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Repellency of six plant essential oils against *Periplaneta americana* L. and *Blattella germanica* L.

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Abstract Six essential oils (EOs) from *Cinnamomum cassia*, *Citrus sinensis*, *Mentha piperita*, *Syzygium aromaticum*, *Zingiber cussumunar*, and *Zingiber officinale* at 10% in soybean oil were evaluated for their repellent activities against *Periplaneta americana* L. and *Blattella germanica* L. adults and compared to that of naphthalene (1 g of sublimating ground powder), a common insect repellent. All six EOs exhibited a significantly higher effective repellency against *B. germanica* than against *P. americana*. Among all EOs tested, *C. cassia* EO exhibited the highest repellent activity against adult *B. germanica* (90.0%) and adult *P. americana* (76.0%). Naphthalene, on the other hand, showed 88% repellency against *B. germanica* and 98% against *P. americana.* It can repel *P. americana* better but repel *B. germanica* worse than *C. cassia* EO. *C. cassia* EO has a good potential to be developed into an effective, and safe insect repellent for controlling *P. americana* and *B. germanica* populations.

Keywords: *Cinnamomum cassia*, *Periplaneta americana* L., *Blattella germanica* L., Repellency

Introduction

In Thailand, American cockroach (*Periplaneta americana* L.) and German cockroach (*Blattella germanica* L.) are two common insect pests in residential buildings (Dingha *et al*., 2016; Ubulom *et al*., 2021). They are one of the major sources of potent allergens against sensitive populations, especially children. In addition, they carry pathogens of cholera, diarrhea, and dysentery (Etim *et al*., 2013; Chang *et al*., 2017; Lee *et al*., 2017). Cockroach allergens and pathogens found throughout a house, such as in the kitchen, are associated with the saliva, feces, secretions, and fragments of their body parts (Chang *et al*., 2017). These pathogens are some of the most serious global public health problems (Dingha *et al*., 2016). To control American and German cockroaches, several repellents and contact synthetic insecticides, such as

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pyrethroids, carbamates, and organophosphates, have been used. However, these insecticides have many serious side effects to human health, cause environmental pollution, and are susceptible to insect resistance after an extended use (Chang *et al*., 2017; Lee *et al*., 2017). Therefore, safe and effective cockroach control agents have been continuously developed.

Among natural plant products, plant essential oils (EOs) and their constituents are good candidates for controlling cockroaches (Chooluck *et al*., 2019; Lee *et al*., 2017; Yeom *et al*., 2015). They are eco-friendly and potent insecticides and repellents. Moreover, insect pests are not likely to develop resistance against them as easily as against synthetic insecticides (Pavela and Benelli, 2016). Good repellency activities against *P. americana* and *B. germanica* of several EOs and constituents from *Anethum graveolena*, *Citrus hystrix*, *Cymbopogon citratus*, *Cymbopogon winterianus*, *Eucalyptus globulus*, *Cyperus rotundus*, *Rosmarinus officinalis*, and *Trachyspermum ammi* have been reported (Zibaee *et al*., 2016; Chang *et al*., 2017; Lee *et al*., 2017; Chooluck *et al*., 2019).

All six EOs (*Cinnamomum cassia*, *Citrus sinensis*, *Mentha piperita*, *Syzygium aromaticum*, *Zingiber cussumunar*, and *Zingiber officinale*) in this study have been reported previously to exhibit insecticidal activity against cockroaches and other insect pests. Namely, *C. cassia* exhibited an adulticidal activity against *Aedes aegypti* and *Ae. albopictus* (Aungtikun and Soonwera, 2021). *C. sinensis* EO exhibited a strong oviposition deterrent activity against *Musca domestica* (Sinthusiri and Soonwera, 2014). *M. piperita* and *Z. officinale* EOs showed a strong insecticidal activity against *B. germanica* (Sittichok *et al*., 2013b). *S. aromaticum* EO showed a repellency activity against *P. americana* adults (Sittichok *et al*., 2013a). *Z. cussumunar* EO exhibited a strong insecticidal activity against *M. domestica* (Sinthusiri and Soonwera, 2014). In addition, these EOs have been used as medicine for humans, with the following activities: antioxidant, anti-bacterial, and anti-flammatory (Sinthusiri and Soonwera, 2014; Pavela and Benelli, 2016; Aungtikun and Soonwera, 2021).

The objective of this study was to determine the repellent activities of six EOs from *C. cassia*, *C. sinensis*, *M. piperita*, *S. aromaticum*, *Z. cussumunar*, and *Z. officinale* against *P. americana* and *B. germanica* adults under laboratory conditions.

