Contact and Repellant Activities of Zerumbone and Its Analogues from the Essential Oil of *Zingiber zerumbet* (L.) Smith against *Lasioderma serricorne*

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Abstract: The contact toxicity and repellent activities of the essential oil extracted from the rhizomes of *Zingiber zerumbet* (L.) Smith (Zingiberaceae) was evaluated against cigarette beetles (*Lasioderma serricorne*). The essential oil obtained by hydrodistillation was investigated by GC-FID and GC-MS. The main constituents of the essential oil were zerumbone (40.2%), α -caryophyllene (8.6%), humulene epoxide II (7.3%), camphene (5.9%) and fenchene (4.7%). Zerumbone and its analogues totally are accounting for 60.3% of the essential oil. It was found that the essential oil possessed contact toxicity against *L. serricorne* adults with a LD₅₀ value of 48.3 μ g/adult. α -Caryophyllene (LD₅₀ = 13.1 μ g/adult) exhibited stronger contact toxicity against *L. serricorne* than humulene oxide (LD₅₀ = 31.2 μ g/adult), β -caryophyllene (LD₅₀ = 35.5 μ g/adult) and zerumbone (LD₅₀ = 42.4 μ g/adult). Moreover, α -caryophyllene possessed strong repellent activity (Class IV and V, respectively) against the beetles at 78.63 nL/cm², after 2 and 4 h treatment. The results indicate that zerumbone and its analogues might be developed into natural insecticides or repellents for control of cigarette beetles, but their bioactivities are affected by their structures.

Key words: *Zingiber zerumbet*, zerumbone analogues, *Lasioderma serricorne*, essential oil, bioactivities

1 INTRODUCTION

Lasioderma serricorne Fabricius (Coleoptera: Anobiidae), variously known as the cigarette beetle or the tobacco beetle, is cosmopolitan and occurs frequently in tropical and subtropical areas. This insect is usually found in perishable food products such as cereals, legumes, tobacco and traditional Chinese medicinal materials in warehouses¹. Most notably, this species is the most injurious pest to stored tobacco. It can attack both the unprocessed commodity and the finished products². Many problems (*i.e.*, insecticide resistance, toxicity to mammals and other non-target animals, residue problems, environmental pollution) have occurred for the overuse of synthetic insecticides during the control of stored product insects³. These problems have necessitated a search for alternative ecofriendly insect pest control methods. Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-

resistance as well as offering new leads for design of targets-specific molecules^{4,5}. Among the botanicals, essential oils are a major category that began to develop with research in the 1980s⁶. They are derived from aromatic plants that, in the course of evolution, developed myriad constitutive and induced chemical defenses against herbivorous insects⁷. The percentage of the components of essential oils varies amongst species and plants parts; these components are chemically derived from terpenes and their oxygenated derivatives, terpenoids, which are aromatic and aliphatic acid esters and phenolic compounds⁸.

Plants in the ginger family (Zingiberaceae) produce essential oils which are toxic to insects^{9,10}. *Zingiber*

Abbreviations: *Z. zerumbet*: *Zingiber zerumbet*; *L. serricorne*: *Lasioderma serricorne*; MeBr : methyl bromide; RI: Retention Index; MS: mass spectrum; DEET: N,N-diethyl-3-methylbenzamide; LD₅₀: 50% of lethal dose; FL: fiducial limits; SE: standard error.

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zerumbet (L.) Smith widely distributed in the tropical region of Asia and is native to Guangdong, Guangxi, Yunnan provinces in China. The rhizomes of *Z. zerumbet* can be used to treat stomachache and diarrhea, and can play the role of expelling wind and detoxication as well as traditional Chinese medicine¹¹. Its extracts have been showed a wide range of bioactivities such as anti-insect toxicity^{12, 13}, anti-hypersensitive, anti-inflammatory¹⁴, antimicrobial¹⁵, anti-tumor¹⁶, nephroprotective¹⁷, antioxidant¹⁸. Moreover, anti-allergic activity¹⁹, antifungal²⁰, antinociceptive²¹, anti-insect^{22, 23}, anti-inflammatory²⁴, antibacterial²⁵ and antimicrobial²⁶ of the *Z. zerumbet* essential oil have also been reported. However, the literature survey has shown that there is no report on insecticidal and repellent activities of essential oil from *Z. zerumbet* against cigarette beetles. In addition, zerumbone, a sesquiterpene found in this plant, has been shown to possess antiproliferative, anticancer, antioxidant, and anti-inflammatory activity against various types of human carcinoma²⁷. Some analogues of zerumbone have been found to exhibit bioactivities against *L. serricorne* adults in our previous study²⁸, whereas the bioactivities of zerumbone were seldom mentioned. Thus, in this paper, we evaluated the contact and repellent activities of the essential oil *Z. zerumbet* and zerumbone for the first time, meanwhile, we compare their bioactivities against *L. serricorne* adults with other three analogues of zerumbone (α -caryophyllene, β -caryophyllene and caryophyllene oxide, Fig. 1) and tried to analyze relationship of structure-bioactivity of them.

