Response of Acetylcholinesterase to Insecticides in *Reticulitermes chinensis* Snyder (Isoptera: Rhinotermitidae)[1]

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Abstract The subterranean termite *Reticulitermes chinensis* Snyder is an important pest in China. We determined the inhibition of six selected insecticides to acetylcholinesterase (AChE) extracted from *R. chinensis*. Our results yielded half maximal inhibitory concentrations (I_{50}) for each of the insecticides to be 3.49×10^{-3} M for methomyl, 3.87×10^{-2} M for phoxim, 2.18×10^{-3} M for triazophos, 1.89×10^{-3} M for profenosos, 1.10×10^{-3} M for malathion, and 4.39×10^{-2} M for omethoate. Furthermore, the inhibitory activity of AChE by the six insecticides was increased with the increase of insecticide concentration from 3.3×10^{-7} to 5×10^{-3} M. These results provide a theoretical basis for the management of *R. chinensis*.

Key Words *Reticulitermes chinensis* Snyder, acetylcholinesterase, I_{50} values

Reticulitermes chinensis Snyder, like many of the species of termites, provides an ecological service but is a serious pest of wooden structures. It is widely distributed in China (Li et al. 2010, Wei et al. 2007). Management in structures is often with the use chemical insecticides, including organophosphates and carbamates whose mode of action is inhibition of acetylcholinesterase (AChE, EC3.1.1.7) (Corbett 1984, Fournier and Mutero 1994, Gunning et al. 1998). Although both are AChE inhibitors and cause very similar symptoms, they differ in the stability of the complex with AChE. Indeed, organophosphates phosphorylate serine residues of AChE in a nonreversible way, whereas the carbamylation of the same serine residue is less stable (Kuhr et al. 1976).

Acetylcholinesterase degrades acetylcholine (ACh) in the synaptic cholinergic system to terminate the excitatory effect of the neurotransmitter on the postsynaptic membrane, thus ensuring conduction of the neural signal within the organism. Acetylcholinesterase also is the target enzyme of organophosphate and carbamate insecticides in insects. Inhibition of AChE by these insecticides is through the phosphorylation and carbamoyl esterification of the active site of AChE that extends the duration of acetylcholine activity within neural synapses. This causes repeated firing of the neurons via hyperexcitability of acetylcholine receptors (AChRs) on postsynaptic membrane, thus inducing critical failures of nervous system by

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disturbing the choline-mediated synapse eventually resulting in death of the insect (Lin et al. 2005, Siegfried and Scott 1990).

To date, we have found no report on the inhibition of AChE by insecticides in *R. chinensis*. Therefore, the purpose of this study was to provide a theoretical basis for the management of *R. chinensis* by defining the inhibition of AChE by selected insecticides.

Materials and Methods

A colony of *R. chinensis* was established by collecting termites on Nanjing Forestry University campus. After collection, the termites were transported to the laboratory where they were maintained at 22 ± 1°C, a relative humidity of 90 ± 3%, and in constant darkness.

The insecticides were obtained from various companies: acetylthiocholine iodide (ATCh) from Fluka Chemical Company (Buchs, Switzerland); 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); methomyl (98%) from Hubei Sanonda Agrochemical Co., Ltd. (Hubei, China); phoxim (99%) and profenofos (90%) from Tianjin Pesticide Co., Ltd. (Tianjin, China); triazophos (92%) from Jiangxi Kaifeng Chemical Co., Ltd. (Jiangxi, China); malathion (95%) from Hebei Century Pesticide Co., Ltd. (Hebei, China); and omethoate (92%) from Hangzhou Qingfeng Agrochemical Co., Ltd. (Hangzhou, Zhejiang, China). All other chemicals were of analytical grade and were purchased from commercial sources.

For the assays, a termite sample was homogenated using ice-chilled phosphate buffer (pH 7.5, including 0.1% Triton X-100) in an ice bath and then centrifuged at 15,000 × *g* for 20 min at 4°C. The resulting supernatants were used for the subsequent AChE activity assay.

Acetylcholinesterase activity was measured using the Ellman method (Gao 1987). Briefly, in a final volume of 145 μl, 100 μl enzyme and 45 μl ATCh were incubated at 40°C for 10 min. The reaction was stopped with 1.45 ml DTNB as the thiol indicator. The optical density (OD) was read immediately at 412 nm using a spectrophotometer.

