COMPARATIVE EFFICACY OF APHID EXTRACTS AND SOME JUVENOIDS AGAINST THE DEVELOPMENT OF MOSQUITOES

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ABSTRACT. Comparative efficacy of natural juvenile hormones extracted from *Aphis craccivora* and *A. gosypii* and 5 juvenoids, i.e., methoprene, Neporex, OMS 3007, OMS 3019 and DPE-28 on the development of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* have been evaluated. OMS 3007, OMS 3019, DPE-28 and Neporex show species specific related activities, while methoprene and aphid extracts do not show such activity against these mosquito species. Treatment of mosquito eggs with an EC_{50} dose of these compounds caused mortality while an EC_{90} dose completely ceased adult emergence. The fecundity and fertility rates of mosquitoes emerged shows significant reduction ($P < 0.001$) when treated with EC_{50} doses of all the compounds.

INTRODUCTION

Environmental hazards and increasing cases of resistance/refractory behavior of mosquitoes to synthetic organic insecticides (Das and Rajagopalan 1980) have warranted a newer approach in the use of chemicals for mosquito control. The juvenile hormones (JH) and juvenile hormone analogues represent potential biorational chemical alternatives. Compounds belonging to various chemical classes such as amides, carbamates and oxime ethers have been shown to possess juvenile hormone-like activity (George et al. 1989). Slama and Williams (1966) had shown that the JH analogue Juvabione disrupts embryogenesis when applied exogenously to insect eggs. The ovicidal action of methoprene (a synthetic juvenoid) in *Lycoriella mali* was demonstrated by Keil and Othman (1988). "It has been the experience of the past 35 years that whenever a potent synthetic organic insecticide was repeatedly applied for mosquito control, resistance usually supervened after a period of 2–10 years of its uninterrupted use" (Brown 1983). Therefore, at present, much attention is being paid to the biological activities of substances from animal organisms and to their synthetic bioanalogues (Paulov and Paulovova 1983). In aphids, a strong juvenilizing and apterizing effect due to their own juvenile hormone was observed by Hardie (1981). Paulov and Paulovova (1983) and Ranjit et al. (1988, 1990) obtained encouraging results on emergence suppression while studying the effects of acetone extracts of aphids on the development of culicine mosquitoes.

Our objective was to assess the comparative efficacy of juvenile hormones extracted from aphids and 5 juvenoids, methoprene, Noporex (a juvenoid produced by Ciba Geigy, Ltd.), OMS 3007, OMS 3019 and DPE-28, having different structural and functional groups against 3 mosquito species of India, *Aedes aegypti* (Linn.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say.

MATERIALS AND METHODS

The aphids, *Aphis craccivora* Koch and *A. gosypii* Glover, were collected from the field from their infested host plants, *Lablab purpurens* (Linn.) (bean) and *Momordica charantia* (Linn.) (bitter gourd) plants, respectively, and were subjected to solvent extraction for the isolation of juvenile hormones. This followed the method of Bergot et al. (1981) with slight modification; i.e., instead of using a high speed blender, the aphids were homogenized with mortar and pestle in an ice bath for 15–20 min, since the quantity of aphids used was more (80–90 g). The extracts of *A. craccivora* and *A. gosypii* were designated TAEac and TAEag, respectively. The extracts were analyzed with thin layer chromatography (solvent benzene:ethylacetate, 15:1) and compared with Sigma JH standards for the presence of JHs.

The juvenoids: methoprene (Isopropyl E,E (RS)-11 methoxy 3,7,11 trimethyl dodeca 2,4 dienoate), OMS 3007 (0-2-4 phenoxy phenoxy ethylpropional deoxime), OMS 3019 (2-(1-methyl 2,4 phenoxy phenoxy ethoxy pyridine) and DPE-28 (2-4 Dinitro phenyl-2, 6-ditertiarybutyl phenyl ether), were obtained through the Vector Control Research Centre (ICMR), Pondicherry a collaborative center of the World Health Organization and Neporex (2-cycloprophylamino 4,6 diamino-S triazine) was donated by Ciba Geigy, Ltd., Bombay, India, for the study.

