Repellent and Contact Toxicity of *Alpinia officinarum* Rhizome Extract against *Lasioderma serricorne* Adults

Jianhua Lü*, Dan Ma

School of Food Science and Technology, Henan University of Technology, Lianhua Street, Zhengzhou High-Tech Development Zone, Zhengzhou, 450001, Henan, China

* jianhlv@163.com

Abstract

The repellent and contact toxicities of *Alpinia officinarum* rhizome extract on *Lasioderma serricorne* adults, and its ability to protect stored wheat flour from *L. serricorne* adults infestation were investigated. The *A. officinarum* extract exhibited strong repellent and contact toxicities against *L. serricorne* adults. The toxicities enhanced significantly with the increasing treatment time and treatment dose. The mean percentage repellency value reached 91.3% at class V at the dose of 0.20 μL/cm² after 48 h of exposure. The corrected mortality reached over 80.0% at the dose of 0.16 μL/cm² after 48 h of exposure. The *A. officinarum* extract could significantly reduce *L. serricorne* infestation level against stored wheat flour. Particularly, the insect infestation was nil in wheat flour packaged with kraft paper bags coated with the *A. officinarum* extract at the dose of above 0.05 μL/cm². The naturally occurring *A. officinarum* extract could be useful for integrated management of *L. serricorne*.

Introduction

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), also called as the tobacco beetle, is one of the most destructive insects of many stored food products including flours, dried fruits such as raisins and dates, cocoa, cereals, herbs, spices, nuts, dry pet foods, tobacco and other products worldwide [1–3]. Methyl bromide and phosphine fumigation had been an effective method to control stored product insects in the world [4,5]. However, methyl bromide has been restricted due to its depleting the ozone layer [6,7]. Phosphine fumigation has almost been the only method to control stored product insects [8]. Repeated use of phosphine fumigation for decades has resulted in serious negative effects, such as environmental threat, pesticide residue in food, lethal effects on non-target organisms and insecticide resistance [9–12], which could threaten the future use of phosphine [13,14]. Therefore, it is urgent to develop alternative control methods [4,15]. Plant-derived insecticides might be potential replacement candidates [16–18].

In fact, much effort has been focused on plant-derived materials as alternatives to synthetic pesticides or as lead compounds for many years [19,20]. Moreover, many plant-derived
materials have been recently researched as insect-resistant packaging materials based on their strong repellent activity. Natural botanical antifeedants, citronella, protein-enriched pea flour, *Citrus reticulata* oil, *Pimpinella anisum* oil, *Anethum graveolens* oil, *Allium sativum* oil and *Ocimum basilicum* oil, etc. have been confirmed to have strong potential for preventing packaging materials from insects infestation, and some of them are being applied on packaging materials for their effect on avoiding insect penetration [21–24].

*Alpinia officinarum*, a traditional Chinese herbal plant, is widely cultivated in southern China, and its pungent and aromatic rhizome is usually used to treat epigastric pains, nausea, indigestion, duodenal ulcer, gastroenteritis and tinea versicolor infection due to its antioxidant, anti-inflammatory, anticancer, anti-proliferative and antiemetic activities [25–27]. Like other traditional Chinese herbal plants, *A. officinarum* has been used as a traditional method by farmers to protect stored grains from insect infestation for many centuries in China [28–30]. Here, we firstly evaluated the potential repellent and contact activities of *A. officinarum* rhizome extract against *L. serricorne* adults, and its ability to prevent *L. serricorne* adults from infesting stored wheat flour in the laboratory.

**Materials and Methods**

**Insects**

Laboratory cultures of the cigarette beetle, *L. serricorne*, were maintained on sterilized diet (wheatfeed/yeast, 95:5, w/w) at 27 ± 2°C, 75 ± 5% r.h. and a 12:12 light:dark photoperiod. Healthy *L. serricorne* adults (three–five days old) were used for bioassays.

**Preparation of the extract**

The *A. officinarum* rhizome is a common Chinese medicine plant grown in China. We bought it from a farmer at Xuwen (20.2335, 110.2110), Guangdong, South China, October 2012. No specific permissions are required for getting *A. officinarum* rhizome in China. The *A. officinarum* rhizome was identified by the Biology Department of Zhengzhou University, then dried at room temperature and finely ground to powder. Each 50 g of the powder was extracted by Soxhlet method with 250 mL anhydrous diethyl ether (analytical purity) until the distilled liquid was colorless. The solvent was evaporated under vacuum in a rotary evaporator, then the extract (in the remainder of this paper referred to as “*Alpinia* extract”) was stored in airtight fuscous glassware at 4°C.

