CHAPTER - 4
BIOCONTROL ON MOSQUITO LARVAE OF *CULEX QUINQUEFASCIATUS* SAY

CHAPTER 4

BIOCONTROL ON MOSQUITO LARVAE OF *CULEX QUINQUEFASCIATUS* SAY

4.1 Introduction

Biological control methods by using insects are the most effective and natural method for controlling the pest and vectors. In the aquatic ecosystems, different species of aquatic insects including heteropterans co-occur with other prey and mosquitoes (Das *et al*., 2006; Bambaradeniya *et al*., 2004) and the mosquito larvae are fed by the predatory aquatic insects as well as other vertebrates (Gurumoorthy *et al*., 2013; Kundu *et al*., 2014). The water bugs are one of the prominent groups in freshwater habitat. The aquatic and semi aquatic bugs play an important role in the food web of these ecosystems (Rajan, 2015). This quality favours the water bugs are used as biological control agents.

Mosquito larvae are the major vectors living in aquatic habitat and the adult forms causes many health problems. Mosquito-borne diseases such as Malaria, filariasis, chikungunya, and dengue fever more prevalent and rising as serious health issues. They cause mortality of human population and economic loss in this region. This is due to unplanned urbanization and many anthropogenic activities. The lymphatic filariasis is predominant because of the principal vector, *Culex quinquefasciatus* Say, 1823. This mosquito is more prevalent in household areas and easily cultured from vegetables wastes. They are commonly called as ‘Southern House Mosquitoes’. So, this species of mosquito larvae was selected for the study. Many synthetic pesticides are commonly used for controlling the larval and adult forms of mosquitoes. However, the destructive effects of a chemical on non-target populations and habitat management and continuing problems with chemical resistance have prompted us to explore alternative, simple and sustainable methods of mosquito control. The application of chemical control of this intermediate host creates many environmental problems such as water pollution and death of other organisms. In this context, it is very urgent to control these vectors for human welfare. Few species under the families of predatory aquatic bugs such as

Belostomatidae and Nepidae are active predators of mosquito larvae (Ohba & Nakasuji, 2006). The selected predatory species of water bugs such as *Laccotrephes ruber* Linnaeus, 1764, and *Ranatra filiformis* Fabricius, 1790 belongs to the Family Nepidae and *Diplonychus rusticus* Fabricius, 1781 coming under the Family Belostomatidae were very commonly found and easily available from this region for the experimental study. They have a specialized forelegs (raptorial) to capture the prey and moreover, these are adapted in highly polluted water. Thus, these three water bugs were selected for the biocontrol experimental study against the mosquito larval species of disease-causing vector, C. *quinquefasciatus*.

4.2 Review of Literature

Many studies have been reported on the predatory behaviour of both aquatic vertebrates and invertebrates. The few studies have been done regarding the biological control experiments on the aquatic vectors such as mosquito larvae and freshwater intermediate vector snails. These kinds of studies were conducted in field as well as in laboratory condition. Raut & Nandi (1984) worked on the effectiveness of the predatory leech, *Glossiphonia weberi* against the freshwater vector snail, *Lymnaea luteola*. This work has been studied experimentally in the laboratory condition. The result was indicated that the predator selected the prey according to its size and this predatory leech, *G. weberi* preferred small sized juvenile snails. Venkatesan & Jeyachandra (1985); Bailey (1989); and Cloarec (1990) reported that the large number of aquatic heteropteran bugs, *Ranatra* sp.*, Diplonychus* sp., and *Anisops* sp. consume mosquito young stars.

