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Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos

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Abstract. Laboratory-bred females of *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes albopictus* from the insectarium, Unit of Medical Entomology, Institute for Medical Research were used in the experiment. The late third stage of the F_0 larvae which survived the high selection pressure of malathion, permethrin and temephos were reared and colonies were established from adults that emerged. *Cx. quinquefasciatus* larvae were subjected to selection by malathion and permethrin for 40 generations, *Ae.aegypti* larvae to malathion, permethrin and temephos for 32 generations and *Ae.albopictus* larvae were selected against malathion and permethrin for 32 generations and 20 generations against temephos.. The rate of resistance development was measured by LC_{50} value . *Cx. quinquefasciatus* larvae developed higher resistance to malathion and permethrin compared to *Ae.aegypti* and *Ae.albopictus.* On the whole, permethrin resistance developed at a faster rate than malathion and temephos.

INTRODUCTION

Culex quinquefasciatus, *Aedes aegypti* and *Aedes albopictus* mosquitoes are cosmopolitan nuisance biting pests and are vectors of diseases. The Southeast Asian region, which is situated in the tropical zone, is a desirable habitat for mosquitoes due to the high temperature and humidity and large area of vegetation. Vector-borne diseases especially classical dengue fever and dengue haemorrhagic fever which are transmitted by *Ae. aegypti* and *Ae. albopictus*, are among the major public health problems in Southern Asian countries (Jahangir *et al.*, 2003). *Cx. quinquefasciatus* mosquitoes are vectors of urban filariasis.

Cx. quinquefasciatus larvae breed and thrive abundantly in stagnant dirty water, while *Ae.aegypti* and *Ae.albopictus* larvae are largely indoor and outdoor container breeders, respectively which thrive in both clean and organically rich water in natural and artificial containers. Both of these species have been known to develop insecticide resistance because chemical insecticides are still used in the control of these vectors.

In some countries the *Cx. quinquefasciatus* breeding sites have been sprayed with organophosphorus insecticides (Ketterman *et al.*, 1993) and this has resulted in the development of resistance. Although there is no control programme designated for *Culex sp.* in Malaysia, this mosquito is highly resistant to organophosphates (Lee, 1990; Lee *et al.*, 1992; Nazni *et al.*, 1998). The control of dengue vectors and other insects of medical importance with insecticides has been hampered by the development of resistance against chemical insecticides, rising costs of these materials and problems of environmental contamination associated with them (Sallehudin *et al.*, 2004).

In Malaysia, the development of resistance could be due to the fogging operations with malathion in early 1970s and with formulation containing permethrin in early 1996 against *Aedes* sp. for dengue control (Nazni *et al.*, 1998). Temephos (Abate™) is an organophosphorus compound widely used as a larvicide in potable water to control container-breeding since 1973 (Lee, 1991). Insecticide resistance is generally believed to arise from selection acting on random variation, i.e. pre-adaptive (Devonshire & Linda, 1991; Nazni *et al.*, 1998). However it has been suggested that insecticides might act both by selection and by increasing mutation rates (Wood *et al.*, 1984). The objective of this study was to determine the resistance rate per generation in *Cx. quinquefasciatus* and *Aedes sp.* in the larval stages with selection pressure from malathion, permethrin and temephos.

MATERIAL AND METHODS

Mosquitoes

Cx. quinquefasciatus and *Ae.aegypti* larvae from a laboratory strain were used and designated as F_0, while *Ae.albopitus* larvae were collected from several localities outside the insectarium of Unit of Medical Entomology, Institute for Medical Research and designated as F_0. The mosquitoes were bread and reared in the insectarium. The F_1 and the subsequent larval stage generations were subjected to selection pressure.

Insecticides

Malathion 93.3% ai (Cynamide), temephos 95.6% ai and permethrin 10.9 ai (Shell) were used in the study.

Selection pressure

The larval stages were subjected to selection pressure against malathion, permethrin and temephos at every generation. For selection of larvae, the insecticides were diluted in ethanol prior to adding into 250 ml water in paper cup containing the larvae. Dosages inducing 50%-70% mortality level were applied to the larvae of each successive generation. Surviving larvae were reared and bred. The first and successive generations of the larvae were tested for susceptibility by the WHO standard bioassay (WHO, 1981) to obtain the 50% lethal concentration (LC_{50}). Bioassay results were subjected to probit analysis (Finney, 1971), using a computerized program of Raymond (1985).

