ABSTRACT. Through a combination of steps including centrifugation, ammonium sulfate gradient precipitation, sephadex G-25 gel chromatography, diethylaminoethyl cellulose 52 ion-exchange chromatography and hydroxyapatite affinity chromatography, carboxylesterase (CarE, EC3.1.1.1) from sixth instar larch caterpillar moth, Dendrolimus superans (Lepidoptera: Lasiocampidae) larvae was purified and its biochemical properties were compared between crude homogenate and purified CarE. The final purified CarE after hydroxyapatite chromatography had a specific activity of 52.019 μmol/(min·mg protein), 138.348-fold of crude homogenate, and the yield of 2.782%. The molecular weight of the purified CarE was approximately 84.78 kDa by SDS-PAGE. Three pesticides (dichlorvos, lambda-cyhalothrin, and avermectins) showed different inhibition to crude CarE and purified CarE, respectively. In vitro median inhibitory concentration indicated that the sensitivity of CarE (both crude homogenate and final purified CarE) to pesticides was in decreasing order of dichlorvos > avermectins > lambda-cyhalothrin. By the kinetic analysis, the substrates alpha-naphthyl acetate (α-NA) and beta-naphthyl acetate (β-NA) showed lesser affinity to crude extract than purified CarE. The results also indicated that both crude homogenate and purified CarE had more affinity to α-NA than to β-NA, and the $K_{cat}$ and $V_{max}$ values of crude extract were lower than purified CarE using α-NA or β-NA as substrate.

Key Words: carboxylesterase, purification, Dendrolimus superans, pesticide sensitivity, biochemical characteristic

Carboxylesterase (CarE, EC3.1.1.1) belongs to the class of serine hydrolases hydrolyzing a wide variety of carboxylic acid esters (Shukla 2012). CarEs are a family of enzymes ubiquitously expressed in all living organisms including animals, plants, insects, and microbes (Cashman et al. 1996, Yu et al. 2009). CarEs have a variety of physio-functional, such as detoxification, allelochemical metabolism and tolerance, degradation of neurotransmitters, metabolism of specific hormones and pheromones, defense, and behavior (Vogt et al. 1985, Taylor and Radic 1994, Vontas et al. 2002, Vogt 2005). Previous researches have shown that CarEs played a key role in defense against xenobiotic compounds in insects (Maymó et al. 2002, Sogorb and Vilanova 2002, Field and Blackman 2003). Some researches have also reported that insects CarEs are involved in metabolism of organophosphates (OPs), carbamates, and pyrethroids and resistance to these pesticides. Increased CarEs activity was associated with pesticide resistance of insects, which had been confirmed in Culex tarsalis, Tribolium confusum, Habrobracon hebetor, Lucilia cuprina, Musca domestica, and Aphis gossypii (Whyard et al. 1995, Perez-Mendoza et al. 2000, Smyth et al. 2000, Wool and Front 2003, Pan et al. 2009). Moreover, different CarE from various insects showed different characteristics and properties to detoxify pesticides, even closely related species. Constitutive expression and protein component of CarEs are highly diversified in insects, resulting in a broad hydroxylation range of carboxylic esters to their component alcohols and acids (Durand et al. 2010, Oakeshott et al. 2010, Ramsey et al. 2010). The biochemical characteristics of CarEs from many insects had been studied including Blattella germanica, Bombyx mori, Culex quinquefasciatus, Tribolium castaneum, and Orozzeriphilus surinamensis (Ketterman et al. 1992, Murthy and Veerabadhrappa 1996, Prabhakaran and Kamble 1996, Haubrueg et al. 2002, Lee 2011). However, characteristics of many insects CarEs such as the larch caterpillar moth, Dendrolimus superans (Lepidoptera: Lasiocampidae), remain unknown.

The D. superans (namely larch caterpillar; Lepidoptera: Lasiocampidae), mainly distributed in Russia (Siberia), North Korea, Hokkaido of Japan, Mongolia, Xinjiang, Hebei Province, and Inner Mongolia of China, is a key lepidopterious pest species of forests, which caused great harm to pines such as Pinus koraiensis Sieb. et Zucc., Pinus tabulaeformis Carrière, Pinus sibirica var. mongolica Litv., Picea asperata Mast., Abies fabri (Mast.) Craib (Li et al. 2002). Many investigations on the occurrence and prediction, physiological ecology, and control of the D. superans had been studied in previous researches (Xiao 2003, Dong et al. 2009, Guo et al. 2011). For example, the dichlorvos, lambda-cyhalothrin, and avermectins insecticide are usually used as chemical control strategy (Lasota and Dybas 1991, Wu 2003, Amweg et al. 2005, Lehner et al. 2011). It also had been confirmed that D. superans larvae could be controlled effectively by three pesticides (Xiao 2003, Tang 2012, Zhou et al. 2012, Song et al. 2013). In this study, the authors further investigated the purification and kinetic characterization of CarE from D. superans larvae in order to understand the physiological and biochemical functions of CarE from D. superans in response to insecticides.

