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Synergism and Detoxification Mechanism of Crude Sugar Apple Seed Extract in *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)

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ABSTRACT

Synergism of maleic acid (MA), piperonyl butoxide (PBO) and triphenyl phosphate (TPP) combined with sugar apple seed extract on the mortality of *T. truncatus* adult females was performed by the spray method. Crude sugar apple hexane extract+0.5% MA induced the highest toxicity to *T. truncatus* (LC_{50}=2.27%), followed by crude hexane extract (LC_{50}=3.52%), crude hexane extract+0.2% PBO (LC_{50}=3.79%) and crude hexane extract+0.2% TPP (LC_{50}=4.13%). *Tetranychus truncatus* mites exhibited similar esterase activity levels (0.09-0.13 n mole/min/mg protein) in all treatments including the control (untreated mites). Mites in the control exhibited relatively higher GST activity (0.32 n mole/min/mg protein) compared to all other treatments (0.14-0.27 n mole/min/mg protein). Both synergism effect and detoxification mechanism suggested that glutathione S-transferase might play an important role in sugar apple seed extract degradation.

Key words: *Tetranychus truncatus*, synergist, detoxification mechanism, sugar apple extract

INTRODUCTION

Insecticidal properties of plant extract, to solve the problem of insecticide resistance of agricultural pests, receive increasing attention in Thailand. Crude seed extract of sugar apple (*Annona squamosa* L.) has insecticidal properties, antimalarial, cytotoxic and immunosuppressive activities (Chungsamarnyart *et al*., 1992; Hopp *et al*., 1997; Morita *et al*., 2000). Crude seed extract of this plant contains many chemical compounds that are able to exhibit high toxicity, repellency against several insect pests and also influence their oviposition (Leatemia and Isman, 2001). In the past, some chemicals were used to combine with insecticides including crude plant extracts in order to increase their effectiveness (Visetson and Milne, 2001). These chemicals are known as synergist forming active intermediates that generate relatively stable complexes with enzymes and making the enzyme inactive. Thus, synergists play an important role in insecticide degradation (Wilkinson, 1983). *Tetranychus truncatus* is a serious pest of many plants in Thailand and feeds on 62 host plants in Asian countries (Bolland *et al*., 1998). Short life cycle and high reproductive rate of mites enable them to develop resistance faster than most insects (Guo *et al*., 1998). Unfortunately, information concerning the resistant mechanisms of mite in Thailand is still limited. Study on the synergism and the interaction with detoxification mechanisms in *T. truncatus* would provide

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information on detoxification enzymes and benefit Thai farmers in terms of increasing environment safety and reducing the insecticidal resistance problems of insect and mite.

MATERIALS AND METHODS

Effects of synergist

Three synergists, 0.5% maleic acid (MA), 0.2% piperonyl butoxide (PBO) and 0.2% triphenyl phosphate (TPP) which were nontoxic to mites were selected for this experiment. The toxicity of each synergist was tested by the spray method using a topical spray tower operated at 15 lb/in^2. *Tetranychus truncatus* females from laboratory colony, Department of Entomology, Kasetsart University, Thailand were used throughout the experiments.

Different concentrations of selected crude plant extract plus the proper concentration of each synergist and 0.5% Tween 80 were then sprayed seperately onto 3 day-old *T. truncatus* adult females (50 mites/leaflet; 3 replications/treatment). Distilled water plus 0.5% tween 80 served as control in this experiment. Treated mites were kept in an incubator set at 28°C for 3 days. Observations were made daily for the numbers of dead mite on leaflets and those fallen into the water. Data in all experiments were analyzed by analysis of variance using SAS program, and Lsd was employed to compare the treatment means.

Determination of detoxification enzyme

Optimization of the numbers of spider mite

Spider mites were minute, therefore the optimum number of mite needed in order to detect the enzyme activity was studied. A total of 30, 50, 100, 300, 500 and 1000 adult female mites of a known age (3 day-old) were used. Mites from each group were placed in a 1.5 ml microtube and homogenized in 1ml of standard homogenizing medium (0.1 M potassium phosphate buffer, pH 7.5 and 1 mM ethylene diamine tetraacetic acid (EDTA)) using a motor-driven grinder (Sigma, Z359947) or mortar and pestle, containing 0.01 g polyvinyl polypyrrolidone (PVPP). Finally, each sample was centrifuged (Refrigerate high speed centrifuge, Hettich Zentrifugen Universal 16R) at 18,000 g, 4°C for 10 min and the supernate (enzyme solution) was used to detect esterase activity.

