A LABORATORY STUDY OF CYROMAZINE ON *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* AND ITS ACTIVITY ON SELECTED PREDATORS OF MOSQUITO LARVAE[1]

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ABSTRACT. In a laboratory study, the insect growth regulator, cyromazine, exerted a high level of biological activity on *Aedes aegypti* and *Culex quinquefasciatus* treated in the 4th larval instar. At 1.5 and 1.0 ppm this IGR produced 97 and 99% inhibition of emergence in adult *Ae. aegypti*, respectively. In *Cx. quinquefasciatus*, there was 99% inhibition at 1 ppm and complete inhibition at 1.5 ppm. The overall pupal mortality was higher than larval or adult stages of both species. This material induced different types of morphogenetic abnormalities in pupae and adults of the 2 species similar to those induced by other IGRs. However, most abnormalities were observed in the pupal stage. Adverse effects were not detected on the 4 mosquito predator species during the acute or posttreatment tests.

INTRODUCTION

In recent years, new materials with different modes of action such as insect growth regulators (IGRs) have increased the options available for the control of a number of insect species including Diptera of medical and public health importance. Insect growth regulators offer a number of advantages compared with conventional broad spectrum insecticides including specificity to target insects, low mammalian toxicity and mode of action that is distinctly different from broad spectrum insecticides (Parrella et al. 1982). In 1984, Schaefer et al. reported that J2931 (2,4-bis(1,1-dimethylethyl)-6-[(4-methoxyphenyl)methyl] 1-methoxybenzene) was highly effective against OPS- and R-strains of mosquitoes in both laboratory and field tests. This material also had negative effects on the development of nontarget aquatic organisms. The effectiveness of IGRs was discussed by several investigators including Mulla and Darwazeh (1975), Dame et al. (1976). Awad and Mulla (1984) reported that cyromazine induced various morphogenetic aberrations in the pupal and adult stages of *Culex quinquefasciatus* Say developed from treated 4th instar larvae. The objective of this study was to evaluate the growth regulating activity of the experimental material, cyromazine, on *Aedes aegypti* (Linn.) and *Cx. quinquefasciatus* and its acute and latent effects on nontarget organisms.

MATERIALS AND METHODS

The *Ae. aegypti* and *Cx. quinquefasciatus* used in these tests were from established laboratory colonies originally obtained from the Centers for Disease Control, Fort Collins, Co. and the U.S.D.A. Mosquito Research Laboratory, Lake Charles, LA, respectively. The nontarget organisms were obtained from the Carolina Biological Supply Company, Burlington, NC and cyromazine was provided by CIBA-Geigy, Greensboro, NC. A 2% stock solution of the technical grade compound (Cyromazine or CGA-72662) (N-cyclopropyl-1,3, 5-triazine-2,4,6-triamine), a derivative of azidotriazine was initially prepared from which lower concentrations of 0.25, 0.5, 1.0 and 1.5 ppm were subsequently made for testing.

TEST INVOLVING TARGET ORGANISMS. Groups of 50 late 3rd or 4th stage larvae of the *Aedes* and *Culex* species were exposed to 1 liter of the aqueous solution of cyromazine in glass culture dishes (250 × 80 mm). The IGR material was tested 4 times on each species and each concentration was run in duplicate at ambient temperature (25°–27°C). Prior to seeding each container, the desired number of larvae was transferred from the rearing trays to a small wire sieve. The sieve was then gently tapped several times on folded pieces of paper towel to remove excess moisture.

Larval diet of finely ground Purina dog chow was added to each treatment and control container (ca. 2 shakes/container from a salt shaker) twice during the exposure period. Each test was monitored daily and dead or moribund larvae, pupae and adults were recorded and removed from the containers. They were subsequently examined under a dissecting microscope to determine any abnormalities resulting from continuous exposures. Data generated from this phase of the study were analyzed for each observation period by using the analysis of variance (ANOVA) and Duncan's new multiple range test (Duncan 1955). Percent inhibition of emergence (EI) was assessed by the formula 100 - T/C x 100, where T = percent emergence or survival in treatment and C = percent emergence or survival in checks (Mulla et al. 1974). Also, the average

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cumulative (pupal) percent mortality values at different concentrations were plotted on log probit scale to determine the LC_{50} levels against the mosquito species.