Materials and methods

Plant materials

Dried barks of *C. cassia* and dried fruits of *S. aromaticum* purchased from Nguan Soon pharmacy, 156-158 Soi Charoen krung 16, Samphantawong, Bangkok 10100, Thailand. Fresh fruits of *C. sinensis* and fresh leaves of *M. piperita* were collected from an organic farm in Nakhon Ratchasima province, Thailand. Fresh rhizomes of *Z. cussumunar* and *Z. officinale* were collected from an organic farm in Chumphon province, Thailand. All parts of plant species were collected during September 2019 to June 2020. They were positively identified by a plant scientist at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL). Parts of these plants were cleaned and cut into small pieces. Then, they were extracted by a water distillation method for 5-8 h. At the end of that time, the EOs were collected and stored at 4 ℃. Each EO was diluted to a 10% solution in soybean oil and kept under general laboratory conditions (25.5±3.0 ℃ and 76.5±3.5%RH) for later uses.

Chemicals

The positive and negative controls in this study were 99.60% (w/w) naphthalene, a common insecticide, and soybean oil. Naphthalene was manufactured by Power Melan Co., Ltd., Soi Chokchai 4, 72, Lat phrao, Bangkok 10230, Thailand, while soybean oil was manufactured by Thai Vegetable Oil Public Co., Ltd., 149 Ratchadapisek Rd (Thapra-Taksin), Thonburi, Bangkok 10600, Thailand.

Adult cockroach rearing

Nymphs and adults of *P. americana* and *B. germanica* were obtained from The National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. The cockroaches were reared in a laboratory under the environmental conditions of 31.5±2 ℃ and 64.5±4% RH, with a photoperiod cycle of 12-h light:12-h-dark, at the Department of Plant Production Technology, Faculty of Agricultural Technology, KMITL. Nymphs and adults of *P. americana* and *B. germanica* were fed with 50 g of dog pellets and 50 g of powdered milk in glass jars (22.5 cm diameter × 35 cm) for food as well as 10% glucose solution soaked in cotton sheets for drink. Eight-month-old *P. americana* and two-month-old *B. germanica* adults, were used in a subsequent repellent bioassay.

Repellency test

An 18.5x26x10.5 cm, open-top, plastic box was used as a cockroach cage for the repellent test called filter-paper choice assay (Chang *et al*., 2017;

Sittichok *et al*., 2013a). All four walls of the box were pasted with a layer of greasy Vaseline to prevent cockroach escaping. A piece of filter paper (Whatman® No.1, 18.5x26 cm) that filled the whole area of the base of the box was placed at the bottom of the box. It was divided into 2 equal areas, a treatment area and a control area. The treatment area was dropped onto with two ml of the treatment EO, while the control area was dropped onto with two ml of distilled water. For the positive control treatment, since naphthalene was a solid substance at room temperature and exerted its action through its sublimated vapor at room temperature, it was ground into powder, and one gram of the powder was placed on the treatment area on the filter paper. Food and drink for the cockroaches, available for all time in the cage, were placed in containers. Each of two identical sets of food and drink containers was placed in the treatment area and the control area to ensure that none of the cockroaches would die from starvation. For each treatment, five adults of *P. americana* or *B. germanica* of both sexes were released at the middle of the filter paper in the box. Repellency was observed and recorded as the number of cockroaches that situated away from the treatment area, or in other words, the number of cockroaches situated on the control area of the filter paper, while attractancy was observed and recorded as the number of cockroaches that situated on the treatment area, compared to the number of cockroaches that situated on the control area. After 24 hours, the outcomes of the repellency test were observed and recorded. Each experiment was repeated ten times. The outcomes were converted into repellency indices (RI) by formula (1) (Thavara *et al*., 2007; Sittichok *et al*., 2013a) below,

$$RI = (NS–NC)/(NS+NC), \quad (1)$$

where NS was the total number of insects situated in the treatment area on the filter paper at the time of observation, and NC was the total number of insects situated in the control area. In general, RI ranges from -1 to +1. A positive RI indicates that the treatment (or control) was an attractant, and conversely, a negative RI indicates that the treatment (or control) was a repellent, while a zero value indicates a neutral response.

The percentage repellency (PR%) (i.e., percentage of insects situated in the control area on the filter paper) for each essential oil was calculated by the following formula (2),

$$PR = [1- (NS)/(NS+NC)] \times 100\%, \quad (2)$$

where NS was the total number of insects situated in the treatment area on the filter paper at the time of observation, and NC was the total number of insects situated in the control area.

