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Research

Laboratory Screening of 26 Essential Oils Against *Cacopsylla chinensis* (Hemiptera: Psyllidae) and Field Confirmation of the Top Performer, *Perilla frutescens* (Lamiales: Lamiaceae)

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Abstract

Similar to other pear psylla species in Europe and America, *Cacopsylla chinensis* (Yang and Li) is one of the most important pests that causes yield loss in commercial pear orchards in China. To investigate effective essential oils as alternatives to conventional pesticides against *C. chinensis*, 26 essential oils derived from commonly used Chinese spices and medicinal herbs were screened for insecticidal activity. Among these, the essential oil from *Perilla frutescens* (L.) Britton leaves was the top performer; it exhibited strong and acute toxicity against pear psylla, with an LD_{50} value of 0.63 μg per adult. Then, we tested the constituents of the essential oil and its toxicity in the field. Field trials showed a 72% corrected reduction in the first–second-instar population 7 d after spraying *P. frutescens* leaf oil solution at a concentration of 1 mg/ml and a 47% corrected reduction at days 3 and 14. This report is the first to document the application of essential oil from *P. frutescens* leaves to control *C. chinensis* under field conditions. Our results suggest that *P. frutescens* oil can be considered a novel potential pesticide for *C. chinensis* control in pear orchards.

Key words: pear psylla, herb pesticide, acute toxicity, pear orchard

Pear psylla (*Cacopsylla chinensis* Yang and Li) is one of the most harmful pests of pear (*Pyrus* spp.) trees in China, especially in northern China (Yang et al. 2004). Adult *C. chinensis* have a large, dark winter form and a small, light-colored summer form (Butt and Stuart 1986). Adults and nymphs in their summer form cause severe damage to pear orchards; they pierce and suck the nutrients from pear leaves and secrete honeydew, which causes black mold and leads to the early defoliation of pear trees. Moreover, they also spread pear pathogens, such as *Erwinia amylovora* Burrill (Hildebrand et al. 2000).

Synthetic pesticides have been heavily applied in China over the past six decades in an attempt to control this pest, resulting in problems (Bai and Ogbourne 2016) such as pest resistance to insecticides, groundwater contamination, ecological disruption, wildlife destruction, and threats to human health (Isman 2006). Because of the development of pest resistance, the use of many insecticides against pear psylla, such as organophosphorus and pyrethroids, has sharply declined. The only insecticide employed from 2005 to 2011 was abamectin (Civolani et al. 2010). Therefore, we feel that it is necessary to investigate more alternatives to conventional pesticides.

Previous studies have indicated that essential oils have significant biopesticide activity and are characterized by their efficacy, low toxicity, and low amounts of residue (Isman 2000). Additionally, essential oils have been used to control insect pests worldwide. Several essential oils and plant extracts, such as garlic oil (Zhao et al. 2013), rapeseed oil (Marcic et al. 2009), clove oil (Tian et al. 2015), and crude alkaloids from *Macleaya cordata* (Willd.) (Sun et al. 2004), possess insecticidal activity against *C. chinensis*.

Perilla (*Perilla frutescens*) is a traditional edible plant in Asia (Yu et al. 2016). In addition, *P. frutescens* has a significant role in medical science. The essential oil from its leaves possesses antibacterial and antifungal activities (Lim and Shin 2011, Lin et al. 2016) and shows strong larvicidal activity against booklice (*Liposcelis bostrychophila* Badonnel; Zhao et al. 2012). The chemical composition of the essential oils derived from *P. frutescens* leaves has been previously determined (Ha et al. 2015, Lin et al. 2016). In addition, an extract of *P. frutescens* oil, which had an LD_{50} value of 3.0 g/kg, showed acute toxicity to mice after intragastric administration (Wen 2006). According to the acute toxicity scale, this LD_{50} value indicates

low toxicity (0.5–5.0 g/kg). This result demonstrates that the oil is relatively safe for human beings.

To investigate more effective essential oils that could serve as alternatives to conventional pesticides against C. *chinensis*, we investigated 26 specific characteristics of Chinese spices and medicinal herbs for insecticidal activity based on their unique smells (such as *Piper nigrum* L., with its pungent odor) and spice flavors (such as *Allium sativum* L. and *Alpinia galanga* Willdenow). The essential oil of perilla leaves was the top performer among them. Then, we tested the constituents of this essential oil and its toxicity in the field to confirm its efficacy.

