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Efficacy of Larvicides against *Aedes aegypti* Larvae in Laboratory Conditions in Lahore, Pakistan

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SN and SR Conception, design of the study, acquisition of data, analysis and interpretation of data and manuscript write, reviewed and approved the final manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: *Aedes aegypti* is a known vector mosquito transmitting world's deadliest disease dengue fever. Vector control by chemo and bio pesticides is popular method for control but due to resistance development in adult and larvae of *Aedes aegypti,* it becomes a serious threat. Present study was planned to assess the efficacy of two insecticides, *Bacillus thuringiensis* var *iaraelensis* and pyriproxyfen against *Aedes aegypti* larvae *Bacillus thuringiensis* is a bacterium that produce toxin and act as a bio pesticide.

Methods: Efficacy of *Bacillus thuringiensis* bioassay was done and mortality was recorded after every thirty minutes for three hours. Larvae were placed in the depression slides with a little amount of water that is sufficient for the larval movement and were examined under microscope. 20 larvaes put in 50ml water in a beaker, and added 100 µl *Bacillus thuringiensis* toxins, average mortality was recorded 9.50%. Efficacy of pyriproxyfen was evaluated by putting it in the 100 ml glass beakers containing 50ml of tap water and maintained at 25±1°C.

Results: *Aedes aegypti* larvae were also exposed to three concentrations (1ppm, 0.5ppm and 0.25ppm) of pyriproxyfen "insect growth regulator" for three weeks, and mortality rate was recorded after every 24 hours. Pyriproxyfen inhibited emergence of *Aedes aegypti* even at very low dose. Comparative analysis between *Bacillus thuringiensis* and pyriproxyfen revealed that highest mortality (13.50%) was recorded by pyriproxyfen, as compared to *Bacillus thuringiensis* (9.50%).

Conclusion: Pyriproxyfen is useful larvicides, especially in fields where larvae have developed resistance against other larvicides.

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Keywords: Bacillus thuringiensis; bacterium; mosquito; pesticides; pyriproxyfen.

1. INTRODUCTION

Mosquitoes are vectors of many diseases in tropical and subtropical regions of the world. *Aedes aegypti* is a world's deadliest mosquito of many diseases, including dengue [1]. Recently, there has been development of alternative methods for control of mosquito vector, use of microorganisms, mostly bacteria as biological control agent against disease causing agent is simpler than the chemical insecticides which cause resistance in insects and pollution in the environment [2]. For the control of larvae of mosquitoes, certain strains of bacteria, especially *Bacillus thuringiensis* var *israelensis* (*Bti*) and *Bacillus (B.) spharicu*have been used and these are called biocides or biolarvicides (Mittal, 2003). Biolarvicides based on the mosquitocidal toxins of *Bacillus thuringiensis* H-14 have great potential in controlling the mosquito vectors of various diseases in an integrated vector control programs, as these bacterial agents are highly specific in action against mosquitoes and are safe to other organisms [3]. Biolarvicides, obtained from mosquitocidal toxins of certain strains of *Bacillus spharicus* and *B. thuringiensis var israelensis* H-14(*Bti*) were very effective against the mosquito larvae at low dose. *B. spharicus* were useful against *Culex* and *Anopheles* Stephensi Plasmodium parasites, but not for *Aedes aegypti*. On the other hand, *Bti* formulations have great activity against *Aedes, Culex* and *Anopheles* Stephensi Plasmodium parasites had no resistance. Pyriproxyfen is called as "insect hormone mimics" or "insects growth regulators" (IGR's) that affects the physiological regulatory processes that are necessary for the normal development of insects or their generation or progeny. An IGR's does not appear too toxic to target insects but may cause abnormalities that cause death of an insect [4-5]. Previous studies have proved that the control of mosquitoes is much easier at the larval stage as compared to adults, so in present study the larvae of *Aedes* were selected to assess the comparative efficacy of *Bacillus thuringiensis* var *israelensis* (*Bti*) and Pyriproxyfen.

