HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.04.008

Evaluation of different formulations of IGRs against *Aedes albopictus* and *Culex quinquefasciatus* (Diptera: Culicidae)

Gul Zamin Khan[1], Inamullah Khan[1*], Imtiaz Ali Khan[2], Alamzeb[1], Muhammad Salman[1], Kalim Ullah[3]

[1]*Entomology Division, Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan*

[2]*Department of Entomology, Faculty of Crop Protection Sciences, the University of Agriculture, Peshawar, Pakistan*

[3]*Pakistan Central Cotton Committee, Cotton Research Station, Dera Ismail Khan, Pakistan*

ARTICLE INFO

Article history:
Received 11 Dec 2015
Received in revised form 22 Dec 2015, 2nd revised form 13 Jan, 3rd revised from 16 Jan 2016
Accepted 22 Feb 2016
Available online 25 Apr 2016

Keywords:
Insect growth regulators
Mortality
Inhibition
Deformities
Insecticides
Environment friendly

ABSTRACT

Objective: To test the relative efficacy of pyriproxyfen and methoprene on mortality, deformity, inhibition and emergence to adult stages of *Culex quinquefasciatus* and *Aedes albopictus*.

Methods: Serial dilutions (0.01–0.05 mg/L) of methoprene, pyriproxyfen 0.5 water dispersible granules (WDG) and pyriproxyfen 1.0 WDG were used to assess mortality and inhibition of 3rd instar larvae of *Aedes albopictus* and *Culex quinquefasciatus*. Each concentration and control was replicated four times in completely randomized design. Data on larval mortality, growth inhibition, deformities and adult's emergence was recorded weekly. On the basis of best comparative performance, the efficacy of pyriproxyfen 1.0 WDG at 0.1 g/m^3 was also tested in the field by collecting treated water samples monthly for 1–6 months after field application. Twenty five 3rd instar larvae of *Aedes* and *Culex* spp. of the same cohorts were used for bioassays and compared with larvae in control cups containing 1 L of untreated tap water.

Results: Results revealed variations in fatality of different insect growth regulators (IGRs) to the 3rd instar larvae of *Culex* and *Aedes* mosquitoes. Among the IGRs, pyriproxyfen 1.0 WDG was found best that exhibited significantly high emergence inhibition against *Culex* and *Aedes* spp. Based on the results, the IGRs were classified in terms of the tested parameters in order of pyriproxyfen 1.0 WDG > pyriproxyfen 0.5 WDG > methoprene. In case of field studies, pyriproxyfen 1.0 WDG, pool data of the entire target treated sites showed minimum adult emergence from water sampled of habitats treated with 0.1 g/m^3 of pyriproxyfen 1.0 WDG.

Conclusions: It is thus concluded that IGRs can be utilized as environment friendly control measures for *Culex* and *Aedes* spp. of mosquitoes on small and large scale. This will reduce the use of conventional insecticides by the public health authorities and help in reducing selection pressure of insecticides.

1. Introduction

Induced hematophagy in mosquito's species of genera *Anopheles*, *Culex* and *Aedes* make them key vectors of pathogens in Pakistan and elsewhere [1–3]. *Anopheles* spp. are responsible for deadly malaria [4,5] while *Culex* spp. breed predominantly in houses [6] and their females while seeking blood meal make irritable bites [7] with the potential vector capacity of Japanese encephalitis virus in Pakistan and else viewer [5,6,8]. *Aedes* spp. that result in the transmission of dengue fever and dengue hemorrhagic fever [9–11] due to Flaviviridae serotypes; Den I to Den IV [12,13] is exotic in Peshawar. A survey on population dynamics of *Aedes albopictus* (*Ae. albopictus*) in different areas of Peshawar Division highlighted that this species is newly introduced in the area. However, its slightly high population in the more dense vegetation of the rural and semi-urban areas shows its

*Corresponding author: Dr. Inamullah Khan, Nuclear Institute for Food & Agriculture (NIFA), Tarnab, G.T. Road, Peshawar, Pakistan.
Tel: +92 903349059180
Fax: +92 912964059
E-mail: inamullah17@gmail.com

Foundation Project: Supported by the Higher Education Commission (HEC) under HEC indigenous fellowship 1061060 AV6038.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

potential for establishment in the near future [14]. Entomologists are therefore, worried about the adoptability of *Aedes* spp. to the urban and rural environment and also their subsequent establishment in the remote cities. The conducive natural habitats in the Peshawar area such as vast agricultural lands, presence of many rivers, several dams and open network of agricultural channels from these reservoirs provide plenty of breeding places for all kind of fresh water mosquitoes [2,15,16] including *Aedes* spp. Moreover, the semi urban and urban communities are overcrowded due to internal displacement caused by devastating floods during 2010 in the area; poverty, insecurity and establishment of temporary camps for internally displaced refugees due to terrorism provide temporary habitats for breeding of *Culex* spp. in Peshawar division. These conditions promote the chances for the spread of vector born diseases [16] and consequently may lead to possible epidemics/outbreaks in different parts of Peshawar division with increased morbidity and mortality. The entomologists and public health authorities are therefore, of more concern to handle the situation in time and avoid the severe sudden outbreak unlike that of Punjab Province in the year 2011–12.

