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ORIGINAL RESEARCH ARTICLE

Biological activity and safety evaluation of monoterpenes against the peach aphid (*Myzus persicae* Sulzer) (Hemiptera: Aphididae)

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Abstract

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is an economically important cosmopolitan crop pest. Essential oils (EOs) are complex mixtures of secondary metabolites that exhibit a wide range of biological activities, including antioxidant, antimicrobial and anti-inflammatory activities. Monoterpenes, as the major constituents of EOs, are well known for having a range of biological activities. In this study, 9 monoterpenes were screened for fumigant toxicity against *M. persicae*, and the safety of monoterpenes to natural enemies was evaluated. The results of the bioassays indicated that 9 monoterpenes showed promising fumigant toxicity against *M. persicae*. Carvacrol was the most toxic compound based on the LC_{50} value, which was 1.566 mg/L, followed by bornyl acetate, terpinolene, and terpinyl acetate, which had higher toxicities towards aphids, with LC_{50} values of 2.648 mg/L, 2.759 mg/L, and 2.832 mg/L, respectively. In addition, the safety evaluation assays of carvacrol against *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) larvae ($LC_{50}=43.851$ mg/L) did not affect nontarget invertebrates at concentrations that effectively controlled aphids. Carvacrol was safer than chlorpyrifos to natural enemy *H. axyridis* larvae (toxicity ratio $=28.00>2.93$). These results identified several high-activity monoterpenes that can be used against this pest in greenhouses as botanical insecticides.

Keywords Monoterpenes · *Myzus persicae* · Carvacrol · *Harmonia axyridiss* · Toxicity ratio

Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is a cosmopolitan and very economically important crop pest that is highly polyphagous (Machado-Assefh and Alvarez 2016). *M. persicae* is remarkably adept at destroying the quality of economically important crops of agricultural, horticultural and greenhouse crops (Sial 2019); this pest has broad host ranges and transmits many plant viruses (Tang et al. 2015; Zeng et al. 2016). The host plants include 400 species in 40 different plant families, such as Solanaceae and Asteraceae. In addition, *M. persicae* feeds on the vascular bundles of plants, including potatoes, cabbages, and peppers, and transmits 100 plant viruses, (Kuhar and Doughty 2010; Tang et al. 2015; Zeng et al. 2016).

Currently, insecticides have become one of the main measures for controlling aphids in field crops (Charaabi et al. 2018). The chemical insecticides included organophosphates, carbamates, pyrethroids, and neonicotinoids (Walgenbach and Schoof 2011; Zeng et al. 2016). In China, an increasing number of insecticides have been restricted because of their environmental impacts. Aphids have developed strong resistance to many different types of chemical insecticides because of the application of insecticides (Elbert et al. 2008; Sial et al. 2018). Currently, an important challenge in developing insecticides is finding novel and effective compounds; this effort is faced with the problems of insecticide resistance and impacts on human health, and the environment (Benelli et al. 2018; Xiao and Wu 2019). Environmentally friendly insecticides were developed for

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pest control purposes to reduce environmental pollution and food safety problems (Cai et al. 2020).

Essential oils (EOs) are a complex of secondary metabolite mixtures (including terpenes, phenolic compounds, and alcohol) that exhibit a wide range of biological activities, including antioxidant, antimicrobial and anti-inflammatory activities (Hanen et al. 2020). Because these complexes have low toxicity levels to vertebrates and nontarget aquatic invertebrates and are fully biodegradable, multifunctional, efficient and environmentally safe (Pavela and Benelli 2016; Pavela et al. 2019), the insecticidal properties of EOs and plant extracts are being studied as new alternative pesticides. A number of plants have been screened as potential natural repellent and insecticide compounds (Singhet al. 2006). For example, Kumar et al. (2011) investigated the repellent, larvicidal and pupicidal activities of the EOs of six plant species for the control of different stages of the housefly lifecycle, and *Mentha piperita* (Linn.) oil has been indicated to be the best repellent, larvicidal and pupicidal agent. The EOs of *Pimpinella anisum* (L.) fruits have been reported to show strong toxicity on *Culex quinquefasciatus* (Say) and did not have any effect on *Daphnia* at short exposure times or low concentrations (Pavela et al. 2014).