The percentage attractant (PA%) (i.e., percentage of insects situated in the treatment area on the filter paper) for each essential oil was calculated by following formula (3),

$$PA = 100\% - PR. \quad (3)$$

Statistical analysis

A paired *t*-test (in SPSS software for Windows, version 16.0) was used to analyze the significant difference at $p<0.05$ in the mean numbers of cockroaches in the treatment and control areas.

Results

All six plant EOs exhibited a significantly higher percentage of effective repellency (PR) against *B. germanica* than against *P. americana* adults (Figure 1). The repellency activity of six plant EOs at 10% in soybean oil and 1 g of sublimating ground powder of naphthalene against *P. americana* adults are presented in Table 1. *C. cassia* EO showed the highest percentage of effective repellency (PR) against *P. americana* at 76.0%, with repellency index (RI) of -0.52. On the other hand, EOs from *C. sinensis* and *Z. cussumunar* showed a lowest PR against *P. americana* at 52.0%, with an RI of -0.04. Moreover, all EOs showed a lower PR% against *P. americana* than that of naphthalene, a positive control (PR = 98.0% and RI = -0.96).

The repellency activity of six plant EOs at 10% in soybean oil and 1 g of sublimating ground powder of naphthalene against *B. germanica* adults are presented in Table 2. *C. cassia* EO and *Z. officinale* EO showed excellent repellency against *B. germanica* adults at the highest PR of 90.0% and an RI of -0.80. On top of that, they also showed a higher percentage repellency than that of a common synthetic repellent naphthalene against *B. germanica* adults (PR = 88.0% and RI = -0.76). At the other end of the spectrum, *S. aromaticum* EO showed the lowest PR against *B. germanica* adults at 78.0%, with an RI of -0.56.

To conclude, ranked according to their PR values against *P. americana* and *B. germanica* adults, the six plant EOs can be ranked as follows: *C. cassia* EO> *Z. officinale* EO> *M. piperita* EO> *C. sinensis* EO> *Z. cussumunar* EO> *S. aromaticum* EO.

Table 1. Repellency activity against *P. americana* adults of six plant EOs at 10% in soybean oil and naphthalene

Treatment	Number of cockroaches ±SD		PR%	PA%	RI [1]
	Treatment	Control			
C. cassia EO	1.2 ±0.7*	3.8 ±2.0	76.0	24.0	-0.52
C. sinensis EO	2.4 ±0.5*	2.6 ±0.5	52.0	48.0	-0.04
M. piperita EO	2.2 ±0.4*	2.8 ±0.4	56.0	44.0	-0.12
S. aromaticum EO	1.7 ±1.8	3.3 ±1.3	60.0	40.0	-0.21
Z. cussumunar EO	2.4 ±0.5*	2.6 ±0.5	52.0	48.0	-0.04
Z. officinale EO	2.2 ±0.4*	2.8 ±0.4	56.0	44.0	-0.12
Naphthalene (1 g of sublimating ground powder; positive control)	0.5 ±0.3*	4.5 ±1.1	98.0	2.0	-0.96
Soybean oil (negative control)	4.8 ±0.5*	0.2 ±0.5	4.0	96.0	0.92

* Significant difference between the treatment and the control by paired *t*-test ($P < 0.05$).
[1] RI ranges from -1 to +1. A positive RI indicates that the treatment (or control) was an attractant, and conversely, a negative RI indicates that the treatment (or control) was a repellent, while a zero value indicates a neutral response.
PR% = Percentage repellency; PA% = Percentage attractancy.

Table 2. Repellency activity against *B. germanica* adults of six plant EOs at 10% in soybean oil and naphthalene

Treatment	Number of cockroaches ±SD		PR%	PA%	RI [1]
	Treatment	Control			
C. cassia EO	0.5 ±1.3*	4.5 ±1.3	90.0	10.0	-0.80
C. sinensis EO	0.9 ±1.4*	4.1 ±1.4	82.0	18.0	-0.64
M. piperita EO	1.0 ±1.9*	4.0 ±1.9	80.0	20.0	-0.60
S. aromaticum EO	1.1 ±1.9*	3.9 ±1.9	78.0	22.0	-0.56
Z. cussumunar EO	1.0 ±1.9*	4.0 ±1.9	80.0	20.0	-0.60
Z. officinale EO	0.5 ±1.3*	4.5 ±1.3	90.0	10.0	-0.80
Naphthalene (1 g of sublimating ground powder; positive control)	0.6 ±0.7*	4.4 ±0.7	88.0	12.0	-0.76
Soybean oil (negative control)	4.8 ±0.5*	0.2 ±0.5	4.0	96.0	0.92

* Significant difference between the treatment and the control by paired *t*-test ($P < 0.05$).
[1] RI ranges from -1 to +1. A positive RI indicates that the treatment (or control) was an attractant, and conversely, a negative RI indicates that the treatment (or control) was a repellent, while a zero value indicates a neutral response.
PR% = Percentage repellency; PA% = Percentage attractancy.

