TO EVALUATE EFFECT OF PYRIPROXYFEN AGAINST ANOPHELES MOSQUITO LARVAE

IMTIAZ HUSSAIN RAJA ABASSI[1]*, FARZANA ABBASI[2], SYED MUHAMMAD NAWAZ [1] AND DILPAT RAI MANGHWAR[1]

1-Department of Veterinary Parasitology and Medicine, Sindh Agriculture University Tandojam - Sindh, Pakistan.
2-Department of Zoology, Shah Abdul Latif University, Khairpur (Mir's) – Sindh, Pakistan

Corresponding author's Email: drimtiazr@gmail.com. +9230000938854

Abstract

The present study was based on a research in the parasitological laboratory, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam in the year of 2013. The main objective of the study to evaluate the effects of Pyriproxyfen against Anopheles mosquito larvae. Anopheles mosquito larvae was collected through fine collection net from stagnant water at livestock experimental station. Then carried out at parasitological laboratory and washed through 3-4 water changes till the debris material was completely removed. After that 10 Anopheles larvae were placed in 7 beakers labeled as A, B, C, D, E, F G were filled with 50ml of distilled water. Beaker A was kept as control (no Pyriproxyfen Chemical). Whereas all other beakers received mentioned concentrations of Pyriproxyfen. T2=0.5µl, T3=1.0µl, T4=1.5µl, T5=2.0µl, T6=5µl, T7=10µl and T8=20µl, respectively. After post application of chemical, the mortality was recorded at 8 hours, 16 hours and 24 hours. Mortality of larvae was confirmed using dissecting microscope. Completely non-motile larvae were considered as dead.

The results showed that in control treatment (T1), there was no mortality of mosquito. Furthermore, T2=0.5 µl Pyriproxyfen concentration were use, the result indicate that out of 10 mosquitoes larvae, 06 mosquitoes larvae were killed. While total mosquitoes larvae were killed, when they were treated with under Pyriproxyfen concentration, T3=1.0µl, T4=1.5µl, T5=2.0µl, T6=5µl, T7=10µl and T8=20 µl, respectively.

It was concluded that Pyriproxyfen was most effective against Anopheles mosquito larvae and result showed that in control, no mortality was observed; while in 0.5µl concentration, 60 percent mortality was observed and in other treatments, where Pyriproxyfen was applied at 1.0 µl, 1.5 µl, 2.0 µl, 5 µl, 10 µl and 20 µl concentration, 100 percent mortality of Anopheles mosquito larvae was observed. It is recommended that for achieving effective control of Anopheles mosquito larvae, Pyriproxyfen may be applied at 1.0 µl to 2.0 concentrations, above this concentration wastage of chemical and pollution in environment.

KEYWORDS: Parasite, Pyriproxyfen, Anopheles Larvae.

INTRODUCTION

The veterinary and medical importance of mosquitoes is mainly determined by their capability of disease transmission. The mosquitoes are a family of small, midge-like flies (Harbach., 1994). Although a few species are harmless or even useful to humanity, most are considered a nuisance because they consume blood from living vertebrates, including humans. The females of many species of mosquitoes are blood-eating pests. In feeding on blood, some of them transmit extremely harmful human and livestock diseases (Michigan Mosquito

Control Organization. 2013). Mosquitoes are members of a family of nematocerid flies. Over 3,500 species of mosquitoes have already been described from various parts of the world (Jaeger, Edmund C 1959). Anophelines exhibit a wide range of host preferences such as humans, livestock, birds, and reptiles (Subbarao SK., 1998). They do not routinely bite humans, but are the vectors for animal diseases, may become disastrous agents for zoonosis of new diseases when their habitats are disturbed, for instance by sudden deforestation (Wilcox, B.A. & Ellis, B 2006). Mosquitoes can act as vectors for many disease-causing viruses and parasites. The parasitic diseases collectively called malaria, caused by various species of *Plasmodium*, carried by mosquitoes of the genus *Anopheles* .Lymphatic filariasis (the main cause of elephantiasis) which can be spread by a wide variety of mosquito species (WHO,2011) Eastern equine encephalitis virus.and Tularemia, a bacterial disease caused by *Francisella tularensis*, is variously transmitted, including by biting flies. *Culex* and *Culiseta* are vectors of tularemia, as well as arbovirus infections such as West Nile virus (Muslu, H. *et al.,* 2011). Potential transmission of HIV was originally a public health concern, but practical considerations and detailed studies of epidemiological patterns suggest that any transmission of the HIV virus by mosquitoes is at worst extremely unlikely (CDC, 2006).