Presently, no vaccine [17] is available for the prevention of dengue virus infection at the world level. Therefore, control of vector mosquito is the only way of dengue management [18]. Mainly, the disease control effort has been made to treat the dengue infected people for minimizing the number of deaths. However, no or very little effort has been made to stop or reduce the number of infected cases through vector breeding control in environmentally safe way. Ever since dengue cases were reported in 2007 [18] and the severe epidemic in 2011, 2012 in Lahore, the local public health authorities of Khyber Pakhtunkhwa (Malaria control program) in collaboration with Non-Governmental Organizations and entomologists have been battling the vectors species by using insecticides and larvicides as the only tool for management. Chemical control is quick and efficient [19], but pose lethal effects on non target organisms and result in environmental contamination [20]. It also poses threats of resistance development in mosquitoes to insecticides [21–25] and therefore, demands for the necessity of developing alternative strategies. Different plant extracts possess lethal characters for suppressing the vector mosquitoes. Oils of cinnamon, eucalyptus and turpentine are fatal to the larvae of *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and act as attractant to the adults for oviposition and therefore, may be good candidates for using in the "attract and kill" strategy of mosquitoes control programs [26]. Similar studies have shown that some commonly available plant extracts are lethal to *Cx. quinquefasciatus* mosquitoes [27].

Insect growth regulators (IGRs) are special new class of insecticides complex in addition to four major chemical groups – chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids, that influence insect mortality and growth inhibition in safe way [28]. Thus the uses of (IGRs) [29–31] in integrated approaches of mosquitoes [32–34] are the key areas to be utilized for the vector control. The physical management of mosquitoes breeding habitats requires huge economics investment and in many cases not practical for low income countries. The current studies were therefore, planned with the aim to monitor and evaluate the efficacy of different formulations of IGRs against the *Culex* and *Aedes* spp. of Peshawar division in Khyber Pakhtunkhwa, Pakistan. In this way, the use of formal insecticides can be minimized and replaced with the safe alternatives in the form of IGRs and ultimately help in resistant management of vector mosquitoes.

2. Materials and methods

The relative efficacy of various formulations of IGRs against *Ae. albopictus* and *Cx. quinquefasciatus* was investigated in the laboratory of Entomology Division, Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan during the year 2013. IGRs with serial dilutions (0.01–0.05 mg/L) of methoprene, pyriproxyfen 0.5 water dispersible granules (WDG) and pyriproxyfen 1.0 WDG were used to assess mortality and inhibition of 3rd instars larvae of *Ae. albopictus* and *Cx. quinquefasciatus* in 500 mL disposable cups containing 100 mL of each concentration and three drops of 1% NIFA larval diet slurry as food [35]. Control treatments comprised of water and food only. Each concentration and the control were replicated four times in completely randomized factorial design.

2.1. Rearing procedures

A laboratory colony was established by collecting the larvae from the different breeding habitats having mix culture. Larval and pupal collections were made with 0.5 L standard iron dippers. The larvae collected were brought into laboratory for rearing using ventilated plastic bottle (2 L) placed in ice chest during transportation. Field collected mosquitoes were artificially blood fed through a flexible membrane (Parafilm M). The culture was established for both *Culex* and *Aedes* species following the standard mosquitoes rearing procedures of Khan *et al.* [35]. Identification to the species level was made with the help of available taxonomic keys [36].

2.2. Bioassays

The granular formulation of IGRs was ground to the uniformity of fine particles with a mortar and pestle and agitated for 1 h in distill water. The IGRs were dissolved by w/v to make stock solution of 10 mg/L. This suspension was subjected in serial dilution and used to derive final concentrations of 0.01–0.05 mg/L in tap water. The evaluations of IGRs were made following the methods of Sihuincha *et al.* [30] and Mulla *et al.* [37] with slight modification according to our requirements. Bioassays experiments in the laboratory were conducted in completely randomized design using different concentration (0.01–0.05 mg/L) of juvenile hormones mimics (methoprene, pyriproxyfen 1.0 WDG and pyriproxyfen 0.5 WDG) separately against 3rd instar larvae of *Aedes* and *Culex* spp. Methoprene was purchased from the market in Analar grade. While two formulations of the pyriproxyfen was supplied by Evyol Chemicals group, Lahore, Pakistan for the trails. An F1 generation of the larvae was used in the bioassays. Following the methods of Sihuincha *et al.* [30] all materials used for containing eggs, larvae, or adults over the course of the experiments were disposed off, after each test for minimizing the potential contamination of experiments with minute doses of IGRs. Further care was taken by handling larvae, pupae, or adults using disposable plastic pipettes. The IGRs were trailed against the batches of 25 (3rd instar) larvae added to 500 mL disposable pots containing 100 mL of the above mentioned

solutions and 0.01 g (3 drops) of larval diet slurry. Controls consisted of tap water and food only. All pots were capped with gauze to prevent the escape of emerging adults and were monitored for 15–21 days. Tests cups were examined daily and molted exoskeletons, dead larvae or pupae, and emerged adults were removed. Pupa formed during the course of experiment were separated through a plastic dropper and put in disposable cups with 2 mL of water. All cups were covered with gauze to prevent the escape of emerging adults and observed for emergence to the adult stage. The whole experiment was run in laboratory maintained at 12:12 h photoperiod and (28 ± 2) °C. The entire bioassay was repeated two times under similar conditions.