RESULT AND DISCUSSION

The larvae have been selected for 40 generations with malathion and permethrin for *Cx. quinquefasciatus*; 32 generations with malathion, permethrin and temephos; for *Ae. aegypti*; 32 generations with malathion and permethrin and 20 generations with temephos for *Ae. albopictus.*

After selection for about 40 generation for *Cx. quinquefasciatus* larvae, the final resistance ratio to malathion and permethrin was 52.7 and 13,130 folds, respectively (Table 1). On the other hand, after selection for about 32 generations for *Ae.aegypti* larvae, the resistance ratio to malathion, permethrin and temephos was 4.97, 64.2 and 51.0 folds, respectively (Table 2). *Ae. albopictus* larvae, after selection for about 32 generation showed resistance ratio of 10.22 and 21.1 folds to malathion and permethrin, respectively; and showed resistance ratio of 4.49 folds to temephos after selection for about 20 generation (Table 3). It is thus obvious that permethrin resistance was developing at a higher rate compared to malathion and temephos. This trend supports a similar study by Nazni *et al.* (1998) where the field collected *Cx quinquiefasciatus* larvae which were already resistant to malathion and permethrin, showed a resistance ratio of 96.2 folds and 9.4 folds, respectively in comparison to a susceptible laboratory strain, developed higher resistance to permethrin compared to malathion after subjecting to selection pressure with malathion (8 generations) and permethrin (9 generations). The final resistance ratio increased to 597 folds and 7,194 folds for malathion and permethrin respectively.

Table 1. LC$_{50}$ value of malathion and permethrin against laboratory selected *Cx. quinquefasciatus* larvae

Generation	Malathion	Permethrin	Generation	Malathion	Permethrin	Generation	Malathion	Permethrin
F0	0.0163 (0.0151-0.0176)	0.00001 (0.00001-0.00002)	F14	0.2675 (0.2503-0.2846)	0.0048 (0.0031-0.0061)	F28	0.3532 (0.2959-0.3865)	0.0514 (0.0432-0.0560)
F1	0.0182 (0.0170-0.0195)	0.0002 (0.00013-0.00038)	F15	0.2626 (0.2447-0.2802)	0.0044 (0.0029-0.0056)	F29	0.3597 (0.3154-0.3869)	0.0547 (0.0465-0.0634)
F2	0.0229 (0.0209-0.0250)	0.00007 (0.00003-0.00011)	F16	0.2824 (0.2356-0.2996)	0.0052 (0.0038-0.0062)	F30	0.5439 (0.5252-0.5670)	0.0621 (0.0540-0.0710)
F3	0.0184 (0.0165-0.0203)	0.00014 (0.00012-0.00016)	F17	0.2653 (0.2470-0.2833)	0.0097 (0.0080-0.0115)	F31	0.4978 (0.4593-0.5234)	0.0453 (0.0365-0.0541)
F4	0.0220 (0.0199-0.0242)	0.00013 (0.00009-0.00016)	F18	0.2653 (0.2470-0.2833)	0.0155 (0.0133-0.0180)	F32	0.6468 (0.6236-0.6778)	0.0537 (0.0454-0.0624)
F5	0.0499 (0.0445-0.0560)	0.00036 (0.00031-0.00042)	F19	0.3092 (0.2891-0.3285)	0.0184 (0.0154-0.0213)	F33	0.6492 (0.6307-0.6671)	0.0584 (0.0493-0.0682)
F6	0.0330 (0.0290-0.0374)	0.00039 (0.00034-0.00046)	F20	0.3776 (0.3472-0.3980)	nd	F34	nd	0.0617 (0.0542-0.0700)
F7	0.0321 (0.0250-0.0387)	0.00056 (0.00049-0.00064)	F21	nd	nd	F35	0.6969 (0.6730-0.7218)	0.0688 (0.0613-0.0772)
F8	0.0904 (0.0778-0.1048)	nd	F22	0.3727 (0.3395-0.3943)	0.0287 (0.0242-0.0335)	F36	nd	0.0890 (0.0772-0.1079)
F9	0.0431 (0.0318-0.0528)	0.0056 (0.0051-0.0061)	F23	nd	0.0255 (0.0233-0.0280)	F37	0.7416 (0.7010-0.7780)	0.0697 (0.0593-0.0793)
F10	0.0681 (0.0606-0.0754)	0.0044 (0.0040-0.0047)	F24	nd	0.0450 (0.0355-0.0555)	F38	nd	0.1209 (0.1088-0.1329)
F11	0.0471 (0.0356-0.0565)	0.0048 (0.0045-0.0052)	F25	nd	0.0285 (0.0218-0.0342)	F39	0.8592 (0.8084-0.9390)	0.1190 (0.1073-0.1306)
F12	0.1722 (0.1563-0.1936)	nd	F26	0.3802 (0.3568-0.3998)	0.0536 (0.0439-0.0666)	F40	0.8598 (0.8252-0.9031)	0.1313 (0.1177-0.1452)
F13	0.1410 (0.1055-0.1725)	0.0041 (0.0026-0.0053)	F27	0.4009 (0.3742-0.4303)	0.0499 (0.0430-0.0578)			