Materials and Methods

Insects. The D. superans larvae were collected from Kecskes Teng Qi, Chifeng city, Inner Mongolia, China, and fed by Larix gmelinii leaves at 25 ± 1°C, photoperiod 14:10 (L:D) h and 70 ± 5% relative humidity. The sixth instar larvae were used in this study.

Chemicals. All the pesticides used were technical grade and their purity in this study was as follows: 93.50% dichlorvos (Zhengzhou Sanonda Weixin Pesticides Ltd., Co., China); 95.00% lambda-cyhalothrin (Heilongjiang Pingshan Forestry Chemical Company, China); 93.80% avermectins (Jilin Tonghua Pesticides Chemical Ltd., Co., China). Alpha-naphthyl acetate (α-NA), beta-naphthyl acetate eserine (β-NA), 2-dichloro-4-nitrobenzene, Coomassie brilliant blue (G-250), bovine serum albumin, Fast Blue B salt, sodium dodecyl sulfate (SDS), 2-ethoxy-1-ethoxy carbonyl-12-dihydroquinoline, Sephadex G-25, ECH Sepharose 4B, poly ethylene glycol 20000 (PEG20000), procainamide, N,N,N′,N′-tetramethylethylene diamine, TEMED, were...
purchased from Sigma Chemical CO., USA. Bio-Gel HTP Hydroxyapatite was purchased from Bio-Rad Laboratories, Inc., USA. Acrylamide, ammonium persulphate (APS), methylene bis acrylamide, Tris (Hydroxymethyl) aminomethane; glycine were purchased from Shenzhen Hua Xinrui Biological Technology Ltd., Co., China. All chemicals and reagents were analytical grade or better.

**Homogenate Preparation.** Ten sixth instar *D. superans* larvae were dissected, and the digesta in the intestine was removed, then the epidermis and other tissues were rinsed using 10 mM cold phosphate buffer (pH 6.8). All tissues were homogenized with 10 mM cold phosphate buffer (pH 6.8), and the supernatant was centrifuged at 4°C, 8,000 × g for 15 min. The final supernatant was centrifuged at 4°C, 12,000 × g for 10 min as crude enzyme and stored into −20°C.

**CarE Activity Test.** CarE activity was determined according to the method of Van Asperen with modification (Asperen 1962). Briefly, 0.05 ml crude enzyme extract and 2.00 ml 3 × 10⁻⁴ mol/liter α-NA containing e-serine (1:1) were mixed and then were incubated at 35°C for 10 min. The catalytic reaction was terminated using 0.90 ml chromogenic reagent (5% SDS:1% Fast Blue B salt for 10 min. The catalytic reaction was terminated using 0.90 ml chromogenic buffer (pH 6.8), and the supernatant was centrifuged at 4°C, 10,000 × g for 15 min. The final supernatant was centrifuged at 4°C, 12,000 × g for 10 min as crude enzyme and stored into −20°C.

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**Purification of the CarE.** CarE from sixth instar *D. superans* larvae has been purified by a combination of ammonium sulfate gradient precipitation, sephadex G-25 gel chromatography, diethylaminoethyl cellulose 52 ion-exchange chromatography, and hydroxyapatite affinity chromatography. All purification steps were carried out at 4°C unless otherwise stated.

**Ammonium Sulfate Precipitation.** Ammonium sulfate gradient precipitation (saturation was from 20 to 90%) was carried out at 4°C, and the obtained precipitate was centrifuged at 8,000 × g for 20 min at 4°C. Each precipitate was dissolved in 5.0 ml 10-mM cold sodium phosphate buffer (pH 6.8), and specific activity and protein content of each precipitate were determined. All assays were repeated three times. The optimal saturation for precipitating objective protein was chosen by the specific activity account.