The sample cuvette was prepared by adding 50 µl of enzyme solution and 50 µl paranitrophenyl acetate (PNPA) into the glass cuvette containing 2.9 ml of 0.1M potassium phosphate buffer pH 7.5. The reference standard cuvette was prepared by placing 2.9 ml of 0.1M potassium phosphate, pH 7.5 into a separate glass cuvette. Afterwards, 50 µl of potassium phosphate, 1 mM EDTA, 0.01M glutathione (GSH) pH 7.5 solution and 50 µl of PNPA were added into each cuvette. The solution in each cuvette was mixed thoroughly and the esterase activity was detected by reading the absorbance in a spectrophotometer (Spectronic Genesys 5) at 400 nm for 3 min.

Optimization of the pH buffer in homogenizing medium

The optimum numbers of spider mite from the previous study was used in this experiment. The pH of homogenizing medium (0.1M potassium phosphate buffer) was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 9.0 and 12.0. Adult female mites were placed in each of 1.5 ml microtubes containing 0.01 g PVPP and 1 ml homogenizing medium at different pH levels before grinding. In this study, the fix concentration of 0.01g PVPP was used in all treatments. After spinning in the centrifuge at 18,000 g, 4°C for 10 min, the supernate of each tube was tested by reading the absorbance in the spectrophotometer at 400 nm for 3 min for esterase activity.

Detection of esterase activity

In order to study the detoxification enzymes of *T. truncatus*, the optimum numbers of mite that survived from the topical spray method from each toxicity bioassay at 3 different concentrations (1,

3 and 5%) were used. The same number of adult female mite randomly picked from the stock colony was used as a reference. Each set of mite sample was placed separately into 1.5 ml microtubes and kept on ice. Supernates were prepared from each sample as described previously. The paranitrophenol product was detected by the spectrophotometer at 400 nm for 3 min. Finally, the esterase activity was calculated from paranitrophenyl produce following the PNPA assay of Mackness *et al*. (1983).

Detection of Glutathione S-transferase activity

To detect glutathione S-transferase activity, the same sample of supernate previously used to detect esterase activity was again utilized. The sample cuvette was prepared by adding 1.15 ml of 0.1 M potassium phosphate buffer pH 7.5 into the sample cuvette. Subsequently, 130 µl of 0.01 M GSH in 0.1 M potassium phosphate buffer pH 7.5, 20 µl of enzyme solution and 10 µl of 0.15 M 1-chlorodinitro-2, 4-benzene (CDNB) were added. The reference standard cuvette was prepared by placing 1.15 ml of 0.1 M potassium phosphate buffer pH 7.5 into the glass cuvette. Later, 20 µl of 1 mM EDTA, 0.01 M GSH in buffer and 10 µl of 0.15 M CDNB were added. The solution in each cuvette was mixed together quickly and thoroughly before the activity of glutathione S-transferase was recorded by spectrophotometer operated at 340 nm for 3 min. Glutathione S-transferase activity was calculated using extinction coefficients of CDNB conjugation products followed the CDNB assay of Booth *et al*. (1961).

RESULTS AND DISCUSSION

Effects of synergist

Preliminary studies of the optimal concentration of three synergists were performed in order to get the highest concentration of each synergist which was non-toxic to the mites. They were 0.5% maleic acid (MA), 0.2% piperonyl butoxide (PBO) and 0.2% triphenyl phosphate (TPP). Toxicity of these synergists on the mortalities of *T. truncatus* adult female was performed by the spray method. The LC_{50} values of the crude sugar apple hexane extract combined with synergists are shown in Table 1. Combination of crude sugar apple hexane extract and MA showed the LC_{50} value of 2.27% which was the lowest compared to 3.79% and 4.13% of the crude hexane extracts combined with PBO and TPP, respectively. This result clearly indicated that the toxicity of sugar apple hexane extract against *T. truncatus* increased when 0.5% MA was added. The slope of the regression line was also the highest in the combination of crude sugar apple hexane extract+0.5%MA.