Materials and Methods

Insects

The winter form of *C. chinensis* adults, which were used for an indoor bioassay, were attracted using corrugated paper bound to 10-year-old pear trees in Chang-ping District (39°37′N, 116°24′E, altitude 22 m), Beijing, China, from late 2014 to early 2015. The overwintered adults were collected on 26 January 2015 (the peak occurrence of winter adults), from pear orchards close to our laboratory (Laboratory of Entomology and Nematology [LEN]), and were transferred by suction implements and brushes into plastic boxes (10 × 10 × 20 cm). Cotton balls wetted with honey water were hung in the boxes to ensure sufficient moisture and food for the insects. The boxes with the winter-form adults were placed in an incubator (27–29°C, 75% relative humidity [RH], under a 15:9 L:D photoperiod) for 24 h prior to experiment initiation (Tian et al. 2015). The summer-form adults and nymphs of *C. chinensis* used in the field trial were obtained from the experimental orchard in early July 2015.

Essential Oils

Allium sativum L., *Alpinia galanga* Willdenow, *Foeniculum vulgare* Miller, *Zanthoxylum bungeanum* Maximowicz, and *Citrus limon* (L.) Burman were purchased from Merry Mart Supermarket (Beijing, China). The remaining samples were purchased from Anguo Chinese Medicinal Herbs Market (Anguo, Hebei Province, China) (Table 1). All samples were identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing, China), and voucher specimens have been preserved in the Department of Entomology, China Agricultural University. The samples were cut into small pieces and subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. Anhydrous sodium sulfate was used to remove water after extraction. The concentrated oils were maintained in a refrigerator at 4°C for subsequent experiments.

Screening for Insecticidal Activity

In laboratory bioassays, the 26 essential oils were diluted in acetone to two concentrations, 1 and 5%. To determine their effective concentrations, the essential oils of the top performers were diluted in acetone to 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml for perilla (*P. frutescens*) and to 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml for garlic (*Allium sativum*) in a range-finding study. Commercial abamectin, purchased from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (six concentrations ranging from 0.005 to 0.16 mg/ml), was used as a positive control, while acetone was used as a negative control. Active adults were transferred using a suction sampler into Petri dishes (9 cm diameter), placed on ice, and anesthetized with ether, after which each adult was dosed with 0.5 µl essential oil, which was applied to the dorsal thorax with an Eppendorf pipette. A group of 10 treated insects was considered one replicate, and each treatment consisted of five replicates. Petri dishes containing treated insects were placed in an incubator (27–29°C, 75% RH, under a photoperiod of 15:9 L:D) for 24 h before mortality was recorded. The insects were considered dead if they did not respond to the touch of a small soft brush. The relative mortality in the laboratory bioassay was control corrected using Abbott's formula (Abbott 1925).

$$R\% = \left[1-(T2\times C1)/(T1\times C2)\right]\times 100$$

where $R\%$ = corrected percent reduction, $T1$ = before treatment, $T2$ = after treatment, $C1$ = before control, and $C2$ = after control.

Chemical Identification and Quantification

The essential oil of *P. frutescens* leaves was subjected to gas chromatography mass spectrometry (GC-MS) on a system consisting of an Agilent 6890N gas chromatograph combined with an Agilent 5973N mass selective detector. The same column and analysis conditions were used for both GC and GC-MS. The flame ionization detector was equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The initial oven temperature was held at 60°C for 1 min and then increased at 10°C/min to 180°C, held for 1 min and then ramped at 20°C/min to 280°C and then held for 15 min. The injector temperature was maintained at 270°C, the injection volume was 1 µl (diluted 1:100 in *n*-hexane), and the split ratio was 1:10. The carrier gas was helium at a flow rate of 1.0 ml/min. Spectra were scanned from 20 to 550 m/z at two scans per second. Most of the components were identified by comparing their retention indices (RIs) with those in the literature or with the authentic compounds available in our laboratory. The RIs were determined in relation to a homologous series of *n*-alkanes (C_8-C_{24}, Sigma-Aldrich, St. Louis, MO) under the same operating conditions, which could be referred to as a Kovats RI. Further identification was performed by comparing the mass spectra with those stored in the NIST 05 and Wiley 275 libraries or with mass spectra data available in the literature (Adams 2007). The relative percentage of each component was calculated by averaging the GC-flame ionization detector peak area percentages.

