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Research

Peppermint Essential Oil Toxicity to the Pear Psylla (Hemiptera: Psyllidae) and Potential Applications in the Field

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Abstract

Chinese pear psylla (*Cacopsylla chinensis* Yang et Li) is a serious orchard pest that causes declines in fruit quality through feeding damage and the spread of pathogens. The rapid development of chemical pesticide resistance has become a severe problem in controlling pear psylla. Thus, the development of natural pesticides to replace conventional chemical pesticides is urgently needed. Here, we found that the essential oil of peppermint (*Mentha haplocalyx* Briq. [Lamiales: Labiatae]) is an ideal agent for controlling pear psylla based on experiments in the laboratory and the field. The major constituents of peppermint essential oil were found including menthol (49.73%), menthone (30.52%), α-pinene (3.60%), and α-terpineol (3.81%). This oil and chemicals in it performed serious contact toxicity against the winter-form adults and nymphs of pear psylla, yielding LD_{50} values of 2.54, 10.71, 2.77, 5.85, and 12.58 μg/adult and 1.91, 9.56, 2.18, 4.98, and 12.07 μg/nymph, respectively. Furthermore, the essential oil strongly repelled the adults of pear psylla with 78% repellence at the highest concentration tested in a Y-tube olfactometer in the laboratory. The combined effect of the two factors made peppermint essential oil a natural pesticide, which achieved a maximum reduction of round to 80.9% in winter-form adult population and round to 67.0% in nymph population at the concentration of 4.0 ml/L in the field. Additionally, it had no effect on the natural enemies of pear psylla in the field. Therefore, peppermint essential oil has potential as an alternative to chemical pesticides for pest control in integrated pest management programs in pear orchards.

Key words: biological sources, natural enemy, pest management

People are becoming increasingly aware of the threats posed to their health and the environment by synthetic insecticides, which have been very important for decreasing damage due to pests (Cardiet *et al.* 2012). Pear is a popular fruit among people worldwide, but the use of chemical pesticides in pear psylla management has led to the serious problem of pesticide residue on fruits (Yamada *et al.* 2007). Therefore, there has been increasing demand for alternative methods of pest management. Recent studies have identified plant essential oils as effective ecological agents with many plant essential oils showing broad spectrum activity against pest insects (Bakkali *et al.* 2008, Isman *et al.* 2011, Campolo *et al.* 2012, Pavela 2015, Campolo *et al.* 2018, Ikbal and Pavela 2019, Santos *et al.* 2019).

Through coevolution, most plants have evolved complex and effective chemical arsenals to limit herbivorous insect-inflicted damage (Hua *et al.* 2013, Liu *et al.* 2017, Hettenhausen *et al.* 2017). Therefore, there is no doubt that plant products can be used to protect plants from insect pests (Isman and Grieneisen 2014). To date, some chemical constituents from several plants have been developed for commercial products, such as pyrethrin, matrine, nicotine, and rotenone. These plant natural products demonstrate mechanisms of insecticidal (Ghasemi *et al.* 2011) or repellent action (Lachance and Grange 2014). Peppermint (*Mentha haplocalyx* Briq.) is a common Chinese medicinal plant, and its compounds include menthone, menthol, isomenthone, menthofuran, 1,8-cineole, pulegone, sabinene hydrate, limonene, and menthylacetate (Lange *et al.* 2011). These compounds have been demonstrated to exhibit toxicity and repellent bioactivity against mosquitoes (Ansari *et al.* 2000), red imported fire ants (Wiltz 2007), olive fruit flies (Pavlidou *et al.* 2004), red flour beetles (Lee *et al.* 2002), and nematodes (Mukherjee and Sinhababu 2014).

The pear psylla, *Cacopsylla chinensis* Yang et Li, is one of the most serious pests on pear tree in the worldwide (Inoue *et al.* 2012, Erler and Tosun 2017). In summer, psylla nymphs and adults cause direct damage to pear plants by sapping from young leaves, shoots, and buds (Erler 2004). During feeding, the honeydew released from psylla adults and nymphs can drop and accumulate on the surfaces of fruit and leaves and lead to russeting disease (Pehlevan and Kovanci 2018). Furthermore, these pests spread pear decline, fire blight, die-back disease, and other diseases (Burckhardt and Hodkinson 1986). In fall, the adults of pear psylla become darker than in summer, becoming winter-form adults and forming the overwintering population. Therefore, it is important to control the winter-form adults and the nymphs that emerge in spring and summer (Zhao *et al.* 2013).

