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Evaluation of bio-efficacy and residual activity of pyriproxyfen against field collected aedes aegypti and aedes albopictus from Gujranwala (Punjab), Pakistan

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Abstract

Background: Dengue disease is a major health problem in tropical and subtropical region of the world. Pakistan has also serious issues on this infectious disease in the form of DF, DHF & DSS since 1994 when it was declared the first epidemic in Pakistan. Since then, many epidemics in different cities of Pakistan have been occurring on regular basis. The ever worst epidemic was occurred in 2011 in Punjab province where more than 286857 suspected cases and 21685 confirmed cases with 350 deaths were reported by Health Department. There is an urgent need for entomological and epidemiological studies on large scale to control the disease. The best known strategy is the effective implication of Integrated Vector Management (IVM) through adaptation of alternative and viable means vector control.

Methodology: The recent study was conducted in district Gujranwala-Punjab, Pakistan to evaluate the Bio-efficacy of pyriproxyfen (a juvenile hormone analogue) against field collected mosquitoes (larvae) of dengue vectors. *Aedes aegypti* and *Aedes albopictus* were collected from three different localities of Aroop Town. Collected mosquitoes were reared to get fresh larvae according to the WHO recommended rearing procedure. Larvae were treated with pyriproxyfen (Predator 0.5 WDG) at the WHO recommended dose of 0.01mg/l of a.i. The tests were conducted in triplicate form along with a control. A fresh lot of 25 larvae were added in all treated as well as in control cups on every ten days interval to determine the residual activity of the tested product.

Result: The bio-efficacy of pyriproxyfen was found effective against field collected *Aedes aegypti* and *Aedes albopictus*. The complete Inhibition of Adult Emergence (EI) was observed 58 days and 47 days for *Aedes aegypti* and *Aedes albopictus* respectively. The results also show that the residual activity of pyriproxyfen against *Aedes aegypti* is greater than *Aedes albopictus* as 68 days and 59 days (up to 80 % EI) respectively.

Conclusions: Pyriproxyfen can be used to control the dengue vectors (*Aedes aegypti* and *Aedes albopictus*) and it can be included in integrated vector control strategy. It is very effective and safer to use for vector control as non-conventional usage of chemical insecticides.

Keywords: Dengue Disease, Vector control, Insecticides, Non- conventional usage of chemicals, Pyriproxyfen.

1. Introduction

Dengue disease has become a major public health concerns worldwide. Over the past three decades, a dramatic increase has been observed in the frequency of the disease. WHO estimated that about 2.5 billion people of the tropical and subtropical countries are at dengue risk and 500 000 are admitted in hospitals each year and die at the rate of 2.5 %. Children are mainly affected ~90% having aged < 5 years [1]. The most affected regions of the world are South-East Asia, Western Pacific and America, among 100 countries [2].

Pakistan is also facing this major public health problem having its morbidities and mortalities due to certain factors like unplanned urbanization, poor water supply system and inadequate waste management. The other important factors are lack of effective vector control activities, lack of financial and human resources. According to the WHO Weekly Epidemiological Monitor, there are 1931 lab confirmed cases with 41 deaths in 2006, 1226 lab confirmed cases with 18 deaths in 2007, 2469 lab confirmed cases with 17 deaths in 2008, 1085 lab confirmed cases with 13 deaths in 2009, 11024 lab confirmed cases with 40 deaths in 2010 and 17057 lab confirmed cases with 219 deaths in 2011 [3]. Dengue cases are still being reported by health department from different localities of the Pakistan.

