COMPARATIVE EFFICACY OF THREE INSECT GROWTH REGULATORS ON THE DEVELOPMENTAL STAGES OF *CULEX TRITAENIORHYNCHUS*

Lucy Sabu[1] and H. Subramanian[2]
Department of Veterinary Parasitology
College of Veterinary and Animal Sciences
Mannuthy-680 651, Thrissur, Kerala

Abstract

The effect of three Insect Growth Regulators (*Diflubenzuron, Methoprene and Bti*) *was studied on the eggs, fourth stage larvae and pupae of Culex tritaeniorhyncus. The LC_{50} of the insecticides was minimum when applied against 4th stage larvae than on eggs or pupae. Methoprene had the least LC_{50} followed by diflubenzuron. High concentrations of Bti were required to produce the desired effect. When maximum retail price was considered, diflubenzuron was the best followed by methoprene. Bti was most expensive.*

Key words: *Insect growth regulators, developmental stages, Culex tritaeniorhynchus*

The term pesticide is an all inclusive term that includes a number of individual chemicals designed specifically for the control of different pests. It also includes chemical compounds known as growth regulators and compounds used for repelling, attracting and sterilising insects. Alternative chemicals which tend to be more compatible with biologicals include insect growth regulators (IGR) and pheromones. These newer alternatives are known as third generation insecticides. All compounds that regulate insect growth and development come under IGRs.

In the present study the efficacy of three IGRs was compared on the developmental stages of *Culex tritaeniorhynchus* colonised in the laboratory. *Culex tritaeniorhynchus* was identified as the most prevalent species of mosquito in cattle sheds in Thrissur, Kerala state (Sabu and Subramanian, 2007).

Materials and Methods

Engorged female mosquitoes collected from cattle sheds at dusk were colonised in the laboratory (Anyanwu *et al*., 2000). Females laid eggs in the water kept in enamel trays in the cages. Hay infusion was used as the rearing medium. The larvae were fed daily or on alternate days with small grains of yeast according to requirement taking care to avoid the development of excess turbidity. The medium was usually changed two times in the course of a life cycle. At L2and L4 stages, the larvae were washed and transferred to fresh medium.

The three insecticides used in the study were

1. Diflubenzuron (HILMILIN 25 % WP)

2. Methoprene (Altosid 5 % aqueous suspension)

3. *Bacillus thuringiensis israelensis* (HIL *Bti* 1.2 % aqueous suspension)

The effects of the above insecticides were studied on the eggs, fourth stage larvae and pupae. Freshly laid eggs not more than twelve hours were used for the study. Each egg raft was dissociated carefully with a fine haired brush on a piece of filter paper and transferred to water in a cavity block. The eggs were counted and pipetted out under a dissection microscope and transferred to plastic

1. Associate Professor
2. Dean

disposable cups in which the various insecticides were added at different concentrations. After 12 hours the eggs were repeatedly washed and replaced in an equal volume of fresh water. They were observed for hatching after 12 hours.

Ten larvae each were transferred to disposable cups containing various insecticides at different concentrations. Ten freshly formed pupae were also collected from the larval pools and used for the study. Each treatment was duplicated with a water control and the whole experiment was repeated three times to obtain mean values.

The medium used for the experiment was 100 ml stored tap water. When the experiment was with larvae, hay infusion was used. Small quantities of dog biscuits and yeast at the rate of 3: 1 were added to the medium according to requirement taking care to avoid excess turbidity in the medium.

The stages were observed for any mortality thereafter. The dead ones were removed. The number of adults emerged were also noted by counting the pupal exuviae. Emerged adults unable to leave the cups were also counted as dead. Percentage of successful pupation and adult emergence were determined until all adults had emerged or all larvae were dead. In each experiment, controls were also kept and the corrected mortality was obtained by using Abbot's formula (Busvine, 1971).

$$Pt = \frac{Po - Pc \times 100}{100 - Pc}$$ where

Pt = Corrected mortality

Po= Observed mortality

Pc= Control mortality, all in percentages.