**Repellency bioassay**

The repellent effect of the *Alpinia* extract against *L. serricorne* adults was evaluated using the area preference method. Test areas consisted of Whatman No.1 filter paper cut in half (Φ12.5 cm). An aliquot of 1.54, 3.07, 6.14 and 12.28 μL of the *Alpinia* extract dissolved in 1 mL acetone (analytical purity) was evenly applied on half-filter paper discs using a micropipette corresponding to the doses of 0.025, 0.05, 0.10 and 0.20 μL/cm² respectively. The other half of the remaining filter paper was treated with 1 mL acetone alone and used as a control. The filter papers were air-dried for about 5 min to evaporate the solvent completely and full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. Each remade filter paper disc was tightly fixed on the bottom of a 12.5 cm diameter Petri dish daubed with polytetrafluoroethylene on the inside wall to prevent the insects from escaping. A filter paper disc with both halves treated with 1 mL acetone alone was tested as a blank control. Then 30 unsexed *L. serricorne* adults (3–5 days old) were released at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in incubators at 27 ± 2°C,
75 ± 5% r.h. and a 12:12 light:dark photoperiod. Each treatment was replicated four times and the number of insects present on the control \((N_c)\) and treated \((N_t)\) areas of the discs was recorded after 12, 24, 36, and 48 h, respectively.

Percentage repellency (PR) values were calculated as follows:

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

The mean percentage repellency value was calculated and assigned to repellency classes from 0 to V [31]: class 0 (PR < 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%), class V (PR = 80.1–100%). The extreme PR values express two extreme conditions: 0 showing no repellency, and 100 showing the strongest repellency.

**Contact toxicity**

An aliquot of 0.6, 1.2, 2.4 and 4.8 μL of *Alpinia* extract dissolved in 0.4 mL acetone (analytical purity) was evenly applied to a Whatman No.1 filter paper (Ø6 cm) corresponding to the doses of 0.02, 0.04, 0.08 and 0.16 μL/cm², respectively. Applying 0.4 mL acetone alone (the dose of 0 μL/cm²) to a Whatman No.1 filter paper (Ø6 cm) was taken as a control. Then, the filter paper was dried in air for 5 min prior to being closely fixed on the bottom of a clean Petri dish (Ø6 cm) by solid adhesive. The Petri dish was in advance daubed with polytetrafluoroethylene on the inside wall to avoid the insects escaping. Thirty treated unsexed *L. serricorne* adults (3–5 days old) were introduced into the Petri dish. The Petri dish was covered and kept in incubators at 27 ± 2°C and 75 ± 5% r.h. and the number of dead insects was recorded after 12, 24, 48 and 72 h. Insects showing any movement were considered to be alive when prodded with a camel’s hair brush. Four replicates were conducted.

**Infestation test**

Kraft paper bags (80 g/m²) and nonwoven cloth bags were made by hand, and their specification was 8 cm × 15 cm. Bags were carefully checked for presence of pores prior to infestation test. The kraft paper bags were sealed with gluewater (Chenguang, Shanghai Chenguang Stationery Co., Ltd.), nonwoven cloth bags were sealed by carefully stitching. Each bag was coated with *Alpinia* extract at the doses of 0 (as a control), 0.025, 0.05, 0.10 and 0.20 μL/cm² respectively. A bag with 50 g of whole wheat flour was put in a glass bottle (500 mL), then 20 unsexed *L. serricorne* adults were released into the glass bottle. The *L. serricorne* adults were outside of the packaged wheat flour. Subsequently, the glass bottles were covered with pieces of cloth, tied with rubber bands and kept at 27 ± 2°C and 75 ± 5% relative humidity. The number of insects (live larvae and adults) in whole wheat flour was recorded after 45 days. Each treatment was replicated four times.

