



EFFICACY OF IGRS COMPOUND TRIFLUMURON AND METHOPRENE AGAINST *Culex quinquefasciatus* MOSQUITO LARVAE AND PUPAL CONTROL IN POOLS, DRAINS AND TANKS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author PR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MM managed the analyses of the study. Author MS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Triflumuron and Methoprene, insect growth regulators (IGRs) were highly effective against *Cx. quinquefasciatus* (Say) larvae and pupae in pools, drains and tanks at the dosages of 0.04 and 0.4 mg/l. To determine the most appropriate field study assessment was made by sampling mosquito larvae and pupae to determine the trends of immature population, weekly after treatments and before treatments.

Keywords: Triflumuron; methoprene; *Cx. Quinquefasciatus*.

1. INTRODUCTION

Lymphatic filariasis is a major vector borne disease making about 120 million people in 83 countries physically disabled [1] and is transmitted by *Cx. quinquefasciatus* (Say) mosquito having cosmopolitan distribution. Designing innovative vector control tools is of paramount importance due to the development of insecticide resistance among disease vectors [2]. Chemical control is an effective

strategy that has been used extensively in vector control for decades. However, the evolution of insecticide resistance among mosquitoes to insecticides has increased in the last two decades [3].

Culex quinquefasciatus (Say) serves as a vector for filariasis and arboviruses [4]. Human filariasis is a major public health problem and remains a challenging problem socioeconomically in most

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tropical countries [5]. Insect growth regulators have shown significant larvicidal efficacy against *Aedes albopictus* mosquito at low doses as compared to microbial, organophosphates and synthetic pyrethroid insecticides [6]. Some studies have disrupted hormonal balance inside the developing embryo [7]. Su and Mulla reported the ovicidal activity of the neem products such as azadirachtin against *Cx. Quinquefasciatus* [8].

Insect growth regulators are comparatively safer to non-target organisms and have been recommended for mosquito control [9,10]. Insect growth regulators (IGR) include chemicals with a unique mode of actions such as juvenile hormone analog, chitin synthesis inhibitor, ecdysone agonist [11,12]. These IGRs have extended effects to the morphology and physiology of mosquito eggs [13,14]. The surface morphology, physical structure and chemical composition of the eggs determine the ability of eggs to adapt and tolerate adverse conditions such as desiccation [15]. Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution and low mammalian toxicity [16]. Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides [17].

The aim of this study was to evaluate the Efficacy of IGRs compound Triflumuron and Methoprene against *Culex quinquefasciatus* mosquito larvae and Pupal control in pools, drains and tanks.

2. MATERIALS AND METHODS

The two IGR compounds Triflumuron 1-(2-chlorobenzoyl)-3-(4-trifluoromethoxyphenyl) urea was received gratis as 10% EC formulation Zhejiang Rayfull Chemicals Co. Ltd, People Republic of China. Methoprene 15% EC chemically known as 1-methylethyl (*E,E*)-11- methoxy-3,7,11-trimethyl- 2,4-dodecadienoate, supplied by Heer Pharma Private Limited, Mumbai, India. The Triflumuron and Methoprene were applied at 0.04 and 0.4 mg/l, in pools, drains and cemented tanks. Application doses were achieved by spraying of pre-calculated amount of Triflumuron and Methoprene with the help of a hand compression sprayer. Prior to spraying, density of immature was estimated by dipper sampling method using a standard dipper of 9cm diameter with 300ml water capacity. Density per dip (larvae and pupae) was monitored in control and treated habitats daily at 24h intervals up to three days and later at weekly intervals. Daily samplings of late instars and

pupae were collected, taken to the laboratory and the percentage of adult emerged from the treated field habitat was recorded in laboratory. The data obtained at different days of observation were pooled to get the weekly means and the data collected in different periods and replicates were pooled together. The per cent reductions in larval and pupal density were calculated Mulla et al. [18] formula given below.

$$\% \text{ Reduction} = 100 - \{(C_1 \times T_2) / (C_2 \times T_1)\} \times 100$$

Where:

C_1 = Pre-treatment immature density in control sites

C_2 = Pre-treatment immature density in control sites

T_1 = Pre-treatment immature density in treated sites

T_2 = Pre-treatment immature density in treated sites

3. RESULTS

The efficacy of Triflumuron and Methoprene against *Cx. quinquefasciatus* was evaluated in waste water drains, pools and tanks in Karumandapam, Tiruchirappalli. The Triflumuron and Methoprene was applied at 0.04 and 0.4 mg/l and the control site was left untreated for comparison *Cx. quinquefasciatus* was the predominant Culicine species found in these habitat.