In recent years, the damage to pear orchards has increased in both intensity and extent. To suppress psylla populations, most orchards are treated with conventional insecticides (abamectin and pyrethroids) eight to nine times during the growing season (Bues *et al.* 2005). However, the development of pesticide resistance in pear psylla is a concern. A study showed that the incidence of pear psylla was increased when orchards were sprayed heavily with chemical pesticides, probably due to reductions in the numbers of the pear psylla's natural enemies (Sanchez and Ortín-Angulo 2012). Similarly, our research has shown that the numbers of natural enemies in pear orchards have been reduced in recent years by the extensive use of synthetic chemical insecticides (unpublished data). The use of several chemical insecticides has been gradually phased out, mainly because of the associated health risks. The increasing challenges of controlling pests that were previously controlled with chemical insecticides prompted us to seek alternatives, such as the pyrethrin, garlic oil, and clove oil which come from medical plants, and they were found to have high toxic to pear psylla (Erler *et al.* 2007, Zhao *et al.* 2013, Tian *et al.* 2015). Nevertheless, there are no reports of the effects of peppermint essential oil on pear psylla.

Hence, this study was trying to evaluate the pesticidal potential of peppermint essential oil, which was screened from nearly 30 medical plants, against pear psylla by laboratory examinations and field trials. In this study, we address the following questions: 1) Does peppermint essential oil exhibit similar toxicity against the nymphs and winter-form adults of pear psylla? 2) What are the effective components in peppermint essential oil? 3) What are the effects of peppermint essential oil on pear psylla and natural enemies in the field?

Materials and Methods

Peppermint Essential Oil Preparation

Peppermint essential oil was obtained according to a previously described method (Tian *et al.* 2015). In brief, we purchased the dried peppermint leaf and stem from Anguo Chinese Medicinal Herbs Market (Anguo, 071200, Hebei Province, China). The sample was identified by Prof. Z. L. Liu (College of Plant Protection, China Agricultural University, Beijing, China) as *Mentha haplocalyx* Brig (Labiatae), based on comparison with a voucher specimen (XBGH009235) in the Herbarium, Xi' an Botanical Garden, Xi'an, Shanxi, China. Five hundred grams were hydrodistilled in a 3-l of flask for 6 h and then was extracted with 1000 ml of *n*-hexane before concentrated at 40°C by rotary evaporators. We obtained approximately 150 ml of essential oil from 6,000-g dry leaves and stem. The oil was stored in a refrigerator (4°C) until it was tested.

Insect Preparation

Pear psyllas were collected from the Hosui pear orchard in Xinji County, Hebei Province, China (37°58′N 115°23′E, altitude 25 m) between July 2015 and January 2016. We collected the adults from corrugated papers which were around the pear tree in winter. We collected the nymphs from the leaves in summer. All the insects were housed in an incubator with 28°C, RH = 90%, L:D = 15:9 h for 24 h before the experiments.

Chemicals Identification in Essential Oil

The GC-MS instrument (Gas Chromatography–Mass Spectrometer, Agilent 7890, Agilent Technologies Inc., CA) was used to identify the contents of peppermint essential oil according to a previously described protocol. In brief, the condition was 60°C for 1 min, increased to 180°C at the speed of 10°C/min, and then maintained for 1 min, continue to 280°C at the speed of 20°C/min, maintained for 15 min; the 1 μl of essential oil (1% in acetone) was injected into a HP-5 capillary column which was 30 m × 0.32 mm and a 0.25-mm film with ionization. The stream injector was 270°C, the ratio was 1:10, the carrier gas was helium with a flow rate of 1.0 ml/min, and the spectra were scanned from 20 to 550 *m/z* at two scans per second. The compounds were identified by comparing their mass spectra with those stored in the NIST 05 and Wiley 275 libraries or with published mass spectra (Bues *et al.* 2005, Wang *et al.* 2011, Tian *et al.* 2015).

The identification and structural elucidation of the main constituents in peppermint essential oil were performed by nuclear magnetic resonance (NMR) according to Figueiredo *et al.* (2018). The spectra of ^{1}H NMR and ^{13}C NMR were obtained in a Bruker AVANCE NEO NMR spectrometer (Germany) with frequencies of 300 and 500 MHz for ^{1}H and 125 MHz for ^{13}C, and the deuterated solvent was used as internal standard (Figueiredo *et al.* 2018)

The Toxicity of Peppermint Oil and Its Constituents Against Pear Psylla in the Laboratory

Seven concentrations (in acetone, 0.075, 0.150, 0.300, 0.600, 1.200, 2.400, and 4.800%) of peppermint oil and its main constituents, such as menthol, menthone, α-pinene, and α-terpineol, were selected for determining the LD_{50} values against the winter-form adults and nymphs, respectively. Seven concentrations (in acetone, 0.015, 0.003, 0.006, 0.012, 0.024, 0.048, and 0.096%) of abamectin were used as a positive control and the pure acetone was used as a negative control in our study. We carried out five replicates per dose of each chemical, ten insects per replicate.

