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Laboratory Evaluation of the Lethal Efficacy of an Insect Growth Regulator, Pyriproxyfen against *Anopheles sinensis* (Diptera, Culicidae) Larvae

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ABSTRACT The granular formulation of 0.5% pyriproxyfen is a mosquito insect growth regulator larvicide. It was evaluated for residual activity for killing and/or emergence inhibition against 3rd stage larvae of *Anopheles sinensis* in the laboratory. Mortality rates ranged from 60.9% to 79.7% at 0.01, 0.05 and 0.1 mg/L of water during the first seven days. Except for the lowest concentration (0.01 mg/L) mortality rates were greater than 80% from day 8 through 28. Mortality rates for 0.01 mg/L concentrations were 70.4% from days 8-14, and thereafter exceeded 80% through day 28. The residual activity gradually increased post-treatment since the granular formulation of pyriproxyfen has a gradual solubility and increases concentration of active ingredient over time. The mortality rates of *An. sinensis* larvae and pupae eventually reached 100% for the three (0.01, 0.05, 0.1 mg/L) concentrations.

Key words : insect growth regulator, pyriproxyfen, residual activity, *Anopheles sinensis*

Introduction

In recent years, the populations of various mosquito species have increased due to warmer temperature and changing of environments in Republic of Korea (ROK) (Kim et al., 1995; Kim et al., 2004). In 1998, extremely high population densities of *Ochlerotatus dorsalis* (Meigen) and *Culex inatomii* (Kamimura et Wada) resulted in excessive biting activity among residents and domestic animals in some coastal areas, such as Ulsan in Southeast Korea and Yeosu in Southwest Korea (Jeong and Lee, 2003). *Anopheles sinensis* Wiedemann and *Culex tritaeniorhynchus* Giles, vectors of malaria and Japanese encephalitis, respectively, developed resistance to organophosphate insecticides resulting from excessive agriculture usage (Shim et al., 1995a, b). Mosquito control programs target mostly in ROK adults through thermal fogging and residual spaying. The effectiveness of these programs have not been evaluated. However, recent evidence suggests that they are not effective (M. Carder, personal communicating). Most public health authorities have came to realize that correct mosquito control strategies must be reexamined and altered if not effective. Therefore, pilot studies for mosquito larval control, in addition to adult control, has been instituted since 1998. However, public health authorities have used only mosquito larvicide formulation such as the microbial larvicide *Bacillus thuringiensis* var. *israelensis*, because it was readily available for purchase in the ROK (Lee and Yu, 1999; Lee, 2000).

Insect growth regulators (IGRs) affect hormonal regulation of immature mosquito growth and development and have little environmental impact on non-target organisms. The primary effect of IGRs is the reduction of adult emergence, but reproduction and ecdysteroid production in surviving females are also affected (Fournet et al., 1993, 1995). The presence of an IGR during the larval-pupal and pupal-adult molts results in the production of abnormal or inter-

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mediate forms that fail to molt normally and soon die (BSTID, 1973). In general, IGRs have high levels of activity and efficacy against various species of mosquitoes and can be applied in a variety of habitats (Mulla et al., 1989). Additionally, IGRs have shown a good margin of safety to personal appling them and to non-target biota, including fish and birds (Miura and Takahashi, 1973, 1975; Mulla et al., 1985). These attributes of IGRs increases the potential for them to be used more widely for mosquito control, in addition to the much used microbial larvicides, pyrethroids and organophosphorus larvicides (Mulla et al., 1989).

Pyriproxyfen is an IGR that affects the physiology of morphogenesis, reproduction and embryogenesis of selected insects (Kawada et al., 1988). This IGR exhibited a high level of activity against a number of nuisance and vector mosquitoes belonging to the genera of *Culex, Aedes, Psorophora* and *Anopheles* (Mulla et al., 1986, 1989; Kawada et al., 1988; Schaefer et al., 1988; Suzuki et al., 1989; Kerdpibule, 1989; Mulligan and Schaefer, 1990; Ishii et al., 1990; Kamimura and Arakawa, 1991). Kamimura and Arakawa (1991), and Lee (2001) reported that pyriproxyfen was extremely effective against *Cx. pipiens pallens, Cx. tritaeniorhynchus*, and *Ochlerotatus togoi* (Theobald) larvae that showed high level of resistance to organophosphate insecticides. However, none of these studies examined the efficacy of pyriproxyfen against *An. sinensis* in ROK. The purpose of the study herein describes the efficacy and residual longevity of pyriproxyfen against *An. sinensis* larvae in the laboratory.