nd (Not done)

Table 2. LC$_{50}$ value of malathion, permethrin and temephos against laboratory selected *Ae. aegypti* larvae

Generation	Malathion	Temephos	Permethrin	Generation	Malathion	Temephos	Permethrin
F0	0.0601 (0.0488-0.0699)	0.0012 (0.0005-0.0018)	0.0002 (0.0002-0.0003)	F17	0.2133 (0.1847-0.2378)	0.0512 (0.0477-0.0548)	0.0092 (0.0084-0.0100)
F1	0.1528 (0.1396-0.1688)	0.0053 (0.0048-0.0059)	0.0003 (0.0003-0.0003)	F18	0.2507 (0.2284-0.2719)	nd	nd
F2	0.1383 (0.1258-0.1535)	0.0019 (0.0005-0.0031)	0.0004 (0.0004-0.0005)	F19	nd	0.0556 (0.0513-0.0598)	nd
F3	0.1584 (0.1461-0.1715)	0.0045 (0.0032-0.0055)	0.0005 (0.0004-0.0005)	F20	nd	0.0547 (0.0504-0.0590)	0.0079 (0.0071-0.0086)
F4	0.1530 (0.1417-0.1650)	0.0163 (0.0146-0.0186)	0.0006 (0.0005-0.0007)	F21	0.2389 (0.2130-0.2624)	0.0507 (0.0467-0.0547)	0.0100 (0.0090-0.0111)
F5	0.1772 (0.1622-0.1932)	0.0180 (0.0165-0.0195)	nd	F22	nd	0.0567 (0.0527-0.0608)	0.0120 (0.0107-0.0138)
F6	nd	0.0127 (0.0109-0.0143)	0.0014 (0.0012-0.0016)	F23	0.2429 (0.2176-0.2661)	0.0532 (0.0484-0.0577)	0.0117 (0.0107-0.0127)
F7	nd	0.0109 (0.0088-0.0126)	0.0026 (0.0022-0.0029)	F24	0.2766 (0.2516-0.3007)	0.0530 (0.0489-0.0569)	0.0110 (0.0094-0.0123)
F8	nd	0.0199 (0.0183-0.0217)	0.0025 (0.0021-0.0028)	F25	0.2617 (0.2347-0.2868)	nd	0.0134 (0.0128-0.0141)
F9	0.1162 (0.0648-0.1532)	nd	nd	F26	0.2307 (0.2066-0.2526)	0.0589 (0.0546-0.0632)	0.0146 (0.0138-0.0153)
F10	0.0987 (0.0803-0.1135)	0.0239 (0.0215-0.0261)	0.0028 (0.0025-0.0031)	F27	0.2687 (0.2458-0.2908)	0.0464 (0.0437-0.0490)	0.0139 (0.0131-0.0146)
F11	nd	0.0229 (0.0208-0.0248)	0.0034 (0.0030-0.0038)	F28	0.2543 (0.2320-0.2740)	0.05044 (0.04619-0.05420)	0.0140 (0.0133-0.0147)
F12	0.0738 (0.0466-0.0994)	0.0207 (0.0183-0.0229)	0.0037 (0.0033-0.0040)	F29	0.2720 (0.2486-0.2928)	0.0541 (0.0505-0.0574)	0.0127 (0.0119-0.0135)
F13	0.1233 (0.1010-0.1451)	0.0168 (0.0049-0.0264)	0.0040 (0.0037-0.0044)	F30	0.2339 (0.2014-0.2604)	0.0545 (0.0517-0.0572)	0.0137 (0.0129-0.0144)
F14	0.1419 (0.1131-0.1699)	0.0550 (0.0508-0.0591)	0.0042 (0.0039-0.0046)	F31	0.3010 (0.2782-0.3222)	0.0611 (0.0579-0.0642)	0.0138 (0.0130-0.0146)
F15	0.1396 (0.1202-0.1598)	0.0521 (0.0475-0.0565)	nd	F32	0.2982 (0.2761-0.3187)	0.0617 (0.0582-0.0651)	0.0160 (0.0153-0.0168)
F16	0.2262 (0.2013-0.2484)	0.0552 (0.0512-0.0593)	0.0046 (0.0038-0.0053)				

