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Effectiveness of Methoprene, an Insect Growth Regulator, Against Malaria Vectors in Fars, Iran: A Field Study

[1]H. Darabi, [2]H. Vatandoost, [2]M.R. Abaei, [1]O. Gharibi and [1]F. Pakbaz
[1]Public Health Center, Bushehr Medical University of Science, Bushehr, Iran
[2]Medical Faculty, Tehran Medical University of Science, Tehran, Iran

Abstract: Methoprene, an insect growth regulator, was evaluated under field conditions against the main malaria vectors in the Islamic Republic of Iran. The effect of 5, 10 and 20 kg ha^{-1} concentration of methoprene granule formulation and 100 and 200 mL ha^{-1} concentration of EC formulation was measured to determine any changes in Anophelini larval abundance and IE ratio in both rice fields and artificial ponds. In artificial ponds, granular methoprene at a dose of 20 kg ha^{-1} inhibited adult emergence by 77.1% after 1 day and 65.9% after 3 days. The emulsifiable concentrate formulation of methoprene at 200 mL ha^{-1} inhibited adult emergence by 83.7% after 1 day and 32.2% after 3 days. In rice fields, inhibition of emergence was 44.3% at 20 kg ha^{-1} granule and 35.8% for emulsifiable concentrate at 200 mL ha^{-1} after 3 days. The results vary depending on the mosquito species, treatment methods, breeding places and type of formulation.

Key words: Larvicide, methoprene, malaria, anophelini, vectors

INTRODUCTION

Despite several years campaign against malaria, around 300-500 million clinical cases occur every year with over 1.2 to 1.7 million deaths that contain over 1 million death among under 5 year- old African children, over 90% of the cases occur in Sub Sahra district of Africa (Vatandoost *et al*., 2005). There are different methods including biological-chemical and environmental management for combating malaria vectors (Davari *et al*., 2006), Larviciding can be useful method for malaria programs, particularly in area where breeding sites are accessible and relatively limited in number and size. Before implementing a larviciding program, survey should be carried out to assessment the insecticide resistance of target species of the area (Vatandoost and Hanafi-Bojd, 2005).

Insect Growth Regulators (IGR), are known as the 3rd generation insecticides and are effective tools for the control of a variety of insect pests and disease vectors. They show an exceptionally high level of activity against mosquitoes and several other groups of noxious and vector insects. IGRs in general have low mammalian toxicity, are quite safe to fish, birds and most non-target biota. However, some IGRs have shown a high level of activity against some crustacean groups, inducing a variety of morphological aberrations. In mosquito control, most IGRs are evaluated and applied against the aquatic stages of mosquitoes. Mosquito larvae are ideal targets for IGRs, most of which have delayed activity, including mortality or morphogenetic anomalies in stages beyond the one treated. In other words, IGRs do not induce immediate mortality in the larval stage (Mulla, 1995). In the ensuing years, its activity as a mosquito larvicide has been clearly shown. The IGRs are selective insecticides and nontoxic to humans and other vertebrate (Graf, 1993). Methoprene, which is a juvenile hormone analog (JHA), are effective additional tools of public health and veterinary importance for controlling pests and disease vectors (Wilson, 2004). Methoprene comes in a granular or pellet form and is applied directly to the water where mosquito larvae are found. When mosquito larvae are exposed to methoprene, their life cycle is disrupted and they are prevented from reaching maturity or reproducing. In the feild studies, Methoprene have shown high level of activity against many species of mosquitoes and related groups (Butler *et al*., 2006; Kuo *et al*., 2010; McCarry, 1996; White and Czajkowska, 2002; Silva and Mendes, 2007).

This study in the Islamic Republic of Iran aimed to evaluate the efficacy different formulations of methoprene against malaria vectors under the field conditions.