Bioassay: Colonies of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were maintained following the method of Dash et al. (1988) at 26–28°C and 86% RH.

Bioassays of the 2 aphid extracts and 5 juvenoids were conducted under the above laboratory conditions against early 4th instar larvae of the above 3 species of mosquitoes following the pro-

Table 1. EC_{50} and EC_{90} values of aphid extracts and juvenoids.

Test materials	*A. aegypti*				*A. stephensi*				*Cx. quinquefasciatus*			
	EC_{50} ($\bar{x} \pm$ SE) ppm*	EC_{90} ($\bar{x} \pm$ SE) ppm	Relative Activity		EC_{50} ($\bar{x} \pm$ SE) ppm*	EC_{90} ($\bar{x} \pm$ SE) ppm	Relative Activity		EC_{50} ($\bar{x} \pm$ SE) ppm*	EC_{90} ($\bar{x} \pm$ SE) ppm	Relative Activity	
			EC_{50}	EC_{90}			EC_{50}	EC_{90}			EC_{50}	EC_{90}
TAEac	0.013 ± 0.008	1.44 ± 0.03	0.09	0.08	0.023 ± 0.001	1.12 ± 0.02	0.1	0.08	0.014 ± 0.009	1.76 ± 0.01	0.1	0.4
TAEag	0.014 ± 0.009	1.38 ± 0.03	0.09	0.08	0.022 ± 0.001	1.07 ± 0.02	0.1	0.09	0.014 ± 0.007	1.68 ± 0.07	0.1	0.04
Methoprene	0.002 ± 0.0005	0.11 ± 0.02	1.0	1.0	0.002 ± 0.001	0.094 ± 0.009	1.0	1.0	0.001 ± 0.0001	0.07 ± 0.002	1.0	1.0
Neporex	0.017 ± 0.007	1.64 ± 0.02	0.07	0.07	0.05 ± 0.001	2.07 ± 0.02	0.04	0.05	0.01 ± 0.002	1.46 ± 0.003	0.14	0.05
OMS 3007	0.001 ± 0.0001	0.02 ± 0.01	1.24	4.10	0.032 ± 0.009	1.52 ± 0.27	0.07	0.06	0.016 ± 0.0001	0.76 ± 0.001	1.5	3.5
OMS 3019	0.007 ± 0.010	0.66 ± 0.02	0.14	0.17	0.002 ± 0.001	0.11 ± 0.02	1.1	0.9	0.016 ± 0.002	0.76 ± 0.02	0.1	0.1
DPE-28	0.001 ± 0.002	2.68 ± 0.15	0.01	0.04	0.07 ± 0.001	2.62 ± 0.02	0.03	0.04	0.002 ± 0.009	0.06 ± 0.02	0.6	1.2

Effective concentrations in mg/liter = ppm.

Table 2. Percentage (mean ± SD) of hatching of mosquito eggs after treatment with EC_{50}/EC_{90} dose of aphid extracts and juvenoids.