Field Trials

Field experiments were conducted in early July 2015 in an experimental pear orchard in which chemical pesticides were typically applied three times per year, i.e., at a much lower frequency than that in other orchards. The last application was carried out in the middle of May, 45 d before the trial.

Garlic oil, perilla leaf oil (the top performer of the 26 tested in the laboratory), abamectin, and detergent (negative control) at concentrations of 1 mg/ml, as well as water (blank control), were applied in the field trial (Tian et al. 2015). The essential oils were diluted with tap water after being dissolved in dishwashing detergent (1:1 v:v as a surfactant, Beijing Goldfish Technology Co., Beijing, China). The solutions (approximately 1.5 liters per tree) were applied using sprinkling cans (Alibaba, China) and a completely randomized block design that included five adjacent parallel replicate blocks. Each block included five plots, one for the blank control (water), one for the negative control (detergent), and three for the garlic oil, perilla leaf oil, and abamectin treatments. The solutions were applied to each plot; the plots contained nine trees per treatment (three rows × three trees). All nine trees were recorded when checking living *C. chinensis* first–second instars and third–fifth instars. The trees in one plot were considered a single treatment (Erler et al. 2014). Observations were conducted

Table 1. Insecticidal activity of 26 essential oils against *C. chinensis* adults

Species	Source	Variety	Active ingredient		Contact toxicity[a]	
			Chemical name	Concentration (100%)	5% (mean ± SE)	1% (mean ± SE)
Acorus calamus Linnaeus	Rhizome	Hangzhou *A. calamus*	β-Asarone	59.41	90.0 ± 0.3%	–
			Isoshybunone	17.77		
Acorus tatarinowii Schott	Rhizome	Zhangjiajie *A. tatarinowii*	β-Asarone	84.76	100%	50.1 ± 0.5%
Agastache rugosa (Fischer and Mey) Otto Kuntze	Aerial parts	Guangdong *A. rugosa*	β-Caryophyllene	59.30	88.2 ± 0.2%	–
Allium sativum Linnaeus	Rhizome	Shandong Jinxiang white garlic	Diallyl trisulfide	50.43	100%	90.3 ± 0.6%
			Disulfide	25.30		
Alpinia galanga Willdenow	Seed	Anguo *A. galanga*	1,8-Cineole	28.30	46.7 ± 0.3%	–
Alpinia katsumadai Hayata	Rhizome	Hainan *A. katsumadai*	n-Hexadecanoic acid	12.31	54.3 ± 0.3%	–
Alpinia officinarum Hance	Rhizome	Yunnan *A. officinarum*	Eucalyptol	28.11	35.6 ± 0.4%	–
Chrysanthemum cinerariifolium (Treviranus) Visiani	Whole plant	Yunnan *C. cinerariifolium*	α-Humulene	25.50	68.5 ± 0.3%	–
Cinnamomum cassia Presl	Aerial parts	Yunnan *C. cassia*	Cinnamaldehyde	30.67	82.3 ± 0.2%	–
Citrus limon (Linnaeus) Burman	Fruit	Meyer lemon	Limonene	92.93	100%	33.3 ± 0.2%
Citrus reticulata Blanco	Pericarp	Guangxi *C. reticulata*	Acetic acid	40.80	58.6 ± 0.5%	–
Cuminum cyminum Linnaeus	Fruit	Xinjiang *C. cyminum*	α-Pinene	29.10	100%	56.7 ± 0.4%
Curcuma zedoaria (Christmann) Roscoe	Rhizome	Kwangsi turmeric	Epicurzerenone	46.60	100%	61.7 ± 0.3%
Curcuma longa Linnaeus	Rhizome	Guangxi Bobai turmeric	Curcuminoids	8.43	85.6 ± 0.3%	–
Cyperus rotundus Linnaeus	Rhizome	Guangdong *C. rotundus*	α-Cyperone	11.00	100%	60.0 ± 0.4%
Dendranthema indicum (Linnaeus) Des Moulins	Flower	Hubei *D. indicum*	Bornyl acetate	15.40	100%	28.3 ± 0.5%
Foeniculum vulgare Miller	Fruit	Gansu *F. vulgare*	β-Anethole	69.87	100%	46.7 ± 0.6%
Illicium verum Hooker	Fruit	Red star	β-Anethole	89.50	52.7 ± 0.5%	–
Kaempferia galanga Linnaeus	Rhizome	Yunnan *K. galanga*	β-P-methoxy-ethyl-cinnamate	59.24	42.9 ± 0.4%	–
Perilla frutescens	Leaf	Kaiyang *P. frutescens*	Sesquiterpenoids	37.30	100%	95.56 ± 0.3%
Piper nigrum Linnaeus	Fruit	Type L *ampong*	β-caryophyllene	57.59	31.1 ± 0.4%	–
Pogostemon cablin Bentham	Aerial parts	Guangdong *P. cablin*	Patchouli alcohol	32.20	72.3 ± 0.2%	–
Tetradium ruticarpum (Jussieu) Hartley	Fruit	Dogwood	β-Basil	67.04	63.7 ± 0.2%	–
Zanthoxylum bungeanum Maximowicz	Fruit	Guizhou *Z. bungeanum*	Linalool	29.00	100%	45.32 ± 0.6%
Zanthoxylum schinifolium Siebold and Zuccarini	Fruit	Chongqing *Z. schinifolium*	Estragole	78.25	18.3 ± 0.3%	–
Zingiber officinale Roscoe	Rhizome	Shandong cuisine ginger	α-Zingiberene	21.24	100%	78.33 ± 0.4%

