EFFECTS OF *Abrus precatorius* L. EXTRACTS ON ACETYLCHOLINESTERASE AND GLUTATHIONE S-TRANSFERASE ACTIVITIES OF *Brevicoryne brassicae* L. (Hemiptera: Aphididae)

Jiajia Li, Rongrong Han, Xueqian Chen, Sihan Lu and Qingfeng Tang

SUMMARY

The insecticidal activities of three organic solvent (methanol, ethanol and petroleum ether) extracts from Abrus precatorius *L. against cabbage aphids with changes in acetylcholinesterase (AChE) and glutathione S-transferases (GSTs) activities were investigated in this study. It was shown that all three* A. precatorius *extracts displayed contact effects on cabbage aphids to varying degrees, with LC_{50} concentrations of 33.04, 8.28 and 2.18mg·ml^{-1} respectively. After 72h treatment at the concentration of 25mg·ml^{-1} the corrected mortality rates were 47.81, 66.90 and 99.07% respectively. The AChE and GSTs activities after treatment with different concentrations of petroleum ether extract and at different durations were strongly dose- and time-dependent. The 24h treatment with the different concentrations of petroleum ether extract showed obvious inhibitory effects on AChE and GSTs activities of cabbage aphids. The LC_{50} of the petroleum ether extract inhibited AChE and GSTs activities with the prolongation of treatment time. The highest inhibition rate of AChE activity was up to 56.43% after 24h treatment, while that of GSTs reached 66.20% after 36h treatment. These results highlight the aphicidal mechanism of* A. precatorius *extracts and contribute to the development of more effective aphicides of botanical origin.*

Introduction

Brevicoryne brassicae L. (Hemiptera: Polygonaceae) is one of the most serious pests of cruciferous vegetables like late cabbage worldwide (Gabrys and Pawluk, 1999). Cabbage aphids often absorb the juice from foliage and stems of plants as a source of nutrition through their piercing-sucking mouthparts (Ellis *et al*., 1998). Due to strong reproductive capacity (parthenogenesis), short life cycle, and overlapping generations, cabbage aphids usually densely gather on leaves of vegetables to seriously affect the crop yield and quality (Pontoppidan *et al*., 2003). Meanwhile, aphids can transmit plant viruses through their oral needles, causing significant crop yield reductions (Blanc *et al*., 2011). Spraying chemical pesticides like pyrethroid insecticides (especially deltamethrin) is the main method used in modern agricultural practices to control aphid populations, and chemical pesticides must be used widely and frequently due to the high population density of cabbage aphids reached in a short time (Dhaked *et al*., 2016). However, prolonged use of chemical pesticides results in a variety of adverse impacts, such as enhanced pesticide-resistance of aphids (Gul *et al*., 2019) and pesticide residues threatening the ecological environment and food safety (Nauen, 2014). Therefore, it is extremely urgent to find low toxicity and environmentally friendly pesticides.

On the one hand, the extraction and separation of new active compounds from plants has become an important approach for the development of new pesticides (Lv *et al*., 2012). On the other hand, the development of botanical pesticides is another new way to solve the above problems (Nukenine *et al*., 2010). Compared with traditional chemical pesticides, botanical pesticides are characterized by low residues, high selectivity and lack of development of resistance. Botanical products are anew type of environment-friendly pesticides (Huang *et al*., 2011).

Abrus precatorius L. is a poisonous legume of the Fabaceae family, native to India with the common name of rosary bean. This species is now distributed in the subtropical regions around the world, and mainly found in Yunnan, Guangdong, Guangxi, and Fujian in China (Garaniya and Bapodra, 2014). *A. precatorius* has been studied and used in medicine and food in China and abroad widely due to its high medicinal and economic values, high toxicity, detox, and sedative properties (Verma *et al*., 2011). The ethanol extract of *A. precatorius* leaves has anti-inflammatory and analgesic effects on rats (Asija *et al*., 2015). Compounds isolated from cotyledons have anti-cancer activity and have provided an alternative option for breast cancer treatment (Sofi *et al*., 2018). In addition, the anti-diabetic effect of the chloroform-methanol extract from seeds is assessed in rabbits with alloxan diabetes mellitus (Monago and Alumanah, 2005). However, there are few reports on the prevention and control of agricultural pests with *A. precatorius* at present. As the main production area of

