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Original Research Article

Chemical composition and toxicity of *Zingiber officinale* (Roscoe, 1807) (Zingiberaceae) essential oil on the aquatic stages of the malaria vector *Anopheles coluzzii*

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Overcoming the phenomenon of insecticides resistance in malaria vectors in Sub-Saharan tropics remains a great challenge to stop the burden of malaria disease. We tested the efficacy of *Zingiber officinale* as an alternative insecticide to synthetic pesticides and as a vector control tool against *Anopheles coluzzii*, the main malaria vector in Cameroon. The biotoxicity of *Z. officinale* essential oil on aquatic stages of *An. coluzzii* was assessed using WHO guidelines, and the essential oil active compounds were identified using Gas Chromatography coupling Mass Spectrometry (GC-MS). The essential oil of *Z. officinale* revealed an ovicidal and larvicidal property against developmental stages of *An. coluzzii*. A median inhibitory concentration (IC_{50}) of 17.81 ppm was obtained for eggs hatching potentials, while LC_{50} of 12.20, 12.12, 12.77 and 13.67 were obtained after exposure to first, second, third and fourth instars larvae respectively. All larval stages exhibited similar and full susceptibility to *Z. officinale* essential oil at 25 ppm. Overall, the essential oil extraction yield was 0.301% and 26 compounds were identified from their retention indices and mass spectra. Hydrocarbon monoterpenes (24.52%), oxygenated monoterpenes (44.93%) and hydrocarbon sesquiterpenes (30.54%) constituted the major classes of the essential oil. β-curcumene (15.24%), geranial (15.16%), camphene (13.79%), neral (11.88%) and α-zingiberene (6.18%) were the most abundant oil compounds. In summary, *Z. officinale* essential oil revealed important ovicidal and larvicidal properties and stands as a promising tool to manage the phenomenon of insecticides resistant vectors in malaria endemic regions.

Key words: *Zingiber officinale,* essential oil, *Anopheles coluzzii*, plant biocides, malaria.

INTRODUCTION

Malaria remains the most important parasitic disease worldwide due to *Hemococcidae* of the genus *Plasmodium* transmitted to human by female mosquito bites (Carnevale et al., 2009; WHO, 2015). *Anopheles coluzzii* (*An. coluzzii*) is known to transmit malaria in Sub-Saharan regions. In Cameroon, malaria remains the main cause of morbidity affecting mostly pregnant women and less than five years old children (PNLP, 2012). Fighting against malaria in the country largely rely on the use of synthetic pyrethroid insecticides for long lasting insecticide treated nets (LLINs) (Zaim et al., 2000, WHO, 2015). However, pyrethroids efficacy is being threatened by the rise of resistance in targeted vector populations (Chandre et al., 2000; Etang et al., 2003 , 2006), mainly due to the use of common insecticide classes for both Public Health and agricultural interventions, as well as the misuse and/or overuse of synthetic pesticides in agricultural settings (Nkya et al., 2013, Dinitz et al., 2015). Vectors resistance to pyrethroid insecticides and the phenomenon of bioaccumulation of insecticide residues revealed in the environment are hindrances for both human and animal health (Dinitz et al., 2015). There is a need to identify new compounds safe for both the environment and non-targeted organisms. Plant-derived extracts and essential oils are relatively non-toxic to the environment and stand as new promising tools to control the burden of insect vectors (Tchoumbougnang et al., 2008, Foko et al., 2011; Akono et al., 2012).

Nowadays, essential oils and their components are gaining increasing interest due to their potential multi-purpose functionality. *Z. officinale* Roscoe (ginger), one of the most important plant spices in the world, is known for its medicinal and flavoring potentials (Onyenekwe and Hashimoto, 1999). It has been used as a medicine since many centuries and the medicinal properties are attributed to its spicy and pungent constituents (Onyenekwe and Hashimoto, 1999). Asian medicine uses dried ginger for stomach problems such as stomach-ache, diarrhoea, and nausea; while the Chinese also use this plant to boost the heart and to help with mucus and phlegm. In Cameroon, 46, 325 tones of ginger are produced yearly, and this culinary plant is widely used as spice and for medicinal values. These wide potentials have been attributed to various chemical constituents found in *Z. officinale* essential oil such as α-pinene, camphene, β-pinene, 1,8-cineole, linalool, borneol, y-terpineol, nerol, neral, geraniol, geranial, geranyl acetate, β-bisabolene and zingiberene (Onyenekwe and Hashimoto, 1999; Sivasothy et al., 2011; Raina et al., 2013; Kumari et al., 2014). Although gingerol, the main constituent of *Z. officinale* essential oil stimulates thermoregulatory receptors, the mechanisms of other specific components of this oil is to be elucidated. Our previous investigations show that *Z. officinale* essential oil has good adulticidal effects on *An. gambiae.* In addition, *Z. officinale* essential oils from India have exhibited larvicidal activity against *Culex Tritaeniorhynchus*, *Culex quinquefasciatus* and *An. Subpictus* (Pushpanathan et al., 2008; Govindarajan, 2011).

