

# LABORATORY SUSCEPTIBILITY TESTS OF AN ARYLTERPENOID INSECT GROWTH REGULATOR, MV-678, AGAINST SIX SPECIES OF MOSQUITOES

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**ABSTRACT.** An arylterpenoid insect growth regulator MV-678 (2-methoxy-9-[4-isopropylphenyl]-2,6-dimethylnonane) was tested against 6 species of mosquitoes from Malaysia. When assayed at early 4th instar larval stage, LC50 values for *Aedes aegypti* (Linnaeus), *Ae. albopictus* (Skuse), *Culex quinquefasciatus* (Say), *Cx. pseudovishnui* (Colless) and *Cx. tritaeniorhynchus* (Giles) were 1.73, 1.86, 0.22, 0.15 and 0.01 ppb respectively. *Armigeres durhami* (Edwards) was the least susceptible with LC50 value of 5.64

ppm. In the same tests, mortality of *Aedes* and *Armigeres* species occurred mostly at the pupal stage, whereas death occurred more often at the larval stage for the *Culex* species. The susceptibility of mosquito immatures to MV-678 varied with age. The 1st and 2nd instar larvae of *Ae. aegypti* were less susceptible than late instar larvae with the pupal stage being the least susceptible. Besides causing mortality, MV-678 also delayed the adult emergence of 3 of the mosquito species tested.

## INTRODUCTION

Increased mosquito resistance to conventionally used organochlorine and organophosphate insecticides has attracted worldwide attention in seeking alternative control methods. Among the new alternatives, chemical compounds which interfere with insect development, the so-called insect growth regulators (IGR) or juvenile hormone mimics, have been shown to have a high potential for mosquito control in laboratory tests (Spielman & Williams 1966, Spielman & Skaff 1967, Nair 1967, Sacher 1971, Jakob & Schoof 1971 and 1972, Jakob 1972, Bransby-Williams 1972, Hsieh & Steelman 1974, Wells et al. 1975, Lowe et al. 1975) as well as outdoor field trials (Wheeler & Thebault 1971, Steelman & Schilling 1972, Schaefer & Wilder 1972 and 1973, Hoppe et al. 1974, Mulla & Darwazeh 1975, Rathburn & Boike 1975, Schaefer et al. 1975 and 1976). Among the newer groups of IGRs, a few arylterpenoid compounds have been found to be effective against several species of flies (Schwarz et al. 1974, Wright et al. 1976) and mosquitoes (Lowe et al. 1975, Schaefer et al. 1976). The present investigation concentrates on laboratory susceptibility evaluation of one of the arylterpenoid compounds, MV-678 (2-meth-

oxy-9-[4-isopropylphenyl]-2,6-dimethylnonane), against 6 species of mosquitoes found in Malaysia.

## MATERIALS AND METHODS

The arylterpenoid IGR selected for the present study was MV-678 (2-methoxy-9-[4-isopropylphenyl]-2,6-dimethylnonane). This compound was formerly tested under the code of AI-3-36206 in the United States and as OMS-1841 by the World Health Organization. Technical grade (88.5%) of the chemical was provided by the Stauffer Chemical Co., California, U.S.A. Analytical acetone was used as solvent for preparation of the test solutions.

The following 6 species of mosquitoes were chosen to evaluate the effectiveness of MV-678: *Aedes aegypti* (Linnaeus), *Ae. albopictus* (Skuse), *Armigeres durhami* (Edwards), *Culex quinquefasciatus* (Say), *Cx. pseudovishnui* (Colless) and *Cx. tritaeniorhynchus* (Giles). *Ae. aegypti* were tested at the 1st to 4th instar larval stages and the pupal stage. The rest of the species were tested only at early 4th instar larval stage. Mosquito larvae of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* were obtained from laboratory colonies established from field populations from Penang Island, Malaysia while late instar

larvae of *Ar. durhami*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* were collected from coconut plantations, stagnant ground pools and rice fields on the Island respectively. The field larvae, if necessary, were reared to the appropriate stages for testing.

Laboratory testing procedures used were essentially those of the World Health Organization for larvicide susceptibility tests (Anonymous 1960, Yap et al. 1968; Yap and Sulaiman 1976). The following modifications were adopted for the present IGR study.