2. MATERIALS AND METHODS

For assessment of efficacy of Bti, strains were isolated and identified according to the standared method as described [6,7] and [8] and test was applied against the *Aedes aegypti* larv Aedes Larvae were placed in the depression slides with a little amount of water that is sufficient for the larval movement and were examined under microscope. Hence, several morphological characteristics were noted such as presence of hair in different parts of body, structure of segments, number of hair in the antenna and siphon etc. All strains of *B.thuringiensis* were grown in the T3-media plates for 48 h. 100µl of the bacterial toxins were added in the 100ml beaker containing 50 ml of water separately. 20 larvae's were introduced in each beaker. There were total 30 treated beakers and one was taken as control. Mortality rate was recorded in each treated and controlled beaker after every thirty minutes for at least three hours [2].

Efficacy of pyriproxyfen was evaluated by putting it in the 100 ml glass beakers containing 50ml of tap water and maintained at 25±1°C in room temperature. Fifteen 3 instar and 15 4 instar larvae of *Aedes aegypti* mosquito were introduced in each beaker along with 22 mg of larval food (6:1 yeast and beef liver powder). Pyriproxyfen formulation was applied at 1ppm, 0.5ppm and 0.25ppm active ingredient against *Aedes aegypti* larvae in each beaker. Three replicates (R_1, R_2, and R_3) of each treatment and untreated beakers to serve as controls were maintained for *Aedes aegypti*. All the beakers were examined daily to count the post treatment larval and pupal mortality or survival and adult emergence. A camel headed brush was used to remove the dead larva, pupa on daily bases. Survival in the treated and controlled was checked by counting the number of pupa in the beakers. After 1 week, when all the larvae and pupa had either died or survived as adults in the control, a fresh batch of 10 3 instar and 4 instar larvae of the *Aedes aegypti* were introduced in each treated and controlled beakers (dead and alive larvae and pupa were collected by brushes and pipette before the introduction of a 2^{nd} batch of larvae) and routine post treatments mortality and survival were continued. In this manner, the residual activities of each concentration were determined. Each beaker was covered with fine mesh cloth to protect from air borne debris, wild insects and any oviposition by wild mosquito [9].

2.1 Statistical Analysis

Microsoft Excel 2010 and Statistical version 8.1 (Analytical Software, 2005) were used for statistical analysis. Data were analyzed for

significant variation by one way ANOVA per day every week for three weeks for three concentrations (1ppm, 0.5ppm and 0.25ppm) and control for pyriproxyfen (IGR). Data were also analyzed for significant variation by one way ANOVA for every thirty minutes up to three hours for *Bti*. Comparative analysis between pyriproxyfen (IGR) and *Bti* were analyzed for significant variation by one way analysis of variance (ANOVA) was used to find out the variations between the efficacies. Probability level <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Soil samples were collected from 26-different localities of Lahore (i.e.,Iqbal town, Doctor Hospital, Expo-center, Industrial Area, L.C.W.U, Gulshan-e-ravi, Defence, Anarkali, Wapda Town, Jahangirabad (NWFP), Faisal Town, CEMB, Jinnah garden, Samnabad, Sandah, Jail road, Dharampura, Model Town, River Ravi, Awan Town, Shadman, Gulshan-e-ravi-A, B, C, D Blocks, Bahria Town). Only five soil samples (from Doctor Hospital, Expo-center, Industrial Area, Jahangirabad (KPK) and Thokar Niazbaig (CEMB) showed positive results. The toxins called Cry-toxins dissolved the midgut of *Aedes* larvae as larvae feed on the toxins. Larvae died due to starvation. It was revealed that mortality rate from *Bti* against *Aedes aegypti* larvae don't vary, it remained same. The completely randomized analysis of variance for different localities of *Bti* against *Aedes aegypti* in 30min, 1 hour, 1:30 min, 2 hours, 2:30 min and 3 hours showed the following results (F=0.83; df=4, 9; P=0.557), (F=0.23; df=4, 9; P=0.911), (F=2.17; df=4, 9; P=0.209), (F=1.17; df=4, 9; P=0.424), (F=0.25; df=4, 9; P= 0.898) and (F=2.75; df=4, 9; P=0.148). Survival probability *Aedes aegypti* larvae against Bti in 3 hours observed after every thirty minutes. It was also revealed from the Table 1 that the mortality rate in 1ppm concentration was high and rapid. The *Aedes* larvae which were exposed to 1ppm concentration died maximum in the first three days. The *Aedes* larvae which were exposed to the 0.5ppm concentration maximum died in the first four days. 0.25ppm concentration exposed *Aedes* larvae took more than five days to become inactive. In the control beakers at 0 ppm, the mortality rate was very low. It was revealed from the completely randomized analysis of variance for different concentrations of Pyriproxyfen against *Aedes aegypti* larvae mortality was observed highest in week-1 Whereas, minimum mortality was observed in control.

