Aphicidal Activity of *Illicium verum* Fruit Extracts and Their Effects on the Acetylcholinesterase and Glutathione S-transferases Activities in *Myzus persicae* (Hemiptera: Aphididae)

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Abstract

This study aims to explore the aphicidal activity and underlying mechanism of *Illicium verum* Hook. f. that is used as both food and medicine. The contact toxicity of the extracts from *I. verum* fruit with methyl alcohol (MA), ethyl acetate (EA), and petroleum ether (PE) against *Myzus persicae* (Sulzer), and the activities of acetylcholinesterase (AChE) and glutathione S-transferases (GSTs) of *M. persicae* after contact treatment were tested. The results showed that MA, EA, and PE extracts of 1.000 mg/l caused, respectively, *M. persicae* mortalities of 68.93%, 89.95% and 74.46%, and the LC_{50} of MA, EA, and PE extracts were 0.31, 0.14 and 0.27 mg/l at 72 h after treatment, respectively; the activities of AChE and GSTs in *M. persicae* were obviously inhibited by the three extracts, as compared with the control, with strong dose and time-dependent effects, the inhibition rates on the whole reached more than 50.00% at the concentration of 1.000 mg/l at 72 h after treatment. The inhibition of the extracts on AChE and GSTs activities (EA extract > PE extract > MA extract) were correlated with theirs contact toxic effects, so it is inferred that the decline of the metabolic enzymes activities may be one of important reasons of *M. persicae* death. The study results suggested that *I. verum* extracts have potential as a eco-friendly biopesticide in integrated pest management against *M. persicae*.

Key words: botanical insecticide, contact toxcity, insecticidal mechanism, acetylcholinesterase, glutathione S-transferase

Introduction

Myzus persicae (Sulzer) (Hemiptera: Aphididae), which is also known as the green peach or peach-potato aphid, is an economically significant pest found in a wide range of field crops and ornamentals worldwide. *M. persicae* is highly polyphagous and attacks hundreds of species from more than fifty plant families, including agroindustrial crops (potato, sugar beets, and tobacco), horticultural crops (plants of Brassicaceae, Solanaceae, and Cucurbitaceae families), and stone fruits (peach, apricot, and cherry). *M. persicae* also transmits more than a hundred viral diseases to more than 400 host plants (Gaspari et al. 2007, Kasprowicz et al. 2008), these viral diseases are responsible for direct and indirect damages that cause significant economic losses.

Control measures against *M. persicae* populations around the world heavily rely on the continued and repeated use of synthetic insecticides (Liu et al. 2007, Rattan 2010). Neonicotinoids, such as imidacloprid, clothianidin, and thiamethoxam, are currently the main class of insecticides used for *M. persicae* control. However, studies have reported varying levels of resistance against imidacloprid in *M. persicae* from Europe, USA, Japan, and China (Nauen and Denholm 2005, Margaritopoulos et al. 2007, Foster et al. 2008), which presents a threat to the long-term efficacy of this insecticide class. Insecticides that can be used specifically against *M. persicae* control are limited, and the frequent use of systemic insecticides to manage insect pests has led to the destabilization of the ecosystem. Thus, alternatives are clearly needed.

Botanicals containing active insecticidal phytochemicals provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages, such as high selectivity, little or no harmful effect on nontarget organisms, rapid degradation, low residual, and minimal cross-

resistance because of their natural complex agents and novel modes of action against insects (Isman 2006, Siskos et al. 2009, Mann et al. 2012, Chae et al. 2014). Furthermore, an increasing number of reports on the negative environmental and health impact of synthetic insecticides and the increasingly stringent environmental regulation of pesticides have resulted in renewed interest in the development and use of natural products from plants, which are rich sources of bioactive chemicals (Nukenine et al. 2010, Huang et al. 2011, Isman, 2014a,b).

