



A METHOD FOR EVALUATING THE EFFICACY OF A SINGLE BIOAGENT ON THREE SPECIES OF MOSQUITO LAREES

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ABSTRACT

The most significant vector for transmission is mosquitoes. Numerous parasites and viruses that cause illness are capable of being carried by it. Diseases such as dengue fever, dengue hemorrhagic fever, malaria, Japanese encephalitis, and filariasis are transmitted by a number of species of the genera *Aedes*, *Anopheles*, and *Culex*. In India, *Anopheles stephensi* is an important vector for malaria. Malaria continues to be a major public health concern, with an estimated 300–500 million cases each year. Nearly 40% of humanity lives in regions where malaria is a common problem. Lymphatic filariasis's vector, *Culex quinquefasciatus*, is prevalent in tropical regions; an estimated 120 million individuals are infected globally, with 44 million experiencing a common chronic form. It is recognized that *Aedes aegypti* carries dengue. This research included *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* mosquitoes, which are known to have three distinct species. The three distinct mosquito larvae were chosen according to their instar numbers: 2, 3, and 4. Researchers selected *Diplonychus indicus* nymphs of the III, IV, and V instars, as well as male and female, to examine the species' predatory abilities and food preferences. Because *Diplonychus indicus* is known to exhibit a selective feeding behavior toward mosquito larvae, it was effective in destroying three distinct types of mosquito larvae. In addition to the predators (*Diplonychus indicus*) that were part of the experiment as a whole, the most active stage of exposure to *Aedes aegypti* during the fourth instar of larvae was compared to the dominating exposure in male predators. A predator's skill in targeting specific prey populations is crucial to the efficacy of natural enemy biological control agents.

KEYWORDS: *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*, *Diplonychus indicus*, dengue.

INTRODUCTION

The most significant vector for transmission is mosquitoes. Numerous parasites and viruses that cause illness are capable of being carried by it. Dengue fever, dengue hemorrhagic fever, malaria, Japanese encephalitis, and filariasis are among the illnesses that may be transmitted by certain species of the *Aedes*, *Anopheles*, and *Culex* genera (Borah et al., 2010; Rahuman 2009; Samuel 2010). In India, *Anopheles stephensi* is an important vector for malaria. Malaria continues to be a major public health concern, with an estimated 300–500 million cases each year. Nearly 40% of humanity lives in malaria-endemic regions at the present time (Wernsdorfer & Wernsdorfer, 2003). Approximately 120 million individuals are afflicted with lymphatic filariasis globally, and 44 million people have common chronic manifestation (Bernhard et al., 2003). The vector of this disease, *Culex quinquefasciatus*, is found in tropical zones. The *Aedes* dengue is a disease that *aegypti* may transmit. Nearly half of the world's population is now at danger due to the fourfold rise in dengue virus prevalence since 1970. Dengue fever was predicted to be more than 50% contagious in some places in 1990, affecting about 30% of the global population, or 1.5 billion people (Hales et al., 2002). Peridomestic mosquitoes like *Culex quinquefasciatus* feast on humans, mammals, and birds. You won't often see these bugs very far from where people live or work. Open ponds, ditches, and drains that contain sewage from humans or other animals are common places to find the larvae in the eutrophic water of man-made impoundments. Because of this, *Culex quinquefasciatus* was well suited to the conditions seen aboard long-distance sailing ships that were equipped to handle livestock and contaminated water. Because mature mosquitoes may only travel short distances to land (Subra, 1981; LaPointe, 2008), and because juvenile mosquitoes may mosquitoes that were brought ashore in water barrels that were being replenished probably spread all over the globe from the 17th to the 19th century by commercial sailing boats that were engaged in the Atlantic slave traffic, the Old China trade, and the American whale oil business (Hardy, 1960; Lounibos, 2002).

