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Synergistic Effects of Sesame Oil with Cypermethrin on the Survival and Detoxification Enzyme Activity of *Plutella xylostella* L. Larvae

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ABSTRACT

Two types of insect-toxicity tests, (1) contact and (2) no-choice leaf dipping test, were conducted using the insecticide cypermethrin without piperonyl butoxide (PB), cypermethrin with PB and cypermethrin with sesame oil against the 2nd –3rd instar larvae of *Plutella xylostella* L. Sesame oil showed good synergism with cypermethrin yielding synergistic ratios (SR) that ranged from 1.54 – 6.33 in the contact method and 2.04-5.88 in the no-choice leaf dipping method and were comparable to using PB, which showed SR's of 6.33 and 6.71, respectively. Both PB and sesame oil mixed together with cypermethrin inhibited monooxygenase activity by approximately two-third but induced glutathione–S-transferase ca. 2-3 folds in both methods. The synergists had no effect on esterase activity (CF ca. 1.2).

Residues of cypermethrin in the larvae increased by 1.29 – 2.57 folds in the treatments with added sesame oil compared to a 2.86 fold increase when PB was added, using the contact method. The no choice leaf dipping method revealed that cypermethrin residue levels increased by 2.82 – 6.91 fold with added sesame oil and 8.27 fold with added PB. This indicated that both PB and sesame oil played the same role in the inhibition of an enzyme, possibly monooxygenase. Field trials with Chinese kale showed the same trends that were evident in the laboratory work. The addition of sesame oil to the insecticide reduced the larval population by 70-80 percent while the addition of PB reduced the larval population by up to 88 percent in the kale crop. Monooxygenase activities of insect larvae collected in the field from kale sprayed with cypermethrin plus synergist (sesame oil or PB) were lower than those from kale treated with insecticide alone. The results in terms of synergism and changes in enzyme metabolism were discussed.

Key words: synergistic effects, sesame oil, cypermethrin, detoxification enzyme, *Plutella xylostella* L.

INTRODUCTION

Although insecticides create many problems such as pollution, insecticide resistant insects, disruption of biodiversity, residues in agricultural products, and most of all, high cost of production, insecticides have been proven to be one of the most effective methods in insect control. In Thailand, of all vegetable insect pests, the diamondback moth (*Plutella xylostella* Linn.) is one of the most serious in vegetable. It is resistant to many insecticides in most parts of Thailand where insecticides are frequently used. Fortunately, Thailand is one of the countries with the most diverse plants in Southeast Asia. Many Thai farmers utilize plant products for insect control. Some

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plants contain secondary plant substances such as azadirachtin from neem seed kernels that inhibit hormone production in lepidopterous larvae (Schmutterer, 1990). Others, like rotenone from derris (*Derris elliptica* Benth) (Visetson and Milne, 2001) and selinnadien from tubers of nutgrass (*Cyperus rotundus* L.) (Visetson *et al*., 2001) have been effectively used in the control of the diamondback moth larvae. However, other uses of plant products in insect control, e.g., as synergists with insecticides have never been investigated. Sesame is widely grown in the northeastern part of Thailand. Thai farmers usually plant sesame crops after the rice has been harvested. Plants use left-over moisture in the soil from the rice crop and grow very fast. Sesame oil has many uses: as a cosmetic for skin protection, in medicine as an adjuvant for many drug emulsions for ulcer, in cooking as a Thai food additive. Furthermore, the chemical structure of the oil is similar to that of the insecticide synergist, piperonyl butoxide, which has played a large role in reducing population of pyrethroid resistance insects (Collins, 1990).

This research was conducted to investigate the synergistic effects of sesame oil and piperonyl butoxide with cypermethrin on the survival and detoxification enzyme activity of *P. xylostella* larvae. The inhibition by cypermethrin of monooxygenase, esterase and glutathione-S-transferase activity after addition of these synergists would show how these synergists interact with detoxification mechanisms. These results could be beneficial to Thai farmers as well as to businessmen who are looking for ways to reduce the cost of insecticides by the addition of an alternative synergist.

