Present status of resistance and susceptibility of four species of West Malaysian Culicine mosquito larvae to insecticides

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INTRODUCTION

IN ADDITION TO being the most common nuisance mosquitoes throughout West Malaysia, **Culex pipiens fatigans, Aedes aegypti, Aedes albopictus** and **Armigeres subalbatus** are natural or experimental vectors of various filarial and viral infections of man and animals.

C.p. fatigans is the most common urban mosquito and the most important natural vector of the "urban" strain of **Wuchereria bancrofti** in India, Ceylon, Burma and other Southeast Asian countries, including Malaysia.

Aedes aegypti, commonly called the yellow fever mosquito, is widely distributed throughout the tropics and subtropics. It is also the primary vector of endemic dengue and of dengue haemorrhagic fever in India, Thailand, Burma, Malaysia, Singapore and other countries of Southeast Asia (Rudnick, 1967). **Aedes aegypti** is predominantly an urban mosquito and is very closely associated with man.

As a result of the medical importance of these two species and their high prevalence rate in various regions of the world, very extensive work has been done on the susceptibility of the larvae and adults of these species. Many strains of both species have developed resistance to various types of insecticides. The number of strains which have developed resistance and the insecticides to which they have developed resistance are discussed by Brown (1967a). He has also reviewed the mode of inheritance of resistance in these species to various compounds (Brown, 1967b). However, published data on the susceptibility or resistance of the Malaysian strains of these mosquitoes to various insecticides are scanty and not up to date. Therefore, the study of these species was made to estimate the susceptibility or the development of resistance of the larvae of these species to the more common insecticides.

Aedes albopictus is also a very common mosquito which coexists with **Aedes aegypti** in urban areas and it breeds in the immediate vicinity of houses. These are present in urban areas as well as in less populated rural areas of Malaysia. In some rural areas near rubber plantations, this species predominates over all other mosquitoes. It has been shown to be a natural vector of dengue (Rudnick, 1966a, 1966b) in Malaysia and Singapore. In spite of its importance as a vector of dengue fever and as a pest, very little work has been done on the susceptibility levels of this species to insecticides.

Armigeres subalbatus is essentially an oriental mosquito with a wide distribution throughout West Malaysia and in neighbouring countries from India to

Japan. In West Malaysia, this species occurs in the urban and the rural areas as well as in forest areas and bites man and animals freely. It has been found to be very efficient experimental vector of **Brugia pahangi** which is predominantly an animal filaria resembling **B. malayi**. Although **B. pahangi** has not been found infecting man in nature, it has been experimentally transmitted to human volunteers (Edeson, et al, 1960). In spite of these facts, there is no information on the susceptibility of adults or larvae of this species to any insecticide.

MATERIALS AND METHODS

Insecticides

Eight insecticides – DDT, methoxychlor, dieldrin, BHC, malathion, fenthion, fenitrothion and diazinon – were tested against the larvae. All except methoxychlor were obtained as standard solutions from the Vector Biology and Control Unit of the World Health Organisation, Geneva. When any intermediate strengths of insecticides were needed, these were prepared from higher does by diluting them with the desired quantity of ethenol. Methoxychlor was obtained from Dr. C.N. Smith, Director, Insect Attractants, Behaviour and Basic Biology Research Laboratory, Florida, as a 95.1% technical grade product. Desired weight of the insecticide was dissolved in ethenol to make a ten p.p.m. stock solution. The weaker dilutions were prepared from the stock solution.

Mosquito larvae

Larvae of **Culex pipiens fatigans** were obtained from eggs which were collected from breeding sites in Kuala Lumpur. These eggs were brought to the laboratory and were reared under standardised laboratory conditions and tests were done on fourth-instar larvae.

Large numbers of larvae of **Aedes aegypti** and **Ae. albopictus** were collected from a common breeding site in Kuala Lumpur. The larvae were brought to the laboratory and reared under standard conditions. When these pupated, the pupae were kept individually in tubes. As adults emerged, these were identified and separated into two species and with these adults, two parent colonies were established. Larvae from these colonies were used for susceptibility tests.