Test were done with 1 ml acetone solution containing the prescribed amount of MV-678 added to 99 ml of tap water containing the mosquito larvae in a slightly tapered, 200 ml glass jar. Twenty individuals of mosquito immatures were used in triplicate for each dose of MV-678. Each test consists of 8 to 11 concentrations plus the untreated checks (control). All tests were conducted under laboratory conditions with a water temperature of  $27 \pm 1^\circ\text{C}$ .

In testing the effect of MV-678 on different immature stages of *Ae. aegypti*, each larval instar and pupal stage was exposed to prescribed concentrations of MV-678 for 3 hr. No food was added during the exposure period. After this, the mosquito immatures were kept under normal rearing procedures until emergence. The early 4th instar larvae of the 6 species tested were exposed to MV-678 continuously without the addition of mosquito food until termination of

experiment. A polyethylene jar with the same rim-diameter as the mouth of the testing glass jar was used for trapping emerging adult mosquitoes.

Total mortality of all stages was recorded for *Ae. aegypti*. In experiments with continuous exposure of 4th instar larvae of the 6 species of mosquitoes to MV-678, individual larval, pupal, pupal-adult intermediates and abnormal adult mortalities as well as total mortalities were recorded daily until the completion of the experiment. The data collected were analysed by probit analysis (Finney 1962) using an IBM 370 computer according to the programs of Daum & Killcreas (1966) and Daum (1970). Where sufficient data were not collected for establishing dosage-mortality regression lines using a computer, the regression lines were eye-fitted using log-probit papers.

## RESULTS

A 3-hr. exposure of MV-678 against immature stages of *Ae. aegypti* indicated that the mosquito was most susceptible at the late instar larval stages. LC50 values for the 3rd and 4th instar larvae were 0.88 and 0.74 parts per million (ppm) respectively. The LC50 value for the pupal stage was about 1,000 times that of the 4th instar larvae (Table 1).

Results obtained from continuous exposure of MV-678 against early 4th instar larvae of 6 species of mosquitoes indicated that the 3 *Culex* species tested were highly susceptible to MV-678 with LC50

Table 1. Susceptibility of *Aedes aegypti* (L.) exposed for 3-hr to MV-678.

Mosquito Stages	Lethal concentration in ppm <sup>a</sup>				Slope	Std. error (±)
	LC <sub>50</sub>	95% C.L.	LC <sub>90</sub>	95% C.L.		
<i>Larval</i>						
1st instar <sup>b</sup>	4.10	—	41.0	—	0.88	0.15
2nd instar	4.10	3.68-4.72	48.56	28.83-110.45	1.19	0.14
3rd instar	0.88	0.59-1.21	6.89	4.23-15.50	1.43	0.10
4th instar	0.74	0.15-20.28	—	—	0.27	0.03
<i>Pupal</i>	650.45	96.25-325868.70	—	—	0.76	0.21

<sup>a</sup> Each test represents a minimum of 4 replications.

<sup>b</sup> Estimation with eye-fitted regression line.

values of 0.01, 0.15 and 0.22 parts per billion (ppb) for *Cx. tritaeniorhynchus*, *Cx. pseudovishnui* and *Cx. quinquefasciatus* respectively (Table 2). Tests on *Ae. aegypti* and *Ae. albopictus* gave LC50 values of 1.73 and 1.86 ppb respectively. *Ar. durhami* was the least susceptible with LC50 and LC90 values in the range of a few ppm (Table 2). The regression slopes of the log dosage-probit mortality lines for all the mosquito species tested were flat.

Besides causing mortality to the mosquito immatures, MV-678 appeared to delay the emergence of the surviving adult mosquitoes. At concentrations of 1, 5 and 7 ppb and above, the surviving immatures of *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus* required at least twice the time to emerge into adults in comparison to the control. Tests on *Ar. durhami*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* did not show any delayed emergence effects from MV-678.

Table 2. Susceptibility of 4th instar larval mosquitoes exposed continuously to MV-678.

