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ORIGINAL PAPER

Chemical composition of *Illicium verum* fruit extract and its bioactivity against the peach–potato aphid, *Myzus persicae* (Sulzer)

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Abstract The chemical components of *Illicium verum* fruit extracts in methyl alcohol (MA), ethyl acetate (EA), and petroleum ether (PE) were determined through GC–MS analysis. The bioactivity of the extracts against *Myzus persicae* was also investigated to assess their roles in the control of aphids. Forty-four compounds with more than 0.20% mass percentage were identified. Trans-anethole was the most abundant component and comprised 41.14, 52.54, and 72.25% of the MA, EA, and PE extracts, respectively. The biological activity assays on *M. persicae* showed that increasing the concentration and prolonging the exposure to the extracts enhanced contact toxicity. After 72 h of treatment, 1.000 mg/L MA, EA, and PE extracts caused 68.93, 89.95, and 74.46% mortality, respectively. The LC_{50} values of MA, EA, and PE extracts against *M. persicae* were 0.31, 0.14, and 0.27 mg/L, respectively. The deterrent effect of 1.0 mg/L EA extract was the highest. The average antifeedant rate reached 76.88% at 48 h. The development period of *M. persicae* nymphs was prolonged to more than 2 days when sprayed with 0.1 mg/L MA extract at 25 °C. Hence, *I. verum* fruit extracts exhibit considerable potential for *M. persicae* control programs.

Keywords Gas chromatography–mass spectrometry · Contact toxicity · Repellency · Antifeedant · Developmental duration

Abbreviations

MA	Methyl alcohol
EA	Ethyl acetate
PE	Petroleum ether
GC–MS	Gas chromatography–mass spectrometry
RT	Retention time

Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is the most common and economically significant aphid pest in greenhouse, field, and horticultural crops of several temperate regions worldwide. This pest infests hundreds of species from more than 50 plant families and is commonly found in agroindustrial crops, horticultural crops, and stone fruits; *M. persicae* transmits more than 100 viral diseases to more than 400 host plants, leads to direct and indirect damage, and causes economic losses (Gaspari et al. 2007; Harmel et al. 2008).

Control of *M. persicae* populations worldwide mainly depends on continuous and repeated use of synthetic insecticides (Liu et al. 2007). Consequently, this species has developed multiple resistance to almost all classes of

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insecticides, including neonicotinoids, organophosphates, carbamates, and pyrethroids (Anstead et al. 2007). Studies reported varied resistance levels of *M. persicae* against imidacloprid in Europe, the USA, Japan, and China (Nauen and Denholm 2005; Margaritopoulos et al. 2007; Foster et al. 2008); this phenomenon represents a considerable threat to the long-term efficacy of this insecticide class. The emergence of resistance mechanisms and the revocation of several insecticides in *M. persicae* control have increased the need to develop novel approaches for pest control in several crops (Barker et al. 2007; Saguez et al. 2013).

Botanical pesticides are alternatives to synthetic pesticides because they confer generally low environmental pollution and toxicity to humans; these pesticides also feature high selectivity, minimal or no harmful effect on nontarget organisms, rapid degradation, low residues formed, and complicated cross-resistance development because of their natural complex agents and novel modes of action against insects (Isman 2006; Siskos et al. 2009; Lv et al. 2012; Ladhari et al. 2013; Guo et al. 2014). In addition, the negative environmental and health effects of synthetic insecticides and increasingly stringent environmental regulation of pesticides have gained renewed interest in the development and use of natural products from plants, which are abundant sources of bioactive chemicals (Nukenine et al. 2010; Huang et al. 2011; Li et al. 2011; Usha et al. 2011). Plant extracts containing natural compounds have been increasingly investigated because of their potential in pest control (Mann et al. 2012).

Several natural products exert different biological activities, including insecticidal effects, on *M. persicae*. For example, Saguez et al. (2013) evaluated the biological activities of lignans and neolignans isolated from flax against *M. persicae* reared on an artificial diet. The results showed that secoisolariciresinol diglucoside significantly increased the mortality and doubling time of the aphid population, whereas anhydrosecoisolariciresinol prolonged the prereproductive period. Dehydrodiconiferyl alcohol-4-β-D-glucoside altered all of the life history parameters. Therefore, lignans and neolignans are potential novel bioinsecticide compounds against *M. persicae* in alternative management programs. Ethanol extracts from selected Brazilian *Annona* species (*A. mucosa*, *A. sylvatica*, and so on) showed aphicidal activity against *M. persicae* (Ribeiro et al. 2014). Ethanol extracts of *Xanthium strumarium* and *Tanacetum parthenium* showed nymphal mortality of more than 80.0% at the highest concentration (12%) against *M. persicae* (Erdogan and Yildirim 2016). Essential oils and volatile compounds obtained from plants, such as *Eucalyptus citriodora*, provide an alternative approach for controlling *M. persicae* (Costa et al. 2015). Faraone et al. (2015) reported that the toxicity of imidacloprid against *M. persicae* was synergized 16- to 20-fold by *Lavandula angustifolia* and *Thymus vulgaris* essential oils. These results demonstrated the potential of plant essential oils as synergists of insecticides. Hence, botanical insecticides could be used in integrated pest management (IPM), especially for organic production (Ribeiro et al. 2014).

