Kasetsart J. (Nat. Sci.) 35 : 284 - 292 (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

Suraphon Visetson[1], **Mantana Milne**[2] **and John Milne**[3]

ABSTRACT

Tubers of nutgrass (*Cyperus rotundus* L.) were collected from various locations of Thailand during the summer and rainy seasons. Toxicity against the diamondback moth (*Plutella xylostella* L.) was observed using different concentrations of the active compound, 4,11-selinnadien-3-one. The toxic effects were also determined on mice (*Mus musculus*), fish (*Poecillia reticulata*) and bee larvae (*Apis florea)*.

It was found that the active principle of nut grass was higher in summer than that in rainy season by ca. 2 folds. This active principle varied according to geographical vareas with Chanthaburi and Chaing Mai producing the highest amounts of 4,11-selinnadien-3-one (0.13-0.16% ai. yield) compared with the other. The LC_{50} against 2^{nd}–3^{rd} instar larvae of diamondback moth were 7-12 ppm. Detoxification enzyme activities as well as synergistic effects revealed that monooxygenase, esterases and some degrees of glutathione-S-transferase played a role in detoxification. Furthermore, synergists, PB and TPP, could raise the effectiveness of the active principle up to ca. 2-6 fold. At 2,000 ppm of 4,11-selinnadien-3-one, exposed mice showed no sign of acute dermal, acute oral or eye irritation effects. However, the active principle was toxic to other non target organisms with LC_{50} of 28.01 ppm and 10.8 ppm to 1-month old guppies and bee larvae, respectively.

Key words: nut grass, *Cyperus rotundus*, diamondback moth, *Plutella xylostella*, detoxification enzymes

INTRODUCTION

Thailand faces several pesticide problems, in terms of residues in food crops, insect resistance to pesticides, toxicity to humans and non target species as well as the high cost of pesticides used in agricultural production (Valleyaluck, 1983).

Currently, Thailand has imported some 20,000 tons of pesticides was imported into the country costing thousands of millions of Thai baht (Katanyukul, 2002). Although pesticide application method of controlling pests, this method is experiencing numerous problems especially in the developing world where knowledge of pesticide toxicity has been ignored (Visetson, 2001).

Apart from toxic residues in food, environmental pollution and the high cost of pest control, pesticide resistance has also become pronounced in terms of cross-resistance and has been a major problem for vegetable producing farmers. The diamondback moth is one pest that has shown a quick development of cross resistance

1 Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

2 Institute of Natural Product Research, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10900, Thailand.

3 Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand.

and is one of the most important pests in the Central Plain of Thailand.

Botanical pesticides derived from neem, lemon grass, galanga, and Siam weed have been proven to be appropriate insecticide alternatives for control of insect pests (Schmutterer, 1990; Singh *et al*. 1989; Visetson and Milne, 2001). At least 5 commercial products from these plants have been introduced into the Thai pesticide market (Visetson, 2001), including products recommended for use against diamondback moth.

Ohsawa*et al*. (1996) found that crude extracts of nut grass (*Cyperus rotundus* L.) tubers gave 80% mortality to diamondback moth larvae in 1 hour post treatment. They indicated that the active compound, 4, 11-selinnadien-3-one, was responsible for killing the larvae. Nut grass also called purple nutsedge, belongs to the Family Cyperaceae. This grass is beneficial as a herb for curing stomach complain (Kiritikar and Basu, 1991). Although Thai farmers regard it as a major weed, it is used as an ingredient in herbal medicine to maintain body function (Anonymous, 1986).

In this research we report the efficacy of the active compound, 4,11-selinnadien-3-one, extracted from the tubers of nut grass for controlling diamondback moth larvae as well as identify its detoxification mechanisms in terms of enzyme reactions, namely esterase, glutathione-S-transferase and monooxygenase activity. Toxicity tests were also conducted for non target organisms, namely the mouse (*Mus musculus*), guppy (*Poecillia reticulata*) and bee larvae (*Apis florea)*.

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. The larvae were cultured for two generations under laboratory conditions at 23 ± 2°C following the method of Leckprayun *et al*. (1999). Nut grass tubers were collected from 5 different locations, Chaing Mai, Ubon Ratchathani, Kanchanaburi, Chanthaburi, Songkhla, in Thailand during the rainy and summer seasons.

