Contact and Repellent 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 (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 µg/adult. α-Caryophyllene (LD₅₀ = 13.1 µg/adult) exhibited stronger contact toxicity against L. serricorne than humulene oxide (LD₅₀ = 31.2 µg/adult), β-caryophyllene (LD₅₀ = 35.5 µg/adult) and zerumbone (LD₅₀ = 42.4 µg/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 products. Many problems (i.e., insecticide resistance, toxicity to mammals and other non-target animals, residue problems, environmental pollution) have occurred for the overuse of synthet ic insecticides during the control of stored product insects². 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 molecules³,⁴. Among the botanicals, essential oils are a major category that began to develop with research in the 1980s⁵. They are derived from aromatic plants that, in the course of evolution, developed myriad constitutive and induced chemical defenses against herbivorous insects⁶. The percentage of the components of essentials 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 compounds⁷.

Plants in the ginger family (Zingiberaceae) produce essential oils which are toxic to insects⁸,⁹. Zingiber

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

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zernumbet (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 medicine. Its extracts have been showed a wide range of bioactivities such as anti-insect toxicity, anti-hypersensitive, anti-inflammatory, antimicrobial, anti-tumor, nephroprotective, antioxidant. Moreover, anti-allergic activity, antifungal, antinociceptive, anti-insect, anti-inflammatory, antibacterial and antimicrobial of the Z. zerumbet essential oil have also been reported. However, the literature survey has shown that there is no report on insecticidal activity against various types of human carcinoma. Some analogues of zerumbone have been found to exhibit bioactivities against L. serricorne adults in our previous study, whereas the bioactivities of zerumbone were seldom mentioned. Thus, in this paper, we evaluated the toxicity of the essential oil/compounds 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 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 specimen (BNU-dushan-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 (Si-no-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 (C₆-C₃₀) 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 literature. 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.
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...and Ho^{32}. 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 LD_{50} values were calculated by using Probit analysis^{33}. The positive control, pyrethrin (pyrethrin 1: 24%; pyrethrin 2: 13%; cinnerin 1: 2%; cinnerin 2: 2%; jasminol 1: 1%; jasminol 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 dishes^{31}. 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 mL/cm^{2}), 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:

$$PR(\%) = \left[ \frac{(N_0 - N_t)}{N_0 + N_t} \right] \times 100$$

Where N_0 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%), caryophyllene (5.9%), followed by fenchone (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 constituents^{31}. 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, Thailand^{21}. 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 compounds^{23}. The main components of it from Tanril 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 components^{31}, as well as curzerenone (14.4%), camphor (12.8%) and zerumbone (12.6%) were the main components in the samples from Siwan district, Bihar, India^{40}. Otherwise, the essential oil of Z. zerumbet collected from Tahiti, Frech, Polynesia was rich in oxygenated derivs 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.
3.2  Bioactivities

3.2.1  Contact activity

The essential oil of *Z. zerumbet* rhizomes exhibited contact toxicity against *L. serricorne* adults with a LD$_{50}$ value of 48.3 µg/adult (Table 2). Compared with the positive control, pyrethrins, the crude essential oil demonstrated almost 241 times less contact toxicity against cigarette beetles because the pyrethrins have acute toxicity to *L. serricorne* with a LD$_{50}$ value of 0.2 µg/adult. Zerumbone demonstrated weakest contact toxicity against the cigarette beetles (LD$_{50}$ = 42.4 µg/adult) among the four individual compounds. The toxicity of α-caryophyllene (LD$_{50}$ = 13.1 µg/adult) was 10 times higher than that of Zerumbone.