Papacek (2001) reviewed the biology and feeding behaviour of small aquatic bug families of Notonectidae, Corixidae, Pleidae, Helotrephidae, Aphelocheiridae; and the ripicolous bugs of Gelastocoridae, and Ochteridae. The predator – prey relationships and their economic importance were also discussed. Aditya & Raut (2002a) worked on the freshwater vector snail, *Indoplanorbis exustus* eggs by using *Pomacea bridgesi*. Aditya & Raut (2002b) also assessed the predatory potential of *Sphaeroderma rusticum* on the Sewage snail, *Physa acuta* in laboratory condition. This study indicated that one *S. rusticum* could destroy a greater number of *P. acuta* and this water bug recognised as biological control agents of the above said Sewage snail. Sukumaran *et al.* (2004) worked on the molluscicide effect of nicotinanilide and niclosamide on the different stages of the same freshwater snail, *Lymnaea luteola*. Aditya *et al.* (2004) revealed the predation efficiency of the water bug *S. annulatum* on the mosquito larvae, *C. quinquefasciatus* and the effect on the adult emergence of mosquito larvae. This laboratory study clearly indicated that the predation rates of this water bug reduce the number of adult emergences of *C. quinquefasciatus*. Aditya *et al.* (2005) also studied the prey selection behaviour of *S. annulatum* and *S. rusticum* on the dipteran larvae, *Armigeres subalbatus*. This study revealed that the prey selection of these water bugs dependant on the number of preys occupied in the population.

Ohba & Nakasuji (2006) described the dietary items of water bugs such as *Lethocerus deyrolli, Appasus japonicas*, and *Laccotrephes japonensis*. Adult forms of *L. deyrolli* mainly feed on the frogs and the nymphs were feed on the tadpoles; *A. japonicas* feed on snails; and *L. japonensis* consume the mosquito larvae. This study was revealed that these bugs feed on medically important pests such as mosquito larvae and freshwater snails. Saha *et al.* (2007) published their work on the comparative account of predation of *Anisops bouvieri* Kirkaldy, *D. rusticus* Fabricius, and *D. annulatus* Fabricius on *C. quinquefasciatus*. Saha *et al.* (2008) also studied the influence of light and habitat structure by the predation of water bugs on *C quinquefasciatus*. The predatory efficiency of *D. annulatus*, *D. rusticus* and *A. bouvieri* was tested in the laboratory. The results indicate that these water bugs consume a greater number of mosquito larvae in the presence of light than in the dark. Chandramohan *et al.* (2008) also reported the predatory performance of *D. rusticus* Fabricius on *C. quinquefasciatus* with different salinity ranges. This was proposed that the predatory behaviour of this water bug was reduced when the salinity level increased. Saha *et al*. (2010) worked on the opportunistic foraging by the heteropteran mosquito predators. The heteropteran bugs, *A. bouvieri* Kirkaldy, *D. rusticus*, and *D. annulatus* Fabricius control the dipteran population, especially chironomids and mosquitoes in different aquatic habitats. *A. bouvieri* and *D. rusticus* showed strong electivity for mosquitoes than chironomids. But *D. annulatus* showed less electivity for mosquitoes. *A. bouvieri* and *D. rusticus* act as potential biocontrol agents for mosquito larvae when the mosquito density was high.

Ghosh & Chandra (2011) worked on the functional responses of *L. griseus* was studied on the mosquito larvae *C. quinquefasciatus* in the laboratory condition. This study revealed that the *L. griseus* consumes a greater number of *C. quinquefasciatus* in laboratory condition. Hazarika & Goswami (2012) monitored the feeding behaviour of the water bug *D. rusticus* on the seven prey individuals of different size such as fish species, mosquito larvae, *Chironomous* larvae, mayfly, small aquatic beetle, and damsel fly as an experimental approach. This study was revealed that the medium sized above said water bug shows more preference to feed the mosquito larvae, *C. quinquefasciatus* followed by *Catla catla* spawn and *Chironomous* larvae, *Tendipes* species. This aquatic bug does not show preference to eat the adult forms of the small water beetles. Mohanraj *et al.* (2012) reported the biocontrol potential of some aquatic insects namely, *Gyrinus natator*, *Nepa cinerea*, and *Cybister tripunctatus* on the dengue vector, *Aedes aegypti*. This was revealed that the consumption rate of predators in 24 hours was higher in *G. natator* than other two insect predators against the mosquito larvae of *Aedes aegypti*. The survivability of these aquatic insects was also tested as trials in sewage water. They show very poor tolerance to sewage. Gurumoorthy *et al.* (2013) assessed the predatory behaviour and the predator – prey relationships of *S. rusticum* on the mosquito larvae, *C. quinquefasciatus*. This study indicated that the rate of predation was increased along with the predator size and the density of prey. The nymphal and adult forms of these bugs feed on the *C. quinquefasciatus*. Brahma *et al.* (2014) reported the mosquito prey vulnerability in the predation by using selected water bugs. The intra-guild predation between *R. filiformis* and *A. bouvieri* was tested on the *C. quinquefasciatus* in the laboratory. This was revealed that the increased density of intraguild predator resulted in increased mortality of intraguild prey and shared prey. Singh (2014) studied the predatory potential of dragon fly nymphs on the larval and adult forms of freshwater snails, *Indoplanorbis exustus and Lymnaea luteola* under laboratory condition. Kumar & Priyadarsini (2014) described the history and histochemistry of the mantle of vector snail, *L. luteola*. Kundu *et al.* (2014) analysed a field study of insect predators of mosquito in paddy field and allied wetlands. The indirect interactions of these aquatic insects such as water bugs, larvae of odonates, and aquatic diving beetles on the larval forms of mosquito were also evaluated. This work indicated that the predator – prey interactions seem to be exaggerated by the biomass and composition of species assemblage.