It has been difficult to collect from nature large numbers of larvae of the same age, size and physiological conditions as are required for the tests. In addition, most of the collections contained larvae of **Aedes aegypti** and **Aedes albopictus** in varying proportions. As such it was laborious, time-consuming and impractical to separate larvae-collected from the field into two species for tests. **Armigeres subalbatus** larvae were obtained from a laboratory colony which was collected from Kuala Lumpur and kept in the insectory of this department for more than a year.

The tests were conducted at room temperatures between 24°C and 28°C. In all tests, only fourth-instar larvae were used. The larval susceptibility levels were determined by the standard method (WHO Expert Committee on Insecticides, 1963). The LC_{50} and LC_{90} levels were obtained from log dosage /probit regression lines as fitted by eye.

RESULTS AND DISCUSSIONS

The results are summarised in the following table. Figures 1 to 8 show the log dosage/probit regression lines for the four species of mosquitoes to various insecticides.

Culex pipiens fatigans

The tolerance of the larvae of this species has been found to be highest to DDT with an LC_{50} value of 0.52 p.p.m. This was slightly higher than the previous record (Thomas, 1962). The larvae have developed 13-fold resistance to BHC during the past few years and the LC_{50} level was 0.35 p.p.m. compared with the previous published value of 0.026 p.p.m. (Reid, 1955). Among the hydrocarbons tested, the larvae were most susceptible to methoxychlor with LC_{50} level of 0.092 p.p.m. The LC_{50} level to dieldrin was 0.24 p.p.m. The larvae were comparatively more susceptible to organophosphorous compounds than to hydrocarbons. Of the four O.P. compounds tested against this species, fenitrothion was the most toxic compound with LC_{50} of 0.023 p.p.m. followed by diazinon (LC_{50} 0.04 p.p.m.) and malathion (0.08 p.p.m.). The larvae have already developed resistance to fenthion (Thomas, 1970). The log dosage/probit regression lines are given in Figures 1 and 2. The regression line for DDT was flat, but others were comparatively steep.

Aedes aegypti

Of the six insecticides tested, the larvae were most susceptible to DDT (LC_{50} 0.072 p.p.m.) and then to methoxychlor and fenthion, both with LC_{50} values 0.11 p.p.m. The LC_{50} level for dieldrin was 0.16 p.p.m. The log dosage/probit regression lines for DDT and dieldrin were flat. The larvae of this species were resistant to BHC and malathion and showed LC_{50} value of 0.66 p.p.m. and 0.64 p.p.m. respectively. The log dosage/probit regression lines are shown in

Mortality Rates, LC50 and LC90 Values for larvae of **C.p. fatigans**, **Ae. aegypti**, **Ae. albopictus** and **Ar. subalbatus** to various insecticides.

Species of Mosquito	Insecticides Tested	No. of Replicates (25 larvae per replicate)	Corrected Percentage larval mortality after 24 hours' exposure to various concentrations of insecticides (p.p.m.)																	LC 50 (p.p.m.)	LC 90 (p.p.m.)
			0.004	0.005	0.01	0.02	0.03	0.04	0.05	0.075	0.08	0.1	0.15	0.2	0.25	0.3	0.5	1.0	2.5		
Culex pipiens fatigans	DDT	14							7.0			12.6			30.0		54.9	71.0	83.7	0.52	2.5
	Methoxychlor	10							14.2		43.6	60.0			92.8					0.092	0.22
	Dieldrin	10										2.4			51.2		95.9			0.24	0.42
	BHC	13										6.1		23.0		35.5	67.6	92.0		0.35	0.97
	Malathion*	11				1.0		12.0	16.0	45.2		67.0		96.0						0.08	0.162
	Fenitrothion	10			3.3	40.0	79.8	96.6												0.023	0.041
	Fenthion*	18							2.0			46.0	91.0	98.2			100			0.10	0.15
	Diazinon*	14				9.2	21.7	45.1	75.1		100									0.04	0.072
Aedes aegypti	DDT	10				24.0			46.0			57.8					86.0	90.0		0.072	0.82
	Methoxychlor	12							14.0		33.0	45.4		80.5			98.3			0.11	0.27
	Dieldrin	9				18.4			30.5			47.1			53.4			78.0		0.16	
	BHC	8												4.1		8.4	42.0	70.5	100	0.66	1.61
	Malathion	12										2.4		10.3			36.8	73.2	100	0.64	2.10
	Fenthion	13							3.2		22.0	39.0	78.0	100						0.11	0.19
Aedes albopictus	DDT	2							9.0			27.0			65.0		83.3	100		0.18	0.63
	Methoxychlor	2							6.6			28.0		79.0	88.0		100			0.13	0.28
	Dieldrin	12	5.1		22.6	44.3			57.1			63.8			83.0		100			0.04	0.43
	BHC	11										20.9		62.2		79.6	88.8	100		0.17	0.45
	Malathion	10							10.2			59.2	74.1	91.5						0.095	0.19
	Fenthion	18										27.3	60.6	85.3	100					0.13	0.23
Armigeres subalbatus	DDT	11				4.9	60.6	89.3	97.4											0.029	0.041
	Methoxychlor	11									8.2	21.3		88.2	95.8		100			0.116	0.21
	Dieldrin	12					16.0	54.4	80.1		100									0.039	0.056
	BHC	15									10.5	16.0		52.0		71.1	100			0.20	0.50
	Malathion	2										17.9	49.4	68.7			100			0.16	0.30
	Fenitrothion	10		6.1		25.0						62.2					100			0.06	0.47
	Fenthion	18										2.1	25.0	51.1		94.0	100			0.19	0.28

