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COMPARATIVE STUDY OF SINGLE POTENT BIOAGENT AGAINST THREE SPECIES OF MOSQUITO LARVAE

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ABSTRACT

Mosquitoes are the most important transmitted vector. It can able to carry many disease-causing viruses and parasites. Several species belonging to genera *Aedes*, *Anopheles and Culex* are vectors for the pathogen of various diseases like dengue fever, dengue hemorrhagic fever, malaria, Japanese encephalitis and filariasis. *Anopheles stephensi* are major malaria vectors in India. With an annual incidence of 300-500 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world s population live in areas where malaria is endemic. *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation**.** *Aedes aegypti* is known to carry dengue. In the present study focused with three-difference mosquitoes were selected, such as *Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus.* The three different mosquito larvae were selected based on instar 2^{nd}, 3^{rd}, 4^{th}. In order to study the Predatory potential of Diplonychus indicus and the prey preference of various nymphal instars like III, IV, V, Male and Female were chosen. Hence the efficacy of three different mosquito larvae were exposed to *Diplonychus indicus* has been reported to show selective feeding behavior on mosquito larvae. 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 4^{th} instars larvae to the dominant exposure in male predators. The success of biological control agents using natural enemies depends on the ability of the predator to select target prey populations.

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

INTRODUCTION

Mosquitoes are the most important transmitted vector. It can able to carry many disease-causing viruses and parasites. Several species belonging to genera *Aedes*, *Anopheles and Culex* are vectors for the pathogen of various diseases like dengue fever, dengue hemorrhagic fever, malaria, Japanese encephalitis and filariasis **(Borah *et al.*, 2010; Rahuman 2009; Samuel 2010**). *Anopheles stephensi* are major malaria vectors in India. With an annual incidence of 300-500 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world s population live in areas where malaria is endemic **(Wernsdorfer and Wernsdorfer, 2003)**. *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation **(Bernhard *et al.*, 2003).** *Aedes aegypti* is known to carry dengue. The dengue fever incidence has increased fourfold since 1970 and nearly half the world s population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50% **(Hales *et al.*, 2002**).

Culex quinquefasciatus is a peridomestic mosquito seldom found far from human residence or activity, and readily feeds on avian, mammalian or human hosts. The larvae are typically found in the eutrophic water of artificial containers or man-made impoundments including open ponds, ditches and drains containing human or animal sewage. As such, *Culex quinquefasciatus* was uniquely adapted to the environs of historical sailing ships outfitted for long voyages where polluted water and livestock were common. Since adult mosquitoes can fly short distances to shore **(Subra, 1981; LaPointe, 2008)** and immature forms could be carried ashore in water casks taken to be refilled **(Hardy, 1960),** it is likely that this mosquito was spread worldwide by commercial sailing vessels involved in the Atlantic slave trade, Old China trade and American whale oil industry between the 17 and 19th centuries **(Lounibos, 2002).**

Dengu virus is primarily transmitted by *Aedes* mosquitos, particularly *Aedes aegypti* **WHO (2009**). *Aedes aegypt*i is a vector for transmitting several tropical fever and only the female bites for blood which she need to mature her eggs**.** They typically bite during the early morning and in the evening, but they may bite and thus spread infection at any time of day **WHO (2012**). An infection can be acquired via a single bite. A female mosquito that takes a blood meal from a person infected with dengue fever, during the initial 2- to 10-day febrile period, becomes itself infected with the virus in the cells lining its gut **Georgiev, Vassil (2009).** About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. *Aedesaegypti* is particularly involved, as it prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed on people rather than other vertebrates. **Gubler (2010)**

Malaria is transmitted by *Anopheles stephensi*. Malaria is caused by plasmodium parasites. It is preventable and curable More than 3.9 Billion people in over 128 countries including India are at risk of contracting dengue, with 96 million cases estimated per year **(WHO, 2017)**. The number of cases reported increased from 2.2 million in 2010 to over 3.34 millions in 2016. After a drop in the number of cases in 2017-18 a sharp increase in cases is being observed in 2019 **(WHO, 2019)**.

