FUNCTIONAL RESPONSE OF *CHRYSOPERLA ZASTROWI SILLEMI* (ESBEN-PETERSON) ON *LIPAPHIS ERYSIMI* (KELTENBACH)

ABSTRACT

Biological control of insect pests depends upon predating potential of natural enemies, which in turn relate to their functional response that is key to selection of a proper species for the biological control programs. The main aim of the study was to access the functional response of second instar grub of *C. zastrowi sillemi* (Neuroptera: Chrysopidae) on Raya (*Brassica juncea*) aphid *Lipaphis erysimi* (Keltenbach). Five different predator: prey densities of aphids (1:50, 1:100: 1:150, 1:200 and 1:250) were taken and results indicated the type-II functional curve response by logistic regression analysis of *C. zastrowi sillemi* on *L. erysimi*. Hollings Type II functional response signifies that the rate of prey consumption by a predator rises as prey density increases, but eventually remains constant in spite of increase in prey density. The aphids consumption, search rate (a`) and maximum predation rate (1/Th) was recorded less (7.36, 0.06 and 0.40) as compared (7.56, 0.068and 0.467) when grubs fed on artificial diet. However, time taken to handle a prey by second instar grub was more (2.55) on natural diet but less (2.17) on artificial diet.

Key words: Functional response, *Chrysoperla* spp, *Lipaphis erysimi*, Aphid, Biocontrol

1. Introduction

The Indian Mustard *Brassica juncea* (L.) Czern & Coss., belongs to family Brassicae. It is most commonly called as Rapeseed-Mustard in the trading oil seed specieses in India (Anonymous, 2015). The continuous effort of crop improvement in Rapeseed-Mustard has resulted nutritionally best edible oil seed and as a rich protein source to animal feed (Banga S S and Labana K S 1984). Among the oilseed crops, the *Brassica* group Indian mustard occupies considerably large acreage placing the country on the top both in acreage and production of rapeseed and mustard in Asia (Rao *et al* 2013). Rapeseed-mustard (*Brassica* species) is the major rabi oilseed crop of India. It is the second most important oil seed crop in India after soyabean accounting for nearly 20- 22% of the total oilseeds produced in the country. India is the fourth producer of mustard seed contributing to around 11 % of world's total production (Kumrawat and Yadav 2018). Rapeseed and mustard are cultivated in an area of 56 lakh ha with a production of 66 lakh tonnes and with an average yield of 1182 kg/ha on national level and "1102 kg/ha" at Punjab state (DACNET, 2009-10).

The average yield of Rapeseed-Mustard in Indian sub-continent is low i.e.1980 kg/ha. It's mainly because of major area of crop is grown under rainfed cultivation and aphid complex of *L. erysimi* infestation as a major drawback. This aphid species start attacking during third week of December and progress upto March end. During these periods both nymphs and adults suck sap from leaves, pods and shoots. That leads to curling of infested leaves, may turn black and dried. In severe infestation sooty mould development seen on pods and leaves respectively (Athhan et al., 2004).The avoidable yield losses caused by aphids vary between 20 to 50 per cent, but in extreme conditions, the yield losses could be as high as 79 per cent (Rao *et al* 2013).

Rapeseed-Mustard (*B. junacea*) is being an edible oil seed crop, use of pesticides to manage these aphid species is not an ecologic sin. But, the previous studies indicating that use of natural enemies may be effective in contrary to aphids in integrated pest management and biocontrol programmes (Mushtaq and Khan 2010). The theory of Biological control as predator-prey interactions has been mainly based upon a community model, composed of discrete tropic levels in the order of autotrophs-herbivores-predators. Among these trophic levels predators act as a top trophic level consumers (Rosenheim et al 1999).

