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Studies on the Biocontrol of Mosquitoes Employing *Poecilia reticulata*

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Abstract: *Mosquitoes spread various vector–borne diseases. Though various methods are available for their control, biocontrol using fish is gaining more importance. Hence the present work has been designed to study the potential of Poecilia reticulata on the larval control of mosquitoes. The fish fingerlings were provided with the larvae of Culex quinquefasciatus for testing the influence of prey density, volume of water, container shape, presence of aquatic plant, predator density and time of the day on the predation of mosquito larvae for one hour and 24 hours. Up to the optimum, rise in prey density and volume of water exhibited an increase in predation of mosquito larvae. Predation was higher in round container and with Hydrilla. Increase in the number of predators resulted in a rise in the rate of predation. Higher rate of predation was noticed in the initial hours of predation. If optimum ecological conditions are maintained, the fish, P. reticulata can be employed in the larval control of Cx. quinquefasciatus.*

Keywords: *Poecilia reticulata, Culex quinquefasciatus, Biocontrol, Mosquitoes, Predation, Hydrilla.*

INTRODUCTION

Health is a very significant and vital factor to identify that the country is prosperous and happy. Promotion of health is essential for national progress. Health is a base on which a country's progress can be measured. A healthy and disease free society is the backbone of a nation's development. Mosquitoes cause more human suffering than any other organisms. Millions of people in developing countries suffer from devastating diseases transmitted by mosquitoes. In India, the disease transmitting mosquitoes come under the genera, *Anopheles, Culex, Aedes* and *Mansonia*. Malaria, Filaria, Japanese Encephalitis and Dengue are transmitted by mosquitoes. Outbreak of mosquito-borne viral disease, Chikungunya, has caused manifold loss in terms of morbidity and economic loss (Sarwar, 2016; Kumar *et al.,* 2017; Annabel *et al.,* 2007).
WHO has announced the mosquito as "Public enemy number one". Each year upto 5 billion cases of clinical illness are reported throughout the world with Africa, having more than 90% of this burden. WHO led effort to rid the world of the disfiguring and disabling tropical disease, lymphatic filariasis. This mosquito transmitted disease is one of the causes of permanent disability globally and currently about 120 million people in Asia, Africa and Latin America are affected. Unabated population growth, ever growing destruction of ecosystems, rapid development in transport facilities and improper management of vector control operations resulted in the resurgence and establishment of the diseases (Joel *et al.,* 2011; WHO, 1996).

New and appropriate vector control strategies are essential for effective disease control. It is our duty to device sound, ecologically safe and eco friendly methods for controlling vector mosquitoes. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides, which were already used in mosquito control, and there are only few adulticides available than there have been in the past (Collins and Blackwell, 2000). The control of mosquito using adulticides is not a prudent strategy, as the adult stage occurs near human habitats and they can easily escape remedial measures. As effective chemical agents are vanishing and suitable replacement chemicals are scarce, we have to resort to other alternatives like using phytochemicals as repellents for protection against mosquito bites, immunological approaches, molecular epidemiology and biological control to mosquito-borne human diseases (Kamareddine, 2012).
Biocontrol encompasses the manipulation, conservation and augmentation of certain organisms as a means of regulating the population of undesirable species. There are several biologicals like bacteria, viruses, protozoans, nematodes, fungi, crustaceans, insects and fishes which can be included in the arsenal against mosquitoes. Biological control using larvivorous fish gained importance in malaria control programmes. It has an important role in the integrated control methodologies (Sumithra *et al.,* 2014; Haq and Yadav, 2003).
Larvivorous fish are being used extensively in biological control all over the world since the early 1900s. *Gambusia affinis* has high larvivorous potential. Guppies are one of the non-aggressive and very friendly fishes. They generally swim in the middle and upper regions of the aquarium. Males are smaller than the females and prefer mosquito larvae. They are very hardy and can survive in extreme conditions (Ragavendra and Poonam, 2008; Govindarao *et al.,* 2017; Phukon and Biswas, 2013; Mohamed, 2003). This present study has been carried out to find the effectiveness of *Poecilia reticulata* (Guppy) in controlling the larvae of *Culex quinquefasciatus*.