MATERIALS AND METHODS

Artificial ponds: In order to evaluate methoprene in the field, 12 artificial ponds, dimensions 100×100 cm and depth 35-50 cm, were dug in pairs alongside each other in

Corresponding Author: H. Darabi, Public Health Center, Bushehr Medical University of Science, Bushehr, Iran
Tel: (98)7712530741 Fax: (98) 7712526741

meadows at southeastern Jades village in Fars province in 2003 and 2004 years. Control ponds were placed on the northern slope of the meadows to minimize the probability of any penetration of methoprene-treated water to the control. The water in these ponds was mostly clear but partly brackish. These artificial ponds received direct sunlight all day and made favourable habitats for a number of aquatic insects (Hydrophilidae, Dytiscidae and aquatic bugs). No larvivorous fish were included.

Rice fields: Jades village is surrounded by rice fields and in the spring the majority of the land in this area is devoted to rice planting, which causes an increase of larval breeding places and correspondingly the density of anophelines. In order to evaluate granule and EC formulations of methoprene in the field, those rice fields which had a high density of anopheline larvae were chosen in both Pirsabz and Jades villages. Control plots were located about 100 m beyond the treated ones which receive the agricultural water first. For evaluation of granular and EC formulations, 2 separate fields were selected.

Treatments: In the artifical ponds, the EC formulation was applied by 1 L hand sprayer in concentrations 100 mL and 200 mL active ingredient per hectare (ai ha^{-1}) with 2 replicates for each dosage. The granule formulation was mixed at a ratio 1:4 with fine sands and manually spread over each plot at concentrations of 5, 10 and 20 kg of active ingredient per hectare (kg a.i. ha^{-1}).

In the rice fields the EC formulation was applied by Hudson X-pert® sprayer at 200 mL a.i. ha^{-1}. The granule formulation was applied as for artificial ponds at 20 kg a.i. ha^{-1}.

All experiments were repeated in 3 rounds from June to September 2003 and 2004. Larval density was determined 1 day pre-treatment and continued up to 7 days post-treatment by dipping methods.

For both artificial ponds and rice fields a simple larval exposure chamber was constructed from a metal cubic frame dimension 5×15×15 cm with netting. The number of cages per experiment unit was 2-3 and 5-6 for artificial ponds and rice fields respectively. Larvae were exposed to treated water for 24 hrs and then transferred to the laboratory in plastic buckets for measuring adult emergence from 4th instar larvae of anopheline species. The field larvae comprised 4 species: *An. stephensi* (64%), *An. fluviatilis* (24%), *An. superpictus* (18%) and *An. d'thali* (5%).

The total mortality of the biotic stages (larvae, pupae and incomplete adults) was considered as percentage inhibition of emergence.

The efficacy of different dosages of granule and EC formulations on larval density (per 10 dips) were analysed by paired t-test and the 2-tailed significance by SPSS 9.2 software. The impact of different dosages of the 2 formulations on anopheline mortality as well as durability of methoprene was also analysed by χ^2 test and the significance of differences were compared using Pearson's test of significance and a-level value.

RESULTS

Artificial ponds: The efficacy of granular formulations of methoprene at 5, 10 and 20 kg a.i. ha^{-1} on inhibition of adult emergence in artificial ponds are summarized in Table 3. All concentrations showed significant differences compared to the control ponds. In general, it was

Table 1: Efficacy of granule formulation of methoprene on mortality and inhibition of adult emergence from 4th instar larvae of anophelines using larval exposure cages in artificial ponds, southern Islamic Republic of Iran