**Sephadex G-25 Gel Filtration.** After ammonium sulfate gradient precipitation, the precipitate were resuspended and dialyzed for 24 h against three changes of 10-mM sodium phosphate buffer at 4°C. The dialyzed solution was filtrated on Sephadex G-25 gel column (15 by 300 mm) equilibrated with 10-mM cold phosphate buffer (pH 6.8). An aliquot of 12 ml sample was added into the column using 10-mM cold phosphate buffer (pH 6.8) as eluent with a flow rate of 30 ml/h, then the elution and fraction (3.0 ml) were begun and collected. CarE activity and protein content from each fraction were assayed, and five fractions containing higher CarE activity were combined and concentrated to 6.0 ml by PEG20000 for next column.

**DEAE-52 Ion-Exchange Chromatography.** The sample from Sephadex G-25 gel filtration was applied into DEAE-52 ion-exchange chromatography column (16 by 300 mm), equilibrated with 50-mM cold phosphate buffer (starting buffer, pH 6.8). The sample column was washed with 120 ml of starting buffer (containing 0.6 M sodium chloride) and 5.0 ml of fractions were collected at a flow rate of 24 ml/h. The CarE activity and protein content from each fraction were assayed; five fractions with higher CarE activity were combined and then dialyzed for 24 h against three changes of 50 mM sodium phosphate buffer at 4°C. The sample solution was concentrated to 10.0 ml by PEG20000 for hydroxyapatite affinity chromatography.

**Hydroxyapatite Affinity Chromatography.** Hydroxyapatite chromatography column was prepared according to the method of Urist et al. (1984). The combined fractions with higher CarE activity through DEAE-52 ion-exchange chromatography were applied to a hydroxyapatite column (15 by 150 mm, Bio-Gel HTP Gel, Bio-Rad Laboratory) equilibrated with 100 ml 50-mM phosphate buffer (pH 6.8). CarE activity fractions were eluted with 400-mM phosphate buffer (pH 6.8) with a flow rate of 36 ml/h. Five fractions with higher CarE activity were pooled and concentrated to 10 ml using PEG20000. An aliquot of 10 ml purified sample was dialyzed for 24 h against three changes of 50-mM sodium phosphate buffer at 4°C. Through above steps, purified CarE was eventually obtained.

**SDS-PAGE.** The molecular weight of CarE was determined by polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out on 10% gels according to the method of Laemmli (1970) using standard proteins (M, 10,000–170,000 Da) (Laemmli 1970). The molecular weight of CarE was derived from the plot of log molecular weight versus relative mobility.

**Kinetic Analysis.** Enzyme kinetics of crude CarE homogenate and purified CarE were compared by recording activity toward five concentrations (3.0 × 10⁻¹, 1.5 × 10⁻¹, 0.75 × 10⁻¹, 0.375 × 10⁻¹, and 0.1875 × 10⁻¹ mol/liter) of α-NA and β-NA. The maximal velocity (V_max) and Michaelis–Menten (K_m) values to the both substrates (α-NA and β-NA) were determined from Lineweaver–Burk plots, and calculated by using Enzfit software (Elsevier) (Pan et al. 2009). The turnover numbers (K_cat) were calculated according to the molecular weights of the enzymes (crude homogenate and purified CarE) and each V_max. Substrate specificity constant (K_cat/K_m) was calculated according to K_cat and K_m.

**In Vitro Inhibition Assay.** In vitro inhibition of CarE in sixth instar *D. superans* larvae by dichlorvos, avermectins, and lambda-cyhalothrin were determined according to the method of Young et al. (2005). The crude and purified CarE were preincubated with 0.0001, 0.0010, 0.0100, 0.1000, and 1.0000 mg/ml of dichlorvos, avermectins, and lambda-cyhalothrin for 10 min at 35°C, respectively. The remaining specific activities of the CarE were assayed using α-NA as substrate, and IC₅₀ values of CarE to each pesticide were calculated by the linear equations. At the same time, crude and purified CarE dealt with distilled water as control, each assay was repeated three times.

**Results**

**Purification.** The result of ammonium sulfate gradient precipitation was shown in Fig. 1A. The CarE showed the maximum specific activities at 60% saturated ammonium sulfate. The CarE precipitated by 60% saturated ammonium sulfate was pooled into the sephadex G25 gel chromatography column. The CarE activity and protein content from each fraction were shown in Fig. 1B. The peak of CarE activity and protein content appeared at the 14th fraction. The CarE from sephadex G25 gel chromatography was pooled into diethylaminoethyl cellulose 52 ion-exchange chromatography column for further purification, 24 fractions had been collected by elution. Two peak values of specific CarE activity appeared at the 7th and 20th fractions while three peak values of protein content appeared at the 7th, 17th, and 19th fractions, respectively (Fig. 1B). The CarE purified by 52 ion-exchange chromatography column was collected and then poured into the hydroxyapatite affinity chromatography column for final purification. Among all 23 fractions collected by elution, both the maximum specific activity and protein content values of CarE appeared at the 19th fraction (Fig. 1D).

