Asian Australas. J. Biosci. Biotechnol. 2022, 7 (3), 82-89; https://doi.org/10.3329/aajbb.v7i3.62338

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
https://www.ebupress.com/journal/aajbb/

Article

Predatory efficiency of dragonfly nymphs, *Crocothemis servilia* and *Rhyothemis variegata* against the mosquito, *Culex quinquefasciatus* Say

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Received: 19 September 2022/Accepted: 04 November 2022/Published: 07 November 2022

Abstract: This study compared the predatory potential of nymphs of two dragonfly species viz. *Crocothemis servilia* (Drury, 1773) and *Rhyothemis variegata* (Linnaeus, 1763) using the different larval instars and pupae of *Cx. quinquefasciatus* (Say, 1823) as preys in normal laboratory settings. Field-collected fed and 24 h starved nymphs of *C. servilia* and *R. variegata* were offered 1st, 2nd, 3rd and 4th instar larvae and pupae of *Cx. quinquefasciatus* to monitor the rate of predation. A 24 h starved nymph of *C. servilia* showed the highest predation on the 2nd instar larvae (92.00±4.06%) followed by the 3rd (83.00±5.61%), 4th (80±6.89%) and 1st (76.00±4.85%) instar larvae and the pupae (26.00±2.91), respectively, whereas, that of *R. variegata* exhibited the highest consumption of the 1st instar larvae (90.00±3.54 %) followed by the 2nd (88.00±5.61 %), 3rd (82.00±3.74 %), 4th (70.00±7.91 %) larval instar and the pupae (23.00±4.63), respectively within 24 h exposure. In the same period, the fed nymphs of *C. servilia* showed maximum consumption of the 2nd instar larvae (77.00±3.54%) followed by the 3rd (76.00±4.58%), 4th (64.00±4.00%) and 1st instar (55.00±3.53%) larvae and the pupae (24.00±3.67), respectively, whereas, that of *R. variegata* exhibited highest consumption of the 1st instar larvae (67.00±5.38 %) followed by the 2nd (65.00±10.12 %), 3rd (58.00±8.46 %) and 4th (53.00±4.06 %) instar larvae and the pupae (21.00±2.92), respectively. The rate of predation was significant on all the larval instars and the pupae compared to their control counterparts ($p<0.05$) and the starved larvae and nymphs of both the dragonfly species showed higher predation compared to the fed nymphs. The aforementioned findings suggest that nymphs of both of the dragonfly species exhibited considerable predation potential against the immature stages of the *Cx. quinquefasciatus* mosquito. The present study recommends assessing the feasibility of using these species in large-scale mosquito control programs.

Keywords: dragonfly; *Crocothemis servilia*; *Rhyothemis variegate*; biocontrol; *Culex quinquefasciatus*

1. Introduction

Mosquitoes are considered the 'deadliest foe' of human and the ongoing battle between them evolved more than a century ago (Tyagi, 2004). They are known as potential vectors for the transmission of various diseases viz. filariasis, dengue, malaria and many kinds of arthropod-borne viral encephalitis (WHO, 2020). In addition, they annoy us by biting and sucking blood. The southern house mosquito, *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae), the most irritating and widespread mosquito in the world is the principal vector of human

lymphatic filariasis (LF), the leading mosquito-borne disease in Asian countries caused by three parasitic nematodes viz. *Wuchereria bancrofti, Brugia timori* and *B. malayi*. LF is one of the major causes of acute and chronic illness worldwide, infecting 51 million people as of 2018 and 863 million people in 47 countries are at risk of infection (WHO, 2022). *Culex quinquefasciatus* is a subtropical mosquito belonging to the *Culex pipiens* complex and acts as a vector of many zoonoses viz. western equine encephalitis, Saint Luis encephalitis, avian malaria, West Nile fever, Rift Valley fever and Zika fever (Ayres *et al*., 2019; Meegan, 1979; Simon *et al*., 2022). Different types of nutrient-rich stagnant and dirty water collections including drains, wells, derelict ponds, septic tanks, marshy swamps, coir pits, pit latrines and wastewater containers act as the breeding habitats of *Cx. quinquefasciatus* as they support the aquatic phases of its life cycle (Das and Shenoy, 2008). Despite the complex life cycle of this mosquito, no single method can reduce the density of this mosquito below reasonable thresholds. The most common types of insecticides used in mosquito control programs include pyrethroids, organochlorines, organophosphates, and carbamates (WHO, 2018). Indiscriminate and repeated use of synthetic chemical pesticides created the emergence of insecticide-resistant insect pests, environmental contamination, and unfavorable impacts on creatures other than the intended targets (Lee *et al*., 2001).