MATERIALS AND METHODS

Insect larvae and plant samples

Diamondback moth larvae were collected from a vegetable producing area in Kanchanaburi province, 150 kms west of Bangkok. Larvae were reared under laboratory conditions (23 ± 2°C) with methods modified from Visetson *et al.* (2001). F_2 –generation was used for all experiments. Sesame pods collected from Nongpai district (Petchaboon province, 230 kms north of Bangkok) were air-dried. Sesame seeds from the pods were ground and screw- pressed to obtain esame oil which was collected into a bottle and stored at -20°C until used in tests.

Efficacy tests

The toxicities of various concentrations (0.01- 0.7% w/v) of cypermethrin with or without 10% piperonyl butoxide (PB) and varied 0.5-10% sesame oil on 2^{nd} – 3^{rd} instar larvae of *Plutella xylostella* L. were determined using a no choice leaf dipping method and a contact method. Five replicates comprising 20 larvae in each replicate were used in each test. A 5% emulsifier, triton X-100, was mixed into each test solution before trials commenced. The no –choice leaf dipping method used a leaf circle disk of Chinese kale with a diameter of 5 cm which was given to the larvae as food. The contact method was done by allowing 20 larvae to move freely in a petri-dish previously sprayed with test solution. A completely randomized design with 5 replicates was used. Mortality was evaluated after 24 hours of exposure. All experiments were run at 23 ± 2°C. In the case of control mortality, Abbott's formula (Matsumura, 1975) was employed. LC_{50} values were calculated from regression equations with cypermethrin used as control groups.

Detoxification enzyme assays

The surviving larvae from each treatment were used in *in vitro* assays to optimize enzyme activity of esterase, glutathione-S-transferase and monooxygenase activities following the method of Visetson and Milne (2001) by using paranitrophenyl acetate, dichloronitrobenzene and aldrin as a substrate for the three enzymes. Protein measurement was done according to the method of Lowry *et al*. (1951) and Bovine Serum Albumin was used to quantify all enzyme activity.

The coefficient of determination (r^2) was determined for both insect larval mortality and enzyme activity. Synergist ratios (SR) and correction factors (CF) were quantified to measure the effectiveness of synergists and changes in enzyme levels, respectively.

Determination of residues

Using the contact and leaf dipping methods with the LC_{50} level, larvae were exposed to various concentration of cypermethrin with or without PB or sesame oil for 24 hours and then transferred to petri-dish. Live larvae (approximately 0.5 g) were assayed for cypermethrin by a method modified from Visetson (1991) using a disposable pasture pipette column. The column was previously packed with 1 g aluminium oxide, followed by 0.5 g silicic acid. Live larvae (0.5 g) were crushed with 10 mg trisodium citrate and 5 mg disodium hydrogen orthophosphate and the mixture was then loaded into the column. The column was eluted with 15 ml of 4% acetone in hexane. The eluent was concentrated to 0.5 ml and injected into a GLC/ (Varian, USA) equipped with a ^{63}Ni electron capture detector and a 20 m capillary column, packed with 5% SE-30 on GAS-Chrom Q 80-100 mesh. The conditions for chromatography were: injector temp. 220°C, column 190°C, detector 285°C, carrier gas flow (N_2) 45ml/min. These conditions gave 99.8% recovery of cypermethrin.

Field experiments

Field trials using Chinese kale were separately undertaken to confirm laboratory results. The experiment used a randomized complete block design with 5 replicates. Plot size was 6 x 2 square meters. Spraying at the rate of 80 litre/rai was done once a week, beginning on 10-day old Chinese kale. Seven sprays at 7 days interval were applied during the experiment. Larval numbers were regularly checked at fixed points before and after spraying for 24 hours. Duncan multiple range test (DMRT) was employed for means comparisons with $p<0.05$ according to Finney (1964).