* Thomas, V., (1970) Southeast Asian J. Trop. Med. Pub. Hlth.

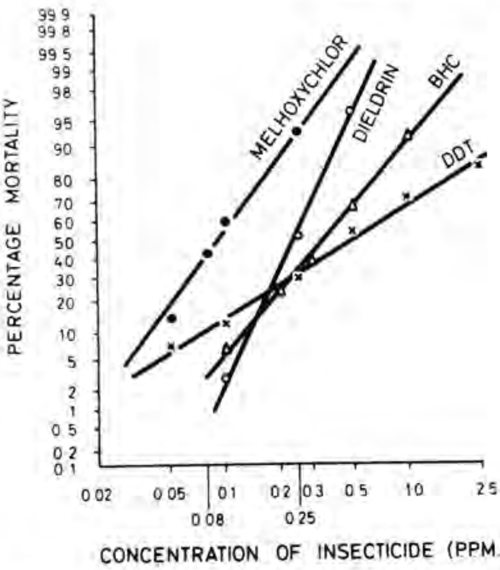

Fig. 1: **Dosage-mortality regression lines to DDT, methoxychlor, dieldrin and BHC for 4th instar larvae of *C.P. Fatigans* from Kuala Lumpur.**

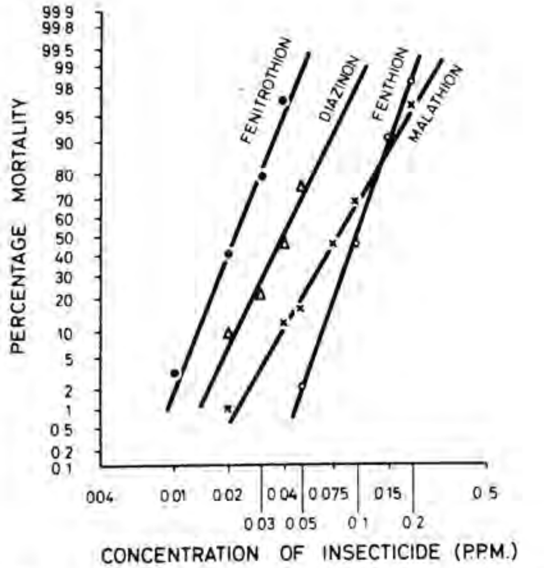

Fig. 2: **Dosage-mortality regression lines to malathion, fenitrothion, fenthion and diazinon for 4th instar larvae of *C.P. Fatigans* from Kuala Lumpur.**