Dengue is a viral disease spread by the biting of female *Aedes* mosquitoes**.** *Aedes aegypti* is considered as primary vector and *Aedes albopictus* is secondary, which breed most likely in artificial containers of in or around the human dwellings depending on certain environmental factors [4]. In case of dengue disease, there is still no availability of vaccine or any effective drugs. The solution to overcome at primarily is "vector control" through four major classes of insecticides in the form of adulticides i.e. IRS (Indoor residual spray), Fogging (Space Spray), ITNs (Insecticide treated nets) as well as larvicides by conventional method of chemical usage [5]. Mostly Pyrethroids are considered the best option for public health. But selection pressure and extensive use of insecticides in agriculture sector are developing insecticidal resistance which causes not only incapability to control the vector but also leave serious adverse effects on the non-target organisms [6]. Another way of vector control is the utilization of Insect Growth Regulators (IGRs) which have shown very effective role in vector control. Few years back some natural and synthetic chemicals like pyriproxyfen and methoprene are being used to inhibit metamorphosis of target vectors which do not harm to non-target organisms [7]. There are two categories of IGRs, the first is Juvenile Hormone Analogues (JHAs) which disturbs normal function of juvenile hormone and the

second is Chitin Synthesis Inhibitor (CSIs) which delays the chitin synthesis and in both cases, it leads to the ultimately death of target species.

Jahan and Shahid (2013) conducted a test against *Aedes aegypti* collected in Lahore Pakistan and indicated a resistance of Deltamethrin 1.5% EC and Cypermethrin 10% EC during spray operation. It was evaluated that Deltamethrin showed greater resistance than Cypermethrin [8]. Temephos (OP) is considered relatively safer larvicide and has many threats by its permanent applications as a mosquito larvicide [9]. Certain reasons that organophosphates are acetyl-cholinesterase inhibitors [10]. Hazardous effect on non-targeted water life [11] and some scientists considered that OP has geno-toxicity effects on human [12].

So, there is an urgent need to evaluate the efficacy of non-conventional chemical like IGRs that could be helpful and have an additive advantage to control dengue vectors as a part of IVM. This recent study has been framed for the evaluation of the bio-efficacy and residual activity of pyriproxyfen against larvae of *Aedes aegypti* and *Aedes albopictus* collected from Gujranwala, Pakistan.

Method: Experimental tests were carried out in Gujranwala which is situated at 32.16° North latitude, 74.19° East longitude and 231 meters above the sea level [13]. Pyriproxyfen was used as non-conventional insecticide against *Aedes* mosquitoes collected from three different places in Aroop Town of District Gujranwala, Pakistan [14]. The dosage rate was used as @ 0.01 mg/l a.i. as recommended by WHOPES [15]. *Aedes* adult mosquitoes were collection by battery operated CDC Sweeper and by mouth aspirator from different places of District Gujranwala at the timing of 07:00 -11:00 hours. Sorting and identification of collected mosquitoes was done in the entomological lab using standard pictorial key [16]. Each species of collected mosquitoes were kept in separate cages and provided 10 % sugar for their nourishment and albino mice were provided as source of eggs. Rearing techniques and test procedures were conducted as per standard WHO guidelines [17]. Temperature and humidity 25± 5^{C} and 75± 5 % respectively and 12:12 day and dark hours were maintained. In each cage, strips of filter paper in an enamel tray were used for the oviposition. The acquired strips of filter paper with laid eggs were dipped in a large size cup containing tap water to get fresh larvae. A pinch of chicken liver powder added in the cup for nourishment to the young larvae. Experimental tests were conducted in triplicate form. Total 9 cups were used for each species of *Aedes* larvae for treated experiment. 25 healthy and late 3rd or early 4th instars larvae of each species were added into each cup. Unhealthy, damaged and small larvae were discarded. Recommended dose of pyriproxyfen (0.01 mg/l of a.i.) was added into each treated cup. Any contamination was avoided by fine netting. Emergence of adults or mortality was observed on daily basis until no live larva or pupa left over after 24 hours. A total of 8 and 7 experimental tests were conducted against *Aedes aegypti* and *Aedes albopictus* respectively and fresh larvae were introduced on 10 days intervals. Control tests for each species were run on parallel basis and showed no significant % of inhibition of adult emergence (%EI). Bio-efficacy and residual activity of pyriproxyfen was observed from 1 to 80 days for *Aedes aegypti* and 1 to 70 days for *Aedes albopictus*. Data was analysis by using SPSS (v 20) to calculate P-value and Chi square.

Results: All tests were conducted on field collected species of *Aedes aegypti* and *Aedes albopictus* in Gujranwala. The results of these tests have been mentioned as following in the form of tables and graph.