The mixture of inhibitors and enzyme was incubated for 10 min at 40°C and then used to determine AChE enzyme activity. The assays were used at seven different concentrations for each insecticide. The mixture containing the corresponding concentration of acetone was used as a control. The I_{50} values, concentrations of inhibitors required to reduce the reaction rate by 50%, were determined by linear regression of the average percent activity on the log of the inhibitor concentration (Neal and Berenbaum 1989). The experiment was performed in triplicate for each inhibitor.

Results

The dose–effect relationship of six insecticides (methomyl, phoxim, triazophos, profenosos, malathion, and omethoate) to AChE from *R. chinensis* is shown in Fig. 1. The I_{50} values of methomyl, phoxim, triazophos, profenosos, malathion, and omethoate were calculated according to the dose–effect relationship, which were

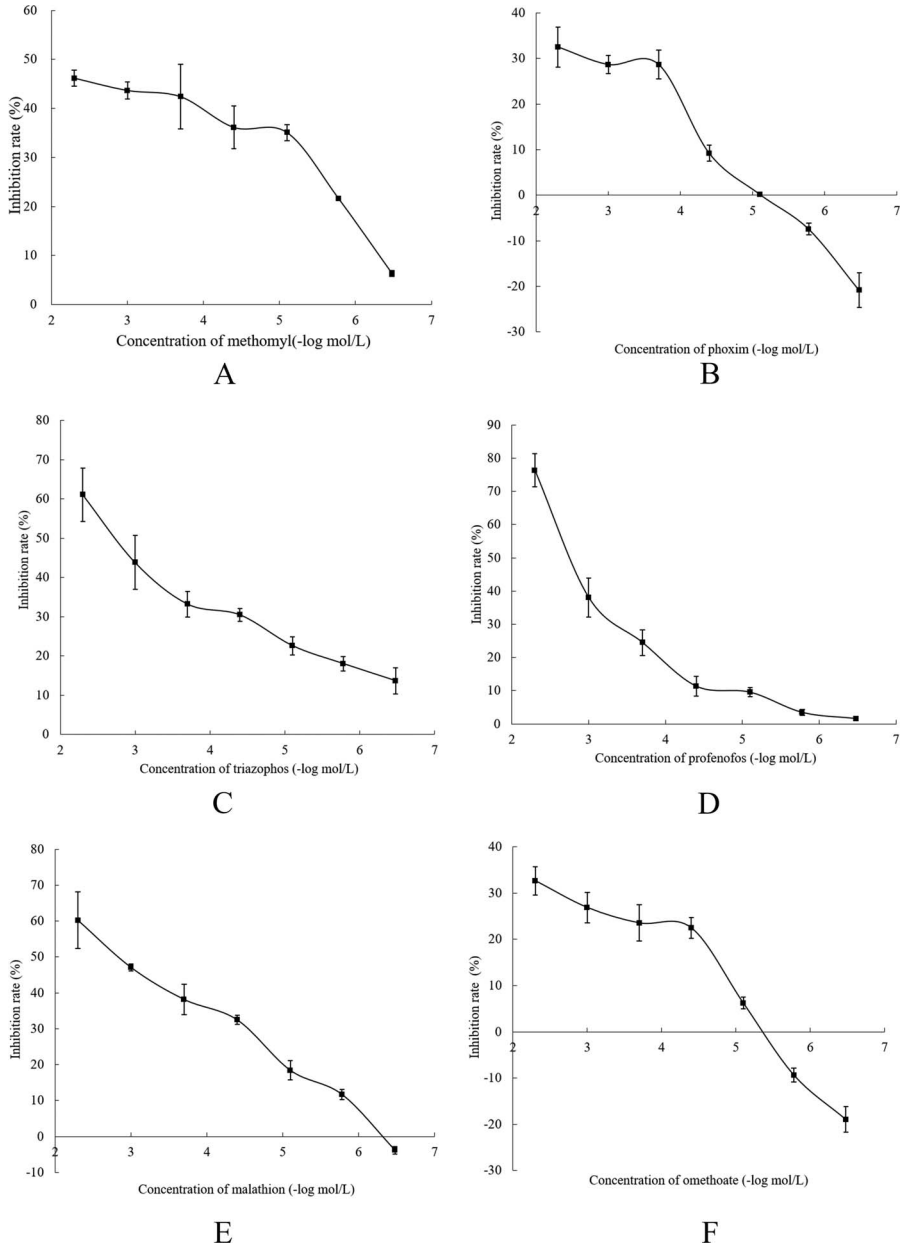

Fig. 1. Inhibitory response of AChE from *R. chinensis* by methomyl (A), phoxim (B), triazophos (C), profenofos (D), malathion (E), and omethoate (F).