Monoterpenes, which are the major components of EOs, are well known for a range of biological activities, such as herbicidal, antifungal and insecticidal activities (Zhang et al. 2016a, b, 2017, 2018; Gouda et al. 2016). In this study, we were interested in appraising the insecticidal properties of 9 monoterpenes, including bornyl acetate, carvacrol, citronellyl acetate, eugenol, geranyl acetate, linalyl acetate, neryl acetate, terpinolene and terpinyl acetate. These monoterpenes have been reported to possess superior insecticidal activity against *Reticulitermes chinensis* Snyder, *Musca domestica* L., *Plutella xylostella* L. (Xie et al. 2014; Zhang et al. 2017; Cai et al. 2020). However, no studies have been conducted to evaluate the insecticidal activities of monoterpenes against vegetable pests in greenhouses. Therefore, we assessed the toxicities of 9 monoterpenes against *M. persicar*. Our results identified several high-activity monoterpenes that can be used against this pest in greenhouses as botanical insecticides. Furthermore, the potential ecotoxicological consequences arising from the high-activity monoterpene were investigated by testing them on the natural enemy of aphids, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae).

Materials and methods

Insect tested and rearing of conditions

The green peach aphid (*M. persicae*) used in this study was obtained from the laboratory of China Agricultural University, Beijing, China. The aphids were a susceptible laboratory strain that had not had exposure to any insecticides since September 2008 (Tang et al. 2015). *M. persicae* was reared on cabbage (*Brassica oleracea* (L.)) seedlings and maintained at 23 ± 1 °C in 65–75% relative humidity (RH) and under a photoperiod of 16:8 (L: D) h.

H. axyridis eggs were purchased from Jiyuan Baiyun Industry Co., Ltd. Following egg eclosion, the larvae were placed on plastic Petri dishes and provided with aphids daily. The larvae were maintained at 25 ± 2 °C and 75% ± 5% relative humidity and under a 16-h photoperiod.

Chemicals

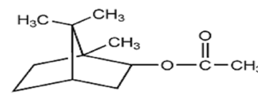
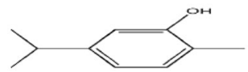
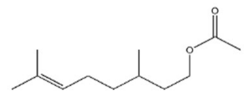
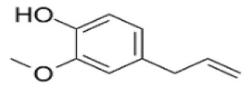
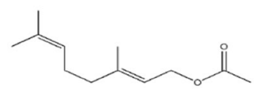
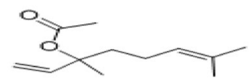
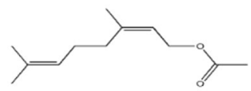
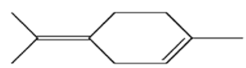
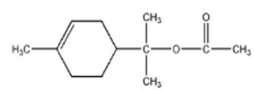
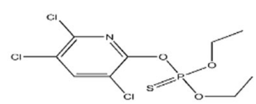
The 7 from 9 experimental monoterpenes compounds, included bornyl acetate, carvacro, citronellyl acetate, geranyl acetate, linalyl acetate, neryl acetate and terpinyl acetate, were purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China). In additional, eugenol and terpinolene were sourced from Sigma-Aldrich Co., Ltd. (Poole, UK). Chlorpyrifos was used as control chemical pesticide, and purchased from Hengdong Chemical Industry Co., Ltd. (Shandong, China). All chemical structures and purity metrics are provided in Table 1. The compounds were stored at -20 °C until use.

Fumigation bioassay

The insecticidal toxicity of 10 volatile compounds to adult apterous aphids was tested according to the method of Zhang et al. (2016b) with slight modifications. Filter papers (1 cm diameter) were soaked in a certain amount of compounds or chlorpyrifos (positive control), and an untreated filter paper (containing acetone; control) was used as a blank control. All were placed in 500 mL glass jars. In every test system, approximately 30 individuals of *M. persicae* were evaluated. The concentration for each test is shown in Fig. 1. For the liquid monoterpenes, the compounds were directly taken at a certain amount for the experimental study. Chlorpyrifos was configured in a series of concentrations with acetone, and the final volume per treatment was 15 μL. The aphid mortalities were noted after 24 h according to agricultural industry standard of the people's Republic of China (NY/T 1154.3–2006), and all tests were kept at 23 ± 1 °C, with 65–75% RH. The aphids were considered dead only when they were probed with a soft paintbrush and did not display any movement, even of their legs (Moores et al. 1996). Three replicates of each control and treatment were tested.

