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Le T Huong
School of natural science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, Vietnam.

Trinh T Huong
[1] Graduate University of science and technology, Vietnam academy of science and technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.
[2] Faculty of Natural Science, Hong Duc University, Thanh Hoa City, Thanh Hoa Province, Vietnam.

Nguyen T Bich
School of natural science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, Vietnam.

Nguyen T Viet
School of natural science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, Vietnam.

Isiaka A Ogunwande
University Road, Aleku Area, Osogbo, Nigeria.

Corresponding Author:
Le T Huong
School of natural science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, Vietnam.

Larvicidal efficacy of essential oils from the rhizomes of *Zingiber castaneum* against *Aedes albopictus*

Le T Huong, Trinh T Huong, Nguyen T Bich, Nguyen T Viet and Isiaka A Ogunwande

Abstract
In this paper, we report the larvicidal activity of essential oils from the rhizomes of *Zingiber castaneum* Škorničk & Q.B Nguyễn growing in Vietnam. Essential oils were obtained from the pseudo-stem by hydro distillation conducted by using Clevenger apparatus. The mortality and larvicidal effects of the essential oil on fourth-instar larvae of *Aedes* were evaluated by the established protocol of the World Health Organization (WHO). The rhizome oil of *Z. castaneum* exhibited 100% mortality towards *Ae albopictus* at concentration of 100 µg/mL after 24 h and 48 h test period. In addition, the essential oil displayed larvicidal activity against *Ae albopictus* with minimum lethal concentration LC_{50} values of 49.85 µg/mL and 43.93 µg/mL at 24 h and 48 h respectively.

Keywords: *Zingiber castaneum*, essential oil, larvicidal activity

1. Introduction

The *Zingiber* species are noted for their economic importance mainly due to volatile and non-volatile constituents and the various biological activities they exhibited. *Zingiber castaneum* Škorničk. & Q.B. Nguyễn is easily recognized among other terminally flowering species by its upright inflorescence with reflex bracts. The plant is also a rhizomatous herb forming small clumps. The creeping aromatic rhizome which grows up to 1.5 cm in diameter is externally light brown and internally cream white [1]. The translucent light green leaves are glabrous. Flowering starts in July and extends to September. It was found growing in Ninh Bình Province [1]. A recent report [2] identified β-pinene (30.6%), α-pinene (9.5%), β-caryophyllene (9.4%) and bicycloelemene (9.1%) as the main constituents of *Z. castaneum* leaf oil. The compounds occurring in higher quantity in the stem oil were β-caryophyllene (14.7%), δ-cadinene (9.8%), bicycloelemene (8.4%) and α-cubebene (7.8%) while large amount of camphene (15.1%), 1,8-cineole (13.6%), linalool (11.3%) and δ-3-carene (8.5%) were present in the root oil, with (*E*)-nerolidol (23.2%), (*Z*)-9-octadecenamide (17.3%) and β-caryophyllene (10.8%) occurring in of the fruit oil [2]. Sabinene (22.9%) occurred as the compound occurring in higher amount in the rhizome oil [3]. Vietnam is classified as a hyperendemic dengue country with present throughout the year and dengue fever epidemics have increased in frequency [4]. Mosquitoes have been and continue to be the most deadly creatures on earth. *Aedes albopictus* (Skuse) (Diptera Culicidae) is ranked among the most invasive mosquito species in the world [5]. Besides its aggressive daytime biting behaviour, the medical importance of *Ae albopictus* is due to its ability to transmit many human pathogens and parasites (e.g. yellow fever, dengue fever, West Nile, Japanese encephalitis, St. Louis encephalitis, chikungunya viruses, filarial nematodes). *Culex quinquefasciatus* Say, commonly known as the southern house mosquito, is a medium-sized brown mosquito that exists throughout the tropics. It is a vector of many pathogens of humans, domestic and wild animals. Viruses transmitted by this species include lymphatic filariasis, West Nile virus, St. Louis encephalitis virus, Western equine encephalitis virus and Zika virus [6]. The yellow fever mosquito, *Aedes aegypti* (Linn), has been a nuisance species for centuries. *Aedes aegypti* is the primary vector of yellow fever, a disease that is prevalent in tropical South America and Africa, and often emerges in temperate regions during summer months. All four dengue viruses are spread primarily through the bite of an infected *Aedes* species (*Ae. aegypti* and *Ae. albopictus*) mosquito [7]. The control of adult mosquitoes commonly relies on the use of synthetic insecticides and repellents, but treatments with such chemicals are expensive, show scarce efficacy and have a strong environmental impact associated to relevant human health risks. For these reasons, alternative natural insecticides and repellents are now very appreciated by consumers. Essential oils of aromatic plants are considered among the most promising alternative to synthetic chemicals [8]. Essential oils are generally recognized as environmentally friendly, easily biodegradable, minimally toxic to mammals and have shown repellent activities against different mosquito species. As part of our ongoing research aimed at the identification of the chemical constituents and larvicidal and antimicrobial potentials of essential oils from plant (especially *Zingiber* species) grown in Vietnam [2-3, 9-11], we have obtained essential oils from *Z. castaneum* and examined the mosquito larvicidal activity.