2 EXPERIMENTAL PROCEDURES

2.1 Material

2.1.1 Plants

Fresh rhizomes of *Z. zerumbet* were harvested in 2014

Fig. 1 Zerumbone and its analogues from the essential oil of *Zingiber zerumbet* (L.) Smith.

from Xishuangbanna (northern latitude: 21° 08' ~ 22° 36'; east longitude: 99° 56' ~ 101° 50'), Yunnan Province, China. The aerial parts were air dried for 1 week. The plant species was identified and voucher specimens (BNU-dushushan-2014-06-12-08) were deposited at the Herbarium of college of Resources Science and Technology, Beijing Normal University (Beijing, China).

2.1.2 Insects

The cigarette beetle (*L. serricorne*) was obtained from laboratory cultures maintained in the dark in incubators at 29-30°C and 70-80% relative humidity. They were reared on wheat flour mixed with yeast (10:1, w/w) at 12-13% moisture content. Unsexed adult beetles and booklouse used in all the experiments were about 1 week old. All containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Sino-rich®, Beijing Sino-rich Tech Co., Ltd., Xuanwu District, Beijing, China).

2.2 Extraction of the essential oil

Rhizomes of *Z. zerumbet* (2 kg) was subjected to hydrodistillation using a modified Clevenger-type apparatus for 8 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in an airtight container in a refrigerator at 4°C.

2.3 GC-MS and GC-FID analysis

The GC and GC/MS analyses were carried out with an Agilent 6890N apparatus equipped with FID and an Agilent 5973 N mass selective detector, resp., and a HP-5MS Column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The initial oven temperature was programmed isothermal at 60°C for 1 min, rising from 60°C to 180°C at 10°C/min, held isothermal at 180°C for 1 min, rising from 180°C to 280°C at 20°C/min, and then held isothermal at 280°C for 15 min; injector temp., 270°C; detector temp., 280°C; carrier gas, He (1.0 mL/min). The sample (1 µL) was injected neat, with a split ratio of 1:10. The spectra were scanned from 20 to 550 *m/z* at 2 scans/s. The retention indices were determined in relation to a homologous series of *n*-alkanes (C₈-C₂₄) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from literature²⁹⁻³¹. Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area% reports.

2.4 Bioactivity

2.4.1 Contact activity

The contact toxicity of the essential oil/compounds against *L. serricorne* adults was tested as described by Liu

and Ho³²). Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five concentrations) was prepared in *n*-hexane. Aliquots of 0.5 µL of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Five replicates were carried out for all treatments and controls. Both treated and control insects were then transferred to glass vials (diameter 2.5 cm, height 5.5 cm, volume 25 mL) (10 insects/vial) with culture media and kept in incubator (29-30°C and 70-80% r.h.). Mortality of insects was observed after 24 h. The LD₅₀ values were calculated by using Probit analysis³³. The positive control, pyrethrins (pyrethrin 1: 24%; pyrethrin 2: 13%; cinnerin 1: 2%; cinnerin 2: 2%; jasmolin 1: 1%; jasmolin 2: 1%) were purchased from Dr. Ehrenstorfer, Germany. Zerumbone, α-caryophyllene, β-caryophyllene and caryophyllene oxide were all obtained from TCI Shanghai Development Co., Ltd. Shanghai, China.