Bio-Efficacy and Residual Activity of Pyriproxyfen against *Aedes Aegypti* at All 3 Localities in Gujranwala

Table 1: (Cumulative): % Inhibition of Adult Emergence (%EI) Against Field Collected *Aedes Aegypti* From 1 To 80 Days of Treatment of Pyriproxyfen 0.5% WDG In Gujranwala.

Days Of Treatment	Dates Of Data Collection	Mall Khana								Junk Market								Model Town								Total No. Of Larvae	Total No. Of Adult	% EI	X2	P-Value
		R1 Treated		R2 Treated		R3 Treated		R4 Control		R1 Treated		R2 Treated		R3 Treated		R4 Control		R1 Treated		R2 Treated		R3 Treated		R4 Control						
		No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult	No. Of Larvae	No. Of Adult					
1-10	30.10.15 To 08.11.15	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	25	225	0	100	-	-
11-20	09.11. 15 To 18.11.15	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	24	225	0	100	-	-
21-30	19.11. 15 To 28.11.15	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	25	225	0	100	-	-
31-40	29.11.15 To 08.12.15	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	24	225	0	100	-	-
41-50	09.12. 15 To 18.12.15	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	25	225	0	100	-	-
51-60	19.12.15 To 28.12.15	25	0	25	1	25	0	25	25	25	0	25	1	25	0	25	25	25	1	25	1	25	1	25	24	225	5	97.8	4.09	0.84
61-70	29.12.15 To 07.01.16	25	5	25	4	25	4	25	25	25	4	25	5	25	5	25	25	25	4	25	5	25	4	25	25	225	40	82.2	0.60	1.00
71-80	08.01.16 To 14.01.16	25	24	25	25	25	24	25	24	25	24	25	25	25	24	25	24	25	24	25	23	25	24	25	25	225	217	3.6	3.37	0.90

% EI= Percentage Inhibition of adult's emergence

P-Value= level of significance P<0.05, P>0.05 Non significance, $\chi 2$ = Chi-square value.

Bio-efficacy and residual activity of pyriproxyfen was observed from 1 to 80 days. A total of 8 experimental tests at 10 days interval were conducted against *Aedes aegypti* as shown in table No. 1

The result from 1-10 days reveals the complete effectiveness of the pyriproxyfen against field collected *Aedes aegypti* that can used for the vector control in the field operation. Its complete % EI was continued up to 1-50 days treatment. Residual activity was dropped to 98.7 % EI on 51-60 days and dropped to 82.7 % EI on 61-70 days. Where, up to 80 % EI was considered as cut of values for its effectiveness of the treated product. On days 71-80, the % EI dropped to 3.6, which indicates ineffectiveness of the treated product. The test results from 51-80 days of the treatment, evaluated non-significant difference within the replicates. The value of chi square and significance (p-value) for 51-60 days was 0.84 and 4.09, while from 61-70 days and 71-80 days it was 1.00 and 0.60, 0.90 and 3.37 respectively. This indicates that both values are greater than 0.05 and there is non-significant difference within replicates which shows that tested population was homogeneous. The difference in results from previous days is because of reason that pyriproxyfen efficacy was drop down day by day as shown in results. However, by counting total days in series for residual activity, pyriproxyfen was found complete effective for 58 days and up to 80 % EI till 68 days of treatment against *Aedes aegypti.* In control test there was no significant percent of % EI) because percent of adult emergence in control was > 95.

2. Bio-Efficacy and Residual Activity of Pyriproxyfen against *Aedes Albopictus* at All 3 Localities in Gujranwala.

Bio-efficacy and residual activity of pyriproxyfen was observed from 1 to 70 days. A total of 7 experimental tests at 10 days interval were conducted against *Aedes albopictus* as shown in table No. 2. The result from 1-10 days reveals the complete effectiveness of the pyriproxyfen against field collected *Aedes albopictus* that can used for the vector control in the field operation.

Table 2: (Cumulative): % Inhibition of adult emergence (%EI) against field collected *Aedes albopictus* from 1 to 70 days of treatment of pyriproxyfen in Gujranwala.

