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Repellency of essential oils against the cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae)

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Abstract

The behavioral responses of the female cigarette beetle to 57 plant essential oils commonly used for the flavoring of foods and cosmetics, were investigated with an olfactometer. Forty-eight of these essential oils exhibited repellency and only patchouli oil exhibited attractiveness at a dose of 1 μl. The repellency of the 28 essential oils that strongly repelled the beetles at this dose were furthermore evaluated at a dose of 0.1 μl. Six plant oils (shiso, savory, cassia, thyme, peppermint and *Litsea cubeba* oils) strongly repelled the beetles at this dose. Then, the dose-responses of these six oils on repellency against the beetles were investigated. These oils exhibited attractiveness at lower doses than each threshold except shiso oil, which did not attract the beetles at any doses tested. The first major components of these six oils repelled the beetles at a dose of 1 μl or 1 mg. However the repellency of these components was less than that of each essential oil. The repellency of shiso oil in the presence of cured tobacco odor, which attracted the beetles, was further evaluated. Shiso oil repelled the beetles at a dose of 10 μl even in the presence of the tobacco odor.

Key words: Cigarette beetle; repellent; shiso oil; essential oil; olfactometer

INTRODUCTION

The cigarette beetle, *Lasioderma serricorne* (Fabricius), damages a wide range of stored products including cured tobacco leaves, cigarettes, cocoa beans, cereals, cereal products, oilseeds, pulses, spices, dried fruits, and some animal products (Hill, 1990). This pest is the most serious insect threat to stored tobacco (Ryan, 1995) and is controlled mainly by sanitization, remodeling equipment and fumigation at present. There are few control methods that can directly prevent the beetles from invading the products. Repellents must be useful tools in an integrated management program of this pest; however, no working repellent has been available.

The repellency against stored product pests has already been demonstrated for some plant essential oils and their components: the essential oil of *Evodia rutaecarpa* Hook f. et Thomas against *Sitophilus zeamais* Motsculsky and *Tribolium castaneum* (Herbst) (Liu and Ho, 1999); the essential oil of *Artemisia annua* L. against *T. castaneum* (Tripathi et al., 2000); and zimtaldehyde, eugenol and thymol against *T. confusum* (Ojimelukwe and Adler, 1999). Behavioral responses of *L. serricorne* to plant materials and their chemical components has also been reported: the attractiveness of the pest's favored food sources such as cured tobacco leaves, toasted coffee meal (Kohno et al., 1983), turmeric and fennel (Yadav and Tanwar, 1985; Jha and Yadav, 1991, 1998); and the repellency of some plant leaves such as *Rauwolfia canescens*, *Vinca rosea*, *Tamarindus indica*, *Euphorbia neriifolia*, *Acalypha hispida*, *Coleus barbatus*, and so on (Ambadkar and Khan, 1994) and of the crude leaf extracts of *Tecoma stans*, *Datura matel*, *V. rosea*, *C. barbatus* and *Vitex negundo* (Ambadkar and Khan, 1989) against the pest.

Females of the cigarette beetle attracted by odor of the stored products oviposit in these products. Larvae that hatched there feed on the products and damage them. If females of the beetle can be prevented from entering into the products, the risks of product losses caused by the beetles will be reduced. In this study, the repellency of common plant essential oils used for the flavoring of foods and cosmetics against females of the beetle was

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evaluated with an olfactometer in order to find the repellents that can be utilized in the control of *L. serricorne*.

MATERIALS AND METHODS

Insects. The cigarette beetles used for the tests were obtained from cultures that had been maintained for several years in our laboratory. They were reared in plastic cases (130 mm in diameter, 73 mm high) containing ca. 50 g of cured tobacco powder, at 27±2°C and 70±5% relative humidity. Four to 8-day-old adult female beetles were collected from the rearing cases and used for all experiments. Adult females were selected by the method described by Kohno (1982).