Third instar larvae of *H. axyridis* were used to test the toxicity of the most toxic compound. The concentration and application method were utilized in the fumigation assay. The mortalities of *H. axyridis* larvae were noted after 24 h. The concentration used in each test is shown in Fig. 2. The larvae of *H. axyridis* were provided aphids as food during each fumigation assay.

Table 1 The 10 compounds for fumigation bioassay

Code	Compounds	Purity (%)	Structure	Source
1	Bornyl Acetate	70		TCI
2	Carvacrol	95		TCI
3	Citronellyl Acetate	95		TCI
4	Eugenol	98		Sigma-Aldrich
5	Geramyl Acetate	70		TCI
6	Linalyl Acetate	95		TCI
7	Neryl Acetate	95		TCI
8	Terpinolene	85		Sigma-Aldrich
9	Terpinyl Acetate	78		TCI
10	Chlorpyrifos	80		HCI

Data analysis

Statistical analyses were conducted with POLOPlus 2.0 (LeOra Software, Petaluma, CA, USA). The criterion of non-overlapping 95% confidence intervals (CIs) was used to consider significant differences in the LC_{50}s and LC_{90}s.

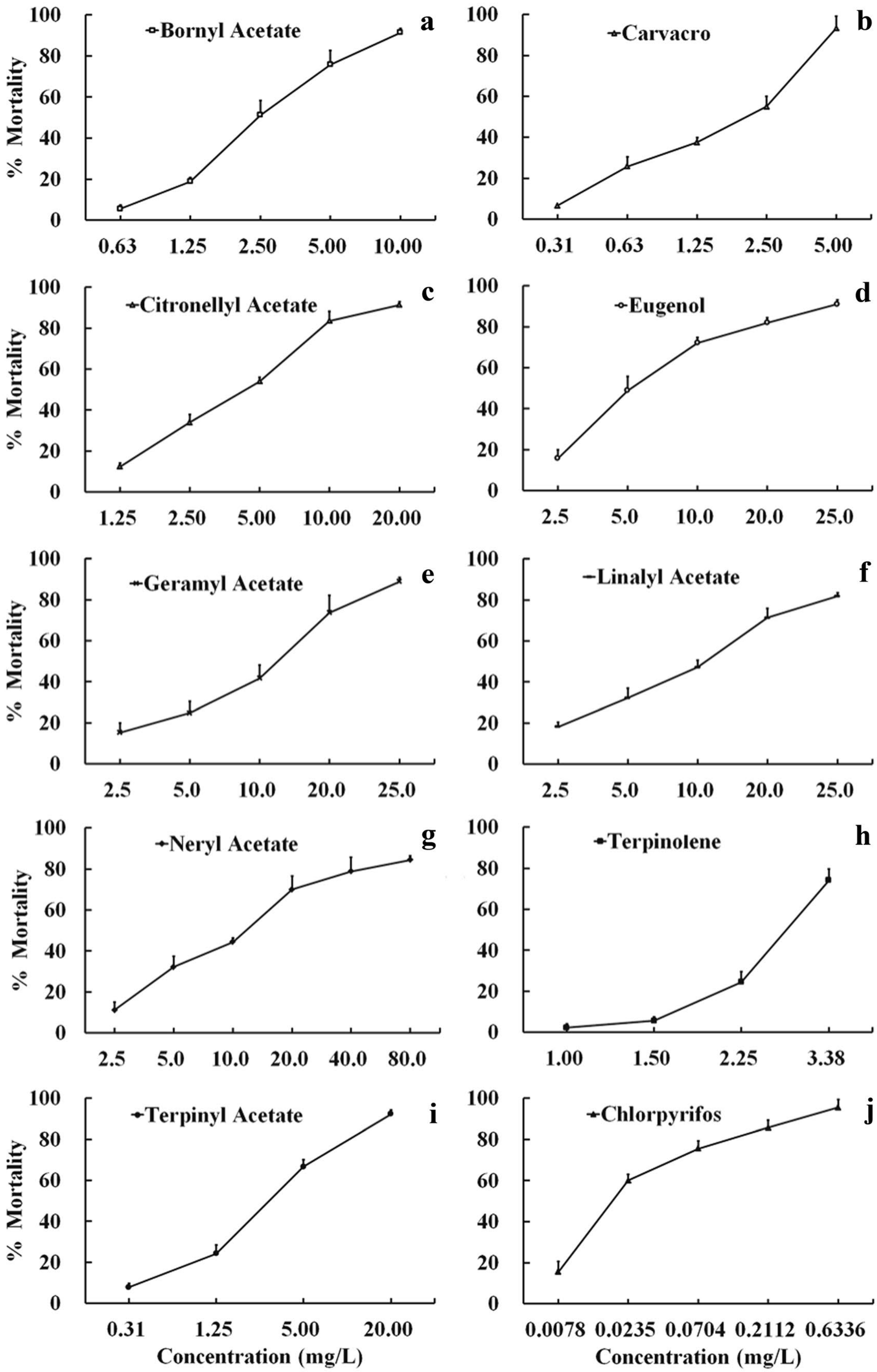
To evaluate the safety of the insecticides to the natural enemy, the ratio of the LC_{50} of insecticide to natural enemy to the LC_{50} of insecticide to insect pest was determined (Wang and Shen 2002). The calculation formula is as follows:

$$\text{Toxicity} = \frac{LC_{50}\text{ of insecticide to natural enemy}}{LC_{50}\text{ of insecticide to insect pest}}$$

Results

The toxicities of 9 pure commercial monoterpenes, including 1 hydrocarbon, 2 aldehydes, and 6 acetate esters, were determined against adults of *M. persicae* (Fig. 1). Carvacrol and terpinolene had greater than 80% mortality against aphids at concentrations of 5 mg/L (Fig. 1b, h). The mortality of geranyl acetate and linalyl acetate was between 20 and 40% at a concentration of 5 mg/L (Fig. 1c, e). Weak toxicity against aphids was shown by neryl acetate, with 80% mortality against aphids at concentrations greater than 40 mg/L (Fig. 1g). The LC_{50} values of the 9 monoterpenes against aphids were determined and shown in Table 2. The insecticidal activity of carvacrol, bornyl acetate, terpinolene, and terpinyl acetate had higher toxicities towards aphids, with LC_{50} values of 1.566 mg/L, 2.648 mg/L, 2.759 mg/L, and 2.832 mg/L, respectively. With the exception of the positive control insecticides, all other tested compounds had LC_{50} estimates ranging from 4.03 mg/L to 9.64 mg/L. The positive control chlorpyrifos showed considerably higher toxicity than any of the monoterpene compounds ($LC_{50} = 0.028$ mg/L).

The effects of carvacrol and chlorpyrifos on the natural enemy were evaluated, and the LC_{50} values of these



Bornyl Acetate
a
% Mortality
0.63 1.25 2.50 5.00 10.00
Carvacro
b
0.31 0.63 1.25 2.50 5.00
Citronellyl Acetate
c
1.25 2.50 5.00 10.00 20.00
Eugenol
d
2.5 5.0 10.0 20.0 25.0
Geramyl Acetate
e
2.5 5.0 10.0 20.0 25.0
Linalyl Acetate
f
2.5 5.0 10.0 20.0 25.0
Neryl Acetate
g
2.5 5.0 10.0 20.0 40.0 80.0
Terpinolene
h
1.00 1.50 2.25 3.38
Terpinyl Acetate
i
0.31 1.25 5.00 20.00
Chlorpyrifos
j
0.0078 0.0235 0.0704 0.2112 0.6336
Concentration (mg/L)

◂**Fig. 1** Toxicity of 10 compounds against *M. persicae*. **a** Bornyl Acetate, **b** Carvacro, **c** Citronellyl Acetate, **d** Eugenol, **e** Geramyl Acetate, **f** Linalyl Acetate, **g** Neryl Acetate, **h** Terpinolene, **i** Terpinyl Acetate, and **j** Chlorpyrifos, respectively

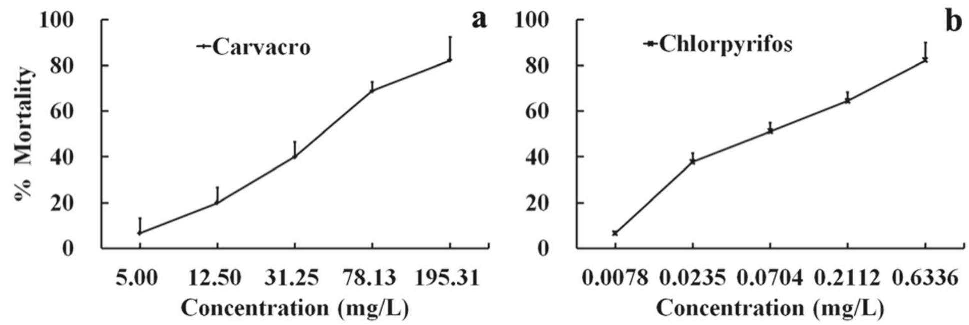


Fig. 2 Safety evaluation assays of two compounds against *H. axyridis*. **a** Carvacro, and **b** Chlorpyrifos, respectively