Illicium verum Hook. f. (Austrobaileyales: Schisandraceae), which is more commonly known as star anise, is a medium-sized evergreen tree native to southwest China. *I. verum* is widely cultivated in the tropical and subtropical areas of Asia and is used as both food and medicine according to a publication made by the Ministry of Health of the People's Republic of China on March 2002, thereby implying the low or nontoxicity of *I. verum* to humans (Li et al. 2013). Previous studies on *I. verum* have mainly focused on its applications in food and medicine (Ohira et al. 2009, Yang et al. 2010), but a few studies have demonstrated that the essential oil of *I. verum* is biologically active and can be used to control *Sitophilus zeamais* (Ho et al. 1995), *Blattella germanica* (Chang and Ahn 2002), *Lasioderma serricorne*, *Callosobruchus chinensis* (Kim et al. 2003), *Aedes aegypti* (Dana and Wej 2006), and *Culex pipiens* (Kimbaris et al. 2012).

An important aspect of insecticide toxicology is the elucidation of insecticidal mechanisms by determining the activities of detoxifying enzymes after insecticides have entered the target insects (Vanhaelen et al. 2001, Francis et al. 2005). Acetylcholinesterase (AChE; EC 3.1.1.7) is widely found in insect nervous systems and is the target of organophosphate and carbamate insecticides. AChE is an important enzyme during the excitation phase of nerve conduction in the insect body. Target pests die when AChE activity in vivo is inhibited to a particular extent (Ramsey et al. 2010, Li et al. 2013). Glutathione S-transferases (GSTs; EC 2.5.1.18) has an important function in the detoxification of many endogenous and exogenous toxic substances, including allelochemicals from plants. GSTs also catalyze the conjugation reaction of endogenous glutathione (GSH) and electrophilic compounds (Francis et al. 2005). AChE and GSTs generally have key functions in the interaction between insects and host plants or chemical pesticides. Studies on enzyme inhibition significantly enhance the current understanding of enzyme specificity and the mechanisms by which enzymes and toxic agents work (Li et al. 2007, Zhang et al. 2012).

At present, chemical composition and biological activity of *I. verum* fruit extracts against *S. zeamais* adults have been studied (Li et al. 2014). In the present study, the contact toxicity of *I. verum* fruit extracts with different solvents against adult *M. persicae* and their effects on the activities of two important enzymes (AChE and GSTs) were tested to explore the aphicidal potential and underlying mechanism of the extracts. The results of this study would be useful for future applications of the indigenous plant source *I. verum* to control aphid pest.

Materials and Methods

Aphids

Experiments were conducted using 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

$(45 \times 45 \times 50 \ cm^3)$ maintained at a controlled temperature $(22 \pm 2°C)$ under light and dark (LD) photoperiod of 16 and 8 h, respectively, with $50 \pm 5\%$ relative humidity (RH). Identical-sized wingless adult aphids used in the experiments were collected from the cabbage on April 2013.

Plant Material

The *I. verum* fruits from Guangxi (China) were purchased from a local supermarket in Hefei, China.

Extract Preparation

I. verum fruits were dried in an oven (GRX–9071B; Yiheng Scientific Instruments Co. Ltd., Shanghai, China) at 40°C for 2 d, ground into powder by using an electric grinding mill (DD–120B; Linda Machinery Co. Ltd., Zhejiang, China), and sifted through a 40 mesh sieve. The dry powder (150 g) was placed into a 1.0 liter 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 then filtered (Whatman No. 2, Whatman Inc., Clifton, NJ, USA). The samples were leached twice via the above same procedure. The final filtrates were collected from each solvent to obtain the crude extracts. The combined filtrate was dried and concentrated using a vacuum rotary evaporator (Buchi rotavapor R–124; Flawil, Switzerland) then weighed using an electronic balance (FA2104; Hangping Co., Shanghai, China). All samples were stored in air-tight brown bottles at 4°C in a refrigerator until needed.