The green lacewing *Chrysoperla zastrowi sillemi* (Esben-Peterson) (Neuroptera: Chrysopidae) is one among the very important biocontrol agents, having wide range of tolerance capacity to withstand different ecological factors namely pesticide molecules in field conditions, potent host searching capacity and voracious feeding (Tassan *et al* 1979). These all characteristics preferably attracted entomologist towards their exploitation as a potent predator of different sucking and eggs of lepidopteron pests respectively (Ridgway and Jones, 1969). It is also well known that before exploitation of any predator, we must know the functional response of that animal.

The functional response of a predator is the capability of a predator kills its prey at different prey densities by which it regulates the population dynamics of predator-prey system in an ecosystem (Mahzoum *et al* 2020; Parvez and Omkar, 2005; Khan and Mir, 2008). The very important components of the functional response of a predator are the searching rate (a`), handling time (Th) and maximum predation rate (1/Th) (Hassel *et al* 1976). So that based on responses, the information we came across the number of prey consumed per predator in per unit time and maximum prey capacity per day, by this information entomologist can able to estimate the doses of a predator and can take decision in pests management programmes (Holling 1959). The predatory green lacewing grub possess an excellent prey searching capacity, high dispersal ability, less prey handling time and maximum predation capacity against aphid pests (Holling 1961). Therefore the study of functional response is an important factor in understanding the highlighting mechanism in predator-prey interactions in revealing the evolutionary relationship and

contributing toward biocontrol of crop pests (Khan 2009). The main objective of the study was to determine the potential of *C. zastrowi sillemi* preying on mustard aphid *L. erysimi* through the study of functional response.

2. Materials and methods

The studies on the functional response of *C. zastrowi sillemi* reared on best semi-synthetic (Diet B) and laboratory host (Diet E) was investigated under Dr G S Kalkat Laboratory, Biocontrol Unit, Punjab Agricultural University, Ludhiana, Punjab, India. Under laboratory conditions with five treatments of aphid densities 1:50, 1:100, 1:150, 1:200 and 1:250 (Predator: Prey ratio) were taken and replicated ten times, where each grubs were kept separately in vials. Mustard aphid complex of *L. erysimi* (Kaltenback) was studied.

2.1Rearing of *C. cephalonica* eggs as a laboratory host to *C. zastrowi sillemi*

In order to carryout investigations on *C. zastrowi sillemi*, its laboratory host, *C. cephalonica* was sustained throughout the period of study by mass culturing in the laboratory. *C. cephalonica* was reared following the procedure described by (Sharma et al 2016). Larvae of *C. cephalonica* were reared on bold grains of white sorghum. The required quantity of sorghum was milled to 3–4 pieces in milling machine and heat sterilized in an oven at 100 °C for 30 minutes. To prevent bacterial infection, streptomycin sulphate was added to the crushed sorghum at the rate of 0.2 gm and mixed thoroughly. Rearing boxes, measuring 43 × 23 × 12 cm made of medium density fiberboard (Rescholar Equipment, India), were filled with 2.5 kg of milled and heat sterilized sorghum. These rearing boxes were charged with *C. cephalonica* eggs at 0.5 cc (=8000 eggs; considering 1.0 cc = 16,000 eggs; Jalali and Singh 1989) per box. The volume of the eggs was measured using measuring cylinder. After charging, the boxes were covered with perforated lids having iron mesh (20 mesh) both externally and internally. They were kept on iron racks (90 cm length × 45 cm breadth × 180 cm height) in rearing laboratory at temperature 27 ±2°C and 70±5 per cent RH. The moths emerging from these boxes were collected daily and transferred to the specially designed oviposition cages (35 × 25 × 18 cm Rescholar Equipment, India). The eggs were collected manually and passed through 30 mesh sieve to remove moth scales. These eggs were further passed through 40 mesh sieve to eliminate dust particle. The embryonic development of eggs was stopped by exposing to freezing temperatures and then these eggs were used for the culturing of *C. zastrowi sillemi*.