Plant essential oils. The essential oils shown in Table 1 were used in this study.

Chemicals. (−)-Perillaldehyde and cinnamaldehyde were supplied by Sigma-Aldrich Co. (St. Louis, Missouri). Carvacrol, γ-terpinene, thymol, (−)-menthol and citral were supplied by Wako Pure Chemical Ind. Ltd. (Osaka, Japan).

Bioassay. The olfactometer described by Kohno et al. (1983) was used in all the experiments (Fig. 1). A glass dish with a 133 mm inner diameter and a 41 mm height was furnished with eight glass tubes (choice tube, length: 25 mm; inner diameter: 5 mm) on the side wall. Each tube was plugged distally with a piece of nylon mesh. A pair of two connected glass jars (inner diameter: 38 mm; height: 60 mm) with two glass tubes (length: 15 mm; inner diameter: 4 mm) were connected to each glass tube of the glass dish. The outer glass jars were filled with activated charcoal and the inner ones were used as sample jars (hereafter referred as control or treatment jar). A 10 μl acetone solution of each sample was applied to a piece of filter paper (ADVANTEC, No. 2, 10×20 mm) and a paper was placed in each of the treatment jars. As a control, a piece of filter paper applied with acetone was placed in each of the control jars. When 10 μl of the sample was tested, 10 μl of the sample was directly applied to the filter paper, which was compared with the filter paper without treatment as the control. In the test for evaluating the attractiveness of cured tobacco, cured tobacco powder (2 g) was put in each of the treatment jars and no substance was put in any of the control jars. In the assays in the presence of tobacco odor, a piece of filter paper treated with the samples was placed on tobacco powder in each of the treatment jars and a piece of control filter paper was put in each of the control jars without tobacco powder. The treatment jars and the control jars were arranged alternately in all the experiments. An air pump was connected to a vent opening at the center of the upper lid of the olfactometer and the air was sucked out at a rate of 50 ml/min. *L. serricorne* has a propensity to enter a narrow space. Therefore, almost all of the beetles released in the olfactometer chose and entered the choice tube by chemotaxis. Fifty adult females were released in the glass ring (inner diameter: 25 mm; height: 15 mm) at the bottom center of the olfactometer. All the tests were carried out under light conditions at 24–26°C. The inner ceiling and the upper part of the inner wall were coated with polishing agent (CRC®, KURE POLY-

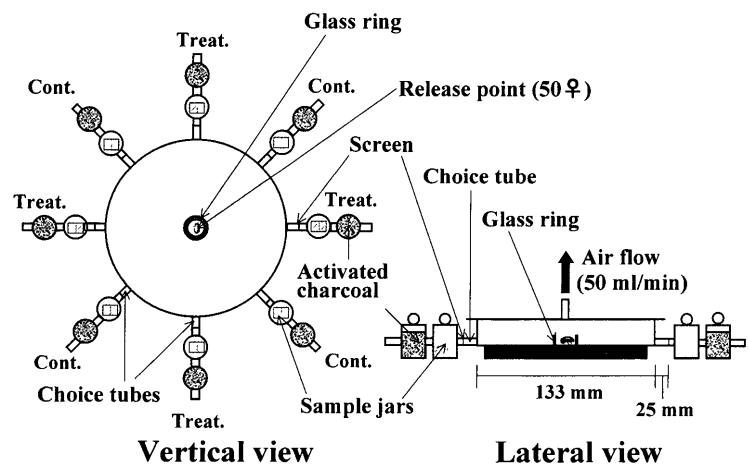

Fig. 1. Olfactometer used for evaluating the repellency of essential oils.