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 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) by using 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 $25 \pm 1°C$, $75 \pm 5\%$ RH, and an LD photoperiod of 14:10. The mortality in each treatment 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 treated with 1:4 (v:v) of acetone aqueous solution only. All treatments and control experiments were replicated six times.

Determination of Enzyme Activity

Based on the method used to perform the contact toxicity assay, approximately 300 aptery adult aphids were collected from the cabbage leaves and placed on a nylon yarn net. The nylon yarn nets with aphids were then immersed in different dilutions with concentrations of 0.063, 0.125, 0.250, 0.500, and 1.000 mg/l for 5 s and sampled 24 h after treatment to create the first set of enzyme extracts. Subsequently, another set of test insects was treated with the respective lethal concentration LC_{50} after 72 h of treatment (the LC_{50} values of the MA, EA, and PE extracts were 0.31, 0.14, and

0.27 mg/l, respectively) and sampled at 12, 24, 48, 60, and 72 h after treatment to create the second set of enzyme extracts.

All test insects were accurately weighed and rinsed twice or thrice with physiological saline. The insects were then placed in physiological saline with a mass-to-volume ratio of 10% and then homogenated. The supernatant was separated from the homogenate by using a refrigerated centrifuge (TGL–18R, Hema Medical Instruments Co., Ltd., Zhuhai, China) at 4°C and 3500 rpm for 10 min. The supernatant was used in the subsequent enzyme assays. The enzyme samples were stored at –70°C in an ultra-low temperature freezer (Sanyo, Tokyo, Japan). The enzyme extraction step was performed in an ice bath, and the physiological saline and homogenizer were precooled before each experiment to prevent enzyme inactivation.

The total protein content was determined using Coomassie brilliant blue. The activities of AChE and GSTs were measured according to the instructions of the AChE and GSTs detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with a DU730 spectrophotometer (Beckman Coulter, Brea, CA, USA). Each treatment was performed thrice.

Statistical Analysis

Treatment mortality rates were corrected using the mortality rate of the control sample (Abbott 1925). The mean values and standard errors of enzyme activities were counted using the DPS software (Tang and Feng 2007), and the means of corrected mortalities and of enzyme activities were compared using 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 the analysis of variance (ANOVA). The relationship between mortality and concentration was modeled using the DPS program

(Tang and Feng 2007). Untransformed data were presented as mean ± SE.

Results

Contact Toxicity

The results of the contact toxicity experiment indicated that the *I. verum* fruit extract in different solvents were highly toxic to *M. persicae* adults (Table 1). The contact toxicity effects of the extract in three solvents were distinctly enhanced by increasing concentrations. The MA, EA, and PE extracts at the highest concentration 1.000 mg/l caused *M. Persicae* mortalities of 68.93%, 89.95%, and 74.46% at 72 h after treatment, respectively.

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 the treatment concentrations and the arcsine square-root values of the mortalities. The LC_{50} values of the MA, EA, and PE extracts were 0.31, 0.14, and 0.27 mg/l, respectively, 72 h after treatment (Table 2). Therefore, the highest contact toxicity was demonstrated by the EA extract, followed by the PE extract, and the MA extract.

Effects of Different Extracts on AChE Activity in *M. persicae* Adults

The activity of AChE in *M. persicae* adults was measured 24 h after treatment with five concentrations of the three extracts. The results (Fig. 1) indicated that these extracts had different inhibition effects on AChE activity. The AChE inhibition rates of the three extracts were in the following order: EA extract > PE extract > MA extract. The inhibition became more intense with increasing extract concentrations. AChE activity was notably inhibited by the three extracts compared with the control sample. The inhibitions of the MA, EA,

Table 1. Contact activity between *I. verum* fruit extracts and *M. persicae* (mean values ± SE)[a]

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

[a]The mortalities of the control samples were less than 5%. Data followed by different letters in the same column show significant differences by ANOVA followed by Tukey's test at 5% level of significance.

