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STUDIES ON BIOLOGICAL ACTIVITY OF PLANT EXTRACTS ON *Dysdercus cingulatus*

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Pelargonium graveolans @ 420 /ug, *Vetiveria zizanioides* and *Ocimum basilicum* @ 490 /ug/nymph resulted in 90, 85 and 80 per cent malformations respectively. No adult emergence was observed in all the three extracts at the doses of 420 and 600 /ug/nymph. The percentage of malformed adults was in the order of efficacy in *P. graveolans* (180 /ug/nymph > *O. basilicum* (420 and 600/ug/nymph) > *V. zizanioides* (600 /ug/nymph) treatments. *P. graveolans*, *V. zizanioides* and *O. basilicum* affected the ovarian development in juvenilised adults. *P. graveolans* induced 86.2 and 87.5 per cent sterility @ 60 /ug/adult in the mating combinations of treated male and untreated female and normal male and treated female respectively. Total fat and total free amino acid contents of the ovaries of adults treated with *P. graveolans*, *V. zizanioides* and *O. bacilicum* were less than in untreated adults.

The effectiveness of acetone extracts of roots of *Iris ensata* Thunb. against nymphs of *Dysdercus koenigii* (G) (Saxena and Srivastava, 1972); extracts of *Azadirachta indica* and *Melia azadirachta* Willd. against grubs of *Epilachna varivestis* Mulsant and *Presma quadratum* (Fieb) (Steets, 1975) and of *Parthenium hysterophorus* L. against *D. koenigii* and *Aedes aegyptii* (Sharma *et al.*, 1977) for their JH activity. Hence attempts were made to study the juvenilising effect of *Pelargonium graveolasn*, *Vetiveria zizanioides* and *Ocimum bailiscum* against the nymphs of *Dysdercus cingulatus* and the results are furnished.

MATERIALS AND METHODS

Dysdercus cinglatus Fabricius was reared to test the plant extracts for juvenomimetic activity. Nymphs were collected from the cotton field and cultured on water soaked cotton seeds in the insectary at room temperature of 27± 0.5° C. Glass chimneys of 15 cm height were used for mass multiplication of this bug. Moist sterilised sand was placed to a depth of 2 cm at the bottom of the chimneys over which the soaked cotton seeds were kept and the other end with a muslin cloth. The moisture in the sand prevented desiccation of eggs as well as the seeds. On oviposition, the eggs were carefully removed from the chimneys and placed in separate vials for further observations

Plant materials of *P. graveolans* *V. ziznioides* and *O. basilicum* were, air dried, finely ground and conti-

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nuously extracted with ether (Reagent grade) in soxhlet extractor for 48 hours and filtered. The filtrate was freed of the solvent completely on a rotary evaporator at 30° C (Jacobson *et al.*, 1975) and fresh materials were steam distilled for volatile fraction (Deshpande *et al.*, 1974). The residue was weighed in required amounts and dissolved in acetone in appropriate concentrations.

The plant extracts were topically applied on the abdominal tergites of freshly moulted 5th instar nymphs (0-4 hours) of *D. cingulatus* with the help of a microsyringe applicator designed by Regional Research Centre, Council of Scientific and Industrial Research, Jammu-Tawi.

The extracts of *P. graveolans*, *V. zizanioides* and *O. basilicum* at five doses were also applied on the abdominal tergi of newly moulted adults. Pure acetone was applied as check. Twenty adult bugs were used for each treatment and replicated thrice. The longevity of adults was observed.

The adultoids produced by the application of extracts of *P. graveolans*, *V. ziznaioides* and *O. basilicum* were dissected five days after moulting when vitellogonesis was known to be completed (Prabhu and John,1975). The number of normal ovarioles and fully formed ova were recorded in both healthy and malformed insects. For every treatment, the dissection was done in four individuals and the treatments were replicated thrice.

To study the effect on fecundity and hatchability of eggs, the plant extracts were tested on the adult bugs at two doses and a check (acetone treatment). Both the sexes of the newly formed adults were treated with plant extracts and the following combination of mating were allowed i. e. (i) treated female x treated male, (ii) treated female x untreated male, (iii) treated male x untreated female (iv) untreated female x untreated male (control). Each treatment was replicated thrice. The sterility percentage was worked out as per the following formula (Outram, 1973),

Where

$S = 100 - (axb) \times 100/AxB$

S = Sterility percentage

a = number of eggs laid/female in treatment

b = percentage of hatch in treatment

A = number of eggs laid in control (acetone)

B = percentage of hatch in control

In order to know the changes in the contents of fat, total amino-acids and free amino acids induced in the ovaries following the treatment with *P. graveolans*, *V. zizanioioides* and *O. basilicum* newly moulted adults of *D. cingulatus* were topically applied with these extracts. The dissection of the ovary was done five days after the moulting when vitellogenesis was known to be completed (Prabhu and John,

1975). The insects were dissected in ice cold saline solution and the ovaries were removed, weighed in a monopan balance and preserved at a temperature of 0° C until further use.