Data were rejected in experiments in which the control mortalities exceeded 20 per cent. For each insecticide the concentrations giving at least two values each below and above 50 percent mortalities were obtained by repeated experiments. Lethal concentration (LC) LC_{50} and LC_{90} were estimated from the mortality data by probit analysis of log dosage, using SPSS (Statistical Package for Social Sciences).

p = a+bx, where p = probit

a = intercept

b = regression coefficient

x = log dose

The field dosages were calculated as four times the LC_{50} on 4^{th} stage larvae (Ponce *et al*., 2002). Based on this, the quantity of the preparations required for applying in $1x10^5$ litres of water and its cost was calculated.

Results and Discussion

In normal hatch, a portion of the anterior end of the egg shell was forced open transversely at a line of dehiscence forming an egg cap that was not completely detached but hinged to the remaining shell (Figs. 1& 2).

Typical symptoms of toxicity on eggs in general were observed as

1. Unhatch – failed to hatch, apparently embryo died before hatching (Fig.3)
2. Abnormal hatch – larvae eclosed from a longitudinal line of weakness at the mesal dorsum of the eggshell (Fig.4)
3. Partial side hatch – larvae died during eclosion (Fig.5 & 6)

Such eggs had

a. Head capsule free, but caudal end still in the shell

b. Caudal end free, but head capsule still in the egg shell, or

c. Thorax and abdomen free, but head and caudal end in eggshell.

Unhatched eggs were found to contain fully developed embryos that failed to hatch or apparently died just before hatching. Segmentation, eyespots and setae were visible through the eggshell. In some cases, larvae eclosed from a longitudinal line of weakness at the mesal dorsum of eggshell, which was different from normal hatch. All the control eggs completed hatching by 36 hours while the exposed eggs required more time for hatching.

The LC_{50} and LC_{90} values of various insecticides on the eggs, 4^{th} stage larvae and pupae are given in Tables 1, 2 and 3. Methoprene was not found to have any effect on the eggs. A concentration of 500 ppm (10,000 times the LC_{100} on 4^{th} stage larvae) was found to induce no influence on hatchability.

The treated larvae died due to various types of deformities caused in the course of development or had delayed development often resulting in mortality in emerging adults. Bulk of the mortality occurred at the larval stage. Delayed larval development and

Fig.1. Egg before hatch

Fig.2. Normal hatch

Fig.3. Unhatch

Fig.4. Abnormal hatch

Fig.5. Partial side hatch

Fig.6. Partial hatch

discoloration of the cuticle of immatures observed in the present study are in agreement with the findings of Mulla *et al.* 1975, Bakshi *et al.* 1982 and Prakash (1992).

The LC_{50} and LC_{90} values for the pupae were much higher than those for the pupae. Insect growth regulators are generally advised for use against larval stages. *Bti* protoxins are insecticidal crystal proteins requiring solubilization and activation in the insect midgut. Hence their effect on the pupae will be minimum as pupae are non-feeding.

From Table 4 it can be seen that the least concentration of insecticide to produce 50 per cent mortality was when they were applied against 4^{th} stage larvae. The concentrations of IGRs to produce 50 per cent mortality in larvae were minimum while several thousand multiples of these insecticide concentrations were needed to produce the same effect on pupae and eggs.

While methoprene had no action on the eggs of *Cx. tritaeniorhynchus*, three hundred and thirty nine times the concentration of LC_{50} on larvae was needed to produce the same effect on pupae.

Field dosages were calculated four times the LC_{50} values on larvae (Ponce *et al*. 2002). The cost of application of the various insecticides in $1x10^5$ litres of water was calculated based on this and are shown in Table 5.