Table 2. Regression analysis on the contact toxicity of *I. verum* fruit extracts to *M. persicae* (72 h)

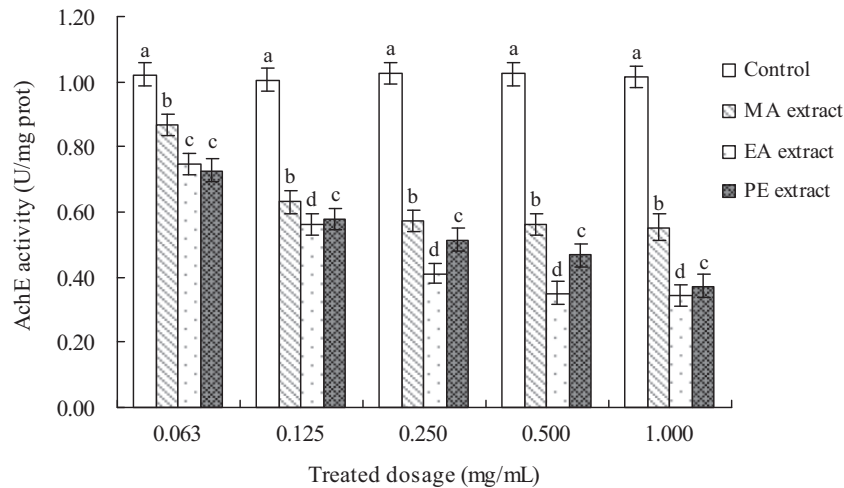| Extracts	Regression equation of toxicity	LC_{50} of extract (mg/l)	Relative coefficient (r)	95% confidence interval (mg/l)	Chi square (χ^2)
MA extract	$y = 0.91x + 5.47$	0.31	0.9885	0.23–0.43	1.84
EA extract	$y = 1.08x + 5.91$	0.14	0.9806	0.10–0.19	0.58
PE extract	$y = 1.03x + 5.59$	0.27	0.9832	0.21–0.31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

Fig. 1. Effect of the different concentrations of *I. verum* fruit extracts on the AChE activity in adult *M. persicae* at 24 h. Columns indicate mean ± SE. Varying letters at the top refer to the mean significant difference of different extracts at the 0.05 level (Tukey's test). The same below.

and PE extracts at 1.000 mg/l reached as high as 45.62%, 62.75%, and 63.32%, respectively.

The activity of AChE in *M. persicae* adults treated with LC_{50} concentrations of the three extracts (72 h after treatment; MA, 0.31 mg/l; EA, 0.14 mg/l; PE, 0.27 mg/l) were tested at 12, 24, 36, 48, 60, and 72 h after treatment. The results showed that AChE activity was significantly inhibited compared with that in the control sample (Fig. 2). The effects of the three extracts on AChE activity are time-dependent, wherein the AChE activity was initially inhibited (from 12 h to 48 h for the MA and EA extracts; from 12 h to 36 h for the PE extract) but was slightly restored when the treatment time was prolonged. The highest inhibition ratios reached 55.68% and 51.23% at 48 h after treatment with the MA and PE extracts, respectively, and 55.76% at 36 h after treatment with the EA extract.

Effect of Different Extracts on GST Activities in *M. persicae* Adults
The activity of GSTs in *M. persicae* adults treated with concentrations of 0.06, 0.13, 0.25, 0.50, and 1.000 mg/l for each extract were tested 24 h after treatment. The results indicated that the PE, EA, and MA extracts had distinct inhibitory effects on GSTs activity (Fig. 3). The low concentration (0.06 mg/l) of the MA extract induced GSTs, and the activity of GSTs increased by 19.56% compared with that in the control sample. Other concentrations also inhibited the activity of GSTs, but the activity improved when the concentration was increased. The GSTs activity levels in the treated insects were significantly lower than in the control sample, and different inhibitory effects on GSTs activity were observed for the different extracts. Moreover, the inhibition of the EA extract was notably higher than that of the other two extracts. The GSTs activity was more significantly inhibited with increasing extract concentration. The inhibition of the three extracts on the GSTs activity was in the following order: EA extract > PE extract > MA extract. The activity of GSTs was notably inhibited by the three extracts compared with the control sample. The inhibition of the MA, EA, and PE extracts reached as high as 50.13%, 60.34%, and 52.76%, respectively, at a concentration of 1.000 mg/l.