nd (Not done)

Table 3. LC_{50} value of malathion, permethrin and temephos against laboratory selected *Ae. lbopictus* larvae

Generation	Malathion	Temephos	Permethrin	Generation	Malathion	Temephos	Permethrin
F0	0.1243 (0.1054-0.1453)	0.0154 (0.0137-0.0174)	0.0022 (0.0019-0.0028)	F17	0.6653 (0.5975-0.7384)	0.0665 (0.0638-0.0692)	0.0404 (0.0381-0.0425)
F1	0.1633 (0.1419-0.1893)	0.0263 (0.0234-0.0291)	0.0027 (0.0025-0.0030)	F18	nd	0.0676 (0.0638-0.0709)	0.0413 (0.0398-0.0429)
F2	0.2619 (0.2344-0.2923)	0.0216 (0.0179-0.0248)	0.0030 (0.0028-0.0033)	F19	0.7245 (0.6650-0.7928)	0.0630 (0.0570-0.0673)	0.0407 (0.0391-0.0424)
F3	0.3206 (0.2915-0.3509)	0.0198 (0.0150-0.0237)	0.0024 (0.0021-0.0028)	F20	0.8191 (0.7927-0.8390)	0.0692 (0.0648-0.0730)	0.0362 (0.0319-0.0386)
F4	0.1496 (0.1060-0.1822)	nd	0.0027 (0.0024-0.00300)	F21	0.8290 (0.8074-0.8463)	nd	nd
F5	0.2108 (0.1417-0.253)	nd	0.0207 (0.0189-0.0227)	F22	0.8725 (0.8577-0.8866)	nd	0.0385 (0.0370-0.0398)
F6	0.3480 (0.3122-0.3833)	0.0446 (0.0397-0.0489)	0.0210 (0.0175-0.0235)	F23	0.8861 (0.8718-0.9002)	nd	nd
F7	nd	0.0471 (0.0424-0.0514)	0.0171 (0.0130-0.0198)	F24	nd	nd	0.0392 (0.0381-0.0403)
F8	0.3835 (0.3172-0.4350)	0.0477 (0.0431-0.0518)	0.0212 (0.0185-0.0233)	F25	1.0127 (0.9705-1.0443)	nd	0.0456 (0.0421-0.0552)
F9	0.2952 (0.2561-0.3332)	0.0372 (0.0320-0.0415)	0.0280 (0.0265-0.0294)	F26	1.0477 (1.0074-1.0787)	nd	0.0386 (0.0368-0.0400)
F10	nd	0.0529 (0.0488-0.0568)	0.0255 (0.0240-0.0269)	F27	nd	nd	0.0422 (0.0408-0.0438)
F11	nd	0.0474 (0.0435-0.0511)	0.0333 (0.0325-0.0340)	F28	1.1394 (1.1238-1.1532)	nd	0.0422 (0.0408-0.0437)
F12	0.4345 (0.3537-0.4960)	0.0550 (0.0525-0.0574)	0.0355 (0.0341-0.0364)	F29	1.1552 (1.1363-1.1718)	nd	0.0438 (0.0426-0.0450)
F13	0.4056 (0.3464-0.4533)	0.0535 (0.0509-0.0560)	0.0352 (0.0330-0.0367)	F30	1.1869 (1.1710-1.2017)	nd	0.0424 (0.0411-0.0436)
F14	0.5048 (0.4505-0.5522)	0.0628 (0.0591-0.0662)	0.0366 (0.0350-0.0378)	F31	1.2047 (1.1517-1.2364)	nd	0.0445 (0.0436-0.0455)
F15	0.5547 (0.4918-0.6109)	0.0645 (0.0611-0.0678)	0.0385 (0.0365-0.0401)	F32	1.2700 (1.2442-1.2917)	nd	0.0460 (0.0450-0.0471)
F16	0.5385 (0.4734-0.5952)	0.0641 (0.0608-0.0673)	0.0402 (0.0380-0.0422)				

nd (Not done)

