EFFECTS OF CYROMAZIN AND METHOPRENE ON THE DEVELOPMENTAL STAGES OF *ANOPHELES DIRUS*, *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA : CULICIDAE)

THUMRONG PHONCHEVIN*, E. SUCHART UPATHAM, BOONLUAN PHANTHUMACHINDA*, CHUSAK PRASITTISUK** and NOUWARATH SUKHAPANTH

Center for Applied Malacology and Entomology, Department of Biology, Faculty of Science, Mahidol University, *Division of Medical Entomology, Department of Medical Sciences, and **Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Bangkok, Thailand.

INTRODUCTION

In Thailand, *Anopheles dirus* Peyton and Harrison, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say, are vectors of human malaria, dengue haemorrhagic fever, and viral diseases, respectively (Scanlon and Sandhinand, 1965; Wilkinson *et al.*, 1978; Peyton and Harrison, 1979; Halstead *et al.*, 1969; Simasathien and Olson, 1973).

Several measures have been taken to control mosquitoes. Chemical insecticides are used extensively as the major method of control. This approach tends to cause many serious environmental problems. Moreover, the resistance to chemical pesticides by mosquitoes has caused failure of many vector control campaigns (Anonymous, 1970). Therefore, research on control strategies using biological, environmental and integrated approaches has been encouraged. In addition, it seems reasonable to look for substances which will damage vital systems that are peculiar to arthropods. Among these substances are insect growth regulators which may be potential replacement compounds in some vector control situations, or as a complementary measure in integrated control operations.

Some insect growth regulators exhibit selective properties promising enough to be introduced for practical uses. Field tests with insect growth regulators have been successful against blackfly (Lacey and Mulla, 1979) and house-fly larvae (Ables *et al.*, 1975).

This paper reports on the effects of cyromazin and methoprene on the developmental stages of *Anopheles dirus*, *Aedes aegypti*, and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Two chemicals, crystal cyromazin (OMS-2014 or Neporex^{R}) (2% a.i) and liquid methoprene (Altosid^{R} SR 10) (10% a.i) were used in this study. A 100 mg/l stock solution of each chemical was prepared by dissolving it in water. From the stock solultions, the desired concentrations were prepared by serial dilutions.

Biological effects of cyromazin and methoprene were studied. Concentrations of cyromazin used were 0.5, 0.1, 0.02, 0.004 and 0.0008 mg/l against *An. dirus;* 2.5, 0.5, 0.1, 0.02 and 0.004 mg/l against *Ae. aegypti;* 0.5, 0.1, 0.02, 0.004 and 0.0008 mg/l against *Cx. quinquefasciatus;* while those of methoprene were 0.1, 0.02, 0.004, 0.008 and 0.00016 mg/l against *An. dirus*, *Ae. aegypti*, and *Cx. quinquefasciatus*. At each concentration, 20 each of the 2nd, 3rd and 4th stage larvae of *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to the chemicals in 250 ml glass jars. All mosquito larvae were exposed to

the chemicals continuously. Each concentration was replicated 5 times, with an untreated control each for *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus*. Dead mosquitoes were checked daily and were kept in 70% alcohol for morphological study. The morphological aberrations of the dead mosquitoes were determined using a stereo-microscope.

Persistence of cyromazin and methoprene was studied. The LC_{50} dosages of cyromazin and methoprene of the larval stages of mosquitoes obtained from the above studies were used to study persistence of the chemicals against the second stage larvae of *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus*. The second stage larvae of each species were exposed to the chemicals in 1,000 ml glass jars. Each dosage was replicated 4 times, plus 4 untreated controls.

Firstly, 25 larvae were introduced into each glass jar. They were fed daily with a small quantity of ground hamster food and were allowed to develop up to the adult stage. After all larvae of the first group developed into adults, a second group of 25 larvae were then introduced into each glass jar. Larval introductions were continued as before until none of them died or they had the same mortality rate as the untreated control. Mortality rates of all stages were recorded daily.