[a]Contact toxicity, expressed as corrected mortality using the Abbott equation. The control treatment was acetone.

on four randomly selected 80-cm-long branches: approximately 25 × 4 leaves per tree were observed using a handheld magnifier (10 ×) immediately before treatment and 3, 7, and 14 d after treatment.

The corrected percent reduction in nymphs ($R\%$) in the different essential oil treatments was calculated using Henderson and Tilton's equation (Henderson and Tilton 1955):

$$R\% = [1-(N\text{ta}/N\text{ca})\times(N\text{cb}/N\text{tb})]\times 100$$

where N = number of nymphs per shoot, t = treated plots, c = control plots, a = after treatment, b = before treatment.

Data Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test using SPSS Statistics 20.0 software.

Significant differences in LD_{50} were based on the non-overlap of 95% fiducial limits.

Results

Screening Essential Oils for Insecticidal Activity

Among the 26 essential oils, 11 showed strong insecticidal activity (100% mortality) against *C. chinensis* at a concentration of 5% (Table 1). However, at a lower concentration of 1%, only perilla oil and garlic oil exhibited strong acute toxicity against *C. chinensis* adults, with corrected percent reductions of 96 and 90%, respectively (Table 1). The adult percent reduction of the control was 2%.

Laboratory Bioassays for Dilution Determination

Perilla oil and garlic oil exhibited insecticidal activity against *C. chinensis*, with LD_{50} values of 0.63 and 1.38 μg/adult, respectively (Table 2).

Chemical Constituents of the Essential Oil of *P. frutescens*

Analysis of the essential oil of *P. frutescens* leaves via GC and GC-MS resulted in the identification of 34 components, constituting 98.2% of the total oil (Table 3). The dominant components in the essential oil were geranial (30.4%), neral (18.1%), β-caryophyllene (9.7%), nerol (5.7%), and *cis*-α-bisabolene (4.0%) (Table 3). Monoterpenoids represented 17 of the 34 compounds, corresponding to 60.0% of the total oil, while 15 of the 34 constituents were sesquiterpenoids (37.3% of the crude essential oil).

Field Trials

The results of the field trials on first–second instars of *C. chinensis*, to which the two essential oils were applied, are shown in Fig. 1. The corrected percent reductions were 85, 81, and 73% at 3, 7, and 14 d, respectively, after the application of garlic oil. There was no significant difference in the response to abamectin over time. The corrected percent reductions in third–fifth instars were 47, 72, and 47% at 3, 7, and 14 d, respectively, after the application of perilla oil. The corrected percent reductions measured in response to garlic oil and abamectin did not differ significantly at 7 d (Fig. 1).

The results of the field trials on third–fifth instars of *C. chinensis*, to which the two selected essential oils were applied, are shown in Fig. 2. The corrected percent reductions measured in response to garlic oil were 60, 34, and 11% at 3, 7, and 14 d, respectively. The corrected percent reduction with perilla oil was 40% at 7 d, which was significantly higher than that measured at 3 and 14 d, i.e., 15 and 14%, respectively ($F = 2.237$; df = 14, 30; $P < 0.05$) but was not significantly different from that measured in response to garlic oil at 3 and 7 d.

