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Original Article

Acaricidal activity of essential oils for the control of honeybee (*Apis mellifera*) mites *Tropilaelaps mercedesae* under laboratory and colony conditions

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Abstract – *Tropilaelaps* spp. mites are considered a major parasite of honeybees. In this study, essential oils (EOs) of 11 plant species were evaluated for acaricidal activity under laboratory and field conditions. Five adult mites per petri dish and ten adult worker bees per plastic cup cage were exposed to different concentrations of each essential oil (0.1, 0.5, 1.0, 5.0, and 10.0% (v/v)). The essential oil of *Piper betle* (betel) showed the highest acaricidal activity in laboratory testing. Additionally, most EOs showed low toxicity to adult honeybees in a lab assay. *Piper betle*, *Amomum krervanh*, and *Zanthoxylum limonella* were then tested in whole colonies using cardboard strips soaked in 10 mL of each essential oil at various concentrations, every week for 4 weeks. *Amomum krervanh* at 10% (v/v) and *P. betel* at 5.0% (v/v) decreased mite populations after 30 days. We further tested grease and a sponge as application methods to deliver *A. krervanh*, with no significant impact on mites. Although several essential oils demonstrated acaricidal activity against *Tropilaelaps* under laboratory conditions, the EOs tested did not significantly reduce mites under colony conditions. Therefore, the concentration and colony delivery methods of these promising EOs warrant further investigation.

Tropilaelaps **/ acaricidal activity / LC_{50} /** ***Apis mellifera*** **/** ***Piper betle*** **/** ***Amomum krervanh***

1. INTRODUCTION

Tropilaelaps spp. are ectoparasitic mites of *Apis* honeybees in Asia. The original host of *Tropilaelaps* mites is recognized as the giant honeybee, *Apis dorsata*, but recently they have become a serious pest of managed honeybees, *Apis mellifera*, in many Asian countries (Anderson and Morgan 2007). A tropical or sub-tropical climate is considered to be a major factor in the prevalence of *Tropilaelaps* (Sammataro et al. 2000). In Thailand, *Tropilaelaps* are considered to be a more problematic parasite of honeybees than another honeybee mite, *Varroa* (Burgett et al. 1983; Buawangpong et al. 2015). *Tropilaelaps* reproduce rapidly and have a shorter phoretic stage than *Varroa*; thus, they may outcompete *Varroa* mites when both mites are present (Burgett et al. 1983; Ritter and Schnieder-Ritter 1988; Buawangpong et al. 2015). Mite parasitism can cause brood mortality and colony decline (Anderson and Morgan 2007; Ritter 2008). Although there are no specific products to control *Tropilaelaps* mites, the synthetic chemical acaricides used to

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control *Varroa* mites such as Apistan® (fluvalinate), Checkmite+® (coumaphos), and Bayvarol® (flumethrin) are also effective on *Tropilaelaps* (Burgett and Kitprasert 1990; Camphor et al. 2005; Kongpitak et al. 2008). Additionally, several commercial products known to control *Varroa* were also tested for efficacy against *Tropilaelaps*. The results showed that the formic acid product, Mite-Away Quick Strips®, was the only product tested that significantly reduced mite population after 8 weeks of treatment without side effect. However, these acaricides used to control *Varroa* are not always effective against *Tropilaelaps* mites (Pettis et al. 2017). Apivar® (amitraz) was also tested to control *Tropilaelaps* but had little to no effect on mite populations in bee colonies (Pettis et al. 2017). Moreover, the application of some acaricides in bee colonies can affect queen performance and sperm viability in drones and impact colony growth (Atwal and Goyal 1971; Haarmann et al. 2002; Burley et al. 2008; Pettis et al. 2017). Also, the presence of chemical residues in the hive and honeybee products can be a negative effect of acaricidal use (Mullin et al. 2010; Chaimanee et al. 2019). Hence, the development of alternative mite control methods is needed. Many natural compounds and organic acids have shown promise in controlling pests and diseases in honeybees. Formic acid and thymol have both been shown to have efficacy against *Tropilaelaps* (Raffique et al. 2012; Pettis et al. 2017). Also, several essential oils have demonstrated acaricidal efficacy in controlling *Varroa* mites, such as *Syzygium aromaticum*, *Acantholippia seriphioides*, *Schinus mole*, *Citrus aurantium*, *Cymbopagon flexuosus*, *Thymus vulgaris*, and *Salvia officinalis* (Ruffinengo et al. 2005; Abd El-Wahab and Ebada 2006; Damiani et al. 2009; Gashout and Guzmán-Novoa 2009; Bendifallah et al. 2018). As alternative control methods, essential oils have shown high acaricidal activity and low toxicity to bees. Low residues and less accumulation in honeybee products are also reported as advantages of natural compounds (Rosenkranz et al. 2010). However, the degree of mite control by essential oils in beehives is not always consistent. Efficacy is often affected by environmental conditions such as temperature and relative humidity (Bacandritsos et al. 2007).

