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POLYTOMOUS QUANTAL RESPONSE OF *CROCIDOLOMIA PAVONANA* (F.) (LEPIDOPTERA: PYRALIDAE) TO EXTRACTS OF *AGLAIA* SPP. AND *DYSOXYLUM* SPP. (MELIACEAE)

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ABSTRACT

Polytomous Quantal Response of* Crocidolomia pavonana *(F.) (Lepidoptera: Pyralidae) to Extracts of* Aglaia *spp. and* Dysoxylum *spp. (Meliaceae). This work was done to study the polytomous binary response of the cabbage head caterpillar *Crocidolomia pavonana* (F.) to extracts of two species each of *Aglaia* and *Dysoxylum* (Meliaceae). Second-instar larvae *C. pavonana* were fed extract-treated broccoli leaves for 2 days and then the surviving larvae were maintained on untreated leaves until pupation. Relationship between extract concentration and the number of dead larvae in different instars was analyzed using polytomous quantal response analysis based on the conditional logit model. The results showed that twig extract of *Dysoxylum acutangulum* and seed extract of *D. mollissimum* possessed strong insect growth regulating activity against *C. pavonana* larvae as reflected by highly significant responses in the later insect life stages after the feeding treatment was removed, including the significant occurrence of malformed pupae. On the contrary, the feeding treatment with twig extract of *Aglaia odorata* and seed extract of *A. odoratissima* resulted in highly significant responses only in the treated larval instar, and after the feeding treatment was removed, responses in the later life stages were insignificant or much less significant. Overall, the above results suggest that *Dysoxylum* extracts interfered with hormonally-controlled insect development and metamorphosis, whereas the activity of *Aglaia* extracts was more insecticidal rather than insect growth regulating.

Key words: botanical insecticides, insect growth regulator, Meliaceae

INTRODUCTION

In the last 15 years or so there has been an increasing interest in the study of insecticidal activity of plants in the genera *Aglaia* and *Dysoxylum* (Meliaceae). The account on insecticidal activity of *Aglaia* first appeared in the world literature in 1985 when Chiu (1985) reported, among others, the antifeedant activity of acetone extracts of leaves and twigs of *Aglaia odorata* against three species of lepidopterous larvae, i.e. *Pieris rapae*, *Spodoptera litura*, and *Mythimna separata*, as well as the growth inhibitory effect of those extracts against *P. rapae* larvae. Isolation of an insecticidal compound rocaglamide (belonging to the benzofuran class of compounds) from *A. odorata* twigs was first reported in 1993 by Janprasert *et al.* (1993). Actually, this compound had previously been isolated from *Aglaia elliptifolia* in 1982 and reported as having antileukemic activity against murine cell lines *in vitro* (King *et al.*, 1982), but its insecticidal activity was then not tested. Since 1993 the work on insecticidal activity of *Aglaia* spp. has been rapidly expanding and presently more than 50 rocaglamide derivatives have been isolated from various species of *Aglaia* (Proksch *et al.*, 2001).

The first report on insecticidal activity of *Dysoxylum* appeared in the world literature in 1987 when Mikolajczak and Reed (1987) described the strong insecticidal activity of ethanolic seed extracts of *Dysoxylum binectariferum*, *D. malabaricum*, *D. reticulatum*, and *D. spectabile* against the fall armyworm *Spodoptera frugiperda* and the strong antiffedant effect of those extracts against the striped cucumber beetle *Acalymma vittatum*. In a further study, Mikolajczak *et al.* (1989) reported that the treatment with ethanolic extracts of *D. malabaricum* and *D. spectabile* at 0.2% in artificial diet caused a complete kill in *S. frugiperda*. Russell *et al.* (1994) isolated a sesquiterpenoid ant repellent (2S,3R)–2,3-dimethy l – 3-(4–methyl–3–pentenyl)–2–nor-bornanol from the fruits of *D. spectabile*. Isman *et al.* (1995) reported that extracts two other species of *Dysoxylum*, i.e. *D. acutangulum* and *D. guadilaidianum*, were sufficiently active against the variegated cutworm *Peridroma saucia*. Recently, Prijono *et al.* (2004) reported that methanolic extracts of *D. acutangulum* and *D. arborescens* twigs and *D. mollissimum* seeds at a concentration of 0.5% had strong insecticidal activity against *C. pavonana* larvae, causing more than 90% mortality.