Test materials	EC_{50}						EC_{90}					
	Ae. aegypti	DMRT	*An. stephensi*	DMRT	*Cx. quinque-fasciatus*	DMRT	*Ae. aegypti*	DMRT	*An. stephensi*	DMRT	*Cx. quinque-fasciatus*	DMRT
TAEac	97.8 ± 2.1	a*	92.0 ± 3.3	a	93.6 ± 2.8	b	97.0 ± 2.4	a	90.1 ± 2.7	a	96.1 ± 2.4	a
TAEag	96.7 ± 2.0	a	91.5 ± 3.2	a	95.3 ± 3.0	ab	96.8 ± 2.6	a	92.5 ± 2.7	a	96.4 ± 2.3	a
Methoprene	68.2 ± 3.2	c	79.5 ± 4.7	b	68.4 ± 4.1	d	62.0 ± 4.0	b	72.3 ± 4.8	b	64.0 ± 3.9	c
Neporex	97.5 ± 2.3	a	90.0 ± 3.2	a	95.5 ± 3.1	ab	96.9 ± 2.3	a	93.1 ± 2.7	a	96.4 ± 2.4	a
OMS 3007	64.6 ± 3.7	c	68.7 ± 4.8	d	64.9 ± 4.7	d	61.0 ± 4.4	c	63.2 ± 4.4	c	62.6 ± 3.9	c
OMS 3019	73.7 ± 2.9	b	73.1 ± 5.2	c	77.8 ± 4.9	c	61.9 ± 2.5	b	64.6 ± 5.1	c	77.3 ± 3.1	b
DPE-28	97.5 ± 2.3	a	94.8 ± 3.3	a	97.1 ± 3.2	a	96.9 ± 2.6	a	92.8 ± 2.2	a	96.3 ± 2.5	a
Control	97.8 ± 2.4	a	94.0 ± 3.1	a	95.9 ± 2.9	a	97.8 ± 2.4	a	91.6 ± 2.2	a	97.8 ± 3.1	a

DMRT = Duncan's multiple range test.

* Any two means having a common letter are not significantly different at the 5% level of significance.

Table 3. Percentage of effective emergence inhibition (mean ± SD) of mosquitoes in EC_{50}/EC_{90} of aphid extracts and juvenoids treated at the egg stage

Compounds tested	*Ae. aegypti*			*An. stephensi*			*Cx. quinquefasciatus*		
	EC_{50}	DMRT	EC_{90}	EC_{50}	DMRT	EC_{90}	EC_{50}	DMRT	EC_{90}
TAEac	76.9 ± 0.6	c*	100	85.3 ± 0.8	d	100	78.3 ± 0.8	c	100
TAEag	75.3 ± 0.7	c	100	85.7 ± 0.6	d	100	78.7 ± 0.9	c	100
Methoprene	90.0 ± 0.9	b	100	93.3 ± 0.9	b	100	87.8 ± 0.8	b	100
Neporex	77.5 ± 0.5	c	100	89.3 ± 0.8	c	100	79.0 ± 0.4	c	100
OMS 3007	90.9 ± 0.6	b	100	93.0 ± 0.8	b	100	91.5 ± 0.8	b	100
OMS 3019	89.1 ± 0.4	b	100	90.8 ± 0.8	c	100	78.3 ± 0.8	c	100
DPE-28	75.0 ± 0.8	c	100	89.2 ± 0.8	c	100	90.7 ± 1.0	b	100
Control	1.5 ± 0.7	a	1.5 ± 0.7	0.5 ± 0.4	a	0.6 ± 0.5	1.5 ± 0.8	a	1.5 ± 0.8

DMRT = Duncan's multiple range test.

* Any 2 means having a common letter are not significantly different at the 5% level of significance.

cedure of the World Health Organization (1981). There were 15 replicates, each with 20 larvae in 500 ml glass beakers with tap water. The mortality was corrected using Abbott's formula (Abbott 1925) when there was mortality in an untreated control. The EC_{50} and EC_{90} doses were calculated by a probit regression equation (Finney 1953), and relative activity between test compounds were computed using the United Nations Environment Programme (1982).

Treatment of eggs with aphid extracts/juvenoids: Test concentrations of EC_{50} and EC_{90} doses were prepared for all compounds by adding appropriate volumes of stock solutions (1 mg/10 ml) and vigorously stirring in 1 liter of tap water kept in 12 × 12 × 3″ white enamel trays. Five replicates were set up for each concentration of each compound. Eggs of all mosquito species were placed into trays, immediately after laying and observed until adult emergence in the same water.

