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Laboratory evaluation of insect growth regulators against several species of anopheline mosquitoes

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Abstract: Larvicide efficacy of insect growth regulators (pyriproxyfen, methoprene and diflubenzuron), in comparison with the larvicidal and adulticidal efficacy of conventional insecticides, against several species of Anopheline mosquitoes including several insecticide resistant strains were evaluated in laboratory conditions. In all species, no cross resistance between IGRs and the other kinds of insecticides, such as organophosphate, organochlorine, carbamate and pyrethroid, was observed. Relative effectiveness of pyriproxyfen to methoprene ranged from several to 40 times and that to diflubenzuron ranged from 19.5 times to more than 400 times.

INTRODUCTION

Anopheline mosquito is one of the most important vectors of tropical diseases. Residual spray on the wall surface and treatment of breeding areas with chemicals, such as organochlorine, organophosphate and carbamate insecticides, have been employed as the best and appropriate ways of controlling these mosquitoes. However, as a result of over-use or inappropriate treatment of insecticides, many cases of insecticide resistance development have been reported (Hemingway and Georghiou, 1983; Scott and Georghiou, 1986; Hemingway *et al.*, 1986). The adoption of insecticides with different modes of action from conventional ones and the combination use of them could provide a solution to overcome this problem.

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In this respect, IGRs (insect growth regulators) seem to be useful insecticides. Pyriproxyfen, a juvenile hormone mimic, had high activities to mosquitoes in the field (Kawada *et al.*, 1988; Suzuki *et al.*, 1989; Okazawa *et al.*, 1991). In this study, we evaluated the larvicide efficacy of pyriproxyfen against several species of Anopheline mosquitoes in laboratory conditions, comparing with other insecticides, and discuss the possibility of its use as a new vector control agent.

MATERIALS AND METHODS

1. Test chemicals. Pyriproxyfen: 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether (5% emulsifiable concentrate). Methoprene: isopropyl-(2*E*, 4*E*)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (5% emulsifiable concentrate). Diflubenzuron: 1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl) urea (25% wettable powder formulation in com-

Table 1 Anopheline mosquitoes used in the study.

Species	Strain	Site of collection	Institution introduced
A. stephensi	SUS	—	St. Marianna Univ.
	MLT-R	Pakistan	London Univ.
A. gambiae	SUS	Tanzania	London Univ.
	DLD-R	Burkina fuso	London Univ.
	DDT-R	Africa	London Univ.
A. albimanus	SUS	—	London Univ.
	OPC-R	El-Salvador	London Univ.
A. farauti		Solomon Islands	Ministry of Health, Solomon Islands

SUS, strain which shows normal susceptibility to insecticides; MLT-R, malathion resistant; DLD-R, dieldrin resistant; DDT-R, DDT resistant; OPC-R, organophosphate and carbamate resistant.

mercial (Dimilin®, Sankyo Co., Ltd.) was used). Temephos: *O, O'*-(thiodi-4, 1-phenylene) bis(*O, O'*-dimethyl phosphorothioate) (5% emulsifiable concentrate). Fenitrothion: *O, O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate (5% emulsifiable concentrate). Malathion: diethyl mercaptosuccinate S-ester with *O, O*-dimethyl phosphorodithioate (5% emulsifiable concentrate). Propoxur: 2-(1-methylethoxy) phenol methylcarbamate (5% emulsifiable concentrate). DDT: dichloro diphenyl trichloroethane. Dieldrin: (1*R*, 4*S*, 4*aS*, 5*R*, 6*R*, 7*S*, 8*S*, 8*aR*)-1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-6, 7-epoxy1, 4:5, 8-dimethanaphthalene. Permethrin: 3-phenoxybenzyl (1*RS*, 3*RS*: 1*RS*, 3*SR*)-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate (5% emulsifiable concentrate).