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EFECTOS DE EXTRACTOS DE *Abrus precatorius* L. EN LAS ACTIVIDADES OF ACETILCOLINESTERASA Y GLUTATIÓN S-TRANSFERASA DE *Brevicoryne brassicae* L. (Hemiptera: Aphididae)

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RESUMEN

Se investigó la actividad insecticida de tres extractos obtenidos de Abrus precatorius *L. con diferentes solventes orgánicos (metanol, etanol y éter de petróleo) sobre áfidos del repollo con cambios en las actividades de aceticolinesterasa AChE) y de glutatión S-transferasas (GSTs). Se mostró que todos los tres extractos de* A. precatorius *tuvieron efectos, en grado variable, al estar en contacto con áfidos del repollo, con concentraciones LC_{50} de 33,04; 8,28 y 2,18mg·ml^{-1} respectivamente. Después de tratamiento de 72h a la concentración de 25mg·ml^{-1} las tasas corregidas de mortalidad fueron de 47,81; 66,90 y 99,07% respectivamente. Las actividades de AChE y GSTs después del tratamiento con diferentes concentraciones de éter de petróleo y con diferentes duraciones fueron fuertemente dependientes de la dosis y el tiempo. El tratamiento por 24h con las diferentes concentraciones del extracto con éter de petróleo mostraron efectos inhibitorios obvios sobre las actividades de AChE y GSTs de los áfidos del repollo. La LC_{50} del extracto con éter de petróleo inhibió la actividad de AChE y GSTs al prolongar el tiempo de tratamiento. La más alta tasa de inhibición de la actividad de AChE alcanzó 56,43% después de 24h de tratamiento, mientras que la de GSTs alcanzó 66,20% tras 36h de tratamiento. Estos resultados resaltan el mecanismo aficída de los extractos de* A. precatorius *y contribuye al desarrollo de aficidas de origen botánico más efectivos.*

EFEITOS DE EXTRATOS DE *Abrus precatorius* L. NAS ATIVIDADES DA ACETILCOLINESTERASE E DA GLUTATIONA-S-TRANSFERASE DE *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae)

Jiajia Li, Rongrong Han, Xueqian Chen, Sihan Lu e Qingfeng Tang

RESUMO

Foi investigada a atividade inseticida de três extratos obtidos de Abrus precatorius *L. com diferentes solventes orgânicos (metanol, etanol e éter de petróleo) sobre pulgões do repolho com mudanças nas atividades da acetilcolinesterase (AChE) e glutationas-S-transferases (GSTs). Foi observado que todos os três extratos de* A. precatorius *tiveram efeitos, em grau variável, quando em contato com pulgões do repolho, com concentrações LC_{50} de 33,04; 8,28 e 2,18mg·ml^{-1} respectivamente. Após o tratamento de 72h em concentração de 25mg·ml^{-1}, as taxas corrigidas de mortalidade foram de 47,81; 66,90 e 99,07% respectivamente. As atividades da AChE e GSTs, após o tratamento com diferentes concentrações de éter de petróleo e diferentes durações, foram fortemente dependentes da dose e o tempo. O tratamento por 24h com as diferentes concentrações do extrato com éter de petróleo mostraram efeitos inibitórios óbvios sobre as atividades da AChE e GSTs dos pulgões do repolho. A LC_{50} do extrato com éter de petróleo inibiu a atividade da AChE e GSTs ao prolongar o tempo de tratamento. A maior taxa de inibição da atividade da AChE atingiu 56,43% após 24h de tratamento, enquanto a dos GSTs atingiu 66,20% após 36h de tratamento. Estes resultados destacam o mecanismo inseticida dos extratos de* A. precatorius *e contribui para o desenvolvimento de inseticidas para pulgões de origem botânico mais efetivos.*

A. precatorius medicinal materials and being one of the countries with the highest demand for pesticides in the world, it is necessary to better promote the development and utilization of this unique medicinal plant resource and deeply examine the prospects for the agricultural applications of *A. precatorius* in China.