Days of Treatment	Dates of data collection	Mall Khan								Fattomund Graveyard								DHDC Hostel								Total No. of larvae tested	Total No. of adult emerged	% EI	$\chi 2$	P-value
		R1 Treated		R2 Treated		R3 Treated		R4 Control		R1 Treated		R2 Treated		R3 Treated		R4 Control		R1 Treated		R2 Treated		R3 Treated		R4 Control						
1-10		No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult	No. of Larvae	No. of Adult					
11-20	30.10.15 to 08.11.15	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	25	225	0	100	-	-
21-30	09.11.15 to 18.11.15	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	25	225	0	100	-	-
31-40	19.11.15 to 28.11.15	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	25	25	0	25	0	25	0	25	25	225	0	100	-	-
41-50	29.11.15 to 08.12.15	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	24	25	0	25	0	25	0	25	24	225	0	100	-	-
51-60	09.12. 15to 18.12.15	25	0	25	2	25	1	25	25	25	2	25	1	25	1	25	25	25	1	25	2	25	1	25	24	225	11	95.1	3.05	0.93
61-70	19.12. 15to 28.12.15	25	5	25	4	25	5	25	25	25	5	25	5	25	5	25	24	25	5	25	6	25	5	25	25	225	43	80.9	0.80	0.99

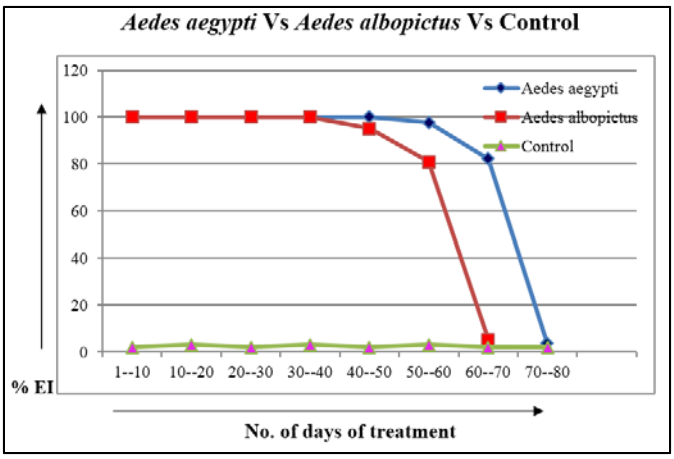
% EI= Percentage Inhibition of adult's emergence

P-Value= level of significance P<0.05, P>0.05 Non significance, $\chi 2$ = Chi-square value

Its complete % EI was continued up to 1-40 days treatment. Residual activity was dropped to 95.1 % EI on 41-50 days and dropped to 80.9 % EI on 51-60 days. Where, up to 80 % EI was considered as cut of values for its effectiveness of the treated product. On days 61-70, the % EI dropped to 5.3, which indicates ineffectiveness of the treated product. The test results from 41-70 days of the treatment, evaluated non-significant difference within the replicates. The value of Chi square and significance (P-value) for 41-50 days was 0.93 and 3.05, while from 51-60 days, and 61-70 days it was 0.99 and 0.80, 0.92 and 3.16 respectively.

This indicates that both values are greater than 0.05 and there is non-significant difference within replicates which shows that tested population was homogeneous. The difference in results from previous days is because of reason that pyriproxyfen efficacy was drop down day by day as shown in results. However, by counting total days in series for residual activity, pyriproxyfen was found complete effective for 47 days and up to 80 % EI till 59 days of treatment against *Aedes albopictus.* In control test there was no significant percent of % EI because percent of adult emergence in control was > 95.