Table 2. Insecticidal activity of *Perilla* oil against *C. chinensis* adults

Treatment	LD_{50} (μg per adult)	95% Fiducial limit	Slope ± SE	χ^2
Garlic oil	1.38	1.22–1.55	3.30 ± 0.33	14.89
Perilla oil	0.63	0.42–0.87	1.04 ± 0.13	7.78
Abamectin	1.87×10^{-3}	1.36×10^{-3}–2.33×10^{-3}	3.83 ± 0.43	14.35

Discussion

Laboratory bioassays indicated that at high concentrations, 11 of the 26 essential oils possessed insecticidal activity against *C. chinensis*. In previous studies, ginger and fennel oils exhibited strong insecticidal activity against mosquitoes (Gomes et al. 2016, Pavela et al. 2016). In addition, the essential oil of *Cyperus rotundus* showed

Table 3. Chemical constituents of the essential oil derived from *P. frutescens*

Peak no.	Compound	RI[a]	Percent composition
	Monoterpenoids		60.0
1	α-Pinene	931	0.3
2	β-Pinene	981	0.1
3	(d)-Limonene	1030	0.2
4	1,8-Cineole	1032	0.3
5	*cis*-β-Ocimene	1037	0.4
6	Fenchone	1088	0.2
7	Linalool	1094	1.3
8	Perillene	1097	0.8
9	exo-Fenchol	1112	0.6
10	4-Terpineol	1177	0.2
11	α-Terpineol	1188	0.2
12	Nerol	1229	5.7
13	Neral	1238	18.1
14	Carvone	1254	0.1
15	Geranial	1270	30.4
16	Phellandral	1279	0.2
17	*cis*-Geranyl acetate	1364	0.9
	Sesquiterpenoids		37.3
18	Copaene	1374	1.3
19	(Z)-Caryophyllene	1405	1.6
20	β-Caryophyllene	1420	9.7
21	α-Bergamotene	1433	3.1
22	*trans*-β-Farnesene	1452	1.9
23	α-Caryophyllene	1454	3.3
24	Germacrene D	1458	2.5
25	*cis*-α-Bisabolene	1504	4.0
26	γ-Cadinene	1514	2.1
27	Germacrene B	1561	1.3
28	Spathulenol	1578	3.0
29	Caryophyllene oxide	1589	1.9
30	Humulene-1,2-epoxide	1606	0.7
31	τ-Cadinol	1624	0.2
32	β-Eudesmol	1645	0.7
	Others		0.8
33	3-Octanol	993	0.1
34	*trans*-Chrysanthemal	1153	0.7
	Total		98.2

[a]RI, retention index, determined on an HP-5MS column using a homologous series of *n*-hydrocarbons.