MATE, KURE Engineering Ltd., Tokyo, Japan) to prevent the beetles from climbing the wall. The number of beetles in the treatment and control choice tubes was counted 3 h after the beginning of the test and the total number of the beetles in the four treatment choice tubes was compared with that in the four control choice tubes. The olfactometer was washed with soapy water and the treatment and control choice tubes were alternated after every trial. Each experiment was replicated four times. The data of four replications were pooled, and then transformed to the excess proportion index (*EPI*) according to the following formulation:

$$EPI=(nt-nc)/(nt+nc)=2PT-1,$$
$$PT=nt/(nt+nc)$$

where *nt* and *nc* represent the total number of beetles in the treatment choice tubes and that in the control choice tubes, respectively, and *PT* represents the proportion of beetles in the treatment choice tubes (Sakuma and Fukami, 1985). The 95% fiducial limits of *PT* were calculated from the critical values of the variance ratio *F*, and then transformed into those of *EPI*. When the upper limit of 95% fiducial limit was below 0, it was concluded that the beetles were repelled. When the lower limit of 95% fiducial limit was above 0, it was concluded that the beetles were attracted. When the upper limit of 95% fiducial limit was 0 or above and its lower limit was 0 or below, it was concluded that the behaviors of the beetle were neutral.

RESULTS

Behavioral responses to plant essential oils

The response to the 57 plant essential oils is summarized in Table 1. Forty-eight of the essential oils repelled and patchouli oil attracted the beetles at a dose of 1 μl. The 28 essential oils that exhibited objective high repellency ($EPI \leqq -0.70$) at a dose of 1 μl were further evaluated at a dose of 0.1 μl. Twenty of these essential oils repelled the beetles, while laurel bay, pennyroyal and clary sage oils attracted the beetles. Shiso, savory, cassia, thyme, peppermint and *Litsea cubeba* oils exhibited an especially high repellency ($EPI \leqq -0.50$) at a dose of 0.1 μl. The dose-responses of these six oils on repellency against the beetles are shown in Fig. 2. Peppermint and *L. cubeba* oils attracted them at doses of 0.01 and 0.001 μl, and savory, cassia and thyme oils did so at a dose of 0.001 μl. Only shiso oil did not attract the beetles at any doses tested.

Behavioral responses to the first major components of shiso, savory, cassia, thyme, peppermint and *L. cubeba* oils

The contents of the main components of shiso, savory, cassia, thyme, peppermint and *L. cubeba* oils are shown in Table 2. The first main components of shiso, cassia, thyme, peppermint and *L. cubeba* oils are (−)-perillaldehyde, cinnamaldehyde, thymol, (−)-menthol and citral, respectively. The first main component of savory oil is carvacrol or γ-terpinene. These components repelled the beetles at a dose of 1 μl or 1 mg except γ-terpinene (Table 3). (−)-Perillaldehyde, carvacrol, cinnamaldehyde and thymol repelled the beetles at a dose of 0.1 μl or 0.1 mg, too, while citral attracted them at a dose of 0.1 μl and (−)-menthol neither repelled nor attracted them at a dose of 0.1 mg. At a dose of 0.01 μl or 0.01 mg, (−)-perillaldehyde, cinnamaldehyde, thymol and (−)-menthol attracted the beetles.

Behavioral responses to shiso oil in the presence of cured tobacco

The repellency of shiso oil was revealed even in the presence of cured tobacco powder, which possesses an attractive odor for this beetle (Table 4). The shiso oil reduced the attractiveness of cured tobacco at a dose of 1 μl and repelled the beetles even in the presence of cured tobacco at a dose of 10 μl.