However, the ovicidal and larvicidal effects of this oil should also be evaluated with the Cameroonian species of *Z. officinale,* in order to identify stage-specific target for integrated intervention strategy, and to correlate information from different countries. Although the variety and the age of the rhizome at harvest, as well as the distillation process affect the yield and composition of the essential oil and hence its flavour, the major factor that determines its value is its origin. In view of the applications of *Z. officinale* essential oil in the medicine and food industries and the compositional differences in various geographic settings, there is a need for better understanding of the composition of this plant in other climatic zones, as well as its additional properties such as insecticidal potentials. The study aimed to evaluate the chemical composition and biocide properties of *Z. officinale* essential oil against developmental stages (eggs and larvae) of the malaria vector *An. coluzzii.*

MATERIAL AND METHODS

Plant material and extraction of essential oil

Rhizomes of *Z. officinale* were collected in 2015 from local markets across Yaoundé, the capital city of Cameroon. Collected materials were identified at the National Herbarium with the certification number 43146/HNC, dried and subjected to hydro distillation in Clevenger type apparatus as described previously (Nyegue, 2006). Extracted essential oil was characterized and used in experimental bioassays with mosquitoes.

Characterization of the essential oil

The analysis of *Z. officinale* essential oil was conducted at the Max Mousseron Institute of Biomolecules, Montpellier National Higher School of Chemistry, France, by Gas-Chromatography coupling Mass Spectrometry (GC-MS) as described previously (Riwom et al., 2015). The oil components were identified based on their relative retention indices with either those of authentic samples or with published data in the literature (Adams, 2012).

Mosquito's collection and rearing

The laboratory susceptible strain *An. coluzzii* was collected from OCEAC and reared in laboratory conditions (27°C, 78-80% RH, 12:12 photoperiod) as described previously (Foko et al., 2007). Mosquito developmental stages (Eggs and larvae) were collected and exposed to different doses of *Z. officinale* essential oil according to WHO bioassay procedures.

Toxicological tests of *Z. officinale* essential oil against aquatic stages of *An. coluzzii*

Prior to toxicological bioassays, a stock solution (500 ppm) was prepared by diluting the extracted essential oil with absolute alcohol. Serial dilutions were then performed from the stock solution and a set of six concentrations (5, 10, 15, 20, 25 and 30 ppm) were prepared for eggs and larval bioassays. Both absolute alcohol and 20% Temephos were used as controls during toxicological tests. 20% Temephos is known with a killing effect on susceptible aquatic stages of mosquitoes and was used as positive control, while pure absolute alcohol does not affect the viability of mosquito's developmental stages and was used as solvent control.

Ovicidal activity

A colony of 100 mature eggs was exposed to each dose of *Z. officinale* essential oil using the WHO guidelines provided for larval activities (WHO, 2005). Briefly, each breeding bowl was filled in with 99 ml spring water and 1 ml of the required concentration of essential oil. *An. coluzzii* eggs were then added into the mixture and incubated in laboratory conditions (27°C, 78-80% RH, 12:12 photoperiod). Each concentration was tested in 4 replicates with 25 individuals and the overall experiment was repeated thrice for data accuracy and validation. Absolute alcohol was used as control and the hatching rates were recorded after 24 and 48 hours of contact with the essential oil.

Larvicidal activity

Hundred individuals (4 replicates of 25 individuals) of first, second, third and fourth instars larvae of An. coluzzii were exposed to each concentration of *Z. officinale* essential oil according to WHO bioassay procedures (WHO, 2005). The overall experiment was repeated thrice for data accuracy and validation. Absolute alcohol was used as solvent control, while 20% Temephos was used as positive control to ascertain the effect of the essential oil on mosquito's larvae. Exposed mosquitos were considered dead or moribund if they were immobile and unable to reach the surface of water in the breeding bowl. The mortality rates were read 24 and 48 hours post-exposure.