2. *Test insects.* Adults and larvae of Anopheline mosquitoes, *A. stephensi, A. gambiae, A. albimanus, A. farauti,* were used. Table 1 shows their collection sites and the institutes which provided colonies to us. Insects were reared at 25°C and 60% RH, under 16L-8D photoperiodic regime. Resistant strains were selected periodically with insecticides at the larval stage in OPC-R strain of *A. albimanus* and adult stage in the other strains.

3. *Bioassays.* All experiments were conducted under the same conditions described above.

(1) Adult susceptibility: A 0.3 μl acetone solution of the test chemical was topically applied to the dorsal mesothorax of a 2- to 3-days-old female adult. Insects treated were kept under the conditions noted above and fed with 3% sugar solution. Mortality was observed 24 hr after treatment. The data obtained were corrected by the mortality of untreated adults and LD_{50} was calculated by Bliss' probit method (Bliss, 1934).

(2) Larval susceptibility: An emulsifiable concentrate of each chemical and wettable powder of diflubenzuron were used. The test formulation was diluted with deionized water at appropriate concentrations. Thirty 4th instar larvae were released into 150 ml of each test solution in an aluminum cup. After 24 hr, mortality was observed and LD_{50} was calculated in the same manner noted above. In the case of IGRs, larvae were reared in the solution until adult emergence. The percentage of emergence was corrected by that of control to calculate IC_{50} in the same manner noted above.

Results and Discussion

1. *Adult and larval susceptibility to insecticides*

Adult and larval susceptibility to insecticides is shown in Figs. 1 and 2. Adult MLT-R strain of *A. stephensi* showed higher resistance to malathion (R/S ratio=71) than larvae (R/S ratio=2.6). The degree of cross resistance between malathion and fenitrothion was small in this strain. This is due to the resistance factor of malathion carboxyleste-

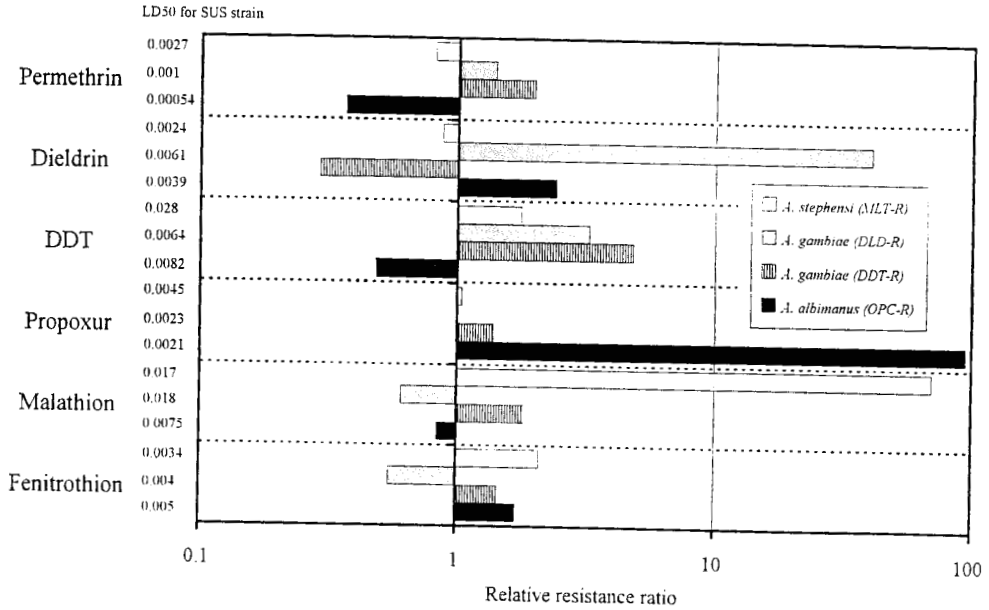

Fig. 1 Efficacy of insecticides to adult Anopheline mosquitoes by topical application.