In Thailand, there are many plant species that contain active compounds with high biological activity, including acaricidal and insecticidal activity. Plants in the family Piperaceae are members of traditional pharmacopeia in Asia and many other countries. These plants have been used to control mites, ticks, and other arthropod pests (Park et al. 2002; Silva et al. 2009; Ferraz et al. 2010). *Cinnamomum* spp. have shown some acaricidal activity against *Varroa* mites (Imdorf et al. 1999; Conti et al. 2020). Additionally, crude extracts of *Amomum krervanh* showed antibacterial activity against *Paenibacillus larvae* (Chaimanee et al. 2017).

Therefore, in this study, the bioactivity of several plant essential oils on *Tropilaelaps* was screened and evaluated under laboratory conditions based on their previously reported acaricidal and biological activities. The most promising essential oils were also tested for adult bee toxicity. Finally, the most promising essential oils were tested at the whole colony level to control *Tropilaelaps* mites using several different delivery methods under field conditions in Thailand. Our goal was to develop more environmentally sensitive control options for honeybee mites.

2. MATERIALS AND METHODS

2.1. Plant species and essential oil extraction

Eight plant species were collected fresh from Phrae province in northern Thailand and three species were purchased from an herbal store in Chiang Mai, Thailand. Different plant parts of each species were used for essential oil extraction (Table I). Plant materials were washed and dried at room temperature. Two hundred grams of each plant species was added to 1.5 L of distilled water, then the essential oil was extracted using a hydrodistillation method for 3 h or until no more essential oil was obtained. The essential oil was collected and anhydrous sodium sulfate added to remove excess water (Damiani et al. 2009). The extraction was run several times for each plant until the desired quantity of oil was obtain for the experiments. The extracts were stored at 4°C until used.

Table I. Plant species were used in this study

Plant species	Common name	Part used	Origin
Cinnamomum camphora	Camphor laurel	Leaf	Phrae province
Citrus hystrix	Kaffir lime	Leaf	
Cymbopogon citratus	Lemon grass	Stem	
Eucalyptus globulus	Eucalyptus	Leaf	
Ocimum basilicum	Basil	Leaf	
Ocimum sanctum	Holy basil	Leaf	
Piper betle	Betal	Leaf	
Zanthoxylum limonella	Ma-khwaen	Fruit and seed	
Amomum krervanh	Cardamon	Fruit	Herbal store
Cinnamomum sp.	Cinnamon	Stem	
Syzygium aromaticum	Clove	Flower	

2.2. Mites

Female mites of *T. mercedesae* were collected from honeybee colonies (*A. mellifera*). The hives were located at Maejo University Phrae Campus, Phrae, Thailand, and had been left untreated for *Tropilaelaps* in the previous 4–6 months. Sealed brood combs were removed from colonies and transported to the laboratory. Brood cells were individually uncapped and inspected for *T. mercedesae*. The female mites were lifted from the cells and comb surface with a fine brush and placed on honeybee larvae in petri dishes to avoid starvation. Mites that appeared newly moulted (light in color), abnormal, and/or weak (slow-moving) were removed from the experiment (Damiani et al. 2009). Healthy mites by comparison are fast moving, a darker tan color and very active and agile.

2.3. Honeybee adults

Frames of sealed brood were removed from healthy *A. mellifera* colonies and held in a wire mesh-made cage and placed in an incubator at 34 ± 1 °C and 70% RH. Ten newly emerged bees were placed in plastic cup cages (Evans et al. 2009) and were fed with 50% (w/v) of sugar solution and water for 3 days at room temperature before exposure to essential oils.

2.4. Acaricidal activity of essential oils on *T. mercedesae* under laboratory conditions

The complete exposure method is described by Ruffinengo et al. (2005) and was modified to evaluate the bioactivity of the essential oils on *Tropilaelaps* mites. Each essential oil was dissolved in acetone to obtain concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0% (v/v). Our experience with other essential oil research (Nuanjohn and Chaimanee 2019) indicated that this range of dosages would cover the most likely range for acaricidal activity. Then, 100 μl of each concentration was placed onto filter paper that filled the bottom of a standard 60 × 15mm petri dish. After 5 min of evaporation, five mites and two larvae were placed in each petri dish. Three replicates were run for each treatment for a total of 15 mites tested against each treatment and dose. Acetone and fluvalinate at concentration of 0.1, 0.5, 1.0, 5.0, and 10% (w/v) were included as negative and positive control, respectively. The experiment was carried out at room temperature and all treated mites were incubated at 28 ± 1 °C and 70% RH. Mortality of mites was checked at 4, 24, and 48 h following initial exposure.

2.5. Toxicity of essential oils on *A. mellifera* adults

The vapor toxicity of essential oils to adult bees was tested using a method modified from Evans et al. (2009). Three-day-old emerged worker bees were used in this study. Concentrations of each essential oil were made at 0.1, 0.5, 1.0, and 10.0% (v/v) by dilution in acetone and a 200 μl aliquot of each was applied to filter paper placed on the bottom of plastic cup cages (Evans et al. 2009). Ten adult bees were placed into each cage after 5 min of solvent evaporation and held at 30 ± 1 °C. Three replicate cages were run for each treatment for n =30 bees total. A 50% (w/v) sugar solution and water was provided on the top of the cage throughout the experiment. Acetone was run as a negative control group. Dimethoate at concentration of 0.01, 0.05, 0.1, 0.5, and 1.0% (w/v) was used as a positive control. Mortality was checked at 4, 24, 48, and 72 h following initial exposure.