The activity of GSTs in *M. persicae* adults treated with the LC_{50} concentration (at 72 h) of the three extracts were tested 12, 24, 36, 48, 60, and 72 h after treatment. The results showed that the GSTs activity in *M. persicae* after treatment with the three extracts were

clearly inhibited as compared with the control sample (Fig. 4). The effect of the three extracts on GTSs activity are time-dependent, wherein the activity was initially inhibited (within 36 h for the MA extract; within 48 h for the EA and PE extracts) but was slightly restored with longer treatment time. The highest inhibition ratios of the MA, EA, and PE extracts to GSTs activity were 55.87% (after 36 h of treatment), 60.02% (after 60 h of treatment), and 56.58% (after 48 h of treatment), respectively.

Discussion

The aphicidal activity and influence of the MA, EA, and PE extracts of *I. verum* fruit on the activities of AchE and GSTs in *M. persicae* were studied. The results of this study confirmed that the MA, EA, and PE extracts of *I. verum* fruit possessed significant contact activity against *M. persicae* adults. Based on the observed mortality rates and LC_{50} values, the contact toxicity of the three extracts against *M. persicae* is in the following order: EA extract > PE extract > MA extract. This result is consistent with the experimental result against *S. zeamais* (Li et al. 2013).

A number of studies have shown that trans-anethole had insecticidal activity against *Blattella germanica* (Chang and Ahn 2002), *Euproctis chrysorrhoea* (Erler and Cetin 2008), *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae* (Chang et al. 2009), *Aedes aegypti* (Waliwitiya et al. 2009), *Liposcelis bostrychophila* (Zhao et al. 2012), *Spodoptera littoralis* (Pavela 2014a), and *Culex quinquefasciatus* (Pavela 2014b). Also, other studies have indicated that hexadecanoic acid had larvicidal activity to *Aedes aegypti* (Ozek et al. 2014), larvicidal and pupicidal activity to *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* (Ragavendran and Natarajan 2015); and Benzyl alcohol had pediculicidal activity to *Pediculus humanus capitis* (Yang et al. 2005). Our previous GC-MS analysis result (Wei et al. 2014) have highlighted that the most abundant component was trans-anethole, whose percentage was 41.14%, 52.54%, and 72.25% in the MA, EA, and PE extracts, respectively; that of hexadecanoic acid was 0, 4.07%, and 2.15%, respectively; that of benzyl alcohol was 0, 4.04%, and 2.80%, respectively. That is, among the three kinds of extracts, EA extract contains more trans-anethole than MA extract, and the most hexadecanoic acid and the most benzyl alcohol; percentage of trans-anethole is lowest, and no hexadecanoic acid and benzyl alcohol in the MA extract. PE extract
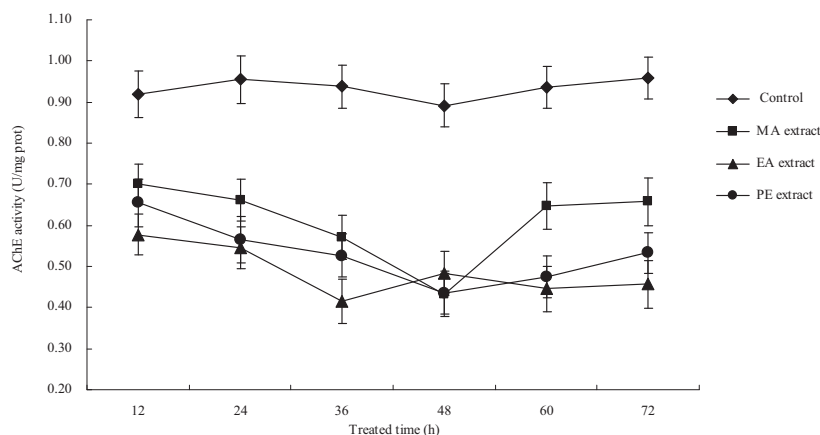
Fig. 2. Effect of LC_{50} (at 72 h) concentrations of *I. verum* fruit extracts on the AChE activity in adult *M. persicae* at different exposure times.
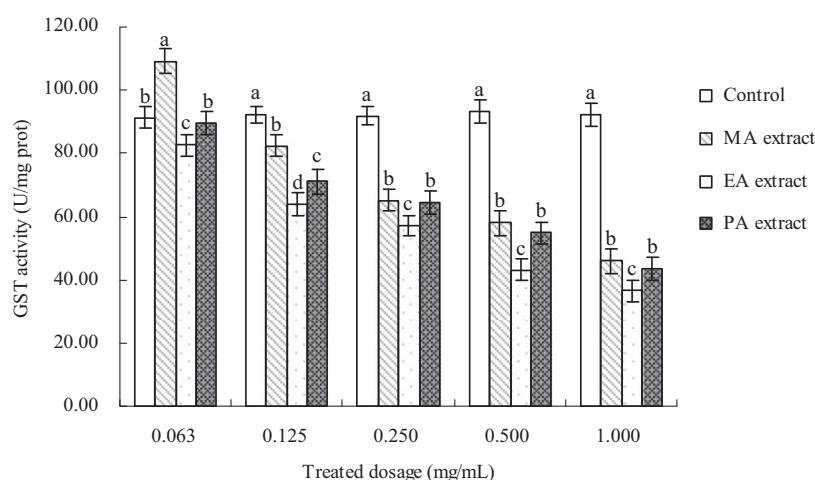

