IN VITRO STUDIES ON THE BIOCONTROL POTENTIAL OF FISH SPECIES *CARASSIUS AURATUS* AGAINST MOSQUITO *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Mosquito control using synthetic insecticides is accompanied with many problems. Thus biological control measure are been widely used recently. In this context present investigation was carried out to test the predatory efficacy and to determine the predatory index of the fish Carassius auratus against the fourth instar larvae and pupae of the mosquito Culex quinquefasciatus cultured using Hay infusion method. Predation potential of the fish C. auratus was evaluated in laboratory condition by the methods of National Vector Borne Disease Control Programme with slight modifications using artificial fish food as control treatment. Predation nature of fish was tested and data were statistically represented as mean ± SD. Initial and final weight gained by the fish species and predatory index was also determined. The results of present study showed that the larval predation efficacy of fish C. auratus is higher than its pupal predation efficacy. The predation potential was found to be high in first few hours and as the experiment proceeds it was found to fall down. The predatory index of C. auratus which showed an excellent larval predation rate was recorded as 47.84. Results revealed that the fish was interested to eat on larvae than pupae and artificial fish food. Thus the fish can be used as a bio control measure in integrated vector control by considering the ecology and epidemiological condition of area to be used.

Keyword: *Fish; Mosquito; Larvae; Pupae; Bio control; Predation efficacy; Predatory index*

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1. INTRODUCTION

In humans and mammals, some of the infectious diseases are transmitted by certain vectors, for example mosquito, flies (sand and tsetse), tics, mites and lice [1]. Mosquito borne diseases continue to be a major problem in almost all tropical and subtropical countries [2]. They are responsible for the transmission of pathogens causing some of the most life threatening and debilitating diseases of man, like malaria, yellow fever, dengue fever, chickengunya, filariasis, encephalitis, etc. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides, which were formerly used in mosquito control programmes, and there are now fewer adulticides available than there have been for the last 20years [3].

Culex quinquefasciatus mosquito is a vector transmitting filariasis disease in India. The eradication of mosquito using adulticides is not a prudent strategy, as the adult stage occurs alongside human habitation, and they can easily escape remedial measures [4,5]. The harmful effects of chemicals on mosquito, as well as non-target, populations, and the development of resistance to these chemicals in mosquitoes along with recent resurgance of different mosquito-borne diseases have promoted use to explore simple sustainable methods of mosquito control [6].

In India, chemical, physical and biological control measures have been in practice to reduce the incidence of mosquito borne diseases [7]. However, chemical control measures pollute nearby aquatic ecosystems, expensive and require much skilled manpower for constant surveillance of mosquito breeding habitats [8]. Integrated vector management (IVM) is described as a rational decision making process for vector control and includes methods based on knowledge of local vector biology, disease transmission, utilization of range of interventions often in combinations with health sector and other public and private sector by involving local communities and stakeholders. In order to control mosquito, mainly chemical and biological approaches are employed.

Biological control is expected to play an increasing role in vector management strategies of the future. In developing countries like India, success of such strategies depends on developing simple technology backed by a campaign of public education (2). Biological control refers to the introduction or manipulation of organisms to suppress vector population. As biological mosquito control agents, larvivorous fish i.e., those that feed on immature stages of mosquitoes are being used extensively all over the world since the early 1900s [9].

Although significant advancements have been made in developing therapeutics and vaccines for mosquito-borne pathogens over the last few decades, efficient vector control strategies are still the primary method used for control and prevention of mosquito-borne diseases [10].The biological control is environmental friendly and not hazardous for plants, beneficial to insects and humans health. The present investigation has been undertaken to find the predation efficacy of the larvivorous fishes *Carassius auratus* against the fourth instar larval and pupal stage of the mosquito *Culex quinquefasciatus*.

2. MATERIALS AND METHODS

2.1 Laboratory Culture of Larvae

Hay infusion method was adopted for culturing mosquito larvae in the College Laboratory. After one or two days eggs were laid by female mosquitoes in cultures forming an egg raft. The egg rafts were collected and maintained in the laboratory. The third instar larvae were collected, reared in enamel trays containing culture medium and provided with powdered dog biscuits and yeast in the ratio of 3:1 as the nutrient source. Immediately after moulting, the fourth instar larvae and pupae were used for the bioassay studies. Larvae were collected by a small scoop net to feed the experimental fish.

2.2 Predatory Potential of Fishes

2.2.1 Collection and Acclimatization of Fish

The fishes were purchased from shop in Coimbatore locale. The fish species *C. auratus* was assessed for its predatory potential. The average weight of *C. auratus* fishes used was 4.18 g. After a period of 7 days of acclimatization, the experiment was conducted in laboratory conditions.

2.2.2 Feeding Assay

Predation potential of the fishes *C. auratus* was evaluated against fourth instar larvae and pupae of *Cx. quinquefasciatus* in laboratory condition by the methods of [11] with slight modifications using artificial fish food as control treatment.