To reduce the extensive use of pesticides, which are now the main approach for mosquito control, biocontrol solutions for diseases spread by mosquitoes are required. It is important to create eco-friendly, economical, secure, and long-lasting techniques that can kill a variety of mosquito species. Utilizing different lineages of predators has shown some promise in lowering the number of mosquito larvae (Knight *et al*., 2004). In addition to attacking mosquito larvae, they also kill and consume a variety of other coexisting creatures. Despite this, the presence of other prey does not negatively affect the role of predators in controlling the population of mosquito larvae (Stav *et al*., 2005). Some aquatic bugs (Saha *et al*., 2007; Valbon *et al*., 2018, 2019), larvivorous fishes (Das *et al*., 2018; Riaz *et al*., 2018), diving beetles (Choo *et al*., 2021; Lundkvist *et al*., 2003), and odonate nymphs (Córdoba-Aguilar *et al*., 2021; Cozzer *et al*., 2022; Samanmali *et al*., 2018) are among the natural predators that can help control the population of mosquito larvae. Odonate nymphs are voracious predators that use unique protractible labium to grab their prey, which includes mosquito larvae, various smaller aquatic invertebrates, and even larvae of fish and amphibians (Zia *et al*., 2011). They have drawn attention to their application in environmentally friendly mosquito control because of their predatory role against mosquito larvae (Subramanian, 2018).

To the best of our knowledge, limited research works have been carried out regarding the efficacy of odonate nymphs in mosquito control in the world (Córdoba-Aguilar *et al*., 2021; Cozzer *et al*., 2022; Riaz *et al*., 2018). In Bangladesh, 102 species of odonates including 57 species of dragonflies and 45 species of damselflies have been recorded so far (Shah & Khan, 2020). However, literature regarding their immature stages (nymphs) as well as their biocontrol potential of mosquitoes are missing. Therefore, the present study was conducted to evaluate the biocontrol potential of nymphs of two species of dragonflies viz. *Crocothemis servilia* and *Rhyothemis variegata* on the larval and pupal stages of *Cx. quinquefasciatus*.

2. Materials and Methods

The experiments were conducted in laboratory conditions (air temperature 28-29°C, water temperature 26-32ºC and relative humidity 71-74 %) from December 2021 to June 2022 in the Insect Rearing and Experimental Station (IRES), Entomology Laboratory, Department of Zoology, Jahangirnagar University, Bangladesh.

2.1. Procedure of larva collection and rearing

2.1.1. Collection and rearing of mosquito

Wild populations of mosquito larvae were collected from the drains near area Dhaka, Bangladesh using a dipper. The larvae were collected in the morning (10:00 am-11:00 am) and put in plastic jars along with water from the larval habitat, covered with fine netting, and transported to the laboratory. The larvae were washed with distilled water in an earthen pot. The larvae of *Cx. quinquefasciatus* were identified following suitable taxonomic keys (Barraud, 1924; Bram, 1967). Finally, the larvae were reared in the laboratory. A mixture of fish food and dried yeast powder was used as supplemental feeding. The adult stages were given cotton balls soaked in a 10 percent glucose solution as food, and they also received periodic blood transfusions from a raised pigeon. Inside the cage, in a jar with water, the female adult mosquitoes lay their eggs. Larvae of every instar were produced when the eggs hatched. Every instar of larvae was continuously available for the experiments. Larvae and pupae from the F2 generation were utilized in the experiments.