3.1 Triflumuron

The efficacy of Triflumuron on adult emergence (EI) of *Culex spp* under field conditions was evaluated at 0.05 and 0.5 mg/l in three different breeding habitats (Table 1). In drains, the density of late dedined trend from day three at both dosages. At 0.04 mg/l the reduction of late instar ranged between 92%, 94% and 98% upto sixth week and then declined to 69.28% in seventh week. The pupal reduction was observed in 86% in sixth week. At the dose of 0.4mg/l, 100% pupal reduction remained upto the fifth week. In pools, the reduction of late instar ranged from 47.05 to 85% upto the seventh week at both dosages. In tanks the effect of Triflumuron was slightly better than in the pools, and 100% reduction of late instars was observed after three weeks at 0.04 mg/l and 0.4 mg/l. However, reduction in pupal density was high at both dosages and reached upto 85% between third days to sixth week in the different habitats.

Table 1. Field evaluation of Triflumuron against immature of *Cx. Quinquifasciatus*

Duration after treatment	Mean \pm SD and percentage reduction of <i>Cx. quinquifasciatus</i> immature per 10 dips											
	Drains				Pools				Tanks			
	0.04 mg/l		0.4 mg/l		0.04 mg/l		0.4 mg/l		0.04 mg/l		0.4 mg/l	
	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P
Control	57.12 \pm 5.30	2.65 \pm 0.25	63.24 \pm 5.87	3.06 \pm 0.28	17.34 \pm 1.64	4.59 \pm 0.43	11.22 \pm 1.04	3.06 \pm 0.28	68.34 \pm 6.34	3.57 \pm 0.33	24.48 \pm 2.27	7.14 \pm 0.66
3 Days	15.30 \pm 1.42 (41.82)	2.00 \pm 0.02 (42.00)	31.82 \pm 2.95 (49.68)	2.04 \pm 0.19 (33.33)	9.18 \pm 0.85 (47.05)	2.55 \pm 0.24 (44.44)	4.08 \pm 0.38 (63.63)	0.51 \pm 0.05 (83.33)	66.80 \pm 8.52 (34.32)	2.14 \pm 0.20 (80.05)	13.26 \pm 1.23 (45.83)	3.06 \pm 0.28 (57.14)
1 Week	13.26 \pm 1.23 (92.78)	1.5 \pm 0.01 (82.00)	15.50 \pm 1.44 (75.49)	1.84 \pm 0.26 (74.20)	6.12 \pm 0.57 (94.70)	2.04 \pm 0.19 (55.55)	3.06 \pm 0.28 (72.72)	0.31 \pm 0.03 (89.86)	11.24 \pm 5.87 (98.46)	1.12 \pm 0.10 (78.62)	11.22 \pm 1.04 (54.16)	2.14 \pm 0.20 (70.02)
2 Weeks	11.22 \pm 1.04 (80.35)	1.0 \pm 0.05 (78.24)	12.04 \pm 1.12 (80.96)	1.54 \pm 0.03 (81.00)	5.10 \pm 0.47 (70.58)	1.53 \pm 0.14 (66.66)	3.0 \pm 0.28 (72.54)	0.25 \pm 0.03 (88.85)	45.53 \pm 3.45 (57.60)	1.00 \pm 0.10 (98.00)	9.00 \pm 0.50 (90.40)	1.90 \pm 0.20 (87.04)
3 Weeks	9.18 \pm 0.85 (83.92)	0.68 \pm 0.00 (81.00)	7.14 \pm 0.66 (88.70)	1.02 \pm 0.09 (66.66)	4.10 \pm 0.47 (76.35)	1.46 \pm 0.14 (66.01)	2.04 \pm 0.19 (81.81)	0.20 \pm 0.02 (93.46)	25.50 \pm 2.37 (62.68)	1.02 \pm 0.09 (81.42)	7.14 \pm 0.66 (80.83)	1.73 \pm 0.16 (85.70)
4 weeks	7.14 \pm 0.66 (87.50)	0.31 \pm 0.03 (88.30)	5.10 \pm 0.47 (91.93)	0.51 \pm 0.05 (83.33)	3.06 \pm 0.28 (82.35)	0.71 \pm 0.07 (84.53)	1.33 \pm 0.09 (90.90)	0.10 \pm 0.01 (96.73)	13.26 \pm 1.23 (80.59)	0.61 \pm 0.06 (82.91)	5.10 \pm 0.47 (79.16)	1.22 \pm 0.11 (82.91)
5 Weeks	4.08 \pm 0.38 (92.85)	0.12 \pm 0.01 (95.47)	3.06 \pm 0.28 (95.16)	0.00 \pm 0.0 (100.00)	2.24 \pm 0.21 (87.08)	0.41 \pm 0.04 (91.06)	1.03 \pm 0.12 (88.14)	0.00 \pm 0.00 (100.00)	10.20 \pm 0.95 (85.07)	0.31 \pm 0.03 (91.31)	3.06 \pm 0.28 (87.50)	0.92 \pm 0.08 (87.39)
6 Weeks	5.10 \pm 0.47 (91.07)	0.10 \pm 0.03 (86.79)	2.04 \pm 0.19 (96.77)	0.10 \pm 0.01 (96.73)	1.12 \pm 0.10 (94.54)	0.32 \pm 0.03 (93.02)	1.00 \pm 0.09 (90.73)	0.00 \pm 0.00 (100.00)	7.14 \pm 0.66 (89.55)	0.10 \pm 0.01 (97.19)	1.02 \pm 0.09 (95.83)	0.51 \pm 0.05 (92.85)
7 weeks	3.12 \pm 0.57 (69.28)	0.08 \pm 0.01 (95.84)	1.06 \pm 0.28 (93.58)	0.06 \pm 0.01 (96.40)	0.86 \pm 0.20 (87.65)	0.20 \pm 0.02 (95.64)	0.84 \pm 0.19 (81.81)	0.06 \pm 0.00 (100.00)	5.18 \pm 0.85 (86.56)	0.05 \pm 0.01 (96.63)	0.52 \pm 0.28 (83.41)	0.22 \pm 0.08 (88.51)