2.3. Efficacy of IGR under field conditions

Pyriproxyfen 1.0 WDG was found the best inhibitor for both the species in laboratory studies and therefore, its efficacy was further evaluated under field conditions. For this purpose, three natural mosquito breeding sites of sizes 10.10 m^3, 11.00 m^3 and 12.53 m^3 were used for treatment with pyriproxyfen 1.0 WDG and 8.00 m^3 area as control was selected at Kalamandi (Peshawar). All these sites had mix culture of *Aedes* and *Culex* spp. The sites were treated according to the recommended filed dose at the rates of 0.1 g/m^3 in 1 000 L of water. The approximate volume of water in the site was calculated by length (m) × width (m) × average depth (m) = cubic meter (m^3) water volume. Pyriproxyfen 1.0 WDG was applied in a pouch bag of muslin cloth in each replication and suspended in the body of the habitat with a building wire. Five liters samples of water from the treated sites were collected and taken to the laboratory for testing the efficacy as described by Sihuincha *et al.* [30]. Twenty five 3rd instar larvae of *Aedes* and *Culex* spp. of the same cohorts were used for bioassays. These cohorts were compared with larvae in control cups containing 1 L of untreated tap water. NIFA larval diet solution at (1%) was added to all cups as a food source as mentioned above. Percent larval mortality, adult emergence inhibition, deformities and adult emergence were recorded for 1–6 months after the treatment.

2.4. Statistical analysis

Mean percent larval mortality, deformities and reductions of adult emergence, in each batch of mosquito species caused by the IGRs formulations were subjected to the analysis of variance technique and means were further separated through least significant difference test using the statistical package (Statistix 8.1).

3. Results

The mean values pertaining to percent larval mortality, deformities, inhibition and emergence of *Aedes* and *Culex* species are presented in Tables 1 and 2 respectively.

3.1. Ae. albopictus

The inhibition percentage as calculated against different IGRs, concentrations and their interaction is expressed in Table 1. Highest inhibition was recorded for methoprene followed by pyriproxyfen 0.5 WDG while lowest was recorded for pyriproxyfen 1.0 WDG (Table 1). The inhibition percentage as observed against various concentrations was maximum at 0.02 mg/L which was statistically similar to 0.01, 0.03 and 0.04 mg/L while 0.05 mg/L concentration depicted lowest inhibition percentage against *Aedes* spp. Pyriproxyfen 0.5 WDG × 0.02 mg/L exhibited highest interaction for inhibition percentage with the value of 63% followed by methoprene at 0.05 mg/L concentration.

In the course of studies, it was observed that minimum emergence percentage (19.67%) was recorded in pyriproxyfen 0.5 WDG followed by pyriproxyfen 1.0 WDG (21.87%) whereas maximum in methoprene (Table 1). In testing various concentrations, no emergence was observed at 0.05 mg/L followed by 0.04 mg/L (0.67%) as compared to control where maximum emergence percentage (91.83%) was noticed. Most of the treatments exhibited minimum interaction for emergence percentage except methoprene × 0.01 mg/L where the interaction was 49.00% followed by methoprene × 0.02 mg/L.

The mortality percentage as calculated against different IGRs was significantly different. Highest mortality of 33.91% was recorded for pyriproxyfen 1.0 WDG which was statistically at par with pyriproxyfen 0.5 WDG. Lowest mortality was recorded in methoprene (Table 1). Similarly, various concentrations also

Table 1

Deformity, mortality, inhibition and emergence of *Aedes* species as influenced by various formulations of IGRs at different concentrations. %.

IGRs	Deformity	Mortality	Inhibition	Emergence
Methoprene	10.83^{b}	21.67^{b}	38.00^{a}	30.21^{a}
Pyriproxyfen 0.5 WDG	11.00^{b}	32.67^{a}	36.83^{a}	19.67^{c}
Pyriproxyfen 1.0 WDG	14.16^{a}	33.91^{a}	29.50^{b}	21.87^{b}
LSD 0.05	2.42	2.03	1.95	1.75
0.00 (control)	0.00^{d}	7.50^{e}	0.00^{c}	91.83^{a}
0.01 mg/L	17.33^{b}	8.67^{e}	43.67^{a}	30.67^{b}
0.02 mg/L	21.33^{a}	16.67^{d}	45.67^{a}	17.33^{c}
0.03 mg/L	17.00^{b}	34.67^{c}	45.33^{a}	3.00^{d}
0.04 mg/L	9.66^{c}	46.67^{b}	43.00^{a}	0.67^{de}
0.05 mg/L	6.67^{c}	62.33^{a}	31.00^{b}	0.00^{e}
LSD 0.05	3.42	2.87	2.76	2.47
Methoprene × 0.01 mg/L	12.00^{de}	11.00^{hi}	31.00^{i}	49.00^{b}
Methoprene × 0.02 mg/L	20.00^{bc}	15.00^{gh}	37.00^{h}	31.00^{c}
Methoprene × 0.03 mg/L	15.00^{cd}	27.00^{f}	49.00^{de}	9.00^{e}
Methoprene × 0.04 mg/L	12.00^{de}	33.00^{e}	53.00^{cd}	2.00^{f}
Methoprene × 0.05 mg/L	6.00^{f}	36.00^{de}	58.00^{b}	0.00^{f}
Pyriproxyfen 0.5 WDG × 0.01 mg/L	16.00^{cd}	8.00^{i}	55.00^{bc}	21.00^{d}
Pyriproxyfen 0.5 WDG × 0.02 mg/L	18.00^{c}	16.00^{g}	63.00^{a}	3.00^{f}
Pyriproxyfen 0.5 WDG × 0.03 mg/L	16.00^{cd}	40.00^{d}	44.00^{f}	0.00^{f}
Pyriproxyfen 0.5 WDG × 0.04 mg/L	9.00^{ef}	52.00^{c}	39.00^{gh}	0.00^{f}
Pyriproxyfen 0.5 WDG × 0.05 mg/L	7.00^{ef}	73.00^{b}	20.00^{j}	0.00^{f}
Pyriproxyfen 1.0 WDG × 0.01 mg/L	24.00^{ab}	7.00^{i}	45.00^{ef}	22.00^{d}
Pyriproxyfen 1.0 WDG × 0.02 mg/L	26.00^{a}	19.00^{g}	37.00^{h}	18.00^{d}
Pyriproxyfen 1.0 WDG × 0.03 mg/L	20.00^{bc}	37.00^{de}	43.00^{fg}	0.00^{f}
Pyriproxyfen 1.0 WDG × 0.04 mg/L	8.00^{ef}	55.00^{c}	37.00^{h}	0.00^{f}
Pyriproxyfen 1.0 WDG × 0.05 mg/L	7.00^{ef}	78.00^{a}	15.00^{k}	0.00^{f}
LSD 0.05	5.93	4.98	4.78	4.29