**CarE from sixth instar *D. superans* larvae was sequentially purified by 60% sulfate ammonium precipitation, G25 Sephadex, DEAE-52 ion exchange chromatography, and hydroxyapatite chromatography. By using α-NA as substrate, the specific activity and total activity of CarE crude homogenate were 0.376 μmol/(min-mg protein) and 287.284 μmol/min, respectively. After the CarE was purified with 60% sulfate ammonium precipitation, the specific activity was 0.571 μmol/(min-mg protein) and 1.528-fold higher than crude homogenate. As the CarE being purified by G25 Sephadex, 9.486-fold higher activity than crude homogenate and 3.567 μmol/(min-mg protein) of specific activity were obtained. However, the specific activity of CarE reached 6.979 μmol/(min-mg protein) through the third step purification with DEAE-52 ion exchange chromatography and 18.561-fold of the crude
homogenate after the purification. After CarE was purified by final hydroxyapatite chromatography, the special activity reached $52.019 \mu$mol/(min/mg protein), which was 138.348-fold of the crude homogenate (Table 1).

**SDS-PAGE and Molecular Weight Estimation.** SDS-PAGE of crude homogenate and final purified CarE was showed in Fig. 2. For purified CarE, one clear protein band appeared in the lane3 which molecular weight was between 70 and 100 kDa. The molecular weight of the purified CarE from sixth instar *D. superans* larvae was estimated about 84.78 kDa by the rate-flow value method. Analysis of crude homogenate by SDS-PAGE revealed several vague protein bands in the lane 1. The results showed that CarE in crude homogenate from sixth instar *D. superans* larvae had been purified using SDS-PAGE analysis.

**Kinetic Analysis.** Three kinetic parameters including the maximum reaction rate ($V_{\text{max}}$), Michaelis–Menten constant ($K_m$) and turnover number ($K_{\text{cat}}$) for the sixth instar *D. superans* larvae were listed in Table 2.

Using $\alpha$-NA and $\beta$-NA as substrates, $V_{\text{max}}$ values of final purified CarE were $5.041 \times 10^1 \pm 3.508$ and $1.756 \times 10^2 \pm 1.819 \times 10^1 \mu$mol/(min-mg protein), which were 205.503- and 143.113-fold compared with the crude homogenate, respectively. $K_m$ values of final purified CarE were $4.325 \times 10^{-5} \pm 1.310 \times 10^{-5}$ and $1.615 \times 10^{-4} \pm 2.315 \times 10^{-5}$ mol/liter, which were only 0.503- and 0.428-fold of crude homogenate. The $K_{\text{cat}}$ values of final purified CarE were 106.084- and 135.012-fold compared with the crude homogenate, respectively. Two substrates of both $\alpha$-NA and $\beta$-NA showed lesser affinity to crude extract than purified CarE. Three parameters determined by using $\alpha$-NA as substrate were lower than using $\beta$-NA as substrate. The results demonstrated that crude homogenate and purified CarE had more affinity to $\alpha$-NA than to $\beta$-NA.

**Inhibition Sensitivity of CarE to Pesticide.** The inhibitions of pesticides, dichlorvos, avermectins, and lambda-cyhalothrin, showed distinct dose–effect relationships between CarE activity and pesticide. The CarE activity decreased with the concentration increase of pesticides.