Statistical analysis

The data were computed in Excel datasheet of Microsoft Office 2013 and analyzed using SPSS v20.0. Non-parametric Chi-square test of Kruskal-Wallis was performed to evaluate the toxicological effect of the oil on mosquito larvae. Results were expressed as percentage mortality, corrected for untreated (check) mortality using Abbott's formula. Dose-mortality regressions were computed within STATISTICA v6.0. The median inhibitory concentration (IC_{50}) and the median lethal concentration (LC_{50}) respectively for eggs and larvae of *An. coluzzii* were recorded. The level of significance was set at p < 5%.

RESULTS

Chemical content of *Z. officinale* essential oil

Rhizomes of *Z. officinale* afforded essential oil (yellowish) in the yield of 0.301% (w/w). The retention indices, relative percentages and the identities of the constituents of the essential oil are provided in Table 1. Overall 26 compounds were identified from their retention indices and mass spectra. Hydrocarbon monoterpenes, oxygenated monoterpenes and hydrocarbon sesquiterpenes constituted 24.52, 44.93 and 30.54% of the essential oil respectively. oxygenated sesquiterpenes were not detected in the essential oil. camphene (13.79%), α-pinene (4.14%) and myrcene (2.24%) were the most abundant hydrocarbon monoterpenes. Geranial (15.16%), neral (11.88%), borneol (3.59%), 1,8-Cineole (3.4%) and geranol (2%) constituted the most abundant oxygenated monoterpenes. β-curcumene (15.24%), α-zingiberene (6.18%), (E)-α-bisabolene (6.07%) and (E)- γ-bisabolene (2.36%) were the most abundant hydrocarbon sesquiterpenes found. Other substantial numbers of compounds were detected in minor amounts as indicated in Table 1.

Ovicidal activity of *Z. officinale* essential oil on *An. coluzzii*

The egg-hatching rates varied significantly within the concentrations of the essential oil (p=0.0001). The median inhibitory concentration (IC_{50}) was 17.81 ppm. No hatching was recorded 24-hours post-exposure to 25 ppm and 30 ppm oil concentrations. The hatching rates ranged from 3.33% to 21.5% within 5-20 ppm (Table 2). These values did not vary significantly after 48 hours incubation of *An. coluzzii* eggs in *Z. officinale* essential oil.

Larvicidal activity of *Z. officinale* essential oil on *An. coluzzii*

The lethal effect of 20% Temephos was confirmed on aquatic stages of *An. coluzzii* in laboratory bioassays. This compound affects all the larval stages (L1-L4) of mosquitoes and its potentials were compared with effects caused by *Z. officinale* essential oil. All data were validated in comparison with the controls.

Larvicidal activity of Z. officinale essential oil on An. coluzzii first instars larvae

Essential oil of *Z. officinale* revealed high larvicidal potentials against first instars larvae of *An. coluzzii* in laboratory bioassays (Table 3). The mortality rates decreased significantly with the oil concentrations (p=0.0150) with LC_{50} of 12.20 ppm. All mosquito's larvae were killed at 20 ppm and the mortality rates ranged from 20% to 74% within 5-15 ppm.

Larvicidal activity of Z. officinale essential oil on An. coluzzii second instars larvae

Essential oil of *Z. officinale* also revealed to be efficient against second instars larvae of *An. coluzzii*. The larvicida property here is comparable with the effect observed on first instars larvae with LC_{50} of 12.12 ppm. Overall, the larval mortality rates decreased significantly within the oil concentrations in comparison with the controls (p=0.0069). No larvae survival was recorded after exposure to 25 ppm or above. However, the mortality rates ranged from 13% to 80% within 5-20 ppm (Table 3).

Larvicidal activity of *Z. officinale* essential oil on *An. coluzzii* third instars larvae

Z. officinale essential oil exhibited high larvicidal potentials

Table 1. Chemical composition of *Z. officinale* essential oil

Elution order	Compounds	Retention Indices	% Compounds
Hydrocarbon monoterpenes			**24.52**
1	α-Thujene	925	0.17
2	α-Pinene	938	4.13
3	Camphene	956	13.79
4	Sabinene	981	0.47
5	ß-Pinene	988	1.92
6	Myrcene	993	2.24
7	α-Phellandrene	1008	0.76
Oxygenated monoterpenes			**44.93**
8	1,8-Cineole	1039	3.4
9	Linalool	1091	0.94
10	Camphre	1102	2.6
11	Pinocarvone	1154	0.47
12	Borneol	1175	3.59
13	Terpinen-4-ol	1183	1.05
14	α-Terpineol	1197	1.41
15	Citronellool	1238	1
16	Neral	1253	11.88
17	Phellandral	1261	0.14
18	Geraniol	1270	2
19	Geranial	1285	15.16
20	Carvacrol	1297	0.57
21	Geranyl acetate	1369	0.72
Hydrocarbon sesquiterpenes			**30.54**
22	Aromadendrene	1491	0.69
23	ß-Curcumene	1510	15.24
24	α-Zingiberene	1517	6.18
25	(E)- γ-Bisabolene	1520	2.36
26	(E)-α-Bisabolene	1537	6.07