Correction factor (CF) was used to compare

Table 1 Responses of *Tetranychus truncatus* to sugar apple seed crude hexane extract combined with synergists.

Treatments	LC_{50} (%)	CF[1/] (%)	95% confidence intervals (%)	Slope±SE	Chi-square(df)[2/]
Crude hexane	3.52	1.00	3.05-4.01	0.37±0.04	2.52*(3)
Hex+0.5% MA	2.27	0.64	1.97-2.62	0.53±0.05	2.73*(4)
Hex+0.2% TPP	4.13	1.17	3.65-4.66	0.35±0.04	4.83*(3)
Hex+0.2% PBO	3.79	1.07	3.31-4.29	0.36±0.04	4.98*(3)

1/ CF = Correction Factor = LC_{50} of treatments / LC_{50} of crude hexane extract

2/ Significance of Chi-square for goodness of fit.

* P>0.10 since goodness of fit of Chi-square is significant, a heterogeneity factor is used in the calculation of confidence intervals.

the efficacy of crude plant extract with synergists. Crude hexane extract combined with MA exhibited lower CF (0.64%) followed by PBO and TPP (1.07 and 1.17%) (Table 1). Maleic acid (MA) is known to inhibit Glutathion S-transferase (GST) (Maa and Liao, 2000). This result clearly indicated that crude hexane extract+0.5% MA was able to block the GST activity by 36%. Therefore, the sugar apple seed extract could move directly to the target site of *T. truncatus* resulting increasing mite's mortality. Hence, glutathione S-transferase might play an important role in this plant extract degradation. In contrast, Visetson and Milne (2001) showed that *Plutella xylostella* L. larvae treated with cubè root extract+MA had correction factor of 1.07-2.61% which indicated that MA was not important in rotenone detoxification.

Determination of detoxification enzyme

Optimization of the numbers of spider mite

In order to optimize the numbers of mite to be used in the experiments, 3-day old female mites were used in batches of 30, 50, 100, 300, 500 and 1000 mite. The highest paranitrophenol product/min/mg protein was 0.27 n mole for 500 mites, but 100 and 300 mites gave similar paranitrophenol products at 0.24 n mole. There were not a significant differences in the amount of paranitrophenol produced by 100, 300 and 500 mites crushed with motorized homogenizer. In contrast, paranitrophenol product of 1000 mites homogenated with mortar and pestle was only 0.16 n mole (Table 2).

Proper equipment for sample homogenization is needed when dealing with specimens of small size. Cohen (1982) stated that major problems with insect samples arose because of the small size and consequently small amounts of tissue available. To avoid this problem, homogenate or centrifugal fraction should come from whole insect. Knowles and Hamed (1990) used mortar and pestle to homogenate 250 bulb mites, *Rhizoglyphus echinopus* (Fumouze and Robin) for a propargite metabolism study while Kulpiyawat (2001) homogenized 900 *Eutetranychus africanus* Tuker adult in the same way to find the esterase activity. In this finding, the results clearly demonstrated that different methods of homogenization gave different esterase activities. Homogenated samples of 100 mite used a motor driven homogenizer exhibited significantly higher esterase activity (0.24 n mole/min/mg protein) as compared with 1000 mites (0.16 n

Table 2 Detection of esterase activity: influence of mite number.

Number of mites[1/]	Mean (S.E.)*of paranitrophenol product (n mole/min/mg protein)
30	0.15 (2.77) b
50	0.13 (0.66) b
100	0.24 (1.76) a
300	0.24 (0.86) a
500	0.27 (0.86) a
1000	0.16 (3.65) b

* Means followed by the same letters are not significantly different at 0.05 level as determined by Lsd.

1/ Different methods of specimen homogenization:
30-500 mites using motorized homogenizer (Sigma, Z359947).
1000 mites using a mortar and pestle for homogenization.

mole/min/mg protein) homogenated with mortar and pestle.