The growth and development of insects are influenced by the activity and content of various enzymes (Ramsey *et al*., 2010). In addition, the defense and self-protection responses to unhealthy growth environments and exogenous substances are also regulated by enzymes (Yang *et al*., 2013). Acetylcholinesterase (AChE) is an important hydrolase in the insect nervous system that blocks nerve impulses by catalyzing the hydrolysis of the neurotransmitter acethylcholine (Yeom *et al*., 2012). Glutathione S-transferases (GSTs) are also important enzymes that develop metabolic resistance to insecticides and are involved in the detoxification mechanisms of many molecules (Enayati *et al*. 2005). In plant-insect interactions, these metabolic enzyme activities will change after feeding on non-hosts thus reflect the effect of exogenous toxic and harmful substances in insects, ultimately elucidating the insecticidal mechanism of botanical insecticides by determining the impact of active compounds (Liu *et al*. 2018).

In this study, the insecticidal activities and action mechanism of three organic solvent (methanol, ethanol and petroleum ether) extracts of *Abrus precatorius* L. against cabbage aphids were investigated by detecting changes in acetylcholinesterase (AChE) and glutathione S-transferase (GSTs) activities at the physiological and biochemical levels. It was shown that the petroleum ether extract of *A. precatorius* was the optimal one among the three extracts, as the changes of AChE and GSTs activities in cabbage aphids treated with it were strongly dose- and time-dependent. After 24h treatment of petroleum ether extract with different concentrations, the cabbage aphids displayed significant inhibitory effects on the enzyme activities. After treatment with the LC50 dose, the activities of AChE and GSTs in the insects were further inhibited with the prolongation of treatment time. The obtained results can contribute to the development of new botanical aphicides, the more effective control of aphid

damage, and the reduced pesticide pollution. These results will also provide references for the efficient use of *A. precatorius* as a botanical insecticide resource.

Materials and Methods

Insects and plant material

Specimens of *Brevicoryne brassicae* were collected from the Agricultural Cropping Garden of Anhui Agricultural University and transferred to cabbage seedlings cultivated in the greenhouse without pesticide contact during the growing season. Insects were used for testing after four generations of indoor rearing. The feeding conditions included a growth photoperiod of 16:8h light: dark, a constant temperature of 25 ±1°C, and constant humidity of 70-80%. Apterous adult aphids with similar individual development were selected for testing. *Abrus precatorius* was purchased from the traditional Chinese medicine market in Bozhou, Anhui Province, China.

Reagents

Methanol, ethanol, petroleum ether and acetone were all pure analytical grade. Sterile double distilled water was used. Normal saline0.86%, a total protein (TP) determination kit (Coomassie Brilliant Blue), and AChE and GSTs assay kits were purchased from Nanjing Jiancheng Materials Biological Engineering Research Institute.

Extract of A. precatorius

Seeds of *A. precatorius* with bright color, full-development and of similar sizes were quickly washed with water, drained and then dried at room temperature. The seeds were crushed and passed through 40 mesh sieves. Using the cold dipping method, *A. precatorius* (100g) was placed into a 1.5liter round-bottomed flask and eight times the volume of organic solvent was added. The mixture was incubated in the dark for 72h. After three days, vacuum filtration was performed using a Buchner funnel. The solution was immersed in an eight-fold volume of organic solvent for three days, and this operation was repeated three times in total. Afterwards, the three filtrates were collected. The extracts were concentrated by vacuum decompression using a rotary evaporator and then concentrated into a paste. Three kinds of organic solvent extraction pastes were weighed to 1g, and acetone was added to dissolve the pastes. Then, a 0.1% Tween-80 aqueous solution was added to prepare solutions with 50, 25, 12.50, 6.25, 3.15, and 1.56mg·ml^{-1} of active ingredient, and stored at 4°C.