Rajan (2015) monitored the predatory ability of *D. rusticus* against the *Culex* mosquito larvae. This was revealed the predatory performance of this water bugs were high in well-fed and starved condition. They prefer big sized larvae than smaller ones. Younes *et al.* (2015, 2016) assessed the predatory capacity of Odonata nymph, *Hemianax ephippigger* as biological control agent of intermediate vector snail, *Lymnaea natalensis* and also studied on the *Schistosoma* intermediate host, *Bulinus truncates* and *Biomphalaria alexandrina*. These laboratory studies revealed that the Odonata nymphs consume the freshwater vector snail, and the predation rate was different according to their snail type, size, and density of the snail. Rajan (2016) also studied on the predatory efficacy and prey shift behaviour of *D. rusticus* Fabricius against the *Chironomous* larvae and larvae of *Culex* mosquito. The results of the study indicated that the water bug, *D. rusticus* Fabricius prefer more on *Culex* larvae than *Chironomous* larvae. Rawal (2019) studied on the review of various strategies used for the biological control of mosquitoes. This study revealed that the information regarding the biocontrol agents such as fishes, nematodes, fungi, bacteria, planarians, anurans, and beetles and reviewed the integrated biocontrol methods to reduce the number of mosquito larvae. This study mentioned about the water bug *Diplonychus* also act as biocontrol agents of mosquito larvae. Vassou (2021) studied the water bug, *D. rusticus* as a biocontrol agent for *Culex* mosquito. A total of 275 adults of *D. rusticus* were added to a pond containing 275 litres of water, and 27500 *Culex* larvae were added to it. At the end of the 24 hours the live larvae were noted. This study revealed and proved that the importance of *D. rusticus* in the mosquito control management. Aneha *et al.* (2022) described the biological mosquito control measurers from past, present, and future. This study reviewed that many water bugs were consumed the larvae of mosquitoes. From this, the families such as Naucoridae, Notonecidae, Nepidae, Gerridae, Pleidae, and Hydrometridae were predatory in nature, and these can be used to control the larvae of mosquitoes.

4.3 Materials and Methods

4.3.1 Collection of water bugs

For the experimental study, the predators were collected from different selected aquatic habitats of Palakkad District, Kerala (Plate 39: Fig. 73). The adults of *L. ruber* were collected from agricultural ditches (Fig. 73a) at Thiruvalathur (Longitude 76°41'22.36" E and Latitude 10°43'59.5" N)*, R. filiformis* from quarry pools (Fig. 73b) of Kuzhalmannam (Longitude 76°35'32" E and Latitude 10°41'35" N) and *D. rusticus* from pond (Fig. 73c) of Kodumbu (Longitude 76°41'22.36" E and Latitude 10°43'59.5" N) by using hand picking method, pond net, and small sieve.