Larval control through larviciding and environmental management are the main intervention methods for malaria vector control around the world. Methods used to prevent the spread of disease, or to protect individuals in areas where disease is endemic, include, Vector control aimed at mosquito control or eradication, Disease prevention, using prophylactic drugs and developing vaccines, Prevention of mosquito bites, with insecticides, nets, and repellents. Source reduction (like, removing stagnant water) biocontrol (e.g. importing natural predators such as dragonflies) trapping, and/or insecticides to kill larvae or adults (The Economist, 2009). Malaria vector control has been largely dependent on the use of chemical insecticides. Only 12 insecticides belonging to four insecticide classes are recommended for public health use either for indoor residual spraying or to treat mosquito nets (WHO., 2005). Present study is designed address following objectives:

Objective

- Collection, mounting and identification of mosquito larvae.
- Evaluation of Decis super against L1, L2, and L3 larval stages of Anopheles mosquito under laboratory conditions.

MATERIALS AND METHODS

Study Area

Present study was conducted by collecting Anopheles mosquito larvae from stagnant water bodies at livestock experimental station, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

Assortment and screening of larvae.

Larvae were collected using fine collection net. The larvae were washed through 3-4 water changes till the debris material was completely removed. For identification of larvae, sample larvae were subjected to mounting using Canada Balsam. The identification was done using published keys.

Preparation of concentration of chemical.

Concentrations from the chemical pyriproxyfen were prepared by diluting 0.5ul, 1.5ul, 2ul, 5 ul, 10 ul and 20 ul of pyriproxyfen in 50 ml of water in beaker.

Protocol:

10 Anopheles larvae were placed in 7 beakers labeled as A, B, C, D, E, F G were filled with 50ml of distilled water. Beaker A was kept as control whereas all other beakers received above mentioned concentrations. The larvae were not fed during experimental phase and the mortality was recorded at 8 hours, 16 hours and 24 hours post application of chemical. Mortality of larvae was confirmed using dissecting microscope. Completely non-motile larvae were considered as dead.

RESULTS

Pyriproxyfen, a novel juvenile hormone mimic, is a potent suppressor of embryogenesis and adult formation of the mosquito and certain other insect pests. Intensive research has been carried out for evaluating insecticides with novel modes of action against whiteflies and with minimum danger to humans and natural enemies and numerous insecticides have such mode of action which also includes Pyriproxyfen. The Pyriproxyfen compound affects the hormonal balance in insects, acting as a juvenile hormone, resulting in strong suppression of embryogenesis, metamorphosis and adult formation in some insect pests.

The present study is based on a research in the laboratory, with the main objective to evaluate the effects of Pyriproxyfen against Anopheles mosquito larvae. In the laboratory, a total of 8 treatments were developed to feed mosquito and examine the mortality out of 10 in each treatment and percentage mortality under each treatment.

The treatments included T1=control, T2=0.5 µl Pyriproxyfen concentration, T3=1.0 µl Pyriproxyfen concentration, T4=1.5 µl Pyriproxyfen concentration, T5=2.0 µl Pyriproxyfen concentration, T6=5 µl Pyriproxyfen concentration, T7=10 µl Pyriproxyfen concentration and T8=20 µl Pyriproxyfen concentration.

Mortality of mosquito as affected by Pyriproxyfen concentration

The mortality of mosquito as affected by different concentrations of Pyriproxyfen concentration was investigated and a total of 10 mosquito larvae were kept in each of the jars and treated with Pyriproxyfen at different concentrations in the laboratory. The results on the efficacy of Pyriproxyfen are presented in Table-1. The data showed that in control treatment, there was no mortality of mosquito, where the mosquito larvae were not treated with Pyriproxyfen (T1). It was noted that the under treatment T2=0.5 µl Pyriproxyfen concentration, out of 10 mosquitoes, 06 mosquitoes were killed while 10 mosquitoes out of 10 were killed when they were treated with Pyriproxyfen under T3=1.0 µl Pyriproxyfen concentration, T4=1.5 µl Pyriproxyfen concentration, T5=2.0 µl Pyriproxyfen concentration, T6=5 µl Pyriproxyfen concentration, T7=10 µl Pyriproxyfen concentration and T8=20 µl Pyriproxyfen concentration.