against *An. coluzzii* third instars larvae. This oil killed all third instars larvae at 25 ppm as observed with second instars larvae, and the LC_{50} value of 12.77 ppm was computed. A significant difference in the mortality rates was recorded within the oil concentrations tested in comparison with the controls (p=0.0430). The larval mortality rates ranged from 12% to 85% within the working concentrations of 5-20 ppm (Table 3).

Larvicidal activity of *Z. officinale* essential oil on *An. coluzzii* fourth instars larvae

The larvicidal property of *Z. officinale* essential oil on fourth instars larvae of *An. coluzzii* was lower than activities recorded with young aquatic developmental stages (L1-L3). In fact, the larvae resisted to the highest concentrations of 30 ppm tested. However, the mortality rates decreased significantly with the oil concentrations (p=0,0091) as recorded with previous developmental stages, and ranged from 10% to 99% within 5-30 ppm (Table 3).

DISCUSSION

Z. officinale is among the healthiest and most delicious spices worldwide. It is loaded with nutrients and bioactive compounds that have powerful benefits on human's health. Rhizomes of *Z. officinale* (yellow variety) collected from market settings in Yaoundé (Cameroon) and subjected to hydrodistillation revealed to contain 26 compounds with 0.301% yield. The oil content as found in this study is close to the 28 and 29 chemicals identified in oils extracted from four varieties of ginger rhizomes in India (Raina et al., 2013). However, the oil content from Cameroon is lower than the 54 and 89 compounds identified in ginger essential oil from Malaysia and Nigeria, respectively (Onyenekwe and Hashimoto, 1999; Sivasothy et al., 2011). It is well known that the origin, the variety and the age of the rhizomes at harvest, as well as the extraction process might affect the yield and composition of the essential oils. β-curcumene (15.24%), geranial (15.16%), camphene (13.79%), neral (11.88%) and α-zingiberene (6.18%) were the most abundant oil compounds found. Although this oil content is like that of oils extracted in Nigeria, Malaysia, China and India, the composition order quietly differs between these countries (Onyenekwe and Hashimoto, 1999; Sasidharan and Menon, 2010; Sivasoth et al., 2011; Raina et al., 2013). In fact, Onyenekwe and Hashimoto (1999) reported that the origin of the oil is the major factor that determines its value. The oil components of *Z. officinale*

Table 2. Eggs' hatching rates of *An. coluzzii* after exposure to *Z. officinale* essential oil in laboratory bioassays

An. coluzzii		**Essential oil concentrations (ppm)**							**P-value**	**χ2**	**IC_{50} (ppm)**
		30	**25**	**20**	**15**	**10**	**5**	**0**			
Hatching rates (%)	After 24 hours	0	0	3.33 ± 0.92	7.17 ± 0.68	15.13 ± 1.05	21.67 ± 0.4	92 ± 0.84	**0.0001**	**83.47**	**17.81**
	χ2	-	-	145.94	113.43	108.26	55.56	13.20			

Hatching rates are explained by mean ± SD (standard deviations). p-value<0.05 are statistically significant. IC_{50}: Median inhibitory concentration

Table 3. Mortality rates of aquatic stages (L1, L2, L3 and L4) of *An. coluzzii* after exposure to *Z. officinale* essential oil in laboratory bioassays.

Larval mortality rates (%)		**Essential oil concentrations (ppm)**							**P-value**	**χ2**	**LC50 (ppm)**
		30	**25**	**20**	**15**	**10**	**5**	**0**			
L1	After 24 hours	100	100	100	74±1.21	53.81±1.21	20.0±0.97	0	**0.0150**	**29.89**	**12.20**
	χ2	-	-	-	37.42	45,34	5,95	-			
L2	After 24 hours	100	100	80±1.08	72.28±1.34	43.12±0.56	13±0.09	0	**0.0060**	**25.99**	**12.12**
	χ2	-	-	55.56	42.45	96.41	3,63	-			
L3	After 24 hours	100	100	85±0.56	62±0.90	34±0.81	12±0.99	0±0.47	**0.0430**	**16.22**	**12.77**
	χ2	-	-	66.41	51.86	14.71	3.02	-			
L4	After 24 hours	99±0.30	98±0.65	82±1.44	60±0.30	28±0.77	10±0.08	0±0.09	**0.0091**	**75.34**	**13.36**
	χ2	125	113.43	80.36	50.13	5.95	2.72	-			