Levels of esterase activity varied among stages of insect or mite species. McEnroe (1965) stated that the esterase activity of two-spotted spider mite, *Tetranychus urticae* Koch was low in the larval stage, then increased in 2-3 day-old adult and declined in the older age. *Tribolium castaneum* (Herbst) also expressed low esterase activity in the egg stage, which increased in the larval stage and declined in pupal and adult stages. Moreover, Zhu and Brindley (1990) stated that *Lygus hesperus* Knight showed differences in esterase activity between males and females (25% more activity of AchE in male than female). The change in activity among the different stages could be affected by the changing hormone titres in each stage (Cohen *et al*., 1977; Visetson, 1991). In addition, insect organs that have different functions may show different esterase activities such as AchE activity in head homogenate is higher than thoracic or abdominal homogenates (Zhu and Brindley, 1990). In this experiment, 3 day-olds *T. truncatus* adult females had a detectable esterase activity similar to those of *T. urticae* and *E. africanus* females (Kulpiyawat, 2001 and Yang *et al*., 2001). Hence, the number of 100 *T. truncatus* adult females was used throughout the remaining experiments.

Optimization of the pH buffer in homogenizing medium

Tetranychus truncatus females were homogenated in buffer with different pH, ranging from 6-12. The highest activity of esterase was 0.47 n mole/min/mg protein at pH 7.5 (Figure 1). The esterase activity increased slightly from buffer pH 6.0 till buffer pH 7.5, and slightly declined thereafter. This result was similar to Kulpiyawat (2001) who found that the highest peak of esterase activity of African spider mite, *E. africanus* female in the same homogenizing medium occurred at pH 7.5. In addition, Visetson (1991) and Rose and Wallbank (1986) also demonstrated that 7.5 was the optimum pH buffer for *T. castaneum* and *Oryzaephilus surinamensis* (L.). This finding was also suported by Hodgson and Plapp (1970) who stated that a pH of between 7-8 was suitable for most insects.

Suitable buffering conditions should be used during the process of homogenization because several inhibitors such as quinones, phenolics and proteolytic enzymes are released. These inhibitors could denature the enzymes and reduce enzyme activity (Visetson, 1991). These compounds are powerful oxidizing agents and they may polymerize

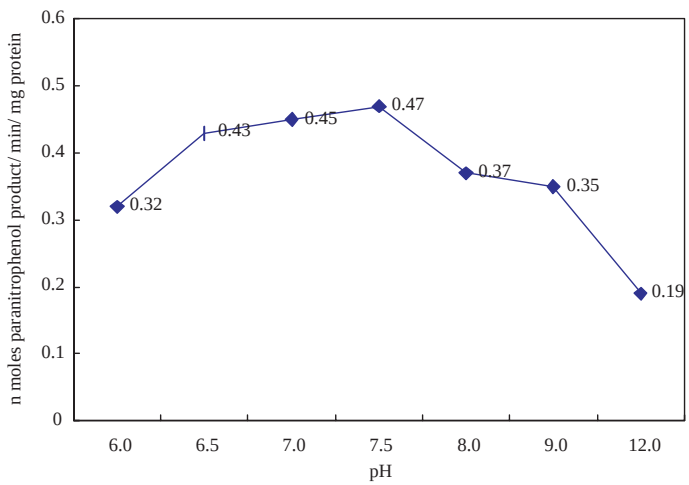

Figure 1 Esterase activity curve at different pH of homogenizing medium.

with themselves or with protein. These reactions caused the inactivation of enzyme activity (Walker, 1980). Several workers provided several media for homogenizing specimens such as distilled water (Ishaaya and Degheele, 1988), phosphate buffer containing sucrose and KCl (Cohen, 1982), Bovine serum albumin (BSA) (Walker, 1980), phosphate buffer containing DTT and EDTA (Willadsen *et al*., 1987) and phosphate buffer without adding any other chemicals (Price, 1984). Walker (1980) showed that PVPP was a powerful absorbent for inhibitors such as phenolics and tannins. EDTA was a stabilizing agent in the homogenizing medium (Krieger and Wilkinson, 1970). In this experiment, PVPP and EDTA were added in the 7.5 selected pH phosphate buffer for homogenization of enzyme in order to avoid the inhibitors.

Detection of esterase activity

Esterase activities were detected from the survived *T. truncatus* females after treated with 1, 3 and 5% sugar apple crude hexane extracts and crude extracts combined with synergists. Results of esterase activity showed no significant differences in all treatments which indicated that there were no changes in esterase activity no matter what kinds of synergist were added (Table 3). This indication clearly illustrated that the extract with or without synergists did not involve in this kind of mechanism in this mite.