RESULTS AND DISCUSSIONS

Efficacy tests

LC_{50} values for cypermethrin in both types of test methods, (1) contact and (2) no choice leaf dipping methods, against 2^{nd} – 3^{rd} instar larvae of the *Plutella xylostella* L were 0.57 and 0.47% w/v, respectively (Table 1). The addition of 10% PB resulted in synergist ratios (SR) of 6.33 and 6.71 folds for first and second methods, respectively whereas addition of 10% sesame oil gave the SR of 5.7 and 4.27 folds, respectively. In addition, sesame oil plus PB added to cypermethrin did not increase SR values. The results indicated that both PB and sesame oil played the same role in increasing cypermethrin efficacy. These results were similar to those of Visetson and Milne (2001) who found that addition of PB and diethyl maleate to rotenone increased rotenone efficiency. A larger SR was obtained when 20% sesame oil was added but at this point it was not very economical in terms of the cost. The correlation between concentration and mortality in most experiments indicated r^2 of 0.87 – 0.99 except that the addition of 0.5% sesame oil showed r^2 of 0.69 only in the contact method. Hence, this low concentration of sesame oil added might not be sufficient to deplete the detoxification mechanisms in the larvae. This result was similar to that of Visetson (1991) who worked with PB added to cyfluthrin in the control of *Tribolium castaneum*. Furthermore, the results showing higher insecticide efficiency after addition of PB confirmed Collins (1990)'s works who showed that PB may inhibit one of detoxification enzymes possibly monooxygenase in insects.

Detoxification enzyme assays

Cypermethrin alone gave little increased monooxygenase activity by ca. 1.12 folds and give no change in esterase and glutathione-S-transferase activity in both test methods (Table 2). Elevated monooxygenase levels after application of pyrethroids have been reported by a number of workers (Rose, 1985; Hung and Sun, 1989;

Table 1 LC_{50} values of cypermethrin (0.01 – 0.7% w/v) with or without piperonyl butoxide (PB) and sesame oil against 2nd – 3rd instar larvae of *Plutella xylostella* L. using no choice leaf dipping method and contact method.

Treatment (% w/v)	Application methods[1]					
	Contact			Leaf dipping		
	LC50 (%w/v)	r^2	Regression eq.[4] [SR] [SR]	LC50 (%w/v)	r^2	Regression eq.
None[3]	0.57 ± 0.02c[2]	0.98	Y = 22.64 + 48.0X	0.47 ± 0.05b	0.97	Y = 33.92 + 34.22X
10% PB	0.09 ± 0.01a	0.99	Y = 46.24 + 41.80X [6.33] [6.71]	0.07 ± 0.02a	0.99	Y = 46.98 + 43.16X
0.5% oil	0.37 ± 0.23bc	0.69	Y = 36.93 + 35.32X [1.54] [2.14]	0.22 ± 0.25b	0.76	Y = 41.80 + 37.26X
1.0% oil	0.21 ± 0.22b	0.87	Y = 39.89 + 48.10X [2.71] [2.04]	0.23 ± 0.12b	0.84	Y = 39.62 + 45.12X
10.0% oil	0.10 ± 0.06a	0.91	Y = 45.72 + 42.80X [5.70] [4.27]	0.11 ± 0.09a	0.97	Y = 45.36 + 42.16X
10% oil + 10% PB	0.11 ± 0.03a	0.97	Y = 43.81 + 56.21X [5.18] [3.92]	0.12 ± 0.09a	0.93	Y = 44.04 + 49.64X
20.0% oil	0.09 ± 0.03a	0.89	Y = 46.24 + 41.80X [6.33] [5.88]	0.08 ± 0.09a	0.99	Y = 46.54 + 43.16X

1 means followed by different letters within the same column are significantly different at $P < 0.05$

2 means ± SD, 5 replicates, 20 individual /replicate, 24 hours check per batch from F2-generation for all experiments.

3 "None" means no PB or sesame oil was added to the various concentration of cypermethrin. SR = (LC_{50} none)/ (LC_{50} with PB or sesame oil) while r^2 was a correlation determination between concentration and mortality.