Graphically representation of comparative results of *Aedes aegypti* and *Aedes albopictus* in respect to control:



The results indicate that pyriproxyfen is effective against dengue vectors *Aedes aegypti* and *Aedes albopictus* collected from field in Gujranwala on WHO recommended dosage of 0.01 mg/l. The residual activity of this product for several weeks supports its feasibility to control the vector. The complete % EI of *Aedes aegypti* dropped down after 58 days to 68 days (up to 80 % EI) and *Aedes albopictus* dropped down after 47 days to 59 days (up to 80 % EI) which shows the effectiveness of pyriproxyfen.. In control tests there was no significant percent of % EI because the percent of adult emergence in control was > 95.The results also show that the residual activity of pyriproxyfen against *Aedes aegypti* is greater than *Aedes albopictus* as 68 days and 59 days (up to 80%) respectively.

Discussion

Dengue disease is a major public health issue in the world and Pakistan is also facing a severe concern over this emerging disease. World Health Assembly (WHA) in 2010 stressed to reduce the reliance on insecticides for vectors control through promotion and approaches of Integrated Pest Management (IPM). It was also stressed to adopt alternatives and viable methodology for disease vector control [18]. A number of scientists conducted trials on IGRs and evaluated its effectiveness and residual effect for several weeks. Invest and Lucas (2008), Belinato *et al.* (2013) and Nayar *et al.* (2002) evaluated the effectiveness of IGRs [19-21]. They also considered that utilization of IGRs is the effective way to control the vectors.

The results of our study are in accordance with Nayar *et al.* (2002) as bio-efficacy and residual effects of pyriproxyfen has shown EI for several weeks. The result shows complete % EI up to 6 weeks. In our results pyriproxyfen remained effective (complete) up to 58 (about 8 weeks) and 47 days (about 7 weeks) and effective (80% EI) up to 75 and 68 days of *Aedes aegypti* and *Aedes albopictus* respectively. Our results have accordance with Okazawa *et al.* (1991) as he has shown % EI up to 60 days [22]. Our results showed the affinity with Hemingway and Bonning (1988) as they evaluated the complete efficacy and residual activity of IGR up to 71 days [23]. However, our results showed the difference to some extent with Darriet and Corbel (2006) and Mbare *et al.* (2013) as they have evaluated 97 % EI and 85 % EI up to six weeks at the dosage rate of 1 mg ai/ m^2 respectively [24, 25]. The difference may be due to dosage rate, local mosquito species, environmental factors, treated breeding places and change in the formulation. However, large scale experimental tests are recommended in the field for further assessment/studies of this product.

Conclusions

Results indicate that the field collected dengue vectors *Aedes aegypti* and *Aedes albopictus* are fully susceptible to WHO recommended dosage of 0.01 mg/l of pyriproxyfen. The results also show that the residual activity of pyriproxyfen against *Aedes aegypti* is greater than *Aedes albopictus* as 68 days and 59 days (up to 80%) respectively. On the basis of recent studies, we can conclude that the granular formulation of pyriproxyfen @ 0.01mg/l provides significant control on larvae of dengue vectors for reasonable time. The residual activity of this product remains for several weeks which support its practicability for an effective tool to control the dengue vector as a part of IVM strategy. This product can safely be used for larviciding against dengue vector which could be an alternative and viable strategy over temephos and other larviciding techniques in Pakistan.

References

1. WHO Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Hemorrhagic Fever. 2011; 60:16, ISBN 978-92-9022-387-0.
2. Nogueira RM, Miagostovich MP, Schatzmayr HG, Santos FB, Araujo ES, Filippis AM, *et al.* Dengue in the state of Rio de Janeiro, Brazil, 1986-1998. Memorias do Instituto Oswaldo Cruz. 1999; 94(3):297-304.
3. WHO Weekly Epidemiological Monitor. Editorial note on Dengue fever in Pakistan. 2013; 6(37): ISSN 2224-4220.
4. WHO Fact Sheet- Dengue and severe dengue, http://www.who.int/mediacentre/factsheets/fs117/en/, 2015:117.
5. Spiegel JM, Bennett S, Hattersley L, Hayden MH, Kittayapong P, Nalim S, *et al.* Barriers and bridges to prevention and control of dengue: the need for a social-ecological approach. Eco Hlth *J*. 2005; 2: 273-290.
6. Chandre F, Darriet F, Manga L, Akogbeto M, Fay O, Mouchet J *et al.* Status of pyrethroid resistance in *Anopheles gambiensis*. Bull. Wld. Hlth. Organ. 1999; 77: 230-234.
7. Mulla MS, Majori G, Darwazeh HA. The future of insect growth regulators in vector control. J. Am. Mosq. Contr. Assoc. 1995; 11:269-273.
8. Jahan N, Shahid A. Evaluation of Resistance against Deltamethrin and Cypermethrin in Dengue Vector from Lahore, Pakistan. The Journal of Animal & Plant Sciences. 2013; 23(5):1321-1326, ISSN: 1018-7081.
9. Sihunincha M, Perea EZ, Rios WO, Stancil JD, Sifuentes VL, Ore CV *et al.* Potential use of Pyriproxyfen for Control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. Journal of Medical Entomology. 2005*;* 42(4):620-630.
10. World Health Organization. Environmental Management of Dengue and Dengue Hemorrhagic Fever in Pakistan Context. World Health Organization, 2012.
11. Braga IA, Lima JBP, Soares Sd, Valle D. *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe, and Alagoas, Brazil. Mem. Inst. Oswaldo Cruz. 2004; 99(2):199-203.