2.1.2. Procedure of odonate nymph collection and rearing

Nymphs of the dragonfly *C. servilia* (Drury, 1773) and *R. variegata* (Linnaeus, 1763) were collected in the morning between 9.00 am to 11.00 am from transport lake in Jahangirnagar university campus (Latitude 23°53′1.246″ N, Longitude 90°16′8.760″ E). They were collected using D-framed hand dip net at a depth of one to two feet in stagnant water. The nymphs along with their breeding place water were poured into containers with water including weed, debris, and leaf litter, and taken into the laboratory in the shortest possible time. The collected nymphs were maintained in an earthen tank filled with unfiltered pond water. Earthen tanks were placed in the IRES, filled with pond water collected from breeding places and well at 1:1 ratio. A netting cage was placed above each tank to protect the nymphs from predators. Although pond water provides natural food, the nymphs were regularly fed with a diet consisting of chironomid larvae. Furthermore, dead individuals were removed each time and pond water was changed every alternate day. The identification of specimens was carried out following suitable taxonomic keys of Fraser (1936) and Mitra (2002). Nymphs of each species used in this study were allowed to grow up to the final instars.

2.1.3. Bioassay experiment

To evaluate the extent of predation on *Cx. quinquefasciatus* by *C. servilia* and *R. variegata*, a series of laboratory experiments were conducted. For the experiments, the nymphs of both dragonfly species were starved for 24 h or fed the diet mentioned above. One nymph was provided with 20 individuals of each of the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval instars and pupae of *Cx. quinquefasciatus*. A similar pot with only prey population and without any dragonfly nymphs was used as the control for each of the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} instar larvae and pupae for comparison. Five replications were maintained for each treatment and control. For each replication of treatment, 300 ml water was taken in a pot and mosquito larvae and nymph were simultaneously introduced to the treatment environment. But for the control replication, only the prey populations were introduced. In the first set of the experiment the prey: predator ratio was maintained at 20:1. In the case where more than 75% of mosquito larvae were predated, a new batch of larvae was introduced into the pot to maintain the 20 individuals per pot larval density throughout a day. The number of preys killed was noted every 24 h interval. At each 24 h the water of the experimental sets was transferred to a white plate for counting the larvae consumed. At the end of the day, the number of preys consumed and those that had died in control were recorded. However, only the numbers of mosquito larvae consumed were noted in this experiment. To avoid contamination, the water of each cup was covered with a net. The temperature of water ranged from 29-34ºC, pH from 6.2-6.7 and dissolved oxygen from 5.5-6.3 mg/during the period of the experiment.

2.1.4. Data interpretation and statistical analysis

The predation rates were calculated as the deducted product of remaining mosquito larvae for their initial surviving larvae. The predation rates of each studied dragonfly nymph on *Cx. quinquefasciatus* larvae were entered into a Microsoft Excel work sheet. The significance in the total and average predation rate after 24 h of each instar with five replications was evaluated by using the general Linear Model in SPSS (version 22). One way ANOVA was employed to determine any significant differences in predation by odonate predators.

3. Results

The present study was conducted to investigate the predatory potentiality of two dragonfly species viz. *C. servilia* and *R. variegata* against different larval instars and pupae of *Cx. quinquefasciatus* Say (Figure 1). Aquatic stages of Odonata (nymphs) and different developmental stages of mosquitoes were used in this experiment. Consumption of the different developmental stages viz. 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval instar and pupae by odonate nymphs were recorded.

Exposure of the larval instars and pupae of *Cx. quinquefasciatus* to 24 h starved nymphs of *C. servilia* showed the highest predation on the 2^{nd} instar larvae (92.00±4.06%) followed by the 3^{rd} (83.00±5.61%), 4^{th} (80±6.89%) and 1^{st} instar (76.00±4.85%) larvae and the pupae (26.00±2.91), respectively (Figure 2). The predation rate on all the larval instars and pupae was significant ($p<0.05$) compared to the control as shown by the General Linear Model (GLM) univariate in SPSS. Next, it was tested whether the fed nymphs show variation in predation. After 24 h exposure of the larval instars and pupae of *Cx. quinquefasciatus* to the fed nymphs of *C. servilia*, the maximum predation was observed on the 2^{nd} instar larvae (77.00±3.54%) followed by the 3^{rd} (76.00±4.58%), 4^{th} (64.00±4.00%) and 1^{st} instar (55.00±3.53%) larvae and the pupae (24.00±3.67), respectively (Figure 2). Again, the predation rate was significant on all the larval instars and the pupae compared to their control counterparts ($p<0.05$). Thus, mean larval predation varied between starved and fed nymphs. As we expected, the starved

nymphs showed higher predation on all the larval instars and pupae compared with fed nymphs and significant differences were observed in the case of 1st, 2nd and 4th larval instars (p>0.05) (Figure 2).