Among the predacious insects promising as biological control are Hemipteran predators which are cosmopolitans and locally available. The backswimmers (Family: Notonectidae) are the most common bugs preying upon mosquito larvae, important factor in reducing immature mosquito population and considered promising in mosquito control. The role of hemipteran predators in controlling mosquito larvae has been recognized since 1939 in New Zealand, when stock troughs with *Anisops Assimilis* were found to be free of mosquitoes whereas puddles in depressions surrounding the troughs contained mosquitoes **(Kumar and Hwang 2006)**.

For instance, emergent vegetation in ponds and other water bodies provide partial protection for mosquito immatures. This effect was experimentally investigated and confirmed by (**Shaalan, 2005**) and (**Shaalan *et al.*, 2007**) whereas predation potential of *Anisops* and *Diplonychus bugs* was significantly reduced by the presence of vegetation.

Diplonychus indicus (**Venkatesan& Rao**), a hemipteran water bug, has been reported to be an efficient predator of mosquito larvae in many laboratory studies (**Venkatesan and Jeyachandra 1985; Victor and Ugwoke 1987; Cloarec 1991; Robert and Venkatesan 1997; Saha *et al.*, 2007**). Although *Diplonychus indicus* has been reported to show selective feeding behavior on mosquito larvae, its feeding behavior in relation to larval size and species has not yet been quantified. The success of biological control agents using natural enemies depends on the ability of the predator to select target prey populations.

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 quinquefaciatus*, *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, Chinnachokkikulam, 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

On observation of the experiment, the predatory potential of the predator – *Diplonychus indicus* was recorded. The belostomatids encountered the larvae and grasped it with its pro- and mesothoracic legs, punctured their body using its sharp rostrum, and sucked its internal body fluid. The efficacy of the nymphal stages (III, IV and V) of predator is shown in the table 1 and the adult stages (Male and female) were depicted in table: 2. Simultaneously, in overall III, IV and V nymphal stages of the water bug selected the 4th instars of mosquito larvae at a fixed prey density of 25. From this predacious habit of *Diplonychus indicus*, it is clear that the predator prefers the prey with high amount of fluid quantity.

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

Predator Stages	Prey	Prey killed					
		2nd prey age		3rd Prey age		4th Prey age	
		Mean±S.D	% mortality	Mean±S.D	% mortality	Mean±S.D	% mortality
III - Nymphal	*Aedesaegypti*	4.8±0.789	19	6.8±1.033	27	11.4±1.955	46
	Culex quinquifaciatus	4.5±0.971	18	5.8±1.032	23	10.1±0.737	40
	Anopheles stephensi	4.2±0.632	17	5.3±0.675	21	7.2±1.032	29
IV-Nymphal	*Aedesaegypti*	6.8±0.789	27	7.7±0.823	31	11.9±0.738	48
	Culex quinquifaciatus	6.0±0.816	24	7.2±0.788	29	11.3±0.948	45
	Anopheles stephensi	5.2±0.788	21	6.9±0.876	28	8.1±0.875	32
V-Nymphal	*Aedesaegypti*	7.0±1.054	32	13.7±2.058	55	14.4±2.591	58
	Culex quinquifaciatus	7.1±0.994	28	12.4±1.430	50	13.4±1.350	54
	Anopheles stephensi	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.

