



Influence of Chlorpyrifos on the Functional Response of *Diplonychus indicus* Venkat & Rao

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ABSTRACT

Mosquitoes are important vectors of several tropical diseases such as Dengue, Chickungunya and Malaria. Vector borne diseases are the most important cause of public health problems in the developing countries. Aquatic insects play an important role in mosquito control since both of them are aquatic D. indicus belonging to the family Belostomatidae, can be used as a biological control agent against the dengue vector A. aegypti. Generally, D. indicus responded positively to increase in prey density. As the prey density increased, there was a corresponding increase in the prey intake. But on exposure to Chlorpyrifos, the predatory potential gets limited and showed type II functional response.

Key words: Mosquitoes, Vector, Diseases, Malaria, Filariasis

INTRODUCTION

Vector borne diseases such as malaria and filariasis are not uncommon in tropical countries like India. The incidence of such endemic diseases is increasing day by day due to improper maintenance of our environment where the mosquitoes -carrier of parasites- are proliferating enormously and pose great threat to human kind. To control ever increasing mosquito population, pesticides are being applied massively and indiscriminate application of chemicals leads to resistance in the insects with residual deposits of the pesticides. Mostly the accumulated pesticides bio-magnify through the food chain to an

alarming level. DDT showed highest potential chronic risk to freshwater arthropods⁸.

Some aquatic insects and crustaceans are susceptible to carbaryl and Malathion pesticides²³. The defective growth in the larval stages of caddis fly was due to the ingestion of pesticide¹³. The pesticide toxic properties and its obvious risk to non-target organism, either at the application site or due to unintentional spreading, at nearby or even distant area. Indirect effects are fundamental to bio-complexity of ecological systems and provide severe challenges to predict the impact of environmental changes^{25,17}.

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As an alternative to the chemical method, a biological phenomenon called integrated pest management has come into force. Nowadays, this has gained momentum in controlling vectors. The vectors particularly mosquitoes, spend their larval period in the aquatic environment during development. When the developmental stages proceed from 1st instar to the adult, some of the aquatic insects prefer such development stages of the vectors as prey. This observation gives a clue to utilize them to feed on mosquito larval stages. There is an increasing evidence that certain species of aquatic bugs such as Notonectids, Nepids, Lethocerines and belostomatids have been utilized against mosquito larva. Generally, the water bugs are voracious feeders of mosquito larvae. Based on the earlier reports, the belostomatid bug *Diplonychus indicus* is tested against the mosquito larvae. Using these aquatic communities, attempts have been made to achieve greater success in the control of mosquito larvae in the laboratory and field conditions. *D. indicus* is a voracious feeder of larval mosquitoes¹⁵. It is found in temporary and permanent ponds found clinging to the rootlets of *Eichhornea* and *Hydrilla*. It belongs to the order Hemiptera and family Belostomatidae. Generally when the prey density increases the prey intake also increases.

The control of a pest by a predator depends strongly on the predator – prey interaction such as the predator's numerical and functional responses¹⁰. Functional response tests show the potential of a predator's ability to suppress the different densities of prey. In fact, it describes the way a natural enemy responds on the changing densities of its prey and it is a commonly measured attribute of natural enemies. Holling^{10,11} proposed three types of functional responses. In type I, number of killed prey rises as linear to a plateau; type II, a curvilinear rise to a plateau which then levels off under the influence of handling time or satiation and in

type III predator response by a sigmoid increase in prey attacked¹⁶. Many factors such as pesticides influence the functional response of a predator. Several studies provided a strong evidence that insecticides affect the functional response of natural enemies^{26,6,7,3,4,20,21,1}. The functional response of *A. spinidens* to different densities of larvae of *N. aenescens* has been studied by Javadi and Sahragard¹². The present work was carried out to study the effect of chlorpyrifos on the functional response of *D. indicus* against *A. aegypti*.