4.3.2 Identification and separation of water bugs

All the adult forms of collected species of water bug predators were transferred to the bottles covered with nylon net. These aquatic bugs were identified with the help of available published literature and taxonomic keys (Kumari & Nair, 1984; Chandra & Jehamalar, 2012b; Basu & Subramanian, 2014a, 2014b; Basu *et al.*, 2018c). They were kept in a separate container made up of mud filled with same pond water (7.5 litres in total volume) in the garden for a period of ten days before the commencement of experiments (Plate 39: Fig. 74a). To provide the natural conditions, some quantity of mud was collected from the field, and it was given in the bottom of the container and some aquatic plants such as *Ceratophyllum demersum* Linnaeus and *Pistia stratiotes* Linnaeus were also placed inside the container. The container was covered with nylon net to prevent the escape of water bugs. Prior to the experiment, the predators were starved for 24 hours. Before the actual experiment, a preliminary study was made in the laboratory. All the trials as well as the actual experimentations were accomplished in the room temperature of 28±2°C, 50-60% RH, water temperature (29-30°C) and pH (7-7.5).

4.3.3 Rearing of mosquito larvae

The prey *C. quinquefasciatus* was sufficiently reared by using the water mixed with decayed vegetable leaves in a plastic bucket (Plate 39: Fig. 74b) and kept for one week in the household areas of Palakkad District to get fourth instars' larvae and identified by using the standard taxonomic keys (Barraud, 1934; Reuben *et al*., 1994) and confirmed by taxonomic experts. They were used as prey for the experimental studies.

4.3.4 Experimental design

Three sets of same-sized eight mud pot, 600 ml in total volume, containing 500 ml of pond water was used in each experiment (Plate 40: Fig. 75). Among these, the experimental group was comprised of four mud pots, each containing a predator and experimental mosquito larvae. This experimental group was considered as test. The remaining four mud pot constituted the control with only mosquito larvae and the same quantity of water. Before the actual experiments, the trial experiments were made.

To find out the predation potential of adults of three selected water bugs against the different densities of fourth instars larvae of *C. quinquefasciatus* such as 25/500 ml, 50/500 ml, 75/500 ml, and 100/500 ml were kept in each mud pots of test and control. Each predator by means of same size was introduced into each set of the test pots with different densities of mosquito larvae and they were allowed to prey for a period of 12 hours. The control pots constituted only the above densities of mosquito larvae. The parameters such as room temperature, relative humidity, water temperature and P^H were noted. The remnant bodies of mosquito larvae (Plate 41: Fig. 77) were removed every three hours. The number of mosquito larvae remained alive at the end of 12 hours was counted. Five replications of the experiments were carried out. This experimental set up was followed by some modified method of Younes *et al.* (2015, 2016).

4.3.5 Data analysis

The experimental data was statistically analysed and performed with the computer software "SPSS 22". Two-way ANOVA was used to test the significance and One-sample Kolmogorov-Smirnov test was used to assess the normality of the data. This was followed the normal distribution. The post-hoc test was used to compare the significant difference in the predatory potential of three selected water bugs. Spearman's correlation coefficient was also used to investigate the relation among density, time of exposure and death of prey.

4.4 Results

The predatory potential of the above water bugs shows a significant difference in various prey densities. Statistically, significant difference ($P < 0.05$) was obtained for the prey density, time, and the different predators. The graph shows the estimated marginal means of death of prey was more by the predator, *L. ruber* (Species 1), followed by *D. rusticus* (Species 3), and *R. filiformis* (Species 2) with respect to the time (hours) and the density (numbers) of prey (Fig. 2 & 3). This study observed that the three selected water bugs were the predacious carnivore of mosquito larvae.

In the density of 25/500 ml mosquito larvae, it shows a significant difference ($P < 0.05$) in *L. ruber* & *R. filiformis*; and *D. rusticus* & *R. filiformis*, but no significant difference ($P > 0.05$) in *L. ruber* & *D. rusticus*. Similar results were obtained from the data analysis in the prey densities of 50/500 ml and 75/500 ml. In the case of 100/500 ml prey densities, it shows the significant difference ($P < 0.05$) between these three water bugs (Table 2).