Days after application/ Dose	Larvae tested (%)	Stage-related mortality (%) Larvae	Pupae	Adults	Adults completely emerged (%)	Cumulative mortality (%)	Corrected inhibition of adult emergence[a] (SE) (%)
1 day							
Control	140	3.1	9.2	2.7	85.0	15.0	
5 kg ha^{-1}	130	4.6	50.0	16.5	28.8	71.0	66.1 (4.1)
10 kg ha^{-1}	121	5.8	59.6	11.0	23.2	77.0	72.7 (4.1)
20 kg ha^{-1}	150	8.1	54.4	17.9	19.5	80.0	77.1 (3.4)
2 days							
Control	148	3.1	8.7	2.6	85.3	15.0	
5 kg ha^{-1}	124	10.5	40.1	12.5	36.7	63.0	57.0 (4.4)
10 kg ha^{-1}	141	11.6	42.2	16.3	29.9	70.0	65.0 (4.0)
20 kg ha^{-1}	139	8.4	41.4	26.8	23.3	77.0	72.7 (3.8)
3 days							
Control	159	3.3	3.7	4.5	88.5	11.0	
5 kg ha^{-1}	145	10.4	31.2	17.0	41.3	59.0	53.3 (4.1)
10 kg ha^{-1}	160	9.3	31.7	23.9	35.0	65.0	60.5 (3.9)
20 kg ha^{-1}	165	11.9	37.9	19.8	30.2	70.0	65.9 (3.7)

[a]Abbott's formula. SE : standard error.

Table 2: Efficacy of emulsifiable concentrate (EC) formulation of methoprene at 2 rates on mortality and inhibition of adult emergence from 4th instar larvae of anophelines using larval exposure cages in artificial ponds, southern Islamic Republic of Iran

Days after application/ Dose	Larvae tested (%)	Stage-related mortality (%) Larvae	Pupae	Adults	Adults completely emerged (%)	Cumulative mortality (%)	Corrected inhibition of adult emergence[a] (SE) (%)
1 day							
Control	132	3.8	9.0	3.6	83.6	16.0	
100 mL ha^{-1}	93	10.7	40.3	27.6	21.2	79.0	74.6 (4.2)
200 mL ha^{-1}	133	10.5	49.6	24.7	13.6	86.0	83.7 (3.0)
2 days							
Control	156	3.2	8.3	1.8	86.7	13.0	
100 mL ha^{-1}	124	13.2	35.3	19.2	31.9	68.0	63.2 (4.2)
200 mL ha^{-1}	155	14.6	42.0	23.1	20.3	80.0	76.6 (3.2)
3 days							
Control	147	3.5	4.9	4.1	87.5	12.0	
100 mL ha^{-1}	139	9.9	16.6	10.1	63.4	36.0	27.5 (4.1)
200 mL ha^{-1}	118	9.7	19.7	11.2	59.3	41.0	32.2 (4.5)

[a]Abbott's formula.SE : Standard error.

Table 3: Efficacy of two formulations of methoprene on mortality and inhibition of adult emergence from 4th instar larvae of anophelines[1] using larval exposure cages in rice fields, southern Islamic Republic of Iran

Days after application/ Dose	Larvae tested (%)	Stage-related mortality (%) Larvae	Pupae	Adults	Adults completely emerged (%)	Cumulative mortality (%)	Corrected inhibition of adult emergence[a] (SE) (%)
1 day							
Control	181	0.9	12.7	2.2	84.2	16.0	
Gr: 20 kg ha^{-1}	227	8.3	47.8	21.1	22.8	77.0	72.9 (2.5)
EC: 200 mL ha^{-1}	173	8.2	44.6	20.0	27.1	73.0	67.8 (3.4)
2 days							
Control	166	1.2	9.2	4.4	85.2	15.0	
Gr: 20 kg ha^{-1}	178	13.4	32.3	24.7	29.6	70.0	65.3 (3.4)
EC: 200 mL ha^{-1}	183	7.4	35.3	25.0	32.2	68.0	62.2 (3.5)
3 days							
Control	159	2.4	13.2	2.4	81.8	18.0	
Gr: 20 kg ha^{-1}	193	10.4	25.5	18.5	45.6	54.0	44.3 (3.6)
EC: 200 mL ha^{-1}	139	6.4	23.7	17.3	52.5	47.0	35.8 (4.2)

[a]Abbott's formula. Gr: granule formulation; EC: emulsifiable concentrate formulation. SE: standard error.

considered that there was no significant difference of granular dosages on inhibition of adult emergence ($p<0.05$) and all dosages had nearly similar effects. The corrected inhibition of adult emergence at a concentration of 20 kg a.i. ha^{-1} was 77.1% 1 day after treatment, 72.7% after 2 days and 65.9% after 3 days.

