Pakistan J. Zool., pp 1-12, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20211022051038

Acute Toxicity, Synergistic and Antagonistic Effects of 12 Monoterpenoids Against the *Poratrioza sinica* Yang et Li (Hemiptera: Psyllidae)

Wei Wan[1], Xiao-Li Wang[1], Shu-Juan Wang[2], Qin Si[2] and Li-Qing Duan[1]*

[1]*Forestry College, Inner Mongolia Agricultural University, Hohhot 010019, China*
[2]*Hohhot Gardening Plant Protection Station, Hohhot 010020, China*

Article Information
Received 22 October 2021
Revised 20 March 2023
Accepted 17 May 2023
Available online 20 June 2023
(early access)

Authors' Contribution
Conceptualization, methodology: WW and L-QD. Investigation: S-JW, QS. Data curation, formal analysis, writing original draft preparation: WW. Supervision, software, validation: X-LW. Validation, writing reviewing and editing: L-QD. All authors have read and agreed to the published version of the manuscript.

Key words
Monoterpenoids, *Poratrioza sinica*, Binary mixtures, Acute toxicity, Synergistic effect, AChE inhibition, GST inhibition

ABSTRACT

Poratrioza sinica (Hemiptera: Psyllidae) is a highly destructive pest that infests of wolfberry. To screen biodegradable and safe insecticides, 12 representative monoterpenoids in essential oils were evaluated for their acute toxicity, synergistic or antagonistic effects on adult *P. sinica*. The modified residual film method was used to test the toxicity of individual and binary mixture of these monoterpenoids against adult *P. sinica.* The effects of these monoterpenoids on acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities in *P. sinica* were assessed *in vitro*. Correlation between numbers of synergistic or antagonistic binary mixtures and AChE or GST activities was analyzed. The results showed that 2-ethylimidazole had the strongest acute toxicity against *P. sinica* adult, with a median lethal concentration (LC_{50}) value of 0.52 g/L. Among the 66 binary mixtures, 19 showed strong synergistic effects, while 21 showed antagonistic effects. The most profound synergistic effect was the mixture of l-carvone and dihydrocarvone, with an expected mortality of 35.2% and actual mortality of 98.4%. Estragole had the highest frequency of antagonistic effect (7 combinations), and the most significant antagonism was observed when combining β-pinene and estragole, with an expected mortality of 29.0% and actual mortality of 3.8%. Furthermore, AChE inhibition was observed with estragole, cuminaldehyde, and 1,8-cineole displayed high potency. L-carvone showed the highest GST inhibition activity, followed by cuminaldehyde. Pearson correlation analysis revealed a significant negative correlation between GST inhibition rate and number of antagonistic binary mixtures. In conclusion, our findings suggest that 2-ethylimidazole and cuminyl alcohol have high toxicity against *P. sinica.* L-carvone was the best synergist, and binary mixtures of l-carvone with dihydrocarvone, cuminaldehyde, cuminyl alcohol, d-carvone, and estragole showed potential as control agents against *P. sinica*. This study provides insights for identifying safe and biodegradable insecticides and potential solutions for controlling *P. sinica*.

INTRODUCTION

Poratrioza sinica Yang et Li (Hemiptera: Psyllidae) is a destructive pest that infests Chinese wolfberry (*Lycium barbarum*) in the northwest regions of China. This insect species feeds on the sap of young leaves, shoots, buds and fruits, causing premature leaf drop, diminish plant growth, and reduce fruit quality and yield when the population density is too high. In addition, the honeydew secreted by *P. sinica* promotes the growth of sooty blotch on leaves and fruits. Currently, controlling *P. sinica* mainly relies on extensive use of synthetic insecticides. However, the use of synthetic insecticides has brought several serious problems, including negative impacts on environment and non-target organisms, such as humans (Hodgson and Levi, 1996; Singh *et al*., 2012), as well as development of resistant *P. sinica* populations. These issues have driven the search for environmentally safe alternative control measures. Amongst alternative strategies aimed at reducing insect populations, the use of essential oils is a promising strategy.

Essential oils are secondary metabolites of plants that possess significant biological activity and various pesticidal effects (Abdelfattah *et al*., 2018; Hennia *et al*., 2019), including insecticidal activity (Ebadollahi *et al*., 2021; Said-Al Ahl *et al*. 2017). Despite numerous

* Corresponding author: duanlq2013@163.com
0030-9923/2023/0001-0001 $ 9.00/0

studies demonstrating their insecticidal effects, only a few essential oil insecticides are commercially produced (Isman and Grieneisen, 2014) due to limited production, quality and quantity issues, and high prices of some essential oils. These factors have hindered the production and wider expansion of essential oil insecticides.

The insecticidal properties of essential oils are primarily attributed to their main active constituents. Thymol is the main insecticidal active ingredient in essential oils extracted from *Trachyspermum ammi*, and has been found to be effective against *Aethina tumida* (Bisrat and Jung, 2020). Moreover, dihydrocarvone, carvone and cuminaldehyde are active constituents of essential oils extracted from *Anethum graveolens*, *Cuminum cyminum* and *Carum carvi* that have shown efficacy against *Sitophilus oryzae* adults and *Aedes albopictus* larvae (Kim *et al*., 2013; Seo *et al*., 2015). Essential oils extracted from *Erechtites hieraciifolius* and *E. valerianifolius* have been shown to possess good mosquito larvicidal properties, attributed to the presence of limonene and α-pinene in the essential oil of *E. hieraciifolius*, as well as α-pinene, β-caryophyllene, and myrcene in essential oil of *E. valerianifolius* (Hung *et al*., 2019). Recent research suggests that many of the active substances contained in essential oils can be produced synthetically with high quality and at a lower cost. However, the use of a single active substance with a single mechanism of action could contribute to the development of resistant insect populations, as observed with other synthetically produced insecticides (Ranson *et al*., 2009). In contrast, essential oils contain complex mixtures of active substances with different mechanisms of action, which may prevent the development of resistance in insects, thus providing a major advantage of essential oils as insecticides (Regnault-Roger *et al*., 2012; Sutthanont *et al*., 2010). Individual components contained in essential oils can exhibit diverse synergistic or antagonistic effects, which significantly influencing their biological efficacy (Hummelbrunner and Isman, 2001; Pavela, 2008, 2014). Therefore, a thorough understanding of this phenomenon is essential for developing essential oils insecticides with standardized mixtures and declared activity, while maintaining relatively lower costs.

Acetylcholinesterase (AChE) is a crucial enzyme that helps break down acetylcholine into choline and acetate at the neuromuscular junction. The choline produced by AChE activity is recycled by being transported back to the presynaptic neuron for the synthesis of new acetycholine. Inhibition of AChE activity may hinder neurotransmission, ultimately leading to insect death (López and Pascual-Villalobos, 2010). Several essential oils from aromatic plants and monoterpenes have been identified as inhibitors of AChE isolated from different insect species (Abdelgaleil *et al*., 2009; Kim *et al*., 2013). Glutathione-S-transferase (GST) functions in the detoxification of foreign substances by conjugating glutathione (GSH) with electrophilic molecules. It plays a crucial role in detoxifying harmful compounds and developing insecticide resistance (Cisse *et al*., 2017; Li *et al*., 2019; Piccoli *et al*., 2019).