**Statistical analysis**

The percentage mortality was corrected by the Abbott formula [32]. The percentage mortality was determined and transformed to arcsine square-root values for repeated measures analysis of variance (ANOVA). The percentage repellency value of *Alpinia* extract against *L. serricorne* adults was also analyzed using repeated measures analysis of variance. The number of *L. serricorne* population in whole wheat flour packaged with nonwoven cloth bags and kraft paper bags coated with *Alpinia* extract was analyzed using two-way analysis of variance. Treatment means were compared and separated by Scheffe’s test at \(p = 0.05\). The LD₅₀ values were calculated using probit analysis. These analyses were performed using SPSS Version 16.0 software.
Results

Repellent activity
The repellent activity of *Alpinia* extract progressively increased with increasing exposure dose and exposure period (Table 1), while the *L. serricorne* adults randomly moved during the whole testing period in the blank control arenas. The mean percentage repellency value reached 91.3% at class V at the dose of 0.20 μL/cm² within 48 h of exposure (Table 2). The interaction dose × exposure time was not significant at *p* < 0.05 level (Table 3).

Contact toxicity
The contact toxicity of *Alpinia* extract significantly increased with increasing exposure dose (Table 4). The corrected mortality reached over 80.0% at the dose of 0.16 μL/cm² after 48 h of exposure (Table 5). The LD$_{50}$ value of *Alpinia* extract was 0.05 μL/cm² with the Confidence Interval 95% from 0.02 to 0.08 μL/cm² after 48 h of exposure. The regression line equation of *Alpinia* extract was $Y = 7.34 X + 1.80$, and the correlation coefficient (*r* value) was 0.98. The interaction dose × exposure time was not significant at *p*<0.05 level (Table 6).

---

Table 1. Repeated measures analysis of variance between subjects effects for the repellent activity of *Alpinia* extract against *L. serricorne* adults at the doses of 0.025, 0.05, 0.10 and 0.20 μL/cm² after 12, 24, 36 and 48 h exposure, respectively.

| Source          | df | Type III SS | Mean square | F-value | p-value |
|-----------------|----|-------------|-------------|---------|---------|
| Dose            | 3  | 18351.418   | 6117.139    | 17.76   | 0.0001  |
| Error           | 12 | 4132.728    | 344.394     |         |         |

doi:10.1371/journal.pone.0135631.t001

Table 2. The repellent activity of *Alpinia* extract against *L. serricorne* adults. Each datum in the table is percentage repellency (mean ± SE, %). The data in a column followed by different letters indicate significant difference tested by Scheffe’s test at *p* = 0.05. The same as below.

| Dose (μL/cm²) | Exposure time (h) |
|---------------|-------------------|
|               | 12                | 24                | 36                | 48                |
| 0.025         | 10.5 ± 3.2a       | 29.3 ± 5.1a       | 28.7 ± 7.3a       | 36.3 ± 4.5a       |
| 0.05          | 18.9 ± 2.6a       | 31.9 ± 6.1a       | 56.3 ± 6.2b       | 67.6 ± 6.4b       |
| 0.10          | 27.7 ± 3.1a       | 52.2 ± 3.6ab      | 55.2 ± 4.8b       | 76.6 ± 8.8bc      |
| 0.20          | 44.2 ± 4.2b       | 74.6 ± 8.9b       | 81.4 ± 9.9c       | 91.3 ± 10.4c      |

doi:10.1371/journal.pone.0135631.t002

Table 3. Repeated measures analysis of variance within subject effects for the repellent activity of *Alpinia* extract against *L. serricorne* adults at the doses of 0.025, 0.05, 0.10 and 0.20 μL/cm² after 12, 24, 36 and 48 h exposure, respectively.

| Source           | df | Type III SS | Mean square | F-value | p-value |
|------------------|----|-------------|-------------|---------|---------|
| Exposure time    | 3  | 15605.744   | 5201.914    | 53.08   | 0.0000  |
| Dose × Exposure  | 9  | 1653.606    | 183.734     | 1.87    | 0.0880  |
| Error            | 36 | 3528.180    | 98.005      |         |         |

doi:10.1371/journal.pone.0135631.t003

Table 4. Repeated measures analysis of variance between subjects effects for the contact toxicity of *Alpinia* extract against *L. serricorne* adults at the doses of 0.02, 0.04, 0.08 and 0.16 μL/cm² after 12, 24, 48 and 72 h exposure, respectively.