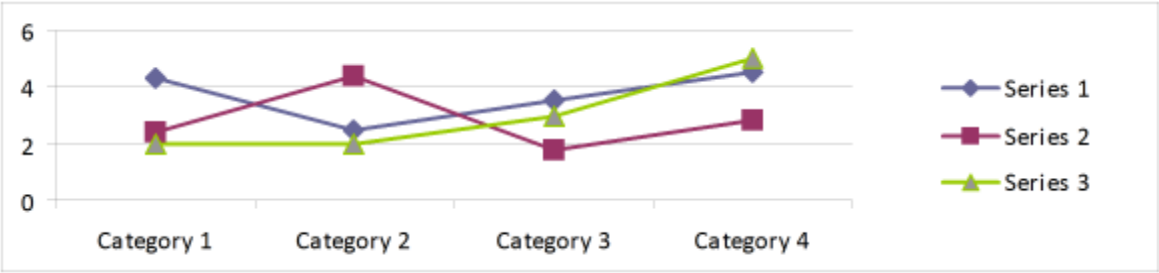
Mortality percentage observed in control

It was further observed that no mortality was observed in control; while under treatment T2=0.5 µl Pyriproxyfen concentration, 60 percent mortality was observed; while 100 percent mortality of mosquito larvae was observed in treatments T3=1.0 µl, T4=1.5 µl, T5=2.0 µl, T6=5 µl, T7=10 µl and T8=20 µl

Pyriproxyfen concentration. This indicated that for achieving 0.5 µl Pyriproxyfen concentration was adequately effective to kill the mosquito larvae 100 percent.

Table-1 Mortality at various time points and concentrations

Concentrations ul/50ml	Larvae used	Mortality number	Mortality %		
			8hrs	16hrs	24 hrs
0.5 ul/50ml	10	06	60%	60%	60%
1 ul/50ml	10	10	100%	100%	100%
1.5 ul/50ml	10	10	100%	100%	100%
2 ul/50ml	10	10	100%	100%	100%
5 ul/50ml	10	10	100%	100%	100%
10 ul/50ml	10	10	100%	100%	100%
20 ul/50ml	10	10	100%	100%	100%
Control	10	0	0%	0%	0%



Line graph showing mortality at various time points and concentrations

Trial-1

Concentration ul/50ml	Total	Mortality number	Mortality %		
			8 hrs	16 hrs	24 hrs
5 ul/50ml	10	10	100%	100%	100%
10 ul/50ml	10	10	100%	100%	100%
20 ul/50ml	10	10	100%	100%	100%
Control	10	0	0%	0%	0%

Trial-2

Concentration ul/50ml	Total	Mortality number	Mortality %		
			8 hrs	16 hrs	24 hrs
2 ul/50ml	10	10	100%	100%	100%
1.5 ul/50ml	10	10	100%	100%	100%
1 ul/50ml	10	10	100%	100%	100%
Control	10	0	0%	0%	0%

Trial-3

Concentration ul/50ml	Total	Mortality number	Mortality %		
			8 hrs	16 hrs	24 hrs
0.5 ul/50ml	10	06	60%	60%	60%
Control	10	0	0%	0%	0%

01 **02** **03**

No. 01 showing stagnant water behind hostel Gymkhana.

NO. 02 live Anopheles mosquito larvae floating on stagnant water.

NO. 03 Anopheles mosquito larvae in glass beaker during experiment period.

DISCUSSION

Pyriproxyfen is highly active against a variety of insects of public health importance including cockroaches, fleas, the tsetse fly and mosquitoes. The Pyriproxyfen could be used to treat animal waste lagoons for *Culex quinquefasciatus* larvae and that single treatments provided up to 2 months. A laboratory study was performed with the objective to evaluate the effects of Pyriproxyfen against Anopheles mosquito larvae. The treatments included 0, 0.5, 1.0, 1.5, 1.0, 1.5, 2.0, 5, 10 and 20 µl Pyriproxyfen concentrations.