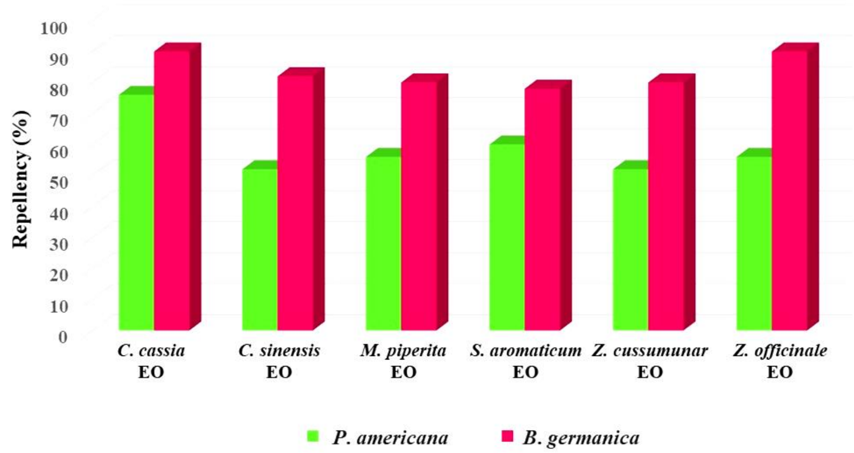

Figure 1. Percentage repellency activity of six plant EOs against *P. americana* and *B. germanica*

Discussion

C. cassia EO exhibited a better-than-positive-control repellency activity against *P. americana* and *B. germanica* adult, as compared to the other EOs. Findings from Chang *et al.* (2006) support our findings. They reported a good repellency activity of *C. cassia* EO against adults of *Ae. aegypti*. Five percent of *C. cassia* EO in ethyl alcohol showed a high adulticidal activity against *Ae. aegypti* and *Ae. albopictus*, with a KT_{50} of 8.6-9.6 min (Aungtikun and Soonwera, 2021). Our results are also supported by a study by Khan (2021), which reported that *C. cassia* EO exhibited a strong insecticidal activity against one-day-old pupae of *M. domestica*, with an LC_{50} of 298 ppm. Chang *et al.* (2006) and Aungtikun and Soonwera (2021) reported that their GC-MS analysis of *C. cassia* EO indicated that cinnamaldehyde was its major constituent. They also reported its strong adulticidal and repellency activities against *Ae. aegypti*. Against insect pests, the mode of action of *C. cassia* EO was permeability inhibition of cell membrane and disruption of intracellular enzymes (Aungtikun and Soonwera, 2021; Benelli *et al.*, 2018). For Cinnamaldehyde, the mode of action against insect pests was respiratory system inhibition caused by inhibition of enzymes involved in cytokinesis. It also reduced cell membrane's ATPase activity (Aungtikun and Soonwera, 2021). Furthermore, it causes loss of membrane integrity and membrane depolarization (Benelli *et al.*, 2018). Historically, *C. cassia* EO has been widely used as an insecticidal, antifungal, and anti-bacterial agent, as a treatment for stomach disorders and for controlling glucose in the blood (Jyoti *et al.*, 2019).

In this study, naphthalene was able to repel *P. americana* better but repel *B. germanica* worse than *C. cassia* EO. Regarding its toxicity and side effects, it has been reported to have an acute oral LD_{50} of 2649 mg/kg against male and female rats and an inhalation LC_{50} of > 0.4 mg/L (77 ppm) against albino rats (USA EPA, 2008). Long time exposure to naphthalene by inhalation, ingestion, or dermal contact may result in hemolytic anemia, liver toxicity, and neurological damage in infants (Thavara *et al*., 2007; Zibaee *et al*., 2016). Furthermore, naphthalene is highly toxic to soil organisms as well as other terrestrial and aquatic animals (USA EPA, 2008). In contrast, EOs are quickly degraded in the environment and relatively harmless to non-target organisms. They are also non-persistence and non-mobile in soil (Benelli and Duggan, 2018).

As demonstrated by the findings in this study, the potential of *C. cassia* EO as a commercial green repellent and adulticide against *P. americana* and *B. germanica* is great. A further study on its long-term toxicity and a development into a spray-formulation repellent are strongly recommended.

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