MATERIAL AND METHODS

The predator *D. indicus* was collected from the permanent pond in Scott Christian College campus Nagercoil. The water bugs found clinging to the rootlets of *Eichhornea* and other vegetation were handpicked. They were then brought to the laboratory and maintained in an aquarium. Larvae of mosquitoes were collected from the stagnant water bodies. The pesticide used in this study were technical material of Chlorpyrifos (ACO, USA, 99% purity). In order to evaluate the searching efficiency of *D. indicus* the functional response of this predator to different densities of *A. aegypti* in pesticide contaminated water was studied. Twenty four hours after treatment with the Sub lethal 0.5ml concentration of chlorpyrifos LC50 of the surveying bugs were kept separately without food for 12 hrs. Then they were individually transferred to Petri dishes (60 mm in diameters) and were fed with different densities (2, 4, 8, 16, 32 and 64). After 24 hrs, the predators were removed and the number of consumed prey was evaluated. The experiment in the same concentration was replicated five times.

In the present study (disc) equation of Holling¹⁰, was used to describe the functional response of *D. indicus* on *A. aegypti*. The various parameters in the (disc) equation are as show below:

- (i) x _____ prey density
- (ii) y _____ total number of prey killed in given period of time Tt.
- (iii) x _____ attack ratio (QAR)

- (iv) T_t _____ total time in days when prey was exposed to the predator.
 (v) b _____ time taken for handling each prey by the predator.
 (vi) a _____ 'rate of discovery' per unit of searching time.

Usually the prey discovery was instantaneous with little of searching time being required. Although the parameter "rate of discovery" (a) was theoretically infinite, the predator did spend sometime in searching for the prey at lower prey density but not at higher density. The extent of searching was closely related to the degree of satiation.

Assuming that the predation is proportional to the prey density and to the time spend by the predator in searching prey (T_s), the expression of relationship is

$$Y = aT_t x \quad \dots\dots (1)$$

But time available for searching is not a constant. It is reduced from the total time, (T_t) by the time spend for handling the prey. If we presume that each prey required, a constant amount of time 'b' for consumption, then

$$T_s = T_t - by \quad \dots\dots (2)$$

Substituting (2) in (1)

$$Y' = a(T_t - by) x \quad \dots\dots (3)$$

$$Y' = Tax \quad \dots\dots (4)$$

$$1 + abx$$

Which is the Holling's (disc) equation¹⁰.

Linear regression was used to establish the relationship between the prey density and the number of prey consumed by the predator and between observed attack ratio and predicted attack ratio.

RESULT

D. indicus- Number of prey killed (*Aedes aegypti*)

The relationship between number of prey density and number of prey consumed is illustrated in Fig.1. In this figure, the response curve rises in a negatively accelerating manner to a plateau that showed type 1 of functional response. The proportion of preys consumed by a predator with a type 1 function response decreases exponentially as the prey density increases. Type 1 is curvilinear and the saturation level is reached in a gradual manner. Comparison of functional response curves

revealed that functional response curve of *D. indicus* in chlorpyrifos treatment was significantly lower than the other treatment.

Table -1 shows the prey death (y) imposed by the adult *D. indicus* on *Aedes aegypti* larvae prey population at different prey densities. The number of prey killed increased with increasing prey density from 1 prey to 32 prey. The adult of *D. indicus* killed the maximum number of *Aedes aegypti* larvae 118.07 for 72 hrs exposures when the prey density level was 32 prey/predator. A regression analysis of prey density(x) against number of prey attacked (y) for 72 hrs observations yielded a positive slope.

$$\text{Slope } y = 3.744x + 14.61$$

The number of *A. aegypti* larvae killed by the adult of *D. indicus* progressively increased of prey density level ,decreases slowly and reached its peak at 32 prey density level.(Table -1 and figure -1).

Attack ratio

The number of prey killed or consumed (y) by *D. indicus* in a given time (y) did not differ significantly from the calculated (y) on the basis of Holling's disc equation.

K would represent the maximum predation at higher prey density, which was 118.07 in *D. indicus* fed on *A. aegypti* larvae at 32 prey density level.