The efficacy reduction ratio of granular methoprene was estimated at 19.4, 16.8 and 14.5% at concentrations of 5, 10 and 20 kg a.i. ha^{-1}

The highest efficacy of granular methoprene on inhibition of adult emergence occurred 1 day after application in artificial ponds (Table 1).

The effects of EC formulation of methoprene at 100 and 200 mL ha^{-1} on inhibition of adult emergence are summarized in Table 2. The corrected inhibition of adult emergence at the concentration of 200 mL ha^{-1} was 83.7% 1 day after treatment, 76.6% after 2 days and 32.2% 3 days post-treatment Upon 2-fold application of EC formulation (200 a.i. mL ha^{-1}) in artificial ponds, inhibition of adult emergence only increased on an average of 16.8%. Two applied dosages of EC formulation (100 and 200 mL ha^{-1}) were statistically compared and the results did not show any significant difference ($p>0.01$), but there was significantly reduced adult emergence compared with untreated artificial ponds ($p<0.01$).

Rice fields: In the rice fields the effectiveness of both granular and EC formulations on mortalities of different biotic stages of 4th instar larvae and inhibition of adult emergence were examined at concentrations of 20 and 200 a.i. mL ha^{-1} respectively using larval exposure cages (Table 3). The granule formulation inhibited adult emergence 10.3% better than the EC formulation. There was no significant difference between the effect of mentioned formulations in all 3 days ($p>0.01$), although the granule form was more effective on population reduction (15.3% in average then compared with the EC form ($p<0.05$).

The greatest effect of both granule and EC formulations was seen on 1 day after treatment and there was a distinct decline in the methoprene effect after the 2nd day (Table 3) the average effect decline during the 2nd and 3rd day for the granule formulation was estimated as 10.4 and 39.2% and for EC 8.3 and 47.2%, respectively.

DISCUSSION

There are a number of different studies using IGRs in field conditions against a variety of mosquito species. Other studies in central and southern parts of Islamic Republic of Iran and other countries have evaluated certain IGRs against various anopheline and other insect's species in field conditions

Three applications of Altosid XR-G (1.5% s-methoprene) were made at application rates of 9.0, 4.5 and 2.3 kg ha^{-1}. Pupae were collected from control and treated sites after inundation. Under field conditions, Altosid XR-G gave 44.6% control at 67 days posttreatment at Site 1 (9.0 kg ha^{-1}), 43.7% control at 67 days posttreatment at Site 2 (4.5 kg ha^{-1}) and 38% control at 53 days posttreatment at Site 3 (2.3 kg ha^{-1}). All treatments taken together achieved an adjusted 10-week cumulative mosquito emergence inhibition of 69% (Qualls and Xue, 2007).

Larvae of Culex pipiens and Cx. restuans in catch basins were exposed to Altosid pellets (4% active ingredient, [S]-methoprene) applied at a rate of 11.3 kg ha^{-1} (7 g of pellets per catch basin). Under field conditions, the pellets yielded an average 82% emergence inhibition of adult mosquitoes over the 15-week trial period (McCarry, 1996).

The efficacy and persistence of sustained-release methoprene (Altosid) pellets were evaluated at rates of 3.4 and 9.0 kg ha^{-1} against Aedes mosquitoes through 7 flood cycles (126 days) in an irrigated pasture. At both rates, the pellets provided greater than 98% control through 2 flood cycles, or 20 days posttreatment and greater than 80% control through 5 flood cycles, or 69 days post treatment (Kramer and Beesley, 1991).

