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Efficacy and persistence of essential oil of *Monodora myristica* against *Anopheles gambiae,* the main vector of malaria in sub-Saharan Africa

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Abstract

Background: *Monodora myristica* known as calabash or nutmeg, is a tree of the Annonaceae family whose seeds are mainly used as a spice. The control of malaria is still a challenge partly due to mosquito resistance to conventional insecticides. The present work aimed to evaluate the insecticidal effect of the essential oil of *M. myristica* seeds to enlarge the list of local plants that can be used as insecticides.

Methods: Essential oil was extracted by hydrodistillation using the Clevenger apparatus, then analyzed by gas chromatography to determine the chemical composition. using absolute alcohol, the essential oil was solubilized to obtain the stock solution which was used to prepare concentrations ranging from 10 to 250 ppm. Larvicide and adulticide tests were carried out with *Anopheles gambiae* larvae and females. The persistence of the *M. myristica*'s essential oil was determined by introducing the larvae into the prepared concentrations at regular time intervals and exposing them to the open air for 28 days.

Results: The essential oil of *M. myristica* contained 20 chemical compounds and the major was the α-phellandrene (35.20%). This essential oil-induced total mortality of *A. gambiae* larvae at all the concentrations after 24 h with concentration-dependent and larval age-dependent efficacy. In females, total mortality was recorded at 250 ppm and the LC_{95} was 168.47 ppm. The essential oil of *M. myristica* is still toxic towards *A. gambiae* after 21 days at the concentration of 150 ppm.

Conclusion: These results led us to conclude that the essential oil of *M. myristica* could be a very good larvicide for vector control campaigns.

Keywords: *Monodora myristica*; malaria; insecticide; plant; larvae; adults

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Background

The history of humanity is closely associated with diseases. Man, wherever he may be and whatever his living conditions, has among his main enemies, disease. The Covid-19 pandemic, which has shaken the whole world since 2019, is the most illustrative proof of this. The fight against the disease is, therefore, a priority for all states and governments around the world. Although vector-borne diseases are better controlled by countries outside the tropics, these diseases are real scourges for countries in the intertropical zone of the globe in general, and particularly for the African continent. Of all the vector-borne diseases in the world, malaria is undoubtedly the most important. According to the WHO report [1], about 40% of humanity is exposed to malaria, mainly the populations of poor African countries. Thus, according to the same report, since 2000, there have been nearly 1.5 billion cases of malaria with more than 7.5 million deaths; sub-Saharan Africa alone accounts for nearly 92% of these cases. Malaria, therefore, remains a major public health problem for humanity. The fight against Anopheles mosquitoes, the vectors of malaria, faces many difficulties, notably their resistance to conventional insecticides, the negative impact of these chemical insecticides on the environment, and their high cost. These facts lead to the use of biological insecticides as an alternative. These natural insecticides are derived from extracts and oils of certain local plant organs. Recent studies show that more than 2000 plant species are capable of killing or repelling crop pests and disease vectors. Some studies carried out with plants from the Cameroonian flora of Cameroon, namely *Lippia rugosa* [2], *Eucalyptus saligna* [3], *Capsicum annuum* [4], *Ocimum canum* [5], *Ocimum basilicum* [6], *Callistemon citrinus* [7], *Cucurma longa*, *Afromomum polyanthum* and *Afromomum daniellii* [8], *Curpressus macrocarpa, Lantana camara* and *Psidium littorale* [9] have shown significant insecticidal properties of botanicals. To enlarge the list of plants with insecticidal potential, we were interested in *Monodora myristica*, whose essential oil contains compounds whose insecticidal effect has been noted by previous studies. This plant, better known as calabash or nutmeg, is a member of the Annonaceae family. It is common in the tropical rainforests of West Africa, from Liberia to Angola. In the Central, Southern, and Eastern Regions of Cameroon and in Gabon, this plant is called "Nding". Outside Africa, it is also found in Asia, Central, and South America, and Australia [10] where it has been domesticated and is even cultivated. The nutmeg-scented seeds are mainly used as a spice by people in West Africa. In traditional pharmacopoeia, they are used in the treatment of several diseases: stomach aches, headaches, fevers, diarrhea, and hypertension [10]. The present work aimed to evaluate the insecticidal effect of the essential oil of seeds of this common plant.

Methods

Study sites

The work was carried out respectively in Yaoundé and Dschang for logistic and professional reasons.