Materials and Methods

Healthy *P. reticulata* fingerlings were collected from private fish farm, Madurai, Tamil Nadu, India. The fingerlings were acclimated to laboratory conditions for ten days in a well aerated and illuminated tank and maintained under room temperature till the commencement of the experiment. Fishes of uniform weight were chosen for the study. Fishes were fed on fish feed before the experiment, but the fishes were subjected to starvation for one day before the commencement of the experiments.
Mosquito larvae were collected from open drainages at Usilampatty, Madurai district, Tamil Nadu, India and the species was identified as *Cx. quinquefasciatus*. From the collections, third instar stage was selected for the experiment.

Prey density

In the first set up of experiments, 500ml of water was taken separately in five different transparent containers. In containers, 100, 150, 200, 250 and 300 larvae were introduced for one hour experiment. Then one *P. reticulata* fingerling was introduced in each container and the time was noted. After one hour the fishes were removed and the remaining larvae were counted. From this, the number of larvae consumed was determined. The experiments were conducted in triplicate sets. Similar experiments were conducted for a period of 24 hours but the numbers of larvae introduced in the containers were 150, 200, 250, 300 and 350.

Effect of volume of water

In the second set of experiments 400, 500, 600, 700 and 800ml of water was taken separately in five different transparent containers. One hundred *Culex* larvae were introduced in each container for one hour experiment. Then one *P. reticulata* fingerling was allowed in each container and the time was noted. After one hour, the fishes were removed, the remaining larvae were counted and the number of larvae consumed was determined. Experiments were conducted to find out the influence of water volume. The same experiment was carried out for 24 hours with the prey density of 200.

Container shape

In the next set of experiments, influence of the shape of the container on the predation was studied. Different shapes of containers such as square, round, rectangle and oval were taken with 500ml of water. Two hundred *Culex* larvae and one guppy were introduced in each container and the time was noted. After one hour the fishes were removed, the remaining larvae were counted and the number of larvae consumed was determined. The same experiment was conducted for 24 hours with 200 larvae.

Influence of aquatic plants

In the fourth set of experiments 500ml of water was taken in two different transparent plastic containers. Two hundred *Culex* larvae were introduced in each container. In each container, one *P. reticulata* was allowed. Few branches of *Hydrilla* plants were put in one container and the time was noted.

After one hour the fishes were removed from the respective containers, the remaining larvae were counted and the number of larvae consumed was determined. The experiments were conducted thrice. Similar experiments were conducted for a period of 24 hours to find out the influence of *Hydrilla* plant on the predation.

Number of predators

In another set of experiments, the influence of number of predators on the predation was studied. For this, 500ml of water was taken in three separate container and two hundred larvae were introduced in each container. Then, one, two, three and four *P. reticulata* fingerlings were allowed individually in each container and the time was noted. After one hour, the fishes were removed, the remaining larvae were counted and the number of larvae consumed was determined. The same experiment was conducted for 24 hours, but the number of larvae introduced in each container was kept as 300.

Influence of time of the day

In the last set of experiments, the effect of time of the day on the predation of the fish was studied. For this, 500ml of water was taken in one container with two hundred larvae and one *P. reticulata* fingerling. The experiment was started at 8 am and continued till 8 am the next day. After every one hour the fishes were removed, remaining larvae were counted and the larvae consumed by the fish were determined. Again the fishes were reintroduced.

Results

Biocontrol of common house mosquito *Cx. quinquefasciatus* employing larvivorous fish, *P. reticulata* was studied. The efficacy in controlling *Cx. quinquefasciatus* was experimented in different, prey densities, volumes of water, shapes of the containers, in the presence of *Hydrilla* plant and by increasing the predator densities.

