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Mosquito Control Evaluations of an Insect Growth Regulator, Pyriproxyfen against *Culex pipiens pallens* (Diptera, Culicidae) Larvae in Marsh Area, Korea

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ABSTRACT **Mosquito control evaluations of the granular formulation of 0.5% pyriproxyfen were undertaken in marshes and a pond in Busan during the period of July through September, 2001. In marsh condition where *Culex pipiens pallens* were predominantly breeding, the treatment of pyriproxyfen produced over an average of 95% mosquito larval reduction at a concentration of 0.05 mg/l for the first 4 weeks in spite of heavy precipitation (total 274.4 mm) during the period. Reduction rates of 72.6% and 32.3% appeared in the 5th and 7th weeks probably due to heavy input of sewage and flood. However, the reduction rate increased again at the 8th week, showing a 94.5% reduction, probably because of the slow releasing of pyriproxyfen granular formulation. In the second treatment of pyriproxyfen, complete mortality was obtained at the 1st and 3rd weeks. Also, a satisfactory level of *Cx. pipiens* larval reduction of 96.6% was observed at the 4th week. In a pond assessment, the treatment of pyriproxyfen at a concentration of 0.05 mg/l produced a complete reduction of *Cx. pipiens pallens* from the 1st week through the 4th week after treatment. In the second treatment, 100.0% reduction was obtained at the 1st and 2nd weeks in the same sites. The treatment resulted in 84.4% and 97.9% reduction of mosquitoes at the 3rd and 4th weeks, respectively.**

Key words : Mosquito Control, Insect Growth Regulator, Pyriproxyfen, *Culex pipiens pallens*, Marsh, Pond

Culex pipiens pallens Coquillett is recognized as the dominant pest mosquito in the urban areas in South Korea. The main larval breeding sites are water pools, sewer system with sewage water, marshes, and ponds. The primary reason for this distinction of being one of main pests is that this mosquito feeds during all seasons in urban area in South Korea (Lee and Lee, 1992), whereas, other species found in the area are not active during winter season. Control measures against *Cx. pipiens pallens* as well as other species in South Korea are primarily with adulticides. *Anopheles sinensis* Wiedemann and *Cx. tritaeniorhynchus* Giles, vectors of malaria and Japanese encephalitis, respectively, became resistant to organophosphorus insecticides by repeated applications (Shim et al., 1995a, b). Since the mosquitoes in South Korea are currently being controlled using only adulticides through thermal fogging and/or residual spray, effective and high level of control is not achievable.

Insect growth regulators (IGRs) affect hormonal control of mosquito growth and development. The main effect of IGRs is the inhibition of adult emergence, but reproduction and ecdysteroid production in surviving females are also affected (Fournet et al., 1993, 1995). In general, IGRs have high levels of activity and efficacy against various species of mosquitoes in a variety of habitats (Mulla et al., 1989; Lee, 2001). Additionally, it has been known that they have shown a good margin of safety to non-target biota including fish and birds. Residue and non-target studies indicated that IGRs have no prolonged residues and are an environmentally safe compound with minimal impact on non-target organisms although they are not much safe to some aquatic insects (Miura and Takahashi, 1973, 1975; Mulla et al., 1985, 1986). On the basis of these attributes, IGRs are likely to provide additional tools for mosquito control, supplementing microbial larvicides, pyrethroids and organophosphorus larvicides (Mulla et al., 1989).

Kamimura and Arakawa (1991) reported that pyriproxyfen was extremely effective against larvae of *Cx. pipiens pallens* and *Cx. tritaeniorhynchus* which showed high resistance to organophosphorus insecticides. Complete inhibition of adult emergence continued for 3 weeks or more in open containers and irrigation ditches at a concentration of

0.01 ppm, in cesspools at 0.05 ppm and in sewers with inflow of house wastewater at 0.1 ppm (AI). The present study was conducted to evaluate pyriproxyfen against *Cx. pipiens pallens* in water of marshes and a pond mixed with sewage in South Korea.

MATERIALS AND METHODS

Pyriproxyfen (Sumilarv®, 0.5% granular formulation; Sumitomo Chem. Co., Osaka, Japan) was evaluated against *Cx. pipiens pallens* larvae in water mixed with sewage and aquatic weeds in 3 submerged marshes (avg. 14.3 m × 0.8 m by 0.2 m deep) and a pond (3.2 m × 2.0 m by 1.0 m deep) in a small park beside Oncheon-cheon stream near a residential area in Busan city from July through September, 2001 (Table 1). At these sites, water was relatively stable during the study except for a flood in the middle of August.

