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Comparative insecticidal efficacy of a new pyrethroid, metofluthrin, against colonies of Asian *Culex quinquefasciatus* and *Culex pipiens pallens*

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Abstract: Comparative insecticidal efficacy of metofluthrin, a newly synthesized pyrethroid, and other pyrethroids against several colonies of Asian *Culex quinquefasciatus* (from Indonesia, Thailand, Vietnam and Malaysia) was evaluated by topical application. Metofluthrin was the most effective against the four colonies of *Cx. quinquefasciatus*. The LD_{50}-based relative effective ratio of metofluthrin against *d*-allethrin was higher in *Cx. quinquefasciatus* (33.3 to 78.8) than in *Cx. pipiens pallens* (27.8). The Vietnam colony was the most susceptible among the colonies in the study.

Key words: metofluthrin, transfluthrin, *Culex quinquefasciatus*, topical application, susceptibility

INTRODUCTION

Culex quinquefasciatus Say is an ubiquitous species and is abundant in tropical and subtropical countries. *Cx. quinquefasciatus* is highly anthropophilic, feeds readily both indoors and outdoors and has its peak biting period around midnight (Kettle, 1995). Lack of proper and adequate disposal of wastes, poor city planning and resource limitations appear to be the contributing factors encouraging propagation of this mosquito (Ali et al., 1999). It has been playing an important role as a biting nuisance in urban areas as well as its main role as a vector of bancroftian filariasis.

Metofluthrin (S-1264) is a newly synthesized pyrethroid, which has strong knockdown and lethal activities against mosquitoes (Shono et al., 2004; Sugano et al., 2004; Ujihara et al., 2004). The vapor pressure of metofluthrin is ca. >2 times and >100 times higher than that of *d*-allethrin and permethrin, respectively (Kawada et al., 2004a), making it vaporize at room temperature, while the other conventional pyrethroids need heating for evaporation. Kawada et al. (2004a, 2004b) reported the promising efficacy of new mosquito-controlling devices using metofluthrin (metofluthrin impregnated multilayer paper strip), which needs no external energy for vaporization, at low cost against *Cx. quinquefasciatus* in Lombok Island, Indonesia. Transfluthrin also belongs to a pyrethroid group which has high volatility and high knockdown and lethal activities. Pates et al. (2002) reported unique attempts using a transfluthrin-mixed kerosene oil lamp to repel the biting of *Cx. quinquefasciatus* in the typical houses in Dar es Salaam, Tanzania.

The two unique pyrethroids mentioned above will constitute a new era in the field of spatial repellent for insects, as well as in the field of conventional mosquito-proofing devices, such as mosquito coil, mat, and vaporized liquid. In this paper, we report on the comparative insecticidal activity of metofluthrin, transfluthrin and other pyrethroids against several colonies of Asian *Cx. quinquefasciatus*.

Materials and Methods

Three colonies of field-collected (from Indonesia, Thailand and Vietnam) and one laboratory colony of *Cx. quinquefasciatus* and one laboratory colony of *Culex pipiens pallens* L. were used for the study. The Indonesia colony (IN) was collected at Mataram, Lombok Island, in February, 2003; the Thailand colony (TL) was collected at Mae Joh, Chiang Mai, in March, 2003; the Vietnam colony (VN) was collected at Catque, Hatay, in March, 2003. After collection, they were reared for several generations in the laboratory and the colonies of the fourth to sixth generations after collection were used for the study. The Malaysia colony (ML) of *Cx. quinquefasciatus*, introduced from School of Biological Sciences, Universiti Sains Malaysia (Foo and Yap, 1982), and the Gose colony of *Cx. pipiens pallens*, collected at Gose, Nara, Japan (Mukai et al., 1974; Shinjo et al., 1981), were standard colonies which are susceptible to most insecticides. Mosquitoes were reared in the laboratory at 25℃, 70% RH and 16L8D photoperiod regime.

Technical grades of metofluthrin, (2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (*E*,*Z*)(1*R*,3*R*)-2,2-dimethyl-3-(prop-2-enyl) cyclopropanecarboxylate), transfluthrin, *d*-allethrin, permethrin, and fenitrothion were used for the study.