DISCUSSION

L. serricorne is repelled by fresh and dried leaves of such plants as *Rauwolfia canescens*, *Vinca rosea* and *Tamarindus indica* (Ambadkar and Khan, 1994). However, olfactory responses of the beetle to plant essential oils and plant components have been poorly reported. This study revealed that many kinds of plant essential oils repelled the female beetles at higher doses. In particular, many essential oils from labiate or lauraceous plants strongly repelled the beetles at a dose of 1 μl. Furthermore, the essential oils that strongly

Table 1. List of the plant essential oils tested and their repellency against *Lasioderma serricorne* at the doses of 1 and 0.1 μl

Essential oil					*EPI*[c]	
Name	Plant source: Scientific name	Plant source: Family	Plant source: Extracted part[a]	Supplier[b]	1 μl dose	0.1 μl dose
Ajowan oil	*Carum ajowan*	Umbelliferae	S	F	−0.57 R[d]	
Angelica root oil	*Angelica archangelica*	Umbelliferae	R	F	−0.21 R	
Anise oil	*Pimpinella anisum*	Umbelliferae	S	F	−0.85 R	−0.36 R[d]
Basil oil	*Ocimum basilicum*	Labiatae	L, FL	S	−0.43 R	
Bergamot oil	*Citrus bergamia*	Rutaceae	P	F	−0.69 R	
Black pepper oil	*Piper nigrum*	Piperaceae	FR	T	−0.14 N	
Cardamon oil	*Elettaria cardamomum*	Zingiberaceae	FR	T	−0.38 R	
Carrot seed oil	*Doucus carota*	Umbelliferae	S	F	−0.24 R	
Cascarilla oil	*Croton eluteria*	Euphorbiaceae	B	F	−0.54 R	
Cassia oil	*Cinnamomum cassia*	Lauraceae	B	F	−0.84 R	−0.88 R
Cedar wood oil	*Juniperus virginiana*	Cupressaceae	HW, SD	F	0.03 N	
Celery seed oil	*Apium graveolens*	Umbelliferae	S	F	−0.17 R	
Citronella oil	*Cymbopogon nardus*	Gramineae	L	T	−0.75 R	−0.41 R
Clary sage oil	*Salvia sclarea*	Labiatae	WP	F	−0.83 R	0.16 A
Clove oil	*Eugenia caryophyllata*	Myrtaceae	C	T	−0.52 R	
Coriander oil	*Coriandrum sativum*	Umbelliferae	S	F	−0.95 R	−0.29 R
Cumin oil	*Cuminum cyminum*	Umbelliferae	S	F	−0.87 R	−0.07 N
Eucalyptus camaldulensis oil	*Eucalyptus camaldulensis*	Myrtaceae	L, T	T	−0.02 N	
Eucalyptus citriodora oil	*Eucalyptus citriodora*	Myrtaceae	L, T	T	−0.17 R	
Fennel oil	*Foeniculum vulgare*	Umbelliferae	FR	F	−0.71 R	−0.46 R
Geranium oil	*Pelargonium graveolens*	Geraniaceae	L, T	S	−0.65 R	
Ginger oil	*Zingiber officinale*	Zingiberaceae	RHI	T	−0.44 R	
Grapefruit oil	*Citrus paradis*	Rutaceae	P	F	−0.10 N	
Hiba oil	*Thujopsis dolabrata*	Cupressaceae	STB, R, SD	F	−0.02 N	
Ho oil	*Cinnamomum camphora*	Lauraceae	L, T	F	−0.86 R	−0.18 R
Hyssop oil	*Hyssopus officinalis*	Labiatae	L, FL	T	−0.20 R	
Juniper berry oil	*Juniperus communis*	Cupressaceae	FR	F	−0.47 R	
Laurel bay oil	*Laurus nobilis*	Lauraceae	L	F	−0.91 R	0.33 A
Lavender oil	*Lavandula officinalis*	Labiatae	L, FL	S	−0.67 R	
Lemon oil	*Citrus limone*	Rutaceae	P	F	−0.79 R	−0.45 R
Lemongrass oil	*Cymbopogon flexuosus*	Gramineae	L	F	−0.90 R	−0.28 R
Lime oil	*Citrus aurantifolia*	Rutaceae	P	F	−0.72 R	−0.35 R
Litsea cubeba oil	*Litsea cubeba*	Lauraceae	FR	T	−0.94 R	−0.50 R
Mace oil	*Myristica fragrance*	Myristicaceae	A	F	−0.78 R	0.01 N
Marjoram oil	*Origanum majorana*	Labiatae	WP	S	−0.71 R	−0.35 R
Mint oil	*Mentha arvensis*	Labiatae	WP	S	−0.88 R	0.10 N
Nutmeg oil	*Myristica fragrance*	Myristicaceae	S	F	−0.74 R	−0.37 R
Orange oil	*Citrus sinensis*	Rutaceae	P	T	−0.43 R	
Oregano oil	*Origanum vulagare*	Labiatae	L	T	−0.39 R	
Parsley leaf oil	*Petroselinum sativum*	Umbelliferae	L, STM	F	−0.09 N	
Patchouli oil	*Pogostemon patchouli*	Labiatae	L	T	0.29 A	
Pennyroyal oil	*Mentha pulegium*	Labiatae	L, FL, STM	S	−0.77 R	0.24 A
Peppermint oil	*Mentha piperita*	Labiatae	L	S	−0.84 R	−0.56 R
Petitgrain oil	*Citrus aurantium*	Rutaceae	L, T	F	−0.86 R	−0.34 R
Pimento oil	*Pimenta dioicalis*	Myrtaceae	FR	F	−0.77 R	−0.41 R
Pine needle oil	*Pinus* spp.	Pinaceae	L	F	−0.53 R	
Rose oil	*Rosa centifolia*	Rosaceae	FL	S	−0.62 R	
Rosemary oil	*Rosmarinus officinalis*	Labiatae	L, FL	S	−0.45 R	
Sage oil	*Salvia officinalis*	Labiatae	L	S	−0.79 R	0.02 N
Sandalwood oil	*Santalum album*	Santalaceae	HW, R	S	−0.09 N	