Materials and Methods

A laboratory experiment was conducted in a glass aquarium ($1.2 \times 0.8 \times 0.5$ m) to evaluate the effectiveness of pyriproxyfen against *Anopheles sinensis* larvae. Ten liter of unchlorinated underground water were placed in each of four uncovered aquariums. Water was added daily to maintain a 10 L water level that was reduced through evaporation. Larvae were maintained at a photoperiod (14L : 10D) (fluorescent lighting) and temperature (26 ± 2°C) in a controlled insect rearing room. Three aquariums were treated with pyriproxyfen (Sumilarv®, 0.5% granular formulation; Sumitomo Chem. Co., Osaka, Japan). The fourth one (control) was not treated with pyriproxyfen. All four aquariums were aerated using a standard aquarium aerator during experimental period. Each of the three treated aquariums had concentrations of pyriproxyfen at 0.01, 0.05 or 0.1 ppm active ingredient (AI). Twenty-five laboratory-reared 3rd instar *An. sinensis* larvae were placed in each aquarium. After each seven day period, larvae and pupae were transferred into plastic bottles with 250 mL of the treated water using a pipette and another 25 larvae were added in the same treated water to evaluate residual effect through day 28.

The treated water from each aquarium were transferred into 500 mL beakers (in triplicates). The larvae and pupae from each treatment were placed separately in each beaker in their respective water using pipettes. Test organisms were placed in two incubators where conditions were maintained at 27°C in temperature, 65% R.H and 14 hour illumination a day. Larvae in each aquarium and each beaker were provided a diet of lab chow once daily. The number of dead larvae and/or pupae in each aquarium and each beaker were recorded daily. The data were corrected using Abbott's formula (Abbott, 1925) to calculate mortality rate and emergence rate. Also, LT_{50} and LT_{90} values were estimated for each three concentrations. The mortality rates were analyzed using the log-probit computer program (Finney, 1971). The data were analyzed using a SPSS PC (ver. 10.0) and a repeated measures analysis of variance (ANOVA). Differences among the means for each treatment were tested for significance using Duncan's multiple range test. All differences cited were significantly different at $p = 0.05$.

Results and Discussion

IGR pyriproxyfen exhibited high levels of biological activity against 3rd instar larvae of *Anopheles sinensis* in the laboratory. The results of bioassay testes with *An. sinensis* mosquitoes after treatment of pyriproxyfen is shown in Fig. 1. Mortality rates ranged from 60.9% to 79.7% at 0.01, 0.05 and 0.1 mg/L of water during the first seven days. Except for the lowest concentration (0.01 mg/L) mortality rates were greater than 80% from day 8 through 28. Mortality rates for 0.01 mg/L concentrations were 70.4% from days 8-14, and thereafter exceeded 80% through day 28. The residual activity increased over time after treatment because the slow-release pyriproxyfen formulation has a gradual solubility. Mortality rates of all groups did not reach 100% levels at the three dosages tested for the seven day period after each introduction of new larvae. However, all pupae and