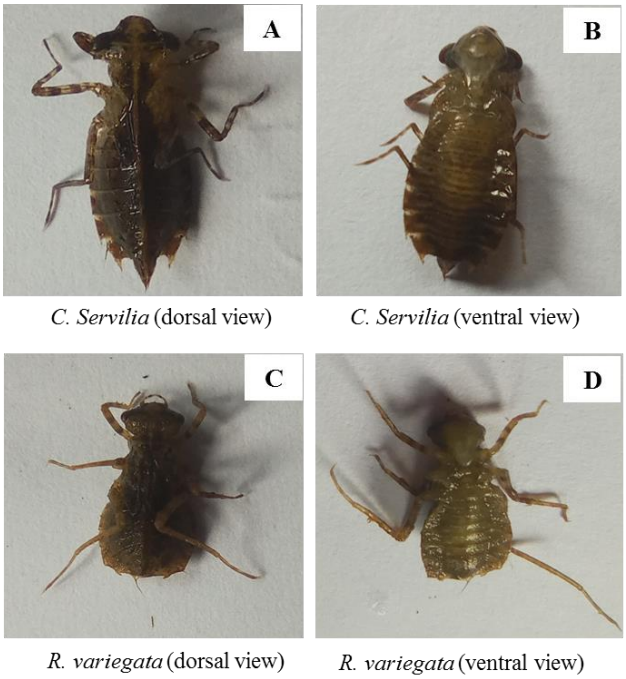

Figure 1. Nymphs of *C. servilia* and *R. variegata* used in predation experiments.

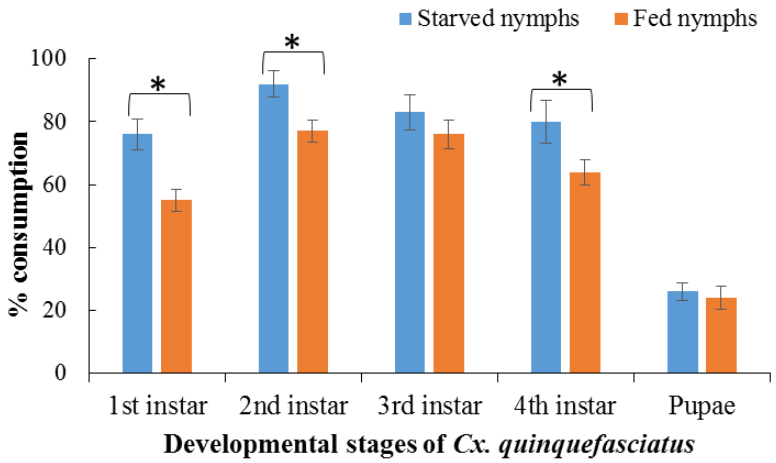

Figure 2. Mean consumption of different larval instars and pupae of *Cx. quinquefasciatus* when exposed to starved and fed nymphs of *C. servilia*. Values are the mean of five replicates. Y-error bar represents the value of the standard error of means. *Significance at p<0.05.