Impact of prey density, volume of water and container shape on predatory activity

The maximum predation of *P. reticulata* was at 200 prey density and it consumed 30 and 54 third instar larvae for a period of 1 hour and 24 hours respectively. The minimum predation was noticed at 100 prey density consuming 14 and 35 larvae for one hour and 24 hour period respectively (Fig.1). The predation of *P. reticulata* was the maximum in the prey density of 100. Feeding rate decreased with the rise in prey density.

With reference to volume of water, highest predation was at 700ml of water for *P. reticulata* and it consumed 39 larvae after one hour. Within 24 hours it could devour 120 larvae (Fig.2). The minimum predation was at 400ml of water and the consumed larvae were 96 after 24 hours. The predation of *P. reticulata* was conducive up to 700ml.

With reference to shapes of container like square, round, rectangle and oval, maximum number of larvae consumed by *P. reticulata* was only 11 in round container after one hour and 122 after 24 hours (Fig.3). Minimum predation was in oval shaped container in one day. Round shape container favoured predatory activity of *P. reticulate* when compared with other types of containers.

Impact of aquatic plants and number of predators on predatory activity

In the presence of *Hydrilla, P. reticulata* consumed only 58 larvae within a day. In the absence of *Hydrilla* it could consume 140 larvae with a percentage of 70 within 24 hrs (Fig.4). *P. reticulata* showed that in the absence of aquatic plants effective control of mosquito larvae can be done. This shows that they feed on mosquito larvae to a certain extent when chance permits them.

In *P. reticulata,* feeding showed an increase with the rise in predator densities. The group of four *P. reticulata* showed maximum predation of 44 after one hour and consumed 196 larvae with a percentage of 98 (Fig.5). Figure 5 indicates that in *P. reticulata* the role of predation was found to be dependent on predator densities.

Predatory Pattern of *P. reticulata*

Figure 6 illustrates the predatory efficacy of *P. reticulata* when exposed to the larvae of *Cx. quinquefasciatus* during 24 hours. At initial hours 8-9 and 9-10 a.m. predation was maximum, where the number of larvae devoured was 21 and 10 respectively. Later predation was little slow and came to stand at 12-13hr. After 12-13 hours the predation was high at 14-15 hours and no predation was found during 16-17, 19-20, 21-22, 01-02, 02-03, 05-06 and 06-07 hours. Figure 6 indicates the total number of larvae eaten by *P. reticulata* being 90 out of 150 introduced with a percentage of 66. *P. reticulata* was slow in devouring larvae when the time increased.

Discussion

Insects are the most important invertebrate group which serve as beneficial to human beings and other animals on one hand and on the other hand they also act as vectors of several devastating diseases. The disease vectors such as tsetse, ticks, mosquitoes and sand flies and the parasites they carry, become more and more resistant to chemical spraying and drug therapy. Among these, mosquitoes are the most important blood sucking groups of insects. Mosquitoes transmit diseases to more than 700,000,000 people each year. No single method will provide adequate control of mosquitoes and so combination of biological, chemical and environmental management and repellents is needed at present to attack mosquitoes. Biological control is expected to play an increasing role in vector management strategies of the future (Kumar and Hwang, 2006).

The introduction of DDT in indoor residual spraying for malaria control led to the gradual decline in the use of environmental management and biological control methods. In the fifties attention was diverted to control mosquitoes using synthetic insecticides until insecticide resistance assumed prominence (Benelli *et al. ,* 2016). The importance of climate and weather is obvious since mosquitoes are cold blooded animals and their activity cycles as well as virus replication in their tissues are influenced by temperature. Temperature and relative humidity of the season favour dengue virus multiplication in the mosquito and is one of the contributing factors to the occurrence of DHF outbreaks (Watts *et al.,* 1987). The predation of *P. reticulata* was the maximum in the prey density of 100. Feeding rate decreased with the increase in prey density. Predation of *P. reticulata* was conducive at 700ml (Misha *et al.,* 2017).

