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Acetylcholinesterase-Inhibiting Insecticide Residues in Commonly Consumed Fried Edible Insects

Thanatchaya Kanthawongwan[1], Wandee Wattanachaiyingcharoen[2], and Pantip Hinhumpatch[1]*

[1]*Division of Environmental Health, Faculty of Public Health, Naresuan University, Phitsanulok, Thailand*
[2]*Department of Biology and Center of Excellence for Biodiversity, Faculty of Science, Naresuan University, Phitsanulok, Thailand*

* Corresponding author : pantiph@nu.ac.th
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Abstract

Insecticide contaminations in food are increasing public health concern. In Thailand, fried edible insects are commonly taken as snacks and probably part of main dishes in some communities. However, the origins of insect which use as raw ingredient in fried edible insects are in concern as it would be collected from insecticide contaminated areas. Therefore, the extent of insecticide residues contained in commonly consumed fried insects should be considered, especially from those frequently use insecticide; organophosphate and carbamate residues. Five commonly consumed insect species (grasshopper, house cricket, short-tailed cricket, giant water bug, and silkworm pupae) were collected from five street vendors in the city area of Phitsanulok Province, Thailand. The degree of toxicity of organophosphate and carbamate insecticides was determined using enzyme inhibition reaction with GT-insecticide residual test kit. The results showed that the degree of carbamate and organophosphate toxicity differed between insect types. The highest percentage of enzyme inhibition was found in grasshoppers (53.67%) followed by house crickets (37.90%), giant water bugs (27.17%), short-tail crickets (23.33%), and silkworm pupae (20.23%). The majority of commonly consumed fried insects (72%) contained levels safe for human exposure. Grasshoppers and house crickets were the only types of insects with toxicity levels unsafe for human exposure (unsafe levels found in 80% and 60% of vendors, respectively). The degree of insecticide residue toxicity detected in consumed insects in Phitsanulok, Thailand, could potentially increase the risk of poisoning from human consumption.

Keywords: Organophosphate and carbamate; Insecticide; Commonly consumed insect; Acetylcholinesterase inhibitor

1. Introduction

Insects and their products have been recognized as food and medicine for long period. Globally, more than 2 billion people, including a great number of children and adults, consume insects as part of a traditional diet (FAO, 2013). It has long been reported that insects are highly nutritious and could be consumed as an alternative food source of protein, vitamins, fiber, and minerals (Menozzi *et al*., 2017). The FAO suggested that for countries which short of protein sources, insects consumption should be alternatively considered for food security (FAO, 2013). The consumption of insects remains traditionally typical, as snacks or additional foods, in many part of the world, including Central America, Africa, Europe, and Asia (Mlcek, *et al*., 2014). However, this tradition is changing as insect consumption has played a major role in daily life consumption in many regions of the world.

In Thailand, consumption of insects used to be limit only in some rural areas. Nowadays, fried edible insects vendors can be seen throughout the country. It is well known that the most commonly consumed fried insects in Thailand were silkworm pupae (order Lepidoptera), giant water bugs (order Hemiptera), grasshoppers (order Orthoptera), short-tail crickets (order Orthoptera), and house cricket (Order Orthoptera) (Siriamornpun and Thammapat, 2008). Some types of commonly consumed insects, such as silkworm pupae and crickets, can be farmed commercially and directly supply to the sellers. In contrast, grasshoppers have been recognized as one of the major pests in Thailand. The uses of insecticides are common and practical for most farmers, hence, increasing chance of insecticide residues in both insect pests and their products. As some suppliers collect grasshoppers from agricultural areas therefore, risks of insecticide contaminations in those insects are increasing.

The amount of agrochemical use in Thailand has increased in recent years, with more than 100,000 tons of insecticides being imported into Thailand (Panuwet *et al*., 2012; Jantawongsir *et al*., 2015). The two main classes of frequently imported insecticides are organophosphates and carbamates. Several reports have confirmed that organophosphate and carbamate toxicity can manifest in both short term and long term health effects, especially in children who are more susceptible to those insecticide-related syndromes. Previous reports have shown that organophosphate exposure may have a substantial impact on the growth and development of children, symptoms of insecticide poisoning from food include abdominal cramps, vomiting, nausea, and headache (Raanan *et al*., 2016; Yu *et al*., 2016). When consumed in large quantities or with repeated exposures, the toxicity can lead to death. Hence, health risks related to insecticide poisoning in food have been deliberated at the international level.

The organophosphates and carbamates insecticide were known as a cholinesterase inhibitor and the inhibition process exerted their effects rapidly in both insects and humans. Cholinesterase is one of the most important enzymes which involve in maintaining a proper function of nervous system. Enzyme inhibition results in a greater amount of acetylcholine that cannot be hydrolysed to cholin. The degree of toxicity of organophosphate and carbamate insecticides can be simply determined via the GT-insecticide residual test kit, which is based upon an enzyme inhibition assay in order to detect functional inhibition of the cholinesterase enzyme. The colorimetric assay can be used to quantify the degree of toxicity as a percentage of inhibition. A sample with toxicity less than 50% is considered as safe for consumption (Thoophom, 2007; Buakham *et al*., 2012).