The 24 h starved nymphs of *R. variegata* exhibited maximum predation on the 1st instar larvae (90.00±3.54 %) followed by the 2nd (88.00±5.61 %), 3rd (82.00±3.74 %) and 4th (70.00±7.91 %) instar larvae and the pupae (23.00±4.63), respectively (Figure 3). The predation rate on all the larval instars and the pupae was significant compared to their control counterparts (p<0.05). It was further checked whether the fed nymphs exhibit variation in predation. After 24 h exposure the fed nymphs of *R. variegata* exhibited the highest predation on the 1st instar larvae (67.00±5.38 %) followed by the 2nd (65.00±10.12 %), 3rd (58.00±8.46 %) and 4th (53.00±4.06 %) instar larvae and the pupae (21.00±2.92), respectively (Figure 3). Again, the predation rate was significant against all the larval instars and the pupae compared to their control counterparts (p<0.05). As we presumed, the

starved nymphs showed higher predation on all the larval instars and pupae compared with fed nymphs and significant differences were found in the case of 1st and 3rd larval instars (p>0.05) (Figure 3).

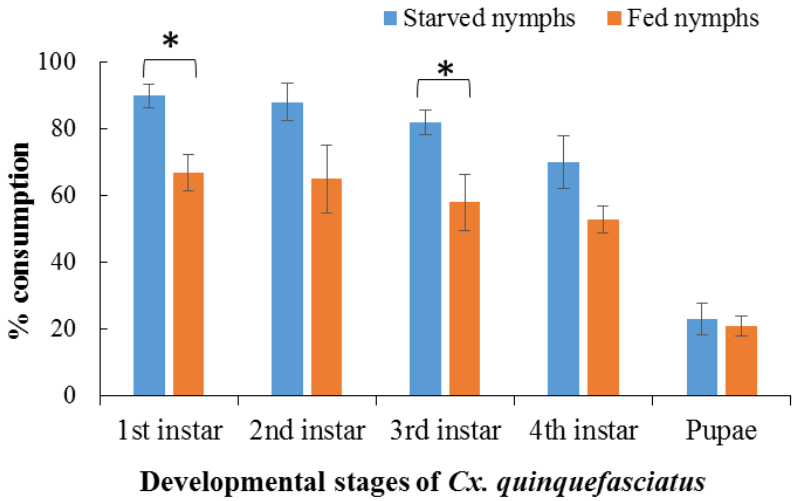

Figure 3. Mean consumption of different larval instars and pupae of *Cx. quinquefasciatus* when exposed to starved and fed nymphs of *R. variegata*. Values are the mean of five replicates. Y-error bar represents the value of the standard error of means. *Significance p<0.05.

The above results indicate that nymphs of both of the dragonfly species used in our study revealed outstanding predation potential against the immature stages of *Cx. quinquefasciatus* mosquito. However, a 24-h starved *C. servilia* exhibited significantly higher predation than that of *R. variegata* on 1st instar larvae (p<0.05), whereas, a fed nymph of *C. servilia* showed significantly higher consumption rate than that of *R. variegata* on the 4th instar larvae (p<0.05) (Table 1).

Table 1. t-test showing equality of means between nymphs of *C. servilia* and *R. variegata* predating on different larval instars and pupae of *Cx. quinquefasciatus*. *Significance at p<0.05).

Larval instars/ pupae		t-test for equality of means						
		t	df	Significance (2-tailed)	Mean difference	Std. error of difference	95% confidence interval of the difference	
							Lower	Upper
Starved nymphs	1st instar	-2.333	8	*0.048	-14.000	6.000	-27.836	-0.163
	2nd instar	0.577	8	0.580	4.000	6.928	-11.976	19.976
	3rd instar	0.148	8	0.886	1.000	6.745	14.554	16.554
	4th instar	0.953	8	0.368	10.000	10.488	-14.185	34.185
	Pupae	0.548	8	0.599	3.000	5.477	-9.630	15.630
Fed nymphs	1st instar	-1.863	8	0.100	-12.000	6.442	-26.855	2.855
	2nd instar	1.112	8	0.299	12.000	10.793	-12.889	36.889
	3rd instar	1.872	8	0.098	18.000	9.617	-4.178	40.178
	4th instar	3.375	8	*0.01	27.000	8.000	8.551	45.448
	Pupae	1.213	8	0.260	5.000	4.123	-4.507	14.507

4. Discussion

Dragonfly and damselfly nymphs have notable predatory potential and they share the same aquatic environment with the larval and pupal stages of mosquitoes (Acquah-Lamptey & Brandl, 2018; Mitra, 2006). Consequently, they can be a good and efficient candidate for the biological control of mosquitoes.