2.4.2 Repellency tests

The repellent effects of the essential oil and some of their individual components against *L. serricorne* were assessed by using assays on Petri dishes³¹. Petri dishes 9 cm in diameter were used to confine beetles during the experiment. The essential oil of *Z. zerumbet* and the individual compounds were prepared in *n*-hexane (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm²), and absolute *n*-hexane was used as the control. Filter paper 9 cm in diameter was cut in half and 500 µL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500 µL of absolute *n*-hexane. Both the treated half and the control half were then air-dried to evaporate the solvent completely (30 s). A full disk was carefully remade by attaching the tested half to the negative control half with tape. Each reassembled filter paper after treatment with solid glue was placed in a Petri dish with the seam oriented in one of four randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. Five replicates were used, and the experiment was repeated three times. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil/compounds was then calculated using the formula:

$$PR(\%) = [(N_c - N_t) / (N_c + N_t)] \times 100$$

Where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half. The averages were then assigned to different classes (0 to V) using the following scale (percentage repellency)³². Class, % repellency: 0, >0.01 to <0.1; I, 0.1-20.0; II, 20.1-40.0; III, 40.1-60.0; IV, 60.1-80.0; and V, 80.1-100. As a positive control, a commercial repellent DEET (N, N-

diethyl-3-methylbenzamide), was used under the conditions as the oil. Analysis of variance (ANOVA) and Tukey's test were conducted by using SPSS statistics 20 for Windows 2007. Percentage was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

3 RESULTS and DISCUSSION

3.1 Chemical Composition of the Essential Oil

A total of 44 components were identified in the essential oil of *Z. zerumbet*, accounting for 90.9% of the total oil (Table 1). The main constituents of *Z. zerumbet* essential oil were zerumbone (40.2%), α-caryophyllene (8.6%), humulene epoxide II (7.3%), camphene (5.9%), followed by fenchene (4.7%), (E)-nerolidol (3.1%), camphor (2.7%), eucalyptol (2.5%), caryophyllene oxide (2.3%) and β-caryophyllene (1.9%). Among them, α-caryophyllene, β-caryophyllene, humulene epoxide II, and caryophyllene oxide are analogues of zerumbone. Zerumbone and its analogues are according for 60.3% of the total oil.

However, a literature survey has shown that zerumbone and its analogues were always the main chemical composition of the samples collected from different areas. For example, the essential oil obtained from traditional herb suppliers in Chiang Mai province contained α-caryophyllene (31.9%), zerumbone (31.7%) as its main constituents¹³. Suthisut *et al.* reported that camphene (27%), α-caryophyllene (14%) and camphor (12%) were the major compounds of *Z. zerumbet* essential oil collected from Phang-Nga province, Thailand²². The main compounds of *Z. zerumbet* essential oil purchased from Chow Kit's wet market, Kuala Lumpur, Malaysia were camphene (14.3%) and humulene (10.0%)³⁴, meanwhile, the essential oil collected from Sabah, Malaysia contained α-caryophyllene (5.9%) and camphene (2.8%) as its main compounds²². The main components of it from Tamil Nadu, India was zerumbone (25.4%) and β-pinene (23.7%)²⁶. Moreover, the sample collected from Manipur, India possessed zerumbone (88.5%) and α-caryophyllene (2.3%) as the major components³³, as well as curzerenone (14.4%), camphor (12.8%) and zerumbone (12.6%) were the main components in the samples from Siwan district, Bihar, India³⁴. Otherwise, the essential oil of *Z. zerumbet* collected from Tahiti, Frech, Polynesia was rich in oxygenated derives of α-caryophyllene, in particular zerumbone (65.3%)³⁵. The above findings suggest that zerumbone and its analogues are the main compounds in *Z. zerumbet* essential oil. Further studies on plant cultivation and essential oil standardization are still needed because component content of the essential oil varies with the plant population.

Table 1 The chemical composition of *Zingiber zerumbet* essential oil.