Contact toxicity bioassay

The contact activity of the three organic solvent extracts of *A. precatorius* against cabbage aphids was determined by the microdrip method (Zhong *et al*., 2005). A microsyringe was used to dispense ~0.5µl of the extract solution onto the front chest plate of the cabbage aphids. After dripping, the insects were transferred to the cabbage leaves with a moisturizing treatment. Then, the treated cabbage leaves and insects were placed in a disposable petri dish with wet filter paper. Each concentration was repeated three times, and each treatment was repeated with 30 cabbage aphids. The control group was treated with acetone and 0.1% Tween-80 aqueous solution, and each treatment was repeated three times. After 24, 48 and 72h, the death of cabbage aphids was evaluated. The abdomen and legs of the aphids were touched with a brush, and aphids were considered dead if they did not move. The corrected mortality was calculated as:

$$\text{Corrected mortality (\%)} = (M_1) / (M_0) \times 100$$

where M_1: treatment mortality, the blank control mortality, and M_0= 1: the blank control mortality.

Determination of enzyme activities

To study the changes in AChE and GSTs activities in cabbage aphids treated with petroleum ether extract from *A. precatorius*, the concentration gradients of 0.79, 1.45, 2.23, 3.23 and 4.57mg·ml^{-1} were set at the concentration of the LC_{10}, LC_{20}, LC_{30}, LC_{40} and LC_{50} levels 24h after treatment. After treatment for 12, 24, 36, 48 and 60h at the LC_{50} level, the enzyme solution was prepared separately. After the treatment, a 0.1g sample of insects was weighed and rinsed in precooled physiological saline three times to remove impurities. The insects were then dried and ground to powder with liquid N_2 and in a mortar (pre-cooled). The powder was placed into centrifuge tube and 0.9ml of normal saline (pre-cooled) was added. At this time, a 10% homogenate of the cabbage aphids was centrifuged for 10min (4°C, 3000rpm) and the supernatant was collected as the enzyme solution to be tested. The enzyme extraction step was performed in an ice bath. The physiological saline and homogenizer were pre-cooled before each experiment to prevent enzyme inactivation.

For AChE, 50µl of the enzyme solution was tested, and four groups were established according to the test requirements: blank tubes, standard tubes, control tubes and determination tubes were used. Then, strictly following the instructions of the AChE assay kit, the samples to be determined were added to the enzyme label plate (96 holes, 100µl per hole). The OD value (412nm) of each hole was measured using a microplate reader, and each hole was measured three times with three replicates per sample. The results were calculated using the average values by referring to the kit instructions.

For GSTs, 100µl of the enzyme solution was used to determine the activities of the following four groups according to the test requirements: blank tube, standard tube, control tube and determination tube. Then, strictly following the instructions of the GSTs assay kit according to the corresponding group relationship, the determined enzyme solution was placed into an enzyme label plate (96 holes, 100µl per hole) and the same exact procedure as for AChE was followed.

For total protein, we used 50µl of the enzyme solution to be tested and diluted the enzyme solution to 2% with physiological saline (precooled). Three groups were established according to the test requirements: homogenate determination tube, blank tube, and protein standard tube. Then, strictly following the instructions of the total protein (TP) assay kit according to the corresponding group relationship, the samples to be determined were added to the enzyme label plate (96 holes, 100µl per hole). The OD value (595nm) of each hole was measured using a microplate reader, and each hole was measured three times with three replicates per sample. The results were calculated using the average values by referring to the kit instructions.

Statistical analysis

The test data was systematically counted (Abbott, 1925) in Excel. Data processing software DPS was used to calculate the mean values and standard errors of insecticidal activities and enzyme activities (Tang and Zhang, 2012), to analyze data variance, and to calculate the virulence regression equation and lethal concentration were also counted using the DPS program. Duncan's new repolarization method was used to test and compare the differences between the treatments ($p<0.05$).