The result of bioassays also indicated that tolerance to temephos existed in laboratory selected strains of *Ae.aegypti* and *Ae. albopictus*. Temephos tolerance in *Ae. aegypti* has been reported previously by Lee *et al.* (1984) and Lee & Lime (1989). Comparing the F_1 LC_{50} value of malathion, permethrin and temephos to their respective generations of selections, the resistance level was increasing at each generation (Figure 1-3). Studies by Bisset *et al.* (1991) and Gopalan *et al.* (1996) demonstrated 1,208 fold resistance after 22 generations and 2,036 folds resistance after 25 generations of selection with malathion. It was not possible to calculate the rate of selection in each generation due to the inconsistency in the larval LC_{50} values which could be due to heterozygosity and homozygosity of the gene(s). From the study we observed that resistance gene(s) expression become more active in exposure to insecticidal pressure. According to the Darwinian theory, gene(s) responsible for insecticide resistance exit in a small segment of population. The gene(s) will be activated on exposure to insecticidal pressure. The speed and degree of development of resistance depends on the frequency of resistance gene(s) in the population, the type of gene which is responsible for resistance, the insecticide dosage applied and the frequency of application (Nazni *et al.*, 1998).

The information obtained in this study is useful in mosquito control programmes. It is important to detect and characterize developing resistance problem so that future control strategies can be developed by optimizing current insecticides usage. If resistance is shown to be directly affecting control, other methods such as rotating the insecticides can be considered.

In summary, *Cx. quinquiefasciatus* developed higher resistance to malathion and permethrin compared to both *Ae. aegypti* and *Ae. albopictus*. Permethrin selection for resistance was at a faster rate compared to malathion and temephos based on their resistance ratio.

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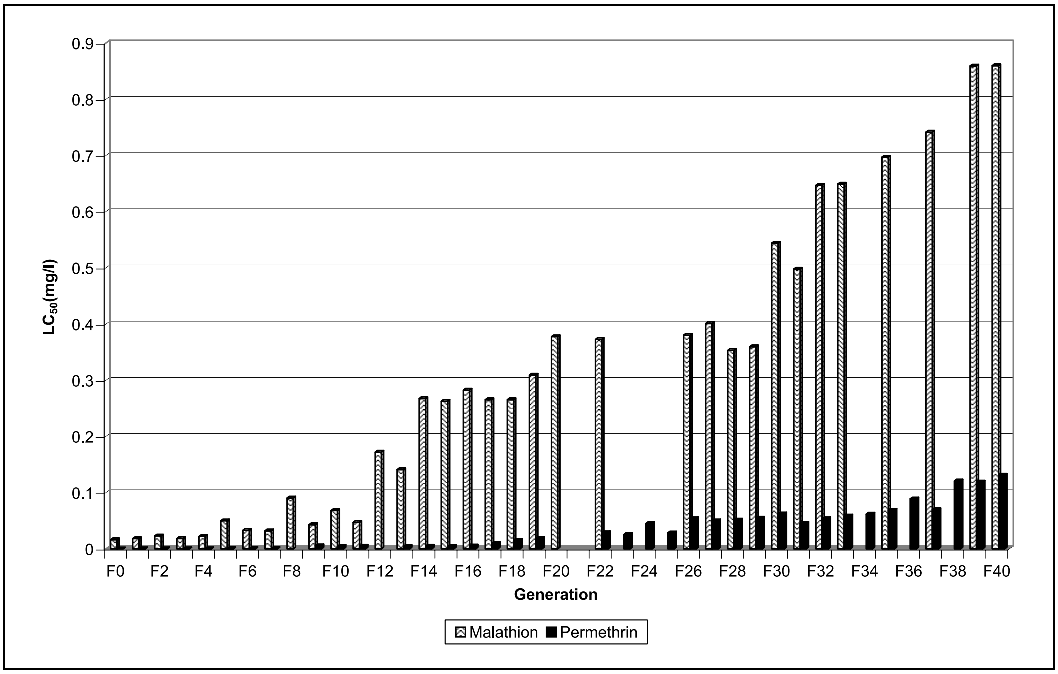

Figure 1. LC_{50} values of insecticide selected *Culex quinquefasciatus* larvae in different generations.