The WHO reports that *Aedes* mosquitoes, and specifically *Aedes aegypti*, are the main vectors for the dengue virus (2009). The female *Aedes aegypti* mosquito bites in order to collect blood, which she uses to fertilize her eggs. The mosquito may transmit many

tropical fevers. Although they are most active in the morning and evening, WHO (2012) states that they may bite and transmit disease at any hour of the day. Even a single bite may spread an illness. The intestinal cells of a female mosquito get infected with the virus when it feeds on the blood of a dengue fever patient during the first two to ten days of the sickness. Vassil Georgiev's work from 2009. After 8 to 10 days, the virus has discharged itself into the mosquito's saliva after spreading to other tissues. Even though it's infectious for life, the virus doesn't appear to harm the mosquito. In particular, the *Aedes aegypti* mosquito is to blame since this species favors humans as a food source, lives in close quarters with humans, and lays its eggs in man-made water containers. I. Gubler (2010) *Anopheles stephensi* is the vector that gives rise to malaria. Plasmodium parasites are the culprits behind malaria. It may be cured and prevented. An estimated 96 million cases of dengue occur annually, putting over 3.9 billion people at danger in more than 128 countries, India included (WHO, 2017). Over 3.34 million cases were recorded in 2016, up from 2.2 million in 2010. The number of cases has risen dramatically in 2019 after a decline in 2017 and 2018. (WHO, 2019).

The worldwide and locally accessible Hemipteran predators are among the predatory insects that show promise as biological controls. The backswimmers, which belong to the family Notonectidae, are among the most frequent insects that feed on mosquito larvae. This makes them a potentially effective tool for controlling mosquito populations. It was discovered in 1939 in New Zealand that hemipteran predators have a role in reducing mosquito larvae. Stock troughs containing *Anisops Assimilis* did not have any mosquitoes, but puddles in depressions around the troughs did (Kumar and Hwang 2006). In ponds and other bodies of water, for example, emerging vegetation partially shields mosquito larvae from predators. The presence of vegetation dramatically decreased the predation potential of *Anisops* and *Diplonychus* bugs, as shown experimentally by (Shalan, 2005) and (Shalan et al., 2007). Several laboratory investigations have shown that the hemipteran water bug *Diplonychus indicus* (Venkatesan & Rao) is an effective predator of mosquito larvae (Venkatesan and Jeyachandra 1985; Victor and Ugwoke 1987; Cloarec 1991; Robert and Venkatesan 1997; Saha et al., 2007). No one has yet measured how *Diplonychus indicus* feeds in relation to the size or type of mosquito larvae, despite reports that it exhibits selective feeding behavior. A predator's skill in targeting specific prey populations is crucial to the efficacy of natural enemy biological control agents.

MATERIALS AND METHODS

Sample collection

One predacious insect – *Diplonychus indicus* in different nymphal instars (III, IV, V, Male and Female) and three types of mosquito larvae (*Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*) were selected in order to study the prey selectivity of the particular predator. For feeding the *Culex*, *Aedes* and *Anopheles* egg crafts were collected from ICMR, Chinnachokkulam, Madurai, India. The egg crafts were cultured in large tray containing de-chlorinated water. For easy observations, 500ml glass beaker was used for the experiments.

Selection of Samples

Diplonychus indicus of various nymphal instars of IIIrd, IVth, Vth, Male and Female were placed separately in plastic trays, due to its synergistic effect. The water bugs were prestarved for 10 days and used in such a way that one predator in each 500ml beaker. The bug of each nymphal instar was exposed to 2nd, 3rd and 4th larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* for each beaker. The prey density was fixed to 25 larvae per predator. Predation by the bug was observed at the end of 1 hour. After predation the bug was transferred to the bucket.