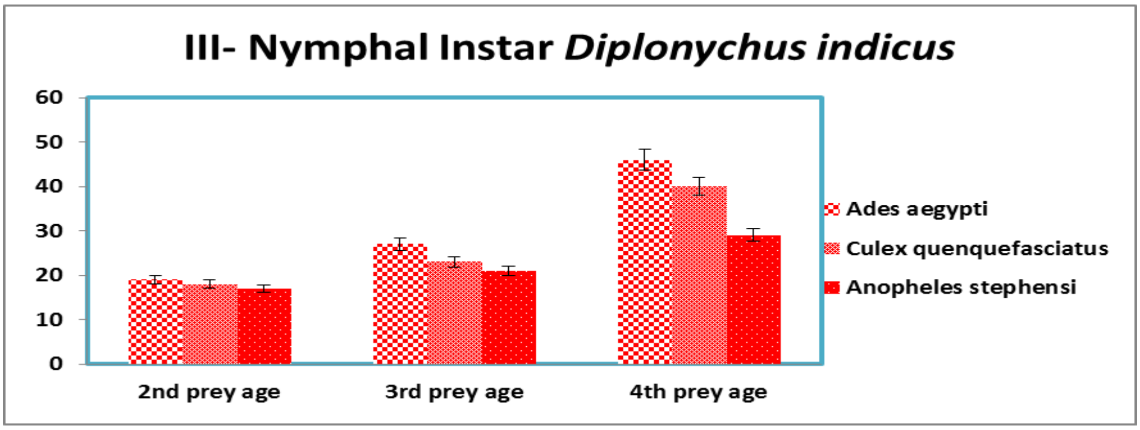

Figure 1: The comparative efficiency of III- Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae.

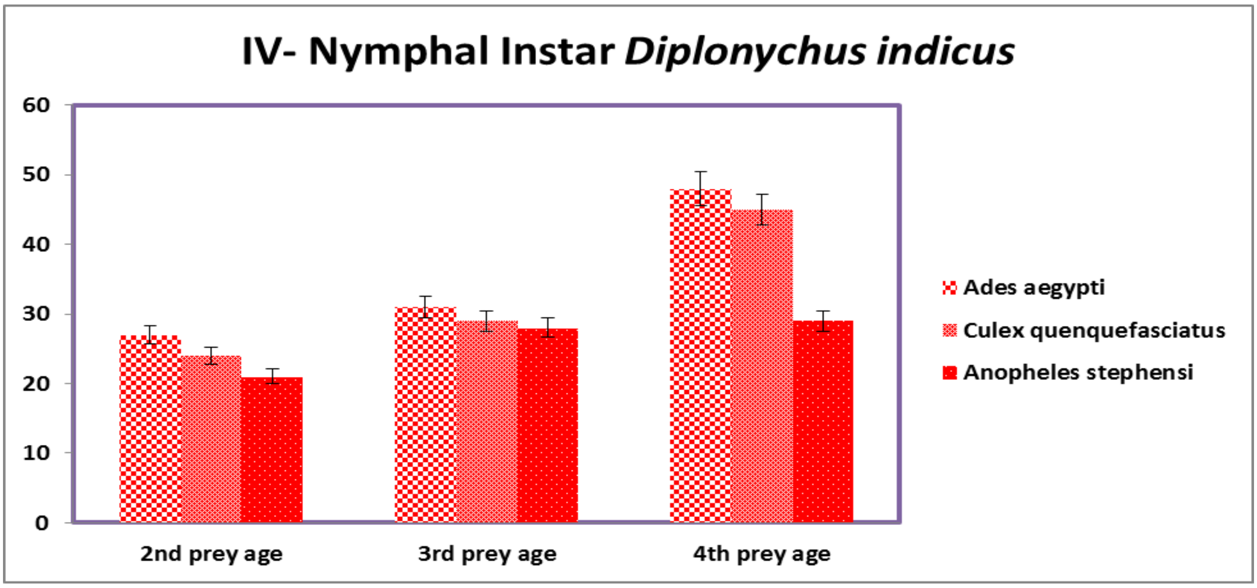

Figure 2: The comparative efficiency of IV- Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae.