Plant extraction and efficacy tests

Five kilogrammes of each sample from each location and each season were ground and dried at room temperature. Ethanolic Soxhlet extraction at 70°C was administered for 48 hours. The crude extracts were then evaporated and freeze dried. The yield of extract was measured. The extracts were carried out by the modified method of Ohsawa *et al.* (1996) with a counter-current distribution method using n-hexane and methanol. The product was separated by silica gel column chromatography using acetone and n-hexane as solvents. The first fraction was used to quantify the amount of 4,11-selinnadien-3-one. Highly purified (99.9%) 4,11-selinnadien-3-one, purchased from Merck Ltd. (Thailand), was used as a standard. The second fraction was employed for preparative TLC using acetone and n-hexane at the ratio of 1:9. The final extracted product was kept at -20°C until required for the following experiments.

The extracts were diluted with acetone and tested against 3^{rd}–4^{th} instar larvae of the diamondback moth. Three replicates, comprising 20 larvae each, were used. A 5% leaf surfactant, triton X-100, was mixed with each concentration prior to use. A no-chioce leaf dipping method was used for the experiments. One leaf circle disk of Chinese kale with diameter of 5 cm was placed for each group of larvae. Mortality was checked after 24 hours exposure. A Completely Randomized Design with 3 replicates was used. All experiments were run at 23 ± 2 °C. Abbott's formula (Matsumura, 1976) was employed to correct control mortality. LC_{50}'s were calculated from regression equations.

Detoxification mechanisms and synergist assays

The life larvae from treatments were used in *in vitro* assays to optimize enzyme activity of

esterase, glutathione-S-transferase and monooxygenase following the methods of Visetson and Milne (2001) and modified from Rose (1985) using paranitrophenyl acetate (PNPA), chlorodinitrobenzene (CDNB) and aldrin to determine enzyme activity. The synergists, piperonyle butoxide (PB), triphenyl phosphate (TPP) and diethyl maleate (DEM), were used at 1% to detect the inhibition mechanisms of activity of the enzymes, monooxygenase, esterase and glutathione-S-transferase, respectively.

Toxicity tests on mice, fish and bee

The 2,000 ppm extract was used to test for acute dermal, oral and eye irritation toxicity on 2 month-old mice (*Mus musculus*) following the method of Ecobichon (1992). Hair on the back of the mice was shaved prior to one treatment of the extract containing 2,000 ppm 4, 11-selinnadien-3-one to be applied. Addition of the extract in the food pellets prior to feed the mice were assayed for acute oral test. The extract was applied topically in the mouse eyes for irritation test. The toxicity was determined by the abnormality of the dermal tissues after 14 days exposures. LC_{50}'s were determined for 1 month old guppies (*Poecillia reticulata*) and bee larvae (*Apis florea)*.

DMRT was employed for all mean comparisons using a significance level of probability > 95% following the method of Finney (1964).

RESULTS AND DISCUSSION

Extraction and 4,11-selinnadien-3-one

The ethanolic Soxhlet extraction of nut grass tuber showed 15-25% more yields in summer than in the rainy season (Table 1). In summer, samples collected from Chaing Mai and Chanthaburi gave the highest contents of 4, 11-selinnadien-3-one (0.13-0.16%w/w) on a dried weight basis. Nut grass tuber extracts from these areas showed 15% less active compound in the rainy season than in the summer. Possible reasons for this included hydrolysis of the active compounds, increased metabolism during the season or the effect of higher moisture content in the tuber prior to extraction. These results were similar to the fluctuations of azadirachtin content of neem seed kernels found in

Table 1 Percentages of 4, 11-selinnadien-3-one in nut grass extracts from samples collected during two seasons from different parts of Thailand.