A value within the parenthesis indicates per cent reduction on control; III + IV – Larvae; P- Pupae

Values are expressed on Mean \pm SD

Table 2. Field evaluation of Methoprene against immatures of *Cx. Quinquifasciatus*

Duration after treatment	Mean \pm SD and Percentage reduction of <i>Cx. quinquifasciatus</i> immature per 10 dips											
	Drains				Pools				Tanks			
	0.04 mg/l		0.4 mg/l		0.04 mg/l		0.4 mg/l		0.04 mg/l		0.4 mg/l	
	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P
Control	60.18 \pm 5.58	3.57 \pm 0.33	65.28 \pm 6.06	5.71 \pm 0.53	19.38 \pm 1.80	6.63 \pm 0.62	13.26 \pm 1.23	5.10 \pm 0.47	59.73 \pm 6.53	4.73 \pm 0.44	27.54 \pm 2.56	6.12 \pm 0.57
3 Days	17.34 \pm 1.61 (70.18)	2.56 \pm 0.52 (68.00)	33.66 \pm 3.12 (48.43)	2.04 \pm 0.19 (64.27)	11.22 \pm 1.04 (42.10)	4.65 \pm 0.43 (29.86)	6.12 \pm 0.57 (53.84)	2.04 \pm 0.19 (60.00)	56.30 \pm 6.15 (10.99)	3.57 \pm 0.33 (24.52)	15.30 \pm 1.42 (44.44)	5.10 \pm 0.47 (16.66)
1 Week	14.28 \pm 1.33 (76.27)	1.78 \pm 0.05 (75.00)	15.50 \pm 1.44 (76.25)	1.84 \pm 0.64 (72.40)	8.16 \pm 0.76 (57.9)	4.08 \pm 0.38 (38.46)	5.10 \pm 0.47 (61.53)	1.38 \pm 0.13 (72.94)	24.26 \pm 5.96 (95.40)	1.91 \pm 0.18 (59.61)	13.26 \pm 1.23 (51.85)	3.71 \pm 0.34 (99.37)
2 Weeks	13.26 \pm 1.23 (77.96)	1.24 \pm 0.24 (84.00)	13.26 \pm 1.23 (79.68)	1.34 \pm 0.24 (76.50)	7.14 \pm 0.66 (63.15)	3.06 \pm 0.28 (53.84)	4.08 \pm 0.38 (69.23)	0.92 \pm 0.09 (81.96)	22.84 \pm 3.98 (98.27)	1.71 \pm 0.16 (63.84)	11.22 \pm 1.04 (59.25)	2.55 \pm 0.24 (98.33)
3 Weeks	10.20 \pm 0.95 (83.05)	0.71 \pm 0.07 (80.11)	9.18 \pm 0.85 (85.93)	1.02 \pm 0.09 (82.13)	6.14 \pm 0.66 (68.31)	2.28 \pm 0.40 (35.44)	3.63 \pm 0.34 (72.62)	0.56 \pm 0.05 (89.01)	19.54 \pm 2.56 (93.89)	1.62 \pm 0.19 (56.87)	9.18 \pm 0.85 (66.66)	2.50 \pm 0.28 (90.00)
4 weeks	8.16 \pm 0.76 (86.44)	0.51 \pm 0.05 (85.71)	7.14 \pm 0.66 (89.02)	0.70 \pm 0.007 (85.00)	5.10 \pm 0.47 (73.68)	1.63 \pm 0.15 (75.41)	2.68 \pm 0.43 (64.70)	0.50 \pm 0.06 (88.03)	17.67 \pm 3.54 (94.64)	1.00 \pm 0.00 (100.00)	6.00 \pm 2.00 (96.00)	2.00 \pm 0.20 (92.00)
5 Weeks	6.12 \pm 0.57 (89.83)	0.20 \pm 0.02 (94.39)	5.06 \pm 0.28 (95.31)	0.51 \pm 0.05 (91.06)	3.52 \pm 0.33 (81.83)	0.61 \pm 0.06 (90.79)	2.0 \pm 0.38 (69.23)	0.45 \pm 0.19 (60.00)	12.24 \pm 1.14 (96.50)	0.53 \pm 0.05 (88.79)	5.10 \pm 0.47 (81.48)	1.90 \pm 0.19 (96.66)
6 Weeks	5.12 \pm 0.57 (91.49)	0.31 \pm 0.03 (91.31)	4.08 \pm 0.38 (93.75)	0.38 \pm 0.04 (93.33)	1.73 \pm 0.16 (91.07)	0.57 \pm 0.07 (88.38)	1.90 \pm 0.28 (76.92)	0.30 \pm 0.14 (70.00)	9.18 \pm 0.85 (97.63)	0.50 \pm 0.09 (78.43)	2.04 \pm 0.19 (92.59)	1.53 \pm 0.14 (95.00)
7 weeks	4.12 \pm 0.57 (93.15)	0.10 \pm 0.01 (97.19)	3.10 \pm 0.47 (92.18)	0.36 \pm 0.03 (93.69)	1.32 \pm 0.31 (82.86)	0.51 \pm 0.05 (92.30)	1.00 \pm 0.38 (69.23)	0.20 \pm 0.09 (80.00)	8.22 \pm 1.04 (95.21)	0.20 \pm 0.19 (56.87)	1.10 \pm 0.47 (81.48)	1.02 \pm 0.09 (93.33)