Mites exhibited low and similar esterase activity levels (0.09-0.13 n mole/min/mg protein) in all treatments including control. This result coincided well with Kulpiyawat (2001) who revealed that *E. africanus* esterase activity was only 0.01-0.15 n mole/min/mg protein. In addition, Ni and Quisenberry (2003) demonstrated that esterase activity of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) fed on different host plants varied between 0.1-0.5 n mole/min/mg

Table 3 Enzyme activities of *Tetranychus truncatus* after treated with different concentrations of crude sugar apple hexane extract or combined crude extract with synergists.

Treatments	Mean enzyme activities (S.E.)[1/] (n mole/min/mg protein)	
	Esterase	Glutathione S-transferase
1% crude sugar apple extract	0.10 (0.020) a	0.23 (0.04) a
3% crude sugar apple extract	0.13 (0.006) a	0.18 (0.04) a
5% crude sugar apple extract	0.13 (0.005) a	0.23 (0.04) a
1% crude extract+0.5%MA	0.10 (0.010) a	0.21 (0.08) a
3% crude extract+0.5%MA	0.13 (0.005) a	0.23 (0.04) a
5% crude extract+0.5%MA	0.11 (0.005) a	0.27 (0.07) a
1% crude extract+0.2%TPP	0.10 (0.005) a	0.23 (0.04) a
3% crude extract+0.2%TPP	0.12 (0.003) a	0.23 (0.09) a
5% crude extract+0.2%TPP	0.09 (0.008) a	0.14 (0.00) a
1% crude extract+0.2%PBO	0.12 (0.003) a	0.23 (0.04) a
3% crude extract+0.2%PBO	0.11 (0.010) a	0.14 (0.00) a
5% crude extract+0.2%PBO	0.11 (0.005) a	0.18 (0.04) a
Control (untreated mites)	0.11 (0.003) a	0.32 (0.04) a

1/ Means in column followed by the same letters are not significantly different as determined by Least Significant Difference test (Lsd) (α =0.05).

protein. Meanwhile, Yang *et al.* (2001) reported that *T. urticae* esterase activity was 0.1-1.2 n mole/min/mg protein. In contrast, the diamondback moth larva, *P. xylostella* had an esterase activity level of 18.16 n mole/min/mg protein (Visetson *et al*., 2003).

Detection of Glutathione S-transferase activity

Mites in the control (untreated mite) exhibited relatively higher GST activity (0.32 n mole/min/mg protein) but not significantly different as compared to all other treatments (0.14-0.27 n mole/min/mg protein) (Table 3). However, TPP and PBO were not involved with GST activity as mentioned by several workers (Collins, 1990; Maa and Liao, 2000; Visetson *et al*., 2001). Therefore, any changes of GST activity in Table 3 might derive from the crude extract itself. The results in Table 3 clearly indicated that the crude extract alone showed ca. 28-44% GST inhibition. Hence, this finding supported that the crude extract itself also inhibited the enzyme system. Moreover, ca. 15-30% decreased in GST activity after addition of MA in the crude extract also indicated that MA plus crude extract played further inhibition. Therefore, glutathione S-transferase in this kind of mite was one of the important role in the crude extract degradation.

Glutathione S-transferase activity of *T. truncatus* might be inhibited by the chemical components within sugar apple seed extract which was similar to the extraction of mangosteen peel that inhibited GST activity in rice weevils, *Sitophilus oryzae* L. (Bullangpoti *et al*., 2004). This finding agreed with Visetson *et al*. (2002) who stated that combination of nut grass extract with maleic acid also inhibited GST activity in golden apple snails, *Pomacea canaliculata* L.

Next experiment on enzyme purification should be done to identify the activity of each fraction because some fractions may exhibit as inhibitor of the other fractions, making GST activity fluctuated. This phenomenon happened with other workers such as Visetson *et al*. (2003) who worked with the diamondback moth larvae and showing the fluctuation of GST activity using crude extract of sesame oil. They also demonstrated that, with the ion exchange purification method, each fraction showed different inhibition to this enzyme. Some fractions were inhibited by another fractions. These trends were similar to those of other workers such as Reidy *et al*. (1989) who purified GST enzyme in the cytosols of the red rust flour beetles, *T. castaneum*.