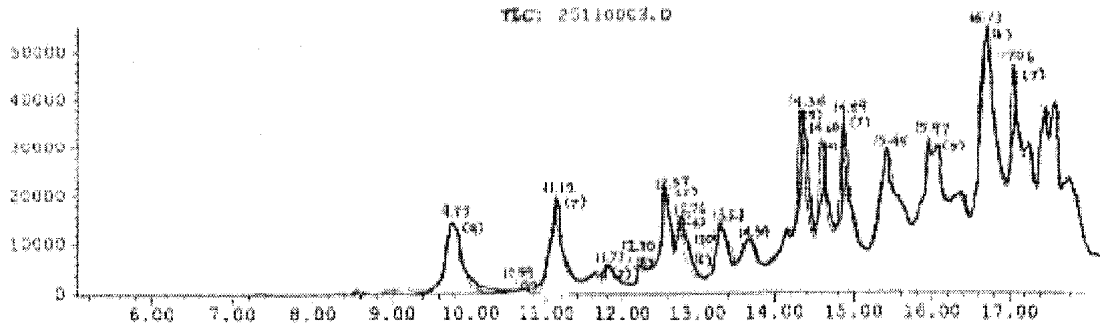
Province	% (w/w) [1,2,3,4]	
	Summer	Rainy
Chaing Mai	0.13 ± 0.07b	0.09 ± 0.02a
Ubon Ratchathani	0.09 ± 0.02b	0.06 ± 0.02b
Kanchanaburi	0.04 ± 0.04a	0.03 ± 0.02a
Chanthaburi	0.16 ± 0.03b	0.09 ± 0.06a
Songkhla	0.03 ± 0.05a	0.03 ± 0.01a
Average	0.09 ± 0.04	0.06 ± 0.03

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

2 means ± SD, 5 replicates for each area and season. Larvae from F_2-generation were used for all experiments at the same period of time.

3 all extracts made using ethanolic Soxhlet extraction at 70-80°C, 48 hours

4 The method of 4, 11-selinnadien-3-one identification was per Ohsawa *et al.* 1996 and the high purity standard of the active ingredient was purchased from Merck (Thailand).



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	9.77	10.88	C:\DATABASE\WILEY275.L			
			Cyperene $$ 3H-3a,7-Methanoazulene, 2	89329	002387-78-2	98
			.alpha.-Gurjunene $$ 1H-Cycloprop[e]a	89468	000489-40-7	91
			(-)-.ALPHA.-GURJUNENE	89794	000000-00-0	91
2	10.09	1.43	C:\DATABASE\WILEY275.L			
			Dispiro(cyclopropane-1,2'-bicyclo[2.2	32938	094348-06-8	25
			1,5-diphenylhex-3-ene	122491	103240-60-4	25
			1,5-Diphenylhex-3-ene	122507	000000-00-0	25
3	11.12	11.50	C:\DATABASE\WILEY275.L			
			trans-Pinocarveol $$ Bicyclo[3.1.1]he	38420	000547-61-5	38
			trans-Pinocarveol $$ Bicyclo[3.1.1]he	38422	000547-61-5	35
			Bicyclo[3.1.1]heptan-3-ol, 6,6-dimeth	38425	005947-36-4	35
4	11.77	0.96	C:\DATABASE\WILEY275.L			
			1-.alpha.-Terpineol $$ 3-Cyclohexene-	40197	010482-56-1	53
			3-Cyclohexene-1-methanol, .alpha.,.al	40185	000098-55-5	50
			.DELTA.3-Carene $$ Bicyclo[4.1.0]hept	25254	013466-78-9	47
5	12.10	1.02	C:\DATABASE\WILEY275.L			
			(1,3E,5E)-Undeca-1,3,5-triene	36322	051447-00-6	43
			(tetrahydroxycyclopentadienone)cricar	164566	117896-75-0	38
			(Tetrahydroxycyclopentadienone)cricar	164567	000000-00-0	38
6	12.37	3.90	C:\DATABASE\WILEY275.L			
			Bicyclo[3.1.1]hept-2-ene-2-carboxalde	35961	000564-94-3	50
			3,4-DIMETHYLPYRIDINE	6885	000000-00-0	49
			Benzenemethanol, .alpha.-methyl- (CAS	15789	000098-85-1	49
7	12.76	3.97	C:\DATABASE\WILEY275.L			
			Myrtenol $$ Bicyclo[3.1.1]hept-2-ene-	38412	000515-00-4	90
			MYRTENOL	27977	000515-00-4	90
			Myrtenol $$ Bicyclo[3.1.1]hept-2-ene-	38413	000515-00-4	89
8	13.09	1.68	C:\DATABASE\WILEY275.L			
			(Cyclopropyl)trivinylsilane	35641	000000-00-0	47
			a-Citral $$ 2,6-Octadienal, 3,7-dimet	37677	000106-26-3	38
			Cyclohexanol, 3,3,5-trimethyl- (CAS)	29939	000116-02-9	35
9	13.28	2.15	C:\DATABASE\WILEY275.L			
			1-Phenanthrenol, 2,4,4a,4b,5,6,7,8,8a	133576	057804-14-7	42
			1-Phenanthrenol, 2,4,4a,4b,5,6,7,8,8a	133574	057804-13-6	42
			METHYL 2,5-DIMETHYLTHIO-9-OXOGERANOAT	171438	060774-84-7	28
10	14.34	8.57	C:\DATABASE\WILEY275.L			
			Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-	35974	000080-57-9	86
			Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-	35969	000080-57-9	76
			Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-	35968	018309-32-5	68