The Chinese medicinal herb star anise, *Illicium verum* Hook. f. (Magnoliaceae), is a medium-sized evergreen tree native to southwest China. The plant is widely cultivated in the tropical and subtropical areas of Asia. *I. verum* was recognized as "both food and medicine" by the Ministry of Health of the People's Republic of China on March 2002, thereby implying its low- or non-toxicity to humans (Li et al. 2013). Previous studies on *I. verum* mainly focused on its applications in medicine and food (Ohira et al. 2009; Yang et al. 2010). A previous screening program for new agrochemicals from Chinese medicinal herbs reported that *I. verum* powder possesses insecticidal activity against *Sitophilus zeamais* and *Cryptolestes pusillus* Schönherr (Li et al. 2013). Other studies also indicated that the essential oil of *I. verum* elicits repellent and fumigant actions on *S. zeamais* (Ho et al. 1995), *Blattella germanica* (Chang and Ahn 2002), *Sitophilus oryzae*, *Callosobruchus chinensis* (Kim et al. 2003), *Aedes aegypti* (Dana and Wej 2006), and *Culex pipiens* (Kimbaris et al. 2012).

Thus far, the biological activities of *I. verum* extracts against the agroforestry pest *M. persicae* have not been elucidated. In this study, components of dried *I. verum* fruits were extracted with three organic solvents. Extract constituents were determined by gas chromatography–mass spectrometry (GC–MS). A series of laboratory experiments were also conducted to investigate and assess the efficacy of controlling aphids as well as the mode of action of different solvent extracts from *I. verum* against *M. persicae*. Results could be used to promote research toward the development of new agents for aphid pest control using bioactive chemical compounds from indigenous plant sources.

Materials and methods

Aphids

Experiments were conducted with clonal lineages of *M. persicae* maintained in a greenhouse at the School of Plant Protection, Anhui Agricultural University (No. 130 West Changjiang Rd., Hefei, Anhui, China). Aphids were reared on greenhouse potted cabbage *Brassica oleracea* var. (Brassicaceae) placed inside vented Perspex cages (45 cm^3 × 45 cm^3 × 50 cm^3) maintained at a controlled temperature (22 ± 2 °C) under a light–dark (LD)

photoperiod of 16 h:8 h, with 50 ± 5% relative humidity (RH). Identical-sized wingless adult aphids used in the experiments were collected from the cabbages on April 2014.

Plant material

The *I. verum* fruits were originated from Guangxi province in China. They are food-grade material which was grown without chemical fertilizers and insecticides. Dried ripe fruit often consists of eight radial ellipsoid follicles integrated under a bending hooked peduncle. The follicle is 10–20 mm long, 5–10 mm tall, and 5 mm wide. The skin surface is brown or reddish brown with wrinkles, inner surface is light brown, shiny, containing one seed. The dry mature fruits used in the experiment are reddish brown with 3.0–5.0% water content.

Extract preparation

Illicium verum fruits were dried in an oven (GRX–9071B; Shanghai, China) at 40 °C for 2 days, ground into powder by an electric grinding mill (DD–120B; Zhejiang, China), and sifted with a 40-mesh sieve. The dry powder (150 g) was placed in a 1.0-L round-bottomed flask. Methyl alcohol (MA; polarity, 5.1; highly polar), ethyl acetate (EA; polarity, 4.4; weakly polar), and petroleum ether (PE; boiling point range, 60–90 °C; polarity, 0.0; nonpolar) were sequentially added at a ratio of 1:5 (w/v) at room temperature (25 °C). The mixture was incubated in the dark for 48 h and filtered (Whatman No. 2). The samples were leached twice via the same procedure. The final filtrates were collected from each solvent to obtain the crude extracts. The combined filtrate was dried, concentrated with a vacuum rotary evaporator (Buchi rotavapor R-124; Switzerland), and weighed with an electronic balance (FA2104; Shanghai, China). All samples were stored in air-tight brown bottles at 4 °C in a refrigerator until further use.