The LC_{50} dosages of cyromazin and methoprene on the larval stage of *Ae. aegypti* were used to study persistence of the chemicals against the second stage larvae of *Ae. aegypti*. They were exposed to the chemicals in 2500 ml earthenwares. There were 4 replications for each dose, with 4 untreated controls. The larval introductions were the same as those performed above. Mortality rates of all stages were recorded daily.

The number of dead mosquitoes in each concentration was corrected using Abbott's formula (Anonymous, 1970). The LC_{50} values of the compounds were determined through logconcentration-probit mortality regression lines (Finney, 1971).

RESULTS

The biological effects of cyromazin and methoprene on mosquito larvae are shown in Tables 1, 2, 3 & 4. The effects of cyromazin and methoprene on the development of the 2nd, 3rd and 4th stage larvae of *An. dirus*, is shown in Table 1. At the highest concentration tested (0.5 mg/l) for both chemicals, the development of all stages of larvae was inhibited completely. At the lowest concentration tested (0.0008 mg/l), mortality rates of larvae ranged from 17.0-34.0% for cyromazin and from 23.4-39.1% for methoprene.

The LC_{50} values obtained for cyromazin were 0.0027, 0.0042 and 0.0114 mg/l for the 2nd, 3rd and 4th stage larvae, respectively, and for methoprene were 0.0110, 0.0041 and 0.0022 mg/l for the 2nd, 3rd and 4th stage larvae, respectively.

The effects of cyromazin and methoprene on the development of the 2nd, 3rd and 4th stage larvae of *Ae. aegypti*, is shown in Table 2.

Mortality rates of larvae ranged from 95.8-100% at 2.5 mg/l to 1.0-8.3% at 0.004 mg/l for cyromazin, and from 100% at 2.5 mg/l to 4.4-19.6 mg/l for methoprene.

The LC_{50} values obtained for cyromazin were 0.1662, 0.2307 and 0.3005 mg/l for the 2nd, 3rd and 4th stage larvae, respectively, and for methoprene were 0.0077, 0.0034 and 0.0025 mg/l for the 2nd, 3rd and 4th stage larvae, respectively.

The effects of cyromazin and methoprene on the development of the 2nd, 3rd and 4th stage larvae of *Cx. quinquefasciatus* is shown in Table 3. Mortality rates of larvae ranged from 95.7-100% at 0.5 mg/l to 22.6-45.9% at 0.0008 mg/l for cyromazin, and from 100%

Table 1

Effects of cyromazin and methoprene on the development of the larvae of *An. dirus* (L = larva; P = pupa; CTM = corrected total mortality (%), using Abbott's formula).

Concentrations (mg/l)	Mortality (%)																	
	Cyromazin									Methoprene								
	2nd stage			3rd stage			4th stage			2nd stage			3rd stage			4th stage		
	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM
0.5	67	33	100	62	37	100	52	42	100	24	75	100	20	80	100	19	81	100
0.1	49	40	88.3	49	33	83.3	44	30	76.6	19	60	78.7	18	69	88.5	17	77	96.7
0.02	43	34	80.9	39	35	76.0	30	29	57.5	16	41	59.6	15	55	70.8	14	63	78.3
0.004	32	22	53.2	28	21	46.9	22	17	38.3	12	17	27.7	11	31	42.7	15	40	54.4
0.0008	24	12	34.0	19	15	31.3	14	7	17.0	8	14	23.4	6	29	33.3	12	31	39.1
Control	5	1	0	4	0	0	3	3	0	4	2	0	3	1	0	5	3	0

at 0.5 mg/l to 26.5-31.9% at 0.0008 mg/l for methoprene.

The LC_{50} values obtained for cyromazin were 0.0015, 0.0068 and 0.0130 mg/l for the 2nd, 3rd and 4th stage larvae, respectively, and for methoprene were 0.0013, 0.0008 and 0.0006 mg/l for the 2nd, 3rd and 4th stage larvae, respectively.