In this study, to gain a better understanding of the mutual relationships between essential oil compounds, we selected 12 representative monoterpenoids found in essential oils. These compounds were tested individually and as binary mixtures for their acute toxicity against *P. sinica*. This will facilitate an improved understanding of the general principles of the mutual relationships of essential oil compounds and determine a suitable mixture of active substances for developing new essential oil insecticides against *P. sinica*. Furthermore, the effects of these 12 monoterpenoids on the activity of AChE and GST of *P. sinica* were assessed *in vitro* to explore the action mechanism of the monoterpenoids.

MATERIALS AND METHODS

Chemicals

Dihydrocarvone (98%) was obtained from Sigma-Aldrich (Saint Louis, MO, USA); L-carvone (98%) and estragole (98%) was obtained from Alfa Aesar (Beijing, China); 2-ethylimidazole (2-MIM, 99%), β-caryophyllene (BCP, 80%), cuminaldehyde (97%), acetylthiocholine iodide (ATCI), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from RHAWN (Shanghai, China); cuminyl alcohol (97%) was procured from Xiya Reagent (Shandong) Co. Ltd. (Linyi, China); 1,8-cineole (99%) and d-carvone (98%) were obtained from Energy Chemical (Shanghai, China); limonene (97%) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China); α-pinene (95%) and β-pinene (95%) were procured from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China); acetone, alcohol, NaH_2PO_4, and Na_2HPO_4 were purchased from the Tianjin Chemical Reagent Factory (Tianjin, China); Coomassie brilliant blue G-250 and bovine serum albumin were procured from Amresco (Solon, OH, USA); the glutathione S-transferase (GST) assay kits were purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China).

Plants and insects

The first generation of *Poratrioza sinica* adult was initially sourced from the Science and Technology Garden of Inner Mongolia Agricultural University (Hohhot, China), and subsequently raised in a laboratory environment without exposure to any insecticides. Chinese

wolfberry (*Lycium barbarum*) seedlings were used as their food source during this process.

Chinese wolfberry seedlings were cultivated in plastic pots with dimensions of 15 cm height and 10 cm diameter. The pots were filled with a mixture of peat soil, perlite, and vermiculite in a 60:20:20 ratio, with a pH range of 6–7. The pots were then placed inside cages covered with an insect-proof netting of 80 mesh size (270 × 170 × 240 cm). The growth conditions were maintained at room temperature (21–26°C) under a photoperiod of 16:8 h (L:D), regulated using a timing socket. Seedlings that grew to be 25–30 cm tall were utilized for subsequent experiments.

To breed *P. sinica*, two pots (containing one seedling for each pot) were transferred into a small insect-proof and net-covered cage measuring 35 cm × 35 cm × 45 cm with a 120 mesh size. Subsequently, 20 pairs of three-day-old *P. sinica* adults were introduced into this cage and removed two days later to ensure hatching of eggs at approximately the same time. The cage was incubated under the same laboratory temperature and photoperiod conditions as described above.

Acute toxicity assessment

To evaluate the acute toxicity of 12 monoterpenoids to *P. sinica*, the modified residual film method was employed as previously described (Shotkoski *et al*., 1990; Shufran *et al*., 1997). The 12 monoterpenoids examined were 2-ethylimidazole, estragole, cuminyl alcohol, d-carvone, l-carvone, cuminaldehyde, dihydrocarvone, β-caryophyllene, β-pinene, 1,8-cineole, α-pinene, and limonene. Serial dilutions of each monoterpenoid were prepared in acetone to generate at least ten concentrations (0.06, 0.12, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mL/L), except for 2-ethylimidazole (solid, with the unit of measurement being g/L). Then, 500 µL of each dilution was added into a glass tube (10 cm length, 1.5 cm diameter), and slowly rolled on a table to ensure the formation of a residual film on the inner surface of the tube until the acetone had completely evaporated. Simultaneously, a piece of wolfberry leaf was immersed in the same solution for 5 s, and then dried on filter paper. The leaf was subsequently transferred into the tube containing the residual film formed by the same tested solution.

Then, 20 *P. sinica* adults **(**newly emerged about 3 days**)** were released into each prepared test tube, and the mortality was recorded after 24 h of exposure. The tube was sealed with Parafilm (PM-996, Bemis, Neenah, WI, USA) to prevent their escape, three replicates were conducted, with n=20 *P. sinica* adults for each concentration, a total n= 60. The assays were performed in a growth chamber with a photoperiod of 16:8 h (L:D) at 26°C.

The values of median lethal concentration (LC_{25}, LC_{50}, LC_{90}), confidence interval of 95% (CI_{95}), slope, and χ^2/df were estimated using probit analysis (SPSS Statistics 22, IBM, New York, NY, USA). Differences among LC_{25}, LC_{50} or LC_{90} values were considered significant when their 95% CI did not overlap (Ebling *et al*., 2004).

Assessment of AChE activity

The inhibitory effect of 12 monoterpenoids on AChE activity in *P. sinica* was evaluated *in vitro* using the modified Ellman's method (Ellman *et al*., 1961). Another 30 healthy *P. sinica* adults were homogenized in an ice bath using a glass tissue grinder with pre-cooled 0.2 M phosphate buffer (PB, pH 7.0). The homogenate was then centrifuged at 10,000 g for 20 min at 4°C, and the collected supernatant was used as the enzyme solution for assessing AChE activity.

To determine the AChE activity, the tested monoterpenoids were diluted in acetone to a concentration of 20 mL/L. Next, 0.02 mL of the diluted monoterpenoid solution, 0.15 mL of the enzyme supernatant, and 0.53 mL of PB (pH 7.0, 0.2 mol/L) were mixed in a tube. After 5 min, 0.2 mL of 0.03 mol/L ATCI was added, and the mixture was incubated at 30ºC for 15 min. Then 2.1 mL of 0.125 mmol/L DTNB was added, and after 2 min, the absorbance (OD at 412 nm) was measured using a TU-1810 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). Three biological replicates were performed for each monoterpenoid, with acetone being used as the control and the dead enzyme (inactivated in boiling water) as a blank. The inhibition rate was calculated as follows:

Inhibition rate (%) = 100 − (Treatment OD − Blank OD)/(Control OD − Blank OD) × 100

Assessment of GST activity

To assess the inhibitory effect of 12 monoterpenoids on the GST activity of *P. sinica*, another 30 healthy adults of *P. sinica* were homogenized using a glass tissue grinder in an ice bath with pre-cooled PB (pH 7.0, 0.2 mol/L). The resulting homogenate was centrifuged at 8000 g for 10 min at 4°C, and the supernatant was collected as the crude enzyme solution for analysis of GST activity. The GST activity was calculated following the instruction of the GST assay kits.

Acute toxicity of binary mixtures

To ascertain the antagonistic or synergistic effects of the 12 monoterpenoids, the mortalities of *P. sinica* caused by these monoterpenoids, both individually and in binary mixtures, using the modified residual film method illustrated above. The two monoterpenoids were combined in a 1:1 ratio volume (concentration in LC_{25} values listed

in Table I). Each binary mixture was replicated 4 times and 20 adults of *P. sinica* for each replication. Expected mortalities of binary mixtures were calculated using the following equation (Hummelbrunner and Isman, 2001; Pavela, 2014, 2015):

$$E = O_a + O_b (1 - O_a)$$

where, E represents the expected mortality of binary mixtures; O_a and O_b are the observed mortalities of the pure monoterpenoid A or B in the binary mixtures of A and B at the given concentration.