After 1 hour, the number of live larvae was counted and the percentage of mortality was calculated for each nymphal stages of the predator.

$$\% \text{ mortality} = \frac{\text{Total No. of prey} - \text{No. of alive prey}}{\text{Total No. of prey}} \times 100$$

RESULT

The predatory capacity of *Diplonychus indicus* was recorded throughout the experiment. After meeting the larvae, the belostomatids grasped them in their pro- and mesothoracic legs. They proceeded to drink the larva's internal fluid after using their pointed rostrum to puncture its body. Table 1 shows the efficacy of the predator's nymphal phases (III, IV, and V), whereas table 2 presents the same data for the adult stages (male and female). Water bugs in their third, fourth, and fifth nymphal stages consistently consumed twenty-five mosquito larvae in their fourth instar. To begin with, this sneak *Diplonychus indicus* is a predator that obviously prefers foods that contain a lot of water.

Table 1: 24-hour observation on consumption pattern of Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*.

Predator Stages	Prey	Prey killed					
		2 nd prey age		3 rd Prey age		4 th Prey age	
		Mean±S.D	% mortality	Mean±S.D	% mortality	Mean±S.D	% mortality
	<i>Aedes aegypti</i>	4.8±0.789	19	6.8±1.033	27	11.4±1.955	46

III Nymphal	<i>Culex quinquefasciatus</i>	4.5±0.971	18	5.8±1.032	23	10.1±0.737	40
	<i>Anopheles stephensi</i>	4.2±0.632	17	5.3±0.675	21	7.2±1.032	29
IV- Nymphal	<i>Aedes aegypti</i>	6.8±0.789	27	7.7±0.823	31	11.9±0.738	48
	<i>Culex quinquefasciatus</i>	6.0±0.816	24	7.2±0.788	29	11.3±0.948	45
	<i>Anopheles stephensi</i>	5.2±0.788	21	6.9±0.876	28	8.1±0.875	32
V- Nymphal	<i>Aedes aegypti</i>	7.0±1.054	32	13.7±2.058	55	14.4±2.591	58
	<i>Culex quinquefasciatus</i>	7.1±0.994	28	12.4±1.430	50	13.4±1.350	54
	<i>Anopheles stephensi</i>	6.5±0.527	26	9.4±0.843	38	10.6±0.516	42

Among the three different stages of predator were studied the highest activity in 4th instars larvae, Female predator exhibited efficacy activity. Beyond which in overall experiment denoted the predators (*Diplonychus indicus*) were studied, comparative between various larvae only the maximum activity exposed to *Aedes aegypti* at 4th instar larvae the dominant exposure in female predators. The results were recorded in Fig.1, 2, 3, 4 and 5 respectively.

In all three instars (2nd, 3rd and 4th) of *Aedes aegypti* larvae, the predator of III, IV, V, Male and female were selected the 4th instar larvae with mortality (Mean±S.D) of 11.4±1.955, 11.9±0.738, 14.4±2.591, 12.8±1.476 and 18.4±3.134 respectively.

Similarly, in all three instars (2nd, 3rd and 4th) of *Culex quinquefasciatus* larvae, the predator of III, IV and V nymphal stages were selected the 4th instar larvae with mortality (Mean±S.D) of 10.1±0.737, 11.3±0.948 and 13.4±1.350, 12.3±1.947 and 15.4±1.430 respectively.

Subsequently, with in all three instars (2nd, 3rd and 4th) of *Anopheles stephensi* larvae, the predator of III, IV and V nymphal stages were selected the 4th instar larvae with mortality (Mean±S.D) of 7.2±1.032, 8.1±0.875, 10.6±0.516, 10.7±1.159 and 14.3±2.406 respectively. Among the three different mosquito larvae showed the maximum activity of *Aedes aegypti* when compared to other two instars.