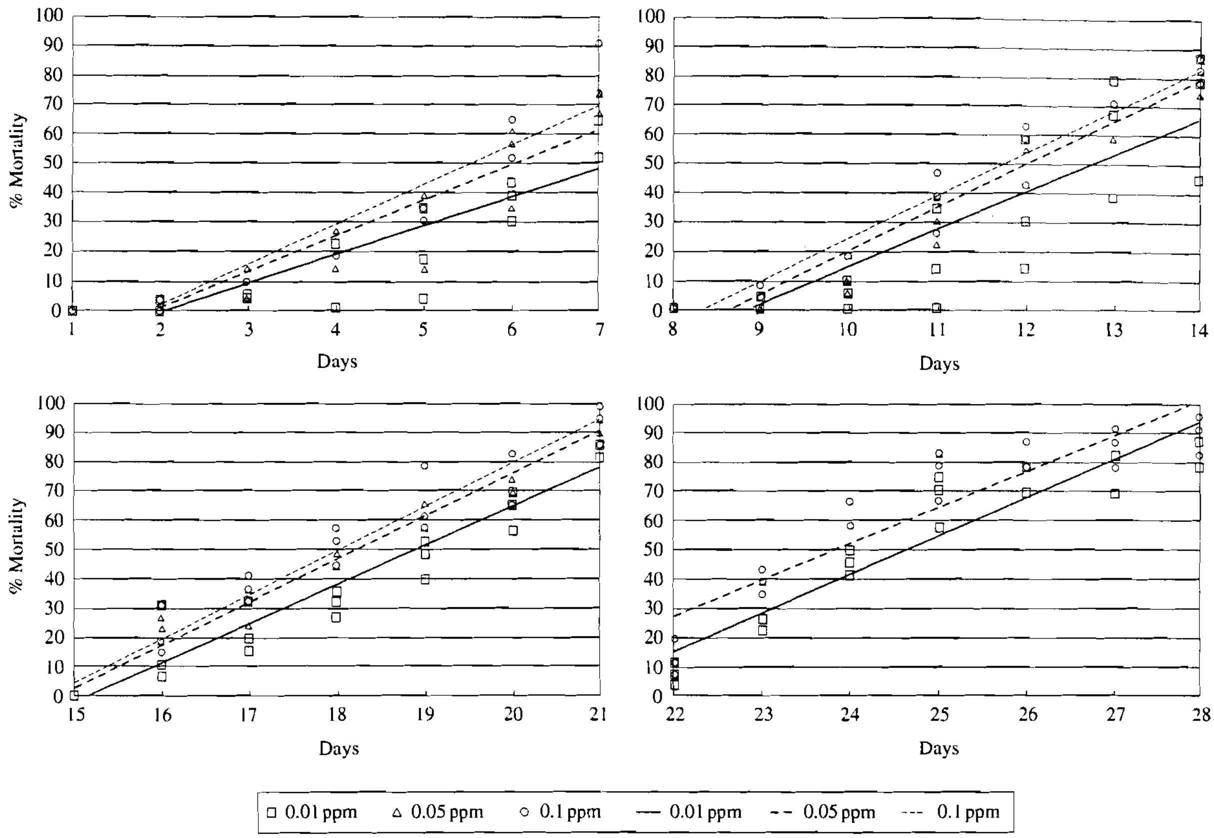

Fig. 1. Mortality effect of 0.5% pyriproxyfen granules against 3rd instars of *Anopheles sinensis* in an aquarium (1.2 ×0.8×0.5 m), three replicates (symbols □△○ = actual morality rates; regression lines based on log-probit analysis adjusted for control mortality) (two lines of 0.05 and 0.1 ppm are same from 22 to 28 days).

Table 1. Mortality effects (LT_{50}, LT_{90} and LT_{99})[1] of 0.5% pyriproxyfen granules against 25, 3rd instars of *Anopheles sinensis* in an aquarium (1.2×8×0.5 m), three replicates at various concentrations

Concentration (mg/L)	Days after introduct.	Slope±SE	LT_{50} (95% CL)	LT_{90} (95% CL)	LT_{99} (95% CL)
0.01	0−7	0.49±0.02	6.55 (6.19−7.04)a[2]	9.18 (8.44−10.29)a	11.33 (10.23−13.00)a
	8−14	0.57±0.25	5.77 (5.33−6.33)ab	8.01 (7.25−9.35)ab	9.85 (8.70−11.93)a
	15−21	0.44±0.18	4.95 (4.61−5.33)b	7.88 (7.24−8.79)ab	10.26 (9.26−11.72)a
	22−28	0.38±0.17	3.62 (3.26−3.97)c	6.96 (6.39−7.75)b	9.68 (8.72−11.04)a
0.05	0−7	0.51±0.23	5.92 (5.64−6.24)a	8.43 (7.89−9.16)a	10.48 (9.67−11.60)a
	8−14	0.57±0.22	5.16 (4.94−5.39)b	7.40 (7.02−7.88)b	9.22 (8.65− 9.98)a
	15−21	0.46±0.18	4.27 (4.00−4.54)c	7.03 (6.56−7.64)bc	9.28 (8.54−10.28)a
	22−28	0.38±0.17	2.77 (2.26−3.18)d	6.11 (5.52−6.96)c	8.83 (7.81−10.40)a
0.1	0−7	0.57±0.02	5.59 (5.41−5.78)a	7.82 (7.49−8.22)a	9.63 (9.13−10.26)a
	8−14	0.51±0.20	4.87 (4.67−5.07)b	7.40 (7.04−7.83)ab	9.46 (8.91−10.15)a
	15−21	0.48±0.19	4.05 (3.73−4.37)c	6.70 (6.20−7.39)bc	8.86 (8.06−10.00)a
	22−28	0.38±0.17	2.71 (2.18−3.14)d	6.11 (5.51−7.00)c	8.89 (7.83−10.55)a