Chart 1: Formulation of grubs/larval semi-synthetic diet

Ingredients	Quantity
Corcyra eggs (Lyophilized powder)	100g
Streptomycin sulphate	0.1g
Hen's egg	80g
Chlortetracycline	0.1g
Sucrose (Sugar)	10g
Agar	15g
Honey	25g
Distilled water	25ml
Brewer's yeast	12g
Acetic acid	5ml
Salt mixture (Wesson's)	0.5g
Vitamin solution	10ml

Semi- sytnthetic diet was prepared in Dr G S Kalkat Laboratory, Biocontrol unit by utilizing different nutrient compositions. The diets formulated for the larvae of *C. zastrowi sillemi* was as follows. The semi-synthetic diet prepared for grubs was the modification of the diet proposed by Sattar *et al* (2007) for the rearing of *C.carnea*.

To enhance the larval life parameter and efficiency for experimental rearing and mass production, all ingredients of different diet combinations were weighed carefully. For diet preparation, ingredients *viz*., sucrose, preservative (streptomycin sulphate and chlortetracycline), salt mixture and Brewers' yeast were mixed in water and were blended in a food processor prior to the addition of the hen's egg. To this mixtures vitamin solution and agar were added and mixed properly by stirring. Egg was mixed in the mixture after boiling it because the raw egg made the mixture smelly, which was not liked by the larvae. Finally, all ingredients were blended for 6-8min until the entire mixture was of a stringy paste-like

consistency. At this point the mixture was a soft wet solid but it retained any shape given to it. After that the diet was ready to be given to the larvae.

2.3 Methodology of rearing aphids

For the rearing of aphids, *Brassica juncea* var. PBR 91 was sown in the earthen pots, which were kept in the open field condition during cropping season. All the agronomic practices were carried without any plant protection measures. After the natural infestation of aphids, their population was maintained on potted plants to get maximum number of aphid colonies for conducting the experiment under laboratory conditions.

To enhance the larval life parameter and efficiency for experiment, all ingredients of different diet combinations were weighed carefully. Diet ingredients, sucrose, preservative (streptomycin sulphate, chlortetracycline), salt mixture and brewers' yeast were mixed in 25 ml water and were blended in a food processor prior to the addition of the hen's egg. To these mixtures vitamin solution, lypolized powder of eggs of *C. cephalonica*, hen's eggs and agar were added, mixed properly by stirring. Finally, all ingredients were blended for 6-8min until the entire mixture was of a stringy paste-like consistency. At this point the mixture was a soft wet solid but it retained any shape given to it.

2.4 Methodology to study the functional response of *C. zastrowi sillemi*

The functional response of second instar larvae of *C. zastrowi sillemi* grub reared on laboratory host *Corcyra* eggs and best semi-synthetic diet B against *L. erysimi* was studied by utilising above mentioned five different densities of aphids in the laboratory conditions. The second.instar larvae were taken from the culture reared on *Corcyra* eggs (standard check) and semi-synthetic diet B, were starved for 12.00 hours before the onset of experiment and then transferred to the experimental arena (9 cm diameter plastic petri dish) with the help of camel hair brush. These were provided with different densities of aphids for feeding. The number of each prey consumed by the predatory larvae was recorded by counting the live prey after 24.00 hours.

2.5 Statistical analysis

The functional response of predatory larvae *C. zastrowi sillemi* reared on the semi-synthetic diets and laboratory host *C. cephalonica* to different prey densities of *L. erysimi* was measured or described by Holling's disk equation to the data (Holling, 1959). Holling's disk equation for Type II functional response was written on confidence interval limits (95%) and asymptotic standard errors are used as

indicators of differences in searching rates, handling time and maximum predation rate for the second instars of *C. zastrowi sillemi* against *L. erysimi*

The functional response of predatory larvae reared on the semi-synthetic diet and laboratory host to prey densities will be expressed by fitting the data to Hollings equation

Na= a' TN/(1+aThN) …… (1)

Where, Na= Number of prey consumed by the predator per unit time

a`= search rate of predator

T= Total exposure period

N= Original number of preys presented to every predator larvae at start of experiment

Th= handling time for each prey caught (proportion of the exposure time that a predator spends identifying, pursuing, killing, consuming and digesting prey).