The part of the work that was done in Yaoundé was carried out at the Biotechnology Centre of the University of Yaoundé I located in Nkolbisson. This locality is situated around 8 km in the West of Yaoundé, between latitudes 3°51' and 3°53' North and longitudes 11°25' and 11°27' East. Nkolbisson is part of the equatorial domain. Two main air masses sweep through the area: the monsoon from the Atlantic Ocean in the southwest and the harmattan from the warm continental regions in the north. Its climate is the Guinean type with four seasons: two rainy seasons alternating with two dry seasons. The big dry season runs from December to February, the small rainy season from March to June, the small dry season from July to August, and the big rainy season from September to November [11]. Annual rainfall varies from 1500 mm to 2000 mm and the average temperature is 25 ± 2.4°C. in this site, the experiment was designed in the small rainy season

The other part of this work was carried out in Dschang at the Vector-borne Diseases Laboratory of the Biology and Applied Ecology Research Unit (VBD- BAEUR) of the University of Dschang. This locality is located between 9°50' and 10°20' East longitude, 5°10' and 7° North latitude, at an altitude of 1400 m. Its vegetation is of the shrubby savannah type and its climate is of the highland Sudano-Guinean type, with two seasons: a rainy season (from March to October) and a dry season (from November to February). The average annual rainfall is 1872 mm with a relative humidity of between 64.3 and 97%. The average daily temperature is 20°C with a thermal amplitude of 2.2°C [12]. The VBD rearing room is equipped with a laboratory thermometer, a radiator to heat the laboratory, and a humidifier that maintains relative constancy of hygrometry. This device allows us to obtain suitable breeding conditions for the mosquitoes.

Collecting of the plant, conditioning, and extraction of the essential oil

The seeds of *M. myristica* were collected in April 2018 in the forests of Mbalmayo, a locality located in the forest area of Southern Cameroon. The collected seeds are dried in the open air and in the shade. The dried seeds are removed from their shells and then crushed in a mortar. This grind will be used for the extraction of essential oil. This is to extract the essential oil. This extraction was an hydrodistillation by using the Clevenger apparatus. The crushed seeds of *M. myristica* are weighed before being introduced with water into the flask and this mixture is boiled for 5 hours. The essential oil collected by decantation at the end of the distillation and freed from all traces of water by filtration on an anhydrous sodium sulphate column was introduced into a dark bottle and then kept at low temperature (around 4°C). The yield of the extraction was determined using the ratio between the mass of essential oil obtained and the mass of the crushed plant seeds introduced into the distillation flask multiplied by 100.

Analysis of the chemical composition of the essential oil

The analysis of the chemical composition of the essential oil is carried out by gas chromatography and by gas chromatography-mass spectrometry (GC/MS).

Gas chromatography of essential oil

The gas chromatography of the oil is done using a Varian CP-3380 type chromatograph equipped with a flame ionization detector and a capillary column (length 30 m, internal diameter 0.25 mm) with an apolar stationary phase of methyl-silicone type (DB-1, film thickness 0.25 µ). Nitrogen is used as carrier gas with a flow rate of 0.8 ml.min-1. The injector temperature is 220°C while the detector is set at 250°C. The oven is programmed from 50°C to 200°C with a

temperature gradient of 5°C.min-1. The retention indices of the different components were calculated in relation to the retention times of a series of n-alkanes and their relative percentages calculated by electronic integration considering that their response factors are all equal to 1 [13].

Gas chromatography-mass spectrometry coupling of essential oil

The gas chromatography-mass spectrometry coupling of essential oils was carried out using a Hewlett Packard HP 5970 A apparatus, equipped with a nonpolar capillary column (30 m x 0.25 mm) made of fused silica of the HP-1 type (film thickness 0.25 µM) and a quadrupole detector (ionization energy 70 eV). The temperature of the injector is 220 °C and that of the interface zone is 210°C (split mode injection (1/100) of 1 µL of a 10% essential oil solution in dichloromethane). The oven temperature is programmed from 70 °C to 200 °C with a gradient of 10 °C.min-1. The carrier gas is helium with a flow rate of 0.6 mL/min [13].

Identification of constituents of essential oil

The identification of constituents of essential oil is done by comparing their retention index and mass spectrum [13]. Retention indices are calculated from C15 - C18 alkanes [13]. The percentages of chemical compounds identified in the essential oils are calculated from the peak areas obtained by gas chromatography without any correction factors.

Biological tests

These were carried out with a colony of *A. gambiae* maintained in the laboratory for several years (approximately 5 years).