Means followed by the same letter(s) in columns are not significant at 5% level of probability.

Table 2
Deformity, mortality, inhibition and emergence of *Culex* species as influenced by various formulations of IGRs at different concentrations.

IGRs	Deformity	Mortality	Emergence	Inhibition
Methoprene	25.50[a]	20.67[c]	26.83[a]	28.00[b]
Pyriproxyfen 0.5 WDG	13.67[b]	31.21[b]	18.00[b]	35.16[a]
Pyriproxyfen 1.0 WDG	11.33[b]	42.71[a]	15.00[c]	28.67[b]
LSD 0.05	2.65	2.38	2.99	2.98
0.00 (control)	2.00[c]	9.16[e]	89.00[a]	0.00[e]
0.01 mg/L	24.00[a]	12.00[e]	12.00[b]	45.67[a]
0.02 mg/L	21.00[a]	26.33[d]	8.67[bc]	43.00[ab]
0.03 mg/L	22.67[a]	31.67[c]	4.67[cd]	40.67[bc]
0.04 mg/L	15.33[b]	45.33[b]	2.33[d]	38.00[c]
0.05 mg/L	16.00[b]	64.67[a]	3.00[d]	16.33[de]
LSD 0.05	3.75	3.37	4.24	4.22
Methoprene × 0.01 mg/L	23.00[ce]	10.00[l]	24.00[a]	
Methoprene × 0.02 mg/L	30.00[b]	19.00[ij]	21.00[b]	
Methoprene × 0.03 mg/L	32.00[ab]	20.00[ij]	9.00[cd]	
Methoprene × 0.04 mg/L	29.00[bc]	28.00[gh]	7.00[ce]	
Methoprene × 0.05 mg/L	37.00[a]	40.00[ef]	9.00[cd]	
Pyriproxyfen 0.5 WDG × 0.01 mg/L	22.00[df]	10.00[l]	10.00[c]	
Pyriproxyfen 0.5 WDG × 0.02 mg/L	17.00[eh]	23.00[hi]	5.00[ce]	
Pyriproxyfen 0.5 WDG × 0.03 mg/L	17.00[eh]	30.00[g]	5.00[ce]	
Pyriproxyfen 0.5 WDG × 0.04 mg/L	13.00[gh]	47.00[d]	0.00[e]	
Pyriproxyfen 0.5 WDG × 0.05 mg/L	11.00[h]	67.00[b]	0.00[e]	Non significant interaction
Pyriproxyfen 1.0 WDG × 0.01 mg/L	27.00[bd]	16.00[jk]	2.00[de]	
Pyriproxyfen 1.0 WDG × 0.02 mg/L	16.00[fh]	37.00[f]	0.00[e]	
Pyriproxyfen 1.0 WDG × 0.03 mg/L	19.00[eg]	45.00[de]	0.00[e]	
Pyriproxyfen 1.0 WDG × 0.04 mg/L	4.00[i]	61.00[c]	0.00[e]	
Pyriproxyfen 1.0 WDG × 0.05	0.00[i]	87.00[a]	0.00[e]	
LSD 0.05	6.50	5.83	7.34	

Means followed by the same letter(s) in columns are not significant at 5% level of probability.

significantly affected the mortality of *Aedes* spp. Maximum mortality of 62.33% was recorded at 0.05 mg/L followed by 0.04, 0.03 while minimum mortality was noted in control where no IGR was applied (Table 1). The interaction of various IGRs with different concentrations was also highly significant. Pyriproxyfen 1.0 WDG exhibited maximum mortality at 0.05 mg/L concentration followed by pyriproxyfen 0.05 WDG at 0.05 mg/L concentration.

According to mean square values (Table 3), highly significant ($P \leq 0.01$) differences were observed among various IGRs, concentrations of IGRs and its interaction regarding deformity of *Aedes* species. Highest deformity (14.16%) for *Aedes* was recorded when treated with pyriproxyfen 1.0 WDG. Among various concentrations maximum deformity was recorded in 0.02 mg/L followed by 0.01 mg/L which was statistically at par with 0.03 mg/L concentration. The interaction of IGRs with various concentrations was also highly significant (Table 3) and thus highest deformity (26.00%) was observed in pyriproxyfen 1.0 WDG × 0.02 mg/L.

3.2. *Cx. quinquefasciatus*

Highly significant ($P \leq 0.01$) differences were observed among various IGRs, concentrations of IGRs and its interaction (Table 3) regarding deformity of *Culex* species.