The type of responses to a certain compound of an insect species over the whole preimaginal period

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may indicate the nature of insecticidal effect of that compound. Prijono *et al.* (2004) reported that *C. pavonana* larvae intoxicated by *Dysoxylum* spp. extracts showed a typical symptom of molting inhibition suggesting hormonal interferences while the treatment with *Aglaia* spp. extracts did not cause that kind of symptom. This suggests that the nature of active compounds in *Aglaia* spp. is different from that in *Dysoxylum* spp. Rocaglamide derivatives have been known as the main insecticidal compounds in *Aglaia* species (Proksch *et al.*, 2001) while the active principles of *Dysoxylum* species have not been firmly established.

One of the quantitative analyses that can be used to differentiate insect growth regulating (IGR) effect from non-IGR is polytomous quantal response analysis based on the conditional logit model (PQRA-CLM) (Robertson & Preisler, 1992). This study was conducted to compare quantitatively, using PQRA-CLM, the responses of *C. pavonana* larvae to extracts of four species of Meliaceae, i.e. *A. odorata*, *A.odoratissima*, *D. acutangulum*, and *D. mollissimum*.

MATERIALS AND METHODS

Insect Culture

C. pavonana larvae were obtained from the laboratory colony maintained at the Laboratory of Insect Physiology and Toxicology (LIPT), Bogor Agricultural University (BAU). The insect colony has been maintained in the laboratory since September 1992 under ambient conditions (25–31.5 ºC, 65-85% RH). The larvae were fed pesticide-free broccoli leaves and the adults were fed 10% honey solution in cotton swab (Basana & Prijono, 1994).

Extraction

Twigs of *A. odorata* and *D. acutangulum* and seeds of *A. odoratissima* and *D. mollissimum* served as sources of extracts used in this study. *A. odorata* twigs were collected from a home garden in Bogor, *D. acutangulum* twigs from a hill forest in Jasinga-Bogor, and *A. odoratissima* and *D. mollissimum* seeds were obtained from Bogor Botanic Garden.

Test plant materials were ground separately and then extracted with methanol or acetone by stirring. The ground twigs of *A. odorata* and *D. acutangulum* and seeds of *A. odoratissima* were stirred separately in methanol in an erlenmeyer flask for at least 24 hours, then the extracts were filtered. The marc was washed repeatedly with methanol until the filtrate was clear. The filtrates were pooled, then the solvent was evaporated in a rotary evaporator (rotavapor) at 50 ºC under reduced pressure. The extracts obtained were partitioned between ethyl acetate and water. The water phase was discarded and the ethyl acetate phase was collected. After evaporation of the solvent, the ethyl acetate fraction was kept in refrigerator (± 4 ºC) until used in the bioassay.

For the extraction of *D. mollissimum* seeds, acetone was used as the extracting solvent instead of methanol, because the results of extract partition with ethyl acetate and water were inconsistent when methanol was used as the extracting solvent.

Extract Bioassay

The bioassay was carried out at the LIPT-BAU under ambient conditions as above. The extracts were tested against second-instar larvae *C. pavonana* using a leaf residual method as previously described (Prijono *et al*. 2000). Each extract was tested at seven concentration levels to cover a range of concentrations which were expected to cause 0-100% mortality as determined in preliminary tests. In this test, ranges of concentrations of *A. odorata*, *A. odoratissima*, *D. acutangulum*, and *D. mollissimum* extracts were 200-1500 ppm, 200-800 ppm, 20-200 ppm, and 100-2000 ppm, respectively.