We prepared the Petri dishes of a diameter of 9 cm, which included a leaf disc of 8 cm diameter, for bioassays. We anesthetized the tested insects by ether on an ice before these bioassays and then applied 0.5 μl of tested oils on their dorsal thorax by using Burkard Arnold micro applicators (Burkard Scientific Supply, Rickmansworth, United Kingdom). Groups of 10 tested insects were put into prepared Petri dishes. All the Petri dishes including tested insects were maintained in an incubator, the incubator was set at 28°C, 90% RH, and a long-day photoperiod (light: dark = 15:9 h). The number of the tested insects was counted after 24 h. The tested insect was considered dead if it failed to respond when probed with a fine camel's hair brush (Tian *et al.* 2015).

Repellence Test Using Peppermint Oil in the Laboratory

We used a Y-tube olfactometer to observe the response of the winter-form to peppermint essential oil. The olfactometer was a Y-tube made of glass with one 27-cm length arm (long arm) and two 7-cm length arms (short arm). The angle was 135 degree and the tube diameter was 2.5 cm. The Y-tube was positioned horizontally at an approximately 15-degree incline (Horton and Landolt 2010). Each short arm was connected with one 1 l of jar by the 5-mm diameter polytetrafluoroethylene (PTFE) pipe (Shenzhen Dankai Technology

Co., Ltd., Dongguan City, Guangdong Province, China). One of the two jars contained 10 μl of peppermint oil and the other one was blank. Airflow, which was cleaned by active carbon and silica-gel desiccant, was pumped through the two jars and each arm of the olfactometer at the speed of 50 ml/min by an air compressor pump for 15 min, and then carried out the assays (Guédot *et al.* 2009).

All the assays were carried out in the midday in a well-ventilated room, because it is the active time for psyllas (Guédot *et al.* 2009). In the experiments, the comparison was performed in which the winter-form pear psylla was offered a choice between the peppermint essential oil and an air control. In each trial, 100, 200, or 400 μl of peppermint essential oil were applied to a filter paper as the test odor source and placed into one of the odor jars. Each dose of peppermint essential oil was tested ten times, and 10 pear psyllas were tested for each replicate. Within each replicate, the olfactometer were rotated horizontally by 180° after 5 pear psyllas were assayed. During an assay, each pear psylla was allowed to enter the long arm of the Y-tube for 10 min and recorded its choice, the effective choice was defined when it arrived at the joint of the short arm and the PTEE pipe. Pear psyllas that did not make choice within 10 min were discarded and excluded from further analysis until we get 10 valid data for every replicate. We cleaned the olfactometer with hot soapy water, water, acetone, and hexane, respectively, and then conducted another replicate with this olfactometer (Guédot *et al.* 2009).

Effects of Peppermint Oil on Pear Psylla in Field Trials

To evaluate the effect of peppermint essential oil on overwintered and summer adults, we carried out the field trials in March (for the winter-form adults) and July (for the nymphs) in 2016, respectively. The experiment was conducted in a Hebei pear orchard (1-hectare acre), which is part of the Xiji Yulong pear specialized cooperatives, development zone in Tianjiazhuang Country, Xinji city, Hebei Province, China (37.90 N, 115.21 E, 30 m altitude. In March, the average temperature and air humidity were around 10.35°C and 30%, respectively; in July, the average temperature and air humidity were around 27.41°C and 70%, respectively). Here, we carried out seven treatments, which contained the controls (positive and negative treatment) and peppermint oil treatment, in 21 plots according the method of Tian (Tian *et al.* 2015).

The negative control plots were treated with tap water. The positive control plots were treated with three concentrations (1000, 2000, and 4000 μl/l) of detergent (purchased from Beijing Goldfish Technology Co., Ltd. Beijing, China) and its HLB value was 13.65 (tested by emulsion method). The peppermint oil plots were treated three concentrations (they were same to the detergent) of peppermint oil which was diluted with tap water after mixing with detergent by the ratio of 1 to 1 (volume). We applied these solutions (1.5 l/tree) in a completely randomized block design by an atomizer (purchased from Alibaba's Taobao, China. https://detail.tmall.com/item.htm?Id=37181994159&cmid=1401053 *35569ed55e27b&abbucket=12*). One plot constituted a single treatment (Sangwan *et al.* 2001).

We counted the numbers of winter-form adults, nymphs, and natural enemies (*Leis axyridis* Pallas and *Chrysoperla sinica* Tjeder) on three trees in the center of each plot before they were treated and at 3, 7, and 14 d after treatment (DAT). To count the winter-form adults, 20 branches (each 80 cm in length) per tree were randomly selected, and the total number of adults was recorded (Frost *et al.* 2016). For the nymphs, 30 randomly selected leaves on each tree were collected, and the number of nymphs on the leaves was determined by using a microscope (80×; Frost *et al.* 2016). To determine the numbers of natural enemies, each entire tree was swept with a sweep net, and the total numbers of natural enemies were counted in the field (Barbour *et al.* 1997, Hu *et al.* 2017).

The effects of peppermint essential oil on the natural enemies were evaluated by comparing the mean number of each insect population among different treatments (Tian *et al.* 2015).