In the course of studies, it was observed that maximum deformity (25.50%) was recorded in methoprene followed by pyriproxyfen 0.5 WDG whereas minimum in pyriproxyfen 1.0 WDG (Table 2). In testing various concentrations, maximum deformity was observed at 0.01 mg/L which was statistically at par with 0.02 and 0.03 mg/L whereas minimum deformity (2.00%) was noticed in control where no IGR was applied. Most of the treatments exhibited minimum interaction for deformity except methoprene × 0.05 mg/L (37.00%) which was statistically similar to the interaction of methoprene × 0.03 mg/L.

The mortality percentage as calculated against different IGRs was also significantly different. Highest mortality of 42.71% was recorded for pyriproxyfen 1.0 WDG followed by pyriproxyfen 0.5 WDG. Whereas, lowest mortality was recorded in methoprene (Table 2). Similarly various concentrations also significantly affected the mortality of *Culex* species. Maximum mortality of 64.67% was recorded at 0.05 mg/L followed by 0.04, 0.03 and so on which were statistically significant to each other, while minimum mortality was noted in control where no IGR was applied (Table 2). The interaction of various IGRs with different concentrations was also highly significant. Pyriproxyfen 1.0 WDG exhibited maximum mortality at 0.05 mg/L concentration followed by pyriproxyfen 0.05 WDG at 0.05 mg/L concentration (Table 2).

The inhibition percentage as calculated against different IGRs, concentrations and their interaction is also expressed in Table 2. Highest inhibition (35.16%) was recorded for pyriproxyfen 0.5 WDG while lowest was recorded for methoprene (Table 2). The inhibition percentage as observed against various concentrations was maximum at 0.01 mg/L which was statistically similar to 0.02 while lowest inhibition percentage against *Culex* species was recorded in control.

In the course of studies, it was observed that minimum emergence percentage (15.00%) was recorded in pyriproxyfen

Table 3
Mean squares of various parameters recorded in *Aedes* and *Culex* species.

Source		*Aedes*				*Culex*			
	df	Deformity	Mortality	Emergence	Inhibition	Deformity	Mortality	Emergence	Inhibition
Replication	3	129.78	48.80	13.90	20.44	15.04	20.54	21.00	7.33
IGRs	2	84.66**	1 090.50**	741.80**	509.56**	1 384.67**	2 916.85**	908.20**	376.22**
Conc	5	764.27**	5 957.03**	15 004.00**	3 840.89**	781.73**	5 273.12**	13 895.20**	4 024.22**
IGRs × Conc	10	36.13*	361.03**	236.30**	706.09**	247.60**	291.73**	67.20**	31.96
Error	51	17.46	12.33	9.10	11.35	211.00	16.90	26.80	26.47
CV%	–	34.83	11.93	12.64	9.69	27.22	13.04	25.94	16.81

CV: Coefficient of variation. *: Significant level of *P* value equal or less than 0.05; **: Highly significant level of *P* value equal or less than 0.01.

1.0 WDG followed by pyriproxyfen 0.5 WDG (21.87%) whereas maximum in methoprene (Table 2). In testing various concentrations, minimum emergence was observed at 0.05 mg/L followed by 0.04 mg/L as compared to control where maximum emergence percentage (89.00%) was noticed. Most of the treatments exhibited minimum interaction for emergence percentage except methoprene × 0.01 mg/L where the interaction was 24.00% followed by methoprene × 0.02 mg/L.

3.3. Efficacy of pyriproxyfen 1.0 WDG treatment in the field

The application of pyriproxyfen, 1.0 WDG in field trials showed significant variation when post treated samples were collected after 1–6 months and tested against the laboratory colony of *Aedes* spp. for mortality and inhibition. Percent mortality (Figure 1) decreased during investigation period and ranged from 46% to 8% within 6 months period. The highest percent inhibition was noted after 4 months duration. Minimum deformity was seen after 1 and 4 months period. No inhibition or malformation was seen at control action. The data showed high percent mortality of *Aedes* larvae after 1 month, inhibition after 2 months and increase in adult emergence after 6 months. Similarly, the efficacy of pyriproxyfen 1.0 WDG treatment in the field showed considerable variations when samples were collected after 1–6 months and tested against the laboratory colony of *Culex* spp. Percent mortality (Figure 2) decreased during experimental time and ranged from 50% to 10%. Lowest mortality was seen after 6 months period. Percent inhibition was also low after 6 months duration. No inhibition or malformation was seen. However, high emergence was recorded at control treatment. The highest manifestation of larval mortality was recorded during first month which decreased after 4 months. The data showed decreasing trend in percent mortality, inhibition and deformity over time from one to six month.

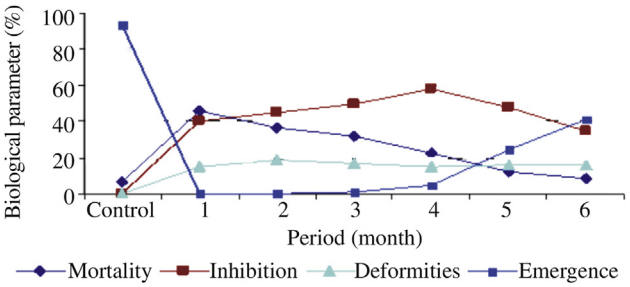

Figure 1. Efficacy of field applied pyriproxyfen 1.0 WDG on mortality, inhibition, deformity and adult emergence of *Aedes* spp. (3rd instar) after 1–6 months period.