The successful search rate of *C. zastrowi sillemi* over the experiment period was computed as:

a` = 1/P ln [N1 / (N1-N2)] ……..... (2)

Where, a = Search rate

ln= Natural logarithm

P = number of predators used

N1= density of prey

N2 = number of prey consumed.

The obtained results of the functional response between second and third instar grubs against *L. erysimi* was fitted to the regression (r2) analysis by using Statistical Package for Social Science (SPSS, IBM version 25 software)

3. Results and Discussion

The aphids (*L. erysimi*) consumption per day by second instar grub varied from 6.30to 8.20 and 6.60 to 8.30 reared on natural diets and artificial diet respectively. The consumption of aphids by second instar grub of *Chrysoperla* recorded an increasing trend from 1:50 to 1:250 predator: prey density (Table

1). The search rate of predator recorded 0.13 to 0.03and 0.14 to 0.03 a decreasing trend from (1:50 to 1:250) predator: prey densities when they offered by natural diet and artificial diet. The time taken to handle the prey by second instar grub was varied between 2.64 to 2.45 and 2.30 to 2.05 at 1:50 to 1:250 predator: prey densities and here the handling time was recorded an decreasing trend when prey densities increases from 1:50 to 1:250 respectively as they offered by natural and artificial diets. The maximum predation rate was recorded an increasing of trend 0.390 to 0.418 and 0.443 to 0.495 from (1:50 to 1:250) predator: prey densities (Table 1) respectively.

The functional response of the second instar grub of *C. zastrowi sillemi* to *L. erysimi* was presented in Figures (1). Obtained results showed that second instar grub of *C. zastrowi sillemi* revealed curvilinear curve Type II functional response on increase of *L. erysimi* from (1:50 to 1:250) predator: prey density. This figure exhibited a significant ($p<0.005$) decline in consumption of aphids at high prey densities, it might be due to attainment of satiation.

The quantity of prey consumption depends upon larval or grub age as well as prey density as supported by Mushtaq and Khan (2010). The relative prey consumption rate was increased initially at lower prey density 50 and it was verified, after which consumption of *L. erysimi* was attained satiation by second instar grub. If further increase in prey density, the prey consumption by grub was not increased further. During the experiment when second instar *C. zastrowi sillemi* grubs were offered with natural host (*C. cephalonica*) and semi-synthetic diet. The curvilinear curve with regression value $r^2=0.885$ and $r^2=0.874$ were obtained with very least difference between two diets.

The search rate of second instar predatory grub completely depends upon the ease of hungriness, density of predator and prey and cohort of prey. The search rate of second instar grub was more at lowest predator: prey density (1:50) and it was started declining as the predator: prey densities increases from 1:100, 1:150, 1:200 and 1:250 ratios. It may be due to the availability of the prey to the second instar grub so grub of chrysoperla expenditure less energy in searching the prey. The grubs fed on artificial diets was more potent in searching its prey than fed on natural diet, this may be due to the dietary requirements or the influence of artificial diet made it to do so. Sultan and Khan (2014) recorded that second instar predatory larvae of green lacewing recorded 0.002 search rate.

Natural enemies when they predate or parasitizes, they take their own handling time to kill the prey or its host by capturing, killing and finally consuming. Usually the time taken to handle a prey was more recorded in second instar predatory grub as compared to the third instar grub of *C. zastrowi sillemi*.

This particular character is due to the stage and age of the predator. The second instar predator is smaller in size and incompletely developed muscular system as well as sensory system to capture a prey and its small appetite. The time taken by second instar grub was recorded a decreasing trend of 2.64 to 2.45 and 2.30 to 2.05 from 1:50 to 1:250 predator: prey densities when they offered by natural and artificial diets respectively. The decrease in the time to handle a prey by predator from lower prey density to higher prey density is mainly due to the availability of more prey or prey density within the 9 cm petriplate indicated in (Table 1). Hassanpour *et al* (2015) recorded handling time of second instar predatory grub was lesser than that of first instar predatory grubs. The rate of maximum predation was recorded at highest predator: prey density (1:250) and lowest at lower predator: prey density (1:50). This was mainly due to the at the lowest prey density and availability of prey was less i.e.50 in number after consuming of all these, there was no prey available to consume furthermore. But at the highest prey density of 250 the availability of prey was more and predator can predate further more aphids until its full of appetite satisfaction. Therefore it was an increasing trend of maximum predation rate was recorded as the prey densities increases from 1: 50 to 1: 250 respectively (Table 1).