Figure 1 GC-mass spectroscopy chromatogram (methods as described in the text) showing the different compounds that constituted the extracts.

various seasons and places in a tropical climate (Schmutterer, 1990).

Chromatograms, from GC-mass spectroscopy following the method of Ohsawa *et al*. (1996), showed that 10 active principles had accumulated in the tubers (Figure 1). The figures also revealed that 4, 11-selinnadien-3-one concentration was higher in our extracts than those of Ohsawa*et al.* (1996). This may indicate location influences, e.g. trace elements which play a role in the accumulation of secondary plant substances in major plants. These results also showed the same trend as rotenone extracted from derris root collected from different locations (Visetson and Milne, 2001).

Efficacy against diamondback moth larvae

The no-choice leaf dipping tests showed significant variation in efficiency against the 3rd-4th instar diamondback moth larvae within the same season (Table 2). On the other hand, differences were pronounced when comparisons were made between two seasons. The extracts showed 1.5-2.0 folds lower LC_{50}'s in the summer than in the rainy season ca. 1.5-2.0 folds. Chantaburi and Ubonratchatani gave the lowest LC_{50} of 7.05 ± 0.03 and 9.06 ± 2.02 ppm in summer but in the rainy season the LC_{50} increased ca. 2 folds. These figures indicated that there were either some substances other than 4,11-selinnadien-3-one in the extracted samples in summer and these substances might have elevated the mortality or some other substances accumulated in tubers in the rainy season might have reduced the mortality. Future experiments should look at other compounds extracted from the tubers that influence the larvae mortality. However, the aims of this experiment were to look at efficacy and detoxification mechanisms in this insect as well as to find the way to use extracts in the field. It is recommended to collect tubers in the summer rather than in the rainy season.

Mechanisms of enzyme activity and synergistic effects

The three synergists, PB, TPP and DEM, increased the efficacy of 4,11-selinnadien-3-one ca. 2-3 folds in samples collected in the summer and increased the efficacy ca. 2-6 folds in samples from the rainy season (Table 3).

Table 2 LC_{50} values for 4,11-selinnadien-3-one in nut grass extracts against 2nd -3rd instar larvae of the diamondback moth (*Plutella xylostella L.*) with a no-choice leaf dipping method for tubers collected during two seasons from different parts of Thailand.

Provinces	ppm [1,2]	
	Summer[3]	Rainy[4]
Chaing Mai	10.05 ±2.03a	17.11 ±2.11a
Ubon Ratchathani	9.06 ±2.02a	19.16±2.12b
Kanchanaburi	12.05± 1.01b	18.12 ±4.07a
Chanthaburi	7.05 ±0.03a	12.11 ±1.03a
Songkla	15.09±1.02b	25.18 ±1.04b

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

2 means ± SD, 5 replicates for each area and season. Larvae from F_2 -generation were used for all experiments at the same period of time.

3 all extracts made using ethanolic Soxhlet extraction at 70-80°C, 48hrs. following the method of Ohsawa *et al*. (1996).

4 no-choice leaf disks tests with 20 individuals of 2nd -3rd instar larvae were employed, 24 hours , LD_{50} from regression equation of discriminating doses from 10-90% mortality.

