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Effect of Three Botanical Oils on Carbohydrate Content in *Cheilomenes sexmaculata* Fabricius and *Chrysoperla carnea* Stephens

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Abstract: The impact of three botanical oils viz., neem, pungam and madhuca each in two dosage levels (1.5 and 3.0 %) on carbohydrate content in two predatory insects *Cheilomenes sexmaculata* and *Chrysoperla carnea* was investigated. In the adult male of *C. sexmaculata*, neem oil 3.0% caused 24.62% decrease in carbohydrate content and in all the other botanical oil treatments it got increased when compared with untreated control. However in it's final instar larva, all the botanical oil treatments had reduced the carbohydrate level. The highest reduction of 44.31-59.31% was observed in neem oil treatment. In *C. carnea* female, all the botanical oil treatments recorded reduction in carbohydrate content except in madhuca oil 3.0%, which showed 8.64% increase over untreated control. In male also, all the botanical oil treatments caused reduction in carbohydrate content. However in it's final instar larva, pungam oil 1.5% and madhuca oil 3.0%, caused an increase of 1.42-44.71% while in all other treatments, the carbohydrate content was decreased.

Key words: Neem, pungam, madhuca, *Cheilomenes sexmaculata*, *Chrysoperla carnea*,

Introduction

Coccinellids are important natural enemies of pest species especially on whiteflies aphids, mealy bugs, scales and mites (Obrycki and Kring, 1998). Similarly the chrysopids also act as effective predators on many soft-bodied insects. Botanical insecticides such as neem, pungam and madhuca exert many adverse effects on various insect pests and hence they are being used in pest management programmes (Krause, 2002; Murugan *et al*., 1999; Rajamohan and Regupathy, 1999; Saikia and Parameswaran, 1999; Schmutterer, 2005; Simmonds *et al*., 2004). In any agro ecosystem, the natural enemies coexist with the incidence of insect pests. In that situation, there is great chance for the natural enemies to get exposed to the applied botanical insecticides targeted against insect pests either directly or indirectly. However the adverse effects of botanical insecticides on natural enemies of insect pests is not yet studied in detail. Some synthetic chemical insecticides, botanical insecticides and biopesticides altered the level of lipid, carbohydrate and protein in treated insects (Badhiya and Hazarika, 1996; Barwal and Kalra, 2001; Ramakoteswara Rao *et al*., 1995; Seyoum *et al*., 2002; Vijayaraghavan and Chitra, 2002; Wang *et al*., 2005). The information and literatures regarding the biochemical changes influenced by the plant products in natural enemies of insects are scanty. In the present investigation, the effects of three commonly available non-edible botanical oils namely, neem (*Azadirachta indica* A. Juss), pungam (*Pongamia glabra* Vent.) and madhuca (*Madhuca indica* J. F. Gmel.) on the carbohydtate levels in two efficient insect predators, a coccinellid *Cheilomenes sexmaculata* and a chrysopid *Chrysoperla carnea* are discussed in detail.

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Materials and Methods

This study was conducted at Agricultural College and Research Institute, Madurai, Tamil Nadu during 2004.

Mass Culturing

Host Insect Aphis Craccivora Koch

The cowpea aphid *A. craccivora* was mass cultured on cowpea seedlings (variety Pusa 152) as described by Gautam (1990).

Test Insect Predators

C. sexmaculata

The adult beetles were collected from cowpea field infested with *A. craccivora* at Government seed farm in Vinayagapuram, near Melur. They were confined in polythene bags and allowed to lay eggs. Both the adults and grubs of *C. sexmaculata* were reared to good numbers on the cowpea aphid *A. craccivora.* Adequate aphids were supplied as food to the grubs till pupation and the prepupae were collected and kept in plastic boxes separately for pupation and adult emergence. Cowpea aphids collected from field and pot culture were alternatively supplied to have virulent insect culture.

C. carnea

The culture of *C. carnea* was maintained in the laboratory from a nuclear culture of 100 cocoons obtained from BASARASS Biocon India (Pvt.) Ltd., Virudhachalam, Tamil Nadu. The neonate larvae were reared on *Corcyra cephalonica* Staint eggs and also on cowpea aphid. The adults were given a semi solid food consisting of equal quantities of honey, yeast, proteinex, fructose and water and water was fed through sponge and they were allowed to lay stalked eggs on the rough surface of brown sheets placed inside galvanized iron oviposition tray as described by Nordlund and Morrison (1992).

Biochemical Analysis

The test insects (Final instar larvae and adults of *C. sexmaculata*) were prestarved for 4 h and given with second instar nymphs of *A. craccivora* sprayed with the respective botanical oils at three and five % concentration levels as food after shade drying. Similarly for biochemical analysis in *C. carnea*, the final instar larvae were fed with the treated *Corcyra* eggs, while the adults were given with the honey mixed with different botanicals at the rate three drops per 10 mL of honey. After 72 h of feeding on the treated foods, standardized biochemical protocol was followed for estimating total carbohydrate by anthrone method (Sadasivam and Manickam, 1992).

