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Chemical composition and larvicidal activity of the essential oil of *Cymbopogon nardus* (L.) Rendle on *Anopheles gambiae*

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ABSTRACT

The increasing resistance toward a number of insecticides of synthetic origin developed by *Anopheles gambiae* (the main vector of malaria in Benin) has prompted us to seek new pesticide formulations in the fight against malaria. In this study, we tested the insecticidal potential of the essential oil of *Cymbopogon nardus* on *Anopheles gambiae* larvae for two genotypes: the sensitive strain Kisumu of Kenyan origin and the wild strain from stagnant water in Cotonou. The essential oil of this plant obtained by steam distillation with a yield of 1.3 ± 0.11% was analyzed by chromatographic method (GC / MS). The main oil compounds were citronellal (37.87%), nerol (19.88%), citronellol (9.11%), elemol (7.40%) and gamma murollene (4.65%). Sensitivity tests performed on *Anopheles gambiae* larvae 3rd instar revealed the larvicidal properties of the oil tested. The lethal concentrations (LC_{50}) figures obtained were respectively 36.83 ppm and 97.33 ppm for the Kisumu strain and the local wild population respectively. The LC_{90} adopted the same trend with 55.72 ppm and 147.9 ppm respectively for sensitive and wild larvae. The essential oil of *Cymbopogon nardus* constitutes thus a bioactive substance for a preventive strategy against malaria vectors by means of green chemistry.

Keywords: *Anopheles gambiae*, *Cymbopogon nardus*, Essential oil, Larvicidal activity.

INTRODUCTION

The fight against malaria is equally concerned with parasites, which consists of the use of antiplasmodial drugs and the vectors of this disease. To combat this vector, several methods can be considered. Among them, we can mention the use of synthetic chemical insecticides to spray the enclosure of the homes as well as the impregnation of the mosquito nets. However, the development of vector resistance to insecticides (Akogbeto et al., 2005; Djogbenou et al., 2011; Rubert et al., 2016) and the impact of their use on humans and the environment (Fontenille, 2008) reduce all efforts made by the scientific community to drastically reduce the prevalence of malaria. There is a growing need for the development and implementation of new mosquito proliferation management programs. Nowadays, many scientists are trying to find effective and accessible alternatives that are beneficial for the

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environment from natural products (El Ouali Lalami et al., 2013; Fagbohoun et al., 2015; Tiendrebeogo et al., 2017). *Cymbopogon nardus* (L.) Rendle shows some propensity for the latter option. Among Poaceae family, *Cymbopogon nardus* is a species of monocotyledonous plant whose essential oil is endowed with anti-fungal activity on the fungus strains of *Aspergillus* and *Penicillium* genus (Kpatinvoh et al., 2017). In addition, this essential oil which contains mainly citronellal, geraniol and elemol (Ouedraogo et al., 2016) has insecticidal effects on *Aedes aegypti* , a species of mosquito vector of dengue (Warikoo et al., 2011). However, no study has been published on the probable inhibitory effect of the essential oil of this plant on the larvae of *Anopheles gambiae*, a major vector of malaria in Africa (Tia et al., 2016). Therefore, in this work, the aim was to evaluate the larvicide activity of the essential oil of *Cymbopogon nardus* on two genotypes of *Anopheles gambiae* of various origins. In addition, the chemical composition of the essential oil was characterized by using a gas chromatography coupled with mass spectrometry (GC / MS).

MATERIALS AND METHODS

Collection of plants and essential oil extraction

The aerial parts of the plant, especially *Cymbopogon nardus* leaves (Figure 1) were harvested in early March 2018 between 7am and 10am at Natitingou. They were then identified and authenticated by the National Herbarium of Benin (the number AA6637 / HNB).

After harvest, the leaves were dried in the laboratory, away from light and moisture for about a week. Then, they were weighed out and grinded (cut into small parts) to increase the area of contact with water and collected in paper bags to preserve them until the moment of experiment. The extraction was carried out by the steam distillation technique using a Clevenger type experimental device (Figure 2). 100 g of *C. nardus* leaves were introduced into the reactor. 500 mL of distilled water was poured into the flask which was heated up until boiling. The heating was maintained at a gentle temperature of 100 °C for 2 hours. The water vapor passes through the reactor containing the plant material and extracts the volatile components of the plant. Once out of the reactor, it was condensed by the water-cooled condenser and the essential oil and water were separated by difference of density. The essential oil obtained was collected in a beaker and dried on MgSO4. The solvent was evaporated under reduced pressure. The oil was recovered and stored in a refrigerator at 4 °C.