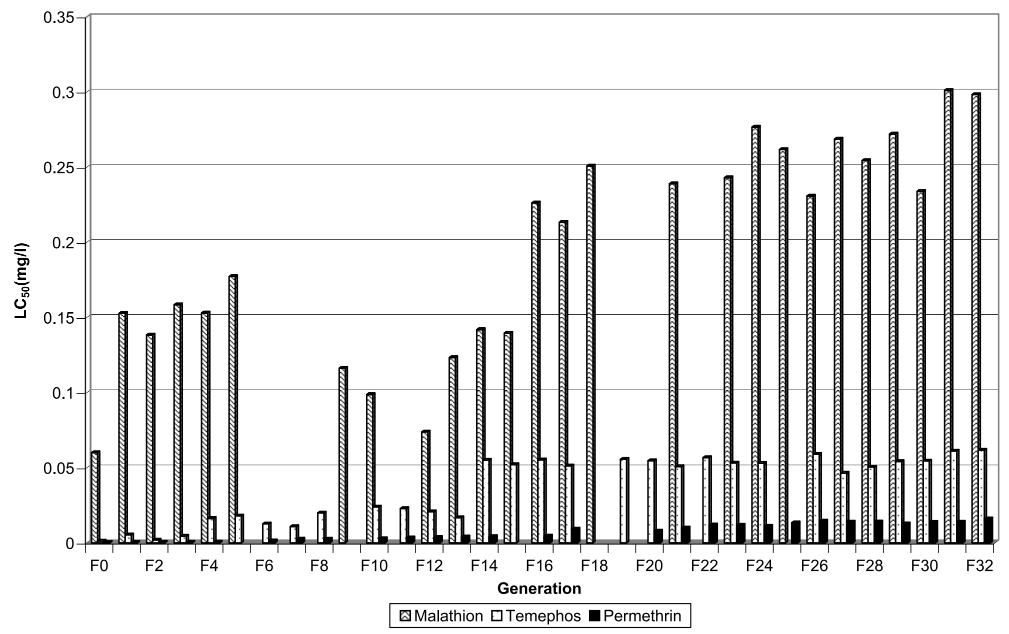

Figure 2. LC$_{50}$ values of insecticide selected *Aedes aegypti* larvae in different generations

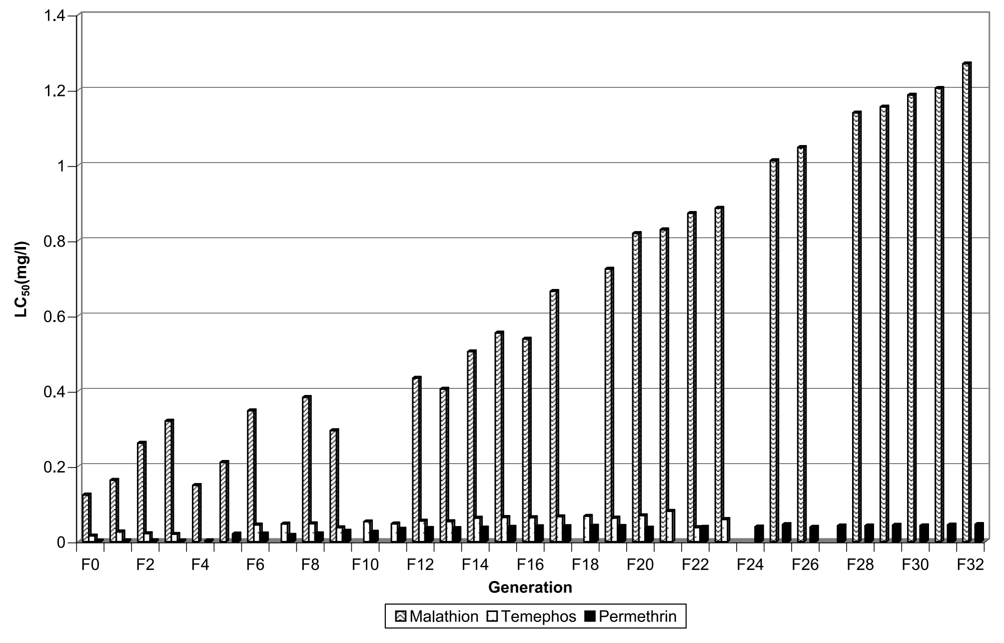

Figure 3. LC$_{50}$ values of insecticide selected *Aedes albopictus* larvae in different generations.