Figures in abscissa indicate resistance ratio to SUS strain (strain which shows normal susceptibility to insecticides). Figures in ordinate indicate LD_{50} (μg/female) for SUS strain: upper, *A. stephensi*; middle, *A. gambiae*; bottom, *A. albimanus*.

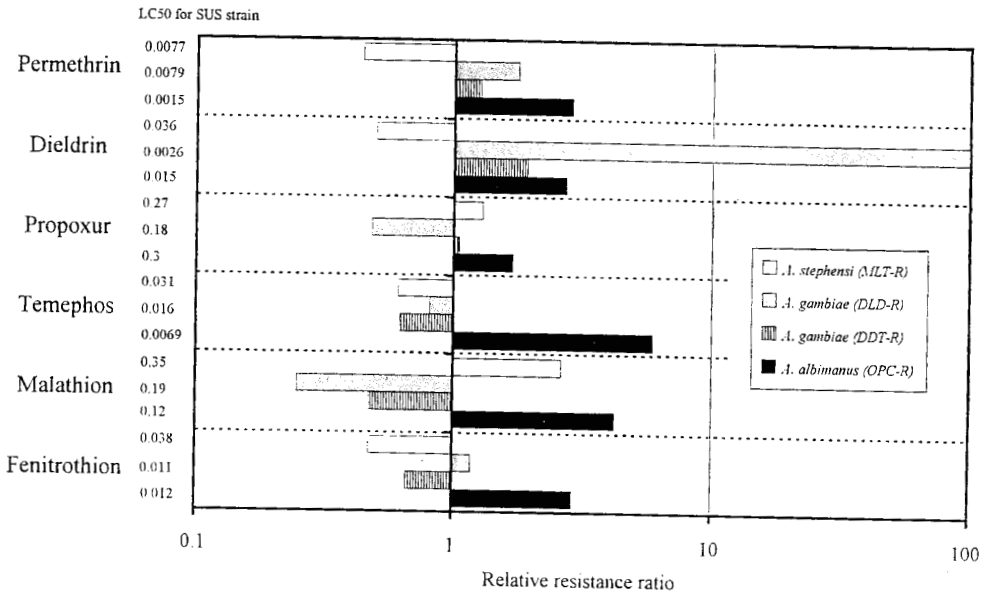

Fig. 2 Efficacy of insecticides to larval Anopheline mosquitoes.

Figures in abscissa indicate resistance ratio to SUS strain (strain which shows normal susceptibility to insecticides). Figures in ordinate indicate LC_{50} (ppm) for SUS strain: upper, *A. stephensi*; middle, *A. gambiae*; bottom, *A. albimanus*.

Table 2 IC_{50} values of IGRs against the last instar larvae of Anopheline mosquitoes.

Species	Strain	IC_{50} (ppb)		
		Pyriproxyfen	Methoprene	Diflubenzuron
A. stephensi	SUS	0.043 (0.011-0.16)	0.54 (0.39-0.77)	0.84 (0.71-0.99)
	MLT-R	0.025 (0.019-0.034)	0.75 (0.099-5.6)	1.6 (1.4-1.7)
A. gambiae	SUS	0.025 —	0.067 (0.022-0.20)	3.7 (2.6-5.2)
	DLD-R	0.0098 (0.0025-0.038)	0.039 (0.011-0.14)	0.87 (0.76-0.99)
	DDT-R	0.0040 (0.0028-0.0057)	0.072 (0.0023-2.3)	1.9 (0.53-7.1)
A. albimanus	SUS	0.016 —	0.16 (0.11-0.23)	0.70 (0.59-0.83)
	OPC-R	0.00042 (0.000035-0.005)	0.016 (0.012-0.021)	0.20 —
A. farauti		0.0017 (0.011-0.16)	— —	— —

Numbers in parentheses are 95% fiducial limits. Blank area indicates that no test was done.

rase (Scott and Georghiou, 1986). Differences in adult and larval susceptibility of three strains of *A. gambiae* to organophosphate and propoxur were not observed. Resistance ratio of DDT-R strain to DDT was significant but moderate (R/S ratio was 4.8 for adult and 8.8 for larva). Cross resistance between DDT and dieldrin or permethrin was not observed. Resistance ratio of OPC-R strain of *A. albimanus* to organophosphate was smaller than that to propoxur. Larvae of this strain showed higher resistance to temephos than the other strains.