compounds against *H. axyridis* larvae are shown in Table 3. According to the toxicity ratio, carvacrol was safer than chlorpyrifos to the larvae of the natural enemy *H. axyridis* (toxicity ratio = 28.00 > 2.93).

Discussion

Our study showed that the 9 monoterpenes tested had varying degrees of insecticidal activity against aphids, and the mortality from the monoterpenes generally increased with increasing doses. Among the tested compounds, carvacrol, bornyl acetate, terpinolene, and terpinyl acetate possessed relatively strong toxicities against *M. persicae*. According to the toxicity ratio, carvacrol was safer than chlorpyrifos to *H. axyridis* larvae.

Some monoterpenes in the EOs and plant extracts have been affirmed to possess very strong toxicity against the various insect species in several studies. Park et al. (2017) reported that carvacrol and thymol from *Thymus vulgaris* L. showed the strongest activity against *Pochazia shantungensis* (Chou & Lu) adults and nymphs. The EOs from *Cuminum cyminum* L. and *Pimpinella anisum* L. were toxic to larvae of *C. quinquefasciatus* and *Spodoptera littoralis* (Bosid) but did not affect nontarget invertebrates (Benelli et al. 2018). In addition, Zhang et al. (2016b; 2017) showed that thymol, carvacrol, (+)-pulegone, citral, (±)-citronellal, and cuminaldehyde had strong fumigation activity against *M. domestica* and *Drosophila melanogaster* Meig. In this respect, we assessed the toxicities of 9 monoterpenes against aphids in the laboratory, and the results showed that monoterpenes were also more effective against *M. persicae* and could be used successfully as a control agent for aphids.

In this study, carvacrol possessed relatively strong toxicity against aphids, and the LC_{50} value against *M. persicae* was 1.566 mg/L. Carvacrol is a phenolic monoterpenoid that is found in the essential oils of some plants, such as *Origanum vulgare* L. and *T. vulgaris* (Sharifi-Rad et al. 2017). Xie et al. (2019) reported that the percentage inhibition rate of carvacrol against pupae of *M. domestica* was 29.5% (0.025 μL/cm^2) according to a contact toxicity assay and 81.8% (1.25 μL/L) according to a fumigation assay. Castilhos et al. (2017) showed that carvacrol was more acutely toxic than the other terpenoids screened, but it had less toxicity than natural pyrethrins. Furthermore, carvacrol has a wide range of bioactivities

Table 2 LC_{50} and LC_{90} values (mg/L) of 10 compounds against *M. persicae* adults over 24 h

Compounds	LC_{50}/(mg/L) (95% CL)	LC_{90}/(mg/L) (95% CL)	Slope ± SE	χ^2 (df)
Bornyl Acetate	2.648 (2.325—3.018)	8.611 (7.054—11.132)	2.503 ± 0.196	4.492 (13)
Carvacro	1.566 (1.309—1.896)	6.483 (4.742—10.176)	2.077 ± 0.180	15.676 (13)
Citronellyl Acetate	4.029 (3.492—4.626)	14.481 (11.673—19.271)	2.307 ± 0.199	2.650 (13)
Eugenol	6.058 (5.136—7.011)	24.049 (19.475 -31.851)	2.140 ± 0.193	7.503 (13)
Geramyl Acetate	9.635 (8.148—11.388)	37.723 (28.569—56.295)	2.162 ± 0.193	15.013 (13)
Linalyl Acetate	9.282 (7.822—11.006)	51.253 (36.945—82.543)	1.727 ± 0.181	4.470 (13)
Neryl Acetate	13.166 (10.479—16.464)	103.395 (69.758—181.761)	1.432 ± 0.125	19.164 (16)
Terpinolene	2.751 (2.514—3.076)	4.594 (3.923—5.913)	5.757 ± 0.587	12.929 (10)
Terpinyl Acetate	2.832 (2.263 to 3.555)	17.317 (12.321—27.150)	1.630 ± 0.140	2.854 (10)
Chlorpyrifos	0.028 (0.019—0.038)	0.234 (0.152—0.447)	1.379 ± 0.127	20.679 (13)