The percentage of hatching of eggs, treated with aphid extracts and juvenoids, were compared with the untreated controls to evaluate ovicidal activity. The number of pupae formed and successful adult (males and females) emergence, if any, after treatment of eggs with different test formulations were counted for both EC_{50} and EC_{90} doses to calculate the percentage of effective adult emergence inhibition.

$$\text{Emergence inhibition (\%)} = 100 - \left(\frac{\text{No. of successful adults}}{\text{No. of pupae formed}} \times 100\right).$$

Fertility and fecundity: All successful adults that emerged after the treatment of eggs were allowed to mate and lay eggs. The number of females that laid eggs was recorded to compute fecundity rate. The eggs laid were counted and allowed to hatch for the evaluation of fertility rate and were compared with control ones. Five replicates were taken and number of adults in each replicate varied from 14 to 25, depending upon emergence in the EC_{50} treatment since there was no adult emergence in the EC_{90} treatment.

Statistical analysis: An arc sine transformation of the data was made before calculation of an F-test and Duncan's multiple range test was done for a mean separation analysis (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

An analysis of the extracts determined that both aphid species contain JHs in addition to the triglyceride ingredients.

The probit analysis suggests that the EC_{50}/EC_{90} of the test compounds range from 0.001–0.017/0.03–2.68 ppm in *Ae. aegypti*, 0.002–0.016/0.06–1.76 ppm in *Cx. quinquefasciatus*, 0.002–0.07/0.094–2.62 ppm in *An. stephensi* (Table 1). Though all the above 3 species are susceptible to JHs and JH analogues applied, the degree of susceptibility varied. Taking the potency of methoprene as standard, the relative efficacy of other compounds were found to be OMS 3007, methoprene, OMS 3019, TAEac/TAEag, Neporex and DPE-28 in *Ae. aegypti*; OMS 3019, methoprene, TAEac/TAEag, OMS 3007, Neporex and DPE-28, in *An. stephensi* and OMS 3007, methoprene, DPE-28, Neporex, OMS 3019 and TAEac/TAEag against *Cx. quinquefasciatus* (Table 1).

When efficacy of the compounds on 3 different species are compared, TAEac/TAEag and methoprene do not show significant differences among the species evaluated.

However, the EC_{50} dose of OMS 3007 is 35.6 times more active against *Cx. quinquefasciatus* and 32.8 times against *Ae. aegypti* than *An. stephensi*, while with increase of the EC_{90}, the same rises to 76 and 56.3 times, respectively. The EC_{50}/EC_{90} value of OMS 3019 is 8/6.9 times more active against *An. stephensi* than *Cx. quin-*

Table 4. Fecundity and fertility rate of mosquitoes (mean ± SD) emerged from EC_{50} of aphid extracts and juvenoids treated at the egg stage.

Compounds tested	*Ae. aegypti*			*An. stephensi*			*Cx. quinquefasciatus*		
	Eggs laid/female	DMRT	Eggs hatched (%)	Eggs laid/female	DMRT	Eggs hatched (%)	Eggs laid/female	DMRT	Eggs hatched (%)	DMRT
TAEac	62.2 ± 2.2	b*	83.6 ± 3.0	51.8 ± 4.6	bc	82.4 ± 2.9	93.4 ± 3.2	b	83.6 ± 3.0	b
TAEag	64.0 ± 4.2	b	84.0 ± 2.6	50.8 ± 5.3	bc	83.4 ± 2.1	93.2 ± 3.4	b	83.0 ± 3.1	b
Methoprene	55.0 ± 4.5	c	77.8 ± 3.7	45.0 ± 5.5	d	72.2 ± 5.6	85.8 ± 2.8	c	74.4 ± 5.0	c
Neporex	65.8 ± 3.6	b	82.8 ± 2.3	53.2 ± 2.9	b	84.0 ± 2.6	94.2 ± 2.6	b	83.4 ± 2.1	b
OMS 3007	52.4 ± 5.9	c	74.6 ± 3.6	39.2 ± 3.8	d	62.8 ± 5.0	80.8 ± 3.8	d	70.4 ± 3.9	c
OMS 3019	55.4 ± 5.5	c	75.8 ± 4.0	48.6 ± 4.4	c	68.2 ± 4.9	87.6 ± 2.8	c	80.2 ± 3.0	b
DPE-28	61.4 ± 2.3	b	81.0 ± 2.3	52.4 ± 4.3	b	81.0 ± 2.4	91.4 ± 4.2	b	82.2 ± 2.6	b
Control	76.0 ± 2.4	a	93.4 ± 2.1	64.0 ± 2.9	a	91.8 ± 2.4	106.6 ± 7.4	a	94.02 ± 2.6	a