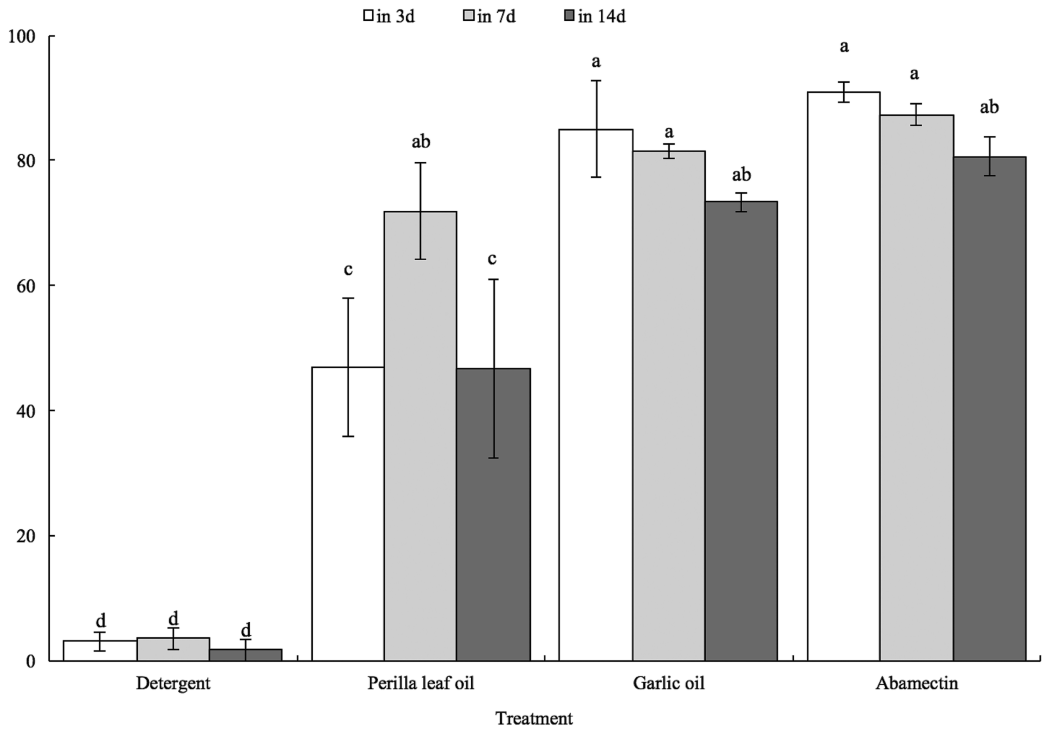

Fig. 1. The corrected percent reduction in *C. chinensis* first–second instars with detergent, perilla leaf oil, garlic oil, and abamectin in the field at 3, 7, and 14 d. The blank control treatment was clear water. Values shown are the mean ± SE calculated according to Henderson and Tilton's equation. Bars indicate SE. Letters indicate significant differences among treatments and the number of days post-application. Means within columns followed by the same letter are not significantly different (ANOVA: Tukey's test; $F = 4.183$; df = 14, 30; $P < 0.05$).

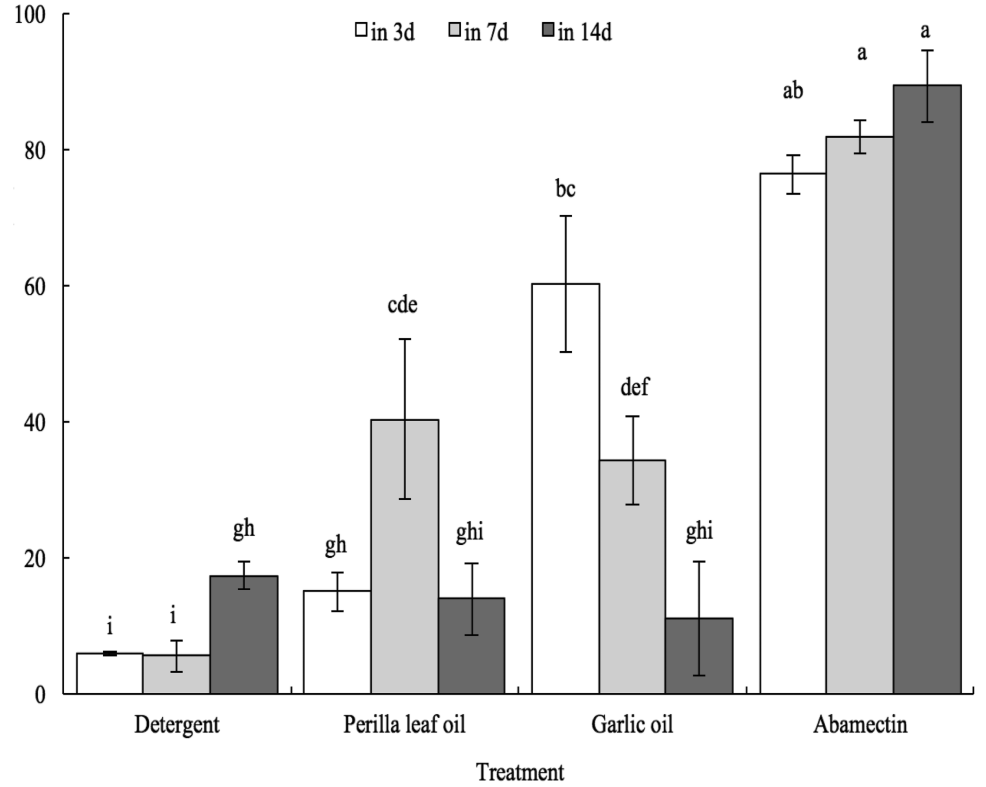

Fig. 2. The corrected percent reduction in *C. chinensis* third–fifth instars with detergent, perilla leaf oil, garlic oil, and abamectin in the field at 3, 7, and 14 d. The blank control treatment was clear water. Values shown are the mean ± SE calculated according to Henderson and Tilton's equation. Bars indicate SE. Letters indicate significant differences among treatments and the number of days post-application. Means within columns followed by the same letter are not significantly different (ANOVA: Tukey's test; $F = 4.183$; df = 14, 30; $P < 0.05$).

