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Prey influence of mosquito larvae: *aedes aegypti* on predatory potential of *diplonychus indicus*

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Abstract
Aedes aegypti is a vector of dengu, which were lives in fresh water habitat and breeding from man made containers. In the presence study using aquatic bug *Diplonychus indicus* as biocontrol agent to eradicate different instars of *Aedes aegypti*. A remarkable increase in the number of prey killed different prey density of 25, 50, 75 and 100 by female predator exhibited a mean value of 28.8 for a prey density of 100 followed by male revealed a similar trent. When the prey density was increased and the predation rate also increased.
Among the different prey ages the adult predator preferred 4th prey age size. Among the different nymphal instar of *Diplonychus indicus* fifth nymphal instar revealed maximum predation rate of 24 percent.

Keywords: Diplonychus indicus, Aedes aegypti, predation, Dengu

1. Introduction
In recent years, many deadly disease were more prevalent in developing countries such as dengue, chikungunya, yellow fever, zika virus, etc., that are transmitted through vectors. These vector-borne diseases account for more than 17% number of all infectious diseases, causing more than 7,00,000 deaths annually. 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 the early reported in 2017-18 a sharp increase in cases is being observed in 2019 (WHO, 2019). Mosquitoes are one of the deadliest animals in the world. Mosquitoes are the most important vector than any other anthropodes that are able to carry many disease-causing viruses and parasites. Among many vectors [mosquitoes] such as *Aedes aegypti*, *Culex quinquefaciatus*, *Anopheles Stephensi* etc. *Aedes aegypti* is the main vector that transmits the viruses that cause dengue, a lethal arboviral disease that has created a new challenge for public health. (WHO 2016).
Dengue is a viral disease caused by a virus of *flaviviridae* family transmitted by female mosquitoes mainly of the species *Aedes aegypti* and to a lesser extent *Aedes albopictus*. This disease is more prevalent in tropics with local variation is risk influenced by rainfall, temperature and unplanned rapid urbanization. Dengue is the 2nd most diagnosed cause of fever after malaria. (WHO 2017).
Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Ae. albopictus*. This mosquito also transmits chikungunya, yellow fever and Zika infection. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization.
Aedes aegypti is the primary vector of dengue. This mosquito lives in urban habitats and breeds mostly in man-made containers such as tyres, drums, disposed plastic cups, coconut shells, etc. (Panicker and Rajagopalan, 1978) [4]. Unlike other mosquitoes, *Aedes aegypti* is a day time feeders its peak biting periods are early in the morning and in the evening before dusk. *Aedes* eggs can remain dry for over a year in their breeding habitat and hatch when in contact with water. Therefore, there is an urge to control these dreadful diseases by effective means that is both efficient and economical. Despite vaccination and effective drugs, vector control provides a promising tool for prevention and control of these diseases. Vector control measures against immature of *Aedesaegypti* are more amenable than adult control (Bang and Tonn, 1993) [5].
Though there are many larvicides of chemical means, their usage has been limited due to the inaccessibility of their habitats (Mahadev *et al.*, 2004) [6] and controversial impact in areas of non-target organisms. Owing to the complications attempted by the application of pesticides, there is increasing evidence that certain groups of aquatic insects may be utilized in the biocontrol of mosquitoes at the larval stages by introducing them in their habitats. (Menke, 1979) [7]
One of the most fascinating characteristics of the aquatic insect population is their diverse pattern of distribution in aquatic habitat coupled with their adaptability and their flexibility to with stand the after extreme and sometimes unpredictable environments (Polhemus, 1979) [8]. *Aedes aegypti* is commonly called yellow fever mosquito active throughout the year, with a peak in abundance from August to October. They preferentially feed multiple times during one gonotrophic cycle (Weaver and Reisen, 2010) [9].
Diplonychus indicus Venk and Rao belongs to *Belostomatidae* family are comparatively small tropical water bugs, where adults measure between 13& 17 mm long. The female bug tends to oviposit on the dorsum of the

male which takes care of the egg mass till their eclosion. These are known as encumbered males. The mode of predation of *Diplonychus indicus* is they grasp prey using their forelegs and consume then by sucking. It also uses different predatory tactics. Venkatesan and Sivaraman (1984) [10] recorded that this species as efficient predator of anophiline and cuilicine mosquito larvae. Thus the aim of this work was to determine the predatory potential of various nymphal stages of *Diplonychus indicus* on different instar of prey *Aedes aegypti*. and methods