2.6. Field experiment

2.6.1. *Essential oils for control of T. mercedesae* under field condition

The field experiment was conducted in the winter during December 2016 to February 2017 (15–20°C of an average temperature and 60–70% of a relative humidity) at Maejo University Phrae Campus, Phrae, Thailand. Langstroth hives with 2–3 frames of sealed brood, 1–2 frames of unsealed brood, and 1 frame of pollen were used in this study. Each hive consisted of a single Langstroth deep box with 10 frames. All hives had a laying queen and the most uniform hives were selected for inclusion in the study. Honeybee colonies that had been left untreated for mite control for the previous 4–6 months were used in the study. Colonies were assessed for adult bee and mite populations. Adult bee population density consisted of a visual inspection of each comb and adult bee coverage estimated to the nearest 0.5 frame coverage (1 = fully covered in adult bees). For the determination of mite infestation rate, 100 cells were opened, groups of 10 cells in a line were opened, and the observer moved at random across the sealed brood area, most often opening 50 cells on two sides of a single brood frame (Pettis et al. 2017). Colonies were then assigned into eight treatment groups with eight colonies per treatment using a stratified random design in which colonies were ranked from high to low mite infestation and treatments assigned down the rank in groups of nine colonies to ensure balanced mite levels across all treatment groups. Three essential oils A. *krervanh*, *P. betle*, and *Z. limonella* were chosen for the field study. Each essential oil was dissolved in liquid paraffin to obtain concentrations of 1.0 and 10.0% (v/v) for A. *krervanh* and *Z. limonella* and 0.5 and 5.0% (v/v) for *P. betle*. Previous research with essential oils had indicated that the 1.0 and 10% dosages should cover a realistic high and low dose. Dosage regimes were designed to compare the acaricidal activity of low and high doses of each essential oil for *Tropilaelaps* control in beehives. Each cardboard strip (5.0 × 22.0 cm) was soaked in 10 mL of each treatment solution. Negative controls consisted of liquid paraffin strips. Fluvalinate strips (Weipeng's manpu; Shanxi Weipeng Pharmaceutical Co., Ltd.) containing 40 mg per strip were used as a positive control (Apistan® strips were not available in Thailand). Two strips of each treatment were applied every week for 4 weeks except the fluvalinate strips, where two strips were applied twice on days 0 and 14. Although the manufacturer recommends one strip of fluvalinate for ten frames of bees and small hives requires only ½ strip, these dosages are recommended for controlling the *Varroa* mites. According to Thai beekeepers, the higher doses are needed to control *Tropilaelaps*. Thus, two strips of fluvalinate were applied every 2 weeks to obtain the effective control in this study.

2.6.2. *Different application methods of essential oil for the control of T. mercedesae* under field conditions

In this experiment, the essential oil tested in the previous experiment that showed a high acaricidal activity *(A. krervanh)* was chosen to evaluate *Tropilaelaps* mite control using different application methods. The experiment was conducted from December 2017 to February 2018. Six

treatments consisted of liquid paraffin strips (negative control), 1.0 and 10.0% (v/v) of essential oil (*A. krervanh*) cardboard strips (5.0 × 22.0 cm), 10% (v/w) of essential oil in semi-solid vegetable grease (Crisco, USA), 10.0% (v/v) of essential oil sponge strips (5.0 × 22.0 cm), (Scotch-Brite, Spain), and commercially available fluvalinate strips (positive control). Colonies were assessed for mite levels and then treatments assigned for the experiment using a stratified random design as described above. Two strips or 10g of grease of each treatment was applied every week for 4 weeks except the fluvalinate strips, where two strips were applied twice on days 0 and 14. The 10g of grease was used based on the experience of Thai beekeepers who use this matrix (grease) to apply other compounds.

In both field experiments, colonies were monitored for adult bee population density and mite infestation rate using the methods as described above at three time intervals, pre-treatment (day 0) and at ca. 30 and 60 days following the initiation of treatments (Pettis et al. 2017).

2.7. Essential oil analysis

The chemical composition of essential oil that showed high acaricide activity (*A. krervanh*) was analyzed by gas chromatography model 6890N (Agilent Technologies, USA) coupled to mass spectrometer detector model 5793 inert (Agilent Technologies, USA). The gas chromatography was equipped with DB5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent Technologies, USA). A 100 mg of essential oil sample was diluted in 1 mL of hexane. The analysis was performed using helium as a carrier gas at a flow rate 1.0 mL/min. The program temperature conditions consisted in a temperature program from 50 until 280 °C, with an increment of 5 °C/min and maintained at this temperature for 5 min. One microliter of the sample was injected at a constant temperature of 250 °C with a split ratio of 20:1. Mass spectrum was scanned from 20 to 350 m/z. The essential oil components were identified by comparing their mass spectra with those stored in the National Institute of Standards and Technology mass spectrometry library (Central Laboratory (Thailand) Co., Ltd.).