Fig. 3. Effect of different concentrations of *I. verum* fruit extracts on the GSTs activity in adult *M. persicae* 24 h after treatment.
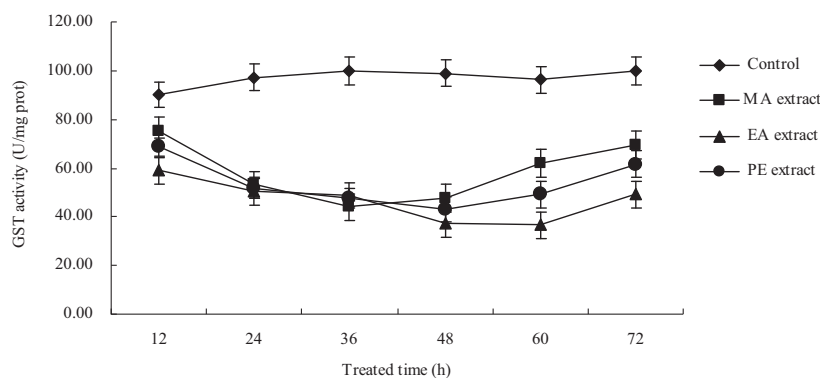

Fig. 4. Effect of LC_{50} concentrations (at 72 h) of *I. verum* fruit extracts on the GSTs activity in adult *M. persicae* at different exposure times.

contains the most trans-anethole, but lower hexadecanoic acid and lower benzyl alcohol than EA extract. The results suggest the major insecticidal active compound in *I. verum* fruit is trans-anethole, but hexadecanoic acid and benzyl alcohol also may play a role in insect pest control. The kinds and percent contents of insecticidal active components in the three kinds of extracts were different, so the three kinds of extracts had different control effects against *M. persicae*.