Female mosquitoes were anesthetized briefly with carbon dioxide and were put on the metal plate, the surface temperature of which was maintained <4℃ with ice, to keep the anesthesia. A 0.3 μg acetone solution of the test chemical was topically applied to the dorsal mesothorax of 4- to 6-day-old female adults by Automatic Microapplicator (Burkard Manufacturing Co., Ltd., Hertfordshire, UK). The same amount of acetone without insecticide was treated to the females as untreated control. Ten insects treated were kept in a plastic cup, the bottom of which was lined with a filter paper, fed with 3% sugar solution under the laboratory conditions noted above. Mortality was observed 24 h after treatment. Data obtained were corrected by the mortality of untreated insects with Abbott's formula and LD_{50} and LD_{90} was calculated by Bliss' Probit

Table 1. LD_{50}s of insecticides for several Asian strains of *Culex quinquefasciatus* and *Culex pipiens pallens* by topical application.

Chemicals	LD_{50} (μg/female) – (95% confidential limit)				
	Culex quinquefasciatus				*Culex pipiens pallens*
	Indonesia	Thailand	Vietnam	Malaysia	Gose
Metofluthrin	0.0012 (0.00086–0.0016)	0.00080 (0.00067–0.00095)	0.00039 (0.00026–0.00055)	0.00033 (0.00029–0.00037)	0.0018 (0.0013–0.0026)
Transfluthrin	0.0053 (0.0033–0.0072)	0.0033 (0.0027–0.0043)	0.0024 (0.0020–0.0028)	0.0024 (0.0018–0.0042)	0.0066 (0.0053–0.0082)
d-Allethrin	0.057 (0.044–0.074)	0.063 (0.049–0.088)	0.013 (0.010–0.016)	0.013 (0.0091–0.016)	0.050 (0.040–0.059)
Permethrin	0.0018 (0.00099–0.0027)	0.011 (0.0093–0.013)	0.0016 (0.0012–0.0020)	0.0044 (0.0035–0.0054)	0.0028 (0.0017–0.0041)
Fenitrothion	0.0081 (0.0066–0.0099)	0.0084 (0.0068–0.010)	0.0033 (0.0022–0.0042)	0.0014 (0.00071–0.0019)	0.0054 (0.0040–0.0065)

Table 2. LD_{90}s of insecticides for several Asian strains of *Culex quinquefasciatus* and *Culex pipiens pallens* by topical application.

Chemicals	LD_{90} (μg/female)−(95% confidential limit)				
	Culex quinquefasciatus				*Culex pipiens pallens*
	Indonesia	Thailand	Vietnam	Malaysia	Gose
Metofluthrin	0.0050 (0.0035–0.0091)	0.0017 (0.0014–0.0025)	0.0014 (0.00096–0.0027)	0.00063 (0.00054–0.00078)	0.0049 (0.0031–0.018)
Transfluthrin	0.019 (0.012–0.086)	0.011 (0.0074–0.020)	0.0053 (0.0044–0.0073)	0.0067 (0.0039–0.034)	0.013 (0.010–0.022)
d-Allethrin	0.16 (0.11–0.38)	0.30 (0.18–0.84)	0.044 (0.033–0.068)	0.034 (0.025–0.061)	0.088 (0.071–0.16)
Permethrin	0.0054 (0.0034–0.013)	0.030 (0.024–0.044)	0.0050 (0.0036–0.0085)	0.015 (0.011–0.026)	0.0057 (0.0038–0.011)
Fenitrothion	0.020 (0.015–0.031)	0.027 (0.020–0.045)	0.012 (0.0091–0.020)	0.0046 (0.0029–0.023)	0.012 (0.0090–0.022)

method (Bliss, 1934). Two to 6 replicates were made for each dosage.