Mosquito Species	No. Tests	Lethal concentration in ppb				Slope	Std. error (±)
		LC <sub>50</sub>	95% C.L.	LC <sub>90</sub>	95% C.L.		
<i>Aedes aegypti</i>	3	1.73	1.52-1.95	29.51	24.15-36.94	1.04	0.04
<i>Aedes albopictus</i>	5	1.86	1.61-2.14	25.97	19.79-35.94	1.12	0.04
<i>Armigeres durhami</i> <sup>a</sup>	4	5.64	5.40-5.91	18.66	16.67-21.31	2.46	0.11
<i>Culex quinquefasciatus</i>	3	0.22	0.17-0.27	21.80	15.50-32.50	0.64	0.03
<i>Culex pseudovishnui</i>	2	0.15	0.10-0.23	10.10	11.52-27.58	0.62	0.04
<i>Culex tritaeniorhynchus</i>	4	0.01	0.004-0.02	4.09	2.09-9.73	0.49	0.04

<sup>a</sup> Values expressed in ppm; all others in ppb.

Observations on the percentage mortalities of larval, pupal, pupal-adult intermediates and abnormal adults when tested at 4th instar larval stage indicated that the majority of *Ae. aegypti*, *Ae. albopictus*, and *Ar. durhami* died at pupal stages both at concentrations closest to LC50 and LC90 values. In contrast, death occurred more frequently at the larval stage for the 3 *Culex* species tested (Table 3).

## DISCUSSION

Of the 6 mosquito species tested, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* were abundant in the urban areas of Malaysia. The 2 *Aedes* species are associated with the transmission of dengue and dengue haemorrhagic fever in many urban centres of Southeast Asia (Yap 1975). *Cx. quinquefasciatus* is a vector of

Table 3. Treatment of 4th instar larvae by MV-678 and the effect on immature and adult stages of mosquitoes

Mosquito Species	Percent mortality at nearest concentration <sup>a</sup>					
	LC <sub>50</sub>			LC <sub>90</sub>		
	L	P	P-A+AA	L	P	P-A+AA
<i>Aedes aegypti</i>	10.4	52.1	37.5	24.4	70.0	5.6
<i>Aedes albopictus</i>	19.4	74.5	5.9	6.0	90.5	3.5
<i>Armigeres durhami</i>	9.1	47.7	43.2	—	—	—
<i>Culex quinquefasciatus</i>	57.7	38.5	3.8	68.8	28.1	3.1
<i>Culex pseudovishnui</i>	58.9	35.7	5.4	66.0	26.6	7.4
<i>Culex tritaeniorhynchus</i>	47.5	42.6	9.9	50.6	49.4	0

<sup>a</sup> Larvae: L; pupae: P; pupal-adult intermediates: P-A; and abnormal adults: AA.

filariasis caused by *Wuchereria bancrofti* in many parts of the tropical world. *Cx. tritaeniorhynchus*, which occurs in rice fields, is associated with the transmission of Japanese B. encephalitis especially in Japan, South Korea and Taiwan (Yap & Ho 1977). *Ar. durhami* is a rural species which breeds mainly in coconut shells containing decaying coconut meat. Larvae of *Cx. pseudovishnui* can be found easily in temporary pools in rural Malaysia. The last 2 species are considered as nuisance mosquitoes which have not been reported as important vectors of human diseases.

From the present study, the susceptibility of *Ae. aegypti* immatures to MV-678 appeared to increase from the 1st to the 4th instar larval stages (Table 1). Schaefer and Wilder (1972) reported similar observations for ZR512 and ZR515 against *Cx. quinquefasciatus*. However, laboratory tests of Dimilin showed a decrease in susceptibility of the 4th as compared to the 3rd instar larvae for *Cx. nigripalpus* and *Ae. tritaeniorhynchus* (Rathburn and Boike 1975).

The laboratory results of tests on the 6 mosquito species show that MV-678 is highly promising for controlling mosquito larvae especially *Culex* and *Aedes* species. *Ae. aegypti* late instar larvae appeared to be more susceptible to MV-678 than to other IGRs such as Altosid<sup>®</sup>, Hercules-24108, Monsanto-0585, and Stauffer-R-20458. The susceptibility values for the 3 *Culex* and 2 *Aedes* species tested were in the range of a few ppb and were more comparable with IGR—Thompson-Hayward-6040 (Hsieh and Steelman 1974). Other laboratory tests on MV-678 also indicated that it was highly effective against *Anopheles* mosquitoes (Lowe et al. 1975). The 3 *Culex* species tested in the present study were more susceptible to MV-678 than the *Aedes* species tested (Table 2). The same tendency was reported when tests were conducted with *Cx. quinquefasciatus* and *nigromaculis* using the same chemical (Schaefer et al. 1976).

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