Handling time (b) and Rate of discovery (a)

The time taken by the predator to feed the captured prey was estimated as 'b' value or feeding time. The adult *D. indicus* fed on *A. aegypti* larvae took 0.9092 days handling time per prey. The rate of discovery 'a' increased with the increase of prey density level. The discovery ability was at its peak at one prey density 3.06 and lowest at prey density level 39.35. The maximum predation was represented by 'k' value and in *D. indicus* the K values were always restricted to the higher prey density levels. The

predicted number of prey killer (y') calculated by using 'disc' equation was more or less similar to the observed number of prey killed at various densities.

The highest attack ratio (y / x) was observed at the density of 1 prey / predator and the lowest attack ratio was found at the density of 32 prey predator. Hence the attack ratio decreased when the prey density increased.

The searching time (Ts) decreased with the increased prey density. At the prey densities below 32 prey / predator, the predator has to spend more time for searching the prey. The searching time (Ts), the attack ratio (y/x) the rate of discovery (a) decreased with increasing prey density, (Table 1 & figure-1).

Summary of calculations used in predicting the functional response (y) for 3 days in *D. indicus* at six different densities of *A. aegypti* (n=6)

Days	x	y	k	b	by	Ts	y/x	k/Tt	a	Disc equation y'= a(Tt-by)X	Y'
3	1	9.2	118.07	0.9092	8.3646	9.6354	9.2	39.35	3.06	$Y'=3.06(3-8.3646)1$	9.2
	2	18.2			16.5474	1.4562	9.1		6.06	$Y'=6.06(3-16.5474)2$	18.2
	4	20.62			18.7477	-0.7477	5.16		6.87	$Y'=6.87(3-18.7477)4$	20.62
	8	49.3			44.8235	-26.8235	6.16		16.43	$Y'=16.43(3-44.8235)8$	49.3
	16	108.22			98.3936	-80.3936	6.76		36.07	$Y'=36.07(3-98.3936)16$	108.22
	32	118.07			107.3492	-89.3492	3.69		39.35	$Y'=39.35(3-107.3492)32$	118.07

Figure – 1

Graph predicting the functional response (y) for 3 days in *D. indicus* at six different densities of *A. aegypti* (n=6)