Many secondary metabolites from deleterious plants have different biological effects, including inhibition and induction of several important enzymes. Determining the inhibitory abilities of exogenous compounds on the activities of enzymes in the insect body is an important method for evaluating insecticidal activities (Li et al. 2007, Tatun et al. 2014a,b). The enzyme AchE, which quickly hydrolyzes the neurotransmitter acetylcholine (ACh) in the synaptic cleft to terminate the conduction of nerve impulses, is one of the most important enzymes that influence the nervous system (Philippou et al. 2010). AChE is the target site of the two major classes of insecticides, namely, organophosphates and carbamates,

which irreversibly inhibit AChE and cause the death of insects (Fournier 2005). The experimental results showed that the *I. verum* extracts significantly inhibited the AChE activity in *M. persicae* adults. Therefore, the extracts probably have neurotoxic effects on *M. persicae*.

The GSTs family is one of the major detoxifying enzyme families found in metazoans (Araujo et al. 2008). GSTs are involved in the detoxification of various plant xenobiotics (Francis et al. 2005) and usually catalyze the conjugation of the thiol group of reduced gluta-thione to electrophilic toxic xenobiotics and endogenously activated compounds molecules, thereby increasing their solubility and promoting rapid excretion or facilitating degradation (Enayati et al. 2005, Li et al. 2007, Ramsey et al. 2010). GSTs are potential drug targets. The inhibitory properties of plant extracts against GSTs may have been the pharmacological basis of their efficacy (Kolawole et al. 2011). Plant extracts enter tissues and organs of target insects and affect the activity of various detoxifying enzymes. Several secondary plant metabolites may inhibit GSTs activity, whereas others can activate GSTs activity (Vanhaelen et al. 2001, Francis et al. 2005, Matthews et al. 2010). The lethal effects of secondary plant metabolites on insects are related to the induction or inhibition of GSTs (Ramsey et al. 2010).

All three extracts showed an obvious concentration effect on the activity of AChE and GSTs in *M. persicae*. The low concentration (0.06 mg/l) of the MA extract induced GSTs, which is probably the stress response of insects. This response enhanced the detoxification metabolism of substances into secondary plant substances. In other treatments with higher concentrations, these two enzymes were characterized by an inhibitory effect, which became more apparent with increasing concentration. At the LC_{50} concentration of each extract, the overall performance of the activities of these two enzymes were first inhibited but was then restored with prolonged processing time.

In addition, all three extracts showed a strong time effect on the activity of AchE and GSTs in the body of *M. persicae*. The activities of the two enzymes were first inhibited and was then induced after treatment with LC_{50s} of the three extracts. This result may be a self-adjusting reaction of an insect to plant xenobiotics, which partially reactivated the two enzymes. As such, the effect of the extracts was continuously eliminated. The effects of the three extracts were slightly different in terms of inhibition speed and extent. Therefore, these extracts may be effective inhibitors of AchE and GSTs in vivo. The important metabolic enzymes in the body of the aphid under normal physiological function were strongly inhibited by the extracts from *I. verum*.

The present study showed that the toxic effects of the three extracts from *I. verum* fruit against *M. persicae* adults were distinctly correlated with the activities of the detoxifying enzymes in *M. persicae*. The extract with a higher contact activity has more inhibitory effects on the GSTs in vivo. The results implied that reduced activities of AChE and GSTs in *M. persicae* accounted for the death of pests after treatment with the extracts. Secondary metabolites present in plants apparently function as a defense mechanism and inhibit different physiological and biochemical processes. The phytochemical biomolecules could be used to maximize the effectiveness and specificity of future insecticide designs with specific or multiple target sites, while ensuring economic and ecological sustainability (Rattan 2010). The yield of Chinese star anise accounts for more than 80% of the global output (Li et al. 2013). Given that star anise fruits are readily available in China, we suggest that *I. verum* extracts be explored as new natural aphicidal agent against *M. persicae* aphids in alternative management programs.

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