Six 1L beakers were used in the experimental setup. One set for assessing the consumption rate of fourth instar larvae to which pre-starved fish (n = 1) was individually placed in five 1L beakers with 800 ml of dechlorinated water. 50 early fourth instar larvae of *Cx. quinquefasciatus* were added individually to the glass containers respectively. Another set for assessing the consumption rate of pupae to which pre-starved fish (n = 1) was individually placed in five 1 L beakers with 800ml of dechlorinated water. 50 pupae of *Cx. quinquefasciatus* were added individually to the glass containers respectively. Remaining 1 L beaker in each set is used as control treatment with 50 artificial fish pellets introduced in it (Fig 1).

Fig 1: Experimental setup on feeding assay of *Carrasius auratus*

Initial length and weight of fish groups were taken and average was calculated. The consumption potential of the fish was noted every 3 h for 24 hours. No food was added in the treatment beakers as per World Health Organization norm. Larval consumption at each point was recorded as the change in the number of larvae in the tank from the previous observation. Five replicates were conducted using this method to evaluate predation efficiency, for each species. Total larval consumption was recorded at the end of 24 h. The experiment was conducted for three consecutive weeks and the mean value was calculated.

Predation nature of fish was tested depending on the consumption of mosquito larvae and pupae. The initial weight and final weight gained by the fish species was determined. Both data were statistically represented as mean ± SD.

2.2.3 Predatory Index

In a separate experiment under the same laboratory conditions, a single *C. auratus* with a body length and body weight of 5.3 cm and 4.14 g respectively was used to determine the predatory index for *Cx. quinquefasciatus* larvae. The predatory index was determined as the number of larvae consumed by the fish per gram body weight per day [12]. Predatory index was calculated using the formula,

$$\text{Predatory index} = \frac{\text{No. of larvae consumed by/fish/day}}{\text{Fish body weight}}$$

2.3 Statistical Analysis

The data on predatory efficacy of fish was subjected to statistical analysis. Standard deviation was calculated for the data which was obtained from the predatory studies. Each value (Mean ± SD) represents average of five replications of the test groups.

3. RESULTS

The feeding behaviour was observed for every 3 h interval for 24 h. Three hourly (24 h) and daily consumption rates for three weeks (mean ± SD) of *C. auratus* against *Cx. quinquefasciatus* larvae and pupae are presented in Table 1 & 2.

3.1 Predatory potential of fish *Carassius auratus*

The results of present study showed that the larval predation efficacy of fish *C. auratus* is higher than its pupal predation efficacy. The fish consumed maximum larvae (43.66±1.97) at end of 3^{rd} h itself and by 6 hours all the larvae (6.33±1.97) introduced were consumed (Table 1). Fish artificial food was not preferred so much by the fish as the larvae and even after 24 h there was some fish food that was left out. Initially the weight of fish was found to be 4.18g and final weight was recorded as 4.69g (Table 3).

Table 1: Larval Consumption Rate of *Carassius auratus*

Sl. No	Treatment	3 h	6 h	9 h	12 h	15 h	18 h	21 h	24 h	Total Larval Consumption
1	Fish food	13	8	7	5	3	2	2	1	-
2	Replicate 1	41	9	-	-	-	-	-	-	50
3	Replicate 2	43	7	-	-	-	-	-	-	50
4	Replicate 3	45	5	-	-	-	-	-	-	50
5	Replicate 4	42	8	-	-	-	-	-	-	50
6	Replicate 5	47	3	-	-	-	-	-	-	50
7	Mean ± SD of treatment	43.66±1.97	6.33±1.97	0±0	0±0	0±0	0±0	0±0	0±0	50±0

Each value (Mean ± SD) represents average of five replicates

The pupae feeding efficacy of *C. auratus* was less compared to larvae feeding (Table 2). The results showed that the fish consumed 26.8% pupae in the first three hours. This was comparatively low than its larvae feeding efficacy. The pupae feeding pattern at the end of 6th and 9th h were 11.4 and 7.6 percentages respectively. The predation was seen to be low in the proceeding hours. The predation potential was found to be high in first few hours and as the experiment proceeds it was found to fall down.

Table 2: Pupal Consumption Rate of *Carassius auratus*

Sl. No	Treatment	3 h	6 h	9 h	12 h	15 h	18 h	21 h	24 h	Total Larval Consumption
1	Fish food	11	8	6	6	4	3	3	2	-
2	Replicate 1	28	12	07	01	01		-	-	50
3	Replicate 2	27	11	07	03	02	-	-	-	50
4	Replicate 3	26	10	10	03	01	-	-	-	50
5	Replicate 4	27	12	07	02	02	-	-	-	50
6	Replicate 5	26	11	04	04	04	01	-	-	50
7	Mean ± SD of treatment	26.8±0.74	11.4±1.49	7.6±1.74	3.2±0.97	2.4±1.35	0.4±0.48	0±0	0±0	50±0