Compounds	RI ^{a)}	Content [%] ^{b)}	Molecular Formula	Mode of Identification ^{c)}
Fenchene	949	4.7	C ₁₀ H ₁₆	RI, MS
Camphene	952	5.9	C ₁₀ H ₁₆	RI, MS
2-Thujene	967	1.1	C ₁₀ H ₁₆	MS
3-Carene	1011	0.1	C ₁₀ H ₁₆	RI, MS
3,6,6-Trimethyl-bicyclo[3.1.1]hept-2-ene	1021	1.8	C ₁₀ H ₁₆	RI, MS
Eucalyptol	1031	2.5	C ₁₀ H ₁₈ O	RI, MS, Co
Ocimene	1041	0.4	C ₁₀ H ₁₆	RI, MS
Sylvestrene	1058	1.1	C ₁₀ H ₁₆	RI, MS
γ-Terpinene	1060	0.2	C ₁₀ H ₁₆	RI, MS
Linalool	1094	1.0	C ₁₀ H ₁₈ O	RI, MS, Co
Fenchon	1117	0.1	C ₁₀ H ₁₆ O	RI, MS
Camphor	1146	2.7	C ₁₀ H ₁₆ O	RI, MS, Co
Terpinen-4-ol	1177	0.4	C ₁₀ H ₁₈ O	RI, MS, Co
α-Terpineol	1189	0.4	C ₁₀ H ₁₈ O	RI, MS, Co
Myrtenal	1193	0.1	C ₁₀ H ₁₄ O	RI, MS
Cinereone	1227	1.4	C ₁₀ H ₁₄ O	RI, MS
Isobornyl acetate	1295	0.3	C ₁₂ H ₂₀ O ₂	RI, MS
β-Caryophyllene	1420	1.9	C ₁₅ H ₂₄	RI, MS, Co
trans-α-Bergamotene	1436	0.2	C ₁₅ H ₂₄	RI, MS
Germacrene D	1474	0.5	C ₁₅ H ₂₄	RI, MS, Co
α-Caryophyllene	1478	8.6	C ₁₅ H ₂₄	RI, MS
(Z,Z)-α-Farnesene	1506	0.1	C ₁₅ H ₂₄	RI, MS
(E)-Nerolidol	1564	3.1	C ₁₅ H ₂₆ O	RI, MS
Caryophyllene oxide	1580	2.3	C ₁₅ H ₂₄ O	RI, MS, Co
Humulene epoxide II	1608	7.3	C ₁₅ H ₂₄ O	RI, MS
1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene	1654	2.4	C ₁₅ H ₂₄ O	RI, MS
Zerumbone	1734	40.2	C ₁₅ H ₂₂ O	RI, MS, Co
Phytone	1827	0.4	C ₁₈ H ₃₆ O	RI, MS
Monoterpenoids		15.3		
Sesquiterpenoids		11.3		
Others		62.0		
Zerumbone analogues (Zerumbone; α-Caryophyllene; β-Caryophyllene; Humulene epoxide II; Caryophyllene oxide)		60.3		
Total		90.9		

a) RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons.

b) Relative area (peak area relative to the total peak area).

c) MS, mass spectrum; Co, co-injection with authentic compound.

3.2 Bioactivities

3.2.1 Contact activity

The essential oil of *Z. zerumbet* rhizomes exhibited contact toxicity against *L. serricorne* adults with a LD₅₀ value of 48.3 µg/adult (Table 2). Compared with the positive control, pyrethrins, the crude essential oil demonstrat-

ed almost 241 times less contact toxicity against cigarette beetles because the pyrethrins has acute toxicity to *L. serricorne* with a LD₅₀ value of 0.2 µg/adult. Zerumbone demonstrated weakest contact toxicity against the cigarette beetles (LD₅₀ = 42.4 µg/adult) among the four individual compounds. The toxicity of α-caryophyllene (LD₅₀ = 13.1

Table 2 Contact toxicity of *Z. zerumbet* essential oil and zerumbone analogues against *L. serricorne*.

Samples ^{a)}	LD ₅₀ (μg/adult)	95 % FL (μg/adult)	Slope ± SE	Chi square (χ ²)
<i>Z. zerumbet</i>	48.3	44.3-52.6	4.58 ± 0.54	10.54
Zerumbone	42.4	35.6-51.2	1.58 ± 0.26	22.24
α-Caryophyllene ^{b)}	13.1	10.6-15.4	1.81 ± 0.29	17.83
β-Caryophyllene ^{b)}	35.5	31.9-39.5	3.07 ± 0.37	15.41
Caryophyllene oxide ^{b)}	31.2	25.1-38.4	1.34 ± 0.25	9.02
Pyrethrins ^{c)}	0.2	0.2- 0.4	1.31 ± 0.20	17.36

^{a)} The mortality of the control was 0 μg/adult for *L. serricorne*.

^{b)} Data from You *et al.*³⁶⁾

^{c)} Data from Yang *et al.*³⁷⁾

Table 3 Percentage repellency (PR) after two exposure times of *Z. zerumbet* essential oil and its main components against *Lasioderma serricorne*^{a)}.