The χ^2 comparisons analysis was utilized to designate the effects of binary mixtures as either antagonistic, additive, or synergistic, using the equation described below:

$$\chi^2 = (O_m - E)^2/E$$

where, O_m is the observed mortality of binary mixtures; E is the expected mortality; $\chi^2 = 3.84$ with df = 1 at $p = 0.05$. If $\chi^2 > 3.84$ and $O_m > E$, it was perceived as synergistic; if $\chi^2 > 3.84$ and $O_m < E$, it was perceived as antagonistic; if $\chi^2 \leq 3.84$, it represented additive effects.

Statistical analysis

Data are presented as mean ± standard deviation (SD). To compare the inhibitory rates of different monoterpenoids on AChE and GST activity, we used one-way analysis of variance (ANOVA) (Duncan's test) at $p < 0.05$. Pearson's correlation coefficient was used to analyze the relationship between the synergistic or antagonistic effects of monoterpenoids binary mixtures and AChE or GST inhibition rates using SPSS software.

RESULTS

Acute toxicity of 12 monoterpenoids

The variability in the toxicities of 12 monoterpenoids against *P. sinica* adults were shown in Table I. At the highest concentration tested (10 mL/L), 2-ethylimidazole, estragole, cuminyl alcohol, d-carvone, l-carvone, cuminaldehyde and dihydrocarvone caused 100% mortality, whereas β-caryophyllene resulted in 88.3% mortality. In contrast, 1,8-cineole, β-pinene, and α-pinene led to less than 50% mortality, with limonene resulting in only 5% mortality. Among these monoterpenoids, 2-ethylimidazole demonstrated the highest toxicity to *P. sinica* adults, with the lowest LC_{25}, LC_{50} and LC_{90} values. The LC_{50} values of estragole, cuminyl alcohol and d-carvone were 2.11, 2.17, and 2.28 mL/L, respectively, with no significant differences in their lethal activities. Similarly, l-carvone, cuminaldehyde, dihydrocarvone and β-caryophyllene exhibited LC_{50} values of 3.06, 3.17, 3.19, and 3.31 mL/L, respectively, with no significant variations in their lethal activities. As for β-pinene, 1,8-cineole, α-pinene and limonene, their LC_{50} values were estimated to be higher than 10 mL/L, as their mortality was less than 50% at the highest concentration tested (10 mL/L).

Table I. Toxicities of 12 monoterpenoids against *P. sinica* adults.

Monoterpenoid	Mortalities (%) at 10 mL/L	LC_{25} (CI_{95}) (mL/L)	LC_{50} (CI_{95}) (mL/L)	LC_{90} (CI_{95}) (mL/L)	Slope	χ^2/df
2-ethylimidazole	100.0 ± 0.0	0.24 (0.17–0.31)a	0.52 (0.41–0.66)a	2.31 (1.63–3.84)a	3.57	34.4/20
Estragole	100.0 ± 0.0	1.36 (1.16–1.55)b	2.11 (1.88–2.36)b	4.82 (4.05–6.15)bc	5.03	12.9/13
Cuminyl alcohol	100.0 ± 0.0	1.74 (1.56–1.89)c	2.17 (2.01–2.35)b	3.29 (2.94–3.95)a	7.08	18.3/13
D-carvone	100.0 ± 0.0	1.62 (1.39–1.82)c	2.28 (2.04–2.54)b	4.34 (3.73–5.40)b	3.66	43.3/22
L-carvone	100.0 ± 0.0	2.25 (2.01–2.46)d	3.06 (2.83–3.28)c	5.47 (4.97–6.21)c	5.07	19.6/18
Cuminaldehyde	100.0 ± 0.0	2.08 (1.63–2.45)d	3.17 (2.71–3.67)c	7.10 (5.79–9.73)e	4.57	19.9/13
Dihydrocarvone	100.0 ± 0.0	2.34 (2.11–2.55)d	3.19 (2.95–3.44)c	5.77 (5.15–6.61)c	2.46	9.6/16
β-caryophyllene	88.3 ± 2.9	1.76 (1.45–2.06)c	3.31 (2.89–3.79)c	11.00 (9.00–14.28)f	1.98	7.9/19
β-pinene	36.7 ± 5.8	8	>10	>10	-	-
1,8-cineole	16.7 ± 2.9	8	>10	>10	-	-
α-pinene	16.7 ± 7.6	8	>10	>10	-	-
Limonene	5.0 ± 5.0	8	>10	>10	-	-

Notes: Serial dilutions of each monoterpenoid were prepared in acetone to generate at least six concentrations (0.06, 0.12, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mL/L), except for 2-ethylimidazole (solid, with the unit of measurement being g/L). LC25, LC50, and LC90 represent the dose necessary to kill 25, 50 and 90% of *P. sinica* adults, respectively; CI_{95}: 95% confidence interval. Differences among LC values were considered significant when their 95% CI did not overlap by 50%. The lowercase letters in the same column represent significant differences.

The slope value indicated the difference of individual sensitivities of *P. sinica* population to the tested solutions. The larger the slope value, the lesser difference in individual sensitivities of the population (Hughes *et al*., 1984). The difference of individual sensitivities of *P. sinica* population to cuminyl alcohol were lesser than that to other monoterpenoids. The results indicated that 2-ethylimidazole was the most acutely toxic monoterpene to *P. sinica*.

In vitro inhibitory effects of 12 monoterpenoids on the activities of AChE and GST in P. sinica

In vitro experiments were conducted to examine the effects of 12 monoterpenoids on the activities of AChE and GST of *P. sinica*. The inhibition rates for AChE varied among the tested monoterpenoids, with estragole displaying the highest inhibition rate of 95.0%, followed by cuminaldehyde (91.4%) and 1,8-cineole (88.0%). The remaining monoterpenoids such as α-pinene (74.2%), d-carvone (66.9%), l-carvone (42.0%), and dihydrocarvone (35.2%) showed lower inhibition rates. Conversely, β-caryophyllene, cuminyl alcohol, 2-ethylimidazole, limonene and β-pinene demonstrated rates under 15% (Fig. 1). It can be inferred that the activity of AChE was significantly inhibited by estragole, cuminaldehyde, and 1,8-cineole.

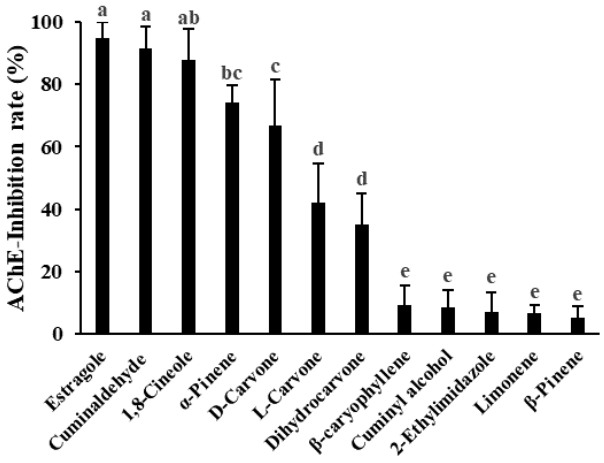

Fig. 1. Inhibition rates of 12 monoterpenoids to AChE activity of *P. sinica in vitro.*
Notes: The concentration of all 12 monoterpenoids tested was 20 mL/L. Data are presented as number or mean ± SD. Columns with the same letters on top are not significantly different based on One-way ANOVA at $p < 0.05$, Duncan's test.