The reduction rate (RR%) after pear psylla were treated by peppermint essential oil was got by using the equation from Henderson and Tilton's (1955):

$$RR\% = (1 - \frac{Nta}{Nca} \times \frac{Ncb}{Ntb}) \times 100,$$

where N is the number of insects; a denotes after treatment; b denotes before treatment; c is the average number in water treatment; and t denotes detergent and oil treatment.

Data Analysis

For the laboratory experiment, we subjected the data to Probit analysis to determine the LD_{50} values by using IBM SPSS Statistics 22.0 software (Nobsathian *et al.* 2019).

For the field trial, we used the one-way ANOVA to analyze the results of RR% of pear psylla and the number of natural enemies. The results are presented as the mean ± SEM (standard error of the mean). The differences of RR% among the time and the concentrations of the detergent and peppermint oil were analyzed at $P < 0.05$ by Tukey's post hoc comparison by using IBM SPSS Statistics 22.0 software, respectively. For the nymph (RR%) and nature enemies (number of insect) in different treatments, the data were transformed by square root before doing analysis.

To make clear the effect of peppermint essential oil on natural enemies, we also compared the numbers of natural enemies in same concentration of detergent and peppermint essential oil plots at same time by paired t-tests ($P < 0.05$) with IBM SPSS Statistics 22.0 software.

The repellence rate of peppermint essential oil and its component against pear psylla is reported as the mean ± SEM. Statistical significance was assessed at $P < 0.01$ using paired t-tests and Tukey's post hoc analysis with IBM SPSS Statistics 22.0 software.

Results

The Constituents in Peppermint Essential Oil

Thirty-six constituents were found in the peppermint essential oil, and they were accounted for 99.13% of the components. The major constituents were menthol (49.73%), menthone (30.52%), α-terpineol (3.81%), and α-pinene (3.60%), followed by isopulegol, 3-octanol, piperitone oxide, dihydro carvone, piperitone, piperitenone oxide, carveol, 4-methyl-isopulegone, α-cubebene, limonene, nerolidol acetate, menthyl acetate, pulegone, cineole, ocimene, thymol, linalool, isomenthone, p-menthane-1,2,3-triol, linalool acetate, isopulegone, dihydro carveol, α-farnesene, carvone, elixene, iso-pulegol acetate, dihydrocarveol acetate, piperitenone, β-myrceneb, carveol acetate, and caryophyllene (Table 1).

The Toxicity of Peppermint Oil Against Pear Pyslla

The peppermint essential oil performed the toxicity against the winter-form adults of pear psylla, yielding with an LD_{50} value of 2.54 μg/adult. Similarly, menthone yielded an LD_{50} value of 2.77 μg/adult, followed by α-pinene, menthol, and α-terpineol, with LD_{50} values of 5.85, 10.71, and 12.58 μg/adult, respectively (Table 2), with 16% of mortality in control. Moreover, the peppermint essential oil had the toxicity against nymphs, yielding an LD_{50} value of 1.91 µg/nymph. Similarly, menthone yielded an LD_{50} value of 2.19 µg/nymph, followed by α-pinene, menthol, and α-terpineol, with LD_{50} values of 4.98, 9.56, and 12.07 µg/ nymph, respectively (Table 3), with 6% of mortality in control.

The Repellence of Peppermint Oil to Pear Pyslla

In this experiment, the results indicated that the majority of pear psyllas chose the air control over the peppermint oil regardless of the dose (100 µl: t = 8.143, df = 9, P < 0.001; 200 µl: t = 10.776, df = 9, P < 0.001; 400 µl: t = 14.000, df = 9, P < 0.001) in the Y-tube test (Fig. 1A), although a total of 85 winter-from adults did not make any choice in the 385 tested insects. The rate of repellence with 400 µl (78.0%) of oil was significantly higher than that achieved with 100 µl (69.0%; F = 8.576; df = 1, 18; P = 0.017 < 0.05), but there was no significant difference in repellence rate between the doses of 400 and 200 µl (73.0%; F = 8.576; df = 1, 18; P = 0.248 > 0.05) or between the doses of 200 and 100 µl (F = 8.576; df = 1, 18; P = 0.402 > 0.05; Fig. 1B).

