# ALTERNATIVE BIO-PESTICIDE FOR GOLDEN APPLE SNAIL (*Pomacea canaliculata*)

**Khobkul Nongnutch[1,*] ana Jakkaphun Nanuam[2]**

*Received: July 24, 2015; Revised: November 24, 2105; Accepted: February 16, 2016*

## Abstract

**As generally known, the expanding of world population requires more food supply. That phenomenon results in the intensive of agriculture in both agricultural area and yield. Consequently, the agriculturist also applied more pesticide to protect their product. Excessive applied pesticide results in negative effects on the organism. Thus, the aim of this study was to evaluate an alternative bio-pesticide to control agro-pests. Sensitive plant (*Mimosa pudica* L.), Bitter bush (*Adriana quadripartita* (Labill.) Muell.Arg.) and Singapore daisy (*Sphagneticola trilobata* (L) Pruski.) are local plant easily found. Some local agriculture uses them to control the pest but it is lack of scientific evidence to support and improve the usage. The ethanol extracted of these three plants was applied as the pesticide to control golden apple snail (*Pomacea canaliculata*) and its effectiveness was then compared to niclosamide and metaldehyde. The results showed that bitter bush results in highest mortality and accompanied by singapore daisy and sensitive plant. After the exposure, the snail which exposed to bitter bush took a long time than singapore daisy and sensitive plant to recover which identified by its acetylcholinesterase level. However, the effectiveness of these three extracts was lower than niclosamide and metaldehyde. Thus, it should improve its effectiveness before applying in the field.**

**Keywords: Bio-pesticide, *Pomacea canaliculata*, acetylcholinesterase**

## Introduction

As world population growing, they also require more food and other supportive compounds. Some studies indicate that world population will reach 7.70 and 8.24 billion in 2020 and 2030, respectively (Rein, 2004) which results in more food being required. In

[1] *School of Science and Mathematics, Faculty of Industrial and Technology, Rajamangala University of Technology Isan Sakon Nakhon Campus, Sakon Nakhon, 47160, Thailand. E-mail: khopkul25@gmail.com*
[2] *Faculty of Science and Social Sciences, Sa Kaeo campus, Burapha University, 27000, Thailand. E-mail: jakkaphu@buu.ac.th*
[*] *Corresponding author*

modern agricultural food production, the agriculturist applies agro-chemical; fertilizer and pesticide, for better yield. In some case, they may apply excessive chemicals especially the pesticide. After pesticide finds its way to the environment in both aerosol and solution forms, it may harm the useful organism (Nanaum *et al.*, 2013).

Metaldehyde and niclosamide are molluscicide generally used for controlling mollusk and snail in paddy field (Coloso *et al.*, 1998; Liu *et al.*, 2006). In excessive metaldehyde application, it makes irreversible damage in mucous cells resulting in excessive mucus production which disturbs absorptive cells of the hepatopancreas and ultimately causing mortality (Coloso *et al.*, 1998). For niclosamide, it is commercially molluscicide recommended by the World Health Organization (Webbe, 1987). However, it is a synthetic compound thus it may cause inadvertent harmful effect on non-target organism. Thus, it is looking for natural molluscicides for using instead on the synthetic one.

Sensitive plant (*Mimosa pudica* L.), Bitter bush (*Adriana quadripartita* (Labill.) Muell.Arg.) and Singapore daisy (*Sphagneticola trilobata* (L) Pruski.) are three plant extract which is locally used for controlling snail pest in Thai paddy field especially golden apple snail (*Pomacea canaliculata*). This useful application may be caused by some compounds in its extracts such as anthraquinone which found in golden shower extracts (Nanuam *et al.*, 2013). This oxygenated polycyclic aromatic hydrocarbon has been identified as weak AChE inhibitor (Kang and Fang, 1997). AChE is an enzyme controlling the level of acetylcholine (ACh) which is the primary neurotransmitter in the sensory and neuromuscular systems. AChE degrades ACh to choline and acetic acid for controlling the level of ACh at cholinergic synapses (Mukherjee *et al.*, 2007). As AChE activity is inhibited, a continuous and excessive stimulation of the nerve/fibers are occurred without control until paralysis, convulsion and death (Ezemonye and Ikpesu, 2011)

However, its effectiveness is quite weak causing the agriculturist has to apply in high volume. Thus, it should be scientific study for improve the effectiveness in pest control. The aim of this study was to evaluate three plant extracts effectiveness in controlling pest snail compared to commercial synthetic molluscicide.