Table 3 Safety evaluation of carvacrol and chlorpyrifos for *H. axyridis*

compounds	LC_{50}/(mg/L) (95% CL)	Slope ± SE	χ^2 (df)	Toxicity ratio
Carvacro	43.851 (33.183—59.172)	1.555 ± 0.190	6.414 (13)	28.00
Chlorpyrifos	0.082 (0.056—0.121)	1.112 ± 0.152	7.661 (13)	2.93

that are putatively useful for insecticide, antimicrobial, and antioxidant activities (Sharifi-Rad et al. 2017; Cai et al. 2020). Kordali et al. (2008) certified that the EOs isolated from *Origanum acutidens* (Hand.-Mazz.) had antifungal, phytotoxic and insecticidal activities; furthermore, the major component, carvacrol, had the potential to be used as fungicide, herbicide and insecticide. Our previous studies have shown that carvacrol possesses good antifungal activities against fungi, such as *Trametes hirsute* (Wulfen), *Schizphylhls commune* (Fr.), *Pycnoporus sanguineus* (L.), *Rhizopus stolonifer* and *Absidia coerulea* (Zhang et al. 2016b; Zhou et al. 2019). Together, these studies proved that carvacrol can be used for the development of antifungal and fungicidal agents and insecticidal agents for controlling agricultural pests and diseases.

In addition, the insecticidal activities of bornyl acetate were generally more toxic than terpinolene, terpinyl acetate, citronellyl acetate, eugenol, linalyl acetate, geranyl acetate, and neryl acetate. Similarly, bornyl acetate has been found to be more effective than other monoterpenes in insecticidal activity against *R. chinensis* (Xie et al. 2014). Bornyl acetate is one major constituent of *Valeriana officinalis* (L.), and the EOs extracted from the roots of *Valeriana officinalis* showed promising fumigant and contact toxicity against *Liposcelis bostrychophila* (Booklouse) (2.8 mg/L air and LD_{50} = 50.9 µg/cm^2, respectively). Furthermore, bornyl acetate also exhibited strong fumigant and contact toxicity against *L. bostrychophila* (LC_{50} = 1.1, 10.1 mg/L air and LD_{50} = 32.9, 701.3 µg/cm^2) (Feng et al. 2019). Moreover, bornyl acetate, as a repellent of *Aedes aegypti* L., was active at 0.28 mg/cm^2 and higher (Hwang et al. 1985). Early research also found that bornyl acetate possessed the strongest activity against fungi, and the effect was correlated most strongly with the content of bornyl acetate and other monoterpenes (Pellegrini et al. 2017). This study highlighted the insecticidal, antifungal and fungicidal potential of bornyl acetate, which has been noted as a natural botanical agent for insecticide and bacteriostasis.

To evaluate the safety of monoterpenes to natural enemies, safety evaluation assays of carvacrol against *H. axyridis* larvae were conducted in this study, which showed that carvacrol was safer than chlorpyrifos to the natural enemies. Our finding was the same as that of Glavan et al. (2020), who showed that carvacrol caused 5% mortality in *Apis mellifera carnica* Pollmann only at the highest concentrations tested. For example, Cai et al. (2020) reported that cuminaldehyde was greater against the adults, larvae, and eggs of *P. xylostella* but did not cause any major damage to their natural enemies *H. axyridis*. Monoterpenes are safe to nontarget aquatic invertebrates at low toxicity levels and are rich sources of botanical pesticides that are easily degradable and exhibit good activity levels against agricultural pests (Copping and Menn 2000).

Conclusions

In this study, the insecticidal activities of 9 pure monoterpenes against aphids were evaluated. The results of the present study showed that carvacrol, bornyl acetate, terpinolene, and terpinyl acetate have good toxicity against aphids. Furthermore, to evaluate the safety of monoterpenes for natural enemies, the safety of carvacrol against *H. axyridis* larvae was studied, and the results showed that carvacrol was safer than chlorpyrifos for natural enemies. Thus, carvacrol, bornyl acetate, terpinolene, and terpinyl acetate could be developed as potential natural insecticides and be useful as new natural insecticidal agents against aphids.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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