Regarding the GST activities of *P. sinica*, 12 monoterpenoids also showed varied inhibition rates (Fig. 2). L-carvone exhibited the highest inhibition rate of 65.4%, followed by cuminaldehyde (58.8%), β-pinene (51.0%), dihydrocarvone (47.2%), 1,8-cineole (46.7%), cuminyl alcohol (41.7%), β-caryophyllene (41.6%), d-carvone (39.0%), α-pinene (35.9%), estragole (28.5%) 2-ethylimidazole (27.6%) and Limonene (22.6%). L-carvone and cuminaldehyde showed the highest GST inhibition activity.

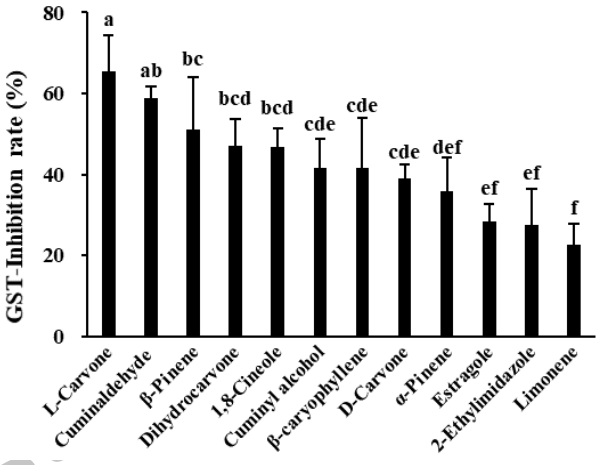

Fig. 2. Inhibition rates of 12 monoterpenoids to GST activity of *P. sinica in vitro* at the concentration of 20 mL/L.
Notes: Data are presented as number or mean ± SD. Columns with the same letters on top are not significantly different based on One-way ANOVA at $p < 0.05$, Duncan's test.

Toxicity of binary mixtures against P. sinica *adults*

A total of 66 binary mixtures were tested for their acute toxicity against *P. sinica* adults (Table II), of which 19 combinations exhibited strong synergistic effects, 21 showed significant antagonistic effects, and 26 displayed additive effects. A higher χ^2 value indicated a stronger synergistic or antagonistic effects. The most profound synergistic effects were observed with the following binary mixtures: dihydrocarvone and l-carvone (χ^2 = 113.6), l-carvone and cuminaldehyde (χ^2 = 108.3), l-carvone and cuminyl alcohol (χ^2 = 109.4), estragole and l-carvone (χ^2 = 112.3). While, limonene and dihydrocarvone (χ^2 = 16.8), α-pinene and dihydrocarvone (χ^2 = 15.4), α-pinene and estragole (χ^2= 19.5), β-pinene and cuminaldehyde (χ^2 = 18.1) exhibited the most profound antagonistic effects. The highest mortalities were achieved for the combinations: dihydrocarvone and l-carvone (98.4%), l-carvone and cuminaldehyde (96.5%), l-carvone and cuminyl alcohol (96.4%), estragole and l-carvone (93.3%), d-carvone and l-carvone (98.5%).

Table II. Effect of binary mixtures of 12 monoterpenoids prepared as LC_{25} combinations on mortality against *P. sinica*.

Monoterpenoid A	Monoterpenoid B	Mortality (%)				χ^2	Effects
		Pure monoterpenoids		Binary mixtures			
		Observed A	Observed B	Expected	Observed		
1,8-cineole	Limonene	3.6	1.8	5.4	9.2	2.7	Additive
1,8-cineole	α-pinene	3.6	5.5	9.0	26.3	33.6	Synergistic
1,8-cineole	β-pinene	3.6	13.7	16.9	9.2	3.5	Additive
1,8-cineole	Dihydrocarvone	3.6	20.8	23.7	17.7	1.5	Additive
1,8-cineole	Estragole	3.6	17.8	20.8	6.1	10.4	Antagonistic
1,8-cineole	D-carvone	3.6	33.6	36.0	28.2	1.7	Additive
1,8-cineole	L-carvone	3.6	18.2	21.1	23.3	0.2	Additive
1,8-cineole	Cuminaldehyde	3.6	20.5	23.4	15.5	2.7	Additive
1,8-cineole	Cuminyl alcohol	3.6	21.1	24.0	5.7	13.9	Antagonistic
1,8-cineole	2-ethylimidazole	3.6	18.4	21.4	5.7	11.6	Antagonistic
1,8-cineole	β-caryophyllene	3.6	17.2	20.2	12.5	3	Additivet
Limonene	α-pinene	1.8	5.5	7.2	13.3	5.2	Synergistic
Limonene	β-pinene	1.8	13.7	15.2	17.9	0.5	Additive
Limonene	Dihydrocarvone	1.8	20.8	22.2	2.9	16.8	Antagonistic
Limonene	Estragole	1.8	17.8	19.2	4.2	11.8	Antagonistic
Limonene	D-carvone	1.8	33.6	34.8	25	2.7	Additive
Limonene	L-carvone	1.8	18.2	19.6	23.3	0.7	Additive
Limonene	Cuminaldehyde	1.8	20.5	22.0	11.8	4.7	Antagonistic
Limonene	Cuminyl alcohol	1.8	21.1	22.5	6.5	11.5	Antagonistic
Limonene	2-ethylimidazole	1.8	18.4	19.9	13.3	2.2	Additive
Limonene	β-Caryophyllene	1.8	17.2	18.7	15.8	0.5	Additive
α-pinene	β-pinene	5.5	13.7	18.5	13.2	1.5	Additive
α-pinene	Dihydrocarvone	5.5	20.8	25.2	5.5	15.4	Antagonistic
α-pinene	Estragole	5.5	17.8	22.3	1.5	19.5	Antagonistic
α-pinene	D-carvone	5.5	33.6	37.3	27.9	2.3	Additive
α-pinene	L-carvone	5.5	18.2	22.7	23.1	0	Additive
α-pinene	Cuminaldehyde	5.5	20.5	24.9	15.4	3.6	Additive
α-pinene	Cuminyl alcohol	5.5	21.1	25.5	15	4.3	Antagonistic
α-pinene	2-ethylimidazole	5.5	18.4	22.9	30.3	2.4	Additive
α-pinene	β-caryophyllene	5.5	17.2	21.8	5.2	12.6	Antagonistic
β-pinene	Dihydrocarvone	13.7	20.8	31.7	25.1	1.4	Additive
β-pinene	Estragole	13.7	17.8	29	3.8	22	Antagonistic
β-pinene	D-carvone	13.7	33.6	42.7	45.1	0.1	Additive
β-pinene	L-carvone	13.7	18.2	29.4	47.5	11.2	Synergistic
β-pinene	Cuminaldehyde	13.7	20.5	31.4	7.6	18.1	Antagonistic
β-pinene	Cuminyl alcohol	13.7	21.1	31.9	40.1	2.1	Additive
β-pinene	2-ethylimidazole	13.7	18.4	29.6	11.9	10.6	Antagonistic

Table continued on next page.....