Collection of *Anopheles gambiae* larvae

Bioassays were carried out on two larval strains: wild larvae collected in larval breeding sites in Cotonou according to the morphological and behavioral criteria of the larvae using taxonomic determination keys (Gillies and Coetzee, 1987) and the sensitive larvae Kisumu of Kenyan origin obtained at the Entomological Research Center of Cotonou (CREC). Kisumu larvae have been kept in culture at the CREC laboratory for several years and their sensitivity is regularly checked.

Chemical analysis of the essential oil

The different chemical compounds were investigated in *C. nardus* by using a gas chromatography method coupled with mass spectrometry (GC / MS). The analysis was carried out on a TRACE GC 2000 series (Thermo Quest, Rodano, Italy), equipped with a AS2000 autosampler (Thermo Quest). The GC system is coupled to a Trace MS (Thermo Quest) type mass spectrometer operating in electronic impact mode. The GC / MS was equipped with a capillary column DB-WAX 122-7032 (Agilent) measuring 30 x 0.25 mm with 0.25 µm internal diameter. The samples are injected in splitless mode (injected volume: 1 µL, inlet temperature: 230 °C.). The coupling temperature of the GC was 260 °C. The energy of the electrons is 70 eV and the electron source was kept 250 °C. Data were recorded and analyzed with Xcalibur 1.1

software (Thermo Quest). The mass spectra of the peaks obtained were analyzed and compared with the reference compounds at the NIST / EPA / NIH 98 library.

Sensitivity tests on the larvae of *Anopheles gambiae*

The larval tests were carried out according to the protocol recommended by WHO (WHO, 1985). Different stock solutions were prepared in ethanol from the crude essential oil of each plant to obtain, after dilution in distilled water, the final concentrations of 300, 200, 100, 50, 10 ppm for the wild-type strain and 80, 60, 40, 20, 10 ppm for the sensitive strain. The tests were carried out in transparent cups 5 cm in diameter each containing 99 mL of distilled water supplemented with one (01) mL of the diluted essential oil solution and 20 larvae of *Anopheles gambiae* 3rd instar of the same category. For each essential oil concentration, the test was repeated 3 times to minimize errors. The control consists of 1% ethanol. This solution was prepared under conditions identical to the other solutions tested. The number of dead larvae was counted after 24 hours exposure to the different concentrations of essential oils. Larvae that remain insensitive to needling or those that are moribund are considered dead. The results of the larval sensitivity tests were expressed as a percentage of mortality as a function of the essential oil concentrations used. If the percentage of mortality in the control is greater than 5%, that of the larvae exposed to the essential oil must be corrected using the Abbott formula: % death = [(Test-Control) / Witness] × 100 (Carballo et al., 2002). If the mortality in the controls exceeds 20%, the test is invalid and must be repeated. The statistical analysis of our data was performed using the GRAPH PAD PRISM 7.0 software, in order to highlight the mortality rate of *Anophelesgambiae* larvae according to the concentrations used and the estimation of the lethal concentrations 50 and 90 (LC_{50} and LC_{90}) was made using the same software.

Figure 1 : *Cymbopogon nardus.*

Figure 2 : Experimental device.

RESULTATS

Content and chemical composition of the essential oil of *Cymbopogon nardus*

With an average extraction yield of 1.3 ± 0.11%, the essential oil of *C. nardus* is mainly composed of citronellal (37.87 %), nerol (19.88%), citronellol (9.11%), elemol (7.40%) and Gamma-cadiene (4.65%) as indicated in Table 1.

Larvicidal activity of the essential oil of *Cymbopogon nardus on Anopheles gambiae*

Figure 3 shows the mortality rates of sensitive and wild *An. gambiae* larvae with the essential oil of *C. nardus*. We note that the control leads to ethanol 1% has no inhibitory effect on wild larvae (B) that continue to live during 24 hours of experience. In addition, a concentration of the essential oil at 80 ppm was sufficient to annihilate the entire population of sensitive larvae while it was necessary to reach 200 ppm to achieve the same effects with wild larvae. The lethal concentrations responsible for 50% and 90% of the mortality of sensitive larvae and wild strains in 24 hours are summarized in Table 2. The values obtained with wild larvae are almost three times higher than doses of sensitive larvae Kisumu whatever lethal concentrations considered.