2.8. Statistical analysis

The LC_{50} values were analyzed by probit analysis using StatPlus:mac for macOS® Version v7 (AnalystSoft Inc.). Statistical analyses were performed using JMP® version 11.2 for Mac (SAS Institute Inc.). Normality of data was checked using the Shapiro-Wilk test. The non-parametric Kruskal-Wallis test was used to determine if there were significant differences in LC_{50} values, number of frames covered with adult bees, and number of mite-infested cells, followed by a Steel-Dwass posthoc multiple comparison to separate means when significance was found. Effect of essential oils on frames covered with adult bees and number of mite-infested cells were compared between treatment groups in each observation time.

3. RESULTS

3.1. Acaricidal activity of essential oils to *T. mercedesae* and toxicity to honeybees under laboratory conditions

The LC_{50} values for essential oils to *T. mercedesae* and adult honeybees at each time interval are shown in Table II. *Piper betle* showed the highest acaricidal activity against *T. mercedesae* mites. All mites were dead at 4 h of exposure at the lowest concentration tested in this study. All essential oils tested had high acaricidal activity with the 4 h of LC_{50} values ranged between 0.00002 − 0.756 (%v/v). Six out of eleven essential oils killed all the mites after 24 h of exposure. Additionally, the essential oils of A. *krervanh*, *C. camphora L.*, *P. betle*, *S. aromaticum*, and *Z. limonella* showed low toxicity to adult bees (4-h LC_{50} = 7.753–26.257 (%v/v)) when compared to dimethoate (0.369 %w/v). *Cinnamomum* sp. was highly toxic to bees with 1.427 %(v/v) of LC_{50} at 4 h. Clove oil had the highest selectivity ratio (760,350) at 4 h followed by oil extracted from *A. krervanh* and *Z. limonella* (133.96 and 94.59, respectively).

Table II. LC_{50} (%v/v) values + (95% confidence intervals) and selectivity ratio of essential oils tested against *Tropilaelaps mercedesae* mites and adult honeybees (*Apis mellifera*) at 4, 24, and 48 h using a vapor exposure method under laboratory conditions. LC_{50} values of mites and honeybees between essential oil treatments in each observation time were analyzed using Kruskal-Wallis ($p > 0.05$)

Essential oil	LC_{50} mites + (95% confidence intervals)			LC_{50} honeybees + (95% confidence intervals)			Selectivity ratio		
	4 h	24 h	48 h	4 h	24 h	48 h	4 h	24 h	48 h
A. krervanh	0.196 (−0.023, 0.415)	0.107 (0.051, 0.163)	-	26.257 (9.959, 42.555)	11.427 (−0.774, 23.628)	10.545 (−0.270, 21.359)	133.96	106.79	210.90
C. camphora	0.756 (0.756, 0.756)	0.231 (0.089, 0.373)	0.021 (−0.069, 0.111)	8.040 (0.250, 15.829)	8.040 (0.250, 15.829)	8.040 (0.250, 15.829)	10.63	34.80	382.86
Cinnamomum sp.	0.021 (−0.069, 0.111)	-	-	1.427 (0.779, 2.076)	0.950 (0.361, 1.539)	1.009 (−0.329, 2.347)	67.95		
C. hystrix	0.042 (−0.048, 0.132)	-	-	Not tested					
C. citratus	0.052 (−0.067, 0.171)	-	-	Not tested					
E. globulus	0.274 (0.157, 0.391)	0.021 (−0.069, 0.111)	-	Not tested					
O. basilicum	0.133 (0.047, 0.219)	-	-	Not tested					
O. sanctum	0.047 (0.013, 0.081)	-	-	Not tested					
P. betle	All mites were dead			7.753 (3.457, 12.048)	1.427 (0.779, 2.076)	1.427 (0.779, 2.076)			
S. aromaticum	0.00002 (−0.000055, 0.000088)	-	-	15.207 (−12.181, 42.595)	1.043 (0.319, 1.767)	1.311 (−0.779, 3.400)	760,350		
Z. limonella	0.247 (0.247, 0.247)	0.084 (0.039, 0.128)	0.021 (−0.069, 0.111)	23.363 (−5.386, 52.112)	14.345 (−21.748, 50.437)	13.806 (−22.734, 50.347)	94.59	170.77	657.43
Fluvalinate (w/v)	2.987 (−8.585, 14.560)	0.043 (−0.094, 0.180)	0.01 (−0.056, 0.090)						
Dimethoate (w/v)				0.369 (−1.218, 1.955)	0.603 (−0.018, 1.224)	0.330 (−0.232, 0.893)			

3.2. Effect of essential oils on *T. mercedesae* control under field conditions

We tested three essential oils, A. *krervanh*, *P. betle*, and *Z. limonella*, under field conditions in colonies with 4–5 frames of adult bees which is a common colony size in the tropics. The adult bees of all treatment groups, including the control group (liquid paraffin), slightly decreased over 60 days of the experiment. Bee population was on average about 2–3 frames of bees at the end of test period. All three essential oils had no significant negative effects on adult bee population (Figure 1a).

The essential oil of *A. krervanh* showed effects on mite populations, with mite populations reduced at 30 and 60 days of treatment when compared to the fluvalinate control group (Steel-Dwass test, $p > 0.05$) (Figure 1b). *Piper betle* (5% v/v) seemed to have some effect on mite levels under field conditions, but without significant differences at 30 and 60 days (Steel-Dwass test, $p > 0.05$) (Figure 1b). However, the mite population increased at day 30 after exposure to 0.5% (v/v) of *P. betle*. Thus, no further evaluation was conducted on these colonies at day 60 based on a lack of control evidence at day 30. The results showed that mite levels were not affected by *Z. limonella* at both concentrations 1% and 10% (v/v) on day 30 (Figure 1b).