## Experimantal to Materials and Methods

Plant materials used in this study was Sensitive plant (*Mimosa pudica* L.), Bitter bush (*Adriana quadripartita* (Labill.) Muell. Arg.) and Singapore daisy (*Sphagneticola trilobata* (L) Pruski.) collected from demonstrating field of Rajamangala University of Technology, Isan Sakon Nakhon Campus, Thailand. Plant extracts preparation was intensively described in Nanuam *et al.* (2013). In brief, plant materials were nicely washed for removing contaminants or unwanted matters. Then, it was shade dried and crushed to coarse powder. For extraction, 500 g of plant powder and 1 L of 95% ethanol was filled in Erlenmayer flask (2 L) capped by aluminium foil and parafilm and leave for 5 days in dark. Then, the solvent was evaporated and the residue was kept in desiccator. Before experiment performed, 1 g/L of stock solution was prepared by mixing 1 g of plant extracts, 200 ml of dimethyl sulfoxide (DMSO) and 300 ml of ethanol in 500 ml of dechlorinated water.

Golden apple snails (*P. canaliculata*) were collected from the demonstration field of Rajamangala University of Technology, Isan Sakon Nakhon Campus. After acclimatization for 3 days, 5 snails were placed in each treatment box (14×40×48 cm) simulating real paddy fields. Sub-lethal level of each agent was identified before apply in the experimental box.

The experiment was divided to 6 groups; (1) adding extracts of sensitive plant (50% v/v of extract/ethanol), (2) adding extracts of bitter bush (50% v/v of extract/ethanol), (3) adding extracts of singapore daisy (50% v/v of extract/ethanol), (4) adding niclosamide and

(5) adding metaldehyde. The exposure times were: 0, 10, and 20 min, and 1, 6, 12, 24, 48, 72, or 96 h. In each tested exposure time, the snail was collected and measured its AChE activity in gill as the method described by Ellman *et al*. (1961). For the control, the experimental box was filled with only dechlorinated water.

For AChE activity recovery testing, exposed snails from each of the 5 treatments and the control (after 96 h of exposure) was transferred to the clean environment filled only with dechlorinated water. The recovery duration tested was 5, 10 and 30 min., and 1, 3, 6, 12, 48, 72, and 96 h., and 7, 15, and 20 d. One way analysis of variance (ANOVA) was used to test the differences between AChE activities from each treatment.

## Reslts and Discussion

After AChE inhibitory capability of plant extracts and two agro-chemicals was tested, the results showed that there was clear significant different ($p>0.05$) in the inhibition between plant extracts and the chemicals whereas there was not found in between plant extracts (Figure 1). For niclosamide, AChE activity decreased from 46.86±0.50 to 4.90±3.33 nmoles ACTC/min/mg protein and from 46.86±0.50 to 5.00±2.53 nmoles ACTC/min/mg protein in metaldehyde exposure.

For plant extracts, the highest decreasing in AChE activity occurred in the snail exposed to bitter bush which decrease from 46.86±0.50 to 5.95±1.12 nmoles ACTC/min/mg protein and the lowest decreasing found in sensitive plant exposure; from 46.86±0.50 to 11.17±1.73 nmoles ACTC/min/mg protein.

From Figure 2, it is clearly shows that AChE inhibitory capability of the strongest plant extracts (bitter bush) was significant lower ($p>0.05$) than that of the stronger commercial molluscicide (niclosamide) in this study.

For recovery, AChE activity level in the exposed snails stayed quite low until 48 h after transfer to the clean environment. After recovery duration (20 days), the lowest AChE activity level for plant extracts found in the snail exposed to bitter bush which was 5.95±1.65 nmoles ACTC/min/mg protein and the highest found in sensitive plant exposure (11.17±1.58). Whereas, it

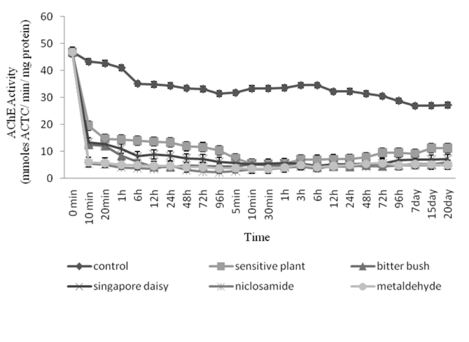


**Figure 1.AChE Ativities (means ± S.D; n = 5) in golden apple snail after exposure**

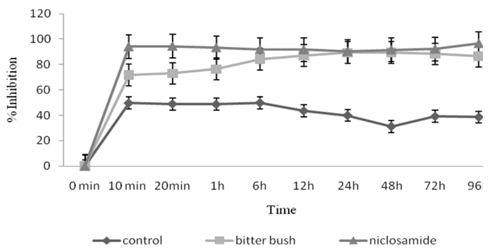


**Figure 2. AChE inhibition percentages (means ± S.D; n=5) in golden apple snail after to bitter bush extracts and niclosamide**