DMRT = Duncan's multiple range test.
* Any 2 means having a common letter are not significantly different at the 5% level of significance.

quefasciatus and 4.35/6 times than *Ae. aegypti*. Similarly, the DPE-28 is 30.4/43.7 times more active against *Cx. quinquefasciatus* than *An. stephensi* and 39.6/44.7 times than *Ae. aegypti*. The EC_{50} dose of Neporex is 5.0 and 2.9 times more potent against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively than *An. stephensi* but interestingly, with the increase of the dose to EC_{90}, the potency decreases to 1.4 and 1.26 times, respectively.

It may be concluded that Neporex, OMS 3007, OMS 3019 and DPE-28 have species specific activities while methoprene and TAEac/TAEag do not have much discrepancy in activities between *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Amalraj et al. (1988) evaluated three OMS compounds (OMS 3009, OMS 3013 and OMS 2015) against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* and found that OMS 3009 showed a higher level of activity than the other 2 compounds. While *Ae. aegypti* was more susceptible to OMS 3009 and OMS 2015, *An. stephensi* was more susceptible to OMS 3013 (Amalraj et al. 1988).

Amongst the 7 compounds studied (Table 2), only methoprene, OMS 3007 and OMS 3019 reduced the hatching percentage significantly ($P < 0.001$) as compared with controls in all the species studied. This shows that these 3 synthetic juvenile hormone analogues possess potential ovicidal activity. The findings are in contrast to Staal (1975), who reported that mosquito eggs are not susceptible to methoprene.

The percentage of emergence inhibition of the 3 species at EC_{50} of all the compounds follows in decreasing order of efficacy:

Ae. aegypti—OMS 3007, methoprene, OMS 3019, Neporex, TAEac/TAEag, DPE-28.

An. stephensi—methoprene, OMS 3007, OMS 3019, Neporex, DPE-28, TAEac/TAEag.

Cx. quinquefasciatus—OMS 3007, DPE-28, methoprene, OMS 3019, Neporex, TAEac/TAEag.

The emergence of adult mosquitoes was completely inhibited with the EC_{90} dose for all the studied compounds, including aphid extracts (Table 3).

Adults which emerged from an EC_{50} treatment by all compounds showed a significant reduction in fecundity ($P < 0.001$) and fertility ($P < 0.001$) rates in treated *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* as compared with their controls (Table 4). Similar observations were made by Robert and Olson (1989) where they recorded reduction of egg production in *Cx. quinquefasciatus* with LC_{50} treatment with methoprene. Iwanga and Kanda (1988) have also shown reduced egg production in *Anopheles balabacensis* Baisas after treatment with 0.005 ppb

S-31183, a juvenoid. In contrast to these findings, Hatakoshi et al. (1986) have shown that the females of *Culex pipiens pallens* (Coq.) emerging after treatment with methoprene and S-21149 (propional dehyde oxime O-2-(4-phenoxyphenoxy) ethyl ether) do not show any significant reduction in their fecundity and fertility rates. Klowden and Chambers (1989) have stated that unlike JH treated females, methoprene treatment never results in viable eggs. Our investigation suggests that aphid extracts (JH), methoprene and juvenoids, irrespective of their chemical structure, have reduced fertility compared with control groups at the sub-lethal dose (EC_{50}).

Our results suggest that juvenile hormones extracted from aphids can check the development of mosquitoes. Methoprene, OMS 3007 and OMS 3019 can also be applied as ovicides against the 3 mosquito species studied.

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