The IGR, methoprene, in 3 solid (Altosid Pellet, Altosid XR Briquet and a granular) and a liquid formulation (A.L.L.) was evaluated against chironomid midges in experimental ponds. The A.L.L. was applied at 0.293 liters ha^{-1} (0.015 kg a.i. ha^{-1}) and at 5.86 liters ha^{-1} (0.28 kg a.i. ha^{-1}). The granules, pellets and briquets were applied at 13 kg ha^{-1} (0.17 kg a.i. ha^{-1}), 5.6 kg ha^{-1} (0.22 kg a.i. ha^{-1}) and one briquet/8m2 (0.82 kg a.i. ha^{-1}), respectively. The low rate of A.L.L. was ineffective, but the high rate produced 84-100% control of Tanytarsini and 30-100% of Chironomini during 2 week posttreatment. The granular formulation gave 61-87% control of total midges in 2 week posttreatment. Altosid Pellets gave initial and prolonged good control of Tanytarsini (64-99% for 7 week), Chironomini (79-94% for 5 week) and total midges (64-98% for 7 week). The briquets at almost 4x the rate of pellets, yielded 38-98% control of midges for 7 week (Ali *et al*., 1991)

Field trials in Tezpur, Assam, India in cemented drains, small ponds and ditches found that methoprene 0.2 ppm (0.020 kg ha^{-1}) eliminated 92-96% of *Cx. quinquefasciatus* and *Ae. albopictus* larvae(Baruah and Das, 1996).

Trials of another IGR, diflubenzuron, in rice fields, riverbeds and artificial ponds in Kazeroun district, southern Islamic Republic of Iran showed a 85-100% reduction in anopheline larval population at concentrations of 25, 35 and 50 g a.i. ha^{-1} by 5 days post-treatment and inhibited the pupal formation up to 2 weeks. There was no significant difference between the concentrate suspension and wettable powder formulations(Farashiani and ladonni, 1999).

The persistence of methoprene (Metoprag S-2G) was evaluated against *Ae. aegypti* late 3rd instar larvae in a semi-field bioassay in Rio de Janeiro, using containers made of plastic, iron, concrete and asbestos, placed in a shaded area. At 1 g per 100 L of water methoprene induced mortality higher than 70% up to 15 days in the plastic and iron containers and only 7 days in the concrete container. In the asbestos container, maximal mortality was achieved on day 1 post-treatment (66%). Their results point to a low persistence of this formulation in the weather conditions of Rio de Janeiro (Lima *et al*., 2005). A high level of methoprene resistance in the mosquitoes Ochlerotatus nigromaculis (Ludlow) and Ae. taeniorhynchus have been reported. Monitoring of levels of resistance to larvicides is highly recommended (Cornel *et al*., 2002).

In the present study , The efficacy of the granular formulation of methoprene at doses of 5, 10 and 20 kg a.i. ha^{-1} against 4th instar anopheline larvae was significantly different compared with controls in artificial ponds. Inhibition of adult emergence with a dose of 20 kg a.i. ha^{-1} was 77.1% 1 day after treatment, reaching 65.9% 3 days post-treatment. The average inhibition of adult emergence methoprene at the high dosage (200 mL a.i. ha^{-1}) was 83.7, 76.6 and 32.2% on days 1, 2 and 3.

In rice fields, the granular (20 kg a.i. ha^{-1}) and EC (200 mL a.i. ha^{-1}) formulations of methoprene were evaluated on the 4th instar larvae of field-collected anopheline. Inhibition of adult emergence (corrected values) was 72.9 and 67.8% for granular and EC formulations on day 1. The granule formulation of methoprene inhibited adult emergence 10.3% better than the EC formulation, although the granule was more effective on population reduction when compared with EC. The greatest effect of both formulations was 1 day after treatment and there was a decline in the methoprene effect after the 2nd and 3rd days. The results of pervious study somewhat conform the finding of this study in

Inhibition of adult emergence according to different dosages and formulations.

CONCLUSION

Results of our study revealed that maximum durability and effetiveness of methoprene under filed condition against mosquito larvae depends on the mosquito species, treatment methods, breeding places and type of formulation. Therefore evaluation and Consideration of monitoring of resistance to such kind of larvicides in different conditions is highly recommended.

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