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REFERENCES

Bisset J.A., Rodriguez, M., Hemingway, J., Diaz, C., Small, G.J. & Ortiz, E. (1991). Malathion and pyrethroid resistance in *Culex quinquefasciatus* from Cuba : efficacy of pirimiphosmethyl in the presence of at least three resistance mechanisms. *Medical and Veterinary Entomology* **5:** 223–228.

Devonshire, A.L. & Field, L.M. (1991). Gene amplification and insecticide resistance. *Annual Review of Entomoogy* **36:** 1–23.

Finney, D.J. (1971). Probit Analysis (3rd. ed.) Cambridge University Press, London.

Gopalan, N., Prakash, S., Bhattacharya, B.K., Anand, O.P. & Rao, K.M. (1996). Development of malathion resistance in *Culex quinquefasciatus* Say (Diptera : Culicidae. *Indian Journal of Medical Research* **103:** 84–90.

Jahangir, K., Yap, H.H., Zairi, J., Lee, C.Y. & Saira Banu, M.M. (2003). The effect of cloth wetted with sugar solution and water on prolonging the life span of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) under laboratory condition. *Tropical Biomedicine* **20(2):** 145–152.

Ketterman, A.J., Karunaratne, S.H.P.P., Jayawardena, K.G.I. & Hemingway, J. (1993). Qualitative differences between populations of *Culex quinquefasciatus* in both the esterases A2 and B2 which are involved in insecticide resistance. *Pest Biochemistry and Physiology* **47:** 142–8.

Lee, H.L., Lee, T.W., Law, F.M. & Cheong, W.H. (1984). Preliminary studies on the susceptibility of field-collected *Aedes* (Stegomyia) *aegypti* (Linneaus) to Abate (temephos) in Kuala Lumpur. *Tropical Biomedicine* **1:** 37–40

Lee, H.L. & Lime, W. (1989). A re-evaluation of the susceptibility of field-collected *Aedes (Stegomyia) aegypti* (Linnaeus) larvae to temephos in Malaysia. *Mosquito Borne Diseases Bulletin* **6:** 91–95.

Lee, H.L. (1990). A rapid and simple biochemical method for the detection of insecticide resistance due to elevated esterase activity in *Culex quinquefasciatus. Tropical Biomedicine* **7:** 21–8.

Lee, H.L. (1991). Esterase activity and temephos susceptibility in *Aedes aegypti* (L.) larvae. *Mosquito Borne Diseases Bulletin* **8:** 127–130.

Lee, H.L., Abimbola, O. & Singh, I.K. (1992). Determining resistance susceptibility in *Culex quinquefascitus* Say adults by rapid enzyme microassays. *Southeast Asian Journal of Tropical Medicine & Public Health* **23:** 458–463.

Nazni, W.A., Lee, H.L. & Sa'diyah, I. (1998). Rate of resistance development in wild *Culex quinquefasciatus* (Say) selected by malathion & permethrin. *Southeast Asian Journal of Tropical Medicine & Public Health* **29:** 849–855.

Raymond, M. (1985). Log – probit analysis basic programme of microcomputer. *Cahiers ORSTOM Entomologie Medicle et Parasitologie* **23:** 117–121.

Sallehudin Sulaiman, Siti Hajar Abdullah & Hidayatulfathi Othman. (2004). Residual efficacy of insect growth regulators pyriproxyfen, triflumuron and s-methoprene against *Aedes aegypti* (L.) in plastic containers in the field. *Tropical Biomedicine* **21(1):** 97–100.

Wood, R.J., Pasteur, N. & Sinegre, G. (1984). Carbamate and organophosphate resistance in *Culex pipiens* L. (Diptera : Culicidae) in Southern France and significance of Est – 3A. *Bulletin of Entomological Research* **74:** 677–687.

WHO Expert Committee on Insecticides (1981). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. World Health Organization mimeograph *WHO/VBC/81.807.*