Fumigant activity of *Elsholtzia stauntonii* extract against *Lasioderma serricorne*

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The repeated use of phosphine over decades for the control of the cigarette beetle (*Lasioderma serricorne*), a significant stored-product insect worldwide, has led to serious negative effects, including strong insecticide resistance, disruption of biological control by natural enemies, and environmental and human health concerns. As an environmentally friendly alternative to synthetic pesticides, plant-derived pesticides have been the focus of much research. We investigated the fumigant activity of whole plant extracts of *Elsholtzia stauntonii*, a Chinese mint shrub, against the adult, larval, pupal and egg stages of *L. serricorne*. *E. stauntonii* extracts exhibited strong fumigant toxicity against *L. serricorne*; larvae and adults were more susceptible to this toxicity than were eggs and pupae. The toxicity significantly increased with increasing dosage. The corrected mortality of larvae, adults, pupae and eggs reached 99.32%, 97.97%, 44.67% and 33.33%, respectively, at a dosage of 40 µL/L air after 48 h of exposure. The declining order of susceptibility of different developmental stages of *L. serricorne* to *E. stauntonii* extracts, as indicated by the concentration at which 50% of the insects died (LC_{50}), was as follows: larvae (LC_{50}= 8.82 µL/L air), adults (LC_{50}= 10.99 µL/L air), pupae (LC_{50}= 45.96 µL/L air) and eggs (LC_{50}= 84.57 µL/L air). The results suggest that *E. stauntonii* extracts show promise as a fumigant for the control of *L. serricorne*.

Introduction

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), is one of the most serious pests of stored tobacco, tobacco products, cereal grains and processed foods throughout the world. Currently, control of *L. serricorne* is primarily dependent upon intensive use of phosphine.[1,2] However, the repeated use of phosphine for decades has led to serious problems including insecticide resistance, disruption of biological control by natural enemies, environmental and human health concerns, the rising cost of production and lethal effects on non-target organisms.[3,4] Development and implementation of alternative control strategies and integrated pest management systems have recently been considered as the only solution to combat these increasingly insecticide-resistant insect pests.

Plant-based insecticides may provide potential alternatives to currently used insect-control agents. They are a natural source of bioactive chemicals with complicated mechanisms of action, which make it difficult for the insect pests to produce resistance against them. In addition, plant-based insecticides are readily biodegradable, often less toxic to mammals, and are less or not dangerous to the environment if used in suitable amounts.[5,6] Particularly because of their unacceptably high cost and the difficulty of researching and developing new synthetic insecticides, recent research has focused on natural product alternatives for pest control in developing countries, as well as for organic food production in industrialised countries.[5,6,7,8]

Many Chinese herbal plants are potential sources of pesticides and have exhibited potent toxic bioactivity to stored-product insects.[7,9] In fact, as a traditional Chinese herbal plant, *Elsholtzia stauntonii* Benth (Lamiales: Lamiaceae) has also been used as a traditional method by farmers to protect stored products from insect infestation in China for many years. However, insecticidal activity of essential oils from *E. stauntonii* against *L. serricorne* has not been investigated thus far. We therefore evaluated the potential fumigant activity of essential oils extracted from whole *E. stauntonii* plants against eggs, larvae, pupae and adults of *L. serricorne* in the laboratory.

Materials and methods

Insects

Cultures of the cigarette beetle, *L. serricorne*, were maintained in the laboratory at the Institute of Stored Product Insects of Henan University of Technology without exposure to any insecticide. Insects were reared on a sterilised diet (wheatfeed: yeast, 95:5, w/w) and kept under the following conditions: a temperature of 27 ± 2 °C, a relative humidity of 75 ± 5% and a photoperiod of 12h:12h. Different developmental stages were randomly chosen from healthy individuals for bioassays.

Preparation of the extract

The *E. stauntonii* whole flowering plant was collected in Henan, central China in October 2008. The plant was identified by the Biology Department of Zhengzhou University, then dried at room temperature and finely ground to powder. Successive 50 g quantities of the powder were extracted by the Soxhlet method with 250 mL anhydrous diethyl ether until the distilled liquid was colourless. The solvent was evaporated under vacuum in a rotary evaporator. The plant extract was then stored in airtight fuscous glassware in a refrigerator at 4 °C.

Fumigant activity

Larvae, pupae and adults

Fumigant activity against *L. serricorne* was investigated by exposing 30 larvae (10–12 days old), 30 pupae (1–2 days old) and 30 unsexed adults (5–7 days old) to *E. stauntonii* extracts in a 250-mL flask tightly sealed with a rubber stopper. Aliquots of 0 µL, 1.25 µL, 2.5 µL, 5 µL and 10 µL of the *E. stauntonii* extract dissolved in 1 mL acetone (analytical purity), corresponding to dosages in air of 0 µL/L (as a control), 5 µL/L, 10 µL/L, 20 µL/L and 40 µL/L, were evenly applied on a Whatman No.1 filter paper strip (7 cm × 9 cm), which was then dried in air for 10 min prior to being fixed on the rubber stopper by a staple at one end. The rubber stopper was tightly stuffed to keep the filter paper suspended in the top of the flask. Care was taken to avoid the filter paper from coming into contact with the flask wall. The flask was placed in an incubator at 27 ± 2 °C and 75 ± 5% relative humidity. Five replicates were conducted. After 48 h of exposure, insects were moved into clean vials. The mortality of *L. serricorne* larvae and adults was determined immediately. Insects showing any movement were considered to be alive. The *L. serricorne* pupae were kept in an insect culture environment. The number of pupae that reached adulthood was recorded every day for the following 10 days. Pupae that did not reach the adult stage were considered to be dead.