| Source           | df | Type III SS | Mean square | F-value | p-value |
|------------------|----|-------------|-------------|---------|---------|
| Dose             | 3  | 49843.386   | 12460.845   | 147.15  | 0.0000  |
| Error            | 15 | 1270.200    | 84.680      |         |         |

doi:10.1371/journal.pone.0135631.t004
**Infesting test**

*Alpinia* extract significantly prevented *L. serricorne* adults from infesting packaged wheat flour (Table 7). The higher the treated dose of *Alpinia* extract, the fewer *L. serricorne* that occurred in the packaged wheat flour after 45 days storage. Specially, the insect infestation was nil in wheat flour packaged with kraft paper bags coated with *Alpinia* extract at the doses of above 0.05 μL/cm\(^2\). The interaction dose × packaging bag was significant at \(p<0.05\) level (Table 8).

**Discussion**

In the present study, *Alpinia* extract showed strong repellent and contact activities against *L. serricorne* adults, and could significantly protect packaged wheat flour from *L. serricorne*. 

---

**Table 5. The contact toxicity of *Alpinia* extract against *L. serricorne* adults.** Each datum in the table is corrected mortality (mean ± SE, %). Mean ± SE mortality on control Petri dish for different doses ranged from 0.0 ± 0.0 to 4.3 ± 2.3%.

| Dose (μL/cm\(^2\)) | Exposure time (h) | 12 | 24 | 48 | 72 |
|---------------------|-------------------|----|----|----|----|
|                     |                   | 16.1 ± 3.8a | 20.7 ± 4.4a | 21.7 ± 4.4a | 27.9 ± 2.1a |
| 0.02                |                   | 30.5 ± 5.2b | 41.4 ± 3.9b | 41.7 ± 3.9b | 43.0 ± 3.6b |
| 0.04                |                   | 33.9 ± 3.3b | 62.9 ± 5.7c | 64.4 ± 6.3c | 65.8 ± 7.8c |
| 0.08                |                   | 53.4 ± 6.1c | 79.3 ± 8.6d | 80.8 ± 9.8d | 83.3 ± 9.3d |

**Table 6. Repeated measures analysis of variance within subject effects for the contact toxicity of *Alpinia* extract against *L. serricorne* adults at the doses of 0, 0.02, 0.04, 0.08 and 0.16 μL/cm\(^2\) after 12, 24, 48 and 72 h exposure, respectively.**

| Source             | df  | Type III SS | Mean square | F-value | p-value |
|--------------------|-----|-------------|-------------|---------|---------|
| Exposure time      | 4   | 3932.386    | 1310.795    | 77.554  | 0.0000  |
| Dose × Exposure time| 12  | 1930.451    | 160.871     | 0.277   | 0.9913  |
| Error              | 45  | 429.082     | 9.535       |         |         |

**Table 7. The number of *L. serricorne* population in whole wheat flour packaged with nonwoven cloth bags and kraft paper bags after 45 days storage at 27 ± 2°C and 75 ± 5% relative humidity.**

| Dose (μL/cm\(^2\)) | Nonwoven cloth bags | Kraft paper bags |
|---------------------|---------------------|------------------|
| 0                   | 120.0 ± 18.5a       | 2.3 ± 0.3a       |
| 0.025               | 31.5 ± 3.3b         | 0.3 ± 0.3b       |
| 0.05                | 10.6 ± 2.8c         | 0.0 ± 0.0c       |
| 0.10                | 5.7 ± 3.7d          | 0.0 ± 0.0c       |
| 0.20                | 3.6 ± 1.3e          | 0.0 ± 0.0c       |

**Table 8. Two-way ANOVA analysis for the number of *L. serricorne* in whole wheat flour packaged with nonwoven cloth bags and kraft paper bags after 45 days storage at 27 ± 2°C and 75 ± 5% relative humidity.**

| Fixed effects       | df  | F-value | p-value |
|---------------------|-----|---------|---------|
| Dose                | 4   | 1165.633| 0.0000  |
| Packaging bag       | 1   | 11348.64| 0.0000  |
| Dose × Packaging bag| 4   | 308.238 | 0.0000  |
| Error               | 30  |         |         |
infestation. Specially, eucalyptol has been determined as the main chemical composition of *A. officinarum* essential oil recently [27], so it will be very useful to further evaluate the repellent and toxic effect of eucalyptol and other compositions on *L. serricorne*. There are other plant extracts or essential oils with obvious toxicities against *L. serricorne* adults. The extracts of *Agastache rugosa* whole plant, *Cinnamomum cassia* bark, *Illicium verum* fruit and *Foeniculum vulgare* fruit as well as horseradish (*Cochleria aroracia*), mustard (*Brassica juncea*) and cinnamon (*C. cassia*) oils have strong fumigant toxicities against *L. serricorne* adults [4]. Moreover, many plant extracts and their constituents have been studied to possess potential as alternative compounds to currently used synthetic insecticides for the management of populations of stored-product insects [20,30,33–36].