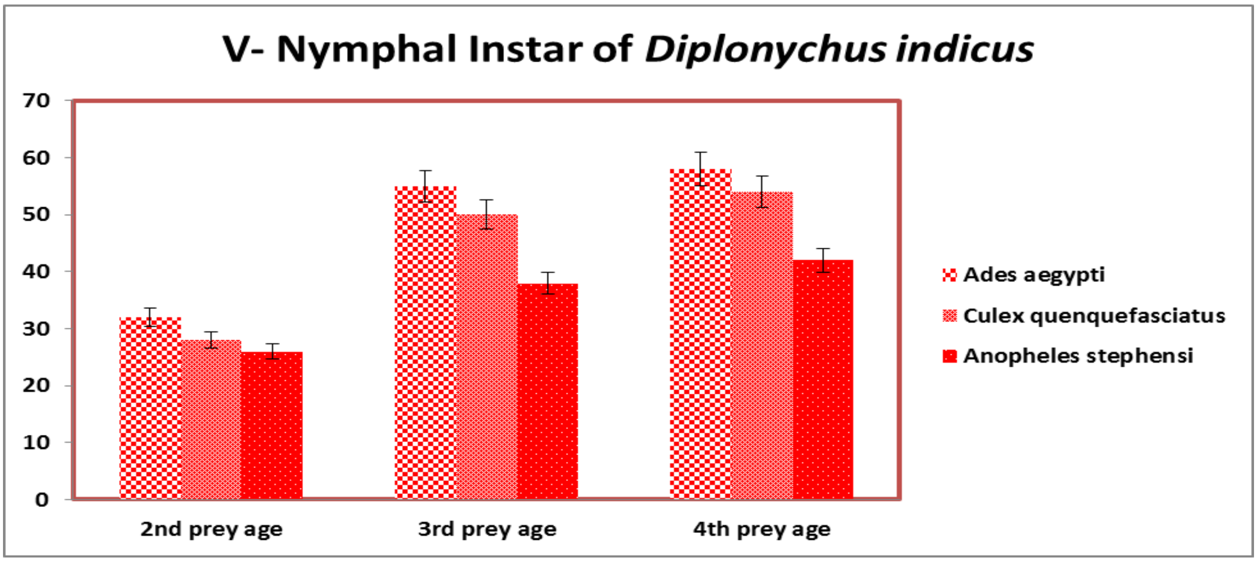

Figure 3: The comparative efficiency of V- Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae.

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 quinquefaciatus, Aedesaegypti and Anopheles stephensi.*

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

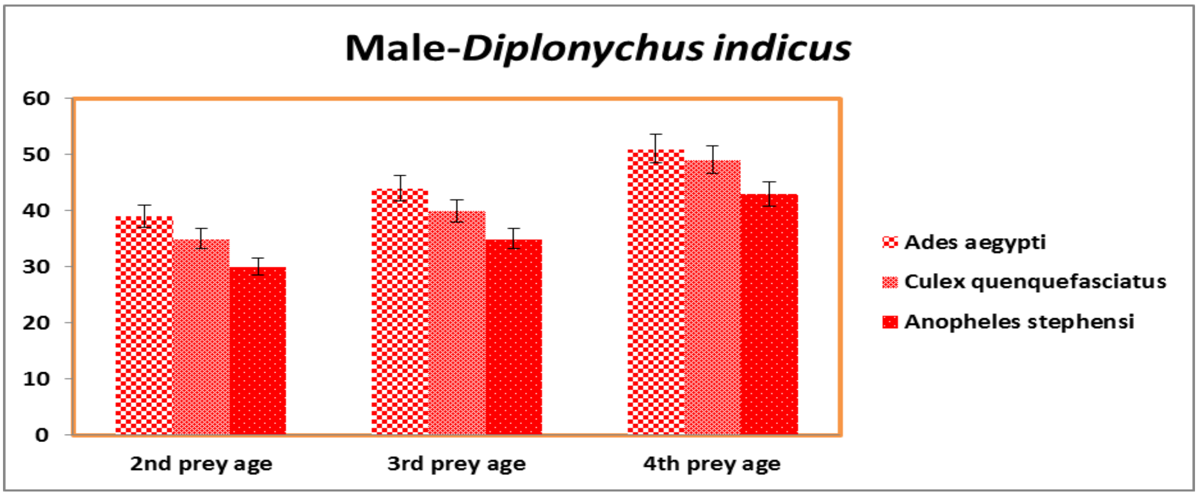

Figure 4: The comparative efficiency of Male *Diplonychus indicus* on three different species and stages of mosquito larvae.