Samples	2 h (nL/cm ²)			4 h (nL/cm ²)		
	78.63	15.73	3.15	78.63	15.73	3.15
<i>Z. zerumbet</i>	72 ± 14 bc IV	50 ± 13 abc III	46 ± 13 ab III	66 ± 16 abc IV	46 ± 13 ab III	42 ± 8 bc III
Zerumbone	34 ± 7 a II	26 ± 10 ab II	42 ± 8 ab III	58 ± 14 ab III	22 ± 8 a II	12 ± 8 a I
α-Caryophyllene ^{b)}	76 ± 11 bc IV	56 ± 7 bc III	66 ± 12 b IV	86 ± 12 bc V	50 ± 10 ab III	52 ± 15 bc III
β-Caryophyllene ^{b)}	42 ± 17 ab III	22 ± 15 ab II	20 ± 17 a II	34 ± 19 a II	22 ± 14 a II	20 ± 9 ab II
Caryophyllene oxide ^{b)}	48 ± 16 ab III	16 ± 12 a I	16 ± 9 a I	46 ± 15 ab III	38 ± 13 a II	34 ± 14 abc II
DEET	88 ± 7 c V	76 ± 14 c IV	28 ± 7 ab II	98 ± 4 c V	78 ± 9 b IV	58 ± 16 c III

^{a)} Means in the same column followed by the same letters do not differ significantly ($p < 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

^{b)} Data from You *et al.*³⁶⁾

μg/adult) is stronger than β-caryophyllene (LD₅₀ = 35.5 μg/adult) and caryophyllene oxide (LD₅₀ = 31.2 μg/adult). In the previous reports, essential oil of *Z. zerumbet* has been demonstrated to possess insecticidal activity against *Aedes aegypti*¹³⁾, *Tribolium castaneum*, *Sitophilus zeamais*²²⁾, *Anisopteromalus calandrae* and *Trichogramma deion* larvae²³⁾. The bioactive testing of the constituent compounds in the essential oil of *Z. zerumbet* rhizomes are utmost importance so that their potential application in controlling stored product pests can be fully exploited.

3.2.2 Repellent activity

In addition to contact activities, the repellent effect of the essential oil of *Z. zerumbet* against *L. serricorne* was also investigated and the result is presented in **Table 3**. Data showed that at the test concentration of 78.63 nL/cm², the essential oil showed weaker repellency (Class IV) against *L. serricorne* compared with the positive control, DEET (Class V) at 2 h and 4 h after exposure. With the decreasing of the sample concentration, the repellent activity of the essential oil of *Z. zerumbet* was decreased. Among the individual compounds, only α-caryophyllene showed stronger repellency (76 and 86% repellency against *L. serricorne* adults, 2 and 4 h after exposure, respectively, at the test concentration of 78.63 nL/cm²) and usually possess

higher repellency classes than the other three ones. However, zerumbone, β-caryophyllene and caryophyllene oxide did not possess significant repellent (lower than Class IV) activity against *L. serricorne* adults.

Although zerumbone and its analogues are all sesquiterpenoids, the contact and repellent activities of them are quite different. α-Caryophyllene possessed obvious contact and repellent activities. It was a naturally occurring monocyclic sesquiterpene with an important biochemical lead structure, consisting of an 11-membered ring, containing three nonconjugated C=C double bonds, two of them being triply substituted and one being doubly substituted³⁸⁾. Zerumbone and α-caryophyllene have similar structures, but differing in an extra carbonyl group in the former, which might lead to its weaker activities against the cigarette beetle. However, α-caryophyllene was less effective than zerumbone against American house dust mite³⁹⁾. Thus, the carbonyl group of zerumbone is a prerequisite component for toxicity against the dust mite but completely opposite for the cigarette beetles. These differences may be due to the diverse mechanism. β-Caryophyllene and caryophyllene oxide also have similar structure. Their C=C double bonds are in different positions and have extra 4-membered rings. We guess it is because α-caryophyllene

has a simpler structure than zerumbone and the other two analogues, so that it has less volatileness. Zerumbone and its analogues are totally accounting for 60.3% of the essential oil, thus they partly determined the activity of the essential oil. The other 40.7% of the essential oil also influenced the bioactivity. Monoterpenoids were accounting for 15.3% of this essential oil. It was demonstrated that some of them have bioactivities against the stored product insects⁴⁰⁾. Thus, the other part of the essential oil also possessed certain bioactivity. In this paper, we report the contact and repellent activities of the essential oil of *Z. zerumbet* and zerumbone against *L. serricorne* for the first time. Further studies are required to best understand its mechanism of action and to find zerumbone analogues with stronger bioactivity.

4 CONCLUSIONS

This work indicates that the essential oil of *Z. zerumbet* and analogues of zerumbone have potential for development into natural insecticides/repellents for control of *L. serricorne*. However, further studies are needed to focus on the improvement of stability of these potential insecticides/repellents for practical use and the mechanism of the active compounds.

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