3.3. Acaricidal activity of essential oil of *A. krervanh* with different applications

To investigate the efficacy of the essential oil of *A. krervanh*, we tested different application methods and compared the acaricidal activity on *Tropilaelaps*. Essential oil (10 %v/v) was added in cardboard, grease, and sponge and applied to colony. In this trial, bees were unaffected by *A. krervanh* at 30 and 60 days (Figure 2a). Mite populations decreased when the essential oil was applied in grease or sponge on day 60 but there were no significant differences between all treatment groups (Steel-Dwass test, $p > 0.05$) (Figure 2b).

3.4. Chemical composition of *A. krervanh* essential oils

Sixty components have been identified in essential of *A. krervanh* (Table III). They are mainly composed of monoterpenes with 1,8-cineole (77.17%), β-Pinene (8.43%), and α-Terpineol (4.58%); their detailed composition is presented in Table III.

4. DISCUSSION

Tropilaelaps mites are another honeybee ectoparasites that has spread in Asia and they are very problematic where *Apis mellifera* is managed in Asia. These mites reproduce rapidly and have a shorter phoretic stage than *Varroa*, making them more problematic. One product, amitraz that is commonly used to control *Varroa* was not effective in controlling *Tropilaelaps* under tropical conditions (Pettis et al. 2017). Therefore, in this study, we tested the acaricidal activity of some promising essential oils in controlling *T. mercedesae* under laboratory and field conditions. The goal was to screen several essential oils and then begin to field test the oils that showed the highest efficacy in lab assays and low adult bee toxicity. The essential oils tested in this research killed mites at a greater rate than fluvalinate after 4 h of exposure under laboratory conditions, especially *P. betle*, which killed all mites in under 4 h of exposure. Previous research with the genus *Piper* has shown acaricidal activity against mites and ticks (Silva et al. 2009; Ferraz et al. 2010; Araújo et al. 2012; da Silva Lima et al. 2014; Vinturelle et al. 2017) but had not been tested on bee mites to our knowledge. The essential oil from *P. betle* leaves is dominated by phenylpropanoids and aromatic compounds (up to 40% of eugenol and up to 40% carvacrol and chavicol) (Salehi et al. 2019) which could be related to its acaricidal activity. Other typical components are terpinen-4-ol, safrole, allyl pyrocatechol monoacetate, eugenyl acetate, hydroxyl chavicol, and piper betol (Pradhan et al. 2013; Dwivedi and Tripathi 2014; Salehi et al. 2019). Some essential oils tested in this study were highly effective against *Tropilaelaps* with low toxicity to adult bees compared to dimethoate which was used as a positive control. Some essential oils were chosen for further testing for mite control at the whole colony level. We must caution, however, that different plant parts and extraction methods could cause variation in both acaricidal and bee toxicity levels.