GC–MS

The composition of the extracts was investigated by GC–MS performed on a Varian (USA). Saturn 2200 GC system equipped with a capillary column containing CP-Sil8CB-MS (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: The initial oven temperature was held at 50 °C for 3 min, ramped at 20 °C/min to 120 °C without reserve, and then ramped at 10 °C/min to 250 °C for 5 min. The temperatures of the injector, interface, and gasification chamber were maintained at 260, 250, and 250 °C, respectively. The samples (0.4 μL) were injected neat, with a split ratio of 1:100. The carrier gas was helium (99.999%). Mass spectra were obtained at 70 eV by an electron impact ionization source. The temperatures of the interface and iron trap were 250 and 150 °C, respectively. The electron multiplier voltage was 2.4 kV. The mass range analyzed was from 20 to 650 amu. Most constituents were identified with GC by comparison of their retention indices based on the literature and MS data obtained from the Saturn and NIST libraries. Component relative percentages were calculated based on the GC peak areas without the use of correction factors (Chu et al. 2011; Gholivand et al. 2009; Li et al. 2014).

Contact toxicity assay

The slide-dip method was utilized to evaluate the contact toxicity of the extracts against aphids (Wang and Shen 2007). Approximately 30 newly adult aphids (1 day old) were attached on their backs to a 2-cm-wide two-sided adhesive plaster on glass slides. Range-finding tests were conducted to determine the appropriate testing concentrations of the extracts. The MA, EA, and PE extracts of *I. verum* were serially diluted into five appropriate concentrations (1.000, 0.500, 0.250, 0.125, and 0.063 mg/L), with 1:4 (v:v) aqueous solution of acetone as the solvent based on the results of preliminary experiments. Glass slides with aphids were immersed in the dilutions for 5 s, and the remaining solution on the slides was absorbed with bibulous paper. The slides were placed on 12-cm diameter glass Petri dishes and maintained in an artificial climate chamber at 25 ± 1 °C with 75 ± 5% RH, and an LD photoperiod of 14 h:10 h. The mortality in each treatment group was recorded at 24, 48, and 72 h after treatment. A test aphid was considered dead if it did not move its legs when its abdomen was probed with a soft brush. The control sample was exclusively treated with 1:4 (v:v) acetone aqueous solution. All treatments and control experiments were replicated six times.

Repellency assay

First, the extracts were diluted to a concentration of 1.0 mg/L with sterile distilled water. Meanwhile, 1% agarose was poured into a Petri dish (9.0 cm i.d. × 1.0 cm), and filter paper (4 cm in diameter) was placed onto the central of the solidified agarose. *Prunus cerasifera* leaves were cut into 2-cm-diameter disks and immersed in the respective diluted extract or sterile distilled water (as control) for 5 s before the disks were dried in air. The front face of the leaf disk was attached to the surface of the agarose surrounding the filter paper. Finally, 40 aphids (1 day old) were placed on the filter paper before the Petri dish was sealed with plastic wrap with multiple vents and placed upside down in a climate-controlled chamber set to optimum growth conditions for *M. persicae*

(22 ± 2 °C, LD photoperiod of 14 h:10 h). The experiments for each extract were performed with ten replications. The total numbers of aphids that moved on the *P. cerasifera* leaf disk were recorded at 12, 24, and 48 h after treatment to calculate repellency control index.

Antifeedant assay

Prunus cerasifera leaves with the midrib were cut into disks (4 cm in diameter) while maintaining the symmetry on either side of the midrib. The left side of the leaf midrib was evenly painted with 1.0 mg/L of the respective extract, whereas the right side was painted with an equal amount of sterile water as a control, and the leaf disk was placed with the leaf face toward the bottom in the central of a Petri dish (12 cm diameter) with 1% agarose culture medium. After the leaf disk was dried in air to form a sensitive layer, the same-sized aphids were gently picked on the back of leaf disk by using a soft brush. Each side had approximately 20 aphids, thereby ensuring an equal number of aphids on both sides of the leaf disk midrib. The method of sealing and breeding was as described above. Each Petri dish represented one extract treatment, and each treatment was replicated 9 times. The number of aphids that inhabited the treated and control sides of the leaf disk was recorded at 12, 24, and 48 h after treatment.