Table 1. I_{50} values of six insecticides to AChE from *R. chinensis*.

Insecticides	I_{50} (M)	Equations	Correlation Coefficients
Methomyl	3.49×10^{-3}	$y = 8.7561x + 71.52$	0.8471
Phoxim	3.87×10^{-2}	$y = 13.372x + 68.89$	0.9499
Triazophos	2.18×10^{-3}	$y = 10.488x + 77.918$	0.9284
Profenofos	1.89×10^{-3}	$y = 15.827x + 93.1$	0.8044
Malathion	1.10×10^{-3}	$y = 14.496x + 92.884$	0.9885
Omethoate	4.39×10^{-2}	$y = 12.539x + 67.02$	0.9126

3.49×10^{-3}, 3.87×10^{-2}, 2.18×10^{-3}, 1.89×10^{-3}, 1.10×10^{-3}, and 4.39×10^{-2} M, respectively (Table 1). The order of inhibition potential of the inhibitors from the highest to the lowest was malathion, profenofos, triazophos, methomyl, phoxim, and omethoate.

Discussion

Acetylcholine-mediated neurotransmission is fundamental for nervous system function, and AChE is one of the most prominent constituents of central cholinergic pathways (Maria et al. 2006). It is involved in the hydrolysis and inactivation of acetylcholine, thereby terminating the synaptic action of acetylcholine and regulating the concentration of the transmitter at the synapse in the central and peripheral nervous system (Mesulam et al. 2002). In a word, modulation of synaptic activity is largely governed by the enzymatic activity of AChE (Quinn 1987).

Organophosphate and carbamate insecticides have been widely used for control of insect pests. Acetylcholinesterase activity is considered as organophosphate toxicity indicator (Aguiar et al. 2004, Chuiko 2000), which works as the action target enzyme of organophosphate and carbamate insecticides in insects. The inhibition of AChE activity by insecticides increased the release of ACh and the cumulation of synaptic cleft. The excess ACh caused an enhancement of the cholinergic transmission through activation of the synaptic nicotinic and muscarinic receptors (Michela et al. 2005), which inevitably upset the balance of physiology and biochemistry, leading to the death of insect. Therefore, study of characteristics of insect AChE and its inhibition by organophosphate and carbamate insecticides is a foundation to comprehension of the mode of action and the resistance mechanism of these insecticides (Guo 2007). In this study, methomyl is a carbamate, whereas phoxim, triazophos, profenosos, malathion, and omethoate are organophosphates.

Organophosphorus compounds were initially developed as warfare nerve agents; their toxicity was caused by the inhibition of the enzyme AChE. A large number of studies have shown that the main difference in AChE reactivators is based on their structural characteristics (Vasil et al. 2013). Molecular asymmetry structure of profenosos represented a development direction of thiophosphate from symmetric structure to asymmetric structure. Triazophos represented another development direction, from original benzene ring structure or general hydrocarbon

to heterocyclic structure, because of its heterocyclic structure (Wang 1995). It is found that heterocyclic compounds play an important role in insect control due to their diversity of structures and extensive ways of action. Approximately 70% of all agrochemicals that have been introduced to the market within the last 20 years bear at least one heterocyclic ring (Clemens 2013).

The degree of toxicity of different insecticides to one species varies considerably, as does the susceptibility of different species to the same compounds. In this study, I_{50} values of six kinds of insecticides to AChE were determined in *R. chinensis*. The I_{50} values expressed the inhibitory degree of AChE by insecticide, for example, the smaller the I_{50}, the higher the inhibitory degree. There were significant differences in the inhibition of AChE from *R. chinensis* by the six insecticides, which meant that the inhibitory degree was affected not only by the dose of insecticides but also by the kind of insecticide. In comparison, the malathion was the most potent inhibitor of AChE with an I_{50} value of 1.10×10^{-3} M. These results suggest that phoxim, triazophos, profenosos, and malathion also were effective AChE inhibitors and have potential in the control of *R. chinensis*.

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