In view of growing concern about safety of chemicals, interest is revived on plant products. Plants act as a rich source of bioactive organic chemicals which serve as defense chemicals against insect attack. These chemicals may serve as attractants. A large number of plant extracts were reported to exhibit mosquitocidal or repellent activity against mosquito vectors (Ignacimuthu, 2000; Sukumar *et al.,* 1991). In the present study it was noticed that predator densities increased predation of mosquito larvae. 24hr continuous study indicated that *P. reticulata* consumed 90 out of 150 3rd instar larvae of mosquito.

In the present study, biocontrol of mosquito employing the larvivorous fish, *P. reticulata* was tested. Larvivorous fishes were in use for over hundred years in mosquito control and many species have proved effective. In the Western Kenyan highlands, the larvivorus fish, *Oreochromis niloticus* is commonly cultured and consumed but has not been previously tested in the field for malaria mosquito control. After *O. niloticus* introduction, mosquito densities immediately declined in the treated ponds (Annabel *et al.,* 2007). It has been found that the average consumption rate during 24 hrs study period was appreciable (90/150, 219/250 respectively). Round shape of container favoured predatory activity. The larvivorous fish devoured more larvae in the absence of the aquatic plant, *Hydrilla*.

Biological control of vector mosquitoes (*Anopheles sinensis*) was demonstrated using fish predators, *Moroco oxycephalus* and *Misgurnus anguillicandatus* in the semi field rice paddy. A sustained control of 55% was reported (Rodríguez-Pérez *et al.*, 2014). Bellini and coworkers (1994) observed the potential of various fish species (*Carassius auratusi, Cyprinus carpio, Gambusia affinis*) in controlling mosquitoes in northern Italy. *O. mossambicus* was effective in controlling mosquitoes in cow dung pits, especially III and IV instar larvae and pupae of *Cx. quinquefasciatus* and *An. Culicifacies* (Ujjania, 2015). *Oreochromis niloticus* is a native African fish possessing mosquito control properties known since 1917. Their fry actively pursue mosquito immatures. Larger fish eat the plant material in which mosquito immatures hide, allowing the fry to find and eat them (Parthasarathi *et al.,* 2016). In the present study *P. reticulata* voraciously consumed mosquito larvae in the absence of aquatic plants. In the presence of vegetation the efficacy towards mosquito larva reduced to a little extent. Field studies conducted by Kumar and coworkers (1998) on the impact of *P. reticulata* on mosquito breeding in drains reported that it can control *Cx. quinquefasciatus* breeding in about three months. There was 100 percent elimination of *Culex* sp in about four weeks. In the case of *G. affinis* breeding was controlled upto 90-95 percent in effluent ponds in about three months where it was able to control 100 percent breeding of *An. culicifacies* and *An. stephensi* in about two weeks in cement tank inside township of Ahmedabad city.

Unlike chemical control, the results are of unpredictable with biocontrol agents. This calls for a better understanding of the biological interactions with the environment. Developing and acquiring the necessary skills assume paramount importance. Another important consideration is the recognition of the fact that, in developing countries like India, success of such strategies depends on developing simple technology backed by a campaign of public education (Spielman *et al.*, 1993).

Health education has always occupied a lower priority in Indian public policy. An activity associated with health education is the security of social support to enable the effective implementation of disease control measures. Community power and potential is combating vectors remain vastly untapped and wherever used under utilized (Saishankar *et al.*, 2013). To promote mosquito and malaria control measures at household and community levels using primary and secondary schools are the entry point for awareness creation. Preventive strategies in the houses and dissemination of all the information on mosquito and malaria control through drama performed by the students will be effective (Adaikalaraj *et al.*, 2015). Towards this end, we require a programme of biological research aimed towards understanding the factors that limit the number of mosquitoes. The two main factors determining the efficacy of the fish are the suitability of fish species to the water bodies where the vectors breed and the ability of the fish to eat enough larvae of vector species to reduce the number of infectious bites. The fish should be of native, able to thrive in breeding sites. Aquatic vegetation can interfere with fish feeding and provide refuge for the mosquito larvae. The effectiveness of larvivorous fish to control mosquitoes may vary due to environmental complexity (Wu *et al.*, 1991; Blaustein and Chase, 2007).