### Table 1. The properties of CarE at every purification stage

| Step                           | Total protein (mg) | Specific activity ($\mu$mol/min-mg protein) | Total activity ($\mu$mol/min) | Purification (-fold) | Yield (%) |
|-------------------------------|-------------------|--------------------------------------------|------------------------------|----------------------|-----------|
| Crude homogenate              | 764.052 ± 58.266  | 0.376 ± 0.008                              | 287.284                      | 1                    | 100       |
| 60 % Sulfate ammonium precipitation | 394.896 ± 73.564  | 0.571 ± 0.004                              | 225.407                      | 1.518                | 78.461    |
| Gel filtration (G25 Sephadex) | 34.912 ± 3.869    | 3.567 ± 0.057                              | 124.521                      | 9.486                | 43.344    |
| DEAE-52 ion exchange chromatography | 10.669 ± 0.661   | 6.979 ± 0.581                              | 74.456                       | 18.561               | 25.917    |
| Hydroxyapatite chromatography | 0.154 ± 0.019     | 52.019 ± 5.898                             | 7.991                        | 138.348              | 2.782     |

The values represented the means and standard errors from three replicates.
The linear regression equation was derived from $\log_{10}$ (pesticide concentration) versus the inhibition rate (%), then the median inhibition concentration ($IC_{50}$) was calculated by the linear equations. Results of $IC_{50}$ values of dichlorvos, avermectins, and lambda-cyhalothrin to crude homogenate and purified CarE were shown in Table 3. Among three pesticides, the $IC_{50}$ values of dichlorvos were $3.309 \times 10^{-3} \pm 2.946 \times 10^{-4}$ and $1.105 \times 10^{-1} \pm 7.344 \times 10^{-2} \mu g/ml$, respectively, showing that CarE from crude homogenate or final purified CarE was the most sensitive to dichlorvos. Sensitivity of CarE to pesticides was in decreasing order of dichlorvos $>$ avermectins $>$ lambda-cyhalothrin.

**Discussion**

Many esterases such as CarE from *B. mori*, *T. castaneum*, *O. surinamensis*, and *C. quinquefasciatus* had been purified by combinatorial methods (Ketterman et al. 1992, Murthy and Veerabhadrappa 1996, Haubruge et al. 2002, Lee 2011). There were dissimilar results for purified CarEs from different insect species by different methods (Murthy and Veerabhadrappa 1996, Haubruge et al. 2002, Lee 2011). In this study, CarE from sixth instar *D. superans* larvae was purified by a series of techniques, with 138.348-fold and 2.782% yield compared with the crude homogenate. The molecular weight of the purified CarE was approximately 84.78 kDa. There was much more impurities in the Lepidoptera insect larvae than in Coleopteran larvae body, which caused lower purification efficiency of CarE from sixth instar *D. superans* larvae.

The kinetic parameters of crude homogenate and final purified CarE were further studied in this study. The crude CarE showed lesser affinity to tested substrates both $\alpha$-NA and $\beta$-NA than purified CarE. Both crude homogenate and purified CarE showed more affinity to $\beta$-NA than to $\alpha$-NA; this result was dissimilar with $K_m$ values of esterase substrates determined on crude supernatants of adult females from susceptible and resistant strains of *Tetranychus urticae* (Van Leeuwen and Tirry 2007). The distinct result was because of enzymes from different organisms which showed different characteristics. As we know, turnover number ($K_{cat}$) indicates catalytic efficiency of the enzyme; the larger $K_{cat}$ indicated more rapid the catalytic events at the enzyme’s active site. In crude extract, non-target protein reduced the catalytic efficiency of CarE to substrates which caused that the $K_{cat}$ of crude extract was lower than purified CarE, whether using $\alpha$-NA or $\beta$-NA as substrate. Moreover, the $V_{max}$ values of crude extract were smaller than purified CarE because the crude extract was with lower catalytic efficiency.

CarEs are hydrolases that involved in the detoxification of many insecticides such as pyrethroid, carbamate, and organophosphorus. Inhibition of CarE activity by organophosphorus and carbamate insecticides has been used as a biomarker of pesticide exposure in many organisms (Wheelock et al. 2008). CarE could be inhibited by many specific pesticides. CarE activities present in *Biomphalaria glabrata*, *Lumbricus variegatus*, and *Lumbricus terrestris* were inhibited significantly by carbaryl and chlorpyrifos-oxon (Sanchez-Hernandez and Wheelock 2009, Vejares et al. 2010). In *Chilina gibbosa*, CarE activity was also inhibited by azinphos-methyl with 1,000 μg/liter of 50% inhibition concentration (Bianco et al. 2013). CarE activity in delta-methrin resistance strain of *A. gossypii* could be inhibited by S,S,S-tributyl phosphorotrithioate (Chang et al. 2010). The dichlorvos also has significant inhibition to CarEs in *Liposcelis bostrychophila* and *Liposcelis entomophila* (Wang et al. 2004), *Dysdercus koenigii* (Singh and Singh 1990), and *Spodoptera litura* (Muthusamy and Karthi 2011). However, the similar results were also obtained in this study. The CarE from sixth instar larvae of *D. superans* was highly inhibited in vitro by dichlorvos. Except for OP pesticides, the pyrethroids and microbial pesticides also affected CarE activity (Sogorb and Vilanova 2002, Heidari et al. 2005). Our results show that the sensitivity of CarE (crude extract and purified CarE) to three pesticides was in decreasing order of dichlorvos $>$ avermectins $>$ lambda-cyhalothrin. The avermectins and lambda-cyhalothrin showed lesser inhibition than dichlorvos. The pesticides with different mechanism and chemical structure showed distinct inhibition to CarE. In addition, CarEs from different source were all inhibited by the same pesticide (Fridovich 1969, Gao 1998,)