Each extract was dissolved in a mixture of methanol-acetone (3:1) to the desired concentrations. Extract solution of a particular concentration was applied uniformly on both sides of broccoli leaf disks (3 cm in diameter) using a microsyringe at a rate of 25 µl/side. Control leaf disks were treated with solvent mixture only. After the solvent mixture had evaporated, two treated or control leaf disks were placed in a glass petri dish (9 cm in diameter) lined with towel paper, then 15 second-instar larvae were introduced into each dish. After 24 hours, treated or control leaf disks were added as necessary, and after additional 24 hours, leftover leaf disks were removed and replaced with untreated leaves. In the treatment with *A. odorata*, *D. acutangulum*, and *D. mollissimum* extracts, each concentration treatment was replicated seven times and in that with *A. odoratissima* extract, each treatment was replicated six times. Larval mortality was recorded daily until the surviving larvae reached the pupal stage.

Data Analysis. Responses of *C. pavonana* larvae to the feeding treatment with each of the test extracts were classified into five categories as previously described (Prijono *et al.* 2000): (1) the second-instar larva died before or during moulting to the third instar, (2) the second-instar larva succeeded in moulting to the third instar but died before or during moulting to the fourth instar, (3) the larva succeeded in developing to the fourth instar but died before pupation, (4) the larva developed into a malformed pupa, and (5) the larva successfully developed into a normal pupa.

Quantitative relationship between extract concentration and the probability of response in various categories was analyzed using PQRA-CLM (Robertson & Preisler, 1992). Detailed procedures of this analysis have previously been described (Prijono *et al.*, 2000).

The probability that an insect exhibited a response in category j, under condition that it did not show any response in all previous categories, was given by the following model:

$$y_{ij} = \alpha_j + \beta_j \log (c_i + c_0)$$

where y_{ij} is the logit of response in category j at concentration c_i, α_j and β_j are model parameters to be estimated, and c_0 is the displaced control value as described by Tukey *et al*. (1985).

The estimated c_0 was calculated using the following formula:

$$X_0 = \log c_i - \frac{c_1 - c_0}{c_2 - c_1} (\log c_2 - \log c_1)$$

where c_1 and c_2 were the lowest and second lowest concentrations, and c_0 was first entered as 0 (zero), then c_0 was calculated back as antilog of X_0.

Parameter estimates of the model were calculated using a computer program GLIM (RSS, 1985). Estimates of the conditional probability $(\hat{p}_{ij})$ of response in particular categories at certain concentrations were calculated from the estimated model:

$$\hat{y}_{ij} = a_j + b_j \log (c_1 + c_0) \text{ and } \hat{p}_{ij} = F(\hat{y}_{ij})$$

where a_j and b_j were estimates of the model parameters, and $F(y) = e^y/(1 + e^y)$ for the logit model. The conditional probability of the last response category is equal to 1 (all insects that did not exhibit any response in all previous categories would belong to the last category).

Estimates of the unconditional probability $(\hat{q}_{ij})$ of response in category 1 to j-1 were calculated as follows:

$$\hat{q}_{ij} = (1 - \hat{p}_{i1}) * (1 - \hat{p}_{i2}) * \cdots * \hat{p}_{ij} \quad \text{for j = J-1}$$

The unconditional response probability in the last response category is equal to $\{1 - (\hat{q}_{i1} + \hat{q}_{i2} + \cdots + \hat{q}_{i(j-1)})\}$.

RESULTS AND DISCUSSION

The treatment with *A. odorata* extract at 200-1500 ppm and *A. odoratissima* extract at 200-800 ppm resulted in highly significant responses only in the instar that was given the feeding treatment, i.e. the second instar (LR statistics for b values were highly sifnificant [$p < 0.001$] only for category 1, Table 1). LR statistic for b value in category 3 for *A. odorata* extract and that in category 4 for *A. odoratissima* extract were significant at the level of 0.05.

LR statistics for b values in categories 2 and 4 for *A. odorata* extract and those in categories 2 and 3 for *A. odoratissima* extract were not statistically significant. The insignificant LR statistics for b values in particular categories means that insect responses in those categories were independent of extract concentration. In such case, the parameter estimates of the model were recalculated by taken b's as zeros (Table 2) and the estimated conditional probabilities of response in various categories were calculated based on the latter estimated model.