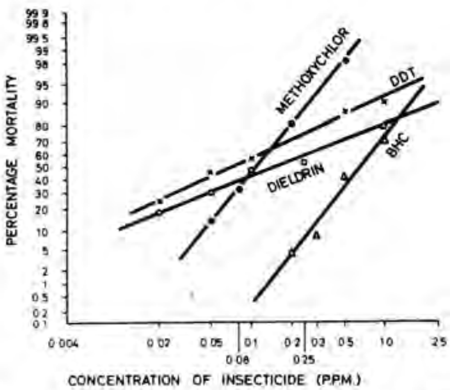

Fig. 3: **Dosage-mortality regression lines to DDT, methoxychlor, dieldrin and BHC to 4th instar larvae of *Ae. Aegypti* from Kuala Lumpur.**

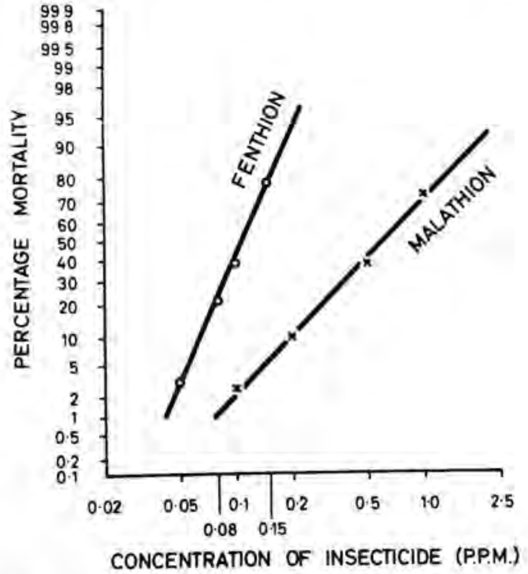

Fig. 4: **Dosage-mortality regression lines to malathion and fenthion to 4th instar larvae of *Ae. Aegypti* from Kuala Lumpur.**

Figs. 3 and 4. The susceptibility level to fenthion was 0.11 p.p.m. A comparison with the previous published data (Wharton, 1955) showed that the larvae have developed a 9-fold increase in tolerance to DDT, 11-fold increase in LC^{50} level to dieldrin and 10-fold increase in LC^{50} to BHC. Since there were no published data on the susceptibility levels of W. Malaysian **Ae. aegypti** larvae to fenthion, malathion or fenitrothion, it is not possible to say how much tolerance the larvae have developed during the past decade or so.

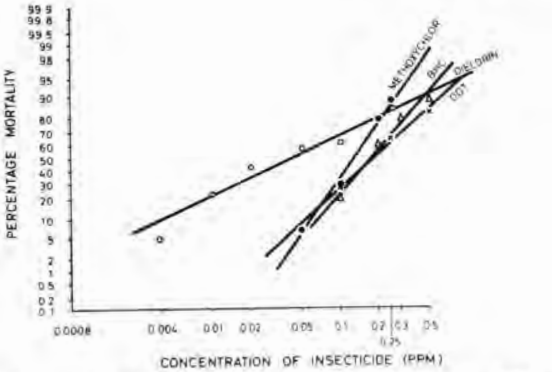

Fig. 5: Dosage-mortality regression lines to DDT, methoxychlor, dieldrin and BHC to 4th instar larvae of *Ae. Albopictus* from Kuala Lumpur.

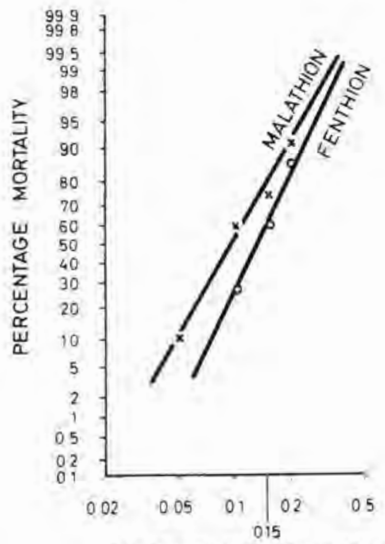

Fig. 6. Dosage-mortality regression lines to malathion and fenthion to 4th instar larvae of *Ae. Albopictus* from Kuala Lumpur.