Phitsanulok Province is located in lower Northern Thailand where agriculture is the principal component of the local economy. One of the province's signature is fried edible insects that attracted many tourists and commonly consume by local people. Although some edible insects are supplied from commercial farms, many of them are collected from agricultural fields. Thus, these collections practice may result in insecticide contamination in those raw edible insects. In Kuwait, high concentrations of organophosphate insecticide residues were found in the edible insects, especially in locusts from food suppliers (Saeed *et al*., 1993). Recent study showed that a single exposure of edible insect larvae to vegetable contaminated with insecticides can result in significant insecticide residues in the insects (Houbraken *et al*., 2016). So, if there are contaminations of insecticide residues in fried edible insects, it would potentially lead to negative health impacts to the consumers. Determining the degree of toxicity of insecticide residues, emphasize on organophosphate and carbamate, in commonly consumed fried insects will lead to the awareness of public in insecticide cycling in our food chain, and will decrease the use of harmful insecticide residues which causes public food safety for the consumers in Thailand and other countries with the similar practices.

2. Methods

2.1 Sample collections

During October 2016, a total of 25 samples of 5 different types of fried edible insects; grasshoppers, silkworm pupae, giant water bugs, short-tail crickets, and house crickets were collected from 5 street vendors located in Muang District, Phitsanulok Province, Thailand. Three hundred grams of each insect sample were kept separately in polyethylene bags and immediately analysed.

2.2 Analysis of toxicity

The degree of toxicity of organophosphate and carbamate was measured using an enzyme inhibition reaction with a commercial kit (GT-insecticide residual test kit,). This method obtained 92.3% sensitivity, 85.1% specificity and 87.1% accuracy with 70.6% positive predictive value and 96.6% negative predictive value. The percentage of acetylcholinesterase inhibition less than 50% is considered as safe for consumption (Thoophom, 2007).

Samples were extracted and processed as previously described by Buakham *et al*. (2012). Briefly, 5 g of sample were chopped and added to 5 mL of dichloromethane. The sample was then shaken for 1 minute and kept at room temperature for 15 minutes to extract insecticide residues from insect specimens. One mL of extracted sample was transferred into the new tube and 1 mL of 5% ethanol in water was added. The extracted sample was then separated. The supernatant was collected by evaporating the bottom layer with an air pump at 34°C for 3 minutes.

The processed sample was then subjected to an enzyme inhibition assay. Two hundred and fifty μL of extracted samples were transferred into a clean glass tube and 500 μL of cholinesterase (GT-1) were added at 36 °C. After 10 minutes, 250 μL of acetylcholine (GT-2) was added and the reaction was set at 36 °C for 60 minutes. One mL of hydroxylamine (GT-3), 500 μL of hydrochloric acid (GT-4), and ferric chloride (GT-5) were added. The sample was mixed thoroughly, filtered and subjected to spectrophotometry at 540 nm wavelength.
The results were expressed as a percentage of acetylcholinesterase inhibition. The calibration curve for acetylcholinesterase inhibition was established using various concentrations of acetylcholine standards, ranging from 0-100% of inhibition, with a correlation coefficient of 0.9992.

2.3 Statistical analysis

Data analysis was carried out with statistical software. The Shapiro-Wilk test was used to evaluate the quantitative variables normality. Consequently, equality of variance was determined using Levene's test. The differences among mean percentages of acetylcholineesterase inhibition were determined using one way analysis of variance (ANOVA), followed by a post-hoc Tukey test. A p-value less than 0.05 was considered to represent statistical significance.

3. Results and Discussion

The present study aimed to determine the degree of carbamate and organophosphate toxicity found in commonly consumed fried insects via enzyme inhibition technique. The degree of toxicity among the insect samples differed between the various types of insects studied (Table 1). Enzyme inhibition was initiated (in decreasing order) in grasshoppers, house crickets, giant water bugs, short-tail crickets, and silkworm pupae. Based on ANOVA, the percentage of acetylcholinesterase was statistically significant among insects studied ($F = 4.215$, $p = 0.026$). Acetylcholinesterase inhibition was significantly higher in grasshoppers than in silkworm pupae (~3 -fold, $p = 0.033$). The majority of grasshoppers and house cricket samples were found to have unsafe levels of acetylcholinesterase inhibition. Unsafe levels were found in insects from 80% of grasshopper vendors and 60% of house cricket vendors (Figure 1).