The concentration-dependent responses of *C. pavonana* larvae to *A. odorata* extract in categories 1 and 3 and those to *A. odoratissima* extract in categories 1 and 4 are shown in Table 3. The mortality of the second instar (response category 1) increased markedly as the extract concentration was increased 4-6 times. In response categories where the LR statistics for b values were not statistically significant, the conditional probabilities of response were constant irrespective of extract concentration (Table 3). In the treatments with *A. odorata* and *A. odoratissima* extracts, the conditional probabilities of response in category 3 (death in fourth instar) and category 4 (malformed pupae), respectively, were also concentration-dependent but much weaker than those in category 1.

Table 1. Parameter estimates of the conditional logit model for polytomous response of *C. pavonana* larvae to extracts of four species of Meliaceae

Extract	Category of response[a]	N[b]	Parameter estimates ± SE[c] a	Parameter estimates ± SE[c] b	LR[d] for H_0: $\beta_j = 0$ (P)
A. odorata	1	836	-27.83 ± 3.80	9.88 ± 1.35	149.75 ($P < 0.001$)
	2	467	-7.12 ± 6.23	1.34 ± 2.39	0.36 ($0.5 < P < 0.7$)
	3	456	-12.86 ± 5.65	3.96 ± 2.09	5.67 ($0.01 < P < 0.02$)
	4	422	-5.17 ± 11.06	-0.07 ± 4.46	0.001 ($0.95 < P < 0.98$)
A. odoratissima	1	715	-27.58 ± 2.84	10.29 ± 1.04	239.70 ($P < 0.001$)
	2	333	-6.63 ± 5.24	0.98 ± 2.06	0.25 ($0.5 < P < 0.7$)
	3	328	-4.97 ± 2.06	1.16 ± 0.81	2.23 ($0.1 < P < 0.2$)
	4	292	-11.00 ± 4.31	3.17 ± 1.62	5.37 ($0.02 < P < 0.05$)
D. acutangulum	1	839	-42.74 ± 7.81	18.57 ± 3.42	130.83 ($P < 0.001$)
	2	770	-20.61 ± 2.36	10.18 ± 1.18	215.98 ($P < 0.001$)
	3	577	-14.45 ± 1.86	8.10 ± 1.06	136.15 ($P < 0.001$)
	4	388	-18.14 ± 4.51	9.29 ± 2.56	23.36 ($P < 0.001$)
D. mollissimum	1	841	-25.31 ± 3.75	7.95 ± 1.20	132.38 ($P < 0.001$)
	2	716	-23.57 ± 3.38	7.99 ± 1.17	134.09 ($P < 0.001$)
	3	581	-30.67 ± 5.19	11.03 ± 1.90	108.18 ($P < 0.001$)
	4	487	-38.81 ± 12.48	13.86 ± 4.65	30.90 ($P < 0.001$)

[a] *See* Materials and Methods for category of response; [b] Initial number of larvae before responding in particular categories; [c] SE = standard error; a and estimates for α and β in the model $y_{ij} = \alpha_j + \beta_j \log (c_i + c_0)$, where c_0 for *A. odorata*, *A. odoratissima*, *D. acutangulum*, and *D. mollissimum* are 8.8889, 88.8889, 88.8889, and 46.8584 (all in ppm), respectively; [d] LR = likelihood ratio statistic.

Table 2. Recalculated parameter estimates of the conditional logit model for polytomous response of *C. pavonana* larvae to extracts of *A. odorata* and *A. odoratissima*

Extract	Category of response	Parameter estimates ± SE	
		A	b
A. odorata	1	-27.83 ± 3.80	9.88 ± 1.35
	2	-3.73 ± 0.62	0 ± aliased [a]
	3	-12.86 ± 5.65	3.96 ± 2.09
	4	-5.35 ± 1.44	0 ± aliased
A. odoratissima	1	-27.58 ± 2.84	10.29 ± 1.04
	2	-4.18 ± 0.59	0 ± aliased
	3	-2.09 ± 0.23	0 ± aliased
	4	-11.00 ± 4.31	3.17 ± 1.62

[a] c_i in category j was set to zero in the calculation of parameter estimates using the GLIM program when b_j was not significantly different from zero ($p > 0.05$; *see* Table 1). This resulted in a zero slope for category j ($b_j = 0$). *See* footnotes of Table 1 for other explanations.