The total fat content was estimated as per the method of Schaeffer (1968) and expressed as mg / g wet body weight.

For analysis of aminoe acids, the sample was homogenised with 80 per cent ethanol, centrifuged, treated with chloroform, dried *in vacuo* and dissolved in one ml of 80 per cent ethanol. Individual amino acids were identified by standard unidimensional paper chromotography (Block *et al.*, 1958). The amino acids were identified in comparison with standard Rf values. The total amino acid content of ovary was assessed by estimating amino nitrogen by ninhydrin method of Moore and Stein (1958).

The data were statistically analysed suitably.

RESULTS AND DISCUSSION

The studies made on the effect of plant extracts on 5th instar nymphs of *D. cingualatus* revealed that *P. graveolans*, *V. zizanioides* and *O. basilicum* resulted in 0.0 per cent normal adult emergence at 420 and600 /u/nymph; 5 to 15 per cent adult emergence at 300 /ug, 15 to 20 per cent adult emergence at 180 /ug/17.5 to 47.5 per cent adult emergence at 60 /ug/nymph as against 100 per cent in control. As the dosage of extract was increased, there was decrease in the percentage of adult emergence (Table 1).

The per cent mortality of 5th instar nymphs was in the order of efficacy of *O. basilicum* (32.5% at 300 /ug/nymph(> *V. zizanioides* (22.5% at 180 /u/nymph)> *P. graeolans* (20.0%) at 600 /ug/nymph)(Table 1).

The attainment of 6th instar nymphs was high in the treatment with *O. basilicum* (27.5% at 600 /ug/nymph) and *V. zizanioides* (20.0% both at 60 and 600 /ug/nymph) *P. graveolans* 57.5% at 420 /ug/nymph) followed. The percentage of malformed adults was high in *P. graveolans* (47.5% at 180 /ug/nymph) followed by that in *O. basilicum* (40% both at 420 and 600 /ug/nymph) and *V. zizanioides* (37.5% both at 300 and 600 /ug/nymph) but *P. graveolans* affected the adults by way of crumpling the wings.

The malformations exhibited in 5th instar nymphs in the treatments with *P. graveolans* and *O. basilicum* were due to the presence of terpene reported to have antibiotic effect (Gupta and Viswanathan, 1956). The juvenilising effect of extracts showed a proportinonate increase with the increase in dosage up to a certain level only. The degree of juvenile characters retained by the post moult individuals depended on the dosage of the extract applied as reported by Sundaramurthy (1976). Deshpande *et al.*, (1974) also reported thati n the evolutionary process, the subsequent

Table 1. Effect of plant extracts on the adult emergence of *D. cingulatus*

Dose ug/ nymph	Normal adults [%] in			Mortality % of 5th instar nymphs			Sixth instar nymphs [%]			Malformed adults [%]		
	P. g.	*V. z.*	*O. b.*	*P. g.*	*V. z.*	*O. b.*	*P. g.*	*V. z.*	*O. b.*	*P. g.*	*V. z.*	*O. b.*
Control	100.0	100.0	100.0	0.0	0.00	0.0	0 0	0.0	0.0	0.0	0.0	0.0
0	(90.00)	(90.0)	(90.00)	(1.81)	(1.81)	(18.1)	(1.81)	(1.81)	(1.81)	(1.81)	(1.81)	(1.81)
60	47.5	22.5	17.5	10 0	20.0	27.5	7.5	20.0	2.5	35.0	20.0	25.0
	(43.56)	(28.30)	(24.61)	(16.38)	(25.90)	(31.61)	(12.19)	(26.64)	(5.99)	(36.28)	(26.64)	(29.96
180	20.0	15.0	20.0	10.0	22.5	30.0	22.5	12.5	0.0	47.5	17.5	25.0
	(26.27)	(22.59)	(26.64)	(16.38)	(28.30)	(33.27)	(22.93)	(20.56)	(1.81)	(43.62)	(24.61)	(29.96)
300	10.0	5.0	15.0	5 0	10.0	32.5	37.5	17.5	7 5	45.0	37.5	2.50
	(18.53)	(10.17)	(22.22)	(8.02)	(16.38)	(34.78)	(37.79)	(24.61)	(14.35)	(42.16)	(37.79)	(29.96)
420	0.0	0.0	0.0	10.0	15.0	20.0	57 7	17.5	25.0	27.5	32.5	40.0
	(1.81)	(1.81)	(1.81)	(18.53)	(22.59)	(26.27)	(49.39)	(24.61)	(29.96)	(31.46)	(34.78)	(39.29)
600	0.00	0.0	0.0	20.0	17.5	17.5	52.5	20.0	27.5	22.5	37.5	40.0
	[1.81]	(1.81)	[1.81]	[25.90]	[23.72]	[23.72]	[46.51]	[26.64]	[31.61]	[27 93]	[37.79]	[39.29]
CD (P=0.05)	16.68	6.01	5.03	13.62	11.84	6 71	9.85	6.15	6.84	7.82	4.00	4.34