In addition, the present results showed that *Alpinia* extract was repellent enough to reduce insect immigration into packaged wheat flour when coated on nonwoven cloth bags and kraft paper bags. Furthermore, the *Alpinia* extract is considered to be safe for human being and the environment because it has been a Chinese traditional pharmaceutical agent for generations. Therefore, *Alpinia* extract had the strong potential to be used in the preparation of insect-resistant and biodegradable packaging materials.

Sound packaging material is an important defence line to protect the stored product from insect infestation during the storage period. Most stored-product insects can effectively find wheat flour by the clue of odour emitted from stored wheat flour, and then enter stored-flour by penetrating through the packaging materials or existing holes in the packaging materials [37–40]. Hence, applying insect repellents to food packaging materials has an important practical interest [23].

Usually, insect repellents are often used to improve the packaging material and design for preventing insects from entering packages by modifying the behavior of insects [22,38,41,42]. In fact, some insect repellents have been approved for use as a treatment for insect-resistant packaging in the USA, such as pyrethrins synergized with piperonyl butoxide [43] and methyl salicylate [44]. (E)-2-hexenal, which has potent repellent activity against stored grain insects, is generally used as a flavoring compound by food industries and is commonly recognized as safe by the U.S. Food and Drug Administration [45].

Although the insect infestation of packaged wheat flour treated with *Alpinia* extract has been reduced to the extremely low level in the present study, any infestation of packaged food is unacceptable to consumers. Therefore, it is necessary to determine whether infestation can be completely prevented by using *Alpinia* extract. Of course, the toxic effect of *Alpinia* extract on *L. serricorne* and its application on the insect-resistant packaging materials depend on several factors among which are the treatment doses of the plant extract, applied methods and the developing stages of the insect, and so on. Thus, the proper formulation, suitable dose, reasonable application strategy and the effect of environmental factors, as well as composition analysis of *Alpinia* extract deserve to be further researched, so that *Alpinia* extract can be exploited for effectively protecting the stored product from infestations by *L. Serricorne* in practice.

**Acknowledgments**

This research was supported by the National Research Plan for the High-tech R & D Program during the Twelfth Five-year Plan Period (National 863 plan, No. 2012AA101705-2), Plan of Nature Science Fundamental Research in Henan University of Technology (No. 11JCYJ01), Key Technologies R & D Program of the Education Department of Henan Province (No. 12A210003) and the Basic and Cutting-edge Technology Research Projects of Henan Province (No. 2015).
Author Contributions
Conceived and designed the experiments: JL. Performed the experiments: DM. Analyzed the data: JL DM. Wrote the paper: JL.