Table 1. (Continued)

Essential oil					EPI[c]	
Name	Plant source			Supplier[b]	1 μl dose	0.1 μl dose
	Scientific name	Family	Extracted part[a]			
Savory oil	*Satureia hortensis*	Labiatae	SP	F	−0.82 R[d]	−0.78 R[d]
Shiso oil	*Perilla frutescens*	Labiatae	L	F	−0.98 R	−0.57 R
Spearmint oil	*Mentha viridis*	Labiatae	WP	S	−0.96 R	−0.16 R
Staranise oil	*Illicium verum*	Magnoliaceae	FR	F	−0.70 R	−0.17 R
Thyme oil	*Thymus vulgaris*	Labiatae	L, FL, STM	S	−0.82 R	−0.83 R
White pepper oil	*Piper nigrum*	Piperaceae	FR	T	0.05 N	
Wintergreen oil	*Gaultheria procumbens*	Ericaceae	L	F	−0.78 R	−0.08 N

[a] A: aril, B: bark, C: clou, FL: flower, FR: fruit, HW: heart wood, L: leaf, P: pericarb, R: root, RHI: rhizome, S: seed, SD: sawdust, SP: spike, STB: stub, STM: stem, T: twig, WP: whole plant.

[b] F: Fuji Flavor Co., Ltd. (Tokyo, Japan), S: Soda Aromatic Co. (Tokyo, Japan), T: Takasago Int. Co. (Tokyo, Japan).

[c] Excess proportion indexes. $EPI=(nt-nc)/(nt+nc)$; nt, nc: total number of *L. serricorne* in the treatment and control tubes, respectively.

[d] R: Repellency (Upper limit of 95% fiducial limits of $EPI<0$), N: Neutral (Upper limit of 95% fiducial limits of $EPI \geqq 0$, and lower limit of 95% fiducial limits of $EPI \leqq 0$), A: Attraction (Lower limit of 95% fiducial limits of EPI>0).