Aedes albopictus

Four hydrocarbons were tested against larvae and these were tolerant to DDT, BHC and Methoxychlor with LC_{50} values of 0.18 p.p.m. 0.17 p.p.m. and 0.13 p.p.m. respectively. The larvae were, however, susceptible to dieldrin (LC_{50} 0.04 p.p.m.). The tolerance levels of DDT, BHC, and dieldrin have increased by 2.7, 1.7 and 2.1 times respectively in comparison with previous results (Wharton, 1955). The LC_{50} levels for malathion and fenthion were 0.095 p.p.m. and 0.13 p.p.m. respectively and the larvae were tolerant to these compounds. The log dosage/probit regression lines are shown in Figs. 5 and 6.

Armigeres subalbatus

Four hydrocarbon insecticides were tested against the larvae of **Ar. subalbatus**. The larvae were most susceptible to DDT (LC_{50} 0.029 p.p.m.) and then to dieldrin (0.039 p.p.m.) but they were tolerant to methoxychlor (0.116 p.p.m.) and to BHC (0.20 p.p.m.). Among the three organophosphorous compounds tested, fenitrothion was the most toxic insecticide, with LC_{50} value of 0.06 p.p.m. followed by malathion (0.16 p.p.m.) and fenthion (0.19 p.p.m.). The larvae were tolerant to all compounds except to DDT and dieldrin and it was rather surprising that these compounds were still potent to the larvae although these have been the most widely used insecticides in West Malaysia. The log dosage/probit regression lines are shown in Figs. 7 and 8. Since there are no published data on the susceptibility of the larvae of this species to any insecticide, either from Malaysia or other parts of the world, it is difficult to say whether the larvae are tolerant in nature before the wide-scale use of the insecticides. The high tolerance levels in larvae, most probably, have developed during the past few years.

Comparative Toxicity of various compounds to these mosquitoes.

It has been found difficult to establish that any one insecticide was most toxic to all four species of mosquitoes. Individual compounds gave varying results to different species of mosquitoes. However, BHC has been found to be the least toxic compound to larvae of all four species tested (Table 1). **Aedes aegypti** has developed a 10-fold resistance to it. DDT was the most toxic compound against **Ar. subalbatus** and **Ae. aegypti** although less potent to other two species. Methoxychlor was toxic to **C.p. fatigans** although less toxic to the other three species.

Of the four organophosphorous compounds, fenitrothion was tested only against **C.p. fatigans** and **Ar. subalbatus.** It was found to be the most toxic compound against **C.p. fatigans** and the second most toxic compound against **Ar. subalbatus.** Diazinon was tested only against **C.p. fatigans** and it is not possible to compare its potency with other species. Malathion was toxic to **C.p. fatigans** and **Ae. albopictus,** but was not very potent to **Ar. subalbatus** larvae. **Aedes aegypti** has developed resistance to it. The toxicity of

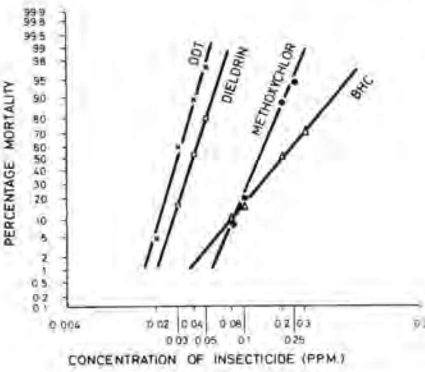

Fig. 7. **Dosage-mortality regression lines to DDT, methoxychlor, dieldrin and BHC to 4th instar larvae of *Ar. subalbatus.***

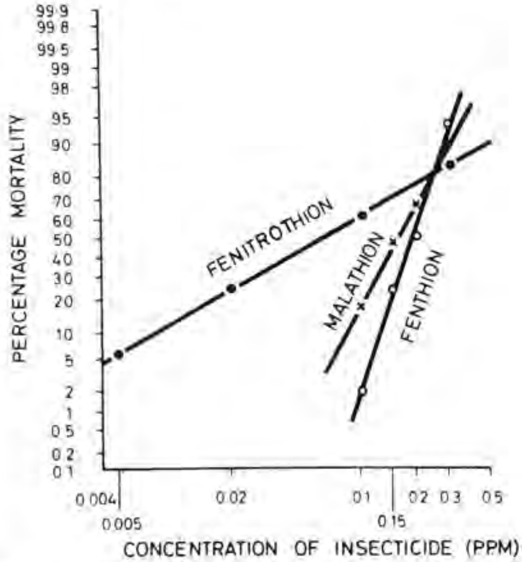

Fig. 8. **Dosage-mortality regression lines to malathion, fenitrothion and fenthion to 4th instar larvae of *Ar. Subalbatus.***

fenthion was more or less uniform to all species tested.