Results

Contact toxicity of A. precatorius *extracts against* B. brassicae

As shown in Table I, all three organic solvent extracts of

TABLE I
CONTACT ACTIVITIES OF THE EXTRACTS FROM *A. precatorius* SEEDS AGAINST *B. brassicae*

Extract	Exposure dosage ($mg \cdot m^{-1}$)	Corrected mortality (%)		
		24h	48h	72h
Methanol extract	3.125	3.42 ±1.36 d	9.61 ±2.51 d	14.99 ±2.54 a
	6.25	18.27 ±5.42 c	23.73 ±4.29 c	26.54 ±3.69 a
	12.5	25.11 ±3.86 bc	29.03 ±3.81 bc	36.30 ±1.79 b
	25	35.43 ±2.58 b	40.36 ±3.07 ab	47.81 ±1.96 c
	50	50.12 ±3.98 a	51.79 ±5.03 a	54.06 ±3.41 d
Ethanol extract	3.125	17.71 ±2.51 d	22.18 ±2.14 d	29.37 ±2.63 d
	6.25	35.56 ±5.31 c	40.99 ±4.41 c	44.69 ±3.12 c
	12.5	52.57 ±5.19 b	57.10 ±5.46 b	61.60 ±4.77 b
	25	57.92 ±5.89 b	63.32 ±5.37 b	66.90 ±5.35 b
	50	82.14 ±2.56 a	83.99 ±3.09 a	85.75 ±2.89 a
Petroleum ether extract	1.5625	24.54 ±2.83 e	32.83 ±3.26 c	39.48 ±7.65 c
	3.125	33.94 ±3.27 d	65.73 ±1.90 b	71.88 ±1.17 b
	6.25	61.81 ±3.93 c	87.53 ±8.50 a	94.61 ±4.66 a
	12.5	74.63 ±5.60 b	95.50 ±3.50 a	98.18 ±2.10 a
	25	90.69 ±1.61 a	99.07 ±1.85 a	99.07 ±1.81 a

Mean values ±SE. The data marked with different letters in the same column indicate significant differences tested by Duncan's new multiple range test method ($p<0.05$)

A. precatorius had contact activities against cabbage aphids. The contact effects of the three organic solvent extracts were related to the extract concentration. After being treated with $25mg \cdot ml^{-1}$ for 72h, the corrected mortality rates of the methanol extract, ethanol extract and petroleum ether extract were 47.81, 66.9 and 99.07%, respectively.

Table I shows the treatment concentrations of the three types of solvent extracts of *A. precatorius* and the mortality rate of cabbage aphids treated for 24, 48 and 72h. The regression equation and LC_{50} values of the three extracts against cabbage aphids were obtained by linear regression analysis of their toxicities. As shown in Table II, longer contact time resulted in the lower LC_{50}. In other words, the best contact effect was achieved with the increase in extract concentration and the prolongation of treatment time. The LC_{50} values of three organic solvent extracts of *A. precatorius* after treatment for 72h were 33.04, 8.28 and $2.18mg \cdot ml^{-1}$ respectively. Based on the observed mortality rates and LC_{50} values, the best contact effect was achieved with the petroleum ether extract followed by the ethanol extract, and then methanol extract.

Effects of petroleum ether extract of A. precatorius *on the AChE activity of* B. brassicae

The experimental concentrations of 0.79, 1.45, 2.23, 3.23 and 4.57 $mg \cdot ml^{-1}$ were used to determine the changes in AChE activity in cabbage aphids treated with five concentrations of petroleum ether extract of *A. precatorius* for 24h. The changes in AChE activity are shown in Figure 1. The extract affected the activity of AChE in cabbage aphids at LC_{10}, LC_{20}, LC_{30}, LC_{40} and LC_{50} concentrations. With the increase in treatment concentrations, the activity of AChE in cabbage aphids showed a significant ($p<0.05$) downward trend compared with the