TEST INVOLVING NONTARGET ORGANISMS. The acute effects on nontarget organisms were determined by exposure to 24 liters of 1 ppm aqueous solution of cyromazine. The brown planaria, *Dugesia dorotocephala* (Woodworth), and nymphs of the dragonflies, *Macromia magnifica* Maclachian and damselflies, *Argia fumipennis* (Hagen) were exposed for 4 days in groups of 20 in 30-liter aquaria, while the mosquito fish, *Gambusia affinis* (Baird and Girard) was exposed for 5 days. The aquaria in which *M. magnifica* and *A. fumipennis* were tested contained autoclaved soil ca. 1.27 cm deep. Air was provided to each aquarium through a rubber tube attached to a compressed air outlet in the laboratory. The top of each aquarium was secured with fine cloth mesh. Test with each species of organism was replicated 4 times and they were allowed to acclimatize to conditions within the aquaria 1 day prior to each test.

At the end of each test, 2 groups of 5 organisms of each species were rinsed 3 times with pond water and held in test containers (250 x 80 mm) in 1 liter of fresh pond water to ascertain any latent or deleterious effects resulting from 4 or 5 days exposure to the different dosages of the IGR. Then, each group was offered 100 late 3rd or 4th stage *Ae. aegypti* larvae. Consumption rates were monitored at one day intervals for 6 days.

RESULTS AND DISCUSSION

EFFECTS ON TARGET ORGANISMS. Cyromazine induced varying levels of biological activity in the larval, pupal and adult stages of *Ae. aegypti* and *Cx. quinquefasciatus*. In this respect, the performance of this IGR is similar to the urea-type compounds HE-24734 and HE-24108 (Mulla et al. 1974). A significant reduction occurred in both the larval and pupal populations compared with the controls. In general, among all 3 stages the highest cumulative mortality occurred in the pupal stage (Table 1). The LC_{50} values for cyromazine (ppm) against pupae of *Ae. aegypti* and *Cx. quinquefasciatus* were 0.45 and 0.84, respectively. Larval mortality figures for *Cx. quinquefasciatus* at the treatment levels do not show significant differences (P = 0.05) whereas for *Ae. aegypti* there were significant differences between the 2 higher and lower dosages.

The accumulated pupal mortality rates of *Ae. aegypti* ranged from 46.5 to 61.0% whereas those of *Cx. quinquefasciatus* ranged from 36.5 to 60.0%. With both species, pupal mortality in the treatments was significantly greater than the controls and the ensuing adults. Emergence of adults from pupae treated in the larval stage was markedly depressed. Dosage levels at 1.5 and 1.0 ppm produced 97 and 99% inhibition of adult *Ae. aegypti* emergence, respectively. However, with *Cx. quinquefasciatus* cyromazine induced complete inhibition of emergence at 1.5 ppm and 99% at 1 ppm. Also, at 0.5 ppm the inhibition of adult emergence exceeded 90% whereas 0.25% yielded moderately high (>77%) inhibitory effects on the emergence of the 2 adult species. The inhibition of emergence in adults developed from untreated larvae declined drastically 21–26%.

Morphogenetic effects were also induced in pupae and adults of both species developed from treated larvae. The following morphogenetic aberrations were observed as earlier described

Table 1. Cumulative percent mortality of *Aedes aegypti* and *Culex quinquefasciatus* treated in the larval stage with cyromazine.