A value within the parenthesis indicates per cent reduction on control; III + IV – Larvae; P- Pupae

Values are expressed on Mean \pm SD

3.2 Methoprene

The efficacy of Methoprene on adult emergence (EI) of *Culex spp* under field conditions was evaluated at two doses at 0.04 and 0.4 mg/l in three different breeding habitats (Table 2). In drains, the density of late instar declined from day three at both dosages. At 0.04 mg/l the reduction of late instar ranged between 70%, 82% and 90% upto sixth week and then declined to 53.15% in the seventh week. The pupal reduction was 94% in the sixth week. At the dose of 0.4mg/l, 100% pupal reduction remained upto the fifth week. In pools, the reduction of late instar ranged from 42.10 to 96% upto the seventh week at both dosages. In tanks the effect of IGR was slightly better than pools, when 100% reduction of late instars was observed after three weeks at 0.04 mg/l and 0.4 mg/l. However, reduction in pupal density was high at both the doses and reached upto 90% from day 3 till the sixth week in different habitats. Overall, higher control levels were obtained for longer time from Methoprene then the Triflumuron.

4. DISCUSSION

Mosquitoes are the most important group of insects in terms of public health importance, a transmitting a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis etc., causing millions of deaths every year [19]. Human beings are compelled to fight against them using available technical ornaments. There was an initial success in controlling vectors by using synthetic insecticides. Since 1900, the World Health Organization has warned about the possible emergence and reemergence of arthropod-borne disease due to combined human, biological, environmental and climatic factors [20].

One of the approaches for control of these mosquito-borne diseases in the interruption of disease transmission is by killing or preventing mosquitoes from biting human. Although numerous synthetic pesticides are presently available for vector control programmes operating in many areas of the world [21,22], the intense and widespread use of these products has caused concerns regarding their impact on both human and environmental health, and has led to a buildup of resistance in pest population [23,24].

Insect growth regulator compounds such as methoprene, diflubenzuron, pyriproxyfen, triflumuron and diflubenzuron have been recommended already by WHO [25] for use against immature mosquitoes. However, none of these compounds are in use for vector control in India [26].

In general IGR compounds do not produce immediate mortality among the larvae at the recommended doses and are therefore, difficult to assess in most situations. Sharma et al. [27] however showed effective control (80 – 100%) of *Culex pipiens fatigans* breeding in polluted drains by using dimilin (Diflubenzuron) at doses of 0.5 to 1 ppm. The residual effect of this larvicide in field application was however, approximately for four days.

5. CONCLUSION

The present study suggests that Methoprene was effective in all the three different fields such as drain, pool and tank in controlling larvae and pupae of *Cx. quinquefasciatus* in Karumandapam, Tiruchirappalli. Suman et al. [28] reported that the diflubenzuron, lufenuron and azadirachtin have the potential IGR activity against different field populations of larval *Cx. quinquefasciatus* mosquitoes. The discovery of the synthetic insecticides for the control of pests as well as human disease vectors has led to concerns about their toxicity and environmental impact and control of pests is becoming increasingly difficult because of increasing resistance to pesticides [29].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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