[Figures in parentheses are arcsin transformed values]

P. g. = *Pealargonium graveolans*

V. z. = *Vetiveria zizanioides*

O. b. = *Ocimum basilicum*

Table 2- Effect of plant extracts on the adult longevity of *D. cingulatus*

Extract source	Dose /ug/ adult	Longevity in days 2-4	4-8	8-12	12-16	16-20	20-24
Pelargonium graveolans	60	78.33	50.00	18.33	0.0	—	—
		[21.67]	[48.27]	[80.00]	[100.0]		
	300	63.33	40.00	8.33	0.0	—	—
		[36.67]	[58.62]	[90.91]	[100.0]		
	600	38.33	21.67	3.33	0.0	—	—
		[61.67]	[77.53]	[96.36]	[100.0]		
Vetiveria zizanioides	70	91.67	63.33	38.33	26.67	8.33	0.0
		[8.33]	[34.43]	[58.18]	[64.44]	[81.90]	[100.0]
	350	81.67	56.57	30.00	11.67	0.0	—
		[18.33]	[41.37]	[67.27]	[84.44]	[10.00]	
	700	68.33	40.67	16.57	0.0	—	—
		[31.67]	[51.72]	[81.81]	[100.0]		
Ocimum basilicum	70	93.33	76.67	55.00	36.67	15.0	0.0
		[6.67]	[20.68]	[40.00]	[51.10]	[84.85]	[100.0]
	350	85.0	65.0	56.67	15.00	0.0	—
		[15.0]	[32.76]	[38.18]	[80.00]	[100.0]	
	700	61.67	40.00	16.57	3.33	0.0	—
		[38.33]	[58.62]	[81.81]	[95.56]	[100.0]	
Control (acetone treated)		100.0	96.67	91.67	75 0	55.0	43 33

production of certain secondary metabolites by these plants could influence various physiological processes in insects resulting in growth inhibition or general toxicity.

The effect of *P. graveolans*, *V. zizanioides* and *O. basilicum* on the longevity of adult bugs revealed that the extract of *P. graveolans* caused cent per cent mortality of bugs on 12th day at a dose of 60 /ug/adult and 61.6 per cent mortality 600 /ug/ adult within 4 days. In case of *V zizanioides*, 100 per cent mortality was evident on 20 th day with 70 /ug/adult and 81.8 per cent reduction in longevity ofadults at 700 /u/g adule on 8th day after treatment. Complete mortality was observed atter 16 - 20 days with 350 /ug of *Ocimum* extract adult (Table 2). The reduction in the longevity up to 84.5% has been reported by Rajendran (1977) when *D. cingulatus* were treated wit h the extracts of *Parthenium hystrophorus*, *Nephrolepis exaltata* and *Polyscias guilfoyled*

Table 3 Effect of plant extracts on fecundity and hatchability of eggs of *D. cingulsatus* [Fingures in parentheses represent percentage decrease over control] Mean of three observations