Influence on growth and development of *M. persicae* test

The test was conducted in the climatic chamber at 25 ± 1 °C of the School of Plant Protection, Anhui Agricultural University (No. 130 West Changjiang Rd., Hefei, China). In the test setup, a fresh cabbage leaf was placed in 12-cm-diameter glass Petri dish, such that the leaf area was less than the bottom area of the dish. The petiole was wrapped with degreasing cotton that contained water to keep leaf fresh. A total of 30 aphids at birth (approximately 4 h old) were gently placed on the leaf with a soft writing brush before spraying 0.1 mg/L of MA extract solution to the leaf with a small spray, enough to wet the leaf without forming droplets. Sterile distilled water was sprayed in the controls. The leaves were dried in air (25 °C) for 1–2 h. Sealing and breeding was performed as described above. The development of aphids and the number of dead and ferriferous aphids were observed and recorded every 12 h after treatment. The used leaves were replaced every 2 days with fresh leaves that were not sprayed with water.

Statistical analysis

Treatment mortality rates were corrected with the mortality rate of the control sample (Abbott 1925), and the means of corrected mortalities were compared by Duncan's new multiple range test with a significance level of 0.05. The corrected data of mortalities were transformed into their arcsine square-root values for ANOVA. The relationship between mortality and concentration was modeled with the DPS program (Tang and Feng 2007). Untransformed data were presented as mean ± SE. The regression equations and LC_{50} for the contact toxicity of the three extracts against *M. persicae* adults were obtained via linear regression analysis of the relationship between treatment concentrations and arcsine square-root values of the mortalities.

The repellency index (RI) of population control was calculated a—$RI = (Nt \times TNc)/(Nc \times TNt)$, where Nc is the number of aphids on the leaf treated with sterile distilled water, Nt is the number of aphids on the leaf treated with extract, TNc is the total number of aphids as control, and TNt is the total number of aphids treated with extract.

The percentage antifeedant (PA) was calculated as: $PA = [(Nc–Nt)/(Nc + Nt)] \times 100\%$, where Nc is the number of aphids on the area treated with sterile distilled water and Nt is the number of aphids on the area treated with extract. Results are presented as the mean ± SE of the PA. PAs were assigned to antifeedant classes (Malik and Muiutaba 1984) from 0 to V, where class 0 was <0.1%, class I was 0.01–20%, class II was 20.01–40%, class III was 40.01–60%, class IV was 60.01–80%, and class V was 80.01–100%. The mean PAs of each treatment were compared and separated by Duncan's new multiple range test with significance levels at $P = 0.05$ and $P = 0.01$.

Results

Effects of solvents on extraction yield from *I. verum*

The highest extraction yield from *I. verum* was obtained with MA at 23.67% (Table 1). The extraction yields obtained from EA and PE were 14.21 and 14.00%, respectively. The polarity values of MA, EA, and PE were 5.1, 4.4, and 0.0, respectively. The extraction yields were dependent on the similarity of the chemical characteristics of the solvent and the polarity of the plant material. The results indicated that the compounds present in *I. verum* were mostly of high polarity.

Chemical composition of the extracts

A total of 44 compounds with concentrations of >0.20% were separated from the MA, EA, and PE extracts and identified by GC–MS (Table 2), thereby representing 70.45, 82.87, and 92.70%, respectively, of the whole composition of each extract. The most abundant

Table 1 Extraction yields of *I. verum* using three types of organic solvents (%)

Solvents	Dry weight of fruit (g)	Volume of solvents (mL)	Dry weight of extracts (g)	Extraction yield[a] (%)
MA	150	750	35.50	23.67
EA	150	750	21.32	14.21
PE	150	750	21.00	14.00

[a] Extraction yield = (dry weight of extract/dry weight of star anise fruit) × 100

component was trans-anethole, which accounted for 41.14% of the MA extract, 52.54% of the EA extract, and 72.25% of the PE extract.

Results of GC–MS analysis showed that the compositions of the three extracts were almost identical (Table 2). However, some minor components differed. The MA extract contained more 4-ethyl benzaldehyde (3.40%) and 1-(4-methoxyphenyl)-2-propanone (3.68%). The EA extract contained more 1-(3-methyl-2-butenoxy)-4-(1-propenyl) benzene (6.22%), cis-3,5-dimethoxy-β-methyl-β-nitrostyrene (4.24%), hexadecanoic acid (4.07%), and benzyl alcohol (4.04%). The PE extract contained more 1-(3-methyl-2-butenoxy)-4-(1-propenyl) benzene (4.73%) and benzyl alcohol (2.81%).

Contact toxicity of *I. verum* extracts on *M. persicae*

Results of the contact toxicity experiment indicated that the *I. verum* fruit extracts in different solvents were highly toxic to *M. persicae* adults (Table 3). The contact toxicity effects of the extract in three solvents were distinctly enhanced by increased concentrations and prolonged exposure time. The MA, EA, and PE extracts with the highest concentration of 1.000 mg/L caused mortality rates of 68.93, 89.95, and 74.46%, respectively, in *M. persicae* adults at 72 h after treatment.