[1] LT = Lethal Time (day). [2] Mean mortalities in the same columns followed by the same letter in each concentration are not significantly different ($p<0.05$; Duncan's multiple range test).

larvae that did not develop into pupae, were killed in the beaker treated with pyriproxyfen at each concentration within 12 days after introduction of larvae (not shows in Fig. 1). Results demonstrate residual activity at 100% mortality against *An. sinensis* larvae in treated water with pyriproxyfen until at least the 4th week after treatment. Mortality occurred in the pupal and larval stages at all concentrations tested (0.01-0.1 mg/L). The higher concentrations overall had more rapid mortality.

Pyriproxyfen was effective against *An. sinensis* larvae, with LT_{90} (90% lethal time) values of 7.0-9.2 days at 0.01 mg/L, 6.1-8.4 days at 0.05 mg/L, and 6.1-7.8 days at 0.05 mg/L in water from 7 days to 28 days after treatment (Table 1). Except for the treatment group of 0.01 mg/L, the values of LT_{50} (median lethal time) for all treatments were significantly different among the four 7-day periods at each concentration (i.e. no overlap in 95% confidence interval). The LT_{50} values at the concentration of 0.01 mg/L were 3.6 to 6.6 days for the 4th week and the 1st week treatment groups, respectively. The slopes of the concentration-mortality curves in tests with pyriproxyfen were 0.38 to 0.57 at 0.01 mg/L for the 4th and 2nd weeks after treatment, respectively. The slopes were 0.38 at both 0.05 and 0.1 mg/L at the 4th week, and the slopes were 0.57 at 0.05 mg/L at the 2nd week and at 0.1 mg/L at the 1st week after treatment. In general, the treatment groups exposed in later assays produced lower slopes than those in the earlier periods.

The foregoing results are similar to these reported by Kamimura and Arakawa (1991) in that complete inhibition of emergence of *Culex pipiens pallens* at 0.1 ppm for 40 days after treatment in polyethylene containers. Ree et al. (1975) and Mathis et al. (1975) reported that one of the juvenile hormone mimics, Altosid (OMS-1804) was effective against larvae and pupae of *Cx. pipiens pallens* with more than 70% mortality for up to 10 days at a dosage rate of 200 gm active ingredient per hectare (0.9 ppm) in parsley fields. Also, Lee (2001) reported that the emergence inhibition rates for *Ochlerotatus togoi* were 100.0% at concentrations of 0.01 and 0.05 ppm of the granular formulation of 0.5% pyriproxyfen through 34 and 48 days after treatment in fresh water in aquariums, respectively. In this study, the residual activity of 0.5% pyriproxyfen granules strongly inhibited emergence of *An. sinensis* larvae in the laboratory. Pyriproxyfen granules may be of benefit to control mosquitoes in flood water environments because concentrations increase over time as indicated by Kamimura and Arakawa (1991). Also, pyriproxyfen formulation demonstrated slow or controlled release of active ingredient significantly increases its persistence at low lethal concentrations required for the control of asynchronous larvae (Mulla, 1995).

In conclusion, 0.5% pyriproxyfen granules provided the greatest initial and residual activities against *An. sinensis* larvae and effectively killed larvae for at least 28 days at concentrations of 0.01-0.1 mg/L. There is sufficient evidence to support that pyriproxyfen may offer excellent potential for control of *An. sinensis*, the principal vector of malaria in Korea, with long residual activity in mosquito breeding sites in fresh water, although it is necessary to carry out further study on their mortality and emergence inhibition rates of *An. sinensis* in field. This preliminary study has identified a promising insect growth regulator such as pyriproxyfen that may be considered in the development of an integrated approach to population management of various species of mosquitoes including *An. sinensis*.

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