2. Materials and methods
2.1 Plant collection and identification
Rhizomes of *Z. castaneum* were collected from Pu Hoat Nature Reserve, Nghệ An Province, Vietnam, in August 2018. Botanical identification was accomplished at Botany Museum, NghệAn College of Economics, Vietnam, where a voucher specimen, LTH741, was deposited for future references.

2.2 Preparation of sample
In the course of preparation for hydrodisitillation process, the rhizomes were air-dried (22°C) under laboratory shade for two weeks to reduce the moisture contents. Moreover, unwanted materials were also removed by handpicking. Afterwards, samples were pulverized to coarse powder using a locally made grinder.

2.2 Hydro-distillation experiment
A total of 1000 g of each of the pulverized samples were used for the experiment at different times. Known weight of samples was separately and carefully introduced into a 5 L flask and distilled water was added until it covered the sample completely. Essential oils were obtained by hydro distillation which was carried out in an all glass Clevenger-type distillation unit designed according to Vietnamese Pharmacopoeia [12] as described previously[2,9-11]. The distillation time was 3 h and conducted at normal pressure. The volatile oils distilled over water and were collected by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4ºC) until the moment of analyses.

2.3 Larvicidal assay
2.3.1 Mosquito larvae
Adults of *Ae albopictus* collected in Hoa Khanh Nam ward, Lien Chieu district, Da Nang city (16°03'14.9"N, 108°09'31.2"E). Adult mosquitoes were maintained in entomological cages (40 x 40 x 40 cm) and fed a 10% sucrose solution and were allowed to blood feed on mice. Eggs hatching were induced with tap water. Larvae were reared in plastic trays (24 × 35 × 5 cm). The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. All stages were held at 25 ± 2°C, 65-75% relative humidity, and a 12:12 h light:dark cycle at the Center for Entomology and Parasitology Research, Duy Tan University.

2.3.2 Larvicidal test
Larvicidal activity of the essential oils from *Z. castaneum* was evaluated according to WHO protocol [13] with slight modifications. For the assay, aliquots of the essential oils from both samples dissolved in EtOH (1% stock solution) was placed in a 200-mL beaker and added to water that contained 20 larvae (fourth instar). With each experiment, a set of controls using EtOH was also run for comparison. Mortality was recorded after 24 h and again after 48 h of exposure during which no nutritional supplement was added. The experiments were carried out 25 ± 2°C. The larvicidal test was conducted with four replicates under four concentrations (100, 50, 25 and 12.5 µg/mL). Permethrin was used as a positive control.