CONCLUSION

Toxicity of three synergists combined with sugar apple crude hexane extract on the mortality of *Tetranychus truncatus* adult female indicated that crude sugar apple hexane extract+0.5% MA induced the highest toxicity to *T. truncatus* (LC_{50}=2.27%). This result clearly indicated that crude hexane extract+0.5% MA was able to block the GST activity. Therefore, the sugar apple seed extract could move directly to the target site of *T. truncatus* resulting in increasing mite's mortality. In contrast, glutathione S-transferase activity of *T. truncatus* might be inhibited by the chemical components within sugar apple seed extract. Hence, the efficacy of sugar apple crude extract to control *T. truncatus* could be increased by adding 0.5% MA. This result should later be tested in the field and it will benefit Thai farmers in terms of increasing environmental safety and reducing production costs.

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LITERATURE CITED

Bolland, H. R., J. Gutierrez and C. H. Flechtmann. 1998. **World Catalogue of Spider Mite Family (Acari: Tetranychidae)**. Brill: Leiden, Boston. 392 p.

Booth, J., E. Boyland and P. Sims. 1961. An enzyme from rat liver catalyzing conjugation with glutathione. **Biochem J**. 79: 516-524.

Bullangpoti, V., S. Visetson, J. Milne and S. Pormbanlualap. 2004. Effects of mangosteen's peels and rambutan's seeds on toxicity, esterase and glutathione-s-transferase in rice weevils, pp. 224-231. *In* **42nd Proc. Kasetsart Univ. Ann. Conf**. Kasetsart University, Bangkok, Thailand.

Chungsamarnyart, N., S. Mahatheeranont, C. Rattanakreetakul, S. Jiwajinda and W. Jansawan. 1992. Isolation of acaricidal substances against tropical cattle ticks from sugar apple seeds. **Kasetsart J. (Nat. Sci. Suppl.)**. 26: 41-45.

Cohen, E. 1982. Studies on several microsomal enzymes in two strains of *Tribolium castaneum* (Tenebrionidae, Coleoptera). **Comp. Biochem. Physiol**. 71: 123-126.

Cohen, E., E. Sverdlov and D. Wool. 1977. Expression of esterase during ontogenesis of the flour beetle *Tribolium castaneum* (Tenebrionidae: Coleoptera). **Biochem. Genet**. 15: 253-264.

Collins, P. J. 1990. A new resistance to pyrethroids in *Tribolium castaneum* (Herbst) **Pestic. Sci**. 28: 101-115.

Guo, F., Z. Zhang and Z. Zhao. 1998. Pesticide resistance of *Tetranychus cinnabarinus* (Acari: Tetranychidae) in China: a review. **Syst. Appl. Acarol**. 3: 3-7.

Hodgson, E. and F. W. Plapp. 1970. Biochemical characteristics of insect microsomes. **J. Agri. Chem**. 18: 1048-1055.

Hopp, D. C., L. Zeng, Z. M. Gu, J. F. Kozlowski and J. L. McLaughlin. 1997. Novel mono-tetrahydrofuran ring acetogenins, from the bark of *Annona squamosa* showing cytotoxic selectivities for the human pancreatic carcinoma cell line, PACA-2. **J. Nat. Prod**. 60: 581-586.

Ishaaya, I. and D. Degheele. 1988. Properties and toxicological significance of diflubenzuron hydrolase activity in *Spodoptera littoralis* larvae. **Pestic. Biochem. Physiol**. 32: 180-187.

Krieger, R. I. and C. F. Wilkinson. 1970. Stabilization of insect microsomal preparations by freezing. **J. Econ. Entomol**. 63: 1343-1344.

Knowles, C. O. and M. Hamed. 1990. Propargite metabolism in bulb mite (Acari: Acaridae). **J. Econ. Entomol**. 83(1): 63-66.

Kulpiyawat, T. 2001. **Resistance and Resistant Mechanism of Some Acaricides in African Red Mite, *Eutetranychus africanus* (Tucker) (Acari: Tetranychidae)**. Ph. D. Dissertation, Kasetsart University, Bangkok.