Results and Discussion

LD_{50}s and LD_{90}s of insecticides for several colonies of Asian *Cx. quinquefasciatus* and *Cx. pipiens pallens* by topical application are shown in Tables 1 and 2. LD_{50}s of *d*-allethrin, permethrin and fenitrothion for *Cx. pipiens pallens* were 0.050, 0.0028 and 0.0054 μg/female, respectively. They correspond well to the data in the prior reports for the same colony, in which LD_{50}s of *d*-allethrin, permethrin and fenitrothion were 0.037 (Shinjo et al., 1981), 0.0031 (Shinjo et al., 1989), and 0.0052 μg/female (Kawada, 1999), respectively. Dose-mortality regression lines for *Cx. quinquefasciatus* and *Cx. pipiens pallens* with metofluthrin, transfluthrin, and *d*-allethrin are shown in Fig. 1. Susceptibility of the standard colony of *Cx. quinquefasciatus* (ML) was slightly higher than that of the standard colony of *Cx. pipiens pallens*; LD_{50}s were ca 5.5, 2.8, and 3.8 times lower in ML colony than *Cx. pipiens pallens* for metofluthrin, transfluthrin, and *d*-allethrin, respectively. Susceptibility of the VN colony seemed to be almost the same as that of the ML colony, indicating that the VN colony has experienced little exposure to pyrethroids and organophosphates, or that the genes conferring resistance were absent in the VN colony. Susceptibility of the IN and TL colonies, on the other hand, was slightly lower than that of the ML and VN colonies; LD_{50}s for the IN and TL colonies were ca. 3.6 and 2.7 times with metofluthrin, 2.2 and 1.4 times with transfluthrin, 4.4 and 4.8 times with *d*-allethrin, higher than ML colony, respectively. Insecticide resistance, however, should not simply be attributed to the above differences in susceptibility, since there is little information on the susceptibility of *Cx. quinquefasciatus* by topical application. Those differences in susceptibility might be deemed as due to variation among the collection sites. Metofluthrin was the most effective for all colonies among the insecticides tested in the study. Table 3 shows LD_{50}-based relative effective ratio of metofluthin and transfluthrin against *d*-allethrin. The relative effective ratio for metofluthrin was higher in *Cx. quinquefasciatus* (33.3 to 78.8) than in *Cx. pipiens pallens* (27.8). On the contrary, differences in the relative effective ratio of transfluthrin against *d*-allethrin between *Cx. quinquefasciatus* (5.4 to 19.1) and *Cx. pipiens pallens* (7.6) seemed to be lower than those in metofluthrin.

Few studies on the insecticide susceptibility by topical application have been made for mosquitoes, since the method

