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

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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

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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