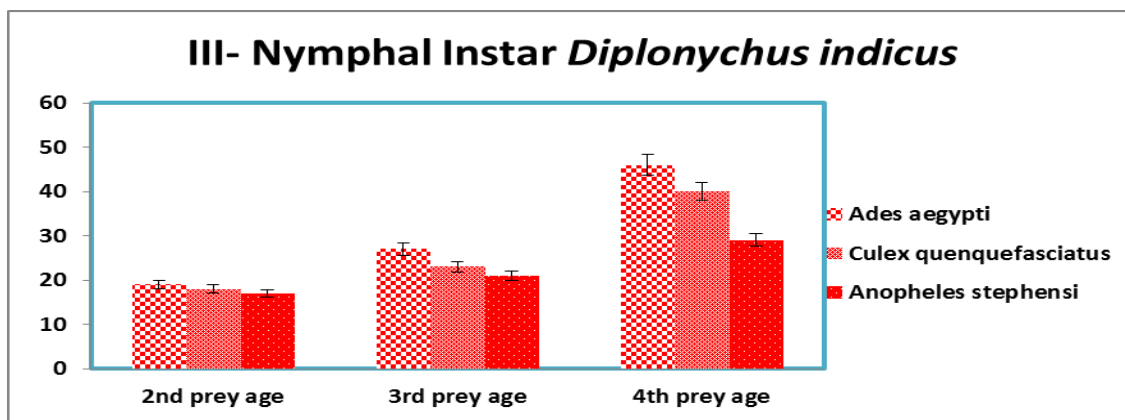


Figure 1: The comparative efficiency of III- Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae.

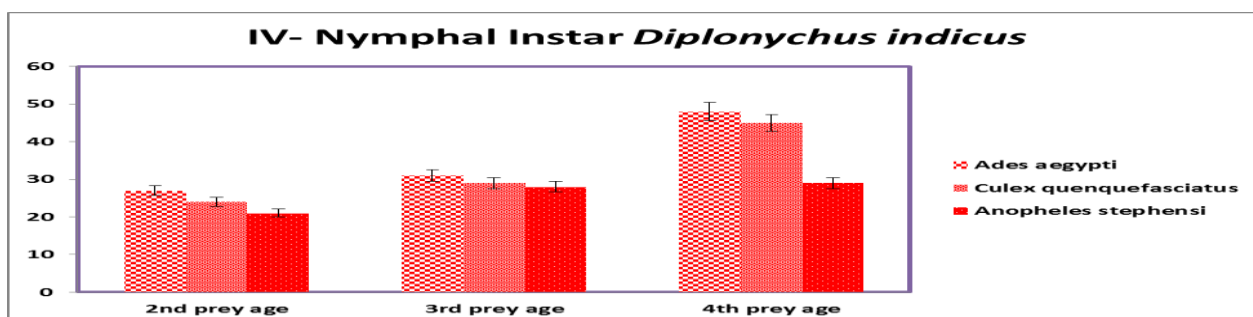


Figure 2: The comparative efficiency of IV- Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae.