4 Y = dependent variable (% mortality), X = independent variable (dose of cypermethrin with or without PB or sesame oil)

Visetson, 1991). Monooxygenase activity was inhibited after PB was added giving a CF of 1.72 fold but giving no change of esterase and glutathione-S-transferase activity in the leaf dipping method. These results were similar to those of Collins (1990) and Visetson (1991) who worked with *Tribolium castaneum*. On the other hand, the CFs for esterase and glutathione-S-transferase were slightly elevated in the contact method. These might be due to slight cross-synergisms of PB with esterase and glutathione-S-tranferase found by Prabharker *et al*. (1988). Although the statistical analysis showed no significant different, the addition of 10% sesame oil to cypermethrin trended to give CF of ca. 1.6 for monooxygenase activity indicating inhibition while no CF change was obtained when more oil was administered. This was an indication that sesame oil was a synergist with cypermethrin that played more or less the same role as PB in monooxygenase inhibition as proposed by Raffa and Priester (1985). The mixed oil - PB treatment did not show differences monooxygenase activity. CF from either PB or oil only treatments is also indicate that both sesame oil and PB gave the same monooxygenase inhibition level.

Determination of cypermethrin residues

The residues of cypermethrin in the larvae from contact and leaf dipping method were 0.07 and 0.11 ppm, respectively (Table 3). The addition of 10% PB and 20% sesame oil increased cypermethrin in the larvae showing "residue increases" (RI) of 2.86 and 2.57 folds in the contact method and 8.27 and 6.91 folds with the leaf dipping method, respectively. The increased

Table 2 Detoxification enzyme activity of 2nd – 3rd instar larvae of *Plutella xylostella* L. after addition of PB or various amounts of sesame oil to cypermethrin at LC_{50} from Table 1.

Treatment (% w/v)	Types of detoxification enzyme activity [1], Monooxygenase[2]		Esterase (product produced/min/mg protein)[3]		Glutathione-S-transferase	
			[CF][4]			
	Contact	Leaf dipping	Contact	Leaf dipping	Contact	Leaf dipping
Control	4,412 ± 187.14ab		18.16 ± 3.23b		1.12 ± 0.46a	
	-		-		-	
None[5]	5,120 ± 231b	5,461 ± 568b	14.24 ± 0.31ab	15.76 ± 1.17ab	1.04 ± 0.10a	2.07 ± 0.33a
	-		-		-	
10%PB	3,671 ± 926ab	3,182 ± 170a	12.14 ± 0.32ab	20.11 ± 1.56ab	0.54 ± 0.16a	2.31 ± 1.12a
	[1.39]	[1.72]	[1.17]	[0.78]	[1.93]	[0.89]
0.5% oil	4,003 ± 243ab	4,678 ± 164ab	12.12 ± 0.96ab	14.79 ± 1.07a	2.43 ± 1.15ab	3.89 ± 1.68 a
	[1.28]	[1.17]	[1.17]	[1.07]	[0.43]	[0.53]
1.0% oil	3,650 ± 331ab	3,783 ± 892ab	10.45 ± 0.47a	17.37 ± 3.54ab	2.17 ± 1.32ab	3.23 ± 1.15a
	[1.41]	[1.44]	[1.36]	[0.91]	[0.48]	[0.64]
10.0% oil	3,200 ± 786ab	3,312 ± 760ab	12.87 ± 0.67ab	19.12 ± 1.23b	3.53 ± 0.11b	3.15 ± 1.12a
	[1.60]	[1.65]	[1.11]	[0.82]	[0.29]	[0.66]
10% oil + 10% PB	3,450 ± 461ab	3,498 ± 312ab	12.88 ± 0.43ab	18.97 ± 3.37ab	3.09 ± 1.11b	3.86 ± 1.09a
	[1.48]	[1.56]	[1.11]	[0.83]	[0.34]	[0.54]
20.0% oil	3,100 ± 879a	3,301 ± 639a	13.54 ± 0.36ab	19.43 ± 7.12ab	3.19 ± 0.12b	3.11 ± 1.14a
	[1.65]	[1.65]	[1.05]	[0.81]	[0.33]	[0.67]

1 means followed by different letters within the same column are significantly different at $P < 0.05$, DMRT

2 means ± SD, 5 replicates, n= 10-15 of 2nd – 3rd instar lavae were employed, 24 hour check per batch from F2-generation for all experiments.