Species	Dosages (ppm)	Percent mortality at indicated stages			
		Larvae	Pupae	Adults	% EI
Aedes aegypti	1.50	37.5 Aa	57.0 Aa	2.5 Ab	97.0
	1.00	43.5 Aa	54.0 Aa	1.5 Ab	99.0
	0.50	26.0 ABb	61.0 Aa	9.5 Ab	96.5
	0.25	22.5 Abb	46.5 Aa	9.0 Ab	78.0
	0.00	14.5 Ba	3.0 Ba	3.5 Aa	21.0
Culex quinquefasciatus	1.50	39.5 Aa	57.0 Aa	3.5 Ab	100.0
	1.00	36.5 Aa	60.0 Aa	3.0 Ab	99.0
	0.50	44.0 Aa	43.5 ABa	8.0 Ab	95.0
	0.25	42.5 Aa	36.5 Ba	5.0 Ab	84.0
	0.00	15.0 Ba	9.5 Cb	1.5 Ab	26.0

[1] EI = Emergence inhibition.

[2] Means not followed by a common capital letter vertically and small letter horizontally differ significantly (P = 0.05) according to Duncan's New Multiple Range Test (1955).

by Awad and Mulla (1984): pupa with dorsal splitting of the thoracic cuticle, anterodorsal thoracic bulbous projection, larval head and portion of the abdominal cuticle and also the appearance of larger than normal pupae. Also, adult aberrations include: those that were incompletely eclosed, only the head and thorax freed of pupal skins and pupal skins attached to a wing, antenna, palps and legs. However, a higher percentage of pupae than adults showed morphogenetic effects. Similar morphogenetic abnormalities were reported for other IGRs, i.e., methoprene (Arias and Mulla 1975), methoprene and diflubenzuron (Kalada et al. 1980).

Data in the present study reveal the greatest mortality in the pupal stage of both species. Thus, the mode of action of this IGR is comparable to mon-0585, methoprene and R-20458 (Hsieh and Steelman 1974); CRD-9499, CRD-9545, R-20458, RO-8-5497, RO-20-3600 and TH-6040 (Mulla et al. 1974). Results of these tests also indicate that the biological activity of cyromazine is superior to a number of benzyphenols and benzyl-1,3-benzodioxoles tested against *Ae. aegypti* and *Cx. quinquefasciatus* (Nelson et al. 1982, 1983). Among these compounds the IGR J2931 exhibited the greatest activity among the 2 species but its performance was slightly less effective than cyromazine (Schaefer et al. 1984).

EFFECTS ON MOSQUITO PREDATORS. In the initial study, cyromazine induced almost complete (99%) inhibition of adult emergence in *Ae. aegypti* and *Cx. quinquefasciatus* at a dosage level of 1 ppm. Based on these findings we decided to evaluate the efficacy of 1 ppm on the nontarget organisms. At this dosage level, no treatment-induced mortality was detected among the 4 nontarget species during the exposure periods. Similar negative treatment effects on population reduction were also reported by Miura and Takahashi (1973) when *Argia* sp. (damselfly naiads) were exposed to methoprene at 1 ppm.

The posttreatment effects are depicted in Table 2. Collectively the majority >84% of prey was consumed in day 1. *Gambusia affinis* was the most effective predator during this period (100%) followed by *D. dorotocephala* (92.5%) in contrast to the controls 63–100 percent. However, each species population consumed all of the *Ae. aegypti* larvae in 6 days of testing. The efficacy of cyromazine in inducing high to complete inhibition of emergence in *Ae. aegypti* or *Cx. quinquefasciatus* and safety to nontarget organisms makes this compound a promising agent for use in a mosquito vector suppression program.

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Table 2. Consumption rates of nontarget organisms on *Aedes aegypti* larvae.

Posttreatment (hr)	Percent larval consumption							
	Fish		Dragonfly		Damselfly		Planaria	
	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.
24	100.0	100.0	81.5	90.0	63.0	69.0	92.5	98.0
48	0.0	0.0	15.5	6.0	6.5	6.0	6.0	2.0
72	0.0	0.0	2.0	2.0	5.5	6.0	1.5	0.0
96	0.0	0.0	0.5	1.5	8.0	9.5	0.0	0.0
120	0.0	0.0	0.5	0.5	7.5	5.5	0.0	0.0
144	0.0	0.0	0.0	0.0	9.5	4.0	0.0	0.0

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