The current study demonstrated that *C. servilia* is a vigorous feeder, capable of consuming a significant amount of *Cx. quinquefasciatus* mosquito larvae in a laboratory setting. All of the predation trials in the current investigation used *Cx. quinquefasciatus* larval instars and pupae. Our results revealed that *C. servilia* exhibits maximum predatory potential against the 2nd instar larvae over other instars and pupae which is compatible with

a previous study showing that nymphs of *C. servilia* prefer to consume 2nd instar larvae of *Cx. quinquefasciatus* over the other instars (Pahari *et al*., 2018). The form and movement of the prey might have an impact on the dragonfly's choice of prey. Since mosquitoes in their 2nd and 3rd instar are more active, their consumption rate is higher. Unlike *C. servilia*, the nymphs of *R variegata* preferred to predate on 1st instar larvae over the other larval instars and pupae. The underlying reason behind such discrepancy is obscure. *C. servilia* nymphs in our study were slightly bigger (20-25mm) than those of *R. variegata* (15-20 mm). The smaller size of *R. variegata* nymphs might be one cause of preferring the smaller, 1st instar larvae of *Cx. quinquefasciatus*. The starved nymphs of both the dragonfly species in our study exhibited higher predation compared to the fed nymphs. Our result is in line with a previous study showing that the consumption rate of *Cx. annulirostris* larvae to the 24-h starved tadpoles was significantly greater than the fed tadpoles (Willems *et al*., 2005).

In addition to size, many variables affect the predation rates and biocontrol effectiveness of dragonfly nymphs on *Cx. quinquefasciatus*. Instar stage, size, and maturity stage of the prey appear to have an impact on the aforementioned predation rates (Blois & Cloarec, 1983; Hassell *et al*., 1976; Venkatesh & Tyagi, 2013). Additionally, some environmental aspects like temperature, container size, and foraging area may have an impact on the dragonflies' ingestion of mosquito larvae (Hampton, 2004). Due to their capacity to eradicate the target species, safety granted to non-target organisms, ease of field application, low cost of production, lack of infectiousness, and lack of pathogenicity in mammals including humans, the use of dragonflies as a biological control agent against *Cx. quinquefasciatus* would essentially be a key solution to control filariasis (Venkatesh & Tyagi, 2013).

The findings of our study clearly revealed that the predatory aquatic dragonfly nymphs of *C. servilia* and *R. variegata* can reduce the density of the larvae and pupae of *Cx. quinquefasciatus* in natural habitats and thereby play a significant role in managing mosquito density in these habitats, suggesting that they may be used as effective biological control agents for mosquitoes in urban and suburban areas.

5. Conclusions

The present study revealed the predatory potential of nymphs of two selected dragonfly species namely, *C. servilia* and *R. variegata* using the immature, aquatic stages of *Cx. quinquefasciatus* under laboratory conditions. Both of the dragonfly species exhibited considerable predation potential against all the larval instars and the pupae of the *Cx. quinquefasciatus* mosquito. Further studies are required to determine the practicability of using these species as well as searching for more species for large-scale mosquito management programs.

Acknowledgements

We acknowledge the Ministry of Science and Technology, Government of Bangladesh for providing partial support for this research project through their ‘Special Allocation Fund’ awarded to KMZ Rahman and MA Razzak (Project Number ES-378, 2021-2022).

Data availability

All the relevant data generated or analysed are provided in the published article. The datasets generated during and/or analysed during the current study are available from the corresponding author on valid request.

Conflict of interest

None to declare.

Authors’ contribution

Khondoker Md Zulfiker Rahman: conceptualization, methodology, insect sampling, bioassay experiments, data collection, data analysis, manuscript writing, manuscript reviewing and editing; Md. Ashikur Rahman and Md. Jillur Rahman: Insect sampling, rearing, bioassay experiments and data entry; Md. Shohag Mia and Md. Mostafa Kamal: Insect sampling and rearing; Mohammad Abdur Razzak and Kabirul Bashar: data analysis, manuscript reviewing and editing. All the authors have read and approved the final manuscript.

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