Table 1. Chemical constituents in the peppermint essential oil

Peak	Compound	RI	Peak area (%)
1	**α-Pinene**	963	**3.60**
2	β-Myrcene	1018	0.11
3	3-Octanol	1023	0.83
4	Limonene	1046	0.42
5	Cineole	1066	0.35
6	Ocimene	1070	0.33
7	Linalool	1120	0.30
8	Isopulegol	1160	1.18
9	**Menthone**	1138	**30.52**
10	Isomenthone	1160	0.29
11	**Menthol**	1179	**49.73**
12	Isopulegone	1187	0.25
13	**α-Terpineol**	1220	**3.81**
14	Dihydro carveol	1228	0.23
15	Dihydro carvone	1235	0.64
16	Carveol	1252	0.49
17	Pulegone	1267	0.38
18	Carvone	1275	0.21
19	Linalool acetate	1300	0.26
20	Piperitone	1304	0.56
21	Piperitone oxide	1309	0.75
22	Iso-pulegol acetate	1320	0.18
24	Nerolidol acetate	1336	0.42
25	Thymol	1339	0.32
26	Menthyl acetate	1345	0.39
27	Dihydrocarveol acetate	1359	0.14
28	Piperitenone	1360	0.12
29	Carveol acetate	1369	0.11
30	4-Methyl-isopulegone	1375	0.45
31	Piperitenone oxide	1391	0.50
32	p-Menthane-1,2,3-triol	1426	0.27
33	Caryophyllene	1454	0.11
34	α-Farnesene	1489	0.23
35	α-Cubebene	1555	0.45
36	Elixene	1592	0.20
Total			99.13

The chemical constituents were selected to assess the bioactivity on insect are shown in bold.

Effects of Peppermint Oil on Pear Psylla in Field Trials

Compared with the control treatment, there were 51.27, 71.29, and 80.92% fewer winter-form adults of pear psylla following treatment with the peppermint essential oil at the concentration of 4000 µl/l at 3 DAT (days after treatment), 7 DAT, and 14 DAT, respectively. The reduction rates relative to the control treatment at 4000 µl/l treatment were significantly higher than did oil at 1000 µl/l, which yielding reduction rates relative to the control treatment of 27.80% at 3 DAT (F = 5.551; df = 2, 6; P = 0.038 < 0.05), 39.54% at 7 DAT (F = 15.784; df = 2, 6; P = 0.003 < 0.05), and 52.85% at 14 DAT (F = 6.201; df = 2, 6; P = 0.031 < 0.05). However, no significant difference was observed between the concentration of 4,000 and 2,000 µl/l treatments, which showed reduction rates relative to the control treatment of 42.94 % at 3 DAT (F = 5.551; df = 2, 6; P = 0.512 > 0.05), 58.65% at 7 DAT (F = 15.784; df = 2, 6; P = 0.146 > 0.05), and 70.83% at 14 DAT (F = 6.201; df = 2, 6; P = 0.470 > 0.05). The reduction rate of the psylla winter-form adult was significantly higher at 14 DAT than at 3 DAT at the oil concentrations of 4,000 µl/l (F = 5.134; df = 2, 6; P = 0.046 < 0.05), 2,000 µl/l ($F_{2,6}$=15.261, P = 0.004 < 0.05), and 1,000 µl/l (F = 9.277; df = 2, 6; P = 0.012 < 0.05). However, no significant difference in RR% was observed between the 7 DAT and 14 DAT at the treatment of 4,000 µl/l (F = 5.134; df = 2, 6; P = 0.592 > 0.05), 2,000 µl/l (F = 15.261; df = 2, 6; P = 0.115 > 0.05), and 1,000 µl/l (F = 9.277; df = 2, 6; P = 0.134 > 0.05; Fig. 2A).

Compared with the control treatment, there were 66.95, 60.11, and 59.96% fewer psylla nymphs following treatment with peppermint essential oil at the concentration of 4,000 µl/l at 3 DAT, 7 DAT, and 14 DAT, respectively. And the mortality in 4,000 µl/l treatments was significantly higher than that in 1,000 µl/l treatments at 3 DAT (F = 10.430; df = 2, 6; P = 0.011 < 0.05), but no significant difference in mortality was observed at 7 DAT (F = 1.037; df = 2, 6; P = 0.410 > 0.05) and 14 DAT (F = 2.416; df = 2, 6; P = 0.170 > 0.05). Similarly, the reduction rate of nymphs did not significantly differ among different times at any concentrations, 1,000 µl/l (F = 1.307; df = 2, 6; P = 0.338 > 0.05), 2,000 µl/l (F = 0.381; df = 2, 6; P = 0.699 > 0.05), and 4,000 µl/l (F = 0.243; df = 2, 6; P = 0.792 > 0.05). However, the mortality was increased with the increasing of the concentration of peppermint essential oil (Fig. 2B).

Table 2. The toxicity of essential oils against the winter-form adults of pear psylla using probit analysis

Treatment	LD_{50} (µg/adult)	Slope ± SE	95%FL[a]	Chi square (χ^2)	P
Peppermint essential oil	2.54	2.095 ± 0.259	1.936–3.194	3.573	0.612
Menthol	10.71	1.685 ± 0.275	7.921–15.539	4.201	0.521
Menthone	2.77	1.788 ± 0.212	2.094–3.561	1.486	0.915
α-Pinene	5.85	1.810 ± 0.237	4.469–7.663	3.037	0.694
α-Terpineol	12.58	1.841 ± 0.316	9.391–18.365	5.729	0.333
Abamectin	0.03	1.793 ± 0.210	0.023–0.039	1.714	0.887

[a]FL denotes fiducial limits.