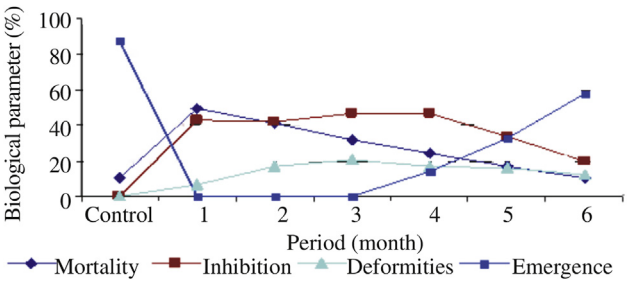

Figure 2. Efficacy of field applied pyriproxyfen 1.0 WDG concentrations on mortality, inhibition, deformity and adult emergence of *Culex* spp. (3rd instar) after 1–6 months period.

4. Discussion

IGRs is a special new class of insecticides which influence insect mortality and growth inhibition in an environment friendly way. This new control strategy was evaluated for the vector control and found effective both in the laboratory and field conditions. Previous researchers have also successfully utilized IGRs as a technique for controlling mosquitoes with pyriproxyfen and methoprene. Pyriproxyfen has been studied by previous investigators, showing high toxicity against *Cx. quinquefasciatus* and *Ae. albopictus* larvae and estimated lethal than methoprene against *Aedes aegypti* (*Ae. aegypti*) larvae. According to Ali *et al.* [38], pyriproxyfen was more effective than diflubenzuron and methoprene, and resulted 21.5 times higher toxicity against *Ae. albopictus* than of S-methoprene, when using the technical grade of each IGR. The superior activity of S-31183 (pyriproxyfen) over S-methoprene against *Anopheles quadrimaculatus* was reported previously by Estrada and Mulla [39]. Thus pyriproxyfen, an IGR, is a juvenile hormone mimic that is highly active against a wide variety of insects of public health importance, including fleas, tsetse flies, houseflies, cockroaches, imported fire ants, chironomid midges, and mosquitoes [40]. The emergence inhibition caused by IGRs at 0.001–0.005 mg/L was also acceptable and in accordance to the study of Trayler *et al.* [41], who reported that pyriproxyfen at 0.01 mg/L caused 90% inhibition of chironomid polypedilum and reduced the emergence of said species. They further stated that pyriproxyfen at 0.01 mg/L significantly reduced the emergence of *Polypedilum nubifer* and *Kiefferrulus intmincrus* (Skuse) for 24 days. Kawada [42] who reported 50% emergence inhibition of *Ae. albopictus* caused by methoprene at 0.0011 mg/L, diflubenzuron at 0.0003 mg/L, and pyriproxyfen at 0.000024 mg/L. Vythilingam *et al.* [43] reported that pyriproxyfen against *Ae. aegypti* at 0.01 and 0.02 mg/L provided 100% control for 4 months.

Some attractive devices contaminated with pyriproxyfen have been shown to attract *Ae. aegypti* towards the station and results in suppression of *Ae. aegypti* populations [44]. In other instances with a fear of vector resurgence due to development of insecticide resistance, IGRs are potential alternative to control mosquitoes, in an environment friendly manner [45–47]. Harburguer *et al.* [48] recorded comparatively low emergence inhibition (20%–40%) and no ovicidal effect on *Ae. aegypti* by releasing pyriproxyfen from a fumigant formulation. However, they reported that the sublethal doses of pyriproxyfen can have effects on fertility and fecundity of *Ae. aegypti* females, which together with its larvicidal activity could contribute to an overall decrease in a given population. Nayar *et al.* [29] reported that pyriproxyfen at comparable treatment rates to S-methoprene and caused very high levels (> 80%–100% in most cases) of initial and residual emergence inhibitions of the tested *Aedes* spp. in the laboratory as well as outdoors.

Our categorization of IGRs in term of efficiency is (pyriproxyfen 1.0 WDG > pyriproxyfen 0.5 WDG > methoprene). This is also in accordance to that of Ali *et al.* [38] who categorized the toxicity ranking of chemicals and microbials tested as IGRs > pyrethroids > organophosphates > microbials.

We observed that 1.0 WDG formulation of pyriproxyfen was highly effective against the larval stages of *Ae. a1bopictus* and *Culex* spp. in the field conditions. In our studies, the mortality, growth inhibition and adult emergence were kept at low from 1

to 4 months period with pyriproxyfen 1.0 WDG in the field. Vythilingam *et al.* [43] reported that pyriproxyfen against *Ae. aegypti* at 0.01 and 0.02 mg/L provided 100% control for 4 months. Sihuincha *et al.* [30] observed that pyriproxyfen prevented adult emergence at extremely low concentrations in the laboratory and field conditions. The decrease in the suppression of the laboratory strain after their exposure to field treated water samples may be due to regular rainfall occurring experiment that has caused the dilution of water in the treated habitats. However, these results were still acceptable up to 6 months period.

IGRs, particularly the two formulations of pyriproxyfen offer an excellent potential for the control of *Ae. albopictus* and *Culex* spp. and require the attention of public health authorities for their use on small scale and area wide control of mosquitoes in the dengue affected areas. This will not only increase in the insecticide free package of the environment friendly program but also help in devising long-term sustainable resistant management strategy for vector mosquitoes of deadly diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors wish to express their profound gratitude to Higher Education Commission (HEC) for financial support under HEC indigenous fellowship 1061060 AV6038 and Pakistan Atomic Energy Commission for study leave. This study form part of Ph.D. thesis of the first author.

References

[1] Suleman M, Arshad M, Khan K. Yellowfever mosquito (Diptera: Culicidae) introduced into Landi Kotal, Pakistan, by tire importation. *J Med Entomol* 1996; **33**(4): 689-93.