SUMMARY

The larvae of four species of mosquitoes were tested against six to eight different insecticides.

The larvae of **Culex pipiens fatigans** from Kuala Lumpur have developed a 13-fold resistance to BHC. A four-fold increase in tolerance has already been noticed to malathion and diazinon. The **Aedes aegypti** larvae have developed a 9-fold increase in tolerance to DDT, an 11-fold increase in tolerance to dieldrin and a 10-fold increase in resistance to BHC over the past 15 years. The larvae were resistant to malathion.

In spite of the increased tolerance, DDT was found to be the most potent insecticide to **Ae. aegypti** larvae tested. **Aedes albopictus** larvae were tolerant to all compounds except to dieldrin. There was a 1.7 – 2.7 fold increase in tolerance to the three hydrocarbons, DDT, dieldrin and BHC, during the past 15 years. The larvae of **Ar. subalbatus** were tolerant to all insecticides tested except to DDT and dieldrin. The potency of each insecticide to larvae of various species of mosquitoes has been evaluated.

This is the first time that the susceptibility levels of the larvae of **Ar. subalbatus** to insecticides have been published. Similarly, there are no other published data on the susceptibility levels of **Ae. albopictus** to methoxychlor, malathion and fenthion. The susceptibility levels of Kuala Lumpur strains of **C.p. fatigans** against methoxychlor and fenitrothium and those of **Ae. aegypti** against methoxychlor, malathion and fenthion are also published for the first time.

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REFERENCES

Brown, A.W.A. (1967a) Presidential address delivered at the opening session of the annual meeting of the Entomological Society of America in New York City, November 27, 1967.

Brown, A.W.A. (1967b) Genetics of Insecticide Resistance in Insect Vectors. *Genetics of Insect Vectors of disease.* Elsevier Publishing Company Amsterdam pp. 505 – 552.

Edeson, J.G.B. Wilson, T., Wharton, R.H. and Liang, A.B.G. (1960) Experimental transmission of **Brugia malayi** and **B. pahangi** to man. *Trans. Roy. Soc. Trop. Med. Hyg., 54:* 229.

Reid, J.A. (1955) Resistance to insecticides in the larvae of **Culex fatigans** in Malaya. *Bull. Wld Hlth Org., 12:* 705.

Rudnick, A. (1966a) Dengue Viruses Isolated from Mosquitoes in Singapore, 1960–61. *Bull. Wld Hlth Org., 35*: 63.

Rudnick, A. (1966b) Mosquito studies in Relation to Haemorrhagic Fever in the Philippines and South-East Asia. *Bull. Wld Hlth Org., 35*: 77.

Rudnick, A. (1967) **Aedes aegypti** and Haemorrhagic fever. *Bull. Wld Hlth Org., 36*: 528.

Thomas, V. (1962) The susceptibility of **Culex pipiens fatigans** Wiedemann larvae to Insecticides in Malaya. *Bull. Wld Hlth Org., 27:* 595.

Thomas, V. (1970) Fenthion-Resistance in **Culex pipiens fatigans** Wiedermann in Kuala Lumpur, West Malaysia. *Southeast Asian J. Trop. Med. Pub. Hlth., 1*: 93.

Wharton, R.H. (1955) The susceptibility of various species of mosquitoes to DDT, dieldrin and BHC. *Bull. Ent. Res., 46*: 301.

Wharton, R.H. (1958) Dieldrin Resistance in **Culex pipiens fatigans** *in Malaya. Bull. Wld Hlth Org., 18*: 657.

WHO Expert Committee on Insecticides (1963) Instructions for determining the susceptibility or resistance of mosquito larvae to Insecticides. *W.H.O. Tech. Rep. Ser., 265:* 41.