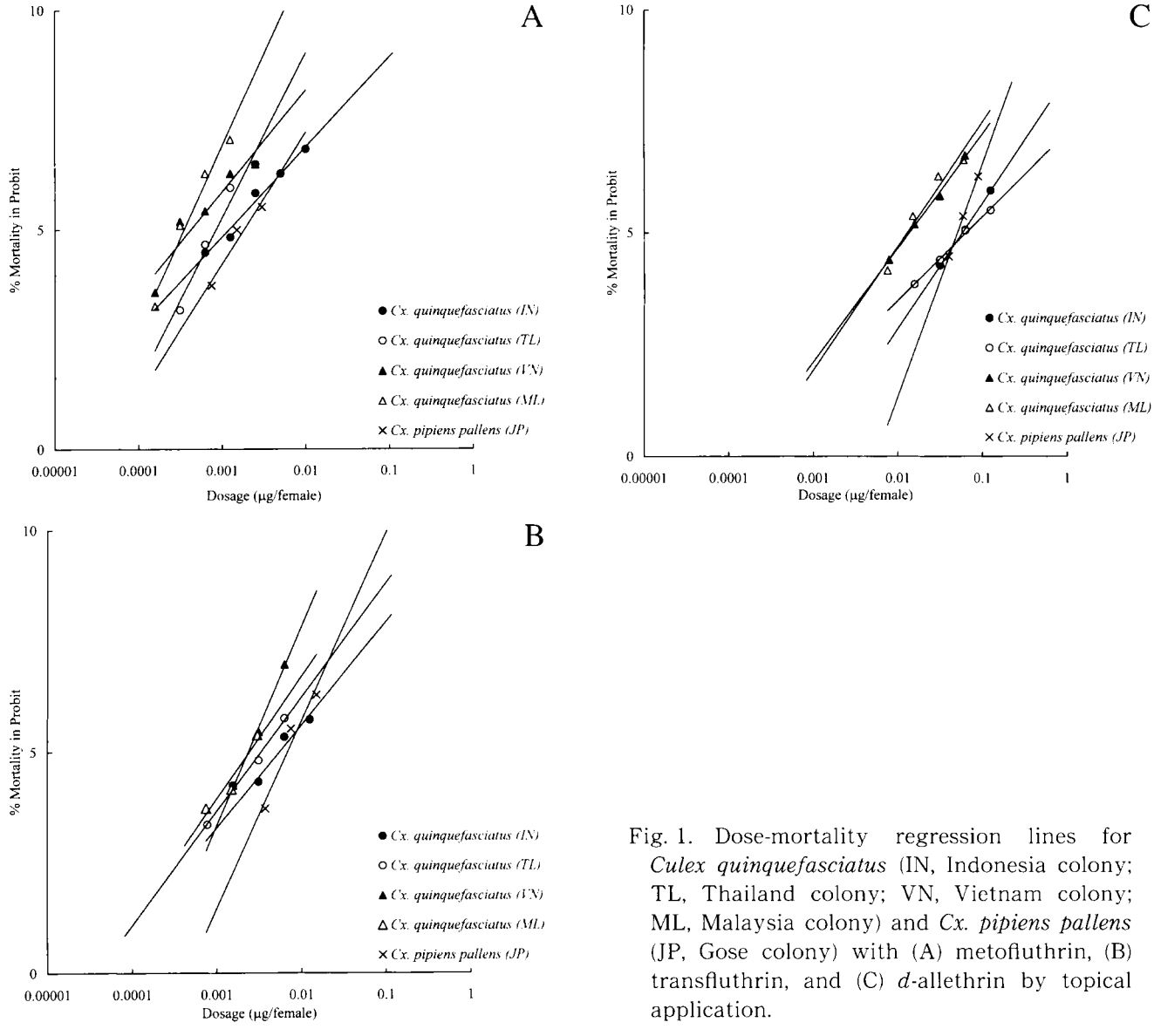

Fig. 1. Dose-mortality regression lines for *Culex quinquefasciatus* (IN, Indonesia colony; TL, Thailand colony; VN, Vietnam colony; ML, Malaysia colony) and *Cx. pipiens pallens* (JP, Gose colony) with (A) metofluthrin, (B) transfluthrin, and (C) *d*-allethrin by topical application.

Table 3. LD_{50} based relative effective ratio of pyrethroids for several Asian strains of *Culex quinquefasciatus* and *Culex pipiens pallens*.

Chemicals	Relative effective ratio (*d*-Allethrin = 1.0)				
	Culex quinquefasciatus				*Culex pipiens pallens*
	Indonesia	Thailand	Vietnam	Malaysia	Gose
Metofluthrin	47.5	78.8	33.3	39.4	27.8
Transfluthrin	10.8	19.1	5.4	5.4	7.6
d-Allethrin	1.0	1.0	1.0	1.0	1.0

requires a well-trained technique and expensive apparatus which will be the most serious obstacles to carrying out such study in rural areas of tropical countries. More convenient and inexpensive methods, such as WHO test kits (Bruce-Chwatt, 1985), are recommendable in such areas. Topical application, however, seems to be the best way to get preliminary information, such as the transition in development of insecticide resistance or the relative efficacy ratio of insecticides (Suzuki, 1963; Kawada et al., 1993). The present results indicate that metofluthrin will be one of the most promising chemicals for controlling mosquitoes with its unique character-

istics and high activity. Kawada et al. (2004a) reported that a single multilayer strip of metofluthrin-impregnated paper caused high knockdown to the caged *Anopheles balabacensis* Baisas, and all insects knocked down in less than 30 min in the indoor conditions. The authors also observed that mosquitoes were affected by airborne metofluthrin vapor and not by direct contact to the chemical, resulting in the spatial repellency (Argueta et al., 2004; Kawada et al., 2004a, 2004b). The spatial repellency is thought to take place with two main modes of pyrethroid action, i.e., knockdown activity and biting inhibition or disruption of orientation to the host. Among them, the latter may be categorized as a sublethal effect that resulted in neural excitement, which probably took place in the earlier stage of treatment or with lower dosage than that required for knockdown or death (MacIver, 1964; Winney, 1975; Birley et al., 1987). Topical application, which is the best way for the evaluation of killing activity, may also be applicable to some extent for the evaluation of knockdown activity of pyrethroids. Topical application, however, seems not to be an adequate way for evaluation of such spatial repellency with the volatile chemicals, since such activity requires a balance of overall factors of chemicals, such as physico-chemical properties as well as biological activity. Another new method, therefore, will be required for further studies on the spatial repellency of pyrethroids.

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