Preparation of essential oil concentrations

The preparation of the concentrations of the essential oil in the laboratory is done according to the WHO protocol [14]. We diluted the essential oil in an organic solvent (pure alcohol). These dilutions were: 10, 30, 50, 100, 150, 200, and 250 ppm by volume. We prepared them from a stock solution obtained by diluting 2000 µL of essential oil in 6000 µL of absolute alcohol. This gave us a 1/4 dilution of the stock solution. Based on this stock solution, we prepared the other dilutions with pure alcohol

Larvicidal tests

Larvicide tests were carried out according to the WHO protocol [14]. They consisted in evaluating the mortality of the larvae induced by different concentrations of the essential oil after 24 hours of exposure. The concentrations chosen for these tests were 10, 30, 50, 100, and 150 ppm. We also prepared a negative control medium (without any trace of essential oil) in which the essential oil solvent (95°pure alcohol) was introduced in the same proportion as in the dilutions. Each medium thus prepared was duplicated 4 times. The tests were performed with young (L1 and L2) and mature (L3 and L4) larvae. We introduced 25 larvae of the same category into each one of the prepared media. We observed the larvae every 5 min for 1 h, then every 20 min for 5 h, and finally after 24 h. Larvae were considered dead if they did not move for 10 seconds after shaking the water. When the percentage of mortality in the controls was less than 5%, the mortality rate of the test larvae was expressed simply as a mortality rate (Mortality rate = Number of dead larvae x 100 / Number of introduced larvae). If this mortality rate for the controls was between 5% and 20% the percentage mortality of the essential oil-treated larvae was corrected using Abbott's formula [15]:

$$CM = (OM - TM) \times 100 / (100 - TM)$$

CM= corrected mortality, OM= observed mortality, TM= Total mortality

In case it was higher than 20%, the test was canceled and repeated.

Adulticidal tests

The concentrations of the essential oil were prepared following the same protocol as mentioned above [14]. For these tests, the concentrations 50, 100, 150, 200, and 250 ppm were used. The first step was to impregnate the net fragments with the different concentrations of *M. myristica* essential oil mentioned above. The impregnation of the net's fragments was done according to the WHO protocol [14]. Each fragment of the net was inserted into the glass Petri dish containing the diluted essential oil solution for 15 min with the help of forceps. The fragment was then taken out and dried horizontally to avoid possible spillage. We also impregnated control fragments in pure alcohol and dried them following the method previously described for each experiment. These fragments were fitted to the two ends of the tube containing the adult females. These were 2 to 5 days old and fasted. Each test series consisted of 6 batches of 25 individuals, of which 4 were exposed to the essential oil and 2 batches were controls. After 1 hour of exposure, the mosquitoes were removed from the test tubes and placed in transparent cups where they were fed with 10% sugar water and observed for 24 hours. Mortality will be assessed using the same protocol as described for the larvicidal tests. We also determined the "knock-down" effect by counting every 5 min the individuals knocked out after 60 min of exposure to the essential oil.

Study of the persistence of the essential oil

The persistence of the substance is generally assessed by determining the degradation time of 50% of active molecules (DT_{50}) of the essential oil (EO) in the sample bottles. However, as we did not have the necessary equipment to quantify the active molecule in the prepared solutions, we proceeded to detect the presence of the essential oil in the solutions. Therefore, we carried out biological tests which also testify to the presence of the active molecule in the medium to evaluate this parameter. This study was carried out only on larvae because we considered that the volatile molecules of the essential oil combined with water would be more stable than those only diluted in pure alcohol and exposed to the air (essential oil used for the impregnation of mosquito netting). Based on the results of the larvicide test, we have retained mature larvae for this part of the study for a reason that is easy to understand. This study was carried out over a period of 30 days. The essential oil solutions were prepared as described above and the larvae were introduced on the same day (D0). Mortality was assessed after 24 h of exposure. These solutions, covered with mosquito net and left in the open, received further waves of larvae on days 1, 5, 10, 14, 17, 21, and 28. Larval mortality is assessed according to the protocol described above.

Statistical analysis

Collected data were entered into Microsoft Excel and analyzed using SPSS for Windows version 22.0. Mortality rates were

compared using the Kruskal-wallis H-test. In the case of the persistence study, the Mann-Whitney U test was used to assess the variation in the efficacy of the essential oil over time, using day 0 data as a reference. The significance level was set at a value of $p < 0.05$. The equations of the regression lines obtained from Henry's simplified table that transforms the mortality percentages into probits were used to determine the LC_{50} and LC_{95} as well as the Tkd_{50} and Tkd_{95}.