Table 3. Estimated conditional probabilities for polytomous response of *C. pavonana* larvae to extracts of four species of Meliaceae at selected concentrations

Extract concentration (ppm)	Estimated conditional probability in category j				
	1[a]	2	3	4	5
A. odorata					
0	1.89×10^{-4}	0.023	0.006	0.005	1
250	0.056	0.023	0.055	0.005	1
550	0.472	0.023	0.148	0.005	1
850	0.823	0.023	0.252	0.005	1
1150	0.939	0.023	0.352	0.005	1
1450	0.975	0.023	0.441	0.005	1
A. odoratissima					
0	5.38×10^{-4}	0.015	0.110	0.008	1
200	0.095	0.015	0.110	0.039	1
350	0.404	0.015	0.110	0.068	1
500	0.716	0.015	0.110	0.098	1
650	0.874	0.015	0.110	0.129	1
800	0.941	0.015	0.110	0.161	1

Table 3 continued.

Extract concentration (ppm)	Estimated conditional probability in category j				
	1[a]	2	3	4	5
D. acutangulum					
0	1.23×10^{-11}	1.75×10^{-5}	0.001	8.91×10^{-5}	1
50	5.17×10^{-5}	0.070	0.472	0.155	1
100	0.007	0.532	0.886	0.686	1
150	0.134	0.858	0.967	0.910	1
200	0.584	0.953	0.987	0.968	1
250	0.888	0.981	0.994	0.986	1
D. mollissimum					
0	5.98×10^{-6}	3.64×10^{-5}	4.83×10^{-6}	1.59×10^{-7}	1
500	0.028	0.155	0.384	0.297	1
850	0.138	0.506	0.870	0.892	1
1200	0.332	0.762	0.970	0.984	1
1550	0.539	0.883	0.991	0.996	1
1900	0.699	0.938	0.996	0.999	1

[a] *See* Materials and Methods for category of response.

The conditional probabilities of response in all categories, except the last one, in the treatments with *D. acutangulum* extract at 20-200 ppm and *D. mollissimum* extract at 100-2000 ppm were highly concentration-dependent as reflected by the highly significant likelihood ratio (LR) statistics for b values in categories 1 to 4 ($p < 0.001$, Table 1). This means that varying extract concentration will have strong effects on the mortality of second-, third- and fourth-instar larvae as well as on the occurrence of malformed pupae. This can be clearly seen in Table 3 where the conditional probabilities of response in categories 1 to 4 increased markedly with the increase in extract concentration. The lower conditional response probabilities for category 1 when compared to probabilities for categories 2 – 4 at the same concentrations suggest that the treatment with *Dysoxylum* spp. extracts at a certain concentration may not exert an immediate effect but could induce responses in the later insect growth stages.

In the treatments with all extracts, unconditional response probabilities in category 1 increased markedly in a concentration-dependent fashion (Table 4). Contrary to response category 1, unconditional probabilities of response categories 2 and 4 in the treatment with *A. odorata* extract and those of categories 2 and 3 in the treatment with *A. odoratissima* extract decreased with the increase in extract concentrations (Table 4). This is because conditional probabilities of response in those categories were not concentration-dependent (Table 3) so that the corresponding unconditional probabilities followed the concentration-survival relationship in the preceding categories, i. e. the rates of survival (= 1 – unconditional response probabilities) decreased markedly with the increase in extract concentrations. Unconditional probabilities of response category 5 represent the survival rates of test insects to the pupal stage (normal pupa).