Results

C. sexmaculata

The carbohydrate level in the insects of *C. sexmaculata* male was 3.936 mg in untreated control. Madhuca oil 1.5% recorded the highest level of carbohydrate (7.342 mg) and it was 86.53% more than that in untreated control. This was followed by 6.443 mg in neem oil 1.5% which was 63.69% higher than control (Table 1). The treatments pungam oil at both concentrations and madhuca oil at higher concentration registered 4.086-5.864 mg in the treated insects and they were on par with untreated control.

The total carbohydrate content in the final instar larva of *C. sexmaculata* was estimated as 15.004 mg in the untreated control. All the treatments registered reduction in carbohydrate quantity in the test insect. Teepol 0.1% recorded the lowest reduction of 1.65% over untreated control. The

Table 1: Effect of botanical oils on carbohydrate quantity in *C. sexmaculata* male

Treatments	Dosage (%)	Carbohydrate Body weight (mg g^{-1})	Reduction over control (%)
Neem oil	1.5	6.443 (2.538)^{ab}	+63.69
Neem oil	3.0	2.967 (1.642)^{bc}	24.62
Pungam oil	1.5	5.864 (2.364)^{abc}	+48.98
Pungam oil	3.0	4.086 (2.004)^{abc}	+3.81
Madhuca oil	1.5	7.342 (2.708)^{a}	+86.53
Madhuca oil	3.0	5.179 (2.264)^{abc}	+31.58
Teepol	0.1	2.655 (1.513)^{c}	32.55
Untreated control	-	3.936 (1.937)^{abc}	-

'+' sign indicates the % increase over untreated control. In a column, the means followed by same alphabets do not differ significantly by DMRT (p = 0.05), The values within parentheses are square root transformed values

Table 2: Effect of botanical oils on carbohydrate quantity in *C. sexmaculata* final instar larva

Treatments	Dosage (%)	Carbohydrate Body weight (mg g^{-1})	Reduction over control (%)
Neem oil	1.5	11.051 (3.324)^{g}	59.31
Neem oil	3.0	10.573 (3.252)^{h}	44.31
Pungam oil	1.5	12.521 (3.538)^{e}	16.55
Pungam oil	3.0	12.028 (3.468)^{f}	19.83
Madhuca oil	1.5	13.957 (3.736)^{c}	6.98
Madhuca oil	3.0	13.596 (3.687)^{d}	9.38
Teepol	0.1	14.757 (3.841)^{b}	1.65
Untreated control	-	15.004 (3.873)^{a}	-

In a column, the means followed by same alphabets do not differ significantly by DMRT (p = 0.05), The values within parentheses are square root transformed values

Table 3: Effect of botanical oils on carbohydrate quantity in *C. carnea* female

Treatments	Dosage (%)	Carbohydrate Body weight (mg g^{-1})	Reduction over control (%)
Neem oil	1.5	29.971 (5.460)^{ab}	1.70
Neem oil	3.0	19.679 (4.440)^{ab}	35.45
Pungam oil	1.5	16.867 (3.910)^{b}	44.68
Pungam oil	3.0	23.946 (4.857)^{ab}	21.46
Madhuca oil	1.5	25.637 (5.023)^{ab}	15.91
Madhuca oil	3.0	33.123 (5.712)^{a}	+8.64
Teepol	0.1	17.981 (4.201)^{ab}	41.02
Untreated control	-	30.488 (5.514)^{a}	-

'+'sign indicates the % increase over untreated control, In a column, the means followed by same alphabets do not differ significantly by DMRT (p = 0.05), The values within parentheses are square root transformed values

carbohydrate level was drastically reduced (44.31-59.31%) in neem oil treatment. Madhuca oil registered the lowest reduction (6.98-9.38%) while the carbohydrate level in the pungam oil treatment ranged between 12.028 and 12.521 mg which was 16.55-19.83% reduction over untreated control (Table 2).

C. carnea

As the concentration of neem oil increased from 1.5 to 3%, the carbohydrate content in the treated *C. carnea* female decreased from 29.971 to 19.679 mg. But this was not true with pungam and madhuca oils in which the carbohydrate level increased from 16.87 to 23.95% as their dosage increased (Table 3). The reduction in carbohydrate level was the highest (44.68%) in the insect fed with honey treated with pungam oil at 1.5% and the lowest reduction of 1.70% was recorded in the case of neem oil 1.5% treated insects when compared with untreated control insects.