The effects of cyromazin and methoprene on the morphology of the larval stages of *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* is shown in Table 4. Insect growth regu-

Table 2

Effects of cyromazin and methoprene on the development of the larvae of *Ae. aegypti* (see detailed legend in Table 1).

Concentrations (mg/l)	Mortality (%)																	
	Cyromazin									Methoprene								
	2nd stage			3rd stage			4th stage			2nd stage			3rd stage			4th stage		
	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM
2.5	32	68	100	30	67	96.8	28	64	95.8	35	65	100	28	72	100	24	76	100
0.5	23	57	79.2	21	42	64.2	18	35	56.3	37	34	69.6	15	59	77.1	26	54	79.4
0.1	11	19	30.2	10	14	24.2	9	14	20.8	12	27	35.9	12	41	54.2	19	37	56.7
0.02	4	9	10.4	4	6	7.4	3	5	6.3	8	12	13.0	6	19	21.9	12	19	28.9
0.004	3	7	8.3	2	4	4.2	2	3	1.0	3	6	4.4	4	10	13.5	8	14	19.6
Control	4	0	0	4	1	0	4	0	0	6	2	0	2	0	0	2	1	0

Table 3

Effects of cyromazin and methoprene on the development of the larvae of *Cx. quinquefasciatus* (see detailed legend in Table 1).

Concentrations (mg/l)	Mortality (%)																	
	Cyromazin									Methoprene								
	2nd stage			3rd stage			4th stage			2nd stage			3rd stage			4th stage		
	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM	L	P	CTM
0.5	58	40	100	55	43	100	48	46	95.7	38	62	100	32	68	100	23	77	100
0.1	50	37	86.7	48	33	83.7	43	31	72.0	24	60	84.7	21	69	91.8	18	75	94.7
0.02	47	27	76.5	39	25	64.3	33	20	52.7	18	36	56.1	14	50	63.9	19	59	76.6
0.004	35	21	55.1	21	14	33.7	18	14	26.9	14	31	46.9	11	41	50.5	14	43	55.3
0.0008	27	19	45.9	18	12	28.6	16	12	22.6	11	17	26.5	8	23	29.9	9	27	31.9
Control	2	0	0	0	2	0	5	2	0	2	0	0	2	1	0	4	2	0

lators (cyromazin and methoprene) induce external morphogenetic changes in larvae, pupae and adult mosquitoes when they are treated at larval stage. These morphological aberrations varied. The dead specimens of the mosquitoes in this study were categorized into 6 major groups according to those described by Aria and Mulla (1975), which are shown in Table 4, as follows:

Larvae (L). This group includes larvae dying before reaching the prepupal stage of development.

Pupae not completely out of larval exoskeletons (P–L). Any partially exuviated pupae die between the prepupal and the pupal stages.

White pupae (WP). Pupae die before hardening and darkening of the cuticle.

Deformed pupae (DP). Dead pupae completely freed from the larvae exuviae, but not normal in appearance.

Black pupae (BP). Dead pupae which are normal in appearance.

Table 4

Comparative percentage morphological effects of cyromazin and methoprene when tested against *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* (L = larvae, P – L = pupae not completely out of larval exoskeletons, WP = white pupae, DP = deformed pupae, BP = black pupae, A – P = adult attached to the pupal cases).

Species	Cyromazin						Methoprene					
	L	P–L	WP	DP	BP	A–P	L	P–L	WP	DP	BP	A–P
An. dirus	38.3	5.0	8.1	2.0	12.0	2.0	15.1	2.0	12.0	3.0	33.9	2.3
Ae. aegypti	13.3	3.0	10.6	2.0	12.0	2.0	16.6	2.0	12.1	5.0	17.3	1.1
Cx. quinquefasciatus	37.1	6.0	6.0	4.0	10.3	1.1	18.3	4.0	12.0	7.0	26.2	0.9

Table 5

Effects of cyromazin (0.0027 mg/l) and methoprene (0.0110 mg/l) on the development of *An. dirus* in 1000 ml. glass jars (100 larvae were used for each stage; NA = normal adult, %; TM/total mortality rate, %; * = for cyromazin; ** = for methoprene).