In Phitsanulok Province, grasshoppers are considered as major agricultural pests with house crickets are classified as occasional pests. Famers commonly apply insecticide, i.e. organophosphate and/or carbamate, for controlling of these pests. Nonetheless, a study showed that house crickets exposed to lipophilic compounds (i.e. polybrominated diphenyl ethers) for 28 days accumulated a large amount of these compounds in the body (Gaylor *et al*., 2012). This finding may indicate increased bioaccumulation of highly lipophilic compounds in house crickets. Nine different insecticide residues were detected in honeybees in Poland; acetylcholinesterase inhibitor was detected most frequently (Kiljanek *et al*., 2017). It has recently been determined that insecticides such as organophosphates that contains a high octanol/water partition coefficients, are taken up rapidly but slowly excreted in insects (Houbraken *et al*., 2016; Chiou *et al*., 1977). This mechanism resulted in bioaccumulation of insecticides in insects, which refer as primary consumer in food chain. Consequently, these insecticides that accumulate in insects are likely entering the human food chain, potentially causing adverse health risks.

In this study, we found all samples of short-tail crickets, giant water bugs, and silkworm pupae were at safe levels of toxicity (figure 1). The short-tail cricket is known as an agricultural pest, but is mostly found in Northeastern Thailand. Collection these insects from fields and prolong transportation, including lengthen time-to-consumer may decrease bioaccumulation of insecticides in these insects. Moreover, the high total lipid content of short-tail crickets (Raksakantong *et al*., 2010) may alter metabolic rate, affecting retention of insecticide residues. However, the retention of insecticides in insects depended upon their chemical properties and insect's xenobiotic metabolism.

Giant water bugs and silkworm pupae are not considered agricultural pests in Thailand. However, they are popularly-consumed insects throughout the country, and some of them might come from commercial insect farming (Ratanachan, 2009). Moreover, the giant water bugs are normally collected from local water sources, while the silkworm pupae are by-product from silk industry as they were discarded after silk reeling. Both of them may have less interact with insecticide applications. These may explain why they contain fewer insecticides residues.

The degree of acetylcholinesterase inhibition in various commonly consumed fried insects from five street vendors located in Muang District, Phitsanulok Province, Thailand was determined. The obtained results indicated that majority of commonly consumed fried insects contained insecticide toxicity at safe level. However, those insects that were recognized as a pest i.e. grasshopper and house cricket contained residues at unsafe level. These findings suggested that the degree of insecticide residue toxicity detected in commonly consumed fried insects could potentially increase the risk of poisoning from human consumption. Therefore, the consumer should take into consideration that dosage and frequency of intake of edible fried insects that labeled as agricultural pest may have the negative impact on health. The toxicity of insecticide exposure can be manifested differently upon various populations, depending on several factors such as frequently of exposure, dose and window of susceptibility.

Although this study used enzyme inhibition as a proxy for organophosphate and carbamate insecticide toxicity, it may be possible that other chemicals exhibit the same effect. Future studies should also address background enzyme inhibition found in insects not exposed to insecticides. These results can be used as a surrogate data for food safety in consumption of commonly consumed fried insects.

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Table 1. Percentage of acetylcholinesterase inhibition among various types of commonly consumed fried insects (n=5)

Type of insect (order)		Acetylcholinesterase inhibition (%)				
		Mean	SD	Median	Min	Max
Grasshopper	(Orthoptera)	53.67	6.22	45.56	36.20	65.20
House cricket	(Orthoptera)	37.90	9.04	34.10	24.50	55.10
Short-tail cricket	(Orthoptera)	23.33	5.54	19.20	16.50	34.50
Giant water bug	(Hemiptera)	27.17	7.11	28.60	14.20	38.70
Silkworm pupae	(Lepidoptera)	20.23	6.87	27.00	6.50	27.00

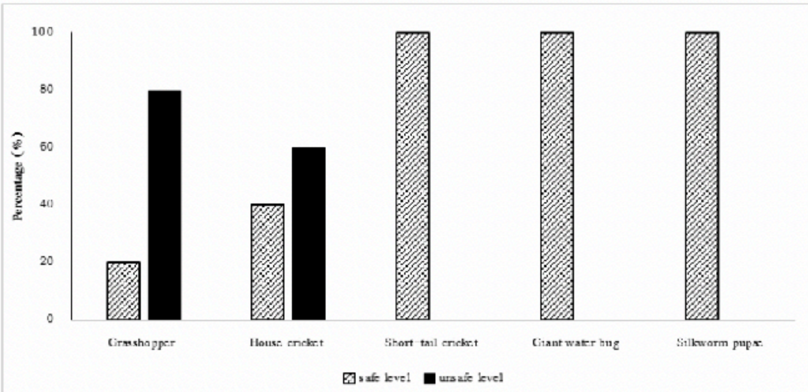

Figure 1. Proportion of safe and unsafe (>50% acetylcholinesterase inhibition)

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