The granular formulation was evenly distributed over each site by hand on July 3 and August 29, 2001. The formulation was tested at a treatment rate (0.05 mg/l A.I.) at the sites. Mosquito samples were taken per site prior to treatment and once every week after treatment. For three months, mosquito pupae and 4th instar larvae in the field water were collected from each site using a 355 ml dipper at each evaluation time. Water samples, containing the larvae and pupae from treated waters, were placed in plastic bottles using a dipper and transported back to a laboratory in styrofoam chests. To reduce mosquito activity and minimize physical damage and mortality during transportation, the chest was chilled with two small ice packs. In the laboratory, 150 ml of water from each treated site were transferred into 250 ml beakers (in duplicates). The wild larvae and pupae from each treatment were placed separately in beakers filled with sampled water using pipettes. Test organisms were placed in an incubator where temperatures were maintained at 27°C. Mortality readings were taken daily and percent inhibition of adult emergence as percent mortality was determined. Air temperatures and precipitation were checked weekly using the data from the Central Meteorological Office during the test period from June 27 through September 26, 2001 to analyze test results. A degree of correlation was observed using a correlation coefficient (r) to determine whether the air temperature and the precipitation affected the activity of pyriproxyfen against mosquitoes in the study periods (Ott, 1984). Percentage mortalities were transformed to arcsine, and subjected to correlation coefficients.

Table 1. Characteristics of pyriproxyfen treatment of three marshes and a pond with sewage, aquatic weeds and naturally bred *Culex pipiens pallens* larvae and pupae, 2001

Site	Measurement (m)			Water volume (t)
	Length	Width	Water depth	
Marsh A	17.5	0.7	0.2	2.45
Marsh B	11.0	1.1	0.2	2.42
Marsh C	14.5	0.7	0.2	2.03
Pond	3.2	2.0	1.0	6.40

RESULTS AND DISCUSSION

Adult inhibition after collection of pupae from pre-treatment samples was not observed in the marshes and a pond where *Culex pipiens pallens* were naturally breeding. In the marshes, an average of 76.9 mosquito pupae and 4th instar larvae in the field water were collected from each site using a 355 ml dipper at each evaluation time for three months (Table 2). The treatment of pyriproxyfen produced over an average of 94.5% mosquito larval reduction at a concentration of 0.05 mg/l for the first 4 weeks in spite of heavy precipitation (total 274.4 mm) during the period (Table 2; Fig. 1). Comparatively low reduction rates of 72.7%, 70.3% and 78.6% appeared at marsh A, B and C in the 5th week (August 9) after treatment, respectively, probably due to heavy input of sewage around the 5th week. Although the main reasons for the lower mortalities in the three marshes in the 7th week (August 22) are not apparent, it is considered to be due to the flood in the 6th week (August 16) after treatment. The reduction rate increased again in the 8th week (August 29), showing a 94.5% reduction probably because of the slow release of pyriproxyfen granular formulation. In the second treatment of pyriproxyfen at the same marshes and concentration on August 29, complete mortalities were obtained at the first and third weeks after treatment (Table 2; Fig. 1). Also, a satisfactory level of *Cx. pipiens pallens* larval reduction was observed at 96.6% at the 4th week.

Table 2. Number of mosquitoes collected and emerged of *Culex pipiens pallens* pupae in three marshes and a pond with controlled release formulation of pyriproxyfen (0.5% granules) applied at the rate of 0.05 mg/l

After treatment		Marsh A		Marsh B		Marsh C		Pond	
Date	Week	Collec.	Emerg.	Collec.	Emerg.	Collec.	Emerg.	Collec.	Emerg.
Jul. 3	Pre-treat	52	52	50	50	86	86	63	63
Jul 10	1	43	2	57	2	45	3	64	0
Jul. 17	2	47	2	61	0	46	2	75	0
Jul. 24	3	131	4	155	8	154	12	146	0
Jul. 31	4	52	0	45	0	38	0	47	0
Aug. 9	5	44	12	37	11	14	3	30	4
Aug. 16[1)]	6	–	–	–	–	–	–	–	–
Aug. 22	7	88	52	50	32	60	50	100	2
Aug. 29	8	57	4	65	3	98	5	50	1
Sep. 5	1[2)]	100	0	100	0	100	0	22	0
Sep. 11	2	100	2	100	0	100	0	35	0
Sep. 18	3	100	0	100	0	100	0	45	7
Sep. 26	4	97	7	96	1	100	2	48	1

[1)] Marshes had been flooded around 16th August.
[2)] One week after re-application of pyriproxyfen at 29th September.

In the pond, the results are given as a number of emerged mosquitoes and average % mortality every week after treatment in Table 2 and Figure 1, respectively. An average of 60.4 mosquito pupae and 4th instar larvae were collected from the pond once a week during the test period (Table 2). Adult emergence in larval and pupal isolations were completely inhibited for four weeks after treatment of pyriproxyfen at a concentration of 0.05 mg/l. Excellent control (>98.0%) was achieved at the concentration tested against larvae and pupae up to 7 weeks post-treatment except in the 5th week (86.7%) most likely due to heavy input of sewage. The mosquito breeding sites held sewage mixed with rain water and were exposed to polluted water with heavy organic materials during the test period. Nevertheless, residual control remained and even increased from 86.7% in the 5th week to 98.0% in the 7th week against larvae in spite of a flood in the 6th week after treatment.