[2] Khan J, Khan I, Amin I. A comprehensive entomological, serological and molecular study of 2013 dengue outbreak of Swat, Khyber Pakhtunkhwa, Pakistan. *PLoS One* 2016; **11**(2): e0147416.

[3] Reinert JF, Harbach RE, Kitching IJ. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zool J Linn Soc* 2004; **142**: 289-368.

[4] Yasinzai MI, Kakarsulemankhel JK. Incidence of malaria infection in rural areas of District Quetta, Pakistan. *J Med Sci* 2003; **3**(9): 766-72.

[5] World Health Organization. World malaria report 2009. Geneva: World Health Organization; 2009. [Online] Available from: http://apps.who.int/iris/bitstream/10665/44234/1/9789241563901_eng.pdf [Accessed on 12th December, 2015]

[6] Jahan N, Hussai N. Susceptibility of laboratory reared *Anopheles stephensi* (Diptera: Culicidae) and field collected *Culex quinquefasciatus* larvae to *Bacillus thuringiensis* serovar. *israelensis* and *Bacillus sphaericus* in Lahore, Pakistan. *Pak J Zool* 2011; **43**(5): 915-9.

[7] Mukhtar M, Herrel N, Amerasinghe FP, Ensink J, van der Hoek W, Konradsen F. Role of wastewater irrigation in mosquito breeding in South Punjab, Pakistan. *Southeast Asian J Trop Med Public Health* 2003; **34**(1): 72-80.

[8] Khan HA, Akram W, Shehzad K, Shaalan EA. First report of field evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. *Parasit Vectors* 2011; **4**(1): 146.

[9] Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. *Clin Epidemiol* 2013; **5**: 299-309.

[10] Directorate of Malaria Control, Ministry of Health. Guidelines for dengue vector(s) control during outbreaks/emergence. Islamabad: Directorate of Malaria Control, Ministry of Health; 2009. [Online] Available from: http://www.dmc.gov.pk/documents/pdfs/Dengue_Vetcor_control.pdf [Accessed on 15th December, 2015]

[11] Wikipedia. Dengue outbreak in Pakistan. San Francisco: Wikimedia Foundation, Inc.; 2015. [Online] Available from: https://en.wikipedia.org/wiki/2011_dengue_outbreak_in_Pakistan [Accessed on 25th October, 2015]

[12] Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998; **11**(3): 480-96.

[13] Gubler D. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. *Rev Panam Salud Publica* 2005; **17**: 221-4.

[14] Khan GZ, Khan I, Khan IA. Surveillance and management of mosquito species complex with special emphasis on the dengue vector(s) in Peshawar Valley [dissertation]. Peshawar: the University of Agriculture; 2014. [Online] Available from:, http://www.prr.hec.gov.pk/Thesis/2897S.pdf [Accessed on 30th December, 2015]

[15] Ali N, Marjan, Khan K, Kausar A. Study on mosquitoes of Swat Ranizai sub division of Malakand. *Pak J Zool* 2013; **45**(2): 503-10.

[16] Naeem-Ullah U, Akram W, Suhail A, Rana SA. Grouping of different mosquito species on the basis of larval habitat. *Pak J Agric Sci* 2010; **47**(2): 124-31.

[17] Riaz MM, Mumtaz K, Khan MS, Patel J, Tariq M, Hilal H, et al. Outbreak of dengue fever in Karachi 2006: a clinical perspective. *J Pak Med Assoc* 2009; **59**(6): 339-44.

[18] World Health Organization. The World Health Assembly Resolution on the "Dengue fever and dengue haemorrhagic fever prevention and control". Geneva: World Health Organization; 2002. [Online] Available from: http://www.who.int/denguecontrol/resolutions/en/ [Accessed on 13th March, 2015]

[19] Wang ZM, Li CX, Xing D, Yu YH, Liu N, Xue RD, et al. Detection and widespread distribution of sodium channel alleles characteristic of insecticide resistance in *Culex pipiens* complex mosquitoes in China. *Med Vet Entomol* 2012; **26**(2): 228-32.

[20] Vreysen MJB, Gerardo-Abaya J, Cayol JP. Lessons from area-wide integrated pest management (AW-IPM) programmes with an SIT component: an FAO/IAEA perspective. In: Vreysen MJB, Robinson AS, Hendrichs J, editors. *Area-wide control of insect pests*. Denmark: Springer Netherlands; 2007, p. 723-44.

[21] Hemingway J, Ranson H. Insecticides resistance in insect vectors of human disease. *Annu Rev Entomol* 2000; **45**: 371-91.

[22] Hemingway J, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem Mol Biol* 2004; **34**: 653-65.

[23] Kulma K, Saddler A, Koella JC. Effects of age and larval nutrition on phenotypic expression of insecticide resistance in *Anopheles* mosquitoes. *PLoS One* 2013; **8**(3): e58322.

[24] Oduola AO, Idowu ET, Oyebola MK, Adeogun AO, Olojede JB, Otubanjo OA, et al. Evidence of carbamate resistance in urban populations of *Anopheles gambiae* mosquitoes resistant to DDT and deltamethrin insecticides in Lagos, South-Western Nigeria. *Parasit Vectors* 2012; **5**: 116.

[25] Jirakanjanakit N, Saengtharatip S, Rongnoparut P, Duchon S, Bellec C, Yoksan S. Trend of temephos resistance in *Aedes* (*Stegomyia*) mosquitoes in Thailand during 2003–2005. *Environ Entomol* 2007; **36**(3): 506-11.