The results showed that in control treatment, there was no larval mortality of mosquito; while under treatment T2=0.5 µl Pyriproxyfen concentration, 60 percent mortality was observed; while 100 percent mortality of mosquito larvae was observed in treatments T3=1.0 µl, T4=1.5 µl, T5=2.0 µl, T6=5 µl, T7=10 µl and T8=20 µl Pyriproxyfen concentration. This indicated that for achieving 0.5 µl Pyriproxyfen concentration was adequately effective to kill the mosquito larvae 100 percent. It was concluded that Pyriproxyfen was most effective against Anopheles mosquito larvae; and in control, zero mortality was observed; while in treatment, where Pyriproxyfen was applied at 0.5 µl concentration, 60 percent mortality was observed and in treatments, where Pyriproxyfen was applied at 1.5 µl, 1.0 µl, 1.5 µl, 2.0 µl, 5 µl, 10 µl and 20 µl concentration, 100 percent mortality of Anopheles mosquito larvae was observed. It is recommended that for achieving effective control of Anopheles mosquito larvae, Pyriproxyfen may be applied at 1.0 µl concentration or above. These results are in accordance with those of Mbare *et al.,* (2013) who reported that larviciding can be an effective tool for integrated malaria vector control. 0.5% pyriproxyfen granule (SurmilarvW0.5G, Sumitomo Chemicals) was assessed for the control of Anopheles gambiae sensu stricto and *Anopheles arabiensis*, the major malaria vectors in sub-Saharan Africa. The minimum dosage that completely inhibited adult emergence was between 0.01-0.03 parts per million (ppm) active ingredient (ai). Compared to the untreated control, an application of 0.018 ppm ai prevented 85% (95% confidence interval (CI) 82%-88%) of adult emergence over six weeks under standardized field conditions. A fivefold increase in dosage of 0.09 ppm ai prevented 97% (95% CI 94%-98%) emergence. Significant sub-lethal effects were observed in the standardized field tests. Female An. gambiae s.s. that were exposed to 0.018 ppm ai as larvae laid 47% less eggs, and females exposed to 0.09 ppm ai laid 74% less eggs than females that were unexposed to the treatment. Furthermore, 77% of eggs laid by females exposed to 0.018 ppm ai failed to hatch, whilst 98% of eggs laid by females exposed to 0.09 ppm did not hatch. Chen *et al.,* (2008) evaluated the effect of a commercially available pyriproxyfen, an insect growth regulator (IGR) on the larvae of a dengue vector, *A. aegypti*. There was a significant difference in the number of emerged adults obtained from the untreated and treated earthen jars up to 25 weeks ($p<0.05$). The duration of effectiveness of pyriproxyfen caused 80% emergence inhibition in earthen jars treated with 10% w/w and 20% w/w pyriproxyfen up to 22 and 25 weeks, respectively. Pyriproxyfen-controlled release block is an effective method of controlling mosquito larvae for several months. The method of application of the block is simple and straight forward and can therefore be used easily. William and Bajomi (2008) reported that, pyriproxyfen is an insect growth regulator that affects the physiology of morphogenesis, reproduction and embryogenesis of insects. It exhibits a high level of activity against mosquito larvae inhibiting adult emergence at very low dose rates. It has low mammalian toxicity and environmental impact. Pyriproxyfen can be transferred by adults to oviposition sites causing effects on egg eclosion and inhibition of emergence. Lee *et al.,* (2005) evaluated that the granular formulation of 0.5% pyriproxyfen which is a mosquito insect growth regulator larvicide. Mortality rates ranged from 60.9% to 79.7% at 0.01, 0.05 and 0.1 mg/L of water during the first seven days. Mortality rates for 0.01 mg/L concentrations were 70.4% from days 8- 14, and thereafter exceeded 80% through day 28. The mortality rates of *An. sinensis* larvae and pupae eventually reached 100% for the three (0.01, 0.05, 0.1 mg/L) concentrations. Moise *et al.,* (2005) argued that although the fecundity of the adult females used as the transfer vehicles in these tests was unaffected, the subsequent enclosion of the eggs that these mosquitoes laid was decreased by 70 to 90%. It also was shown that, at very high concentrations (_30,000 ppb), pyriproxyfen-treated water sources were as likely to be used as oviposition sites as untreated sources. These data suggest that treated sites might act as sinks for mosquito reproduction and moreover that such sites might act as dissemination sources for the horizontal transfer of larvicides to new environments by mature females. We review the literature on the

environmental and human health effects of this compound and discuss its potential for use as a mosquito control agent in the field.

SUMMARY, CONCLUSIONS AND SUGGESTIONS

Summary

The present study was done in the laboratory, with the main objective to evaluate the effects of Pyriproxyfen against Anopheles mosquito larvae. In the study, a total of 8 treatments were developed to feed mosquito and examine the mortality out of 10 in each treatment and mortality percentage under each treatment. The treatments included T1=control, T2=0.5 µl, T3=1.0 µl, T4=1.5 µl T5=2.0 µl, T6=5 µl, T7=10 µl and T8=20 µl Pyriproxyfen concentration,respectivly. The findings of the study are summarized as under

The results revealed that in control treatment, there was no mortality of mosquito, where the mosquito larvae were not treated with Pyriproxyfen (T1). It was noted that the under treatment T2=0.5 µl Pyriproxyfen concentration, out of 10 mosquitoes, 06 mosquitoes were killed while 10 mosquitoes out of 10 were killed when they were treated with Pyriproxyfen under T3=1.0 µl Pyriproxyfen concentration, T4=1.5 µl Pyriproxyfen concentration, T5=2.0 µl Pyriproxyfen concentration, T6=5 µl Pyriproxyfen concentration, T7=10 µl Pyriproxyfen concentration and T8=20 µl Pyriproxyfen concentration, respectively.