Monoterpenoid A	Monoterpenoid B	Mortality (%)				χ^2	Effects
		Pure monoterpenoids		Binary mixtures			
		Observed A	Observed B	Expected	Observed		
β-pinene	β-caryophyllene	13.7	17.2	28.6	30	0.1	Additive
Dihydrocarvone	Estragole	20.8	17.8	34.9	12.6	14.3	Antagonistic
Dihydrocarvone	D-carvone	20.8	33.6	47.4	84.6	29.1	Synergistic
Dihydrocarvone	L-carvone	20.8	18.2	35.2	98.4	113.6	Synergistic
Dihydrocarvone	Cuminaldehyde	20.8	20.5	37.1	80.4	50.5	Synergistic
Dihydrocarvone	Cuminyl alcohol	20.8	21.1	37.5	62.8	17	Synergistic
Dihydrocarvone	2-ethylimidazole	20.8	18.4	35.4	22.3	4.8	Antagonistic
Dihydrocarvone	β-caryophyllene	20.8	17.2	34.5	32.3	0.1	Additive
Estragole	D-carvone	17.8	33.6	45.4	71.8	15.4	Synergistic
Estragole	L-carvone	17.8	18.2	32.7	93.3	112.3	Synergistic
Estragole	Cuminaldehyde	17.8	20.5	34.7	79.0	56.6	Synergistic
Estragole	Cuminyl alcohol	17.8	21.1	35.1	52.9	9.0	Synergistic
Estragole	2-ethylimidazole	17.8	18.4	32.9	18.8	6.1	Antagonistic
Estragole	β-caryophyllene	17.8	17.2	31.9	12.0	12.4	Antagonistic
D-carvone	L-carvone	33.6	18.2	45.7	98.6	61.4	Synergistic
D-carvone	Cuminaldehyde	33.6	20.5	47.2	87.8	34.8	Synergistic
D-carvone	Cuminyl alcohol	33.6	21.1	47.6	70.0	10.5	Synergistic
D-carvone	2-ethylimidazole	33.6	18.4	45.8	33.2	3.5	Additive
D-carvone	β-caryophyllene	33.6	17.2	45.0	42.3	0.2	Additive
L-carvone	Cuminaldehyde	18.2	20.5	35.0	96.5	108.3	Synergistic
L-carvone	Cuminyl alcohol	18.2	21.1	35.4	96.4	104.9	Synergistic
L-carvone	2-ethylimidazole	18.2	18.4	33.2	55.7	15.1	Synergistic
L-carvone	β-caryophyllene	18.2	17.2	32.2	18	6.3	Antagonistic
Cuminaldehyde	Cuminyl alcohol	20.5	21.1	37.3	65.8	21.8	Synergistic
Cuminaldehyde	2-ethylimidazole	20.5	18.4	35.2	28.5	1.3	Additive
Cuminaldehyde	β-caryophyllene	20.5	17.2	34.2	31.9	0.2	Additive
Cuminyl alcohol	2-ethylimidazole	21.1	18.4	35.7	75.2	43.9	Synergistic
Cuminyl alcohol	β-caryophyllene	21.1	17.2	34.7	15.1	11.1	Antagonistic
2-ethylimidazole	β-caryophyllene	18.4	17.2	32.5	20.1	4.7	Antagonistic

L-carvone displayed a synergistic effect with 7 monoterpenoids, while cuminyl alcohol exhibited this effect with 6 monoterpenoids. Cuminaldehyde and d-carvone displayed synergistic effects with 5 monoterpenoids each, and dihydrocarvone and estragole with 4 monoterpenoids each. In contrast, α-pinene, 2-ethylimidazole, limonene, 1,8-cineole, and β-pinene exhibited limited synergistic effects. Estragole, on the other hand, demonstrated antagonistic effects in 7 combinations. The maximal synergic binary mixtures were dihydrocarvone and l-carvone, l-carvone and cuminaldehyde, l-carvone and cuminyl alcohol, d-carvone and l-carvone, and estragole and l-carvone, suggesting that L-carvone could be considered as the most effective synergist (Table III).

Correlation between the number of synergistic or antagonistic binary mixtures and AChE or GST activities

The five compounds, namely l-carvone, cuminaldehyde, β-pinene, 1,8-cineole, and cuminaldehyde, demonstrated a relatively high degree of inhibition against GST activity of *P. sinica in vitro*, and resulted in fewer antagonistic binary mixtures against *P. sinica* mortality. This indicates that there may be a correlation between the number of antagonistic binary mixtures and the GST inhibition ability. Pearson correlation analysis revealed a negative correlation trend between the GST inhibition rate

Table III. The number of monoterpenoids reaching the level of being a synergist or antagonist.

Monoterpenoid	Synergist[a]	Antagonist[a]	Additive[a]	Maximal synergic effect[b]
L-carvone	7	1	3	Dihydrocarvone
Cuminyl alcohol	6	4	1	L-carvone
D-carvone	5	0	6	L-carvone
Cuminaldehyde	5	2	4	L-carvone
Dihydrocarvone	4	4	3	L-carvone
Estragole	4	7	0	L-carvone
α-pinene	2	4	5	1,8-cineole
2-ethylimidazole	2	5	4	Cuminyl alcohol
1,8-cineole	1	3	7	α-pinene
β-pinene	1	3	7	L-carvone
Limonene	1	4	6	α-pinene
β-caryophyllene	0	5	6	-

[a] The number of the monoterpenoid creating the synergistic, antagonistic, or additive effect on mortality of *P. sinica*; [b] Monoterpenoid with which was achieved most significant synergism.

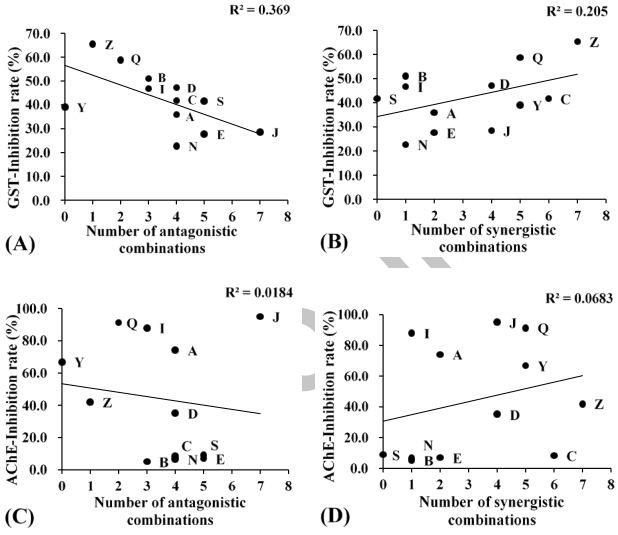

Fig. 3. Correlation analysis between AChE or GST inhibition rates and the number of synergistic or antagonistic combinations

Data analyzed using Pearson correlation coefficient at a significance level of $p < 0.05$. Z, L-carvone; C, Cuminyl alcohol; Y, D-carvone; Q, Cuminaldehyde; D, Dihydrocarvone; J, Estragole; A, α-pinene; E, 2-ethylimidazole; I, 1,8-cineole; B, β-pinene; N, Limonene; S, β-caryophyllene.

and the number of antagonistic binary mixtures (Pearson coefficient= -0.608, R^2= 0.369, p= 0.036, Fig. 3A). However, no significant relationship was found between the GST inhibition rates and the number of synergistic binary mixtures (Pearson coefficient = 0.453, R^2 = 0.205, p = 0.140, Fig. 3B). Similarly, no significant correlation was observed between the AChE inhibition rates and the number of synergistic or antagonistic binary mixtures (Pearson coefficient = −0.136, R^2 = 0.018, p =0.412, Fig. 3C; Pearson coefficient = 0.261, R^2 = 0.068, p = 0.674, Fig. 3D).

In brief, the inhibition of GST activity in *P. sinica* by certain compounds was found to be negatively correlated with the number of antagonistic binary mixtures, while no significant correlation was observed between AChE inhibition and the number of synergistic or antagonistic binary mixtures.