Results and discussion

Yield of the extraction

Hydrodistillation of 2500 g of *M*. myristica seeds produced 30 g of light-yellow essential oil at a yield of 1.20%. This yield is higher than that of the leaves of Zingiberacea *Cucurma longa*, *Afromomum polyanthum,* and *Afromomum daniellii* [7] as well as those of *Cupressus macrocarpa*, *Lantana camara* and *Psidium littorale* [8]. This difference would be related, as suggested [16, 17, 18], to the plant species, the plant organ, the extraction method, the pathophysiological state of the plant, the season of harvest, the geographical location, the climatic conditions, and even the time of harvest.

Chemical composition of the essential oil

Chemical composition analysis (Table 1) reveals that the essential oil of *M. myristica* contains 20 chemical compounds divided into 3 functional classes: 9 molecules of the hydrocarbon monoterpene class (HMT), the dominant compound of which is phellandrene (35.20% of the 74.50% of compounds in the class); 6 molecules from the class of oxygenated monoterpenes (OMT), the dominant compound of which is pinocarveol (6.25% of the 14.83% of compounds in the class); 5 compounds from the class of hydrocarbon sesquiterpenes (HST), the dominant compound of which is germacrene-D (1.31% of the 2.88% of 0001). Thus, according to Table 2, the mortality of these larvae was complete after 120 min of exposure for the lowest concentration, 10 ppm. At the highest concentration, 150 ppm, total mortality of the larvae occurred after only 20 min (Table 2). The LC_{50} and LC_{95} were 9.25 ppm and 27.53 ppm respectively. Mortality of mature larvae also varied significantly with essential oil concentration ($H = 32.564$; $p < 0.0001$) and exposure time ($H = 13.289$; $p < 0.0001$). However, total mortality of these larvae at 10 ppm took 180 min of exposure and 40 min at 150 ppm (Table 3). The LC_{50} and LC_{95} were 16.59 ppm and 42.39 ppm respectively. From the above results, it is clear that young larvae are more sensitive than mature larvae. This fact has been reported [5, 7, 8, 18, 19] and could be explained not only by the fragility of the cuticle of young larvae but also by the low body mass of this category of larvae compared to their older counterparts. According to Tsila et al. [20], the cuticle of L1 and L2 larvae is softer and therefore less rich in chitin (a compound that gives the cuticle of arthropods its rigidity). This same suggestion was made [7]. We also found that the essential oil of *M. myristica* seeds was significantly more toxic to *A. gambiae* larvae than that of the leaves of *O. basilicum* [5], *C. longa*, *A. polyanthum* and *A. daniellii* [7], *C. macrocarpa*, *L. camara* [8] whose LC_{50s} were respectively 17.6 ppm, 70.18 ppm, 75.76 ppm and 130.33 ppm for young larvae, 23.50 ppm, 92.49 ppm, 220.24 ppm, 352.47 ppm, 60.44 ppm and 25.08 ppm for mature larvae This high toxicity of the essential oil of *M. myristica* seeds would be linked to the presence of certain molecules whose insecticidal effect had already been observed on other insects. These are mainly myrcene [21-23], α-pinene [24], and β-pinene [25]. In view of the content of these molecules in the essential oil of *M. myristica*, it is believed that these molecules act rather in synergy to amplify this toxic action [7, 26, 27] had already noted the biological importance of the synergy of minority compounds in the essential oils of several plants.

Adulticide tests

The essential oil of *M. myristica* induced mortality in adults of *A. gambiae*. The toxic effect of this essential oil increases with the concentration ($H = 22.73$; $p < 0.0001$). Thus, Table 4 shows that this essential oil does not cause the knock-down effect nor the death of A. gambiae females at the concentration of 50 ppm. The efficacy of the essential oil of *M. myristica* appears beyond the latter to reach its maximum at the concentration of 250 ppm for which we found that all knocked down *A. gambiae* females died. The equations of the regression lines allow us to understand why the Tkd50 values were higher than the Tkd95 values. This could be explained by the fact that some of the knocked down mosquitoes regained some vitality after some time and flew away. We can say that the essential oil of *M. myristica* is less toxic to the adult anopheles than that of *O. canum* for which Akono Ntonga et al. [28] obtained total mortality of adults at the concentration of 200 ppm. However, it is also toxic to *O. basilicum* essential oil for which the same authors recorded a total mortality at 250 ppm. The toxicity of the essential oil of *M. myristica* would be mainly related to its high content of hydrocarbon monoterpenes. We can also attribute this toxic effect to the presence in this oil of phenolic compounds such as linanol and thymol whose toxic action on the adults of certain insects has been demonstrated [28, 29]. However, the low content of phenolic compounds in the essential oil of *M. myristica* (linalool, 3.15% and thymol, 0.32%) could justify the lesser adulticidal effect of this essential oil compared to that of *O. canum*, which is very richly endowed, particularly linalool, which is 53.8% [28].