Stages of treated larvae	Exposure times (days)	Emergence times (days)	Cyromazin				Methoprene			
			Control		Treated		Control		Treated	
			NA	TM	NA	TM	NA	TM	NA	TM
First	19,* 20**	19*, 20**	95	5	48	52	93	7	44	56
Second	39	20, 19	92	8	91	9	91	9	89	11
Third	58	19, 18	94	6	92	8	92	8	91	9

Table 6

Effects of cyromazin (0.1662 mg/l) and methoprene (0.0077 mg/l) on the development of various stages of *Ae. aegypti* in 1000 ml glass jars (see detailed legend in Table 5).

Stages of treated larvae	Exposure times (days)	Emergence times (days)	Cyromazin				Methoprene			
			Control		Treated		Control		Treated	
			NA	TM	NA	TM	NA	TM	NA	TM
First	17, 18	17, 18	91	9	46	54	94	6	39	61
Second	35, 33	18, 15	92	8	90	10	90	10	89	11
Third	51, 50	16, 17	94	6	92	8	92	8	90	10

Table 7

Effects of cyromazin (0.0015 mg/l) and methoprene (0.0013 mg/l) on the development of various stages of *Cx. quinquefasciatus* in 1000 ml glass jars (see detailed legend in Table 5).

Stages of treated larvae	Exposure times (days)	Emergence times (days)	Cyromazin				Methoprene			
			Control		Treated		Control		Treated	
			NA	TM	NA	TM	NA	TM	NA	TM
First	20, 19	19	93	7	47	53	92	8	38	62
Second	38, 37	18	94	6	76	24	96	4	69	31
Third	58, 57	19	92	8	90	10	94	6	93	7

Table 8

Effects of cyromazin (0.1662 mg/l) and methoprene (0.0077 mg/l) on the development of various stages of *Ae. aegypti* in 2500 ml earthernwares (see detailed legend in Table 5).

Stages of treated larvae	Exposure times (days)	Emergence times (days)	Cyromazin				Methoprene			
			Control		Treated		Control		Treated	
			NA	TM	NA	TM	NA	TM	NA	TM
First	14, 16	14, 16	94	6	48	52	92	8	48	52
Second	30, 31	16, 15	94	6	93	7	92	8	91	9
Third	45, 47	15, 16	94	6	94	6	96	4	94	6

Adults attached to the pupal cases (A–P). Dead adults that are attached to pupal cases by their tarsi, legs, wings, or any part or all of their tarsi, their abdomens still contained with in the pupal cases.

Persistence of cyromazin and methoprene of tests in tests in 1000 ml glass jars and 2500 ml earthernwares are shown in Tables 5, 6, 7 & 8. Mortality rates on the second stage larvae of *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* after continuous exposure to cyromazin and methoprene are shown in Table 5, 6 and 7, respectively.

The first stage larvae of mosquitoes were most affected, followed by the second and third stage larvae, respectively, by cyromazin and methoprene. The emergence times ranged from 16 to 20 days depending on species.

Mortality rates on the second stage larvae of *Ae. aegypti* after continuous exposure to cyromazin and methoprene in 2500 ml earthenwares are shown in Table 8.

The first stage larvae were most affected, followed by the second and third stage larvae, respectively. The emergence times ranged from 14 to 16 days.

DISCUSSION

This investigation reveals that the mosquitoes studied were susceptible to cyromazin in the early larval stage. The effectiveness of cyromazin is similar to diflubenzuron (PH 6040), which is said to be mainly stomach poison that interferes with the formation of insect chitin (Phillips-Dupher, 1972). The results indicate that cyromazin had effectively blocked adult emergence of *Cx. quinquefasciatus*, *An. dirus* and *Ae. aegypti*.