12. Aiub CAF, Coelho ECA, Sodre E, Pinto LFR, Felzenszwalb I. Genotoxic evaluation of the organophosphorus pesticide temephos. Genetics and Molecular Research. 2002; 1(2):159-66.
13. Google map-Gujranwala. www.google.com.pk/?gws_rd=ssl#q=gujranwala.
14. Ishaaya I and Horowitz A. Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweet potato whitefly (Homoptera: Aleyrodidae). Journal of economic entomology. 1992; 85(6):2113-217.
15. WHO: WHOPES-recommended compounds and formulations for control of mosquito larvae Updated 2013. http://www.who.int/whopes/recommendations/en/
16. Rueda LM. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission: DTIC Document, 2004.
17. WHO: Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP 2005, 13.
18. WHO Guidelines for Vector Control Needs Assessment. WHO Regional Office for Africa Division of Prevention and Control of Communicable Diseases Vector Biology, 2003.
19. Invest JF, Lucas JR. Sixth International Conference on Urban Pests, Pyriproxyfen as a mosquito larvicide, eds Robinson WH, Bajomi D OOK-Press Kft, Veszprtim, Hungary. 2008; 239-245.
20. Belinato TA, Martins A, Lima J Bento Valle D. Effect of Triflumuron, a chitin synthesis inhibitor, on *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* under laboratory conditions. Parasites & Vectors 2013; 6:83, doi: 10.1186/1756-3305-6-83,
21. Nayar J K, Ali A and Zaim M. Effectiveness and residual activity comparison of granular formulation of insect growth regulators Pyriproxyfen and S-Methoprene against Florida mosquitoes in Laboratory and outdoor conditions. Journal of the American Mosquito Control Association. 2002; 18(3):196-201.
22. Okazawa T, Bakote B, Suzuki H, Kere N. Field Evaluation of an Insect Growth Regulator Pyriproxyfen against Anopheles punctulatus on North Guadalcanal, Solomon Islands. Journal of the American Mosquito Control Association.1990; 7(4):604-607.
23. Hemingway J, Bonning BC, Jayawardena K, Weerasinghe I, Herath P and Oouchi H. Possible selective advantage of *Anopheles spp*.(Diptera: Culicidae) with the oxidase-and acetyl cholinesterase-based insecticide resistance genes after exposure to organophosphates or an insect growth regulator in Sri Lankan rice fields. Bulletin of entomological research. 1988; 78(3):471-478.
24. Darriet F, Corbel V. Laboratory Evaluation of Pyriproxyfen and Spinosad, Alone and in Combination, Against *Aedes aegypti* Larvaedes J. Med. Entomol. 2006; 43(6):1190-1194.
25. Mbare O, Lindsay SW, Fillinger U. Dose – response tests and semi-field evaluation of lethal and sub-lethal effects of slow release pyriproxyfen granules (Sumilarv 0.5 WG) for the control of the malaria vectors *gambiae* and *An. arabiensis*. Malaria Journal. 2013; 12:94.