3 enzyme assays were followed Visetson and Milne (2001), the unit of monooxygenase, esterase and glutathion-S-transferase are picM aldrin epoxidation/min/mg protein, nM paranitrophenol produced/min/mg protein and nM DCNB conjugated product/min/mg protein.

4 CF is a correlation factor = (enzyme activity of none)/ (enzyme activity of treatment).

5 "None" means no PB or sesame oil was added to the concentration of cypermethrin while control means spraying with water onto the larvae.

cypermethrin in the larvae after addition of sesame oil or PB indicated that might be due to monooxygenase inhibition when the synergists were added. Both synergists might block an active site or bind with apoenzymes of monooxygenase making it inactive resulting in increased cypermethrin levels. Furthurmore, the oil plus PB treatment showed little difference in larval cypermethrin levels from those of either the PB or the sesame oil treatment alone. This was also another indication of monooxygenase inhibition showing RI of 3.0 and 6.55 in the contact and leaf dipping methods, respectively. Higher levels of accumulated insecticides in insects in terms of insecticide metabolism have been reported before by a number of workers using labeled insecticides with synergists: trans-permethrin by De Vries and Georghiou, (1981), diazinon by Forgash *et al.* (1962), DDT and dieldrin by Palpp and Hoyer, (1968).

Field experiments

Cypermethrin alone reduced larval numbers in the field by given reduced number (RN) of 1.67. Greater RN (ca. 8.34) was obtained for cypermethrin with 10% PB and for cypermethrin

Table 3 Residues (ppm) of cypermethrin found in larvae of *Plutella xylostella* L. in the two assay methods (contact and leaf dipping) after exposure to cypermethrin at LC_{50} level with and without PB or sesame oil added.

Treatment (% w/v)	Residue (ppm) of cypermethrin			
	Contact	(RI)[4]	Leaf dipping[3]	(RI)
None[1]	0.07 ± 0.01a[2]	-	0.11 ± 0.06a	-
10% PB	0.20 ± 0.11b	[2.86]	0.91 ± 0.37b	[8.27]
0.5 % oil	0.09 ± 0.06ab	[1.29]	0.31 ± 0.12ab	[2.82]
1.0% oil	0.09 ± 0.08ab	[1.29]	0.54 ± 0.11ab	[4.91]
10.0% oil	0.11 ± 0.06ab	[1.57]	0.62 ± 0.09b	[5.64]
10% oil + 10% PB	0.21 ± 0.07b	[3.0]	0.72 ± 0.08b	[6.55]
20.0% oil	0.18 ± 0.07b	[2.57]	0.76 ± 0.08b	[6.91]

1 "None" means no synergist was added to the cypermethrin.

2 means followed by different letters within the same column are significantly different at P = 0.05, DMRT

3 means ± SD, 5 replicates with 100 individual of 2nd – 3rd instar lavae of F_2 generation per replicate were employed, exposure for 24 hours for all experiments.