Mortality rates are explained by mean ± SD (standard deviations). p-value<0.05 are statistically significant. LC_{50}: Median lethal concentration. L1, L2, L3 and L4 correspond to first, second, third and fourth instars larvae respectively.

as found in this study seems to exhibit an inhibitory effect against aquatic stages of *An. Coluzzii* in laboratory conditions. A high ovicidal effect was recorded with the eggs of this malaria vector. The oil concentration 25 ppm stands as the critical dose to be used in intervention strategies in inhibiting the hatching of *An. coluzzii* eggs in environmental or field conditions. Ovicidal effects of essential oils have often been linked to the presence of oxygenated monoterpenes (Marimithu, 2010). Although the ovicidal effect of each active component of the oil was not assessed here, the inhibitory potentials of *Z. officinale* essential oil could be inferred to the presence of Geranial, neral, borneol, 1,8-Cineole and geranol which constituted the most abundant oxygenated monoterpenes of the essential oil. Essential oil of *Z. officinale* also revealed powerful larvicidal potentials against larvae of *An. coluzzii*. Previous studies have reported this larvicidal effect on *Culex tritaeniorhynchus*, *Culex quinquefasciatus, Aedes aegypti* and *An. subpictus* (Lucia et al., 2007; Pushpanathan et al., 2008; Govindarajan, 2011). However, these species revealed less sensitivity to the oil than the larvae (L1-L4) of *An. coluzzii*. In fact, a low dose (LC_{50} < 14 ppm) of *Z. officinale* essential oil as found here is sufficient to kill 50% of the mosquito's population. Interestingly, all larval stages exhibited similar susceptibility to the oil. An integrated intervention strategy using 25 ppm oil concentration could therefore be implemented to target all larval stages of this malaria vector. This suggests that *Z. officinale* essential oil can affect the shell of old sensitive mosquito's developmental stages and affect their survival capacity. In fact, decrease in mosquito's sensitivity after exposure to essential oils has often been attributed to the shell which helps to withstand the oil activity (Nathan et al., 2006; Nasir et al., 2015). Lucia et al. (2007) attributed the larvicidal activity of *Z. officinale essential oil* against *Aedes aegypti* to α-pinene and β-pinene. These compounds as well as myrcene and E-β-caryophyllene are highly effective against the chitin which constitutes the main constituent of the insect's cuticle (Pavela et al., 2014). The low quantity of these chemicals in *Z. officinale* essential oil as found here suggests the activity of other bioactive components such as oxygenated terpenes against the larval stages of *An. coluzzii*.

The data presented in this study correlate with the repellent effect recorded in our previous investigations with *An. gambiae* (Foko et al., 2011) and further highlight the importance of *Z. officinale* essential oil as alternative tool for Public Health interventions targeting vector-borne diseases. The repellent effect of this oil has also been reported against *Culex tritaeniorhynchus* and *An. subpictus* (Govindarajan, 2011), and *Culex quinquefasciatus* (Pushpanathan et al., 2008). Overall, essential oil of *Z. officinale* revealed efficient ovicidal and larvicidal properties that could be used as alternative to ineffective synthetic insecticides in fighting *An. coluzzii* in Cameroon. However, further studies should investigate the efficacy of individual ingredient of the oil in both laboratory and field conditions, as well as their mode of action in targeted insects of Public Health importance.

Conclusion

Essential oil of rhizomes of *Z. officinale* collected from Yaoundé markets was made up of 26 compounds. β-curcumene (15.24%), geranial (15.16%), camphene (13.79%), neral (11.88%) and α-zingiberene (6.18%) were the most abundant compounds of the oil. This oil exhibited high ovicidal and larvicidal effects against *An. coluzzii* in laboratory bioassays. All larval stages exhibited similar susceptibility to *Z. officinale essential* oil and an integrated Public Health intervention strategy using 25 ppm oil concentration could be implemented to target all aquatic stages (eggs and larvae) of this malaria vector under laboratory and field conditions. Although the active ingredients of *Z. officinale* essential oil are yet to be identified, this oil stands as a promising tool to manage the phenomenon of insecticides resistant vectors in malaria endemic regions.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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