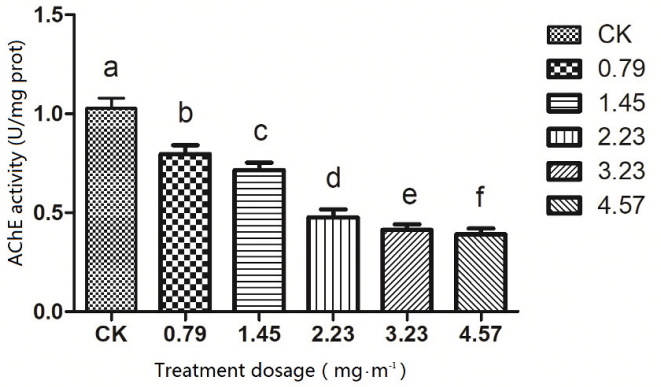

Figure 1. Effect of different concentrations of petroleum ether extract of *A. precatorius* seeds on the AChE activity in *B. brassicae* at 24h after treatment. Columns indicate values of the mean ±SE. Different letters indicate significant differences in the effects of petroleum ether extract of *A. precatorius* seeds at the 0.05 level (Duncan's test). CK: control.

TABLE II
REGRESSION ANALYSIS OF CONTACT VIRULENCE OF THE EXTRACTS OF *A. precatorius* SEEDS AGAINST *B. brassicae*

Extract	Treatment time (h)	Toxicity regression equation	LC_{50} of extract ($mg \cdot m^{-1}$)	Relative coefficient (r)	95% confidence interval ($mg \cdot m^{-1}$)	χ^2
Methanol extract	24	y= 1.23x+2.94	47.91	0.9634	1.53-33.94	3.40
	48	y= 1.61x+3.35	43.46	0.9815	1.48-29.94	1.54
	72	y= 0.92x+3.60	33.04	0.9889	1.37-23.37	0.99
Ethanol extract	24	y= 1.38x+3.46	12.94	0.9824	1.03-10.64	4.17
	48	y= 1.33x+3.65	10.43	0.9858	0.92-8.41	3.04
	72	y= 1.24x+3.86	8.28	0.9864	0.81-6.40	2.45
Petroleum ether extract	24	y= 1.40x+3.83	4.57	0.9914	3.84- 5.35	2.83
	48	y= 1.51x+4.26	3.12	0.9972	2.43-3.80	0.60
	72	y= 1.73x+3.89	2.18	0.9891	1.61-2.73	1.60

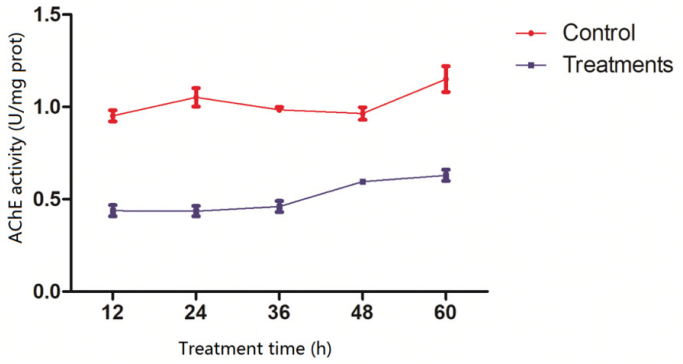

Figure 2. Effect at 24h of LC50 (4.57mg·m^{-1}) of petroleum ether extract of *A. precatorius* seeds on the AChE activity of *B. brassicae* at different exposure times.

control. Therefore, the petroleum ether extract of *A. precatorius* had a significant inhibitory effect on the AChE of cabbage aphids.

The lethal concentration of *A. precatorius* extract for 24h of contact treatment against cabbage aphids was 4.57mg·ml^{-1}. The activity of AChE in cabbage aphids was assayed under different treatment times at 12, 24, 36, 48 and 60h. Figure 2 shows that the AChE activity in cabbage aphids in the treatment group was obviously inhibited compared with the control. The highest inhibitory rate of AChE activity in cabbage aphids was 56.43% after 24h of treatment (Figure 2). After 48h of treatment, the AChE activity was gradually increased, but was generally lower than that in the control group. Therefore, the effect of petroleum ether extract of *A. precatorius* on AChE activity in cabbage aphids was time dependent.