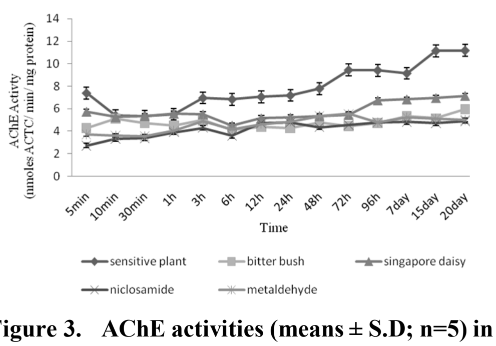


**Figure 3. AChE activities (means ± S.D; n=5) in golden apple snail in recovery period**

was 4.90±2.74 nmoles ACTC/min/mg protein for niclosamide and 5.00±0.56 nmoles ACTC/min/mg protein for metaldehyde.

Our results is in agreement with the study of Bakry (2009) which studied on the usage of some plant extracts to control *Biomphalaria alexandrina* snails and found that plants in the family of Euphorbiaceae in which bitter bush is having AChE activity inhibitory capability. For sensitive plant, the study of Wink (2013) which evaluated secondary metabolites in legumes (Fabaceae) also indicate that the plant in Fabaceae family was classified as AChE inhibitor. And, the study of Seo *et al.* (2014) which studied on Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential oils and their constituents against Japanese termite (*Reticulitermes speratus* Kolbe) indicated that the plant in the family of Asteraceae in which singapore daisy in can inhibit AChE acitivity.

## Conclusions

The AChE activity inhibitory capability of ethanolic extracts of three plants, especially bitter bush, showed in this study can be used as strong supporting scientific evidence for using as alternative plant molluscicides for controlling golden apple snail in the paddy field. However, the effective constituent for AChE inhibition in each plant should be identified for improving its effectiveness.

## References

Coloso, R.M., Boriongan, I.G., and Blum, R.A. (1998). Use of metaldehyde as a molluscicide in semi-commercial and commercial milk fish ponds. Crop Protect., 17(8):669-674.

Ellman GL, Courtney KD, Andres V, and Feathertone RM. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharma., 7:88-95.

Ezemonye, L.I.N. and Ikpesu, T.O. (2011). Evaluation of sub-lethal effects of endosulfan on cortisol secretion, glutathione S-transferase and acetylchloinesterase activity in Clarias gariepinnus. Food Chem. Toxi., 49:1898-1903.

Fayez A. Bakry. (2009). Use of some plant extracts to control Biomphalaria alexandrina snails with emphasis on some biological effects. Pestic. Biochem. Phys., 95:159-165.

Nanuam, J., Nongnutch, K., Somnuek, C., and Cheevaporn, V. (2013). Preliminary Screening a Potential AChE Inhibitor in Thai Golden Shower (Leguminosae mimosoideae) Extracts. EnvironmentAsia, 6(2):47-50.

Kang J.J. and Fang, H.W. (1997). Polycyclic aromatic hydrocarbons inhibit the activity of acetylcholinesterase purified from electric eel. Biochem Biophys Res Commun., 238(2):367-369.

Mukherjee, P.K., Kumar, V., Mal, M., and Houghton, P.J. (2007). Acetylchlolonesterase inhibitors from plants., 14:289-300.

Taagepera, R. (2004). A world population growth model: Interaction with Earth's carrying capacity and technology in limited space. Technol. Forecast. Social Change, 82:34-41.

Seo, S.M., Kim, J., Kang, J., Koh, S.H., Ahn, Y.J., Kang, K.S., and Park, I.K. (2014). Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential oils and their constituents against Japanese termite (*Reticulitermes speratus* Kolb). Pestic Biochem Physiol., 113:55-61.

Webbe, G. (1987). The toxicology of molluscicide screening and evaluation, Bull. World Health Organ, 33:567-581.

Liu, W.-H., Chio, Y.-w., Huang, D.-J., Liu, M.-Y., Lee, C.-C., and Liu, L.-L. (2006). Imposex in the golden apple snail (Pomacea canaliculata) in Taiwan. Sci. Total Environ., 371:138-143

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). South African J. Botany, 89:164-175.