Methoprene is a juvenile hormone analogue. This chemical was based simply upon supplying the hormone to the insect at a time when it should normally be absent (Bower, 1971). The results of our study agree with those of Aria and Mulla (1975) that the action of methoprene was marked on the fourth larval stage, indicating that juvenoids have unusual property of being more toxic to the fourth larval stage than to young larvae, which is contrast to conventional larvicides. The results indicate that methoprene had effectively blocked adult emergence of *Cx. quinquefasciatus*, *An. dirus* and *Ae. aegypti*.

The persistence of cyromazin and methoprene was also investigated in this study. The residual effects of LC_{50} values cyromazin

of the second stage larvae of *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* in glass jars were in the periods of 20-39, 18-35 and 39-58 days, respectively; whereas the residual effects of LC_{50} values methoprene of the second stage larvae of *Cx. quinquefasciatus*, *An. dirus* and *Ae. aegypti* in glass jars were in the periods of 38-57, 21-39 and 19-33 days, respectively. These results indicate that cyromazin and methoprene were effective for controlling the mosquitoes, especially *Cx. quinquefasciatus*.

The residual effects of cyromazin and methoprene persisted longer in glass jars than in earthernwares, probably because the chemical compounds were absorped by earthenware.

The ideal effective compound must persist in active concentration throughout the sensitive stage of all mosquito populations in their habitats. When this is not possible, repeated applications are preferred over the increase of the dosages. This is because the very high dosages of the compounds may affect the non-target organisms. Therefore, cyromazin and methoprene seem to be effective compounds which deserve further studies under field conditions.

SUMMARY

The effects of two chemical compounds, cyromazin and methoprene, on the developmental stages of *Anopheles dirus*, *Aedes aegypti* and *Culex quinquefasciatus* were investigated under laboratory conditions, with the mean temperature of 24° ± 1°C and the relative humidity at 65-75%. Both compounds were tested against the second, third and fourth instar larvae. The concentrations of cyromazin used for *An. dirus* and *Cx. quinquefasciatus* ranged from 0.0008 to 0.5 mg/l; and for *Ae. aegypti* from 0.004 to 2.5 mg/l. The concentrations of methoprene used for *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* ranged from 0.00016 to 0.1 mg/l.

The mortality rates were found to be relatively high in larval and pupal stages when treated with cyromazin and methoprene. The primary toxic effects of cyromazin were on the second stage larvae. The LC_{50} values for cyromazin on the second, third and fourth stage larvae were, respectively, 0.0027, 0.0042 and 0.0114 mg/l for *An. dirus*, and 0.1662, 0.2307 and 0.3005 mg/l for *Ae. aegypti*. *Cx. quinquefasciatus* was the most sensitive species to cyromazin with LC_{50} values for second, third and fourth stage larvae of 0.0015, 0.0068 and 0.0130 mg/l, respectively. The primary toxic effects of methoprene were in the fourth stage larvae. The LC_{50} values for methoprene on the second, third and fourth stage larvae were, respectively, 0.0110, 0.0041 and 0.0022 mg/l for *An. dirus*, and 0.0077, 0.0034 and 0.0025 mg/l for *Ae. aegypti*. *Cx. quinquefasciatus* was the most sensitive species to methoprene, with LC_{50} values for second, third and fourth stage larvae of 0.0013, 0.0008 and 0.0006 mg/l, respectively. Cyromazin persisted as long as methoprene. The effectiveness of cyromazin in glass jars at concentrations of 0.0027, 0.1662 and 0.0015 mg/l for controlling *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* were in the periods of 20-39, 18-35 and 39-58 days, respectively. The effectiveness of methoprene in glass jars at concentrations of 0.0110, 0.0077 and 0.0013 mg/l for controlling *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* were in the periods of 21-39, 19-33 and 38-57 days, respectively. The effectiveness of cyromazin and methoprene in earthenwares at concentrations of 0.1662 and 0.0077 mg/l for controlling *Ae. aegypti* were in the periods of 15-30 and 17-31 days, respectively.

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