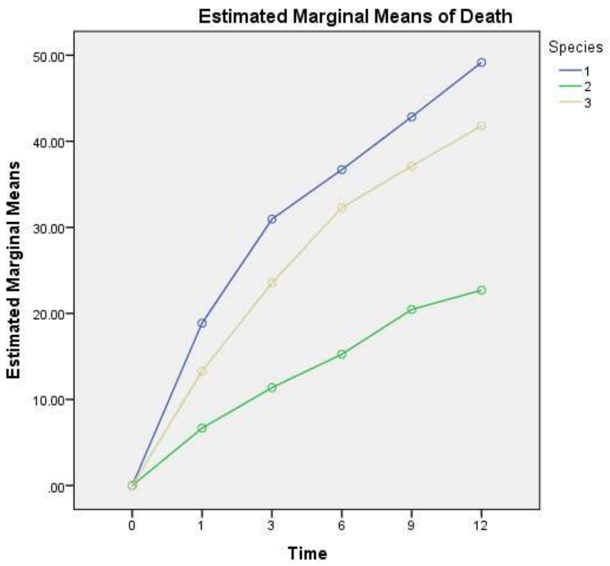

Figure 2. The estimated marginal means of death of prey by different predators with respect to time.

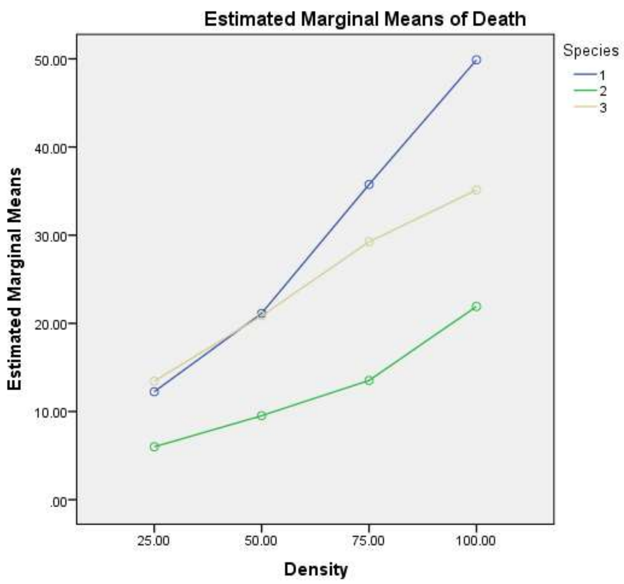

Figure 3. The estimated marginal means of death of prey by different predators with respect to density.

Table 2. Multiple comparison of the predation potential of three selected water bugs.

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.
Density - 25/500 ml	*L. ruber*	*R. filiformis*	9.00000*	1.86389	.000
		D. rusticus	2.16667	1.86389	.263
	R. filiformis	*L. ruber*	9.00000*	1.86389	.000
		D. rusticus	11.16667*	1.86389	.000
	D. rusticus	*L. ruber*	2.16667	1.86389	.263
		R. filiformis	11.16667*	1.86389	.000
Density - 50/500 ml	*L. ruber*	*R. filiformis*	20.00000*	5.39719	.002
		D. rusticus	3.83333	5.39719	.488
	R. filiformis	*L. ruber*	20.00000*	5.39719	.002
		D. rusticus	16.16667*	5.39719	.009
	D. rusticus	*L. ruber*	3.83333	5.39719	.488
		R. filiformis	16.16667*	5.39719	.009
Density - 75/500 ml	*L. ruber*	*R. filiformis*	37.83333*	5.84776	.000
		D. rusticus	10.00000	5.84776	.108
	R. filiformis	*L. ruber*	37.83333*	5.84776	.000
		D. rusticus	27.83333*	5.84776	.000
	D. rusticus	*L. ruber*	10.00000	5.84776	.108
		R. filiformis	27.83333*	5.84776	.000
Density - 100/500 ml	*L. ruber*	*R. filiformis*	39.66667*	8.25945	.000
		D. rusticus	17.66667*	8.25945	.049
	R. filiformis	*L. ruber*	39.66667*	8.25945	.000
		D. rusticus	22.00000*	8.25945	.018
	D. rusticus	*L. ruber*	17.66667*	8.25945	.049
		R. filiformis	22.00000*	8.25945	.018

* The mean difference is significant at the 0.05 level.

There is a positive correlation between the time of exposure and death of prey by predation (Table 3). Also, a high positive correlation between the density and death of preys i.e., the preys died more with the increase of time and density was observed.

Table 3. Correlation coefficient of time, density, and death of prey.