| 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$_{16}$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$_{16}$O | RI, MS, Co                |
| Fenchon                          | 1117   | 0.1             | C$_{10}$H$_{16}$ | RI, MS                    |
| Camphor                          | 1146   | 2.7             | C$_{10}$H$_{16}$O | RI, MS, Co                |
| Terpinen-4-ol                    | 1177   | 0.4             | C$_{10}$H$_{16}$O | RI, MS, Co                |
| α-Terpineol                      | 1189   | 0.4             | C$_{10}$H$_{16}$ | RI, MS                    |
| Myrtenal                         | 1193   | 0.1             | C$_{10}$H$_{16}$ | RI, MS                    |
| Cinerone                         | 1227   | 1.4             | C$_{10}$H$_{16}$O | RI, MS                    |
| Isobornyl acetate                | 1295   | 0.3             | C$_{11}$H$_{20}$O | RI, MS                    |
| β-Caryophyllene                  | 1420   | 1.9             | C$_{11}$H$_{16}$ | RI, MS, Co                |
| trans-α-Bergamotene              | 1436   | 0.2             | C$_{11}$H$_{16}$ | RI, MS                    |
| Germacrene D                     | 1474   | 0.5             | C$_{11}$H$_{14}$ | RI, MS, Co                |
| α-Caryophyllene                  | 1478   | 8.6             | C$_{11}$H$_{16}$ | RI, MS                    |
| (Z,Z)-α-Farnesene                | 1506   | 0.1             | C$_{11}$H$_{14}$ | RI, MS, Co                |
| (E)-Nerolidol                    | 1564   | 3.1             | C$_{11}$H$_{16}$ | RI, MS, Co                |
| Caryophyllene oxide II           | 1580   | 2.3             | C$_{11}$H$_{16}$ | RI, MS, Co                |
| Humulene epoxide II              | 1608   | 7.3             | C$_{11}$H$_{16}$ | RI, MS, Co                |
| 1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene | 1654 | 2.4 | C$_{11}$H$_{16}$ | RI, MS, Co |
| Zerumbone                        | 1734   | 40.2            | C$_{15}$H$_{22}$O | RI, MS, Co                |
| Phytone                          | 1827   | 0.4             | C$_{15}$H$_{22}$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            |                  |                           |

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

Table 2  Contact toxicity of *Zingiber zerumbet* essential oil and its components against *L. serricorne* adults.

| Compounds                        | LD$_{50}$ | Mode of Identification |
|----------------------------------|-----------|------------------------|
| Positive control (pyrethrins)     | 0.2 µg/adult | Co-injection with authentic compound |
| Zerumbone                        | 42.4 µg/adult | Co-injection with authentic compound |
| α-Caryophyllene                  | 13.1 µg/adult | Co-injection with authentic compound |
| Zerumbone analogues              | 45.6 µg/adult | Co-injection with authentic compound |

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

| Samples | LD50 (µg/adult) | 95% FL (µg/adult) | Slope ± SE | Chi square (χ²) |
|---------|-----------------|------------------|-----------|----------------|
| Z. zerumbet | 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 | 13.1 | 10.6-15.4 | 1.81 ± 0.29 | 17.83 |
| β-Caryophyllene | 35.5 | 31.9-39.5 | 3.07 ± 0.37 | 15.41 |
| Caryophyllene oxide | 31.2 | 25.1-38.4 | 1.34 ± 0.25 | 9.02 |
| Pyrethrins | 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.
c) Data from Yang et al.

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

| Samples | 2 h (nL/cm²) | 4 h (nL/cm²) |
|---------|--------------|--------------|
| Z. zerumbet | 78.63 | 15.73 | 3.15 | 78.63 | 15.73 | 3.15 |
| 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 | 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 | 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 | 48 ± 16 ab III | 16 ± 9 a I | 16 ± 9 a I | 46 ± 15 ab III | 38 ± 13 a II | 34 ± 14 ab 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.

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 aegypti11, Tribolium castaneum, Sitophilus zeamais12, Anisopteromalus calandrae and Tribchogromma deon larve23. 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 substituted. Zerumbone and α-caryophyllene have similar structures, but differing in an extra carbonyl group in the former, which might led to its weaker activities against the cigarette beetle. However, α-caryophyllene was less effective than zerumbone against American house dust mite. 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 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 insects. 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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