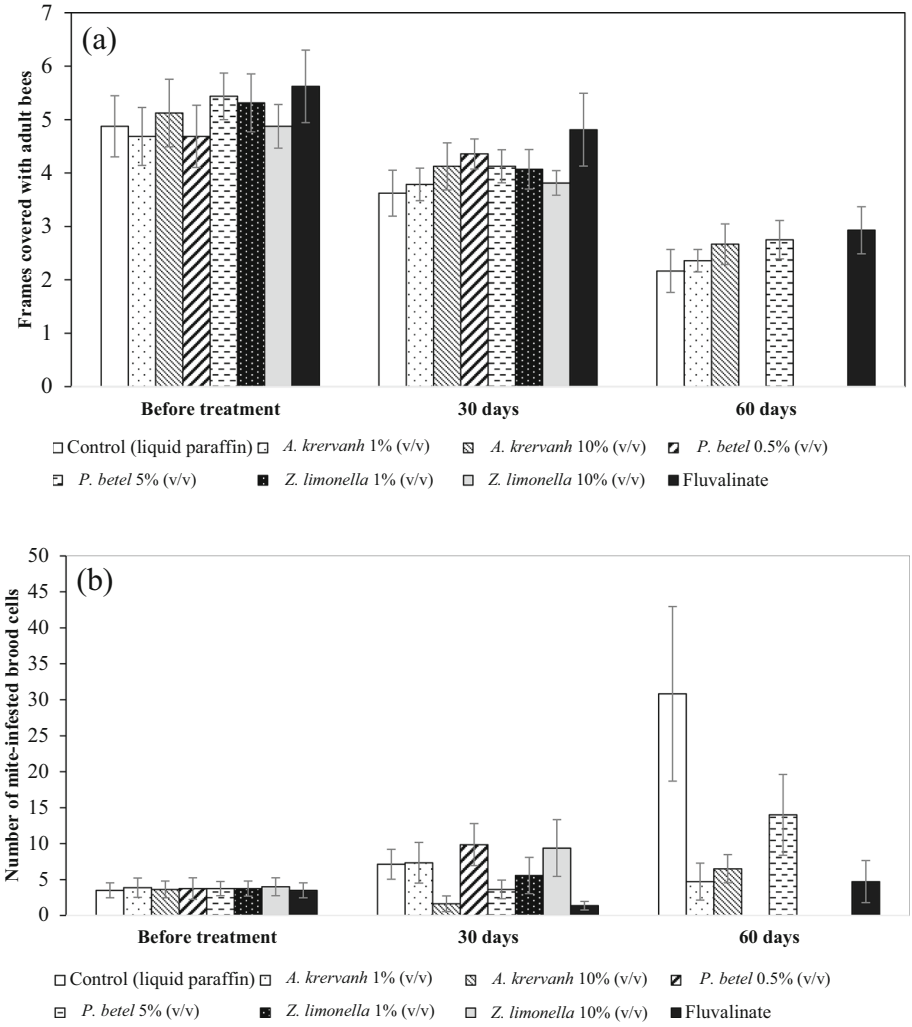

Figure 1. Average number of frames covered with adult bees (**a**) and mite populations (**b**) in honeybee colonies (n = 8/treatment group) after exposure to the essential oil of *Amomum krervanh* , *Piper betle* (L.), and *Zanthoxylum limonella* compared to controls (liquid paraffin) and fluvalinate at each observation period (before treatment and 30 and 60 days after treatment) (Kruskal-Wallis H and Steel-Dwass test, $p > 0.05$).

Thus, more testing is needed and or the identification of the more active fractions of the most promising essential oils. EOs vary in content and concentration of the active acaricidal compounds depending on many factors such as variety of plant used, locality, and the method of extraction (Table IV). These factors can result in variable results. Five essential oils, *C. hystrix* , *C. citratus* , *E. globulus* , *O. basilicum* , and *O. sanctum* , were not tested for adult bee toxicity. Although, high acaricidal effects were observed, the yield of essential oils from these plants was low by this extraction method and thus further testing was limited.

We therefore performed the field experiments with *A. krervanh* , *P. betle* , and *Z. limonella* based on their acaricidal activity, toxicity on bees, and essential oil yield. The mite infestation rate was low in all colonies used in these studies, and this could be due to several factors such as weather and reduced honeybee brood rearing at this time of year. Even with low mite levels, we

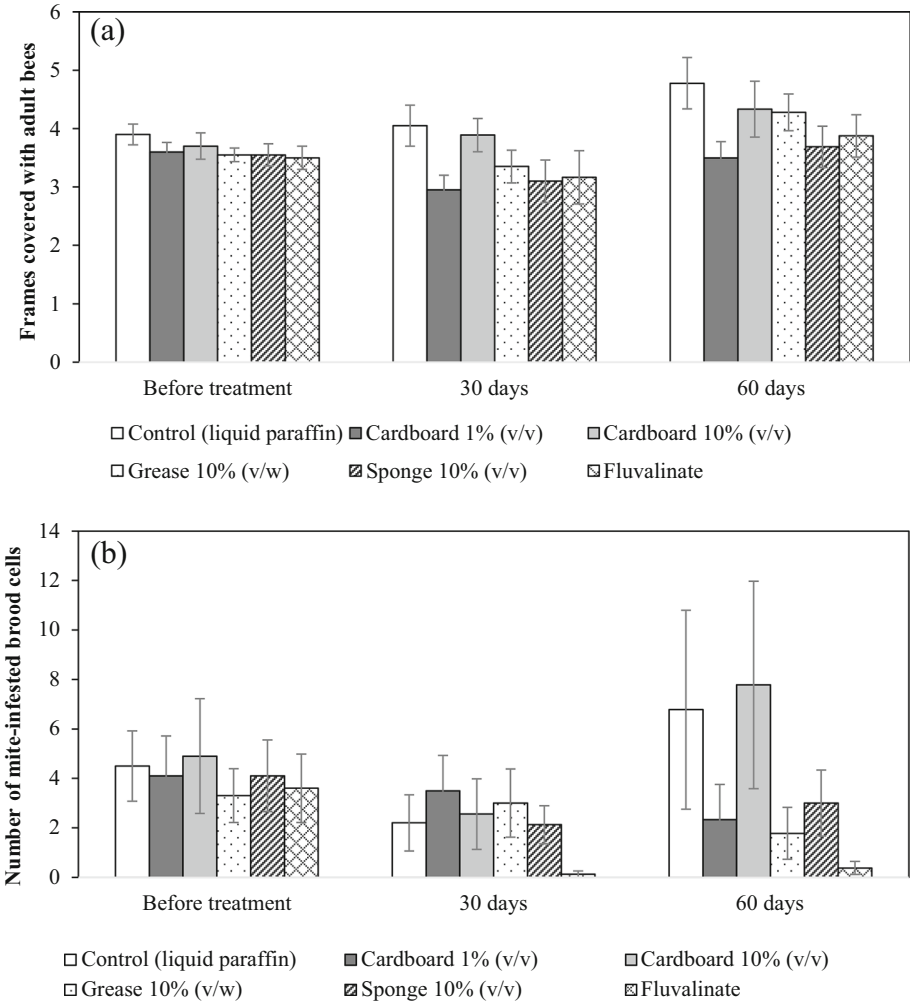

Figure 2. Average number of frames covered with adult bees (**a**) and mite populations (**b**) in honeybee colonies after exposure to the essential oil of *Amomum krervanh* with the different delivery methods (cardboard, grease, and sponge) at each observation period (before treatment and 30 and 60 days after treatment) (Kruskal-Wallis H and Steel-Dwass test, $p > 0.05$).