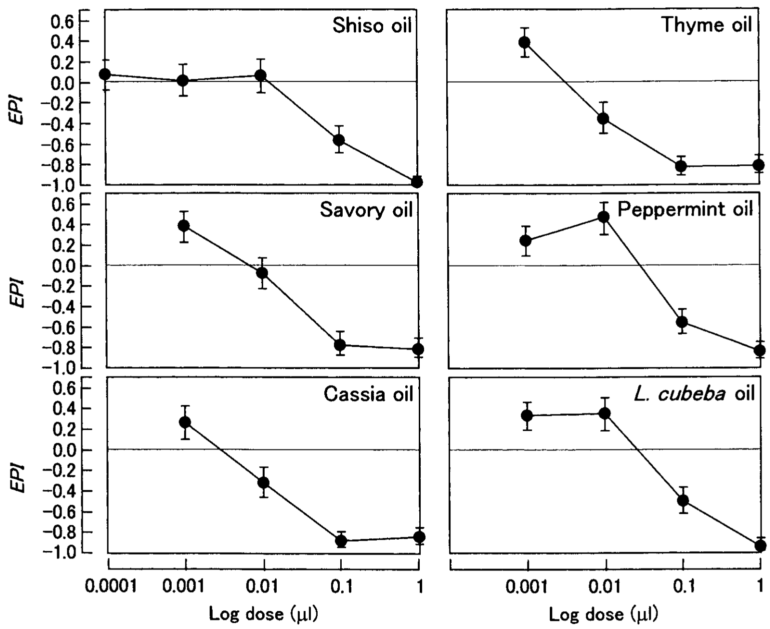

Fig. 2. Dose-responses of shiso, thyme, savory, peppermint, cassia and *L. cubeba* oils on repellency against *Lasioderma serricorne*.

repelled them at a dose of 0.1 μl were from either labiate or lauraceous plants. Considering this, many essential oils from these plant families may be promising repellent sources against *L. serricorne*.

The first main components of shiso, savory, cassia, thyme, peppermint and *L. cubeba* oils repelled beetles at a dose of 1 μl or 1 mg except for γ-terpinene. However, the repellency of these components was less than that of essential oils. It was suggested that the repellency of these oils was not caused only by these main components but also by other components, as well. The repellency of these essential oils may be caused by synergism of plural

Table 2. The main components of shiso, savory, cassia, thyme, peppermint and *L. cubeba* oils

Essential oil	Main components of oil (contents in oil (%))[a]
Shiso oil	(−)-Perillaldehyde (32–55), (−)-Limonene (8–33), Caryophyllene (5–9), *trans*-Shisool (2–9), α-Farnesene (3–7), Linalool (1–9), (−)-Perillyl alcohol (1–5)
Savory oil	Carvacrol (18–51), γ-Terpinene (34–63), *p*-Cymene (3–8)
Cassia oil	Cinnamaldehyde (77), Coumarin (15), Cinnamyl acetate (4), Benzaldehyde (1)
Thyme oil	Thymol (30–71), Carvacrol (2–15)
Peppermint oil	(−)-Menthol (40–45), (−)-Menthone (16–25), 1,8-Cineole (6–8), Isomenthone (3–4), (−)-Menthyl acetate (3–9), Neomenthol (3–4), Limonene (3–4), (+)-Pulegone (1–3)
L. cubeba oil	Citral (67–93), Linalool (3), Methyl heptanone (2)

[a] The data of contents of components in essential oils were quoted from following literature. Shiso oil: Fujita et al. (1970), Savory oil: Deans and Svoboda (1989), Cassia, thyme, peppermint oils: Moroe (1989), *L. cubeba* oil: Gogoi et al. (1997).