In male, all the treatments registered the reduction in carbohydrate quantity in the test insect. Teepol showed the lowest reduction of 4.14% and in the botanical oil treatments, as the concentration

Table 4: Effect of botanical oils on carbohydrate quantity in *C. carnea* male

		Carbohydrate	
Treatments	Dosage (%)	Body weight (mg g^{-1})	Reduction over control (%)
Neem oil	1.5	8.732 (2.955)^{g}	33.09
Neem oil	3.0	8.033 (2.832)^{h}	38.63
Pungam oil	1.5	10.937 (3.309)^{e}	16.25
Pungam oil	3.0	10.519 (3.245)^{f}	19.41
Madhuca oil	1.5	12.014 (3.462)^{c}	7.99
Madhuca oil	3.0	11.945 (3.455)^{d}	8.46
Teepol	0.1	12.524 (3.553)^{b}	4.14
Untreated control	-	13.059 (3.615)^{a}	-

In a column, the means followed by same alphabets do not differ significantly by DMRT (p = 0.05), The values within parentheses are square root transformed values

Table 5: Effect of botanical oils on carbohydrate quantity in *C. carnea* final instar larva

		Carbohydrate	
Treatments	Dosage (%)	Body weight (mg g^{-1})	Reduction over control (%)
Neem oil	1.5	2.409 (1.258)^{c}	77.21
Neem oil	3.0	5.093 (1.826)^{bc}	51.82
Pungam oil	1.5	15.300 (3.845)^{a}	+44.70
Pungam oil	3.0	8.672 (2.908)^{ab}	17.96
Madhuca oil	1.5	7.014 (2.638)^{abc}	33.64
Madhuca oil	3.0	10.720 (3.265)^{ab}	+1.42
Teepol	0.1	6.699 (2.575)^{abc}	36.62
Untreated control	-	10.570 (3.255)^{ab}	-

'+' sign indicates the % increase over untreated control, In a column, the means followed by same alphabets do not differ significantly by DMRT (p = 0.05), The values within parentheses are square root transformed values

increased from 1.5 to 3% there were increased reductions in the quantity of carbohydrate. On the other hand, neem oil 3% showed only 8.033 mg carbohydrate which was 38.63% lower than that estimated in the untreated control insects (Table 4). The carbohydrate content in the pungam oil treatment ranged between 10.519 and 10.937 mg.

The carbohydrate levels observed in the final instar larva of *C. carnea* in all the treatments were reduced except in pungam oil 1.5% and madhuca oil 3%. In the untreated control insects it was estimated as 10.57 mg. Carbohydrate content was highest (15.3 mg) in the treatment pungam oil 1.5% which was quantitatively 44.7% higher than that occurred in untreated insects (Table 5). Neem oil recorded the highest reduction of 51.82-77.21% while pungam oil 3% showed lowest reduction of 17.96% over untreated control.

Discussion

C. sexmaculata

Downer (1979) and Ingalhalli *et al*. (1995) reported that the insects under stress due to disease or other cause had hyperglycemia and hypertrehalosemia in their haemolymph. Similar findings were observed in the present investigation with coccinellid beetle adults fed with botanical oils treated preys except neem oil 3%. The stress due to these botanical oils might have triggered the biochemical change such as glycogenolysis of fat body leading to the increased sugar content (hyperglycemia). But at the higher dosage of neem oil 3%, the result is reverse and this could be correlated with the antifeedant property of neem expressed at higher dosage level. Due to starvation, more sugars might be metabolized to meet out the energy expenses and hence there was decreased carbohydrate level. Similar reduction in the carbohydrate level in the adult desert locust after inoculation with the entomopathogenic fungus *Metarhizium anisopliae* was observed earlier (Seyoum *et al*., 2002). However in the final instar larva of *C. sexmaculata*, the relationship between the higher dosage level of neem and percent reduction in carbohydrate level was negative. This is an unusual phenomenon and this warrants further investigation.

C. carnea

As the concentration of neem oil increased from 1.5 to 3%, the decrease in carbohydrate content in the treated *C. carnea* female may be due to more antifeedant property expressed in the higher dosage. But in the other two botanical oils, the reason for the reverse trend and increased carbohydrate level in madhuca oil 3% is unknown. Regarding males, the carbohydrate level had decreased in varying degrees in all the treatments. This showed that the male adult had feeding deterrence towards various treatments. The larvae would have fed on very few numbers of neem treated aphids and due to more starvation, more depletion of sugars might have occurred.

In conclusion, the tested botanical oils had altered the carbohydrate levels of the two predatory insects and the reason of increased level might be attributed to the glycogenolysis of fat body under stress condition and it was supported by the findings by various authors reported earlier. The decreased carbohydrate content could be correlated with the antifeedant property of botanical oils, which led to more starvation and under such situation, more sugars might be metabolized to meet out the energy expenses. This speculation was also suggested by Seyoum *et al*. (2002) in his study earlier. The alteration caused in the carbohydrate level in the predatory insects due to botanical insecticides may affect their prey searching efficiency and fecundity, which are major limiting factors in biocontrol of insect pests. Hence application of botanical insecticides against insect pests should be carefully carried out with minimum exposure to the natural enemies.

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