Tableau 1: Chemical composition of the essential oil of *Cymbopogon nardus.*

Compounds	SI	Area %	Molecular weight
1,1-dimethoxyethane	892	0.16	90
Tert-butylalcohol	969	0.46	74
Limonene	942	1.21	136
Citronellal	**921**	**37.87**	**154**
Linalool	946	0.31	154
Menthol	948	0.41	154
Beta-elemen	928	1.86	204
Cis-2,6-Dimethyl-2,6-octadiene	**918**	**2.71**	**138**
Neral	917	0.23	152
Gamma-cadiene	**896**	**4.65**	**204**
Germacrene D	**909**	**2.86**	**204**
Alpha-Cadinene	934	0.49	204
Geranial	925	0.36	152
Acetate de geranyle	**932**	**4.41**	**196**
Citronellol	**943**	**9.11**	**156**
Nerol	**878**	**19.88**	**154**
Germacrene D-4-ol	850	0.75	222
Elemol	**948**	**7.40**	**222**
Geranium cyclohexane	819	0.36	172
Eugenol	922	0.57	164
Acetate de gouaiol	875	1.03	222

Tau-Muurolol	909	0.43	222
Alpha-Eudesmol	916	0.64	222
Beta-Eudesmol	938	0.68	222
à-cadinol	935	0.89	222
Farnesol	931	0.15	222
Cis-Z-à-Bisaboleneepoxide	875	0.12	220

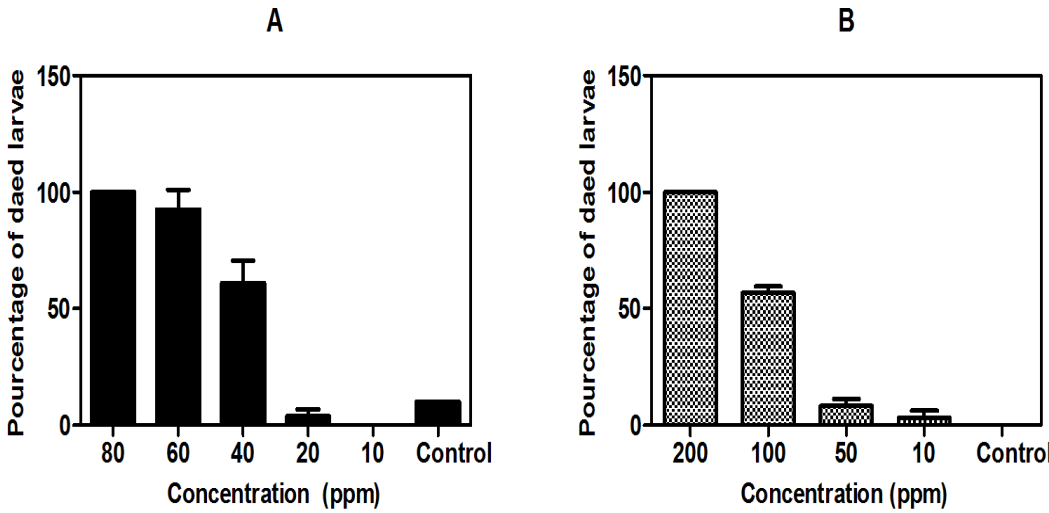

Figure 3: Mortality of larvae with essential oil of *C. nardus* in 24 hours
A: Sensitive larvae Kisumu;
B: Wild larvae

Tableau 2: Lethal concentrations (LC_{50} and LC_{90}) of the essential oil of *C. nardus.*

	Sensitive larvae		Wild larvae	
LC (ppm)	**95% Confidence Intervals**		**LC (ppm)**	**95% Confidence Intervals**
LC_{90}	**55.72**	49.54 to 63.49	**147.9**	135.6 to 161.4
			97.33	93.45 to 101.4
LC_{50}	**36.83**	33.98 to 39.92		