The treatment with *D. acutangulum* extract at 150 ppm and *D. mollissimum* extract at 850 ppm caused only 13.4% and 13.8% mortality in the second instar (100 x unconditional response probabilities in category 1, Table 4), respectively, but could cause

Table 4. Estimated unconditional probabilities for polytomous response of *C. pavonana* larvae to extracts of four species of Meliaceae at selected concentrations

Extract concentration (ppm)	Estimated unconditional probability in category j				
	1[a]	2	3	4	5
A. odorata					
0	1.89×10^{-4}	0.023	0.006	0.005	0.966
250	0.056	0.022	0.051	0.004	0.867
550	0.472	0.012	0.076	0.002	0.437
850	0.823	0.004	0.043	6.10×10^{-4}	0.128
1150	0.939	0.001	0.021	1.83×10^{-4}	0.039
1450	0.975	5.88×10^{-4}	0.011	6.48×10^{-5}	0.014
A. odoratissima					
0	5.38×10^{-4}	0.015	0.108	0.007	0.869
200	0.095	0.014	0.098	0.031	0.763
350	0.404	0.009	0.065	0.035	0.487
500	0.716	0.004	0.031	0.024	0.225
650	0.874	0.002	0.014	0.014	0.096
800	0.941	8.94×10^{-4}	0.006	0.008	0.044
D. acutangulum					
0	1.23×10^{-11}	1.75×10^{-5}	0.001	8.90×10^{-5}	0.999
50	5.17×10^{-5}	0.070	0.439	0.076	0.415
100	0.007	0.528	0.412	0.036	0.017
150	0.134	0.743	0.119	0.004	3.67×10^{-4}
200	0.584	0.396	0.019	2.44×10^{-4}	8.03×10^{-6}
250	0.888	0.110	0.002	1.26×10^{-5}	1.75×10^{-7}
D. mollissimum					
0	5.98×10^{-6}	3.64×10^{-5}	4.83×10^{-6}	1.59×10^{-7}	1.000
500	0.028	0.151	0.316	0.150	0.356
850	0.138	0.436	0.371	0.050	0.006
1200	0.332	0.509	0.154	0.005	7.77×10^{-5}
1550	0.539	0.407	0.053	5.02×10^{-4}	1.88×10^{-6}
1900	0.699	0.283	0.019	6.83×10^{-5}	7.76×10^{-8}

[a] *See* Materials and Methods for category of response.

74.3% and 43.6% mortality in the third instar (category 2) and spared only 0.04% and 0.6% of the initial population of test larvae to develop successfully to the pupal stage (category 5). In the treatments with *D. acutangulum* extract at 250 ppm and *D. mollissimum* extract at 1550 ppm, the survival rates of second instars were 0.112 (= 1 – unconditional probability in category 1 [0.888]) and 0.461 (= 1 – 0.539), respectively, but the respective rates of normal pupa emergence were less than 2×10^{-7} and less than 2×10^{-6} (Table 4). Since the feeding treatment was given only to the second instar, strong responses in the later insect growth stages (mortality of third- and fourth-larvae and occurrence of malformed pupae) suggest that *D. acutangulum* and *D. mollissimum* extracts contain highly active insect growth regulating substances.

In the treatments with *A. odorata* and *A. odoratissima* extracts, the rates of normal pupa formation (unconditional probabilities in category 5, Table 4) did not differ greatly from the survival rates at the initial stage (category 1), whereas in the treatments with *D. acutangulum* and *D. mollissimum* extracts, the rates of pupa formation were much lower than the survival rates at the initial stage. For example, the survival rates for category 1 in the treatments with *A. odorata* and *A. odoratissima* extracts at 550 and 500 ppm were 0.528 (= 1 – 0.472) and 0.284 (= 1 – 0.716), respectively, and the respective rates of normal pupa formation were 0.437 (only 1.2 times lower) and 0.225 (only 1.3 times lower) (Table 4); whereas the survival rates for category 1 in the treatments with *D. acutangulum* and *D. mollissimum* extracts at 200 and 1200 ppm were 0.416 (= 1 – 0.584) and 0.668 (= 1 – 0.332), respectively, but the respective rates of pupa formation were only 8.03×10^{-6} (more than 50,000 times lower) and 7.77×10^{-5} (more than 85,000 times lower) (Table 4). Thus, the nature of activity of *Dysoxylum* spp. extracts against *C. pavonana* was very different from that of *Aglaia* spp. extracts, i.e. *Dysoxylum* spp. extracts possessed strong insect growth regulating activity and the latter extracts were more insecticidal rather than insect growth regulating.