The present findings of the experiment demonstrate that, for any pest management programme utilizing bio-agents. We have to check its predating potential Memon *et al* (2015). The predating potential of a predator can be known by its functional responses. The functional response involves, the search rate, handling time and predation rate respectively. Based on these characteristics of a predator, the doses of a bio-agent can be judged. In this study mustard aphid *L. erysimi* was offered to *C. zastrowi sillemi* to check its functional response. The results came with Type II functional response curve for second instar grubs of *C. zastrowi sillemi*. Saljoqi *et al* (2016) reported that second and third instar larvae of *Chrysoperla carnea* offered with *B. brassicae* at different densities of 10, 20, 30, 40 and 50 resulted Type II functional response curve to second instar predatory grubs. By comparing the effect of natural diet and artificial diet, artificial diet had greater effect on *C. zastrowi sillemi* in consuming the little bit more aphids (*L. erysimi*) than natural diet offered to the predator. Hence from the above experiment it has been concluded that artificial diet had a greater effect on the predator biology and predator developed with potent natural enemy that can better predate and controls the mustard aphid *L. erysimi*.

4. Conclusion

According to the research findings, the use of an artificial diet (meridic diet) for feeding second instar grubs resulted in the fulfillment of all the physiological processes of the larvae. Additionally, the artificial diet had a significant impact on the predator's biology, leading to the development of a highly effective natural enemy capable of effectively preying on the mustard aphid *L. erysimi*. The development of this

semi-synthetic diet (meridic diet) provides a reliable food source for the mass multiplication of natural enemies during seasons when natural hosts are scarce. In the present findings diet plays a crucial role in the functional response as a potent predatory grub. The diet showed type II response curve. It signifies the rate of prey consumption by a predator rises as prey density increases, but eventually remains constant in spite of increase in prey density because of satiation.

5. Future Prospects:

Artificial diets play a great significant role in the future. Throughout the seasons the host or prey is not available for the rearing of predatory grub *C. zastrowi sillemi*. In this case these diets act as alternate source for mass multiplication leads to continuous supply of predator in farming and succeeding of the achievable yield.

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Table 1. Functional response of *C. zastrowi sillemi* second instar larva reared on laboratory host and semi-synthetic diet against *L. erysimi* on *B. juncea* (pooled data of year 2017 and 2018)

Treatments	2nd instar larvae reared on laboratory host				2nd instar larvae reared on semi-synthetic diet			
	Aphids consumed	Search rate (a`)	Handling time (Th)	Maximum predation rate (1/Th)	Aphids consumed	Search rate (a`)	Handling time (Th)	Maximum predation rate (1/Th)
T1-(1:50)	6.30[b] (2.60)	0.13[a] (0.79)	2.64[a] (1.77)	0.39[a] (0.94)	6.60[b] (2.66)	0.14[a] (0.80)	2.30[a] (1.67)	0.44[a] (0.97)
T2-(1:100)	6.60[b] (2.66)	0.06[b] (0.75)	2.59[a] (1.75)	0.40[a] (0.94)	6.90[b] (0.72)	0.07[b] (0.75)	2.24[a] (1.65)	0.45[a] (0.97)
T3-(1:150)	7.70[a] (2.86)	0.05[c] (0.74)	2.56[a] (1.74)	0.40[a] (0.95)	7.90[a] (2.89)	0.05[c] (0.74)	2.18[a] (1.63)	0.46[a] (0.98)
T4-(1:200)	8.00[a] (2.91)	0.04[cd] (0.73)	2.53[a] (1.74)	0.40[a] (0.95)	8.10[a] (2.93)	0.04[d] (0.73)	2.12[a] (1.61)	0.48[a] (0.98)
T5-(1:250)	8.20[a] (2.94)	0.03[d] (0.73)	2.45[a] (1.71)	0.41[a] (0.95)	8.30[a] (2.96)	0.03[d] (0.73)	2.05[a] (1.59)	0.49[a] (0.99)
CD(p=0.05)	(0.70)	(0.01)	(0.67)	(0.11)	(0.70)	(0.01)	(0.45)	(0.09)
r^2	0.885*				0.874*			