Table 3 LD_{50} and enzyme activity after addition of 1% synergist extracts containing 4,11-selinnadien-3-one extracted from nut grass tubers collected during two seasons from Chanthaburi with synergistic ratio (SR), correction factors {CF} and correlation determinations [r^2] also presented.

Synergist added	LD_{50} and Detoxification enzyme activity [1,2,3,4]					
	Summer (SR)[r^2]	Enzyme activity[5]	{CF}	Rainy (SR)[r^2]	Enzyme activity	{CF}
None	7.05 ±0.03b	Est	12.14 ±2.23	12.11 ±1.03c	Est	16.23 ±5.83
		GSH	32.13 ±2.46		GSH	42.45 ±6.48
		Mo	4,320 ±126		Mo	5,550 ±422
PB	2.41 ±1.2a	Mo	2,111 ± 233	2.32±2.05a	Mo	3,232 ±333
	(2.92) [0.8]		{2.04}	(5.22)[0.9]		{1.71}
TPP	2.11 ±1.13a	Est	8.14 ±2.11	2.14±3.02a	Est	9.12 ±3.13
	(3.34)[0.7]		{1.49}	(5.66) [0.8]		{1.78}
DEM	5.13±1.23b	GSH	21.22 ±2.76	5.12±1.10b	GSH	29.15 ±2.24
	(1.37)[0.6]		{1.51}	(2.37)[0.6]		{1.46}

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

2 means ± SD, 5 replicates for each area and seasons. Larvae from F_2 -generation were used for all experiments at the same period of time.

3 all figures for extracts made using ethanolic Soxhlet extraction at 70-80°C, 48 hrs. following the method of Ohsawa et al. (1996)

4 no-choice leaf dipping tests with 20 individuals of 3^{rd} –4^{th} instar larvae were employed. 24 hr checked.

5 Est, GSH and Mo stand for esterase, glutathione-S-transferase and monooxygenase with units of nM paranitrophenol produced/min/mg protein, nM DCNB conjugated product/min/mg protein and picM aldrin epoxidation/min/mg protein, respectively. CF was a correction factor derived from the division of enzyme activity with no synergist by synergistic enzyme activity. The synergistic ratio, SR, was derived from division of the LD_{50} with no synergist by the synergistic LD_{50}. r^2 was a correlation determination between LD_{50} and enzyme activity. "None" means no synergist was added to the active compound.

Moreover, correlations ($r^2 > 0.8$) were found between efficacy and enzyme activity, thus indicating that monooxygenase and some general esterases play a role in detoxification of this compound. Synergistic ratios (SR) and correction factors (CF) for the synergists, PB and TPP, showed that addition of these synergists resulted in a high inhibition of monooxygenase and esterase, respectively, in diamondback moth larvae. These results confirmed those of numbers of work in the area of detoxification mechanisms (Rose, 1985; Mackness *et al*., 1983; Yu and Hsu, 1985 and Visetson and Milne, 2001). The results showed that esterase, glutathione-S-transferase and monooxygenase would be inhibited 2-folds after addition of PB, TPP and DEM. These mechanisms were different from those involved with the detoxification of rotenone extracted from derris and of azadiractin from neem which revealed that monooxygenase was the only enzyme system that played a major role (Visetson and Milne, 2001). On the other hand, esterase had a major role in the detoxification of galanga and Siam weed extracts (Visetson *et al*. 2001). In another case, cholinesterase played a role in detoxifying citronella extracted from lemon grass in the dog tick (Visetson and Chuchouy, 1999). It can be concluded that because more than two mechanisms are involved in the detoxification of the 4,11-selinnadien-3-one in this insect larvae, nutgrass tuber extract should be a better insecticide alternative to control this insect pest. Any pesticide in which more than one

mechanism is involved in its detoxification results in a longer time taken for the pest to produce resistant genes (Yang *et al*., 2001; Yu, 1983; Yu, 1984).

Toxicity to non target species

The concentration of 2,000 ppm of 4,11-selinnadien-3-one in the extract from nut grass tubers gave no signs of growth effects (Figure 3). In addition, no acute dermal, acute oral as well as eye irritation were observed after 14 days of exposure. These results indicated no toxic effects based on the criteria of Ecobichon (1992).