The above results show that PQRA-CLM could be used to delineate the nature of activity of a certain insecticidal preparation whether it has an immediate lethal effect or IGR activity. *D. acutangulum* twig and *D. mollissimum* seed extracts were revealed to possess a strong insect growth regulating activity whereas *A. odorata* twig and *A. odoratiassima* seed extracts had an immediate lethal effect rather than insect growth regulating. The treatment with an insect growth regulator could cause a strong effect not only in the stage that was given the feeding treatment, but also in the much later growth stage as exemplified in this study by the effect of *D. acutangulum* and *D. mollissimum* extracts on the occurrence of malformed pupae. The pattern of insect growth regulating activity such as that resulting from the treatments with *D. acutangulum* and *D. mollissimum* extracts has also been reported for neem and azadirachtin (Prijono & Hassan, 1993; Prijono *et al.*, 2001).

The treatment with an insecticide lacking or having limited insect growth regulating activity causes a strong effect only in the stage that was given the feeding treatment as exemplified in this study by the effect of *A. odorata* and *A. odoratissima* extracts. This result is consistent with that described for *A. elliptica* and *A. harmsiana* extracts (Prijono *et al.*, 2000) as well as for rocaglamide (Prijono *et al.*, 2001).

More than 50 rocaglamide derivatives (benzofuran) have been isolated from various *Aglaia* species, including at least 15 rocaglamide derivatives from *A. odorata* (Proksch *et al*., 2001), but active compounds in *A. odoratissima* have never been reported. Considering the similarity of activity of *A. odorata* twig and *A. odoratissima* seed extracts as shown in this study and that the two species belong to the same genus, it seems that *A. odoratissima* seeds also contain rocaglamide derivatives. Further work is needed to prove this conjecture.

Insecticidally-active compounds in *Dysoxylum* have never been reported. Given the very different nature of insecticidal activity of *Dysoxylum* and *Aglaia* extracts, it can be assumed that the chemical nature of *Dysoxylum* active substances is different from that of *Aglaia*. Moreover, the known active principles of Meliaceae plants that have IGR activity usually belong to a group of compounds (limonoid) that is different from benzofuran (Isman *et al.*, 1995).

It is assumed that the primary target of active compounds of *Aglaia* spp. is not one of the hormones that control insect growth and development since those extracts are more insecticidal than insect growth regulating. The concentration-dependent occurrence of some delayed effects in the treatment with *A. odorata* and *A. odoratissima* extracts (category 3 and 4, respectively; Table 1) was probably due to the

interference of some basic cellular events in organs that produce insect developmental hormones by extract residues in the body of test larvae. Some rocaglamide derivatives from *A. odorata* were reported to inhibit the growth of K-*ras*-NRK tumor cells and specifically inhibit protein synthesis (Ohse *et al*., 1996). Recently, Proksch *et al.* (2001) reported that rocaglamide arrested division of *Spodoptera frugiperda* cells *in vitro*.

Unlike in the treatments with *A. odorata* and *A. odoratissima* extracts, the consistent occurrence of moulting inhibition and malformed pupae in the treatment with *D. acutangulum* and *D. mollissimum* extracts provides strong clues as to the primary biochemical lesion of active substances in those extracts. It has been well established that insect growth inhibitors interfere with the function of some hormones that control insect growth and development, such as juvenile hormone, ecdysone, eclosion hormone, or the preceding stimulating hormones. Further work is needed to determine the precise mode of action of *Dysoxylum* spp. active substances.

In conclusion, *D. acutangulum* twig and *D. mollissimum* seed extracts had strong insect growth regulating activity whereas *A. odorata* twig and *A. odoratissima* seed extracts exhibited more immediate lethal effect on *C. pavonana* larvae. Further work on isolation and identification of active compounds in extracts other than *A. odorata* is worthwhile to be pursued.

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