Table 3. The toxicity of essential oils against the nymphs of pear psylla using probit analysis

Treatment	LD_{50} (μg/nymph)	Slope ± SE	95%FL[a]	Chi square (χ^2)	*P*
Peppermint essential oil	1.91	1.864 ± 0.193	1.504–2.376	2.336	0.801
Menthol	9.56	1.371 ± 0.188	7.053–14.116	1.460	0.918
Menthone	2.19	1.351 ± 0.151	1.632–2.863	0.642	0.986
α-Pinene	4.98	1.648 ± 0.182	3.907–6.425	2.997	0.700
α-Terpineol	12.07	1.412 ± 0.204	8.794–18.547	2.493	0.778
abamectin	0.027	1.710 ± 0.179	0.020–0.033	1.770	0.880

[a]FL denotes fiducial limits.

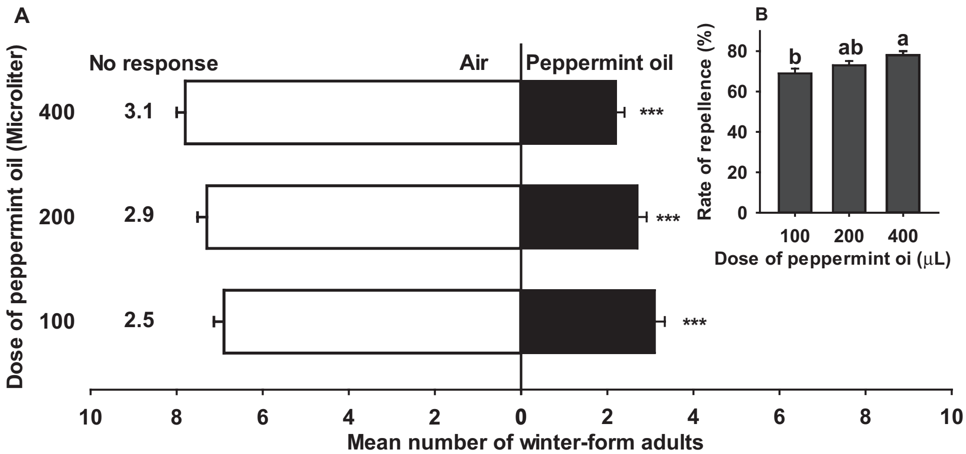

Fig. 1. Mean numbers of winter-form adults of pear psylla selecting different sources (peppermint essential oil vs air) at different doses of oil (A) and the repellence rate of peppermint against pear psylla, determined from the mean number of psylla adults selecting the Y tube arm containing air (B). Values shown are means ± SEM; *** indicates means that are significantly different ($P < 0.01$), and bars with same letter are not significantly different ($P > 0.05$).

Effects of Peppermint Oil on Natural Enemies in Field Trials

There are two natural enemies at the experimental site for control of pear psylla, namely, *Leis axyridis* Pallas in spring and *Chrysoperla sinica* Tjeder in summer. The effects of the peppermint essential oil on the two natural enemies were tested to assess the safety of the use of this oil in pear orchards. It was found that there were no significant differences in the number of natural enemies in the treatments of 1,000, 2,000, and 4,000 μl/l at any time point for either *Leis axyridis* Pallas, 3 DAT ($F = 0.151$; df = 2, 6; $P = 0.863 > 0.05$), 7 DAT ($F = 0.294$; df = 2, 6; $P = 0.756 > 0.05$), 14 DAT ($F = 1.463$; df = 2, 6; $P = 0.304 > 0.05$; Fig. 3A) or *Chrysoperla sinica* Tjeder, 3 DAT ($F = 0.799$; df = 2, 6; $P = 0.492 > 0.05$), 7 DAT ($F = 0.528$; df = 2, 6; $P = 0.615 > 0.05$), 14 DAT ($F = 0.000$; df = 2, 6; $P = 1.000 > 0.05$; Fig. 3B). Similarly, there was no significant difference among different times (Fig. 3A and B). Comparing with the detergent treatment, there were more *Leis axyridis* Pallas in the treatments of peppermint essential oil ($t = -2.096$; df = 26; $P = 0.046$). For the *Chrysoperia sinica* Tjeder, there was no significant difference between detergent and essential oil treatment ($t = 0.000$; df = 26; $P = 1.000$; Supp Fig. 1 [online only]).