The LC_{50} values of the MA, EA, and PE extracts were 0.31, 0.14, and 0.27 mg/L, respectively, at 72 h after treatment (Table 4). Therefore, the highest contact toxicity was demonstrated by the EA extract, followed by the PE extract and the MA extract.

Based on the observed mortality rates and LC_{50} values, the contact toxicity of the three extracts against *M. persicae* was in the following order: EA extract > PE extract > MA extract.

Repellent effect of *I. verum* extracts on the population of wingless *M. persicae*

A value of RI > 1 implies an attracting action to insects, whereas RI < 1 means repellency action to insects; the higher is the index, the weaker is the repellency action (Li et al. 2014). Based on Table 5, all extracts showed evident repellency effects on wingless peach aphids, and the repellency action of the EA extract was strongest with a mean RI of 0.32; the PE extract was the second strongest with a mean RI of 0.51; the MA extract was the weakest with a mean RI of 0.71.

Apastic effect of *I. verum* extracts on *M. persicae*

Table 6 shows the strong antifeedant activities of the three extracts. At a concentration of 1.0 mg/L, the average PA for the MA, EA, and PE extracts was 42.16, 76.88, and 32.47%, which corresponded to repellency levels of III, IV, and II, respectively. The order of the apastia reaction was EA extract > MA extract > PE extract.

Effect of *I. verum* extracts on the *M. persicae* developmental duration

The MA extract with weak performance in the early stage of the biological activity determination during peach aphid growth was tested at a relatively low concentration of 0.1 mg/L.

From Table 7, the developmental period of wingless-type aphids was extended from 5.28 to 7.36 days under 25 °C after spraying with MA extract at a concentration of 0.1 mg/L, whereas that of alate aphids was from 5.35 to 7.53 days; both were prolonged by more than 2 days. *M. persicae* nymphs have a total of four instars, and the average age of aphids was extended by more than 0.5 days.

During the early days before the virgin birth, namely, from when nymphs were born to the adult stage and from when nymphs were born to the next-generation nymphal stage, the treatment groups had different degrees of extension compared with the control group. These results suggested that the MA extract has strong growth inhibition, thereby preventing or delaying the growth of the peach aphid population.

Discussion

Considerable effort has been recently exerted on the potential of plant extracts or phytochemicals as sources of commercial insect-control agents or as new leads for designing target-specific molecules (Ateyyat et al. 2009;

Table 2 Chemical composition of extracts from *I. verum* dried fruits using three types of organic solvents