Larval resistance ratio of each strain was relatively smaller than that of adult except for *A. albimanus* OPC-R. This seems to be explained by the fact that *A. albimanus* OPC-R strain was selected at larval stages with severe contamination of various insecticides for agricultural use (Bruce-Chwatt, 1985). *A. gambiae* DLD-R strain showed high resistance to dieldrin in both stages (R/S ratio was 41 for adult and 100 for larva).

2. *Larval susceptibility to insect growth regulators*

Larval susceptibility to insect growth regulators is shown in Table 2. IC_{50} of pyriproxyfen ranged widely from 0.00042 ppb (*A. albimanus* OPC-R) to 0.043 ppb (*A. stephensi* SUS). In *A. stephensi* there was no difference in susceptibility to pyriproxyfen of SUS strains and that of malathion-resistant strain. In contrast, in *A. albimanus* and *A. gambiae,* susceptibility to pyriproxyfen was rather higher in resistant strains than in SUS strains. These results indicate that there is no cross resistance between pyriproxyfen and the other insecticides, such as organophosphate, carbamate and pyrethroids. Schaefer *et al.* (1988) also reported that IC_{50}s of pyriproxyfen for SUS and organophosphate resistant strain for *Culex quinquefasciatus* were 0.018 and 0.022 ppb, and those for *C.*

tarsalis were 0.021 and 0.052 ppb, respectively. There also seemed to be no cross resistance between methoprene or diflubenzuron and the other insecticides. Relative effectiveness of pyriproxyfen to methoprene was less than 5 times in *A. gambiae* SUS and DLD-R, which ratio is similar to that in *Culex pipiens pallens* (Kawada *et al.*, 1988). Relative effectiveness for the other strains, however, was much higher (ranged from 10 to 40 times) than those for the above two species. The relative activity of pyriproxyfen seems to be higher for *Anopheles* than for *Culex* as Hatakoshi *et al.* (1987) reported. Relative effectiveness of pyriproxyfen to difluzenburon was much higher than to methoprene (ranged from 19.5 to more than 400 times).

The availability of pyriproxyfen as a mosquito larvicidal formulation has been reported in many papers (Schaefer *et al.*, 1988; Kawada *et al.*, 1988; Suzuki *et al.*, 1989; Okazawa *et al.*, 1991). They concluded that a single, low dose application of pyriproxyfen ranged from 0.01 to 0.1 ppm gave a long-term control effect against mosquito larvae. This must be due to the high activity and stability of pyriproxyfen in the field. Because of the lack of cross resistance, pyriproxyfen may compensate the lack of efficacy in conventional insecticides due to the development of resistance. The combinational use of pyriproxyfen with other controlling methods, such as space spray and/or residual spray of adulticide, will provide a new tool for managing tropical diseases by chemical treatment.

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摘　要

ハマダラカ属数種に対する殺虫剤および昆虫成長制御剤の効力評価

殺虫剤抵抗性系統を含むハマダラカ属数種の成虫の殺虫剤感受性，および幼虫の殺虫剤と昆虫成長制御剤に対する感受性を室内条件にて評価した．供試したすべての種において，成虫および幼虫の殺虫剤（有機リン剤，有機塩素剤，カーバメイト剤，ピレスロイド剤）と昆虫成長制御剤に対する交差抵抗性は観察されなかった．昆虫幼若ホルモン様物質であるピリプロキシフェンのメトプレンに対する相対効力比は数倍から40倍の範囲に，また，キチン形成阻害剤であるディフルベンズロンに対する相対効力比は19.5倍から400倍以上の範囲にあった．