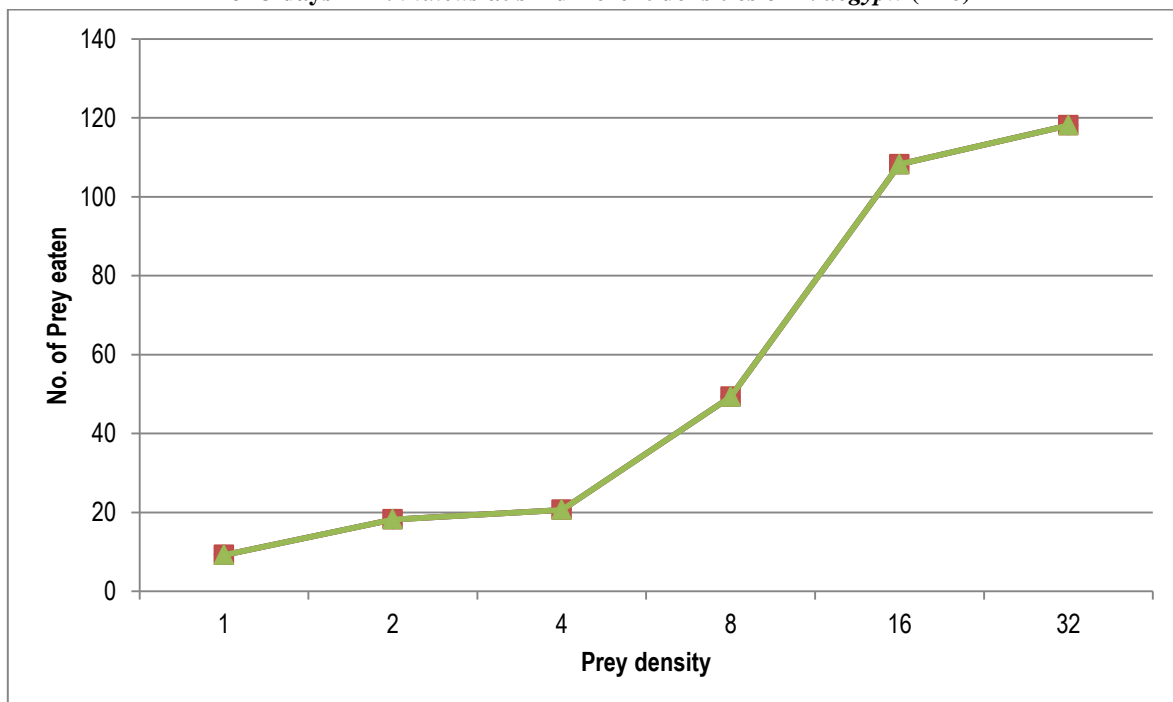
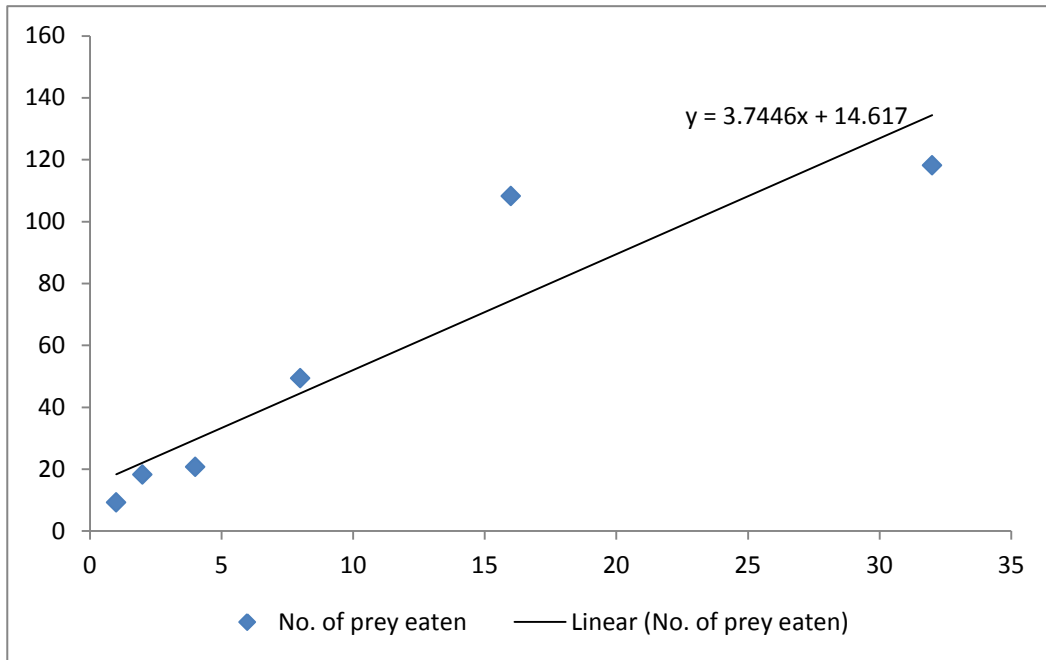