Leatemia, J. A. and M. B. Isman. 2001. Crude seed extract of *Annona squamosa* (Annonaceae) as a potential botanical insecticide. **The ESA 2001** Annual Meeting.

Maa, W. C. and S. Liao. 2000. Culture-dependent variation in esterase isozymes and malathion susceptibility of diamondback moth, *Plutella xylostella* L. **Zool. Studies**. 39(4): 375-386.

Mackness, M. I., C. H. Walker, D. G. Rowland and N. R. Price. 1983. Esterase activity in homogenates of three strains of the rust red flour beetle *Tribolium castaneum* (Herbst). **Comp. Biochem. Physiol**. 74: 65-68.

McEnroe, W. D. 1965. Esterase in the twospotted spider mite, pp. 214-224. *In* J. A. Naegele (ed.). **Advances in Acarology**. Cornell University, New York.

Morita, H., Y. Sato, K. L. Chan, C. Y. Choo, H. Itokawa, K. Takeya and J. Kobayashi. 2000. Samoquasine A, a benzoquinazoline alkaloid from the seeds of *Annona squamosa*. **J. Nat.**

Prod. 63: 1707-1708.

Ni, X. and S. S. Quisenberry. 2003. Possible roles of esterase, glutathione s-transferase, and superoxide dismutase activities in understanding aphid-cereal interactions. **Entomol. Exp. Appl.** 103: 187-195.

Price, N. R. 1984. Carboxylesterase degradation of malathion *in vitro* by susceptible and resistant strains of *Tribolium castaneum* (Herbst). **Comp. Biochem. Physiol**. 77: 95-98.

Reidy, G. F., H. A. Rose, S. Visetson and M. Murray. 1990. Increased glutathione s-transferase activity and glutathione content in an insecticide-resistant strain of *Tribolium castaneum* (Herbst). **Pestic. Biochem. Physiol.** 36: 269-276.

Visetson, S. 1991. **Insecticide Resistance Mechanisms in the Red Rust Flour Beetle (*Tribolium castaneum* Herbst)**. Ph. D. Dissertation, The University of Sydney, Australia.

Visetson, S. and M. Milne. 2001. Effects of root extract from derris (*Derris elliptica* Benth) on mortality and detoxification enzyme levels in the diamondback moth larvae (*Plutella xylostella* Linn.). **Kasetsart J. (Nat. Sci.)**. 35: 157-163.

Visetson, S., J. Milne, M. Milne and P. Kanasutar. 2003. Synergistic effects of sesame oil with cypermethrin on the survival and detoxification enzyme activity of *Plutella xylostella* L. larvae. **Kasetsart J. (Nat. Sci.)**. 37: 52-59.

Visetson, S., S. Naknatti, W. Ruamthum and J. Milne. 2002. Detoxification mechanisms of the golden apple snail against nut grass extracts containing 4, 11-salinnadien-3-one and extract toxicity to some non-target species, pp. 285-294. *In* **Proc. Int. Conf. Biopesticides**. Kuala Lumpur, Malaysia.

Walker, J. R. L. 1980. Enzyme isolation from plants and the phenolic problem. **What's New in Plant Physiology**. 11: 33-36.

Wilkinson, C. F. 1983. Role of Mixed-function oxidases in insecticide resistance, pp. 175-205. *In* G. P. Georghiou and T. Saito (eds.). **Pest Resistance to Pesticides**. Plenum Press, New York.

Willadsen, P., R. V. McKenna and G. A. Riding. 1987. Purification and characterization of an esterase from the tick *Boophilus microplus*. **Insect Biochem**. 17: 659-664.

Yang, X., D. C. Margolies, K. Y. Zhu and L. L. Buschman. 2001. Host plant induced changes in detoxification enzymes and susceptibility to pesticides in the twospotted spider mite (Acari: Tetranychidae). **J. Econ. Entomol**. 94(2): 381-387.

Zhu, K. Y. and W. A. Brindley. 1990. Acetylcholinesterase and its reduced sensitivity inhibition by paraoxon in organophosphate-resistant, *Lygus hesperus* Knight (Hemiptera: Miridae). **Pestic. Biochem. Physiol**. 36: 22-28.