Eggs

The eggs (0–24 hours old) were exposed to the *E. stauntonii* extract on round cloning plates with diameters of 9.5 cm. Each cloning plate had 54 microwells; each microwell was 6 mm in diameter and 5 mm deep. One egg was transferred into each numbered microwell using a moistened fine brush. A total of 30 eggs was used for one replicate. The plates were placed in 5-L glass jars with screw-top lids. *E. stauntonii* extract was applied on a Whatman No.1 filter paper strip (7 cm × 9 cm) which was attached to the lower side of the jar lid by adhesive tape. The tested dosages in air were 0 µL/L (control), 5 µL/L, 10 µL/L, 20 µL/L and 40 µL/L. After 48 h of exposure, the plates were taken out of the jars and placed in an insect culture environment. Eggs were observed for hatching by a stereomicroscope. The number of eggs that hatched into larvae was recorded every day for the following 10 days. Unhatched eggs were considered to be dead. All experiments were repeated five times.

Statistical analysis

Percentage mortality was corrected using the Abbott formula.[10] The percentage mortality was determined and transformed to arcsine square-root values for an analysis of variance. Treatment means were compared and separated by Scheffe's test at $p = 0.05$.[11] The LC_{50} values (the concentration at which 50% of the insects died) were calculated using probit analysis.[12]

Results

Elsholtzia stauntonii extracts showed strong fumigant activity against *L. serricorne* and the toxicity progressively increased with increasing exposure dosage ($p < 0.05$; Tables 1 and 2). The responses varied significantly across developmental stages: larvae and adults were far more susceptible than eggs and pupae. At a dosage of 40 µL/L air, the corrected mortality of larvae, adults, eggs and pupae reached 99.32%, 97.97%, 44.67% and 33.33%, respectively. The declining order of susceptibility of different developmental stages of the insects to the *E. stauntonii* extract was as follows: larvae (LC_{50}= 8.82 µL/L air), adults (LC_{50}= 10.99 µL/L air), pupae (LC_{50}= 45.96 µL/L air) and eggs (LC_{50}= 84.57 µL/L air).

Discussion

Elsholtzia stauntonii showed promise as a fumigant for the control of *L. serricorne*. The toxic effect of the *E. stauntonii*

TABLE 1: Fumigant toxicity of *Elsholtzia stauntonii* whole plant extract against eggs, larvae, pupae and adults of *Lasioderma serricorne* after a 48-h exposure period.

Dosage (µL/L)	Eggs (%)	Larvae (%)	Pupae (%)	Adults (%)
5	6.00 ± 1.25^{d}	23.65 ± 0.83^{d}	9.33 ± 1.25^{d}	16.22 ± 1.26^{d}
10	11.33 ± 0.82^{c}	60.14 ± 1.66^{c}	22.67 ± 1.25^{c}	54.73 ± 1.72^{c}
20	22.67 ± 0.67^{b}	75.68 ± 2.48^{b}	34.67 ± 0.82^{b}	67.57 ± 1.72^{b}
40	33.33 ± 1.05^{a}	99.32 ± 0.68^{a}	44.67 ± 1 .70^{a}	97.97 ± 1.35^{a}

Each value represents the mean corrected mortality (± s.e.) of five replicates (n = 150). Means within a column followed by the same superscript letters are not significantly different at $p < 0.05$.

TABLE 2: Mortality of tested insect stages after 48 h of exposure to *Elsholtzia stauntonii* whole plant extract at different dosages.

Developmental stage	Regression line equation	LC_{50}† (µL/L)	Confidence limit of LC_{50} (µL/L)	Chi-square (χ^2)	Chi-square p-value
Egg	Y = 1.27X + 2.56	84.57	53.50~191.11	2.74	0.99
Larva	Y = 2.75X + 2.40	8.82	7.70~9.88	15.54	0.62
Pupa	Y = 1.25X + 2.92	45.96	33.66~75.82	4.13	0.99
Adult	Y = 2.86X + 2.02	10.99	9.82~12.18	20.52	0.30

†, LC_{50}, the concentration at which 50% of the insects died.

extract was dependent on several factors, such as the treatment dosage and the developmental stage of the insect.

Similarly, extracts of *Agastache rugosa* whole plant, *Cinnamomum cassia* bark, *Illicium verum* fruit and *Foeniculum vulgare* fruit as well as cinnamon (*C. cassia*), horseradish (*Cocholeria aroracia*) and mustard (*Brassica juncea*) oils showed good fumigant activity against *L. serricorne* adults.[13] *E. stauntonii* extracts have also shown strong fumigant activity against adults of the sawtoothed grain beetle (*Oryzaephilus surinamensis*) and rice weevil (*Sitophilus oryzae*), with percentage mortalities of 98.0% and 54.7%, respectively, at a dosage of 160 µL/L air.[14] Moreover, many essential oils and their constituents have been reported to possess potential as alternative compounds for the currently used insect-control agents for the management of populations of stored-product insects.[4,15,16,17,18]

Thus, the *E. stauntonii* extract has great potential as a fumigant against *L. serricorne* for integrated pest management programmes. As a traditional pharmaceutical agent, an *E. stauntonii* extract is also considered to be safe for humans and the environment. The appropriate use of the *E. stauntonii* extract as a fumigant for the control of *L. serricorne* in practice, as well as the plant extract pure constituent levels and structure–activity relationships against different developmental stages of *L. serricorne*, may warrant further investigation.

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Competing interests

We declare that we have no financial or personal relationships which may have inappropriately influenced us in writing this paper.

Authors' contributions

J-H.L. was the project leader and was responsible for the experimental design. J-H.L. also made conceptual contributions and wrote the manuscript. X-H.S. and J-J.Z. performed most of the experiments.

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