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Research Article

Functional Response Of *Chrysoperla Zastrowi Sillemi* Reared On Meridic Diet And Natural Host Against *Brevicorynae Brassicae*

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ABSTRACT

Effective Bio-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 third instar grub of *C. zastrowi sillemi* (Neuroptera: Chrysopidae) on Raya (*Brassica juncea*) aphid *Brevicorynae brassicae* Linnaeus. Five different predator-prey densities of 1:50, 1:100, 1:150, 1:200, and 1:250 were used in the experiment, and the results revealed a type-II functional curve response through logistic regression analysis on *B. brassicae*. The type-II functional response envisage the rate of prey consumption by the predator increases with higher prey density, but eventually reaches a plateau despite further increases in prey density. The consumption of aphids, search rate (a`), and maximum predation rate (1/Th) by third instar grubs were observed to be lower (16.20, 0.16, and 0.47) fed on natural diet in comparison to grubs fed on an artificial diet were higher (16.54, 0.16, and 0.48). Nonetheless, the duration required by third instar grubs to handle prey was longer (2.11) on a natural diet but lesser (2.08) on an artificial diet.

Keywords: Function response, *Chrysoperla zastrowi sillemi, Brevicorynae brassicae and* Meridic diets

1. Background

The Indian Mustard *Brassica juncea* (L.) Czern & Coss is a member of the Brassicae family and is commonly known as Rapeseed-Mustard in the Indian oil seed trading industry (Anonymous, 2024). Extensive efforts in crop improvement have led to the development of this variety as the most nutritionally superior edible oil seed, as well as a valuable protein source for animal feed (Banga S S and Labana K S 1984). Among oilseed crops, Indian mustard from the Brassica group holds a significant share of agricultural land in India, making the country a leading producer of rapeseed and mustard in Asia in terms of both acreage and production (Rao *et al.,* 2013). Rapeseed-mustard (Brassica species) is the primary *Rabi* oilseed crop in India, ranking second in importance after soybean and accounting for approximately 20-22% of the country's total oilseed production (AICRP-Rapeseed-Mustard, 2018). India is the fourth-largest producer of mustard seed, contributing around 11% to the global production (Kumrawat and Yadav 2018). The cultivation of rapeseed and mustard spans an area of 56 lakh ha, with a production of 66 lakh tonnes and an average yield of 1182 kg/ha at the national level, and "1102 kg/ha" in the state of Punjab (DACNET, 2009-10).
The average yield of rapeseed mustard in the Indian sub-continent is reported to be low at 1980 kg/ha. This can be attributed to the fact that a significant portion of the crop is cultivated under rainfed conditions, coupled with the infestation of the aphid complex of *B. brassicae*. The infestation of this aphid species typically begins in the third week of December and continues until the end of March. Both nymphs and adults feed on sap from leaves, pods, and shoots during this period, leading to the curling, blackening, and drying of infested leaves. In severe cases, sooty mould can develop on pods and leaves. The yield losses caused by aphids are estimated to range from 20 to 50 percent, with potential losses reaching as high as 79 percent under extreme conditions.

The cultivation of Rapeseed-mustard (*B. junacea*) as an edible oil seed crop often involves the use of pesticides to combat aphid species, a practice that is generally accepted. Nevertheless, recent studies propose that utilizing natural enemies could offer a more efficient solution in integrated pest management and biocontrol initiatives (Mushtaq and Khan 2010). The theory of Biological control, which revolves around predator-prey interactions, is predominantly structured on a community model comprising distinct trophic levels, including autotrophs, herbivores, and predators. Predators, positioned at the apex trophic level, function as top consumers (Rosenheim *et al*., 1999).

The green lacewing *C. zastrowi sillemi* (Esben-Peterson) (Neuroptera: Chrysopidae) is an important biocontrol agent that can tolerate various ecological factors and pesticide exposure in field conditions. It also has excellent host searching skills and is a voracious feeder, making it a suitable predator of different sucking and eggs of lepidopteron pests (Tassan *et al.,* 1979). However, before using any predator as a biocontrol agent, it's important to understand its functional response (Ridgway and Jones, 1969).