One-month old guppies (*Poecillia reticulata*) were cultured in aquaria containing 10, 20, 30, 40 and 50 ppm of active ingredient. The mortality after 24 hours exposure showed 13.12 ±1.16, 38.28±2.12, 58.11±1.45, 88.35±2.34 and 96.12±1.23%, respectively, and gave an LC_{50} of 28.01 ppm (Y = -6.02 + 2X) (Table 5).

Nut grass tuber extracts at 1, 3, 5, 10 and 20 ppm of 4,11-selinnadien-3-one were applied to 1 week-old bee larvae (*Apis florea)* using a topical mist method and showed 8.12 ±3.36, 10.23±3.56, 34.39±2.51, 67.12±10.44 and 89.32±12.14% mortality, respectively, after 24 hours exposure indicating a LC_{50} of 10.8 ppm (Y = - 6.8 + 4X) (Table 6). Because this $LC_{50}$was more or less similar to the LC_{50} for diamondback moth larvae, then care must be taken with using nut grass extracts in areas of large bee populations. However, toxic levels on adult bee should be done in the future to evaluate the safety level in the bee population.

Table 5 Effects of 4,11-selinnadien-3-one extracted from nutgrass tubers on juvenile guppies (*Poecillia reticulata*).

Concentration (ppm)[1,2]	% mortality
10	13.12 ±1.16
20	38.28±2.12
30	58.11±1.45
40	88.35±2.34
50	96.12±1.23
	(LC_{50} = 28.01ppm, Y = -6.03 + 2X)

[1] means ± SD, n = 20, 0ne-month old juvenile fish.

[2] the active compound dissolved in acetone and then poured into water to make each concentration. Jars containing only acetone in water served as untreated controls, 24 hr check

CONCLUSION

Nut grass tubers in summer showed higher contents of 4,11-selinnadien-3-one compared to tubers collected in the rainy season. Chiengmai and Chantaburi tuber extracts had the highest 4,11-selinnadien-3-one contents. The LC_{50} against 3rd-4th instar larvae of diamondback moth was 7 – 12 ppm. The addition of PB, TPP and DEM to the extracts increased their efficacy. The reduced amounts of esterase, monooxygenase and glutathione-S-transferase after synergists were added indicated that more than one mechanism were involved in the detoxification of this compound. These results show that the insect may

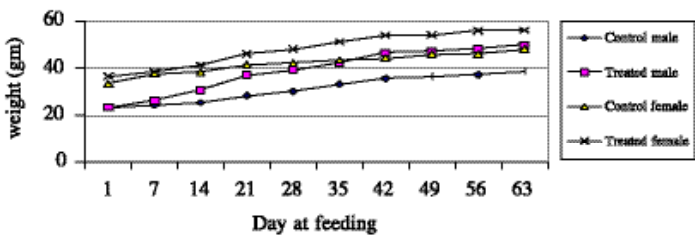


Figure 2 Growth rates of mice after feeding on nut grass tuber extract at 2,000 ppm 4,11-selinnadien-3-one.

Table 6 Effects of 4,11-selinnadien-3-one from nutgrass extracts on bee larvae (*Apis florea).*

Concentration (ppm)	% mortality[1,2]
1	8.12 ±3.36
3	10.23±3.56
5	34.39±2.51
10	67.12±10.44
20	89.32±12.14
	(LC_{50} = 10.8 ppm, Y = -6.8 + 4X)

[1] means ± SD, n = 20 individuals of 1-week old bee larvae.
[2] the active compound dissolved in acetone to a certain concentration then applied to the larvae. Acetone served as untreated controls. 24 hr check

take more time to produce resistance to this substance compared with other plant products. Although the substance did not show acute dermal, acute oral and eye irritation to the mouse, the LC_{50} values for diamondback moth larvae and bee larvae were similar. Therefore care must be taken in the use of this compound where bees are important to an area. This compound expressed a LC_{50} ca. 28.01 ppm for the guppy so the use of this extract near water was quite safe for fish. However, purification of both the enzyme systems and the active compound are crucial for the future in order to determine the exact synergistic relationships. This will lead to improvement of alternative plant substances for production on a commercial scale in the future.

ACKNOWLEDGMENTS

This research was supported by the Kasetsart University Research and Development Institute-(KURDI), Kasetsart University, Thailand.