DISCUSSION

In this study, the toxicity of 12 monoterpenoids was tested against *P. sinica* adults, and the results showed that 2-ethylimidazole had the highest toxicity with the lowest LC_{25}, LC_{50} and LC_{90} values. However, there was limited reports on its insecticidal activity, and further research is needed to determine its pesticide effects and mechanisms. Cuminyl alcohol, estragole, and d-carvone also demonstrated better toxicity against *P. sinica* with low LC_{50} and LC_{90} values. Other monoterpenoids, such l-carvone, cuminaldehyde, dihydrocarvone and β-caryophyllene were also efficient at eliminating *P. sinica*. In contrast, Limonene, α-pinene, β-pinene, and 1,8-cineole showed low mortality, even at the highest tested concentration of 10 mL/L.

It was noted that d-carvone and cuminyl alcohol were more virulent than l-carvone and cuminaldehyde, respectively. This suggests that the molecular structure of these substances may influence their toxicity. However, the relationship between the efficacy of individual substances and their molecular structures was difficulty to define (Pavela, 2015). Previous studies have shown that lipophilicity influenced the insecticidal activity of lipophilic compounds through enzyme inhibition (Ryan and Byrne, 1988; Santos *et al.*, 2010). For example, thymol and carvacrol, which have lipophilic CH chains outside a phenyl ring, displayed higher larvicidal activity against than *Aedes aegypti* larvae than phenol alone (Santos *et al.*, 2010). Exocyclic double bonds have also been found to influence the toxicity of α-pinene and β-pinene to larvae of *A. aegypti* (Lucia *et al.*, 2007; Perumalsamy *et al.*, 2010; Simas *et al.*, 2007).

The insecticidal activities of essential oils and their constituents also depends on the type of insects. Previous study has shown that cuminaldehyde, cuminyl alcohol,

1,8-cineole, limonene, and β-caryophyllene were toxic to *Stomoxys calcitrans*, with cuminaldehyde being the most effective (Hieu *et al*., 2012). However, neither 1,8-cineole nor β-caryophyllene showed insecticidal activity against *P. sinica* in our study. Similarly, the toxicities of essential oils and their constituents against different insects varied. For instance, Yeom *et al*. (2015) found that estragole was more effective than β-caryophyllene against German cockroach (*Blattella germanica*), and our work also revealed that estragole outperformed β-caryophyllene in controlling *P. sinica.* In contrast, l-carvone demonstrated significant toxicity against *S. oryzae*, *Rhyzopertha dominica* adults and *Tribolium castaneum* adults in a previous study (Tripathi *et al*., 2003), but it was not effective against *P. sinica* in our work.

Previous research has shown that mixing essential oil components or chemical pesticides may result in synergistic, antagonistic, or additive effects (Wu *et al*., 2017). Synergistic effects of complex mixtures was important to standardize the formulations of insecticides (Akram *et al*., 2023; Jabbar *et al*., 2022), especially to essential oil insecticides. In our study, 66 binary mixtures were tested, of which 19 resulted in a synergistic effect, and 21 had an antagonistic effect on *P. sinica* mortality. Notably, despite using concentrations that matched the estimated LC_{25} in the tests, pure monoterpenoids often caused significantly lower mortality than expected (25%), but when combined, some mixtures created up to 95% mortality. This phenomenon was observed in 5 binary mixtures: l-carvone and cuminaldehyde, l-carvone and cuminyl alcohol, dihydrocarvone and l-carvone, estragole and l-carvone, d-carvone and l-carvone. Previous studies have also explored the potential for synergistic effects of essential oil components. Pavela (2015) assessed the acute toxicity of 30 aromatic compounds and their binary combinations against *Culex quinquefasciatus* larvae, and found 249 combinations showing significant synergistic effect. The mixture of limonene and transanethole caused the highest mortality, and l-carvone had a synergistic effect with 24 out of the 30 tested compounds. In addition, a study testing binary combinations of 6 monoterpenoids against *Musca domestica* found that p-cymene mixed with γ-terpinene, carvacrol and 1,8-cineole resulted in the most significant synergistic effect (Pavela, 2008). Our study confirmed that l-carvone, with low toxicity, created a synergistic effect with 7 monoterpenoids and an antagonistic effect with 1 monoterpenoid. Comparatively, 2-ethylimidazole, with high toxicity, was found to be antagonized with 5 monoterpenoids and synergized with 2 monoterpenoids.

Inhibition of AChE activity may be a mechanism for causing insect death. Previous studies have shown that the oil extract of *Acalypha wilkesiana* inhibited AChE activity in adult *Callosobruchus maculatus* (Oni *et al*., 2019), estragole inhibited AChE activity in *Tribolium castaneum* (Olmedo *et al*., 2015), and cuminaldehyde, limonene and 1,8-cineole inhibited AChE activity in *S. oryzae* (Abdelgaleil *et al*., 2009). Our study confirmed that estragole, cuminaldehyde, and 1,8-cineole inhibited AChE activity in *P. sinica*, although their insecticidal activities against *P. sinica* was weak. This is similar to the research reported by Kim *et al*. (2013), in which α-pinene showed the highest inhibition rate (97.36%) of AChE activity in *S. oryzae* but had low toxicity. We found that 2-ethylimidazole and cuminyl alcohol had the best toxicity to *P. sinica,* but had almost no effect on AChE activity *in vitro*, implying that AChE was not the target for 2-ethylimidazole or cuminyl alcohol. Furthermore, some essential oils also have inhibitory effects on insect GST activity. For instance, the essential oils of *Acalypha wilkesiana* significantly reduced GST activity in *Callosobrunchus maculatus* (Oni *et al*., 2019). The geranoil, linalool, citral, and 3-carene cause a significant reduction of GST activity in *Sitophilus zeamais* and *Callosobrunchus maculatus* (Oyedeji *et al*., 2020). Our results showed that 12 monoterpenoids tested had inhibitory effects on GST activity in *P. sinica*.

The synergistic effect of synergists is typically attributed to the inhibition of detoxification enzymes (Churcher *et al*., 2016; Shen *et al*., 2016). One substance's ability to inhibit GST activity may protect other toxins from degradation by GST (Metcalf, 1967). Degradation of toxins through multiple detoxification pathways in insects may decrease the antagonism of binary mixtures (Bernard and Philogene, 1993; Ishaaya, 1993). Piperonyl butoxide, a well-known inhibitor of cytochrome P450 monooxygenases and esterases, has a synergistic effect on chlorpyrifos, methomyl, acetamiprid, and spirotetramat by inhibiting the activity of cytochrome P450 monooxygenase (Ullah *et al*., 2017). In this study, it seemed that the higher the GST inhibitory activity of the monoterpenoid, the fewer antagonistic binary mixtures it created. However, the synergistic or antagonistic mechanisms of these monoterpenoids still require further study.

CONCLUSION

Two monoterpenoids, 2-ethylimidazole and cuminyl alcohol, showed high toxicity against *P. sinica.* L-carvone was identified as the best synergist, and the maximal synergic binary mixtures were: l-carvone and dihydrocarvone, l-carvone and cuminaldehyde, l-carvone and cuminyl alcohol, l-carvone and d-carvone, l-carvone and estragole. These binary mixtures may have the potential to be used as effective control agents against *P.*

sinica. The findings of this study provide valuable insights into potential control strategies for prevention and control of *P. sinica*.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No.31360182).

IRB statement

Not applicable, the study did not involve humans or animals.