The functional response of a predator refers to its ability to kill prey at different prey densities, thereby regulating the population dynamics of the predator-prey system in an ecosystem (Mahzoum *et al.,* 2020; Parvez and Omkar, 2005; Khan and Mir, 2008). The searching rate (a'), handling time (Th), and maximum predation rate (1/Th) are important components of a predator's functional response (Hassel *et al.,* 1976). Based on these responses, entomologists can estimate the doses of a predator and make decisions in pest management programs (Holling 1959). The predatory green lacewing grub has excellent prey searching capacity, high dispersal ability, less prey handling time, and maximum predation capacity against aphid pests (Athhan *et al*., 2004; Hany et al 2010; Hassan 1975). Therefore, the study of functional response is crucial to understanding the highlighting mechanism in predator-prey interactions, revealing evolutionary relationships, and contributing to the biocontrol of crop pests (Khan 2009). The main objective of this study was to determine the potential of C. zastrowi sillemi in preying on the mustard aphid B. brassicae through the study of functional response.

2. Materials and methods

The functional response of *C. zastrowi sillemi*, which was reared on two different diets (Diet B and Diet E), was investigated at the Biocontrol Unit of Dr. G S Kalkat Laboratory, Punjab Agricultural University, located in Ludhiana, Punjab, India. The study was conducted under laboratory conditions, where five treatments of aphid densities (1:50, 1:100, 1:150, 1:200, and 1:250 predator to prey ratio were examined. Each treatment was replicated ten times, and individual grubs were kept separately in vials. The focus of the study was on the mustard aphid complex of *B. brassicae* (Kaltenback).

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

Investigations on *C. zastrowi sillemi* were conducted by mass culturing its laboratory host, *C. cephalonica*, throughout the study period. The rearing of *C. cephalonica* larvae followed the protocol outlined by (Sharma *et al.,* 2016). Where white sorghum grains were used as the primary food source. The sorghum was milled into 3-4 pieces using a milling machine and heat sterilized at 100 °C for 30 minutes. To prevent bacterial contamination, streptomycin sulphate was added to the sorghum at a rate of 0.2 gm and thoroughly mixed. Rearing boxes made of medium density fiberboard were filled with 2.5 kg of milled and sterilized sorghum, and charged with C. cephalonica eggs at a rate of 0.5 cc per box. The boxes were covered with perforated lids and placed on iron racks in a rearing laboratory maintained at a temperature of 27 ±2°C and 70±5% RH. Moths emerging from the boxes were collected daily and transferred to specially designed oviposition cages. The eggs were manually collected, sieved to remove moth scales and dust particles, and then exposed to freezing temperatures to halt embryonic development before being used for culturing *C. zastrowi sillemi*.

2.2 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

A semi-synthetic diet was created in the Biocontrol Unit of Dr. G.S. Kalkat's Laboratory, using different nutrient compositions. The diet was specifically formulated for the larvae of *C. zastrowi sillemi*. The semi-synthetic diet used for the grubs was based on a modified version of the diet proposed by (Sattar *et al.,* 2007) for rearing *C. carnea*.
To optimize the larval life parameter and improve efficiency in experimental rearing and mass production, meticulous weighing of all ingredients in various diet combinations was conducted. The diet preparation involved the careful blending of sucrose, preservatives (streptomycin sulfate and chlortetracycline), salt mixture, and Brewers' yeast in water using a food processor, prior to the addition of the hen's egg. Subsequently, vitamin solution and agar were incorporated into the mixture and thoroughly mixed through stirring. To prevent any unpleasant odor caused by raw egg, the egg was added to the mixture after boiling. The blending process continued for approximately 6-8 minutes until the entire mixture attained a paste-like consistency with a stringy texture. Although the resulting mixture was soft and wet, it maintained its shape. Following this, the diet was deemed suitable for feeding the larvae.