![Fig. 2. SDS-PAGE of crude homogenate and final purified CarE. Lane 1: crude homogenate; Lane 2: molecular-mass markers; and Lane 3: final purified CarE.](image-url)

**Table 2. Kinetic parameters of CarE in sixth instar *D. superans* larvae**

| Substrates          | $V_{max}$ (μmol/mg-min protein) | $K_m$ (mol/liter) | $K_{cat}$ (s) |
|---------------------|---------------------------------|------------------|---------------|
| Crude homogenate    |                                 |                  |               |
| $\alpha$-NA         | $4.253 \times 10^{-1} \pm 1.914 \times 10^{-2}$ | $8.601 \times 10^{-5} \pm 1.939 \times 10^{-5}$ | $5.490 \times 10^{-1} \pm 4.917 \times 10^{-2}$ |
| $\beta$-NA          | $1.227 \times 2.057 \times 10^{-1}$ | $3.775 \times 10^{-4} \pm 4.166 \times 10^{-5}$ | $1.708 \times 1.213 \times 10^{-1}$ |
| Final purified CarE |                                 |                  |               |
| $\alpha$-NA         | $5.041 \times 10^{1} \pm 3.508$ | $4.325 \times 10^{-5} \pm 1.310 \times 10^{-5}$ | $5.824 \times 10^{1} \pm 5.901$ |
| $\beta$-NA          | $1.756 \times 10^{2} \pm 1.819 \times 10^{1}$ | $1.615 \times 10^{-4} \pm 2.315 \times 10^{-5}$ | $2.306 \times 10^{2} \pm 1.624 \times 10^{2}$ |

The parameters [$V_{max}$, $K_m$, and $K_{cat}$] were estimated from Lineweaver–Burk plots and used to calculate the specificity constant of each substrate. The values represented the means $\pm$ standard errors from three replicates.
Table 3. Median inhibition concentration (IC₅₀) of pesticides to CarE-specific activity in sixth instar D. superans larvae

| Pesticides          | Crude homogenate | Final purified CarE |
|---------------------|------------------|---------------------|
| Dichlorvos          | 3.309 × 10⁻³     | 1.105 × 10⁻¹        |
| Avermectins         | 1.259 × 10⁻³     | 1.025 × 10⁻¹        |
| Lambda-cyhalothrin  | 4.529 × 10⁻⁴     | 1.951 × 10⁻¹        |

IC₅₀ values were calculated by the linear equations, the values represented the means and standard errors from three replicates; Means followed by a different letter in same column are significantly different by the Dunnet test (P < 0.05); Means followed by ** in same row are extremely significant difference by the t-test (P < 0.001).

Dengholm et al. (1999). The IC₅₀ values of crude homogenate and purified CarE from sixth instar D. superans larvae to three pesticides were different because there was more target protein CarE in purified CarE than crude extract in per unit protein. Non-target enzymes significantly impacted the biochemical characteristics of target enzymes as inhibitors (Mann and Keilin 1938, Vontas et al. 2002, Ma et al. 2011). The IC₅₀ value of dichlorvos to the final purified CarE was 33.39-fold of crude homogenate. IC₅₀ values of avermectins and lambda-cyhalothrin to the purified CarE showed slightly smaller than the crude homogenate. In insect organisms, other various non-CarE enzymes in crude homogenate such as acetylcholinesterase, multi-function oxidase, cytochrome P450 enzymes, and glutathione S-transferase significantly impacted on the biochemical properties of CarE. The competition inhibition mechanism of pesticides to various enzymes needed to be further studied.

In summary, these results will provide some basic information for understanding the physiological and biochemical properties of the CarE in D. superans. We will further study the CarE involved in toxicology mechanism in D. superans.

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