Discussion

The present findings regarding the constituents of peppermint essential oil agree with those of previous studies, which indicated that there are α-pinene, β-pinene, limonene, cineol, isomenthone, menthone, menthofurane, menthyl acetate, caryophyllene, pulegone, and menthol in peppermint essential oil mainly (Gherman *et al.* 2000). However, there are differences in the component concentrations reported among studies (Zade *et al.* 2018). For instance, Li *et al.* (2016) found that peppermint oil mainly contained α-pinene (0.82 ± 0.26%), β-pinene (1.75 ± 0.63%), limonene (4.54 ± 1.33%), pulegone (1.64 ± 0.43%), menthofurane (5.77 ± 1.12%), menthone (47.29 ± 7.43%), isomenthone (6.01 ± 2.15%), menthol (47.29 ± 7.43%), and menthyl acetate (9.85 ± 2.46%; Li *et al.* 2016). The reason is maybe because of the variations in the soil nutrition, growing season, and origin of the peppermint (Sangwan *et al.* 2001, Goswami *et al.* 2015). Although the reported composition of peppermint essential oil is inconsistent among studies, menthone is consistently a major constituent of peppermint oil from different places. Additionally, menthone exhibited the strongest contact toxicity among the constituents, suggesting that menthone may be the active ingredient against pear psylla (Riachi and De Maria 2015, Rajkowska *et al.* 2016).

There is no doubt that the toxicity of peppermint essential oil and its constituent against the adults and nymphs of pear psylla was weaker than the commercial insecticide. For example, compared with the abamectin which with the LD_{50} value of 0.030 μg/adult and 0.027 μg/nymph, the essential oil was about 84 and 70 times less toxic against the adults and nymphs of pear pyslla, respectively (Tables 2), and the menthone was approximately 92 and 81 times less toxic, respectively (Tables 3). Moreover, the toxicities of the oil and menthone against pear psylla were weaker than those of some botanical essential oils, such as garlic oil with the contact toxicity of an LD_{50} value of 1.42 μg/adult (Zhao *et al.* 2013) and clove oil with the toxicity of an LD_{50} value of 1.795 μg/adult (Tian *et al.* 2015). What's more, the nymphs were more susceptible than the adults to the essential oil and its main constituents in the laboratory and in the field, but the reduction of nymphs was less than the adults in

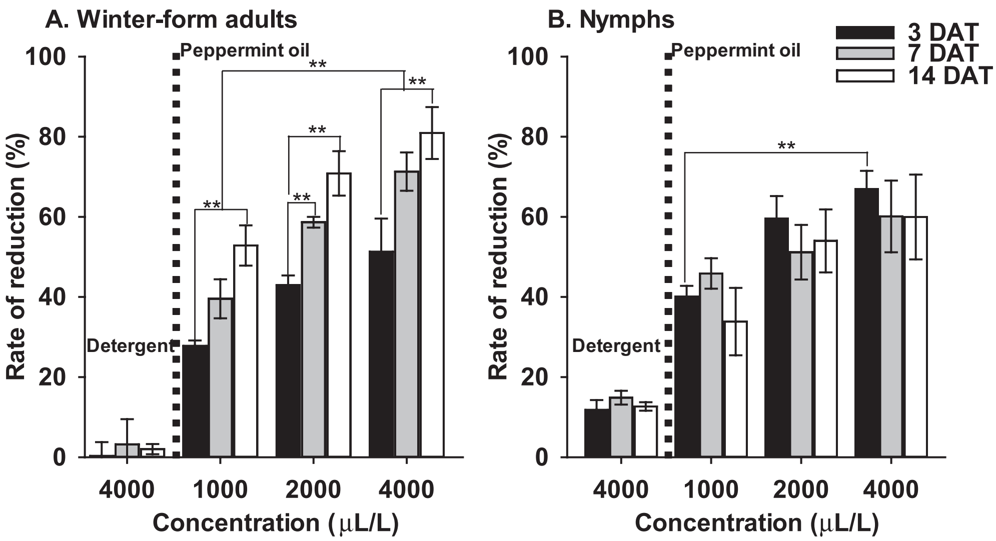

Fig. 2. The corrected reduction rate (RR%) of winter-form adults (A) and nymphs (B) by foliar spray of peppermint essential oil or detergent in a field trial. Values shown are means ± SEM; ** indicates means that are significantly different ($P < 0.05$).