*Significant at 0.05 level of probability

The values in the parentheses are √n+0.5 transformed

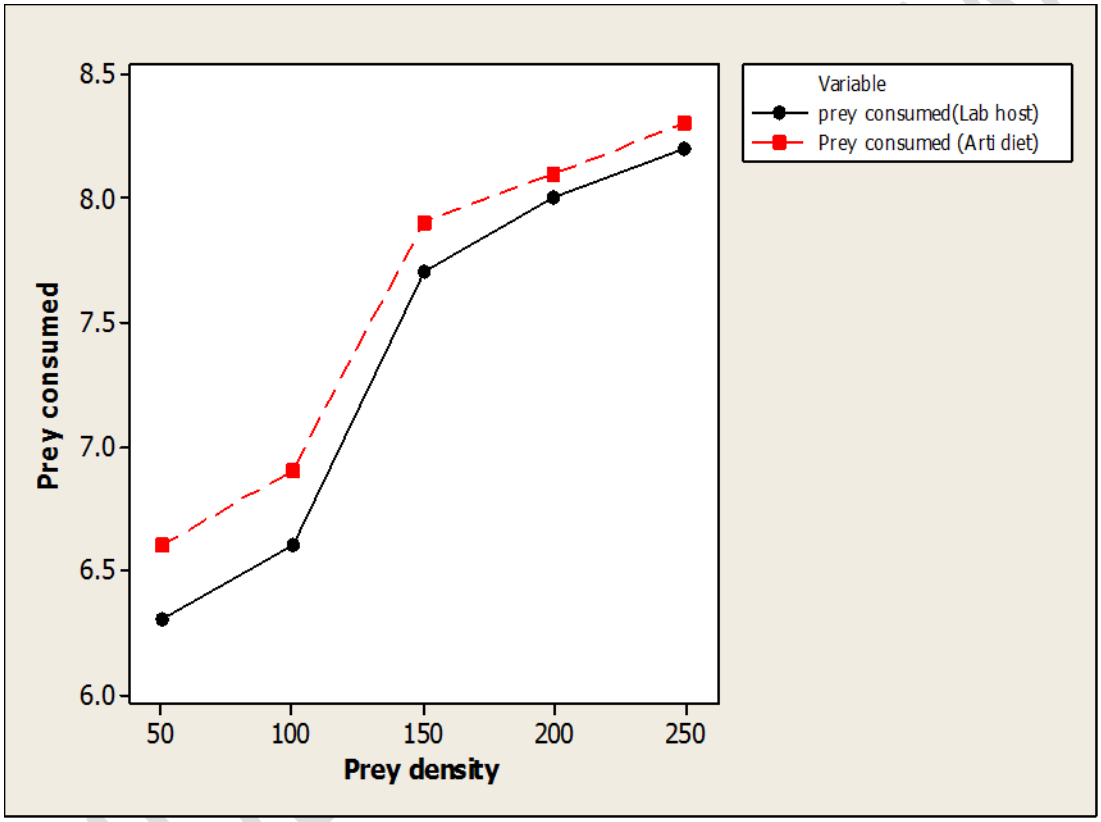

Figure1. Functional response of *C. zastrowi sillemi* (second instar grub) against *L. erysimi* fed on lab host *C. cephalonica* and best semi- synthetic diet