When the maximum retail price alone was considered, diflubenzuron was found to be the cheapest insecticide, costing Rs.1.38/- per $1x10^5$ litres of water followed by methropene (Rs. 13.87/-). The most expensive one was *Bti* (Rs. 2179). Diflubenzuron acts by interfering with the chitin biosynthesis and its deposition during ecdysis resulting in insect mortality due to cuticular malformation. Insect growth regulators are specific and do not interrupt the natural regulatory mechanisms,

Table 1. LC_{50} and LC_{90} values of various insecticides on the eggs of *Cx. tritaeniorhynchus*

Insecticide	LC_{50} in ppm	Range	LC_{90} in ppm	Range
Diflubenzuron	38.26575	25-53	197.98773	126-434
Methoprene	--	--	--	--
Bti	21.72520	14-31	110.55540	67-267

Table 2. LC_{50} and LC_{90} values various insecticides on the 4th stage larvae

Insecticide	LC_{50} (ppm)	Range (ppm)	LC_{90} (ppm)	Range (ppm)
Diflubenzuron	0.00023	0.00014-0.00036	0.00407	0.002-0.009
Methoprene	0.00068	0.00034-0.0014	0.00673	0.003-0.039
Bti	6.22577	4.15-10.74	20.81060	11.7-90.07

Table 3. LC_{50} and LC_{90} values of various insecticides on the pupae

Insecticide	LC_{50} (ppm)	Range (ppm)	LC_{90} (ppm)	Range (ppm)
Diflubenzuron	28.77353	12.33-53.81	489.73381	201-3478
Methoprene	0.22858	0.09-0.43	6.25179	2.89-23.5
Bti	66.68765	34.83-107.86	187.36604	114-907

Table 4. Comparison of LC_{50} values of various insecticides on eggs, larvae and pupae

Insecticide	LC_{50} values in ppm				
	Eggs		4th stage Larvae	Pupae	
		*X			*X
Diflubenzuron	38.265508	167244	0.0002288	28. 7732483	125743
Methoprene	-	-	0.0006738	0. 2285781	339
Bti	21.7253912	3.3	6.6076021	66. 6887168	10

** Multiples of larval LC_{50}*

Table 5. Cost of application of different insecticides in a unit volume of water

Sl. No.	Insecticide	MRP (Rs.)	Field dosage (ppm)	Cost of application/ $1x10^5$ l (Rs.)
1.	**Hilmilin** (Diflubenzuron 25%)	375/100 g	0.00092	1.38
2	**Altosid** (Methoprene 5%)	255/100 ml	0.00272	13.87
3	**HIL *Bti*** (*Bti* 1.2%)	105/100 ml	24.91	2179

they have become one of the integral part of the integrated pest management programme.

Acknowledgements

The authors thank the Dean, College of Veterinary and Animal Sciences, Mannuthy for the facilities provided

References

Anyanwu, I.N., Agbeda, R.S., Ajansui, O.J., Umooh, J.U. and Ibrahim, N.D.G. 2000. The incrimination of *Aedes aegypti* as the vector of *Dirofilaria repens* in Nigeria. *Vet. Parasitol.,* **92**: 319-327

Bhakshi, N., Bhasin, V.K. and Pillai, M.K.K. 1982. Laboratory evaluation of insect growth regulator compounds against mosquitoes. *Entomon.,* **7**: 469- 473

Busvine, J.R. 1971. *A Critical Review of the Techniques for Testing Insecticides*. Commonwealth Agricultural Bureau. England. 375p.

Mulla, M.S., Majori, G. and Darwazeh, H.A.1975. Effect of the insect growth regulator dimilin or TH- 6040 on mosquitoes and some non- target organisms. *Mosq. News,* **35**: 211-216

Ponce, G.G., Flores, A.E., Badi, M.H., Rodrigeez- Tovar, M.L. and Salas, I.F. 2002. Laboratory evaluation of Vectobac against *Aedes aegypti* in Monterrey, Nuevo Leon, Mexico. *J. Am. Mosq. Control Ass*., **18**: 341-343

Prakash, A. 1992. Ovicidal action of certain chitin synthesis inhibitors in mosquitoes. *Entomon.,* **17**: 15-19

Sabu, L and Subramanian, H. 2007. Prevalence and vector potentiality of mosquitoes from Thrissur, Kerala. *J. Vet. Parasitol*., **21**: 165-167.

❑