In the second treatment of pyripoxyfen on August 29 at the same concentration rate as the 1st treatment, complete reduction was obtained at the 1st and 2nd weeks after treatment (Fig. 1). The recorded results showed a continuously high mortality rate of over 97.9% for four weeks after treatment probably due to a residual control effect, although a comparatively lower mortality rate (84.4%) appeared in the 3rd week. Therefore, the residual activity of 0.5% pyriproxyfen granules inhibits the emergence from pupae to adults of *Cx. pipiens pallens* in sewage water for at least four weeks. It is suggested that the concentration for a long term effective control of *Cx. pipiens pallens* in sewage water is 0.05 mg/l (A.I.) of 0.5% pyriproxyfen granules.

The average air temperature was 25.4°C (21.3-26.4°C) during the test period from June 27 through September 26, 2001. It rained for a total of 30 days out of 92 days, and the total precipitation was 468.4 mm in the test period (Fig. 1). It is obvious that precipitation should not be important for mosquito control using pyriproxyfen during the summer months. The mosquito control of *Cx. pipiens pallens* using pyriproxyfen was hardly correlated with weekly precipitation ($r = 0.13$) for the whole mosquito season (July, August and September). On the other hand, the mosquito control rates were slightly correlated with the average weekly air temperatures ($r = 0.45$) during the three summer months. It might be implicated that Sumilarv® 0.5% is a granular formulation. The granular formulation can be slowly released in water, which continuously affects mosquito larvae.

In the treatments of the marshes and the pond, the following observations were made concerning the period of activity, although the mortality of larvae was confirmed with other previous studies such as Mulligan and Schaefer, 1990; Kamimura and Arakawa, 1991; and Lee, 2001. The formulation caused a high mortality during the pupal stage. Emergence inhibition rates were higher in pupal isolates than in larval isolates. Therefore, it is suggested that the actual mortality in the test sites might be higher than the results obtained in the larval isolates and pupal isolates. The effect

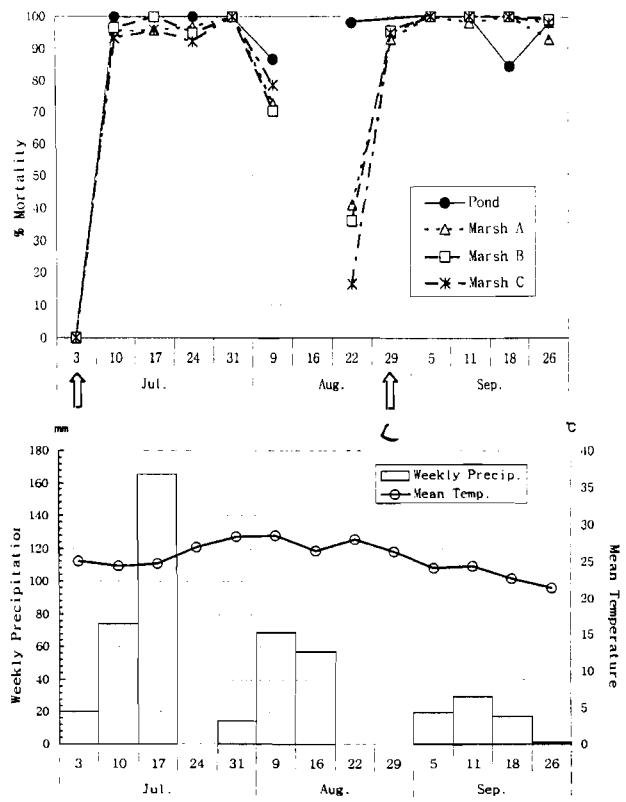

Fig. 1. Weekly mean temperatures (°C), total weekly precipitation (mm) and average percent mortalities of *Culex pipiens pallens* pupae in a pond and three marshes in Busan with controlled release formulation of pyriproxyfen applied (↑) at a concentration of 0.05 mg/l from July through September, 2001.

decreased somewhat when the sites were flooded by the Oncheoncheon stream due to heavy rain (Fig. 1). However, pyriproxyfen granules may be of benefit in controlling mosquitoes in flood water situations because the concentration of this material has been predicted to be higher in the bottom water than the upper water for a long period of time when they were applied as indicated by Kamimura and Arakawa (1991) and Lee (2001). Syafruddin et al. (1990) reported that granules of this IGR sink to the bottom, and the effective component was slowly released; thus, the effective component remained in the substratum for a substantial time, being slowly released in the water.

In conclusion, 0.5% pyriproxyfen granules (Sumilarv®) provided the greatest initial and residual activities against *Cx. pipiens pallens* larvae in marshes and ponds with sewage water. The pyriproxyfen effectively inhibited adult emergence of *Cx. pipiens pallens* for at least 2 months at a concentration of 0.05 mg/l. There is sufficient evidence to support that pyriproxyfen may offer excellent potential for *Cx. pipiens pallens* control with long residual activity in mosquito breeding sites even in sewage water.

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