demonstrated that *A. krervanh* was effective in reducing mite populations at the end of the experimental period (60 days after initial exposure). Higher concentration of 10% (v/v) did not significantly reduced mite levels. It might be due to the variation between colonies such as genetic variation or that the higher concentration of *A. krervanh* caused significant changes in bee behaviors within the colony that limited the distribution of the higher dose (i.e., avoiding the strip). The 1,8-Cineloe was found as a major chemical composition in the essential oil of *A. krervanh* (Diao et al. 2014). Many studies demonstrated the biological activities of *Amomum* species including antibacterial activity and insecticidal activity (Mathew et al. 2003; Pulbutr et al. 2012; Satyal et al. 2012; Singtothong et al. 2013; Diao et al. 2014; Chen et al. 2018). Additionally, the ethanolic extract of *A. krervanh* showed the antimicrobial activity on *Paenibacillus larvae* which is the causative agent of American foulbrood in honeybees (Chaimanee et al. 2017) the most serious bacterial disease of honeybees.

Table III. Chemical composition of *A. krervanh* essential oil obtained by hydro-distillation method using GC-MS

No.	Compound	RT. (Min)	Total area (%)	Relative area (%)
1	2-Methyl-3-buten-2-ol	8.69	0.017	0.02
2	Alpha-Phellandrene	9.05	0.082	0.11
3	Alpha-Pinene	9.31	2.036	2.64
4	Camphene	9.86	0.053	0.07
5	Oxirane, 2,2-dimethyl-3-propyl-Hexane, 2,3-epoxy-2-methyl	10.23	0.016	0.02
6	Sabinene	10.69	0.466	0.60
7	Beta-Pinene	10.89	8.430	10.92
8	2-Propenamide, N-ethyl-	11.05	0.083	0.11
9	Beta-Myrcene	11.28	0.733	0.95
10	Beta-Phellandrene	11.84	0.250	0.32
11	2-Carene	12.21	0.107	0.14
12	1,8-Cineole/Eucalyptol	12.85	77.17	100.00
13	Trans-beta-Ocimene	13.22	0.072	0.09
14	Gamma-Terpinene	13.60	0.414	0.54
15	Gamma-Terpinene	13.99	0.331	0.43
16	1-methyl-4-(1-methylethylidene)-Cyclohexene	14.45	0.127	0.16
17	D-Fenchone	14.58	0.376	0.49
18	Linalool	14.95	0.618	0.80
19	Fenchyl alcohol	15.55	0.042	0.05
20	Delta-3-Carene	15.71	0.021	0.03
21	DL-Camphor	16.42	0.070	0.09
22	Camphene	16.67	0.018	0.02
23	Pinocarvone	16.89	0.027	0.03
24	Delta-Terpineol	17.15	0.332	0.43
25	4-Terpineol	17.46	0.764	0.99
26	Alpha-4-trimethyl-Benzenemethanol	17.67	0.049	0.06
27	Alpha-Terpineol (p-menth-1-en-8-ol)	17.97	4.587	5.94
28	Sabinol	18.15	0.092	0.12
29	Cycloheptane, 1,3,5-tris(methylene)-	19.21	0.026	0.03
30	2-Methyl-5-isopropenyl-2-cyclohexenone	19.31	0.024	0.03
31	Carvotanacetone	19.46	0.057	0.07
32	Phenol, 5-methyl-2-(1-methylethyl)-	20.61	0.029	0.04
33	Phenol, 2-methyl-5-(1-methylethyl)-	20.84	0.159	0.21
34	p-mentha-1(7),4(8)-diene	21.24	0.107	0.14
35	Allo-Ocimene	22.16	0.865	1.12
36	Delta-3-Carene	22.44	0.028	0.04
37	Alpha-Copaene	22.95	0.067	0.09
38	Beta-Elemene	23.31	0.016	0.02
39	Alpha-Santalene	24.07	0.100	0.13
40	Alpha-Bergamotene	24.42	0.057	0.07
41	Beta-Farnesene	24.94	0.032	0.04

Table III (continued)

No.	Compound	RT. (Min)	Total area (%)	Relative area (%)
42	Alpha-Humulene	25.03	0.023	0.03
43	D-Germacrene	25.67	0.017	0.02
44	Beta-Chamigrene	25.73	0.025	0.03
45	Beta-Selinene	25.89	0.159	0.21
46	Naphthalene, 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-	26.08	0.119	0.15
47	Alpha-Bisabolene	26.31	0.216	0.28
48	Gamma-Cadinene	26.46	0.039	0.05
49	Germacrene B	26.57	0.056	0.07
50	(+)-endo-6-methyl-2-methylene-6-(4-methyl-3-pentenyl)bicyclo[3.1.1]heptane	26.70	0.033	0.04
51	Alpha-Calacorene	27.15	0.015	0.02
52	Gamma-Curcumene	27.44	0.026	0.03
53	Beta-Bisabolene	27.57	0.080	0.10
54	1,7-Octadiene, 2,7-dimethyl-3,6-bis(methylene)-	28.00	0.015	0.02
55	Santolinatriene	28.14	0.018	0.02
56	Gamma-Curcumene	28.29	0.061	0.08
57	Alloaromadendrene	28.68	0.021	0.03
58	Clovene	29.32	0.018	0.02
59	Alpha-bergamotene	30.12	0.064	0.08
60	Tricosane	42.37	0.028	0.04
	Total area		100.00	