No.	Compound	RT (min)	Molecular formulae	Relative area (%)		
				MA	EA	PE
1	Phenol	5.66	C_6H_6O	0.34		
2	Carene	5.96	$C_{10}H_{16}$		0.04	0.21
3	Terpinolene	6.20	$C_{10}H_{16}$		0.51	1.07
4	Benzoic acid	7.76	$C_7H_6O_2$	0.43		
5	Terpineol	7.93	$C_{10}H_{18}O$	0.24	0.09	0.12
6	Anethole	8.75	$C_{10}H_{12}O$	0.24	0.12	0.16
7	4-Ethyl benzaldehyde	8.86	$C_9H_{10}O$	3.40	0.67	0.53
8	Benzyl alcohol	9.00	C_7H_8O		4.04	2.81
9	Trans-anethole	9.38	$C_{10}H_{12}O$	41.14	52.54	72.25
10	β-Copaene	10.26	$C_{15}H_{24}$	0.06	0.12	0.21
11	1-(4-Methoxyphenyl)-2-propanone	10.32	$C_{10}H_{12}O$	3.68	1.57	0.80
12	Bergamotene	10.65	$C_{15}H_{24}$	0.11	0.23	0.36
13	Benzoic acid,4-methoxy	10.80	$C_8H_8O_3$	0.87		
14	Longifolene	10.83	$C_{15}H_{24}$	0.08	0.49	0.32
15	Bisabolene	10.89	$C_{15}H_{24}$	0.41	0.58	0.54
16	Acetaldehyde	11.14	C_2H_4O	0.24	0.32	0.22
17	4-Methoxy-benzaldehyde-oxime	11.44	$C_8H_9NO_2$	0.35	0.03	0.04
18	O-Nitrosobenzoic acid	11.53	$C_7H_5O_3N$	0.92	0.06	0.05
19	4-Hydroxybenzoic acid	11.64	$C_7H_6O_3$	2.28		
20	β-Humulene	11.77	$C_{15}H_{24}$		0.17	0.24
21	3,6-Dimethyl-4*H*-furo[3,2-C]pyran-4-one	11.80	$C_9H_8O_3$	0.69		
22	3-Hydroxybenzoic acid	11.88	$C_7H_6O_3$	0.54		
23	3-Hydroxy-1,2-benzisoxazole	12.40	$C_7H_5NO_2$	0.37	0.26	0.11
24	4-Methoxy cinnamaldehyde	12.62	$C_{10}H_{10}O_2$		0.41	
25	4-Ethyl-α-methyl benzyl alcohol	12.64	$C_{10}H_{14}O$	1.41		0.45
26	*Cis*-3,5-dimethoxy-β-methyl-β-nitrostyrene	12.73	$C_{11}H_{16}NO_4$	1.57	4.24	0.30
27	(s)-2,5,5,8-Tetramethyl-1,2,3,6-tetrahydro-azulen-4(5H)-one	13.19	$C_{14}H_{20}O$	0.39		0.32
28	δ-Cadinene	13.62	$C_{15}H_{24}$			0.23
29	9-Methyl-9H-fluorene	13.69	$C_{14}H_{12}$	0.41		0.33
30	1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene	13.82	$C_{14}H_{18}O$	2.52	6.22	4.73
31	Bendazol	14.10	$C_{14}H_{12}N_2$	0.47	1.21	0.47
32	Trans-2-ethoxy-β-methyl-β-nitrostyrene	14.20	$C_{11}H_{15}NO_3$	0.29	0.44	0.41
33	*N*-(4-hydroxyphenyl)-2-methylbenzamide	14.81	$C_{14}H_{13}NO_2$		0.23	
34	Phenol-3-[2-(2-phenylethy)amino]ethyl]	15.52	$C_{17}H_{21}NO$		0.65	
35	1*H*-Benzimidazol-4-ol.5-ethoxy-1(methylphenyl)	15.57	$C_{16}H_{16}N_2O_2$	0.77		
36	2,2-Diisobutyl-1,3-benzodioxole	15.59	$C_{15}H_{22}O_2$		0.37	0.27
37	Hexadecanoic acid, methyl ester	16.27	$C_{17}H_{34}O_2$	2.26	0.07	0.07
38	1,1′-(1-Ethyl-2methyl-1,2-ethenediyl)bis(4-methoxybenzene)	16.80	$C_{18}H_{20}O_2$	2.05	0.77	
39	Hexadecanoic acid	16.88	$C_{16}H_{32}O_2$		4.07	2.15
40	Octadeca-9C, 12C dienoic acid(z,z)	18.51	$C_{18}H_{32}O_2$	0.71		
41	9,12-Octadecadienoic acid(z,z)-methyl ester	18.56	$C_{19}H_{34}O_2$	1.21		
42	1-Acetoxy-6-(1-hydroxy-1-methylethyl)-3,9-dimethyl-2z,8-deca-diene	18.66	$C_{18}H_{34}O_3$			2.15
43	(E,E)-Methyl linolelaidate	18.85	$C_{19}H_{34}O_2$		0.90	0.64
44	1-Methoxy-4-(1-propenyl)benzene	19.94	$C_{10}H_{12}O$		1.45	0.14
Total				70.45	82.87	92.70

Table 3 Contact activity between *I. verum* fruit extracts and *M. persicae* (mean values ± SE)

Treatment	Concentration of extract (mg/L)	Concentration of trans-anethole (mg/L)	Corrected morality (%)		
			24 h	48 h	72 h
MA extract	1.000	0.411	51.11 ± 0.64 a	57.78 ± 0.64 a	68.93 ± 1.39 a
	0.500	0.206	31.04 ± 1.81 b	41.00 ± 3.60 b	54.49 ± 4.21 b
	0.250	0.103	22.15 ± 1.51 c	37.77 ± 0.65 bc	48.88 ± 1.69 bc
	0.125	0.051	15.52 ± 0.89 d	27.68 ± 1.87 c	38.88 ± 0.65 c
	0.063	0.026	8.82 ± 1.52 e	15.31 ± 2.41 d	24.43 ± 0.74 d
EA extract	1.000	0.525	57.94 ± 3.65 a	62.32 ± 2.40 a	89.95 ± 3.26 a
	0.500	0.263	41.11 ± 0.65 b	54.55 ± 3.61 a	70.04 ± 1.20 b
	0.250	0.131	34.44 ± 0.67 bc	42.22 ± 0.65 b	55.60 ± 2.32 bc
	0.125	0.066	27.77 ± 0.71 c	35.55 ± 0.67 bc	47.78 ± 0.64 bc
	0.063	0.033	17.37 ± 3.17 d	28.88 ± 0.70 c	37.77 ± 0.65 c
PE extract	1.000	0.723	42.18 ± 2.32 a	51.11 ± 0.64 a	74.46 ± 0.73 a
	0.500	0.361	27.77 ± 0.71 b	38.88 ± 0.65 b	61.16 ± 1.74 b
	0.250	0.181	22.20 ± 0.77 bc	28.84 ± 1.38 c	44.43 ± 1.69 c
	0.125	0.090	16.67 ± 0.10 c	25.49 ± 1.44 c	34.41 ± 1.35 d
	0.063	0.045	10.89 ± 2.14 d	18.86 ± 0.82 d	28.84 ± 1.38 d