The mortality rate was calculated according to the formula

$$Mc= (Mo)/(Mt) \times 100$$

Mo = number of larvae dead in the treated groups, Mt = number of larvae introduced and Mc = calculated mortality

2.3.3 Statistical analysis
The data obtained were subjected to log-probit analysis [14] to obtain LC_{50} values, LC_{90} values, 95% confidence limits, and chi square values using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

3. Results & discussion
3.1 Yield of the essential oil
The average yields of the essential oils of *Z. castaneum* was 0.31% (v/w, ± 0.01), calculated on a dry weight basis. The oil was light yellow coloured.

3.2 Mortality of the essential oils against vector mosquitoes
The rhizome oil of *Z. castaneum* exhibited potent mortality (100%) against *Ae. albopictus* at concentration of 100 µg/mL, under the test period of 24 h and 48 h as seen in Table 1. There was no mortality in the EtOH used as control for all the tested oil samples. The percentage mortality was dependent on the concentration of the tested oil samples. Thus, higher inhibition of mosquito larvae was observed as concentration increases.

Table 1: Mortality and larvicidal activity of rhizome oil of *Z. castaneum*

Concentration (µg/mL)	Mortality (%) [a,b]	
	24 h	**48 h**
12.5	0	0
25.0	5.0 ±0.00	15.0 ±0.10
50.0	40.0 ±0.50	48.7 ±0.75
100.0	100 ±0.50	100 ±0.50
Minimum lethal concentration (µg/mL)[a]		
LC_{50}	49.85 ±1.20	43.93 ±0.11
LC_{90}	71.71 ±0.50	68.12 ± 0.91
Regression equation	y = -2.921 +0.059x	y = -2.327 +0.053x
$\chi 2$	6.468	7.571
P	0.001	0.001

[a] n =4; [b]no mortality in the EtOH used as control

3.3 Larvicidal tests
From Table 1, the *Z. castaneum* rhizome oil exhibited larvicidal action towards *Ae albopictus* with LC_{50} values of 49.85 µg/mL and LC_{90} 71.71 µg/mL at 24 h. Also, LC_{50} values of 43.93 µg/mL and LC_{90} of 68.12 µg/mL were obtained at 48 h. Permethrin used as a positive control displayed larvicidal activity with LC_{50} and LC_{90} values of 1.32 µg/mL and 0.97 µg/mL, respectively. The results in this study showed that essential oils hydrodistilled from the rhizomes of *Z. castaneum* exhibited good mortality and larvicidal activity on *Ae albopictus* larvae. The results of the present study are also comparable to earlier reports on the larvicidal activities