The morphogenetic effect of pyriproxyfen is primarily seen during larval-pupal transformation. Therefore, death occurs at the pupal stage and adult mosquitoes fail to emerge. Pyriproxyfen can inhibit emergence of Aedes aegypti at very low dose rates. The LC_{50} has been shown to be 0.012 ppb. However, in order to achieve complete inhibition of adult emergence and prolong the duration of control the actual field dose rates are higher than this, with label rates of 0.25ppm, 0.50ppm and 1.00ppm. 1.00ppm concentration of IGR showed highest mortality rate among all concentration and this effect persisted for up to three weeks showing still highest mortality rate in three weeks.

Table 1. Comparison of mean mortality rate of Aedes aegypti larvae against different concentration of pyriproxyfen within twentyone days trial

Days	Concentrations			
	1ppm	0.5ppm	0.25ppm	0ppm
0	8.33 (A)	4.667 (B)	4.333 (B)	0.00 (C)
1	12.33 (A)	8.667 (B)	8.50 ©	0.00 (C)
3	15.00 (A)	11.33 (B)	13.00 (C)	0.00 (D)
5	15.00 (A)	1230 (A)	12.33 (B)	0.00 (C)
7	8.66 (A)	9.88(B)	10.66 (B)	0.00 (C)
9	8.00 (A)	8.28(B)	9.00(B)	0.00 (C)
11	7.00 (A)	8.00(B)	8.88(B)	0.00 (D)
13	7.00(A)	8.00(B)	8.00(B)	0.00 (C)
15	5.00(A)	7.00(B)	7.50(B)	0.00 (C)
17	3.00(A)	5.00(B)	6.00(B)	1.00 (C)
19	1.00(A)	3.00(B)	4.00(B)	1.00 (D)
21	0.00 (A)	0.00 (B)	1.00(B)	2.00 (C)

Comparative analysis of *Bti* and Pyriproxyfen (IGR) against *Aedes* larvae showed that the highest mortality was observed (13.5000 or 13.50%) when the *Aedes* larvae were treated with slow acting insecticide Pyriproxyfen (IGR), whereas minimum mortality was observed (9.5000 or 9.50%) when the *Aedes* larvae were treated with bio-insecticide *Bacillus thuringiensis var israelensis toxins (Bti)*. The comparative analysis for 24-hour showed that the Pyriproxyfen was more effective against the *Aedes aegypti* larvae as compared to Bti. Because the mean mortality were higher in (IGR) and lower in *Bti* treated larvae and also showed that the mortality rate of *Aedes aegypti* larvae from Pyriproxyfen were greater than and lower from *Bti*.