Ethical statement

Not applicable.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdelfattah, E.M., Vezzoli, G., Buczkowski, G. and Makagon, M.M., 2018. Essential oils: Effects of application rate and modality on potential for combating northern fowl mite infestations. *Med. Vet. Ent.*, **32**: 304-310. https://doi.org/10.1111/mve.12300

Abdelgaleil, S.A., Mohamed, M.I., Badawy, M.E. and El-arami, S.A., 2009. Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. *J. chem. Ecol.*, **35**: 518-525. https://doi.org/10.1007/s10886-009-9635-3

Akram, S., Butt, A. and Khan, S.A., 2023. Toxicity of imidacloprid administered alone and in combination with heavy metal lead on silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Pakistan J. Zool.,* **54**: 165-171. https://doi.org/10.17582/journal.pjz/20210623070602

Bernard, C.B. and Philogene, B.J., 1993. Insecticide synergists: Role, importance, and perspectives. *J. Toxicol. environ. Hlth.*, **38**: 199-223. https://doi.org/10.1080/15287399309531712

Bisrat, D., and Jung, C., 2020. Insecticidal toxicities of three main constituents derived from *Trachyspermum ammi* (L.) Sprague ex Turrill fruits against the small hive beetles, *Aethina tumida* Murray. *Molecules*, **25**: 1100. https://doi.org/10.3390/molecules25051100

Churcher, T.S., Lissenden, N., Griffin, J.T., Worrall, E. and Ranson, H., 2016. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. *Elife*, **5**: e16090. https://doi.org/10.7554/eLife.16090

Cisse, M.B.M., Sangare, D., Oxborough, R.M., Dicko, A., Dengela, D., Sadou, A., Mihigo, J., George, K., Norris, L. and Fornadel, C., 2017. A village level cluster-randomized entomological evaluation of combination long-lasting insecticidal nets containing pyrethroid plus PBO synergist in Southern Mali. *Malar. J.*, **16**: 477. https://doi.org/10.1186/s12936-017-2124-1

Ebadollahi, A., Jalali, S.J., Ziaee, M. and Krutmuang, P., 2021. Acaricidal, insecticidal, and nematicidal efficiency of essential oils isolated from the *Satureja* genus. *Int. J. environ. Res. Publ. Hlth.*, **18**: 6050. https://doi.org/10.3390/ijerph18116050

Ebling, P.M., Otvos, I.S. and Conder, N., 2004. Comparative activity of three isolates of LdMNPV against two strains of *Lymantria dispar*. *Can. Entomol.*, **136**: 737-747. https://doi.org/10.4039/n04-012

Ellman, G.L., Courtney, K.D., Andres Jr, V. and Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**: 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

Hennia, A., Nemmiche, S., Dandlen, S. and Miguel, M.G., 2019. *Myrtus communis* essential oils: Insecticidal, antioxidant and antimicrobial activities: A review. *J. Essent. Oil Res.*, **31**: 487-545. https://doi.org/10.1080/10412905.2019.1611672

Hieu, T.T., Kim, S.I. and Ahn, Y.J., 2012. Toxicity of *Zanthoxylum piperitum* and *Zanthoxylum armatum* oil constituents and related compounds to *Stomoxys calcitrans* (Diptera: Muscidae). *J. med. Ent.*, **49**: 1084-1091. https://doi.org/10.1603/ME12047

Hodgson, E. and Levi, P.E., 1996. Pesticides: An important but underused model for the environmental health sciences. *Environ. Hlth. Perspect.*, **104**: 97-106. https://doi.org/10.1289/ehp.96104s197

Hughes, P.R., Wood, H.A., Burand, J.P. and Granados, R.R., 1984. Quantification of the dose-mortality response of *Trichoplusia ni*, *Helioithis zea*, and *Spodoptera frugiperda* to nuclear polyhedrosis viruses: Applicability of an exponential model. *J. Invertebr. Pathol.*, **43**: 343-350. https://doi.org/10.1016/0022-2011(84)90079-X

Hummelbrunner, L.A. and Isman, M.B., 2001. Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep.,

Noctuidae). *J. Agric. Fd. Chem.*, **49**: 715-720. https://doi.org/10.1021/jf000749t

Hung, N.H., Satyal, P., Hieu, H.V., Chuong, N.T.H., Dai, D.N., Huong, L.T., Tai, T.A. and Setzer, W.N., 2019. Mosquito larvicidal activity of the essential oils of *Erechtites* species growing wild in Vietnam. *Insects*, **10**: 47. https://doi.org/10.3390/insects10020047

Ishaaya, I., 1993. Insect detoxifying enzymes: Their importance in pesticide synergism and resistance. *Arch. Insect Biochem. Physiol.*, **22**: 263-276. https://doi.org/10.1002/arch.940220119

Isman, M.B., and Grieneisen, M.L., 2014. Botanical insecticide research: Many publications, limited useful data. *Trends Pl. Sci.*, **19**: 140-145. https://doi.org/10.1016/j.tplants.2013.11.005

Jabbar, A., Tariq, M., Gulzar, A., Mukhtar, T. and Zainab, T., 2022. Lethal and sub lethal effects of plant extracts and green silver nanoparticles against *Culex pipiens*. *Pakistan J. Zool.,* **54**: 1259-1267. https://doi.org/10.17582/journal.pjz/20191226161232

Kim, S.W., Kang, J. and Park, I.K., 2013. Fumigant toxicity of Apiaceae essential oils and their constituents against *Sitophilus oryzae* and their acetylcholinesterase inhibitory activity. *J. Asia Pac. Ent.*, **16**: 443-448. https://doi.org/10.1016/j.aspen.2013.07.002

Li, L., Gao, X., Lan, M., Yuan, Y., Guo, Z., Tang, P., Li, M., Liao, X., Zhu, J., Li, Z., Ye, M. and Wu, G., 2019. *De novo* transcriptome analysis and identification of genes associated with immunity, detoxification and energy metabolism from the fat body of the tephritid gall fly, *Procecidochares utilis*. *PLoS One*, **14**: e0226039. https://doi.org/10.1371/journal.pone.0226039

López, M.D. and Pascual-Villalobos, M.J., 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Ind. Crop Prod.*, **31**: 284-288. https://doi.org/10.1016/j.indcrop.2009.11.005

Lucia, A., Audino, P.G., Seccacini, E., Licastro, S., Zerba, E. and Masuh, H., 2007. Larvicidal effect of *Eucalyptus grandis* essential oil and turpentine and their major components on *Aedes aegypti* larvae. *J. Am. Mosq. Contr. Assoc.*, **23**: 299-303. https://doi.org/10.2987/8756-971X(2007)23[299:LEOEGE]2.0.CO;2

Metcalf, R.L., 1967. Mode of action of insecticide synergists. *Annu. Rev. Ent.*, **12**: 229-256. https://doi.org/10.1146/annurev.en.12.010167.001305

Olmedo, R., Herrera, J.M., Lucini, E.I., Zunino, M.P., Pizzolitto, R.P., Dambolena, J.S. and Zygadlo, J.A., 2015. Essential oil of *Tagetes filifolia* against the flour beetle *Tribolium castaneum* and its relation to acetylcholinesterase activity and lipid peroxidation. *Agriscientia*, **32**: 113-121. https://doi.org/10.31047/1668.298x.v32.n2.16562

Oni, M.O., Ogungbite, O.C., Oguntuase, S.O., Bamidele, O.S. and Ofuya, T.I., 2019. Inhibitory effects of oil extract of green Acalypha (*Acalypha wilkesiana*) on antioxidant and neurotransmitter enzymes in *Callosobruchus maculatus*. *J. Basic appl. Zool.*, **80**: 47. https://doi.org/10.1186/s41936-019-0116-0