4 RI was residue increase derived from (residues found in oil and PB or sesame oil added)/ (residue found in "none").

with 10% sesame oil, RN was 5.74. In other words, the addition of sesame oil to the insecticide reduced the larval population by 70-80 % while the addition of PB reduced the larval population by up to 88 % in the kale crop. These results showed higher RN values than those found by Visetson and Milne (2001) who, using derris extracts with PB, showed that the addition of PB to cypermethrin reduced the larvae in chinese kale by up to 50%. The difference might be due to a variety of ecological effects. Season has often been reported to cause major variation in terms of insect infestation in crops. This research was done in the rainy season when more larvae were found in the area while the work of Visetson and Milne (2001) was done in summer when there were less larvae were found. No further increase in RN was detected when a mixture of PB and sesame oil or a higher percentage of sesame oil was added (Table 4). The detoxification enzyme activity of larvae from the field experiment indicated more or less the same trend as that in laboratory experiments except monooxygenase activity. The monooxygenase activity varying from 1.1- 1.5 folds was obtained while esterases and glutathione-S-transferase activities showed no significant differences from "none". The field results indicated that 10% sesame oil added to cypermethrin exhibited the same level of monooxygenase inhibition as the addition of 10% PB. This result confirmed the experiments of Yu (1986), Brattsten (1988) and Yang *et al*. (2001) who pointed out that plant allelochemicals could either reduce or inhibit detoxification mechanisms in terms of enzyme systems and hence increased or decreased insect susceptibility to insecticides. This was similar to the result of Rose (1985) who found that mid guts of polyphagous lepidopterous larvae showed stronger aldrin epoxidase activity than did those of monophagous lepidopterous larvae. So, this research also gave strong evidence that sesame oil could stop monooxygenase function in diamondback moth larvae and that this oil could be used as a synergist in place of PB. However, the results of enzyme activity of purified enzyme both induced and inhibited forms after sesame oil was applied with other insectcides would reveal the

Table 4 Means numbers ± SD enzyme activities of 2nd – 3rd instar *Plutella xylostella* L. larvae, found on the leaves of Chinese kale after application of cypermethrin at LC_{50} (from Table 1) with or without PB or sesame oil.

Treatment (% w/v)	Number of larvae/10 plants [RN][4,5]	Enzyme activity (product produced/min/mg protein)[2,3] [CF]		
		Monooxygenase	Esterase	Glutathione-S-transferase
Control	35.13 ± 2.24c[1]	4,412 ± 187a	18.16 ± 9.23a	1.12 ± 0.12a
	-	-	-	-
None	21.21 ± 8.23b	5,822 ± 100b	22.14 ± 0.05ab	2.22 ± 0.36ab
	[1.67]	-	-	-
10% PB	4.21 ± 0.89a	3,825 ± 254a	24.67 ± 3.25b	3.12 ± 1.76b
	[8.34]	[1.52]	[0.89]	[0.71]
0.5 % oil	10.12 ± 3.41ab	5,273 ± 385b	24.23 ± 1.56b	3.46 ± 1.11b
	[3.47]	[1.10]	[0.91]	[0.64]
1.0 % oil	5.61 ± 2.32a	4,982 ± 146a	15.24 ± 3.89a	3.33 ± 1.54b
	[6.26]	[1.17]	[1.45]	[0.67]
10.0 % oil	6.12 ± 3.13a	4,481 ± 119a	21.11 ± 1.12ab	3.12 ± 1.11b
	[5.74]	[1.29]	[1.05]	[0.71]
10% oil + 10% PB	5.32 ± 1.67a	3,898 ± 540a	22.33 ± 2.67ab	3.23 ± 1.11b
	[6.60]	[1.49]	[0.99]	[0.69]
20.0 % oil	6.24 ± 2.41a	4,211 ± 931a	21.44 ± 1.11ab	3.98 ± 1.25b
	[5.63]	[1.38]	[1.03]	[0.56]

1 means followed by different letters within the same column are significantly different at P = 0.05, DMRT

2 None means no PB or oil was added to the cypermethrin while control means spraying with water onto the kale.

3 enzyme assays were followed Visetson and Milne (2001), the unit of monooxygenase, esterase and glutathion-S-transferase are picM aldrin epoxidation/min/mg protein, nM paranitrophenol produced/min/mg protein and nM DCNB conjugated product/min/mg protein.

4 RN was reduced number which was derived from the division of larvae found in untreated control and number found in treated one.