The mortalities of the control samples were less than 5%. Data followed by different letters in the same column show significant differences based on Duncan's new multiple range test at 5% level of significance

Table 4 Regression analysis on the contact toxicity of *I. verum* fruit extracts against *M. persicae* (72 h)

Extracts	Regression equation of toxicity	LC_{50} of extract (mg/L)	LC_{50} of trans-anethole (mg/L)	Relative coefficient (r)	95% confidence interval (mg/L)	Chi square (χ^2)
MA extract	$y = 0.91x + 5.47$	0.31	0.128	0.9885	0.23–0.43	1.84
EA extract	$y = 1.08x + 5.91$	0.14	0.266	0.9806	0.10–0.19	0.58
PE extract	$y = 1.03x + 5.59$	0.27	0.195	0.9832	0.21–0.31	1.26

Table 5 Repellent effects of *I. verum* organic solvent extracts against *M. persicae* (Mean values ± SD)

Extracts	Concentration of extract (mg/L)	Concentration of trans-anethole (mg/L)	Repellency index (RI)			Mean repellency index
			12 h	24 h	48 h	
MA extract	1.0	0.41	0.61	0.74	0.77	0.71 ± 0.15 Aa
EA extract	1.0	0.53	0.19	0.28	0.50	0.32 ± 0.02 Bc
PE extract	1.0	0.72	0.41	0.48	0.64	0.51 ± 0.16 Ab

Different small letters and capital letter behind the SD were significantly different in 0.05 and 0.01 level based on Duncan's, respectively

Gaikwad et al. 2010; Li et al. 2011). Previous studies found that dried star anise fruit contains 8–12% essential oil. A total of 49 compounds have been separated and identified in the essential oil, including trans-anethole (81.40%), limonene (6.50%), chavicol (2.10%), and anisaldehyde (1.81%) (Gholivand et al. 2009). In the current study, 44 compounds with concentrations greater than 0.20% were separated and identified from the MA, EA, and PE extracts. According to the extraction yields of the three solvents for dried *I. verum* fruits and the percentages of the main active compound trans-anethole in MA, EA, and PE extracts, the yields of trans-anethole from *I. verum* in the corresponding solvents were 9.7, 7.5, and 10.1%, respectively, which are higher than those obtained by hydrodistillation (Gholivand et al. 2009). Moreover, the extracts obtained from MA contained more high-polarity materials, whereas the extracts obtained from PE contained more nonpolar materials. The polarity of the material is related to its size and

Table 6 Apastia effects of *I. verum* organic solvent extracts against *M. persicae* (mean values ± SD)

Extracts	Concentration of extract (mg/L)	Concentration of trans-anethole (mg/L)	Percentage antifeedant (PA) (%)			Mean PA (%)	Apastia level
			12 h	24 h	48 h		
MA extract	1.0	0.41	48.56	42.64	35.27	42.16 ± 5.13 Bb	III
EA extract	1.0	0.53	86.38	76.00	68.26	76.88 ± 7.45 Aa	IV
PE extract	1.0	0.72	44.15	32.19	21.08	32.47 ± 6.06 Bc	II

Different small letters and capital letter behind the SD were significantly different in 0.05 and 0.01 level based on Duncan's, respectively

Table 7 Effect of *I. verum* extracts to *M. persicae* developmental duration

Biotype of aphid	Treatment	Nymph	PR[a]	TBA[b]	TBR[c]
Apterous aphid	MA extract	7.36 ± 0.14	2.12 ± 0.10	7.28 ± 0.19	8.86 ± 0.24
	CK	5.28 ± 0.16	1.45 ± 0.04	5.19 ± 0.17	6.65 ± 0.22
Alate aphid	MA extract	7.53 ± 0.29	2.25 ± 0.12	7.57 ± 0.26	8.95 ± 0.28
	CK	5.35 ± 0.21	1.56 ± 0.05	5.37 ± 0.16	6.88 ± 0.15

[a] PR for early days before the virgin birth

[b] TBA means from nymph was born to adult stage

[c] TBR means from nymph was born to the next-generation nymphal stage

molecular structure, and the polarity of target active components should be assessed to ensure that the appropriate solvent is selected to separate them. The toxicity and reactivity of solvents with target components should also be considered.