Extract source	Dose /ug/ adult	Method of mating: Treatad male x Normal female — Mean number of eggs/laid female	Mean number of eggs hatched	% hatch	% Sterility	Normal male x Treated female — Mean number of eggs/laid female	Mean number of eggs hatched	% hatch	% Sterility
Pelargonium graveolans	60	5.70	39.5	69.29	86.24	50.0	32.5	65.0	87.4
		(81.0)	(86.2)	—	—	(45.0)	(38.6)	—	
	300	33 3	0.0			20.0	0.0		—
		(88.8)				(93.3)			
Vetiveria zizanioides	70	98.0	80.0	81.64	72.12	80.0	68.0	85.0	73.8
		(67.3)	(72.0)			(73.3)	(76.2)		
	350	66.7	51.5	77.21	82.05	55.0	(40.5)	73.6	84.4
		(77.7)	(82.0)			(81.6)	(85.8)		
Ocimum basilicum	70	102.2	90.5	88.30	73.62	80.2	68.5	85.4	68.5
		(65.9)	(68.49)			(72.9)	(76.1)		
	350	60.2	45.3	75.3	84.20	55.3	40.3	7.29	84.4
		(79.9)	(84.1)			(81.6)	(85.9)		
Control (acetone)	0	300	286.7	95.67	—				

The dissection of five day old adults for their ovarian development indicated that there was not much in the number of ovarioles in each ovary (7 ovarioles/ovary) due to treatment with *P. graveolans*, *V. zizanoiides* and *O. basilicum* extracts but the ovarioldes exhibited abnormalities in their structure, without eggs at the distal end. Similar deformities in the ovaries of *D. cingulatus* and *D. koenigii* due to the application of extracts of plant species showing JH activity have been reported by Prabhu and John (1975) and Saxena and Mathur (1976) respectively. The disturbance in the hormonal metabolism induced by the plant extracts might be responsible for the inhibition of ovarian development in *D. cingulatus*. When the newly moulted adults were treated with the extracts, no mating occurred between treated male and treated female. Mating could be seen only between normal male and treated female as well as normal female and treated male which resulted in a fecundty of 50 and 57 eggs respectively in *P. graveolans* treatment as aginst 300 in control. There was a reduction in egg hatch being 86.2 and 88.6 per cent over control as in the case of treated male and untreated female and normal male and treated female respectively. There was no egg hatch with 300 /ug/adult in both the combinations of mating (Table 3).

Vetiveria and *Ocimum* reduced the fecundity to 67.3 and 65.9 per cent respectively at a dosege of 70 /ug/adult. At 70 /ug/adult, *Vetiveria* induced 81.6 and 85.0 per cent egg-hatch as against 95.6 per cent in control, and induced sterility in *Dysdercus* in the above two mating combinations.

Ocimum with 70 /ug produced 88.3 and 85.4 per cent egg hatch in the two mating combinations and the sterily induced was 73.6 and 68.5 per cent respectively. Of the three plant extracts, *P. graveolans* induced 86.2 and 87.4 per cent sterility at 60 /ug/adult in the mating combinations described and was found to be superior in suppressing the ovarian development of *D. cingulatus* Table 3). Cent per cent sterility was induced in *Dysdercus* spp. when they recived 100 /ug of ethyl farnesoate dihydrochloride (Rens, 1974). The redution in fecundity and hatchig in *Choristoneura occidentalis* and in *D. cingulatus* were also reported by Richmond (1972) and Judson *et al.* (1976), respectively.

The total fat content of the ovary treated with 300 /ug of *P. graveolans* extract was reduced by 78.7 per cent compared to control whereas *Vetiveria* and *Ocimum* with 350 /ug the reduction in total fat content was 68.0 and 57.4 per cent respectively (Table 4). Similar reduction in fat content in malformed insects was reported by Balasubramanian (1978). The reduction in fat content would adversely effect growth and development of reproductive organs and fecundity (Saxena and Mathur, (1976).

Table 4 Effect of plant extracts on total fat and total amino acid content of *D. cingulatus* ovary

Extract source	Dose µg/adult	Fat content mg/gram	Per cent reduction over control	Total amino acid content µg/g gram	Per cent reduction over control
Pelargonium graveolans	60	80.5	42.84	3360	48.14
	300	30.0	78.70	1120	82.17
Vetiveria zizanioides	70	90.0	36.10	3703.2	42.85
	350	45.0	68.05	2468.8	61.90
Ocimum basilicium	70	100.8	28.43	3568.0	44.93
	350	60.0	57.40	2374.0	63.36
Control (acetone)	0	140.8	—	6480.0	—

In all the treatments with three extracts, the reduction in the content of total amino acid was evident. *Pelargonium* at 300 µg reduced the total amino acids to an extent of 82.7 per cent while *Vetivreria* and *Ocimum* with 350 µg reduced the amino acid content of the ovary to 61.9 and 63.3 per cent respectively (Table 4). The reduction in total free amino acids might be principally responsible for the reduction in fecundity and hatchability of eggs of D. *cingulatus* (Rajendran, 1977 and Balasubramanian 1978).

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