Collection and maintenance of Predator (*D. indicus*)

The belostomatid of *Diplonychus indicus* were collected from the study area in a permanent pond enriched with floating vegetation (such as chara sp, Hydrilla sp) in Upparapatti, Theni, India. These vegetation were collected using a hand net and spread over the tray to hand pick the bugs that cling on to roots of the vegetation. Early hours of a day were preferred for collection of the predator. The collected bugs were maintained in an aquarium of 50 Litre capacity along with sample vegetation. The tank was placed under room temperature (31°C±2°C) with natural photoperiod (LD: 12:12).Water in the container was changed on a regular basis and the dead ones were removed in order to avoid the fouling of the medium.

Collection and maintenance of Prey (*Aedes aegypti*)

Larvae of *Aedes aegypti* were taken as the prey species throughout the analysis. *Aedes aegypti* egg crafts were collected from ICMR, Chinnachokkikulam, Madurai, India. There were brought to the laboratory and held for larval hatch in a plastic tray containing clean tap water of 2 litre. The bucket was covered with the nylon mosquito mesh. The larvae were fed with the dog biscuits and yeast powder in the ratio of 3:1 as food until they convert into pupae.

Experimental setup: Predatory efficiency of various nymphal stages of *Diplonychus indicus* at different prey densities

Diplonychus indicus of various nymphal instars of III, IV, V, Male and Female were placed separately in plastic trays, due to its cannibalistic effect. The water bugs were pre-starved 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 *Aedes aegypti* and prey density was fixed as 25, 50, 75 and 100 for each 500 ml beaker. Predation by the bug was observed at the end of 1 hour. After predation the bug was transferred to the bucket.

After 24hrs of starvation, again the predator was fed with mosquito larvae, and then the number of prey killed was noted at end of 1 hour. The experiment was conducted with 10 trials and the mean, median, mode, standard deviation and percent of prey killed were recorded.

The predatory performance of different stages of *D. indicus* in relation to the specific parameters was investigated in order to determine the interaction effects of categorical independent variable which includes prey density, prey size and nymphal stages.

Results

During the course of the experiment, predatory behavior of *Diplonychus indicus* on the prey *Aedes aegypti* with different prey density and prey size were investigated (Fig:1 and 2). Table: 1 depicted when prey density increases, mortality rate gradually decreases. On other hand, when prey age increases from 2nd, 3rd, 4th instar larvae, % of prey killed was also gradually increases.

Fig 1: Predatory potential of Male *Diplonychus indicus* using 4thnymphal instar larvae of *Aedes aegypti*

Fig 2: Predatory potential of Female *Diplonychus indicus* using 4thNymphal instar larvae of *Aedes aegypti*

The predator *Diplonychus indicus* prefer 4th prey age to 2nd prey age, due to its unique predatory tactics of piercing and sucking.

When III-nymphal instar of *Diplonychus indicus* employed for the predation experiment and exposed to 4th prey age of *Aedes aegypti* revealed a remarkable increase in the number of prey killed from a prey density of 25,50,75 and 100 which revealed 46%, 27%, 20% and 19% respectively.

Simultaneously the predatory potential of IV-nymphal instar of *Diplonychus indicus* upon exposed to 4th prey age of *Aedes aeypti*the number of prey killed per hour was found to be maximum mortality that reveals 48%, 29%, 23% and 20% exposed to a prey density of 25, 50,75 and 100 respectively. Since handling time of the predator instars of IV and V stage *Diplonychus indicus* differs.

The study further extended with larger prey size of 4th instar larvae of *Aedes aegypti* exposed to Vth nymphal instar of *Diplonychus indicus* showed comparatively higher predation rate among the prey density of 25 and gradually increased to 28% on analysis of percentage of predation revealed a gradual decrease from a prey density of 25 - 100.