[26] Khan I, Badshah T, Saeed M, Khan GZ. Testing efficacy of botanical and mineral kerosene oils on *Culex quinquefasciatus* mortality and their repellency in field ovitraps. *Sci Postprint* 2015; **1**(2): e00050.

[27] Khan H, Khan IA, Khan IU, Sohail K, Khan A, Hussain SH. The efficacy of some plant extracts against *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *J Entomol Zool Stud* 2015; **3**(3): 212-4.

[28] World Health Organization. Report of the 9th WHOPES working group meeting. Geneva: World Health Organization; 2005. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69236/1/WHO_CDS_NTD_WHOPES_2006.2_eng.pdf [Accessed on 25th January, 2014]

[29] Nayar JK, Ali A, Zaim M. Effectiveness and residual activity comparison of granular formulations of insect growth regulators pyriproxyfen and s-methoprene against Florida mosquitoes in laboratory and outdoor conditions. *J Am Mosq Control Assoc* 2002; **18**(3): 196-201.

[30] Sihuincha M, Zamora-Perea E, Orellana-Rios W, Stancil JD, López-Sifuentes V, Vidal-Oré C, et al. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *J Med Entomol* 2005; **42**(4): 620-30.

[31] Lee DK. Field evaluation of an insect growth regulator, pyriproxyfen, against *Aedes togoi* larvae in brackish water in South Korea. *J Vector Ecol* 2001; **26**(1): 39-42.

[32] Wang S, Jacobs-Lorena M. Genetic approaches to interfere with malaria transmission by vector mosquitoes. *Trends Biotechnol* 2013; **31**(3): 185-93.

[33] Wang Y, Yang S, Sun Y. [The arboviruses of genus *Alphavirus* family Togaviridae were isolated from mosquitoes captured from Yantai]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 2000; **14**(2): 181-3. Chinese.

[34] Delatte H, Paupy C, Dehecq JS, Thiria J, Failloux AB, Fontenille D. *Aedes albopictus*, vector of chikungunya and dengue viruses in Reunion Island: biology and control. *Parasite* 2008; **15**(1): 3-13.

[35] Khan I, Farid A, Zeb A. Development of inexpensive and globally available larval diet for rearing *Anopheles stephensi* (Diptera: Culicidae) mosquitoes. *Parasit Vectors* 2013; **6**: 90.

[36] Rueda LM. *Pictorial keys for the mosquitoes (Diptera: Culicidae) associated with dengue virus transmission*. Auckland: Magnolia Press; 2004.

[37] Mulla MS, Darwazeh HA, Norland RL. Insect growth regulators evaluation procedures and activity against mosquitoes. *J Econ Entomol* 1974; **67**: 329-32.

[38] Ali A, Nayar JK, Xue RD. Comparative toxicity of selected larvicides and insect growth regulators to a Florida laboratory population of *Aedes albopictus*. *J Am Mosq Control Assoc* 1995; **1**: 72-6.

[39] Estrada JG, Mulla MS. Evaluation of two new insect growth regulators against mosquitoes in the laboratory. *J Am Mosq Control Assoc* 1986; **2**(1): 57-60.

[40] Hirano M, Hatakoshi M, Kawada H, Takimoto Y. Pyriproxyfen and other juvenile hormones analogues. *Rev Toxicol* 1998; **2**: 357-94.

[41] Trayler KM, Pinder AM, Davis JA. Evaluation of the juvenile hormone mimic pyriproxyfen (S-31183) against nuisance chironomids (Diptera: Chironomidae), with particular emphasis on *Polypedilum nlcbifer* (Skuse). *J Aust Entomol Soc* 1994; **33**: 127-30.

[42] Kawada H. Can mosquitoes be carriers of larvicides? Potential new strategy for mosquito control using insect growth regulator. In: Proceeding of International Conference on Insect Pests in the Urban Environment; 1993. Cambridge, England.

[43] Vythilingam I, Luz BM, Hanni R, Beng TS, Huat TC. Laboratory and field evaluation of the insect growth regulator pyriproxyfen (sumilarv 0.5 G) against dengue vectors. *J Am Mosq Control Assoc* 2005; **21**(3): 296-300.

[44] Ponlawat A, Fansiri T, Kurusarttra S, Pongsiri A, McCardle PW, Evans BP, et al. Development and evaluation of a pyriproxyfen treated device to control the dengue vector, *Aedes aegypti* (L.) (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health* 2013; **44**(2): 167-78.

[45] Belinato TA, Martins AJ, Lima JB, Valle D. Effect of triflumuron, a chitin synthesis inhibitor, on *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* under laboratory conditions. *Parasit Vectors* 2013; **6**: 83.

[46] Ohba SY, Ohashi K, Pujiyati E, Higa Y, Kawada H, Mito N, et al. The effect of pyriproxyfen as a "population growth regulator" against *Aedes albopictus* under semi-field conditions. *PLoS One* 2013; **8**(7): e67045.

[47] Lau KW, Chen CD, Lee HL, Norma-Rashid Y, Sofian-Azirun M. Evaluation of insect growth regulators against field-collected *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) from Malaysia. *J Med Entomol* 2015; http://dx.doi.org/10.1093/jme/tju019.

[48] Harburguer L, Zerba E, Licastro S. Sub-lethal effect of pyriproxyfen released from a fumigant formulation on fecundity, fertility, and ovicidal action in *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 2014; **51**(2): 436-43.