insecticidal activity against booklice (*L. bostrychophila*) (Liu et al. 2016). The essential oil of *Citrus limon* displayed fumigant toxicity against the red flour beetle (*Tribolium castaneum*) (Abou-Taleb et al. 2016). Furthermore, the essential oil of *Z. bungeanum* exhibited fumigant toxicity against *Lasioderma serricorne* (Zhang et al. 2016).

However, only two of the essential oils (from garlic and from perilla leaves) exhibited strong acute toxicity against *C. chinensis* at lower concentrations. The insecticidal activity of garlic oil has been widely studied in the laboratory and in the field against many insect pests. Several commercial garlic oil products are available in China. Thus, *P. frutescens* oil was chosen for further field experiments to examine its potential for development.

Geranial, neral, β-caryophyllene, nerol, and *cis*-α-bisabolene were the principal constituents of the essential oil of *P. frutescens* leaves; the oil had high concentrations of monoterpenoids and sesquiterpenoids (Table 3). These results are quite different from those presented in previous reports. For example, perilla ketone (35.6%) and isoegoma ketone (35.1%) were the two main constituents of *P. frutescens* oil from Turkey (Baser et al. 2003). In addition, perillaldehyde (72.07%) and limonene (13.15%) were the two major compounds in *P. frutescens* oil from Lithuania (Bumblauskiené et al. 2009). The volatile compounds of *P. frutescens* in Cheonan, Korea, contained mainly perilla ketone (95%) (Seo and Baek 2009), while those harvested from Muhan, Korea, contained mainly perilla aldehyde (19.14–47.28%) followed by β-caryophyllene (4.16–12.3%) (Choi and Min 2003). Perilla ketone and its isomers were also found in oil from India and Japan (Nitta et al. 2006, Gwari et al. 2016) but were not found in our study. These results are consistent with research conducted by Tian et al. (2015), who reported that the essential oil of *P. frutescens* collected from 11 areas across China (Quzhou, Loudi, Ganzhou, Baoding, Pingliang, Nanning, Kaili, Suining, Macheng, Nanyang, and Yixing) did not contain perilla ketone. However, in other research conducted in China, perilla ketone was reported to be one of the main constituents of *P. frutescens* (Ye and Zheng 2009). Because geranial was the major component in the current study, the tested chemotypes of *P. frutescens* oil in the leaves were considered C type (Honda et al. 1994); six chemotypes have been classified according to their main components, geranial (C), phenylpropanoids (PP), perillene (PL), perillaldehyde (PA), perilla ketone (PK), and Elsholtzia ketone (EK).

The above results indicate that the essential oil of *P. frutescens* can vary considerably as a result of geographic location and different growing conditions (Verma et al. 2013, Isman and Grieneisen 2014). Therefore, the standardization of the essential oil is strongly needed for further studies.

With respect to the percent reduction in the first–second instars and third–fifth instars of *C. chinensis* in the field on day 7, the efficacy of the *P. frutescens* oil was not significantly different from that of garlic oil. Garlic oil and extracts have already been formulated into commercial products for pest control, such as Garlic Barrier Ag and ENVI Repel. Similarly, the essential oil of *P. frutescens* has the potential to be formulated into products.

The essential oils could help suppress in insect population when deployed in either a reduced risk program or in the organic market. There are numerous reports on the insecticidal activity of essential oils tested indoors on stored-product insects, mosquitoes, and lepidopterous larvae (Tapondjou et al. 2002; Sim et al. 2006; Tawatsin et al. 2006; Pitasawat et al. 2007; Lima et al. 2010; Liu et al. 2012, 2015; Zhu et al. 2015). However, there are very few results from the field. At this point, field trials are greatly needed to prove the efficacy of essential oils in natural environments and promote their practical application. Moreover, future investigations should pay more attention to low-cost application techniques in pear orchards.

Acknowledgments

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