Study of the persistence of the essential oil

The essential oil of *M. myristica* remained effective against *A. gambiae* larvae during the 28 days of the experiment. Table 5 shows that all concentrations of this essential oil-induced total larval mortality after 24 h of exposure. However, this efficiency decreased relatively significantly with time for the lowest concentrations. Thus, for the 10 ppm and 30 ppm concentrations, we recorded larval mortality of 67.75% and 74.75% respectively on day 14 and two weeks later (day 28), 47.50% and 53.00% (Table 5). On the other hand, this efficacy remained almost unchanged for the highest concentrations of 150 ppm with induced larval mortality of 100% on day 10 and 93.50compounds in the class) This chemical composition is very similar to that of the same plant obtained in the Central African Republic [10].

Larvicide activity

Mortality of young larvae varied significantly with the concentration of *M. myristica* essential oil ($H = 24.639$; $p < 0.0001$) and with exposure time ($H = 10.418$; $p < 0$.
% on day 28. The persistence of the essential oil of *M. myristica* at the concentration of 150 ppm is higher than that of the essential oil of *O. canum* which is 18 days at the most at the concentration of 200 ppm [30] and more largely superior to that of the hydroalcoholic extract of the roots of *Syzygium guineense* and the fruits of *Zanthoxylum heitzii* which is 8 days [31].

Table 1. chemical composition of the essential oil of *M. myristica*

Compounds	**IK**	**Percentage**
Monoterpenes		**89.33**
Hydrocarbon monoterpenes		**74.50**
α-Thujene	933	1.75
α-Pinene	937	6.37
Sabinene	950	0.12
β-pinene	976	0.30
Myrcene	989	4.38
α-Phellandrene	1004	35.20
p-Cymene	1016	21.82
β-Phellandrene	1026	0.61
Limonene	1029	3.95
Oxygenated monoterpenes		**14.83**
1,8-Cineole	1027	0.20
Linalol	1096	3.15
Pinocarveol	1138	6.25
α-Terpinéol	1187	0.85
Thymol	1295	0.32
Carvacrol	1300	4.05
Sesquiterpenes		
Hydrocarbon sesquiterpenes		**2.88**
β-Caryophyllene	1432	0.78
γ-Muurolene	1477	0.32
Germacrene D	1490	1.31
γ-Cadinene	1509	0.22
δ-Cadinene	1525	0.25
Others		**7.79**

IK: retention index by order of dilution on DB-1 column

Table 2. Mortality (%) of young *A. gambiae* s.s. larvae, equation of the regression line and the LC_{50} and LC_{95} in the presence of *M. myristica* essential oil

Concentrations (ppm)	Time						LC
	20 min	**40 min**	**60 min**	**80 min**	**100 min**	**120 min**	
Control	0	0	0	0	0	0	
10	0	13.50 ± 2.32	25.25 ± 3.15	62.25 ± 2.28	81.75 ± 1.78	100	LC_{50} **=** 9.25 ppm
30	26.75 ± 2.41	45.25 ± 2.54	73.75 ± 1.86	94.75 ± 0.58	100		
50	40.50 ± 2.17	75.00 ± 1.65	96.25 ± 0.54	100			
100	79.75 ± 1.65	100					LC_{95} = 27.53 ppm
150	100						
Regression line equation	y = 3.462x + 1.655						

LC50 or LC95, lethal concentration for 50% or 95% of the individuals

Table 3. Mortality (%) of mature *A. gambiae* s.s. larvae, equation of the regression line and the LC_{50} and LC_{95} in the presence of *M. myristica* essential oil