Each value (Mean ± SD) represents average of five replicates

Table 3: Average Initial and Final Length and Weight of Fish in Test Groups

Sl.	Fish Sp.	Length of fishes (cm)	Weight of fishes (g)

No		Initial	Final	Initial	Final
1	*C. auratus*	5.51±1.35	5.86±0.97	4.18±1.10	4.96±0.4

3.2 Predatory index of the fish species

In the present study, *C. auratus* was found to be an efficient biocontrol agent against the larvae of *Cx. quinquefasciatus*. All sizes of *C. auratus* were found to prey upon maximum number of larvae in the first 3h itself. Results revealed that the fish was interested to eat on larvae than pupae and artificial fish food. Therefore the predatory index of the fish was calculated and is tabulated in Table 4. The predatory index of *C. auratus* which showed an excellent larval predation rate was recorded as 47.84.

Table 4: Predatory Index of the Selected Fish Species

Sl. No	Fish Sp.	Length of fish (cm)		Weight of fish (g)		No. of larvae introduced/ day	No. of IV instars consumed/day			Avg. No. of larvae consumed/ fish/day	Predatory index
		Initial	Final	Initial	Final		Day 1	Day 2	Day 3		
1	*C. auratus*	5.51	5.86	4.18	4.96	500	257	228	224	236.33	47.84

4. DISCUSSION

Biological control is considered to be not only the best method but highly effective in vector management as compared to the other used chemical methods as it is effective and safe to human and other non-target populations, producing lower risk of resistance development and offers low cost of production [13]. A number of studies have suggested introduction of fish species, and indigenous species to be effective at suppressing mosquito populations at breeding places [14].

During 3h of experiment in the present study, it was found that the fish *C. auratus* of length 5.51 cm consumed a maximum number of mosquito larvae (43.66%). At a end of experiment the final length of fish is recorded as 5.86 cm. Similar experiments were carried out by Arunachalam *et al* [15] in which it was reported that during 1h of experiment, it was found that an individual *Channa gachua* (9.5cm) consumed a maximum number of mosquito larvae (179±21.21) followed by other two size groups of the same species. Similarly, *P. sophore* (5.7cm) and *T. fasciata* (7.1cm) consumed a maximum of 66.33±1.52/hr and 45.67±0.58 /hr respectively.

In the present study it was evident that the fish *C. auratus* predated upon the mosquito larvae in maximum number during the first few hours and afterwards the consumption rate was found to fall down. Our results were in concordance with the observations of Arunachalam *et al* [15] in which, it was observed that all the fish species consumed maximum numbers of mosquito larvae at first 30 minutes and thereafter the feeding intensity was progressively slowed down.

According to the reports of Sumithra *et al* [16] the feeding efficacy of fishes was found to increase on increasing size groups of the species studied. These findings were in harmony with the results of present study in which the predation rate of fishes is depended on its size. Higher the size greater will be its predation potential. It is recorded that the *C. auratus* actively chased the mosquito larvae and tried to immobilize it by encounter with its jaws and then swallowed the IV instars, while the pupae were further attacked and inactivated before consuming them. The early instars of mosquito larvae were actively predated than the later stages.

In accordance to the present study Saleeza *et al* [17] used five different fish species as predators against *Ae. aegypti* larvae and found that the larger fish are more effective predators, and female guppies are more capable to eradicate *Ae. aegypti* larvae than male guppies. Therefore, larger fish eat more mosquito larvae than smaller fish do. Daily feeding patterns were size-dependent. The largest fish fed at a relatively constant level through the day, and small fish fed most at first and last light. These differences were due probably to the effect of competitive interactions upon fish of different sizes.

Review of WHO [18] has shown that biological control using fish is best achieved as part of an integrated vector control strategy. While larval control by fish, like chemical larviciding, will reduce vector densities, a nearly perfect larval control is required to significantly reduce the risk of mosquito disease transmission. A nearly complete larval control is possible in well-defined situations and would require, among other things, a thorough knowledge of the vector ecology, geographical reconnaissance of larval habitats in targeted areas and a significant degree of skill in breeding, transportation and use of fish.

5. CONCLUSION

In mosquito control programmes, especially those using biocontrol agents, it is highly desirable to have materials and agents that will yield long lasting control with one or few treatments or introductions so as to be cost-effective. Under the alternative strategy of vector control by means of bio-environmental improvement techniques, primary importance is given to antilarval operations. The fish species studied here are very active, and can effectively be used as strong biocontrol agent against immature mosquitoes. Integrated vector mosquito control strategies, using the larvivorous fishes is simple, inexpensive and the commonly available fish can be given first preference to avoid possible undesired implications of introduction of new fish species. Nevertheless, present study suggested that *C. auratus* could be used as an ideal bio-control agent against mosquito larvae in all mosquito-breeding habitats, including urban and sub-urban areas as well as in the backyard of houses. The fish *C. auratus* was found to be quite useful for mosquito control in the present experiment without any ecological and environmental hindrance.

6. REFERENCES

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