P. betle with a high concentration (5% (v/v)) seemed to affect the mite population at day 30. Thus, we only observed this concentration at day 60 and it did not decrease the mite population. For *Z. limonella* treatment, the results indicated that this oil was insufficient to control *T. mercedesae* when concentrations of 1% and 10% (v/v) were applied under field conditions. Environmental conditions can influence the differences observed between the acaricidal activity under laboratory and field conditions. We further tested different application methods to deliver the essential oil of *A. krervanh*. We selected methods that beekeepers can easily apply in the colony. Our results demonstrated that grease nor a sponge acted as good materials to distribute the oils to control mites in the colony under the field conditions in Thailand. This could be due to the surface property of the media or other as yet unknown properties. The viscosity of commercial grease may be a factor that reduce the release rate and distribution of essential oil in colony. On the contrary, the essential oil could be rapidly absorbed into the sponge and quickly evaporate when exposed to the air because the sponge had a large pore size. Therefore, these methods might not be suitable for essential oil delivery or may need more refinement as they have been used by Thai and other beekeepers to deliver some products within the hive. In this study, only liquid paraffin strips were assigned as negative control since liquid paraffin was used to dissolve the essential oil and the number of colonies available to test was limited. However, grease and sponge strips should be included as negative control in future experiments to fully test the effects of these carrier materials if they prove to be effective delivery media. Some essential oils that showed high activity to kill mites under laboratory did not exhibit control potential under whole colony conditions. Control

Table IV. Chemical composition of essential oil used in this study

Essential oil	Chemical composition		Reference
	Compound	%	
Amomum krervanh	1,8-Cineole	77.17	This study
	β-Pinene	8.43	
	α-Terpineol	4.58	
Cinnamomum camphora	D-Camphor	40.5	Guo et al. (2016)
	Linalool	22.9	
	1,8-Cineole	11.3	
Cinnamomum sp.	Cinnamaldehyde	65.0–80.0	Rao and Gan (2014)
	Eugenol	5.0–10.0	
Citrus hystrix	Terpinen-4-ol	13.0	Waikedre et al. (2010)
	β-Pinene	10.9	
	α-Terpineol	7.6	
	1,8-Cineole	6.4	
	Citronellal	6.0	
Cymbopogon citratus	Geranial	51.14–53.2	Pinto et al. (2015)
	Citral	35.21–36.37	
Eucalyptus globulus	1,8-Cineole	55.29	Harkat-Madouri et al. (2015)
	Isovaleraldehyde	10.04	
	α-Terpineol	5.46	
	α-Pinene	4.61	
Ocimum basilicum	Methyl eugenol	39.3	Joshi (2014)
	Methyl chavicol	38.3	
Ocimum sanctum	Eugenol	46.2	Awasthi and Dixit (2007)
	(E)-caryophyllene	27.6	
	β-elemene	16.3	
Piper betle	Eugenol	Up to 40%	Salehi et al. (2019)
	Carvacrol and Chavicol	Up to 40%	
Syzygium aromaticum	Eugenol	71.56	Nassar et al. (2007)
	Eugenol acetate	8.99	
Zanthoxylum limonella	Limonene	31.09	Itthipanichpong et al. (2002)
	Terpin-4-ol	13.94	
	Sabinene	9.13	

failure at the colony level is not uncommon and *Tropilaelaps* control failures for essential oils could be due to numerous variables such as temperature and relative humidity. These variables likely affect evaporation rate and thus can affect the exposure of mites to essential oils. Considering the acaricidal activity under laboratory test and the reduced harm to bees demonstrated by several of these essential oils in the present study, differing application methods and concentrations should be further investigated at the colony level. Essential oils can offer alternative mite control options but are more sensitive to environmental and hive conditions than synthetic miticides in slow release strips. The limitations and challenges of applying essential oils to honeybee colonies should not deter us from trying to develop these promising mite control options.

CODE AVAILABILITY

Not applicable

AUTHOR CONTRIBUTION

VC, NW, and JSP conceived this research and designed experiments; VC, TB, and JSP performed the experiments and data analysis; VC, NW, and JSP wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

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DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

DECLARATIONS

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

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Activité acaricide des huiles essentielles pour la lutte contre les acariens de l'abeille (*Apis mellifera*) *Tropilaelaps mercedesae* en conditions de laboratoire et milieu naturel.

Tropilaelaps* / activité acaricide / LC50 / *Apis mellifera* / *Piper betle* / *Amomum krervanh.

Die akarizide Aktivität essentieller Öle für die Kontrolle der Milbe *Tropilaelaps mercedesae* der Honigbiene (*Apis mellifera*) unter Labor- und Feldbedingungen.

Tropilaelaps* / akarizide AKtivität / LC50 / *Apis mellifera* / *Piper betle / Amomum krervanh.

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