LITERATURE CITED

Anonymous. 1986. Medical Herb Index in Indonesia. PT. Eisai Indonesia, Indonesia, 398 p.

Ecobichon, D.J. 1992. The basis of toxicity testing. CRC press. Inc Boca Raton. 350 p.

Finney,D.J. 1964. Statistical Methods in Biological Assay. 2nd ed., Charles Griffin and Company Limitted, London. 365 p.

Katanyukul, W. 2002. Comparison of pesticide used between Japan and Thailand. J. Home Econ. 26 (2) : 50-51. (in Thai).

Kirtikar, K.R. and B.D. Basu. 1991. Indian Medical Plants. Periodical Experts Book Agency, India. 540 p.

Lekprayun, J., S. Visetson., and M. Pianjalern. 1999. Effects of the leaf of *Chromolaena odorata* on the detoxification enzyme changes in *Plutella xylostella* L, pp.870-873. *In* 3rd BRT-Conference, Songkla. Hadyai, 11-14 October. 1999. (in Thai).

Mackness, M.L., C.H. Walker, D.G. Lowland, 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 (c) : 65-68.

Matsumura, F. 1976. Toxicology of Insecticides. Plenum press. Newyork and London. 503 p.

Ohsawa, D.K., S. Kato, and I. Yamamoto. 1996. Insecticidal compound in tuber *of Cyperus rotundus* L. against the diamondback moth larvae. J. Pestic. Sci. 21 : 444-446.

Rose, H.A. 1985. The relationship between feeding specialization and host plants to aldrin epoxidase activities of midgut homogenates in larval Lepidoptera. Ecol. Ent. 10 : 455-457.

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

Singh, D.,M.S. Siddiqui, and S. Sharma. 1989. Reproduction retardant and fumigant properties in essential oils against the rice weevil (Coleoptera: Curculionidae) in stored wheat. J. Econ. Entomol. 82 (3) : 727-733.

Valeeyaluck, N. 1983. Insect Pests of Vegetables in Thailand. Pahonyothins Pub. Bangkok, 205 p. (in Thai).

Visetson, S. and R. Chuchouy 1999. The efficiency of lemon grass extracts and neem extracts on some detoxification enzyme changes in the dog tick, pp. 847-851. *In* 3rd BRT-Conference. Songkla. Hadyai, 11-14 October. 1999. (in Thai).

Visetson, S., M. Piansiri, and T. Watanasombat. 2001. The effects of the Siam weed (*Chromolaena odorata*) extracts and the alpinia (*Alpinia galanga* Stuntz) extracts on some detoxification enzymes of the diamondback moths (*Plutella xylostella* L.), pp. 55-61. *In* The 5th National Plant Protection Conference: Food Products for the World. Thai association, Chemical businessmen, Plant protection association and Association of Entomology and Zoology of Thailand. 21-23 November 2001. Felix River Kew, Kanjanaburi. (in Thai).

Visetson, S. 2001. Effects of Azadirachtin from various Thai neem extracts on some detoxification enzyme activities in *Callosobruchus maculatus* F., pp.38 – 46. *In* 20th ASEAN/2nd APRC Seminar on Postharvest Technology. 11-14 September 2001. Lotus Hotel Pang Suan Kaew. Chiang Mai.

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.

Yang, X., D.C. Margolies, K.Y. Zhu, and L. Buschman. 2001. Host plant-induced changes in detoxification enzymes and susceptibility to pesticides in the two spotted spider mite (Acari: Tetranychidae). J. Econ. Entomol. 94 (2) : 381-287.

Yu, S.J. 1983. Induction of detoxifying enzymes by allelochemicals and host plant in the fall armyworm. Pestic. Biochem. Physiol. 12 : 330-336.

Yu, S.J. 1984. Interaction of allelochemical with detoxification enzymes of insecticides in susceptible and resistance fall armyworm. Pestic. Biochem. Physiol. 14 : 275-281.

Yu, S. J. and E.L.Hsu. 1985. Induction of hydrolases by allelochemicals and host plants in fall armyworm (Lepidoptera:Noctuidae) larvae. Environ. Ent. 14 : 512-515.

Received date : 8/08/01
Accepted date : 28/09/01