The results of present study coincides with study of [2] the ubiquity of *Bacillus thuringiensis* in soil supports the hypothesis several authors suggesting that this is the normal environment of *Bacillus thuringiensis*. Thus, the positive bacterial strain showed highest larvicidal potency with LC_{50} value of 0.36cfu/ml at 24 h and 0.57cfu/ml at 48 h. The results of the study showed that *Bti* isolated from soil is promising as larvicides against the target Dengue virus carrying mosquito. *B. thuringiensis* acts as a larvicides due to the toxin produced, which is activated by the alkaline midgut of the mosquito larvae and disrupts the midgut epithelia.

As Pyriproxyfen is classified as a juvenile hormone (JH) analog and has been used against a range of arthropods since its introduction to the agrochemical market in the early 1990s. The World Health Organization has recently recommended that it be used for the control of some mosquito species [10].

Nayar [11] determined the effectiveness and residual activity comparison of granular formulations of insect growth regulators Pyriproxyfen and s-methoprene against Florida mosquitoes in laboratory and outdoor conditions. Pyriproxyfen at 0.02 and 0.05ppm rates monitored for 6 weeks after treatment induced almost 100% emergence inhibition of *Aedes aegypti* in the laboratory as well as in the tubes, whereas s-methoprene was less effective, reducing emergence of this species 22.3-93.7% in laboratory and 10.3-100% in tubes, even at higher rate of 0.05ppm. The activity profile of s-methoprene for the 1st two weeks after treatment in laboratory and 1 week after treatment in tubes at high rate of 0.05ppm was similar to that of pyriproxyfen, but thereafter, pyriproxyfen showed much higher levels of sustained residual activity against *Aedes aegypti*. Itoh, 1993 reported that a synthetic slow-releasing formulation of pyriproxyfen (0.05% AI) exhibited prolonged activity against larvae of *Aedes aegypti* even when the treatment was diluted by using and replenishing water in the treated jars. Pyriproxyfen induced 52.7-100% (at 0.02ppm) and 93-100% (at 0.05ppm) emergence inhibition in the laboratory. Pyriproxyfen was distinctly superior over s-methoprene in terms of magnitude and duration. Pyriproxyfen at 0.1ppm and .2ppm completely inhibited adult emergence.

Our results are also coincides with the results of [12,13,14]. From the present study, it can be concluded that Biolarvicides based on mosquitocidal toxins of *B. thuringiensis* H-14 had great potential in controlling the breeding of mosquito vectors of Dengue fever. These bacterial agents were highly specific in action against mosquitoes. *Bti* toxins dissolved the midgut of *Aedes aegypti* larvae act as an enema. *Bacillus thuringiensis var israelensis* results were non-significant (P>0.05), mean mortality rate of *Aedes aegypti* larvae from different soil sample do not vary and remain same. The insect growth regulator (IGR) pyriproxyfen exhibits juvenoid activity and efficiently suppressing adult emergence, resulting in pupal mortality. *Aedes aegypti* larvae were tested under three concentration 1ppm, 0.5ppm and 0.25ppm. The highest mortality was observed when the larvae were treated at 1ppm concentration followed by 0.5ppm and 0.25ppm. Whereas minimum mortality was observed in control group. Pyriproxyfen (IGR) results were highly significant up to three weeks. Mean mortality rate of *Aedes aegypti* larvae differs in different concentrations. The dengue vectors are highly susceptible to the pyriproxyfen (IGR), followed by the microbial control agents *B. thuringiensis var israelensis*. The highest mortality was observed when the *Aedes aegypti* larvae were treated with Pyriproxyfen (IGR), whereas minimum mortality was observed when the *Aedes aegypti* larvae were treated with bio-insecticide *Bacillus thuringiensis var israelensis* (*Bti*) for 24 hours showing significant difference in efficacy.

4. CONCLUSION

The current study concluded that Pyriproxyfen was more effective than the bio-insecticide (*Bti*)

against the *Aedes aegypti* larvae. Especially, in fields where larvae have developed resistance against other larvicides.

ETHICS APPROVAL

The University Institute of Public Health Committee and the Research Ethics group of the University of Lahore gave ethical approval.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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