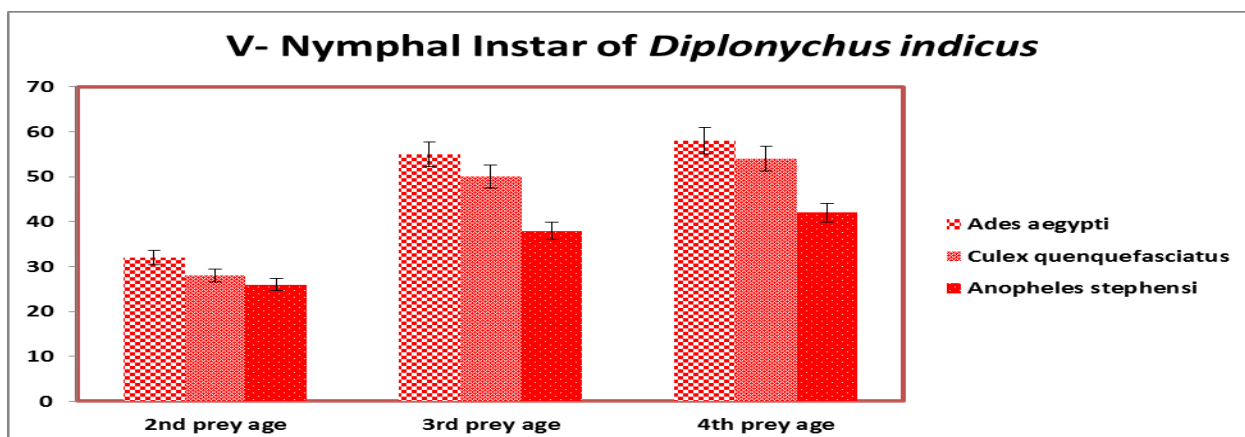


Figure 3: The comparative efficiency of V- Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae.

Table 2: 24-hour observation on consumption pattern of Male and Female Predators of *Diplonychus indicus* on three different species and stages of mosquito larvae, *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*.

Predator Stages	Prey	Prey killed					
		2 nd prey age		3 rd Prey age		4 th Prey age	
		Mean±S.D	% mortality	Mean±S.D	% mortality	Mean±S.D	% mortality
Male	<i>Aedes aegypti</i>	9.7±0.949	39	11.1±0.994	44	12.8±1.476	51
	<i>Culex quinquefasciatus</i>	8.8±0.919	35	9.9±1.663	40	12.3±1.947	49
	<i>Anopheles stephensi</i>	7.5±0.707	30	8.7±0.823	35	10.7±1.159	43
Female	<i>Aedes aegypti</i>	10.2±1.229	41	12.1±1.287	48	18.4±3.134	74
	<i>Culex quinquefasciatus</i>	9.2±0.632	37	10.9±1.524	44	15.4±1.430	62
	<i>Anopheles stephensi</i>	7.9±0.875	32	9.6±0.966	38	14.3±2.406	57

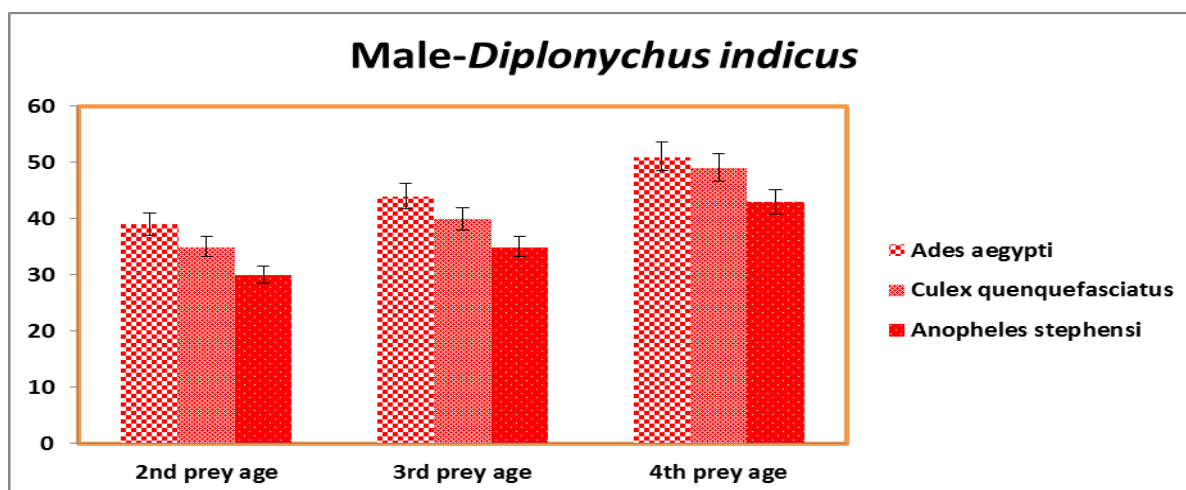


Figure 4: The comparative efficiency of Male *Diplonychus indicus* on three different species and stages of mosquito larvae.