References
1. Papadopoulos SC, Athanasiou CG. Lariophagus distinguendus (F.) (Hyme., Chalcidoidea, Pteromalidae), an ectoparasitoid of Lasioderma serricorne (F.) (Col., Anobiidae), found for the first time in tobacco stores in Greece. J. Pest Sci. 2004; 77: 183–184.
2. Abdelghany AY, Awadalla SS, Abdel-Baky NF, El-Syrafi HA, Fields PG. Stored-product insects in botanical warehouses. J. Stored Prod. Res. 2010; 46: 93–97.
3. Fardisi M, Mason LJ. Influence of temperature, gender, age, and mating status on cigarette beetle (Lasioderma serricorne (F.)) (Coleoptera: Anobiidae) flight initiation. J. Stored Prod. Res. 2013; 52: 93–99.
4. Kim S, Park C, Ohh MH, Cho HC, Ahn YJ. Contact and fumigant activity of aromatic plant extracts and essential oils against Lasioderma serricorne (Coleoptera: Anobiidae). J. Stored Prod. Res. 2003; 39: 11–19.
5. Kljajic P, Peric I. Susceptibility to contact insecticides of granary weevil Sitophilus granarius (L.) (Coleoptera: Curculionidae) originating from different locations in the former Yugoslavia. J. Stored Prod. Res. 2006; 42: 149–161.
6. Butler JH, Rodriguez JM. Methyl bromide in the atmosphere. In: Bell CH, Price N, Chakrabarti B, editors. The Methyl Bromide Issue, vol. 1. Wiley, West Sussex, England; 1996. pp. 27–90.
7. MBTOC. Methyl Bromide Technical Options Committee: Assessment of alternatives to methyl bromide. Nairobi, Kenya, United Nations Environment Programme, Ozone Secretariat, 1998. pp. 374.
8. White NDG, Leesch JG. Chemical Control. In: Subramanyam B, Hagstrum DW, editors. Integrated Management of Insects in Stored Products, Marcel Dekker, New York; 1995. pp. 287–330.
9. Rajendran S, Narasimhan KS. Phosphate resistance in the cigarette beetle Lasioderma serricorne (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. Int. J. Pest Manag. 1994; 40: 207–210.
10. Zettler JL, Keever DW. Phosphate resistance in cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. J. Econ. Entomol. 1994; 87: 546–550.
11. Liu ZL, Ho SH. Bioactivity of the essential oil extracted from Eovida rutaecarpa Hook f. et Thomas against the grain storage insects, Sitophilus zeamais Motsch. and Tribolium castaneum (Herbst). J. Stored Prod. Res. 1999; 35: 317–328.
12. Jovanović Z, Kostić M, Popović Z. Grain-protective properties of herbal extracts against the bean weevil Acanthoscelides obtectus Say. Ind. Crop. Prod. 2007; 26: 100–104.
13. Bell CH, Wilson SM. Phosphate tolerance and resistance in Trogoderma granarium Everts (Coleoptera: Dermestidae). J. Stored Prod. Res. 1995; 31:199–205.
14. Daglish GJ, Collins PJ. Improving the relevance of assays for phosphate resistance. In: Jin X, Liang Q, Butler JH, Rodriguez JM, editors. Proceedings of the Seventh International Working Conference on Stored Product Protection, 14–19 October 1998, Beijjing, China. Sichuan Publishing House of Science and Technology, Chengdu, China, 1999. pp. 584–593.
15. Tapondjoua AL, Adlerb C, Fontemc DA, Boudaa H, Reichmuth C. Bioactivity of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against Sitophilus zeamais Motschulsky and Tribolium confusum du Val. J. Stored Prod. Res. 2005; 41: 91–102.
16. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 2006; 51: 45–66. PMID: 16332203
17. Isman MB. Botanical insecticides: for richer, for poorer. Pest Manag. Sci. 2008; 64: 8–11. PMID: 18022796
18. Paul UV, Lossini JS, Edwards PJ, Hilbeck A. Effectiveness of products from four locally grown plants for the management of Acanthoscelides obtectus (Say) and Zabrottes subfasciatus (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. J. Stored Prod. Res. 2009; 45: 97–107.
19. Hill JM, Schoonhoven AV. The use of vegetable oils in controlling insect infestations in stored grains and pulses. Recent Advances in Food Science and Technology. 1981; 1: 473–481.
20. Shaaya E, Kostjukovski M, Elberg J, Sukprakarn C. Plant oils as fumigants and contact insecticides for the control of stored-product insects. J. Stored Prod. Res. 1997; 33: 7–15.
21. Bioszyk E, Nawrot J, Harmatha J, Drozda D, Chmielewitz Z. Effects of antifeedants of plant origin in protection of packaging materials against storage insects. J. Appl. Entomol. 1990; 110: 96–100.

22. Hou XW, Field P, Taylor W. The effect of repellents on penetration into packaging by stored-product insects. J. Stored Prod. Res. 2004; 40: 47–54.

23. Wong KY, Signal FA, Campion SH, Motion RL. Citronella as an insect repellent in food packaging. J. Agric. Food Chem. 2005; 53: 4633–4636. PMID: 15913337

24. Mikhaiel AA. Potential of some volatile oils in protecting packages of irradiated wheat flour against Ephestia kuehniella and Tribolium castaneum. J. Stored Prod. Res. 2011; 47: 357–364.

25. Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant extracts for antioxidant activity, Life Sci. 2003; 73: 167–179. PMID: 12738032

26. Fan GJ, Kang YH, Han YN, Han BH. Platelet-activating factor (PAF) receptor binding antagonists from Alpinia officinarum. Bioorg. Med. Chem. Lett. 2007; 17: 6720–6722. PMID: 17964782

27. Zhang BB, Dai Y, Liao ZX, Ding LS. Three new antibacterial active diarylheptanoids from Alpinia officinarum. Fitoterapia 2010; 81:948–952. doi: 10.1016/j.fitote.2010.06.015 PMID: 20600688

28. Yang RZ, Tang CS. Plants used for pest control in China: a literature review. Econ. Bot. 1988; 42: 376–406.

29. Wang J, Zhu F, Zhou XM, Niu CY, Lei CL. Repellent and fumigant activity of essential oil from Artemisia vulgaris to Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 2006; 42: 339–347.

30. Liu ZL, Goh SH, Ho SH. Screening of Chinese medicinal herbs for bioactivity against Sitophilus zeamais Motschulsky and Tribolium castaneum (Herbst). J. Stored Prod. Res. 2007; 43: 290–296.

31. Juliana G, Su HCF. Laboratory studies on several plant materials as insect repellents for protection of cereal grains. J. Econ. Entomol. 1983; 76:154–157.

32. Abbott WS. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 1925; 18: 265–267.

33. Lee BH, Annis PC, Tumaalii F, Choi WS. Fumigant toxicity of essential oil from the Mytaceae family and 1,8-cineole against 3 major stored-grain insects. J. Stored Prod. Res. 2004; 40: 553–564.

34. Lee BH, Choi WS, Lee SE, Park BS. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, Sitophilus oryzae (L.). Crop Prot. 2001; 20: 317–320.

35. Lee SE, Lee BH, Choi WS, Park BS, Kim JG, Campbell BC. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, Sitophilus oryzae (L.). Pest Manag. Sci. 2001; 57: 548–553. PMID: 11407032

36. Cosmi S, Rossi E, Cioni PL, Canale A. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: evaluation of repellency against Sitophilus zeamais Motschulsky, Cryptolestes ferrugineus (Stephens) and Tenebrio molitor (L.). J. Stored Prod. Res. 2009; 45: 125–132.

37. Barrer PM, Jay EG. Laboratory observations on the ability of Ephestia cautella (Walker) (Lepidoptera: Phycitidae) to locate and to oviposit in response to a source of grain odour. J. Stored Prod. Res. 1980; 16: 1–7.

38. Mullen MA. Rapid determination of the effectiveness of insect resistant packaging. J. Stored Prod. Res. 1994; 30: 95–97.

39. Mowery SV, Mullen MA, Campbell JF, Broce AB. Mechanisms underlying sawtoothed grain beetle (Oryzaephilus surinamensis (L.)) (Coleoptera: Silvanidae) infestation of consumer food packaging materials. J. Econ. Entomol. 2002; 95: 1333–1336. PMID: 12539851

40. Allahvaisi S. Reducing insects contaminations through stored foodstuffs by use of packaging and repellency essential oils. Not. Bot. Hort. Agrobot. Cluj. 2010; 38: 21–24.

41. Highland HA. Insect infestation of packages. In: Baur FJ, editor. Insect Management for Food Processing. American Association of Cereal Chemists, St. Paul, MN; 1984. pp. 311–320.

42. Mullen MA, Mowery SV. Insect-resistant packaging. International Food Hygiene. 2000; 11: 13–14.

43. Highland HA. Protecting packages against insects. In: Gorham JR, editor. Management of Food-Industry Pests. Association of Official Analytical Chemists, Arlington, VA; 1991. pp. 345–350.

44. Radwan MN, Allin GP. Controlled-release insect repellent device. US Patent 5,688,509. 1997.

45. Germinara GS, Conte A, Cristofaro AD, Lecce L, Di Palma A, Rotundo G, Del Nobile MA. Electrophysiological and behavioral activity of (E)-2-hexenal in the granary weevil and its application in food packaging. J. Food Prot. 2012; 75: 366–370. doi: 10.4315/0362-028X.JFP-11-142 PMID: 22289599