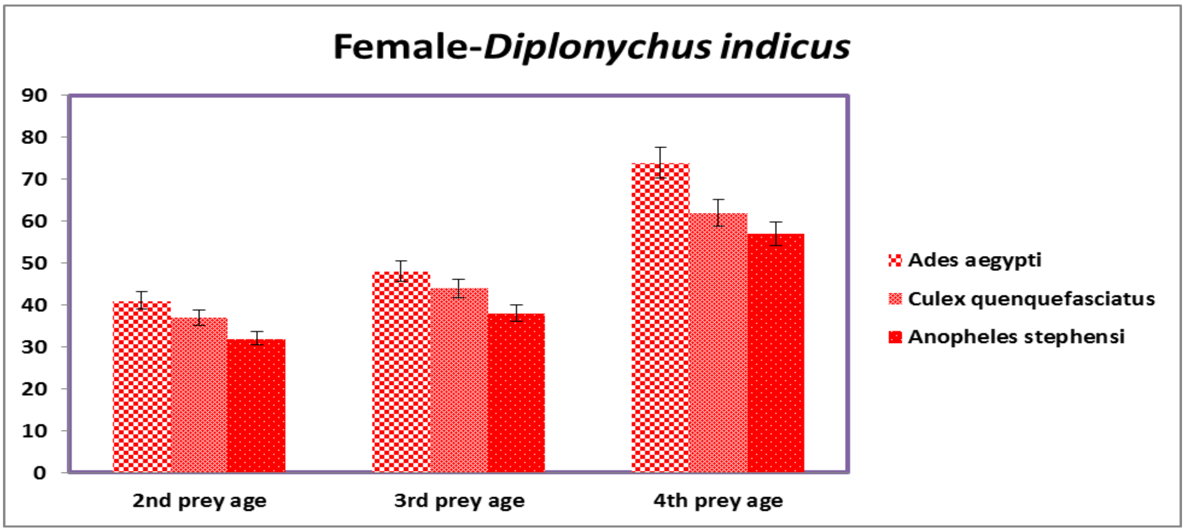

Figure 5: The comparative efficiency of Female Nymphal stages of *Diplonychus indicus* on three different species and stages of mosquito larvae

DISCUSSION

The above results were concurrency with the three mosquitoes was selected and the results were compared with biocontorl agent. All the instars larvae were selection of *Diplonychus indicus in* IV instars of *Aedes aegypti*. The profitability of the prey is judged based on its palatability, ease of handling by the predator, net energy gain obtained and consciousness to the predator. Presumably, predatory behavior of *Diplonychus indicus* may be influenced by differences in movement, shape and size of the larval populations, as evidenced from other aquatic insect predators **(Prichard 1964; Thompson 1978; Chowdury *et al.* 1984).**

Similarly, when different instars of *Diplonychus indicus* were exposed to various densities of *Anopheles stephensi*, the percentage mortality of *Anopheles stephensi* was significantly low at high prey density **(Venkatesan, 1985).** At high prey densities, *D.indicus* exhibited multiple prey capture **(Cloarec, 1991).** Very high densities of mosquito larvae make *Diplonychus indicus* restless resulting in incomplete handling of prey and extensive attacks on mosquito larvae and thereby bring down their density **(Venkatesan, 2005).**

CONCLUSION

Among the three different mosquitoes shown the efficacy of the nymphal stages (III, IV and V) of predator in the adult stages (Male and female) were studied. The III, IV and V nymphal stages of the water bug selected the 4th instars of mosquito larvae at a fixed prey density of 25. From this predacious habit of *Diplonychus indicus*, it is clear that the predator prefers the prey with high amount of fluid quantity. When different instars of *Diplonychus indicus* were exposed to various densities of *Anopheles stephensi*, the percentage mortality of *Anopheles stephensi* was significantly low at high prey density. 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.

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