5 3 replicates with 100 individual of 2nd – 3rd instar lavae were employed, exposure for 24 hours for all experiments.

extent to which sesame oil could be used as a synergist. If sesame oil acts synergistically with all other insecticides, the farmers can reduce the use of insecticides and increase their effectiveness by addition of sesame oil in formulations.

CONCLUSIONS

Sesame oil showed good synergism with cypermethrin. Both PB and sesame oil mixed together with cypermethrin inhibited monooxygenase activity. Residues of cypermethrin in the larvae increased by 1.29 – 2.57 folds in the treatments with sesame oil added. This indicated that both PB and sesame oil played the same role in inhibition, possibly monooxygenase. Field trials involving larvae population in Chinese kale gave similar results as in the laboratory. The addition of sesame oil to the insecticide spray reduced the larval population by 70-80 percent while the addition of PB reduced the larval population in the kale by up to 88 percent.

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LITERATURE CITED

Brattsten, L.B. 1988. Potential role of plant allelochemicals in the development of insecticide resistance, pp 313-348. *In* P. Barbosa and D. Letourneau (eds.). **Novel aspects of insect plant interactions.** Wiley, New York.

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

De Vries, D.H. and G. P. Georghiou. 1981. Decreased nerve sensitivity and decreased cuticular penetration as mechanisms of resistance to pyrethroids in a (IR)-trans-permethrin-selected strain of the house fly. **Pestic. Biochem. Physiol**. 15 : 234-241.

Finney, D.J. 1964. **Statistical Methods in Biological Assay.** 2nd ed. Charles Griffin and Company Limited, London. 238p.

Forgash, A.J., B.J. Cook, and R.C. Riley. 1962. Mechanisms of resistance in diazinon-selected multi-resistant *Musca domestica*. **J. Econ. Ent**. 55 : 544-551.

Hung, C.F. and C.N. Sun. 1989. Microsomal monooxygenase in diamondback moth larvae resistant to fenvalerate and piperonyl butoxide. **Pestic. Biochem. Physiol**. 33 : 168-175.

Lowry O.H., N.J. Rosebough, A.L. Parr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. **J. Biol. Chem**. 193 : 256-275.

Matsumura, F. 1975. Botanical insecticides, pp. 94-99. *In* Matsumura (ed.). **Toxicology of Insecticides.** Plenum, New York.

Palpp, F.W.Jr. and R.F. Hoyer. 1968. Insecticide resistance in the house fly: decreased rate of absorption as the mechanism of action of a gene that acts as an intensifier of resistance**. J. Econ. Ent.** 61 : 1298-1303.

Prabharker, N., D.J. Coudriet, and N.C. Toscano. 1988. Effect of synergists on organophosphate and permethrin resistance in sweetpotato whitefly (Homoptera: Aleyrodidae). **J. Econ. Ent.** 81 : 34-39.

Raffa, K.F. and T.M. Priester. 1985. Synergists as research tools and control agents in agriculture. **J. Agric. Ent.** 2 : 27-45.

Rose, H.A. 1985. The relationship between feeding specialization and host plants to aldrin epoxidase activities of midgut homogenases in larval Lepidoptera. **Ecol. Ent**. 10 : 455-467.

Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. **Annu. Rev. Entomol.** 35 : 271-297.

Visetson, S. 1991. **Insecticide resistance mechanisms in the rust-red flour beetle (*Tribolium castaneum*. Herbst).** PhD-thesis, The University of Sydney, NSW, 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., M. Milne and J. Milne. 2001. Toxicity of 4,11-selinnadien-3-one from nutsedge (*Cyperus rotundus* L.) tuber extracts to diamondback moth larvae (*Plutella xylostella* L.), detoxification mechanisms and toxicity to non target species. **Kasetsart J. (Nat. Sci.)** 35 : 284- 292.

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. Ent**. 94 : 381-387.

Yu, S.J. 1986. Consequences of induced foreign compound-metabolizing enzymes in insects, pp 153-174. *In* L. B. Brattsten and S. Ahmad (eds.). **Molecular aspects of insect-plant associations.** Plenum, New York.