Effects of petroleum ether extract of A. precatorius *on the GSTs activity of* B. brassicae

The experimental concentrations of 0.79, 1.45, 2.23, 3.23 and 4.57mg·ml^{-1} were used to determine the changes in GSTs activity in cabbage aphids treated with five concentrations of petroleum ether extract of *A. precatorius* for 24h. The changes in GSTs activity are shown in Figure 3. The activity of GSTs in the body was affected when cabbage aphids were exposed to the petroleum ether extract of *A. precatorius*. Compared with the control, the extract significantly inhibited the activity of GSTs in cabbage aphids. The activity of GSTs at the concentration of LC_{10} and LC_{20} was first activated and significantly higher than that of the control group ($p<0.05$). It began to decrease at LC_{30} until the LC_{40} and LC_{50} levels were significantly lower than in the control group ($p<0.05$). With the increase in extract concentrations, the activity of GSTs was inhibited more significantly.

The lethal concentration of *A. precatorius* extract was 4.57mg·ml^{-1} for contact treatment of cabbage aphids for 24h. The GSTs activity (Figure 4) was assayed under different treatment times at 12, 24, 36, 48 and 60h. The petroleum ether extract of *A. precatorius* had obvious effects on the activity of GSTs in cabbage aphids. Compared with the control group, the GSTs activity was significantly inhibited after treatment with the extract, and this effect was time dependent. The highest inhibition rate of GSTs activity was 66.20% at 36h. With the prolongation of treatment time, the GSTs activity was gradually increased, but it was inhibited compared with the control group.

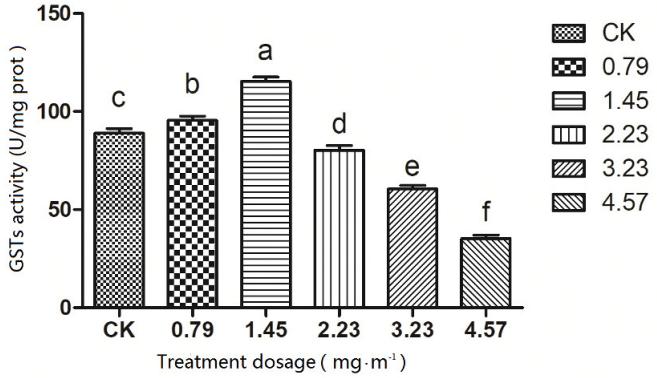

Figure 3. Effect of different concentrations of petroleum ether extract of *A. precatorius* seeds on the GSTs activity of *B. brassicae* at 24h after treatment. CK: control.

Discussion

The insecticidal activities of methanol, ethanol and petroleum ether extracts of *A. precatorius* and the effects of petroleum ether extract of *A. precatorius* on the activities of AChE and GSTs in cabbage aphids were studied. The results showed that these extracts had contact effects against cabbage aphids. However, the petroleum ether extract had the highest contact activity against cabbage aphids. Compared with other botanical aphidicides, the extract of *A. precatorius* showed stronger insecticidal activity against aphids. For example, the essential oil of *Cinnamomum camphora* seeds also showed insecticidal activity against cotton aphids with an LD50 value of 14.68ng/aphid (Jiang *et al.*, 2016). Three extracts of *Illicium verum* fruit were reported to have had significant contact activity against *M. persicae* adults in the following order: ethyl acetate extract > petroleum ether extract > methyl alcohol extract (Zhou *et al.*, 2016).