The results of this study confirmed that the MA, EA, and PE extracts of *I. verum* fruit possessed strong aphicidal activity against *M. persicae* adults. Therefore, the *I. verum* extracts are potential bioinsecticides against aphids.

Verheggen et al. (2013) tested *M. persicae* responses to volatile cues from turnip plants infested with phloem-feeding and chewing herbivores; their results showed that the aphids exhibited a strong preference for the odors of healthy versus plants subjected to herbivore damage. The present findings suggested that *M. persicae* is repelled by the three extracts in a closed space, such as a laboratory; the three extracts also showed strong antifeedant effects. Plant-derived products can be used as volatile repellents (push) or alluring volatile traps (pull) to manipulate and control the distribution of insect pests. The effects of the "push–pull" method on the host selection behaviors of *Bemisia tabaci* in a greenhouse have been demonstrated (Li et al. 2014). *B. tabaci* exhibited a prominent attraction response to (E)-2-hexenal, 3-hexen-1-ol, and mixtures of these compounds. However, limonene had a greater deterrent effect on adults and repelled egg-laying by more than 80%. Azadirachtin at concentrations from 1.5 to 12 mg/L had significant repellent effects on the oviposition of *Phthorimaea operculella*, whereas eucalyptol at concentrations from 3 to 12 mg/L promoted oviposition; the combination of azadirachtin (12 mg/L) with eucalyptol (3.0 mg/L) produced a significant push–pull effect on oviposition (Ma and Xiao 2013). The *I. verum* essential oil possessed weak repellency at higher concentrations against German cockroaches; however, the essential oils from *I. verum* were strongly attractive at a lower concentration (Liu et al. 2011). Therefore, *I. verum* extracts can be used for the "push–pull" method to control *M. persicae*.

In this study, the three extracts from *I. verum* exhibited pest-combating activities, including mortality, repellency, and apastia. The MA extract demonstrated strong growth inhibition by preventing or delaying growth of the peach aphid population. Therefore, *I. verum* fruit-derived materials could be used to manage aphid populations. *I. verum* is widely cultivated in the Guangxi, Yunnan, Guangdong, Fujian, and Guizhou provinces of China; the yield of Chinese star anise accounts for more than 80% of the global output, thereby making star anise fruits readily available in China (Li et al. 2013). The crude extracts of *I. verum* may be directly used in IPM to repel aphid pests (pull) from vegetables and fruit trees, to kill aphids, to prevent aphid feeding, or to inhibit the growth and development of aphids via its toxic components. Therefore, *I. verum* extracts may be explored as novel natural aphicidal agents against *M. persicae* in alternative management programs.

The insecticidal activity against *M. persicae* has been evaluated for several plant essential oils. *M. persicae* was sprayed with *Cymbopogon citratus* essential oil at different concentrations with a Potter tower, and the LC_{50} value for *M. persicae* was 0.28% (Costa et al. 2013). The essential oil of *Cymbopogon winterianus* at 1% (w/v) causes

mortality in *M. persicae* at 96.9%; the LC_{50} and LC_{90} values for *M. persicae* were 0.36 and 0.66%, respectively (Pinheiro et al. 2013). *I. verum* essential oil reduced the oviposition potential, egg hatching rate, pupal formation, and emergence of adults of *Callosobruchus chinensis* F1 fumigated with sublethal concentrations (Chaubey 2008). Therefore, these essential oils are promising natural alternatives for developing pesticides to manage *M. persicae*.

This article only analyzes the results of indoor research; to comprehensively understand the biocontrol effects of star anise extracts to aphids, field trials should also be conducted to validate the efficacy of these extracts for control of aphid pests of vegetables and fruit trees and to determine the optimum dosage. Our future studies will also focus on isolating and purifying biologically active constituents derived from the EA extract of star anise to select for compounds with high biological activity and relatively simple structures for the development of novel repellents or insecticides. Moreover, an in-depth study on the relationship between the chemical composition and insecticidal activity of the extracts should be performed with biological activity tracking to provide a clearer understanding of the compounds responsible for extract bioactivity.

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