* females could be related to a disturbance of oviposition due to the volatile effect of the essential oil.

Regarding the ovicidal and larvicidal effect of the essential oil of *Cananga odorata* leaves, there is a significant difference between the rates of application of the oil for the parameters measured. The essential oil of *C. odorata* significantly reduced the egg hatch rate to 100% from the lowest applied dose of $0.2 \mu\text{L/mL}$. It should be noted that from the high doses of $1 \mu\text{L/mL}$, $3 \mu\text{L/mL}$, we found that the oil completely inhibited the hatching of eggs and the emergence of young adults of *Sitotroga cerealella* and led to a lengthening of the development cycle of the insect by about nine (9) to fifteen (15) days more than the control for the low doses of application of the essential oil. Such a reduction in emergence rates would result from the manifestation of the ovicidal or larvicidal effect of the volatile extract which would have annihilated or prevented larval development.

According to the study of the repellent properties of the essential oil of *C. odorata*, the average repellency obtained is $75.7\% \pm 3.87\%$ and belongs to the repellency class IV according to the classifications of Mc Donald et al., (1970). These results corroborate those of Kaballero-Gallardo et al., (2011) and Olivero-erbel et al., (2013), which showed that the essential oil of *C. odorata* leaves has repellent activity against *Tribolium castaneum*, a red flour beetle that is known to be the pest associated with stored products, thus protecting stored products from insect damage. Most of the studies listed above have indeed shown that the essential oils of *C. odorata* have demonstrated good repellent properties against different insect species, in this case against post-harvest insect pests. This suggests that it can be considered as an excellent candidate for natural repellents.

After the bioassays were carried out, the impact of the treatments on rice grain weight preservation and paddy germination was studied. Indeed at the end of the bioassays, the attack rates and weight losses recorded prove that the activity of the essential oil of *C. odorata* leaves could be due to ovicidal, larvicidal or insecticidal activities, whose main compounds of the volatile extract, would have acted alone or in synergy with other minor constituents in the preservation of rice grain weight throughout the experiment and corroborate the results of other authors such as Ashamo et

al, (2012), Cissokho et al., (2015), Compolo et al., (2018), Thiam et al., (2021) and Demeter et al., (2021).

From the statistical analysis of the germination data, there is a highly significant difference between germination rates as a function of dose. The germination tests showed that the plant material tested against *S. cerealella* had no visible negative effects on the germination capacity of the seeds. Indeed, according to Glitho et al., (2008), the presence of residues in treated seeds does not affect their germination capacity. Contrary to the present results, some oils can be phytotoxic (Werrie et al., 2020, Paranagama et al., 2003).

CONCLUSION

The results obtained in this study show that the essential oil from the leaves of *C. odorata* has potential for development as a new natural insecticide/fumigant for the conservation of stored products. In addition, the essential oil of *C. odorata* has also repellent activity against *Sitotroga cerealella*, a major rice pest in Benin. It may therefore be a safer alternative to modern toxic synthetic chemicals. However, for the applicability of the results in the field, technical aspects such as the availability of leaves to be extracted, the availability of extraction equipment, the availability of production and packaging units, and economic aspects such as the cost of extraction and the profitability of the extract must be taken into account. Indeed, with its relatively low yield, we suggest the use of the plant itself or a crushed fresh leaf to allow the application of the results obtained in the present study directly in the field, especially in rural areas. Further research is also needed on, among other things, the encapsulation of the oil for slow release, its mode of action on insects, the formulation of a quality bio-pesticide to improve the efficacy and stability of the product, and the safety of this essential oil for humans.

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