Table 3. Behavioral response of *Lasioderma serricorne* to the first major component of each essential oil

Chemical	Essential oil	Dose	*EPI*[a]	(95% Fiducial limits)
(−)-Perillaldehyde	Shiso oil	1 μl	−0.71	(−0.80, −0.59) R[b]
		0.1 μl	−0.29	(−0.43, −0.13) R
		0.01 μl	0.25	(0.10, 0.39) A
Carvacrol	Savory oil	1 μl	−0.62	(−0.73, −0.49) R
		0.1 μl	−0.29	(−0.44, −0.14) R
		0.01 μl	0.12	(−0.03, 0.27) N
γ-Terpinene	Savory oil	1 μl	0.28	(0.13, 0.42) A
Cinnamaldehyde	Cassia oil	1 μl	−0.81	(−0.88, −0.70) R
		0.1 μl	−0.18	(−0.33, −0.03) R
		0.01 μl	0.19	(0.04, 0.34) A
Thymol	Thyme oil	1 mg	−0.61	(−0.73, −0.47) R
		0.1 mg	−0.42	(−0.55, −0.27) R
		0.01 mg	0.19	(0.04, 0.33) A
(−)-Menthol	Peppermint oil	1 mg	−0.73	(−0.82, −0.61) R
		0.1 mg	0.04	(−0.11, 0.19) N
		0.01 mg	0.55	(0.41, 0.67) A
Citral	*L. cubeba* oil	1 μl	−0.61	(−0.73, −0.48) R
		0.1 μl	0.31	(0.16, 0.45) A

[a] Excess proportion indexes with 95% fiducial limits in parentheses. $EPI=(nt-nc)/(nt+nc)$; *nt*, *nc*: total number of *L. serricorne* in the treatment and control tubes, respectively.
[b] R: Repellency (Upper limit of 95% fiducial limits of $EPI<0$), N: Neutral (Upper limit of 95% fiducial limits of $EPI \geqq 0$, and lower limit of 95% fiducial limits of $EPI \leqq 0$), A: Attraction (Lower limit of 95% fiducial limits of $EPI>0$).

components.

In this study, savory, cassia, thyme, peppermint and *L. cubeba* oils attracted the beetles at lower doses than each threshold, although these oils strongly repelled them at higher doses. It was suggested that the activity of plant essential oils against the beetles usually depended on their doses. However, shiso oil did not attract the beetles at any doses tested. The dose of shiso oil attracting the beetles may be less than 0.001 μl or shiso oil may be an exception and may not attract them at any lower doses. The quantity of active principles released from dosages usually decreases with the passage of time. Therefore, the chemicals attracting the beetles at lower doses have a difficult problem in practical use. Considering this and the strong repellency of shiso oil at higher doses exhibited in the experiment, shiso oil is a promising repellent against *L. serricorne*.

Shiso is a common herb in Japan and is traditionally used for food preservation (Sasaki et al., 1990). However, there has so far been no report on the bio-activity of shiso oil against insects. In this study, shiso oil showed a strong repellency against

Table 4. Behavioral responses of *Lasioderma serricorne* to the combination of cured tobacco powder and shiso oil

Test material (Dose of shiso oil)	*EPI*[a] (fiducial limits)
Tobacco powder	0.59 (0.46, 0.70) A[b]
Tobacco powder and shiso oil (1 μl)	0.35 (0.19, 0.49) A
Tobacco powder and shiso oil (10 μl)	−0.57 (−0.68, −0.44) R

[a] Excess proportion indexes with 95% fiducial limits in parentheses. $EPI=(nt-nc)/(nt+nc)$; *nt*, *nc*: total number of *L. serricorne* in the treatment and control tubes, respectively.

[b] R: Repellency (Upper limit of 95% fiducial limits of $EPI<0$), A: Attraction (Lower limit of 95% fiducial limits of $EPI>0$).

the beetles, and the repellency was shown even in the presence of cured tobacco odor. Cigarette beetle has a propensity to enter and stay at a narrow opening. If the beetle is prevented from entering a narrow opening by a repellent, it will not be able to easily invade products in the packaging. Application of shiso oil around the opening of the packaging may reduce the risk of the beetles entering it because shiso oil repels them even in the presence of food odors.

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* Translation by author.