Oyedeji, A.O., Okunowo, W.O., Osuntoki, A.A., Olabode, T.B. and Ayo-Folorunso, F., 2020. Insecticidal and biochemical activity of essential oil from *Citrus sinensis* peel and constituents on *Callosobrunchus maculatus* and *Sitophilus zeamais*. *Pestic. Biochem. Physiol.*, **168**: 104643. https://doi.org/10.1016/j.pestbp.2020.104643

Pavela, R., 2008. Acute and synergistic effects of some monoterpenoid essential oil compounds on the house fly (*Musca domestica* L.). *J. Essent. Oil Bear. Pl.*, **11**: 451-459. https://doi.org/10.1080/09720560X.2008.10643653

Pavela, R., 2014. Acute, synergistic and antagonistic effects of some aromatic compounds on the *Spodoptera littoralis* Boisd. (Lep., Noctuidae) larvae. *Ind. Crops Prod.*, **60**: 247-258. https://doi.org/10.1016/j.indcrop.2014.06.030

Pavela, R., 2015. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. *Parasitol. Res.*, **114**: 3835-3853. https://doi.org/10.1007/s00436-015-4614-9

Perumalsamy, H., Chang, K.S., Park, C. and Ahn, Y.J., 2010. Larvicidal activity of *Asarum heterotropoides* root constituents against insecticide susceptible and resistant *Culex pipiens pallens* and *Aedes aegypti* and *Ochlerotatus togoi*. *J. Agric. Fd. Chem.*, **58**: 10001-10006. https://doi.org/10.1021/jf102193k

Piccoli, B.C., Segatto, A.L.A., Oliveira, C.S., D'Avila da Silva, F., Aschner, M. and da Rocha, J.B.T., 2019. Simultaneous exposure to vinylcyclohexene and methylmercury in *Drosophila melanogaster*: Biochemical and molecular analyses. *BMC Pharmacol. Toxicol.*, **20**: 83. https://doi.org/10.1186/s40360-019-0356-0

Ranson, H., Abdallah, H., Badolo, A., Guelbeogo, W.M., Kerah-Hinzoumbé, C., Yangalbé-Kalnoné, E., Sagnon, N., Simard, F. and Coetzee, M., 2009. Insecticide resistance in *Anopheles gambiae*:

Data from the first year of a multi-country study highlight the extent of the problem. *Malar. J.*, **8**: 299. https://doi.org/10.1186/1475-2875-8-299

Regnault-Roger, C., Vincent, C. and Arnason, J.T., 2012. Essential oils in insect control: Low-risk products in a high-stakes world. *Annu. Rev. Ent.*, **57**: 405-424. https://doi.org/10.1146/annurev-ento-120710-100554

Ryan, M.F. and Byrne, O., 1988. Plant-insect coevolution and inhibition of acetylcholinesterase. *J. chem. Ecol.*, **14**: 1965-1975. https://doi.org/10.1007/BF01013489

Said-Al Ahl, H.A., Hikal, W.M. and Tkachenko, K.G., 2017. Essential oils with potential as insecticidal agents: A review. *Int. J. environ. Plan. Manag.*, **3**: 23-33.

Santos, S.R., Silva, V.B., Melo, M.A., Barbosa, J.D., Santos, R.L., de Sousa, D.P. and Cavalcanti, S.C., 2010. Toxic effects on and structure toxicity relationships of phenylpropanoids, terpenes, and related compounds in *Aedes aegypti* larvae. *Vector Borne Zoonotic Dis.*, **10**: 1049-1054. https://doi.org/10.1089/vbz.2009.0158

Seo, S.M., Jung, C.S., Kang, J., Lee, H.R., Kim, S.W., Hyun, J. and Park, I.K., 2015. Larvicidal and acetylcholinesterase inhibitory activities of Apiaceae plant essential oils and their constituents against *Aedes albopictus* and formulation development. *J. Agric. Fd. Chem.*, **63**: 9977-9986. https://doi.org/10.1021/acs.jafc.5b03586

Shen, X.M., Liao, C.Y., Lu, X.P., Wang, Z., Wang, J.J. and Dou, W., 2016. Involvement of three esterase genes from *Panonychus citri* (McGregor) in fenpropathrin resistance. *Int. J. mol. Sci.*, **17**: 1361. https://doi.org/10.3390/ijms17081361

Shotkoski, F.A., Mayo, Z.B., and Peters, L.L., 1990. Induced disulfoton resistance in greenbugs (Homoptera: Aphididea). *J. econ. Ent.*, **83**: 2147-2152. https://doi.org/10.1093/jee/83.6.2147

Shufran, R.A., Wilde, G.E. and Sloderbeck, P.E., 1997. Response of three greenbug (Homoptera: Aphididae) strains to five organophosphorous and two carbamate insecticides. *J. econ. Ent.*, **90**: 283-286. https://doi.org/10.1093/jee/90.2.283

Simas, N.K., da Costa Lima, E., Kuster, R.M., Lage, C.L.S. and de Oliveira Filho, A.M., 2007. Potential use of *Piper nigrum* ethanol extract against pyrethroid-resistant *Aedes aegypti* larvae. *Rev. Soc. Bras. Med. Trop.*, **40**: 405-407. https://doi.org/10.1590/S0037-86822007000400006

Singh, B., Singh, P.R. and Mohanty, M.K., 2012. Toxicity of a plant-based mosquito repellent/killer. *Interdiscip. Toxicol.*, **5**: 184-191. https://doi.org/10.2478/v10102-012-0031-4

Sutthanont, N., Choochote, W., Tuetun, B., Junkum, A., Jitpakdi, A., Chaithong, U., Riyong, D. and Pitasawat, B., 2010. Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of *Aedes aegypti* (Diptera: Culicidae). *J. Vector Ecol.*, **35**: 106-115. https://doi.org/10.1111/j.1948-7134.2010.00066.x

Tripathi, A.K., Prajapati, V. and Kumar, S., 2003. Bioactivities of l-carvone, d-carvone, and dihydrocarvone toward three stored product beetles. *J. econ. Ent.*, **96**: 1594-1601. https://doi.org/10.1093/jee/96.5.1594

Ullah, S., Ejaz, M. and Ali Shad, S., 2017. Study of synergism, antagonism, and resistance mechanisms in insecticide-resistant *Oxycarenus hyalinipennis* (Hemiptera: Lygaeidae). *J. econ. Ent.*, **110**: 615-623. https://doi.org/10.1093/jee/tow302

Wu, L., Huo, X., Zhou, X., Zhao, D., He, W., Liu, S., Liu, H., Feng, T. and Wang, C., 2017. Acaricidal activity and synergistic effect of thyme oil constituents against carmine spider mite (*Tetranychus cinnabarinus* (Boisduval)). *Molecules*, **22**: 1873. https://doi.org/10.3390/molecules22111873

Yeom, H.J., Jung, C.S., Kang, J., Kim, J., Lee, J.H., Kim, D.S., Kim, H.S., Park, P.S., Kang, K.S. and Park, I.K., 2015. Insecticidal and acetylcholine esterase inhibition activity of Asteraceae plant essential oils and their constituents against adults of the German cockroach (*Blattella germanica*). *J. agric. Fd. Chem.*, **63**: 2241-2248. https://doi.org/10.1021/jf505927n