The predation of Male Diplonychus indicus exposure of 2nd, 3rd, 4th prey age of *Aedes aegypti.* The prey death rate by male predator of *Diplonychus indicus* was recorded with 2nd prey age which exhibited 163 number of prey killed for ten replicates of *Aedes aegypti*. Similarly upon exposure to 3rd prey age exposed to a prey density of 100 shown number of prey killed as 232 in *Aedes aegypti*. The study further carried over with 4th prey age of *Aedes aegypti* exhibited a drastic increase in the number of prey killed of 256 per hour by male *Diplonychus indicus* recorded for ten replicates for a period of 1 hour predation during 24 hours of exposure. The above result were calculated by mean value was noted in Table 1.

Before commencement of experiment the predator was pre-starved over night. On observation of percentage of predation it was found to be gradually declined from a prey

density of 25 to 100 exhibiting 51%, 36%, 28% and 26% respectively.

While the predation by female *indicus* on 4th prey age size revealed the number of prey killed per hour was gradually increased from 184, 231, 264 and 288 on increasing prey density 25-100. It is interesting to denote that number of prey killed increased with increased prey density irrespective of the prey species. The predator increased the attack rate and decreased the handling track. Among the all experiment were analyzed calculated by mean values based on the triplicate.

Table 1: Prey Death Rate /hour of Different Prey Ages of *Aedes aegypti* by *Diplonychus indicus* at Prey Density of 100

Diplonychus indicus	**No. of Prey killed (Mean of 10 Replicates)**											
	2nd preyAge				**3rd preyAge**				**4th preyAge**			
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**
III - instar	4.8	7.3	10.1	12.4	6.8	10	12.3	15.4	11.4	13.5	15.1	18.5
IV - instar	6.8	8.5	10.9	13.8	7.7	10.4	13.6	15.7	11.9	14.3	16.9	20.3
V - instar	8	9.3	12.7	14.8	13.7	17.5	20.3	23.2	14.4	19.7	22.3	24.4
Male	9.7	12.3	14.2	16.3	11.1	14.8	18.3	23.2	12.8	18	21.3	25.6
Female	10.2	13	15.4	17.5	12.1	16	21.1	25.1	18.4	23.1	26.4	28.8

Table 2: Prey Death Rate /hour of Different Prey Ages of *Aedes aegypti* by *Diplonychus indicus*

	No. of Prey killed in Percentage											
	2nd preyAge				**3rd preyAge**				**4th preyAge**			
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**
III - instar	19%	15%	13%	12%	27%	20%	16%	15%	46%	27%	20%	19%
IV - instar	27%	17%	15%	14%	31%	21%	18%	16%	48%	29%	23%	20%
V - instar	32%	19%	17%	15%	55%	35%	27%	23%	58%	39%	30%	24%

Table 3: Prey death rate /hour of different prey ages of *Aedes aegypti* by male and female *Diplonychus indicus*

	No. of Prey killed in Percentage											
	2nd preyAge				**3rd preyAge**				**4th preyAge**			
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**
Male	39%	25%	19%	16%	44%	30%	24%	23%	51%	36%	28%	26%
Female	41%	26%	21%	18%	48%	32%	28%	25%	74%	46%	35%	29%

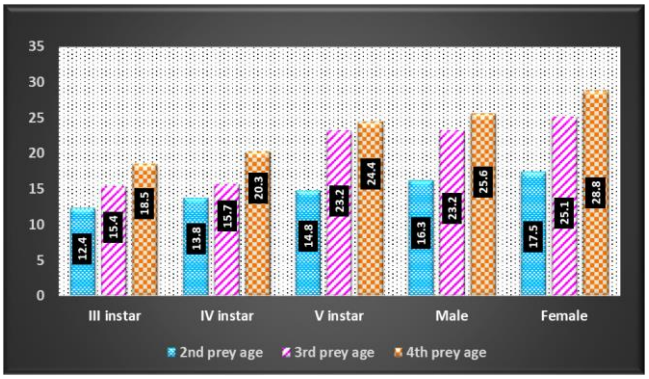

Fig 1: Prey Death Rate /hour of Different Prey Ages of *Aedes aegypti* by *Diplonychus indicus* at Prey Density of 100