		Time	Density	Death
Time	Pearson Correlation	1	.000	.587**
	Sig. (2-tailed)		1.000	.000
	n	432	432	432
Density	Pearson Correlation	.000	1	.464**
	Sig. (2-tailed)	1.000		.000
	n	432	432	432
Death	Pearson Correlation	.587**	.464**	1
	Sig. (2-tailed)	.000	.000	
	n	432	432	432

**. Correlation is significant at the 0.01 level (2-tailed).

Among the three selected water bugs, *L. ruber* was found to have the highest predation efficiency (79.16) followed by *D. rusticus* (61.50) and *R. filiformis* (39.16) (Table 4).

Table 4. Predatory efficiency of the three predators on different prey densities.

Name of the predator	Prey density	Mean value of death rate of prey/12 hours
L. ruber	25/500 ml	19.50
	50/500 ml	38.00
	75/500 ml	60.00
	100/500 ml	79.16
R. filiformis	25/500 ml	10.33
	50/500 ml	17.66
	75/500 ml	23.66
	100/500 ml	39.16
D. rusticus	25/500 ml	21.66
	50/500 ml	34.16
	75/500 ml	49.83
	100/500 ml	61.50

4.5 Discussion

Mosquitoes in general are, carriers of different kinds of diseases such as Dengue fever, Malaria, Chikungunya and Filariasis. The major groups of natural biocontrol agents are fishes (guppies), larvae of odonates, aquatic beetles, and aquatic bugs. They have been used for controlling the mosquito larvae.

Based on the results it can be stated that the predatory bugs, *L. ruber, R. filiformis,* and *D. rusticus* can be used for control the larvae of *C. quinquefasciatus*. Predatory behaviour of these water bugs was noted during the experimental study. These three water bugs captured the prey with the help of their modified forelegs (Plate 41: Fig. 76a-76c) and suck the body fluids of mosquito larvae by its sharp rostrum. The predatory bugs also captured another prey while feeding. After sucking the body fluids of mosquito larvae, the remnant body (Plate 41: Fig. 77) was discarded in the pot. Then they fed another mosquito larva. Venkatesan & D'Sylva (1990) described the similar way of capturing and feeding behaviour in *D. indicus* Venkatesan & Rao.

The results of ANOVA and Correlation revealed that the predation potential of these water bugs varied significantly according to their prey densities and predation time. The death of prey species was more with the increase of exposure time and density. Vimala (1990) and Vassou (2021) reported the similar observations in Belostomatids. It has been found that the predation performance increased with the increase in the period of exposure.

The predation efficiency of the above said water bugs also varied. The predatory efficacy was more in *L. ruber* compared to other two aquatic bugs. It denotes that the three selected water bugs serve as a natural biological control agent, although there was a possible difference in the predation potential occupying in the same habitat. Saha *et al.* (2007) also reported the similar observations in the comparative study of three aquatic heteropteran bugs such as *A. bouvieri, D. rusticus,* and *D. annulatus.*

From the findings of the results, every predator act as natural biological control agent when the density of prey reached at 100/500 ml. This result supports the findings of Saleeza *et al*. (2014) in fishes, guppies. They reported that the female guppies fed the number of mosquito larvae when the prey densities were increased. Thus, these three water bugs play an important role in maintaining the ecological balance in the aquatic habitat; they actively control the larvae of *C. quinquefasciatus* when the number crossed the threshold level.

The present study was concluded and assumed that the predation efficiency was more at highest prey density, and this may be due to the number of availabilities of the prey and the vibrations in water made by the wriggling movement of the same.

PLATE – 39


a


b


c

Figure 73. Predators collected from different aquatic habitats: (a) Agricultural ditches; (b) Quarry ponds; (c) Pond.


a


b

Figure 74. Experimental predators and preys: (a) Water bugs; (b) Mosquito larvae.

PLATE – 40

1

2

3

Figure 75. (1-3): Three sets of experimental groups consist of test and control.

PLATE – 41

a **b** **c**

Figure 76. Predators capturing the mosquito larvae, *Culex quinquefasciatus* Say, 1823: (a) *Laccotrephes ruber* Linnaeus, 1764; (b) *Ranatra filiformis* Fabricius, 1790; (c) *Diplonychus rusticus* Fabricius, 1871.

Figure 77. Remnant bodies of mosquito larvae.