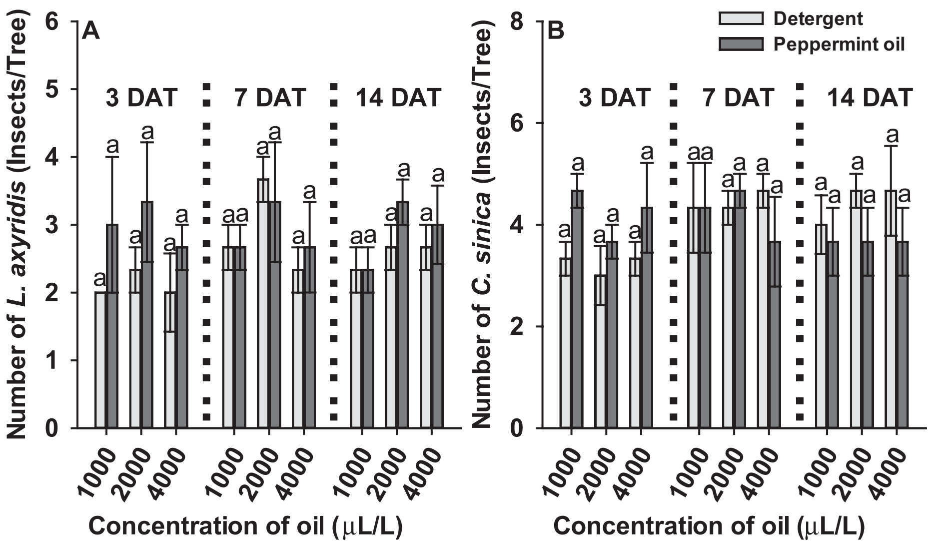

Fig. 3. Mean numbers of *Leis axyridis* Pallas (A) and *Chrysoperla sinica* Tjeder (B) after foliar spray of peppermint essential oil or detergent in a field trial. Values shown are means ± SEM; the bars with the same letter are not significantly different ($P > 0.05$).

the field. The reason is that because the peppermint oil was also repelled 65–75% of adults in the laboratory bioassay in addition to toxicity, whereas nymphs were not repelled. The conclusion is supported by the similar research on the effect of the zerumbone on cigarette beetles (Wu et al. 2017b). In addition, the honeydew produced by nymphs may protect these nymphs from natural enemies (Powell and Silverman 2010, Hogg *et al.* 2018).

Similarly, the effect of peppermint essential oil on pear psyllas in the field was stronger than that in the laboratory, potentially due to the strong toxic and repellant activity of the oil against pear psylla at the same time in the field also (Fig. 1; Tables 2 and 3). This finding is consistent with previous reports demonstrating that peppermint essential oil is both a repellent and toxic agent against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* Fabrichs (Coleoptera: Bostrychidae), and *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) (Ukeh and Umoetok 2011, Renkema *et al.* 2016). Because the oil was safe to natural enemies, they may enhance the efficacy against pear psylla in the field, in particular for the adults. Besides that, it may be also related to the synergistic effect of detergent, which could enhance the toxicity of chemicals when they were mixed, such as the mixture of trichlorfon and sodium dodecyl sulphate (main content in detergent) perform stronger toxicity which enhanced the insecticide's inhibition of acetylcholinesterase (AChE; Feng *et al.* 2008) and the 4-nonylphenol (a detergent metabolite) can enhance the toxicity of diazinon (Zein *et al.* 2015).

As it turned out, it is not strange that peppermint essential oil could reduce the abundances of the winter-form adults and nymphs by up to more than 50% in the field and identified as an ideal alternative agent to control the population of pear psyllas in pear orchards. The reduction rates of the winter-form adults in the 4,000 μl/l treatments were significantly greater than the rate in the 1,000 μl/l treatment, but the significant differences were not found in the reduction rate between the 4,000 and 2,000 μl/l treatments as well as the 2,000 and 1,000 μl/l treatments, so the concentration of

4000 µl/l may be the optimal selection to control the adults in practice. In contrast, for the nymphs, the 4,000 µl/l treatment yield significantly greater reductions in nymph numbers than the 1,000 µl/l treatment at 3 DAT, although there was no significant difference between 1,000 and 2,000 µl/l treatment at any time. But the results of the field tests revealed that treatment with peppermint oil could greatly reduce the numbers of winter-form adults and nymphs of pear psylla in a concentration-dependent manner. For the responding time, peppermint essential oil has strong activity against the adults of pear psylla in a long time and the effect will became stronger with time in 14 d. Whereas the peppermint essential oil performs strong activity against the nymphs of pear psylla in short time but the effect will become weaker with time in 14 d.

Compared with some commercial bioinsecticides, the activity of peppermint essential oil was weaker than the azadirachtin, abamectin, and diflubenzuron in field, which caused mean mortality values exceeding 90% (Marčic *et al*. 2009). But the activity of the peppermint essential oil was similar to those of clove oil at 2400 µl/l (Tian *et al*. 2015) and novaluron at 750 ppm (Erler *et al*. 2014), which caused 62–70% nymph mortality, because of its repellent activity besides the contact toxicity. Therefore, as bioinsecticide, peppermint essential oil was a good optional to develop an agent to protect the pear trees from pear psylla, especially without affecting its natural enemies. Furthermore, the peppermint essential oil is widely used as in food, so it is recognized as no harmful to human health (Wu et al. 2017a). Additionally, because of the positive attributes of these constituents, it is warranted into the development of ecofriendly insecticides not only for pear psylla in further research, although the oil was less effective against the adults and nymphs than some commercial bioinsecticides at present.

Taken together, this study indicates that the essential oil from peppermint plant could be developed as a natural insecticide for controlling pear psylla in the future because of its toxicity and repellent activity against pear psylla, its lack of effects on the natural enemies of this pest, and its environmental safety.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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