The secondary metabolites of insecticidal plants occur in various forms, and their modes of action against target pests are also different; the reaction mechanisms of pests against plant secondary metabolites are

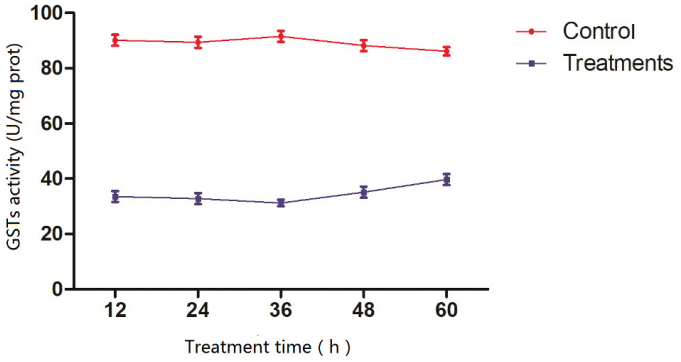

Figure 4. Effect at 24h of LC_{50} (4.57mg·m^{-1}) of petroleum ether extract of *A. precatorius* seeds on the GSTs activity of *B. brassicae* at different exposure times.

also varied (Ramsey *et al*., 2010). Insect pests use different biologically active detoxification enzymes and substance metabolism systems in the body to jointly resist plant secondary metabolites (Li *et al*., 2007). AChE is a very important enzyme in insect neurotransmission and therefore an important target for insecticides. When the activity of AChE is inhibited in vivo, insects die because of excessive stimulation (Yeom *et al*., 2012). GSTs are important detoxifying enzymes involved in the metabolism of endogenous and exogenous substances in insects. The GSTs in insects can be increased or decreased due to the action of toxic and harmful exogenous substances (Enayati *et al*., 2005). Therefore, examination of the indicators of detoxification of metabolic enzymes can be used to quantify the effects of botanical pesticides.

The results of the enzyme activity assay showed that the petroleum ether extract of *A. precatorius* had an obvious dose and time dependent effect on the activity of AChE and GSTs in cabbage aphids. The petroleum ether extract of *A. precatorius* had significant concentration dependent effects on the activities of AChE and GSTs in these aphids. The activity of AChE in cabbage aphids was inhibited by both high and low concentrations of petroleum ether extract of *A. precatorius* 24h after treatment. Therefore, it is speculated that the petroleum ether extract of A. precatorius had a neurotoxic effect. In the treatment with higher concentrations, both enzymes were inhibited, and these effects became more obvious with the increase in concentration. These effects may have been related to the decline in physiological function after insect poisoning, which finally led to the death of the insects (Li *et al*., 2007).

In addition, the petroleum ether extract of *A. precatorius* showed a strong time-dependent effect on the activities of AChE and GSTs in cabbage aphids. Compared with the control group, under the treatment with the LC_{50} of the petroleum ether extract of *A. precatorius*, the activities of the two enzymes in cabbage aphids were first inhibited and then recovered with the prolongation of the treatment time. Generally, the activities were inhibited. The decrease in the GSTs activity may have inhibited the normal detoxification metabolism of cabbage aphids and the accumulation of toxic substances may have caused death of the insects after treatment (Vanhaelen *et al*., 2001).

The petroleum ether extract of *A. precatorius* contains different plant secondary substances, which strongly inhibit the activities of AChE and GSTs after entering the bodies of cabbage aphids. This may have affected their detoxification metabolism, causing the accumulation of toxic substances. At the same time, the extract may have also seriously interfered with the normal physiological and biochemical properties of the insects and accelerating their death (Francis *et al*., 2005). The experimental results showed that the petroleum ether extract of *A. precatorius* may be an effective inhibitor of AChE and GSTs in cabbage aphids. For the practical application of *A. precatorius* extract as a botanical pesticide to control cabbage aphids, metabolic enzyme inhibitors can be considered to improve pesticide efficacy and speed of action. *A. precatorius* is a unique plant in China, is abundant, and is one of the traditional Chinese herbal medicines. Therefore, it is necessary to further explore the insecticidal activity of this plant. In addition, it is possible to develop a new class of safe, highly efficient botanical insecticides. The findings of the current study can contribute to the development of new botanical aphicides to effectively control aphid damage and reduce pesticide pollution into the environment.

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