Figure – 2

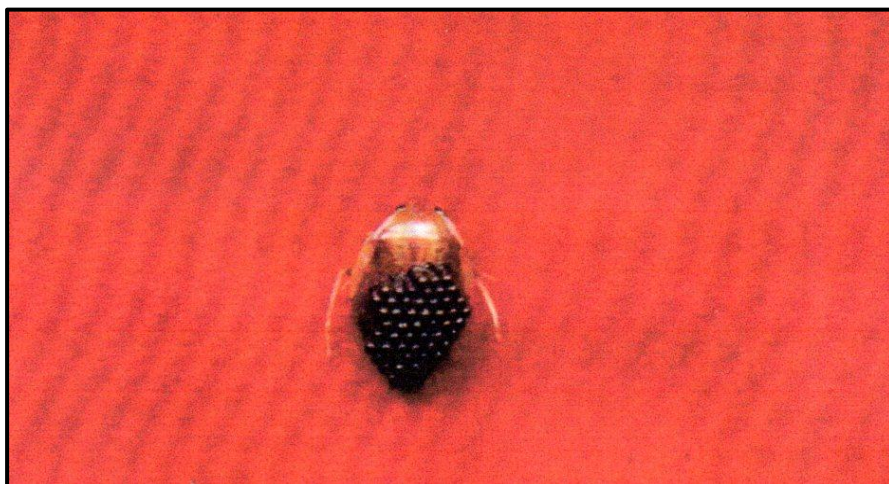
Linear regression analysis predicting the functional response (y) for 3 days in *D. indicus* at six different densities of *A. aegypti* (n=6)



Adult *Diplonychus indicus*



Encumbered male of *Diplonychus indicus*



DISCUSSION

Earlier reports says that *D. indicus* is a good biocontrol agent of mosquitoes especially the dengue vector *A. aegypti*. These aquatic insect are reported to certifie, abundantly with other organisms including the aquatic stages of several species of mosquitoes. Earlier workers have investigated the biological control potential of *D. indicus* at different prey densities. Understanding the prey-predator relationship is an important aspect in community ecology and one principal component of this relationship in the predators rate of feeding upon the prey. Functional response of a predator against prey is the mathematical form of the feeding rate that can influence that the spatiotemporal variation of predators, correlations between nutrient enrichment and the biomass of higher trophic levels⁵. During the present study, the prey-predator relationship exhibited a type II response that describes the average feeding rate of a predator when it spends more time searching processing each captured engaging in encounters with other predators.

Functional response manifests two important parameters including a' and there the used to evaluate the effectiveness of predators and parasitoids. The rate of these parameters are influenced by different factors. One of them in the sublethal concentrations of insecticides^{20,4}. In this study, the sub lethal effects of Chlorpyrifos on the functional parameters of *D. indicus* showed different searching efficiency and handling time compared to control. The present findings showed that the bugs exposed to insecticides had a high handling time. Deng *et al.*⁷, found that contact with organ phosphorus compounds resulted in increased handling time in *Hylyphantes graminicola*. The negative effect of insecticides on functional response have been reported in many natural enemies. Ambrose *et al.*⁴, studied the impact of chlorpyrifos on functional response of *R. marginatus* and reported that the chemical compound caused a less pronounced type II response with reduced numbers of prey killed

attack rate, searching time and prolonged handling time in 4th and 5th nymphal instars, adult and female reflecting reduced predatory potential. Rafiee *et al.*²⁰, demonstrated that profenofosthiodicarb, hexaflumuron and spinosad had a negative effect on functional response of *Habrobracon hebetor*. Furthermore the effect of cypermetrin on the functional response of *A. pedestris* indicated that the pesticide negatively affected the functional response events such as attack ratio, handling time, and rate of discovery and also reduced the predatory efficiency. In addition, Abedi *et al.*¹, showed that the application of cypermethrin on *H. hebetor* caused longest handling time and lowest attack rate. In other study, the functional response of the spider, *P. cespitum* was significantly affected after application of SpinTor, Neem Azal, and Dimilin²¹. Furthermore, the effect of cypermetrin on the functional response of *A. pedestris* indicated that the pesticide negatively affected the functional response events such as attack ratio, handling time and rate of discovery and also reduced the predatory efficiency⁶. Most of the studies reported negative effects of insecticides on the functional response of natural enemies and the presents study too showed negative effects on the functional response of *D. indicus* on *A. aegypti*. And showed type II functional response curve.

Generally, in an integrated control program it is necessary to utilize some insecticides with minimum toxicity to natural enemies of pests. Pesticides are organophosphate compounds and among these chlorpyrifos caused considerable effect on functional response of *D. indicus*, and potentially limited its biocontrol potentiality. So it is recommended to use the pesticides by caution and extensive use should be avoided.

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