DISCUSSION

The yield of essential oil obtained for *C. nardus* is significantly higher than that obtained in Brazil (0.4%) by Rios (Ríos et al., 2017). These results can be explained by the influence of certain particular factors such as the growing season, harvest condition origin, climatic and edaphic conditions of each region on the plant. The results obtained after analysis of this essential oil reveal that it is mainly composed of citronellal (37.07%), nerol (19.88%), citronellol (9.11%) and elemol (7.40%). These results contrast with those previously obtained by Koumaglo in 2004 who reported that citronellal and geraniol are the majority compounds with respective levels of 47.80 and 25.00% on samples of *C. nardus* in Benin (Koumaglo, 2004). Similarly, Silou analyzed the essential oil of *C. nardus* from Congo-Brazzaville (Silou et al., 2017) and found citronellal (44.87%) and geraniol as major compounds (22.99%). Also, Abena obtained citronellal (41.3%) and geraniol (23.4%) which are mainly present in this essential oil in Benin (Abena et al., 2007). Furthermore, Nakahara obtained geraniol (35.7%), trans-citral (22.7%) and cis-citral (14.2%) as major compounds in Japan (Nakahara et al., 2003). This large difference in the composition may be due to seasonal variations in the distribution of the various essential oil components of a plant, related to changes throughout the vegetative cycle of the plant, as well as the prevailing environmental conditions in the regions. Other factors, such as growing conditions (sowing and harvest dates), phytosanitary treatments, fertilizer use, and harvesting techniques, also influence the chemical composition of aromatic plants and thus essential oils derived from it (Anton and Lobstein, 2005; Rajeswara, 2002).

Mortality rates from the action of *C. nardus* essential oil confirm the larvicidal activity of this plant against mosquitoes as reported before (Ilahi and Yousafzai, 2017). The results obtained with wild larvae are much higher values of about three times those of sensitive larvae Kisumu at the same time of exposure. This is justified by the fact that the wild larvae collected in full environment have already been in contact with certain active molecules against which they have developed a certain level of resistance. Moreover, larvae in this category are resistant to synthetic chemical insecticides of the pyrethroid group (Djogbenou et al., 2011) In addition, these same wild larvae had already developed resistance to *Elaeis oleifera* (Ahouansou et al., 2017). These results obtained with *C. nardus* are in agreement with those reported previously (Ríos et al., 2017) in their study on larvae of other culicid species, notably *Aedes aegypti*. The tests showed a larvicidal activity of this oil with an LC_{50} of 75.85 ppm. On the other hand, our results are better than those obtained recently by (Ilahi and Yousafzai, 2017) who have demonstrated the larvicidal activity of the hexane extract of *C. nardus* on 2nd and 4th instar larvae of *Culex quinquefaciatus*. The LC_{50} associated are 451.8 ppm and 599 ppm respectively.

In many studies, *C. nardus* has been considered promising as insecticidal. To this end, we mention the work of (Clemente et al., 2010) testing the larvicidal activity of *C. nardus* on *Amblyomma cajennense* (Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae) and the work of (Warikoo et al., 2011) in which the toxicity of the essential oil of *C. nardus* has been confirmed on eggs and larvae of *Aedes aegypti*. In addition, this species shows a very interesting activity compared with that obtained on larvae of *Anopheles stephensi* through the studies carried out on leaves of *Ajuga remota*: 330 ppm in 24 hours and 290 ppm in 48 hours (Preeti et al., 2004), as well as that carried out by M.J. Muema in Kenya on of *Anopheles gambiae* and *Anopheles arabiensis* larvae with the methanolic extract of *Agerantum conyzoides*, which yielded LC_{50} values of 232.70 ppm and 406.35 ppm respectively after 24 hours of treatment (Muema et al., 2016). The protection of foodstuffs against pests by the essential oil of *C. nardus* has been studied on *Sitophilus zeamais* Motsch and *Rhyzopertha dominica* F (Ouedraogo et al.,

2016). The LC_{50} obtained for this purpose was 1729 ppm. This difference in action is probably related to the variability of the chemical constituents of essential oils of various horizons. Indeed, the active molecules of insecticidal plants can vary from one family to another, within a family and the sensitivity can differ from one species to another and within a species (Guèye, 2015). However, the LC_{50} values obtained show a toxicity lower than that of the essential oil of *C. citratus* against larvae of the same mosquito species (LC_{50} = 18 ppm) in 24 hours of exposure (Tchoumbougnang et al., 2009).

Conclusion

Our study has shown that the essential oil of *C. nardus* has a remarkable larvicidal activity against *An. gambiae* and can serve as a prototype for the development of biolarvicides in the fight against the vectors responsible for malaria. Citronellal and nerol which are the major chemical compounds of this oil could be considered responsible for this activity. It would be interesting to complete this work by evaluating the effect of this essential oil on the morphometry of the larvae.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

ACA: principal investigator, manuscript preparation and analysis supervision. SRMF: elaboration of technical methods and contribution to the writing of the manuscript. JMT: assisted in the development of the technical protocols. HT: realization of chromatographic analyses (GC / MS). YKB: collection of *Anopheles gambiae* larvae and performing larval tests. FAG: initiator and general supervisor.

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