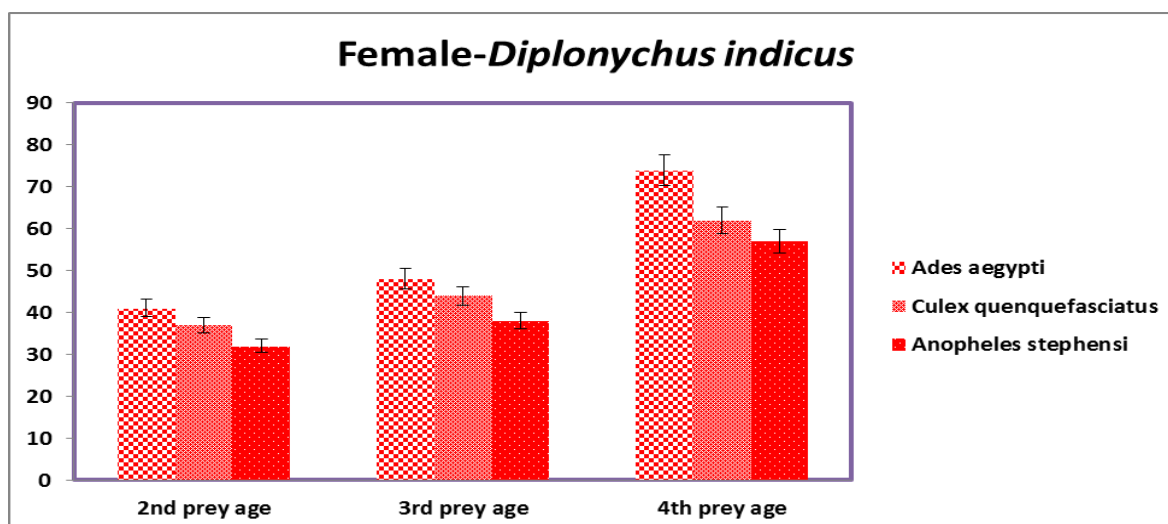


Figure 5: The comparative efficiency of Female Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae

DISCUSSION

After comparing the outcomes with the biocontrol agent, the three mosquitoes used for the study showed concordance with the findings. All of the *Aedes aegypti* larvae were *Diplonychus indicus* instar IV. Considerations such as the prey's flavor, its manageability, the amount of energy gained, and the predator's level of awareness determine the prey's profitability. Similar to other aquatic insect predators, *Diplonychus indicus* is likely impacted by variations in the size, shape, and mobility of its larval populations (Prichard 1964; Thompson 1978; Chowdury et al. 1984).

The proportion of *Anopheles stephensi* mortality was notably reduced at high prey density when different instars of *Diplonychus indicus* were subjected to varied densities of *Anopheles stephensi* (Venkatesan, 1985). According to Cloarec (1991), when the prey density was high, *D. indicus* displayed numerous prey capture behaviors. *Diplonychus* has very large populations of mosquito larvae, which *indicus* agitated, leading to insufficient prey handling and widespread assaults on mosquito larvae, which in turn reduces their density (Venkatesan, 2005).

CONCLUSION

Research on the effectiveness of the male and female mosquito nymphal stages (III, IV, and V) as predators was conducted on three distinct species. At a constant prey density of 25, the water bug's III, IV, and V nymphal stages chose the 4th instar mosquito larvae. *Diplonychus indicus* clearly favors prey with a significant amount of fluid quantity based on this predatory tendency. *Anopheles stephensi* had a very low percentage of mortality at high prey density when exposed to different instars of *Diplonychus indicus* at varying densities. Female predators showed the most effectiveness activity among the three phases of predators tested, with the maximum activity seen in fourth instar larvae. In addition to the predators (*Diplonychus indicus*) that were the focus of the experiment as a whole, researchers compared female predators expose their larvae to *Aedes aegypti* during their peak activity during the fourth instar of development.

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