No mortality was observed in control; while under treatment T2=0.5 µl Pyriproxyfen concentration, 60 percent mortality was observed; while 100 percent mortality of mosquito larvae was observed in treatments T3=1.0 µl, T4=1.5 µl, T5=2.0 µl, T6=5 µl, T7=10 µl and T8=20 µl Pyriproxyfen concentration. This indicated that for achieving 0.5 µl Pyriproxyfen concentration was adequately effective to kill the mosquito larvae 100 percent.

Conclusions

- Pyriproxyfen was most effective against Anopheles mosquito larvae.
- In treatment, where Pyriproxyfen was applied at 0.5 µl concentration, 60 percent mortality was observed.
- In treatments, where Pyriproxyfen was applied at 1.5 µl, 1.0 µl, 1.5 µl, 2.0 µl, 5 µl, 10 µl and 20 µl concentrations, 100 percent mortality of Anopheles mosquito larvae was observed, but excessive from 2.0 µl was economically loss and environmental pollution.

Recommendations/ Suggestion.

- On the basis of findings from the present research, it is recommended that for achieving effective control of Anopheles mosquito larvae, Pyriproxyfen may be applied from 1.0 µl to 2.0 µl concentrations, above this concentration wastage of chemical and pollution in environment.

LITERATURE CITED.

Chen, C.D., W.A. A. Tan, S.R. Loke, H.L. Lee, A.R. Yasmin and M. S. Azirun. 2008. Effectiveness of pyriproxyfen-controlled release block against larvae of *Aedes* (*Stegomyia*) *aegypti* in Kuala Lumpur, Malaysia. Journal of Entomology. 32 (3) : 199-206.

Harbach, R.E. (1994) Review of the internal classification of the genus Anopheles (Diptera: Culicidae): the foundation for comparative systematics and phylogenetic research. Bulletin of Entomological Research 84, 331-342.

Jaeger, Edmund C. (1959). A Source-Book of Biological Names and Terms. Springfield, Ill: Thomas. ISBN 0-398-06179-3.

Lee, J. Won and D. K. Lee. 2005. Laboratory Evaluation of the Lethal Efficacy of an Insect Growth Regulator, Pyriproxyfen against *Anopheles sinensis* (Diptera, Culicidae) Larvae. Journal of Entomology Research. 35 (1) : 39-43.

Mbare, O., S. W Lindsay and U. Fillinger. 2013. Dose–response tests and semi-field evaluation of lethal and sub-lethal effects of slow release pyriproxyfen granules (SumilarvW0.5G) for the control of the malaria vectors Anopheles gambiae sensu lato. Journal of Veterinary Parasitology. 12 (3) : 12-94.

Michigan Mosquito Control Organization (2013). Mosquitoes of Michigan -Their Biology and Control

Moise, S.S., E. Z. Perea, W. O. Rios, J. D. Stancil, V. L. Sifuentes, C. V. Ore and G. J. Devine. 2005. Potential Use of Pyriproxyfen for Control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. Journal of Med. Entomology. 42 (4) : 620-630.

Muslu, H.; Kurt, O.; Ozbilgin, A. (2011). "Evaluation of Mosquito Species (Diptera: Culicidae) Identified in Manisa Province According to Their Breeding Sites and Seasonal Differences". Turkish Journal of Parasitology 35 (2): 100.

Subbarao SK: Anopheline species complexes in South-East Asia. WHO Tec.Pub 1998, 18:82.

The Economist. Resistance is Useless"April 8, 2009.

William, H., R. and D. Bajomi. 2008. Pyriproxyfen as a mosquito larvicide J.F. invest and J.R. lucas. Journal of Entomology. 43 (3) : 239-245

Wilcox, B.A. & Ellis, B. (2006). "Forests and emerging infectious diseases of humans". Unasylva 57. ISSN 0041-6436.

World Health Organization (WHO) 2004. The work of the African network on vector resistance to insecticides. Geneva, Switzerland: African Network on Vector Resistance; 2005.

World Health Organisation (WHO) 2011. Website Lymphatic Filariasis. World Health Organisation (WHO). Retrieved 24 August 2011.

.