Conclusion

P. reticulata exhibits high efficiency in controlling the larvae of *Cx. quinquefasciatus*. If suitable environmental conditions like predator density, presence of aquatic plants and depth of water bodies are maintained, it can perform better in the biocontrol of mosquitoes.

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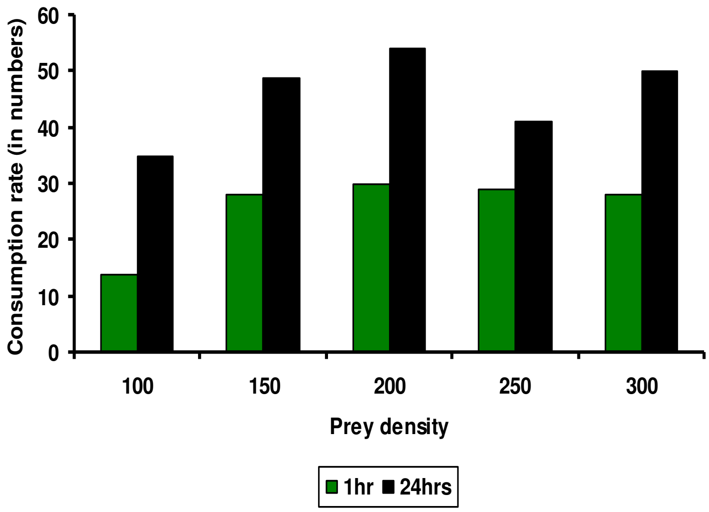