of plant extracts.
The observed larvicidal action of *Z. castaneum* in this study was comparable with findings from *Zingiber* plants analysed for their larvicidal activity from Vietnam and other parts of the world. The essential oil of *Z. collinsii* from Vietnam displayed larvicidal action against *Ae albopictus* (LC_{50} = 25.51 µg/mL; LC_{90} = 40.22 µg/mL) and *Cx. quinquefasciatus* (LC_{50} = 50.11 µg/mL and LC_{90} = 71.53 µg/mL) after 24 h [10]. Essential oil from the rhizome of *Z. zerumbet* exhibited potent larvicidal action against *Ae albopictus* within the 24 h and 48 h tested period having LC_{50} of 55.75 µg/mL and 36.22 µg/mL respectively [11]. In addition, the oil displayed larvicidal activity towards *Cx. quinquefasciatus* with LC_{50} of 33.28 µg/mL and 21.81 µg/mL respectively after 24 h and 48 h test period [11]. The essential oil of *Z. zerumbet* from Malaysia displayed much lower larvicidal action against *Ae aegypti* with LC_{50} of 102.6 µg/mL [15] while the rhizome oil from Thailand also showed larvicidal action against *Ae aegypti* with LC_{50} and LC_{90} values of 48.92 and 62.2 µg/mL respectively [16]. Likewise, *Z. cernuum* was toxic towards *Ae aegypti* (LC_{50} = 44.88 µg/mL), *Ae albopictus* (LC_{50} = 55.84 µg/mL) and *Cx. quinquefasciatus* (LC_{50} = 48.44 µg/mL) after 24 h [17]. *Zingiber officinale* was shown to have larvicidal activity against *Cx. quinquefasciatus* with a LC_{50} value of 50.78 µg/mL [18]. The essential oils from the rhizome of *Z. nimmonii* demonstrated significant larvicidal activity against *Ae aegypti* and *Cx. quinquefasciatus*, with LC_{50} values of 44.46 and 48.26 µg/mL, respectively [19]. Moreover, essential oils from *Zingiber* plants have also demonstrated potential insecticidal and larvicidal activities against other insect pests. For example, *Z. officinale* demonstrated action against *Cx. tritaeniorhynchus* and *Anopheles subpictus* with the LC_{50} and LC_{90} values as 98.83, 57.98 µg/mL and 186.55, 104.23 µg/mL, respectively [20]. Extracts from *Z. officinale* var. *rubrum*, *Z. montanum* and *Z. spectabile* were shown to displayed larvicidal mortality against *Ae albopictus* larvae with LC_{50} of 96.86, 99.04 and 93.35 mg/L respectively [21].
Since the WHO has not established a standard criterion for determining the larvicidal activity of natural products, several authors have developed individual criteria to characterize the potency of mosquito larvicides developed from natural products[22, 23]. For example, considered products showing $LC_{50} \leq 50$ mg/L to be active, 50 mg/L < $LC_{50} \leq 100$ mg/L to be moderately active, 100 mg/L < $LC_{50} \leq 750$ mg/L to be effective, and $LC_{50} > 750$ mg/L to be inactive[22]. Likewise, considered compounds with $LC_{50} < 100$ mg/L to exhibit a significant larvicidal effect [23]. It should be stressed that these criteria must be directly correlated with the time of exposure and the origin of larvae, which are variables that can alter the LC_{50} values. The results obtained in this study showed that the essential oils of *Z. castaneum* had promising effects, according to the criterion established previously[22, 23]. In summary, *Z. castaneum* essential oils from Vietnam revealed important toxicity and larvicidal properties on *Ae. Albopictus* larvae and stands as a promising tool to manage the phenomenon of insecticides resistant vectors in malaria endemic regions.
The variations in toxicity of essential oils against different species of mosquitoes are common, due to qualitative and quantitative variations of chemical constituents. The main compounds of the essential oil were described as sabinene (22.9%), α-pinene (7.8%), β-pinene (6.5%), bornyl acetate (6.1%) and γ-terpinene (5.5%) making up the major constituents [3]. Interestingly, the larvicidal activity of the main compounds in this work, including, α-pinene, β-pinene, sabinene and limonene are well known. The essential oils of *Cupressus macrocarpa* aerial parts and their constituents, including sabinene, α-pinene, and terpinen-4-ol, provided good larvicidal effect against *Ae albopictus*, with LC_{50} of 54 mg/L and LC_{90} of 84 mg/L[24]. Sabinene exhibited significant larvicidal activity against *Ae aegypti* and *Ae albopictus* with LC_{50} values of 74.1 and 39.5 µg/mL, respectively [25]. In addition, α-pinene and β-pinene demonstrated strong larvicidal potential against *Ae aegypti* with LC_{50} of 15.4 and 21.1 µg/mL, respectively [26]. The isolation and purification of active compound which could be responsible for the larvicidal activity against mosquito vectors of would be an important step in the development of novel mosquitocidal agents. Production of larvicides from the locally available plants, could be a new acceptable alternative to employ which may lead to decreasing dependence on imported synthetic insecticides and be beneficial for developing countries such as Vietnam.

4. Conclusions
Assessment of larvicidal efficacy demonstrated that the rhizome oils of *Z. castaneum* were toxic against susceptible and resistant *Ae albopictus* larvae at reasonable LC_{50}, and LC_{90} levels. In conclusion, we have documented the promising larvicidal potentials of essential oils from *Z. castaneum* from Vietnam, which could be considered as a potentially alternative source for developing novel formulation for controlling diseases.

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