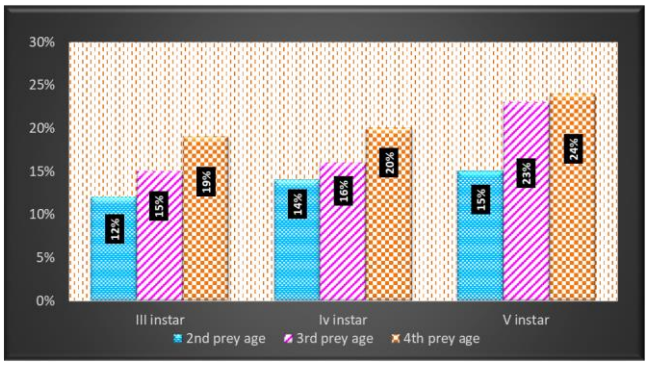

Fig 2: Prey Death Rate /hour of Different Prey Ages of *Aedes aegypti* by Different instars of *Diplonychus indicus*

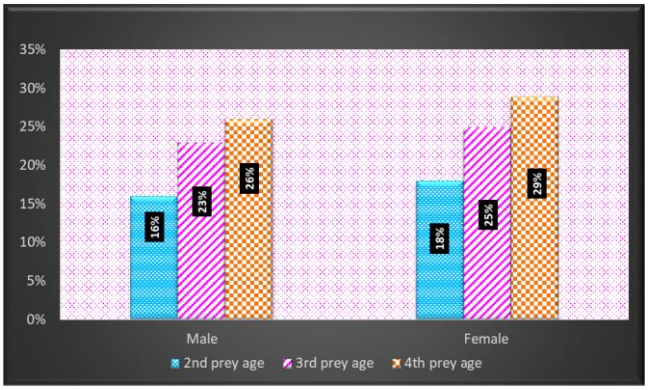

Fig 3: Prey Death Rate /hour of Different Prey Ages of *Aedes aegypti* by Male and Female Predator *Diplonychus indicus*

Discussion

Diplonychus indicus is consider as an efficient predator even at high density which similar to back swimmer notonecta undulate where the predation rate was found to be high at high prey density. *Diplonychus indicus* more efficient than copepods that are currently advocated for the control of *Aedes aegypti* a freshwater breeding such as discarded automobile tyres and stagnant rain water. During rainy season there would be an increase in the number of containers in and around house hold which is a breeding source of *A.aegypti* (Nam *et al.*, 2000) [11].

Earlier report suggested the *D.indicus* is a good biocontrol agent of mosquito especially the dengue vector *A.aegypti*.

The present study highlighted to understand the prey predator relationship which is an important aspects in community ecology besides, principle components of this relationship in the predators rate of reading upon the prey. *D.indicus* preferred predominantly late instars such us IVth prey age of *A.aegypti*. Hence emergence of pupae can be prevented. So, these predators have to be released directly in breeding grounds of *A.aegypti.* Our results coincide with the study of (Dieng *et al.,* 2002) [12].

In our studies were coincide with Sivgananame (2000) on *D. indicus* used with increasing prey density and reached a plateau at and beyond the density of 80, thereby sowing optimum prey density for its predation.

Conclusion

In our research were concluded with *Diplonychus indicus* is consider as an efficient predator even at high density which similar to back swimmer notonecta undulate where the predation rate was found to be high at high prey density. *Diplonychus indicus* more efficient than copepods that are currently advocated for the control of *Aedes aegypti.* The *D.indicus* is a good biocontrol agent of mosquito especially the dengue vector *A.aegypti*. The present study highlighted to understand the prey predator relationship which is an important aspects in community ecology besides, principle components of this relationship in the predators rate of reading upon the prey. *D.indicus* preferred predominantly late instars such us 4th prey age of *A.aegypti*. Hence emergence of pupae can be prevented.

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