Figure 1. Effect of prey density on the predation of *Poecilia reticulata*

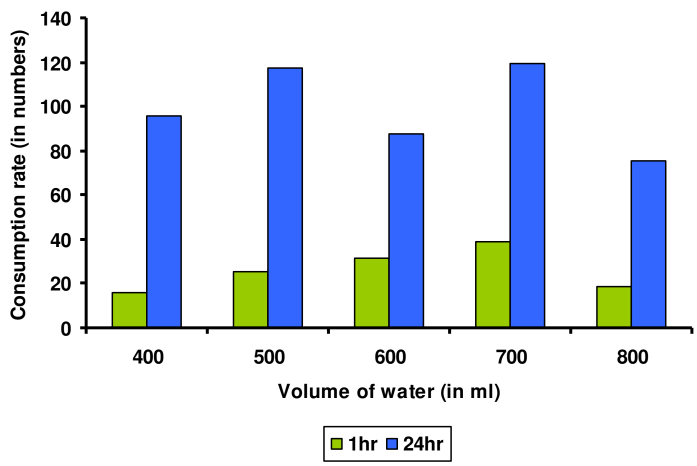

Figure 2. Effect of volume of water on the predation of *Poecilia reticulata*

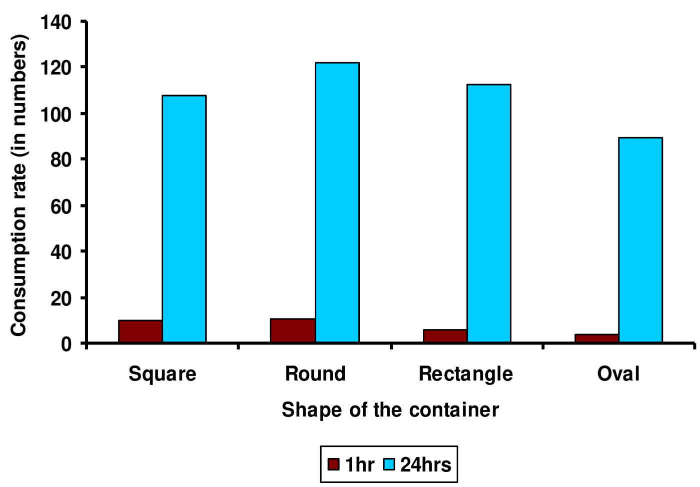

Figure 3. Effect of shape of container on the predation of *P. reticulata*

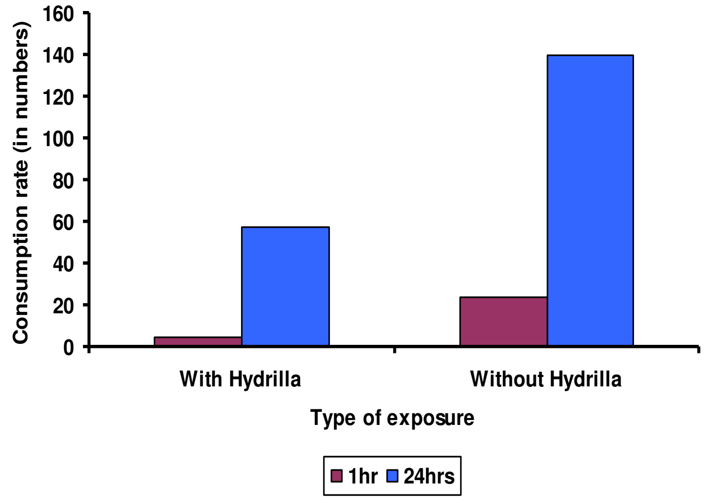

Figure 4. Influence of *Hydrilla* plant on the predation of *P. reticulata*

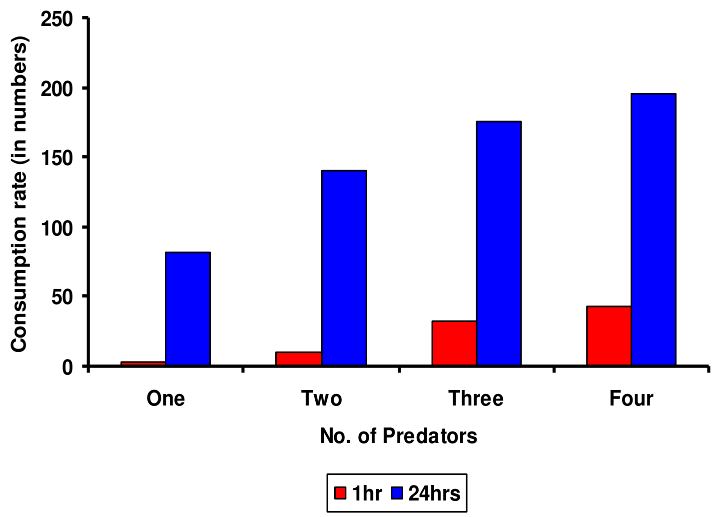

Figure 5. Effect of number of predators on the predation of *P. reticulata*

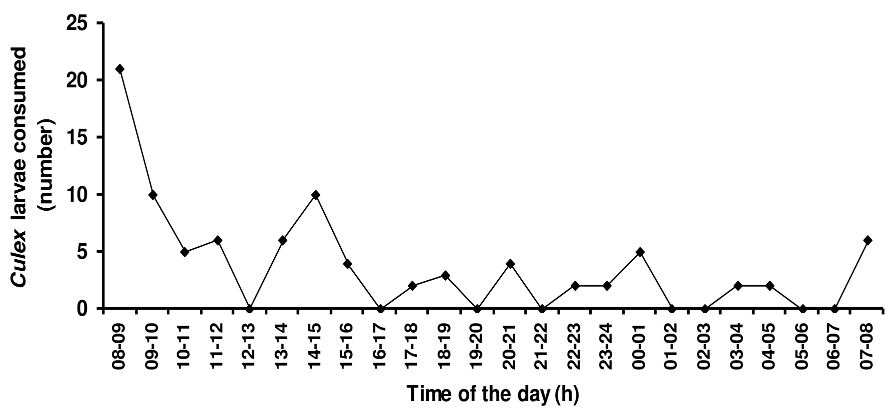

Figure 6. Predatory pattern of the fish, *Poecilia reticulata*