Concentrations (ppm)	T**ime**						LC
	20 min	**40 min**	**60 min**	**100 min**	**140 min**	**180 min**	
Control	0	0	0	0	0	0	
10	0	0	10.00 ± 2.25	27.75 ± 2.50	66.00 ± 2.75	100	LC_{50} **=** 16.59 ppm
30	16.00 ± 2.50	33.00 ± 2.25	54.00 ± 2.75	79.75 ± 1.50	100		
50	24.75 ± 2.75	46.00 ± 2.50	65.00 ± 2.00	97.00 ± 0.50	100		LC_{95} = 42.39 ppm
100	35.00 ± 2.50	82.00 ± 1.75	100				
150	81.00 ± 1.50	100					
Regression line equation	y = 4.025x + 0.090						

LC50 or LC95, lethal concentration for 50% or 95% of the individuals

Table 4. *A. gambiae* s.s. female mortality, knock-down time (Tkd), regression line equations and the LC50 and LC95 in the presence of *M. myristica* essential oil

Concentrations (ppm)	Mortalités (%)	Tkd			LC
		Tkd_{50}	**Tkd_{95}**	**Regression line equations**	
Control	0	/	/		LC_{50} = 95.28 ppm
50	0	/	/	y = 1.91	
100	60.50 ± 2.25	54 min 37 s	6 min 36 s	y = - 0.034x + 6.840	
150	87.50 ± 1.75	87 min 08 s	12 min 20 s	y = - 0.021x + 6.830	LC_{95} = 168.47 ppm
200	98.75 ± 0.25	194 min 33 s	12 min 20 s	y = - 0.009x + 6.751	
250	100	/	/	y = 8.72	

LC50 or LC95, lethal concentration for 50% or 95% of the individuals; Tkd 50, 95 : knock- down time of 50% or 95% of the individuals

Table 5. Mortality of aged *A. gambiae* s.s. larvae during four weeks of exposure of *M. myristica* essential oil in the open air (Mann-Whitney U test with significance at $p < 0.05$)

Concentrations (ppm)	Temps						
	D0	**D5**	**D10**	**D14**	**D17**	**D21**	**D28**
10	100^{a}	72.75 ± 3.25^{g}	70.00 ± 2.25^{h}	67.75 ± 2.50^{i}	59.00 ± 2.00^{k}	53.00 ± 2.25^{m}	47.50 ± 2.25^{n}
30	100^{a}	78.00 ± 2.50^{f}	76.00 ± 2.75^{fg}	74.75 ± 1.50^{g}	66.50 ± 2.50^{i}	62.75 ± 2.50^{j}	53.00 ± 2.50^{m}
50	100^{a}	91.5 ± 2.50^{c}	90.00 ± 2.00^{c}	88.00 ± 0.50^{d}	82.00 ± 2.25^{e}	78.50 ± 2.25^{f}	73.50 ± 2.75^{g}
100	100^{a}	100^{a}	98.00 ± 2.25^{a}	97.00 ± 1.25^{ab}	94.50 ± 125^{b}	92.00 ± 1.25^{b}	88.00 ± 1.25^{d}
150	100^{a}	100^{a}	100^{a}	98.75 ± 0.50^{a}	96.75 ± 0.75^{ab}	95.00 ± 1.50^{b}	93.50 ± 2.25^{b}

* Same letters in superscript means no significant difference; D: day

Conclusion

This work, whose main goal was to bring our contribution to enlarge the list of local plants with insecticidal properties, allowed to conclude that the essential oil of *M. myristica* has insecticidal properties. The latter proved to be a very good larvicide with a toxic effect at a very low dose and more or less lasting over time, at least under laboratory conditions. This last aspect seems more interesting to us insofar as if this persistence of toxicity does not change or changes very little in the natural environment, the essential oil of *M. myristica* would undoubtedly be a serious potential candidate for the alternative substances that could be recommended in campaigns against the aquatic stages of malaria vectors and more specifically for *A. gambiae*, whose larvae are particularly found in temporary breeding sites.

Abbreviations

CM, corrected mortality; DT_{50}, degradation time of 50% of active molecules; EO, essential oil; HMT, hydrocarbon monoterpene; HST, hydrocarbon sesquiterpenes; LC_{50} or LC_{95}, lethal concentration for 50% or 95% of the population; OM, observed mortality; OMT, oxygenated monoterpenes; Tkd_{50} or Tkd_{95}, knock-down time of 50% or 95% of the population;TM, Total mortality; VBD, Vector-borne Diseases; WHO, World Health Organization

Authors' Contribution

HGT designed and carried out the part of the work that deals with the collection of plant material, the extraction of the essential oil and the biological tests with the assistance of PAN, SCLM carried out the persistence tests. TT was the team supervisor of this work.

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Conflict of interest

The authors declare no conflict of interest

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