2.3 Methodology of rearing aphids

To rear aphids, *Brassica juncea* var. PBR 91 was sown in earthen pots placed in open field conditions during the cropping season. All agronomic practices were conducted without the application of any plant protection measures. Following the natural infestation of aphids, their population was sustained on potted plants to ensure a sufficient number of aphid colonies for the laboratory experiment.
To enhance the larval life parameter and improve the efficiency of the experiment, a precise measurement of all ingredients was undertaken. The diet components, namely sucrose, preservatives (streptomycin sulfate, chlortetracycline), salt mixture, and brewers' yeast, were meticulously combined in 25 ml of water and processed in a food processor prior to the addition of the hen's egg. Following this, a mixture of vitamin solution, lyophilized powder of *C. cephalonica* eggs, hen's eggs, and agar was added to the blend and thoroughly mixed through stirring. The blending process was continued for duration of 6-8 minutes until the entire mixture attained a cohesive, paste-like consistency. At this point, the mixture exhibited a soft, moist solid state while retaining its shape.

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

The functional response of third-instar larvae of C. zastrowi sillemi grub, which were raised on laboratory host Corcyra eggs and semi-synthetic diet, against *B .brassicae* was investigated using five different densities of aphids in laboratory conditions. Third instar larvae, obtained from the culture reared on Corcyra eggs and semi-synthetic diet was starved for 12.00 hours prior to the experiment. Subsequently, they were transferred to the experimental arena (9 cm diameter plastic petri dish) using a camel hair brush. The larvae were exposed to varying densities of aphids for feeding, and the number of prey consumed by the predatory larvae was recorded by counting the live prey after 24.00 hours.

Fig.A.Experimental Set Up of Functional Response of *C.zastrovi sillemi* Under Laboratory Conditions

2.5 Statistical analysis

The functional response exhibited by predatory larvae *C. zastrowi sillemi*, which were raised on semi-synthetic diets and laboratory host *C. cephalonica*, towards varying prey densities of *B. brassicae* was quantified using Holling's disk equation (Holling, 1959 and 1961). The Type II functional response model proposed by Holling was applied to the data, with confidence interval limits (95%) and asymptotic standard

errors utilized to assess differences in searching rates, handling time, and maximum predation rate of second instar *C. zastrowi sillemi* against *B. brassicae*.
The expression of the functional response of predatory larvae, which were raised on a semi-synthetic diet and laboratory host, towards prey densities can be determined by fitting the data to the Hollings equation (Version 1).

$$Na= a' TN/(1+aThN) \quad \text{..} \quad (1)$$

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:

$$a` = 1/P \ln [N1 / (N1-N2)] \quad \text{...} \quad (2)$$

Where, a = Search rate
ln= Natural logarithm
P = number of predators used
N1= density of prey
N2 = number of prey consumed.
The functional response results obtained from the interaction of third instar grubs and *B. brassicae* were subjected to regression analysis using the Statistical Package for Social Science (SPSS, IBM version 25 software).

3. Results and Discussion

The daily consumption of aphids (*B. brassicae*) by third instar grubs ranged from 15.20 to 17.00 and 15.70 to 17.15 reared on a natural diet (Laboratory host, *C. cephalonica*) and an artificial diet, respectively. The consumption of aphids by third instar grubs of *C. zastrowi sillemi* exhibited an upward trend as the predator-to-prey density ratio increased from 1:50 to 1:250 (Table 1). The search rate of the predator showed a declining trend, ranging from 0.362 to 0.070 and 0.382 to 0.073 as the predator-to-prey densities increased from 1:50 to 1:250 provided with a natural diet and an artificial diet. The time taken by third instar grubs to handle the prey varied between 2.21 to 2.01 and 2.17 to 1.99 at predator-to-prey densities of 1:50 to 1:250, respectively. Notably, the handling time exhibited a decreasing trend as the prey densities increased from 1:50 to 1:250, regardless of whether they were offered a natural or artificial diet. The maximum predation rate displayed an increasing trend, ranging from 0.45 to 0.49 and 0.45 to 0.50 as the predator-to-prey densities increased from 1:50 to 1:250 (Table 1), respectively. The graph in Figure (I) illustrates the functional response of the third instar grub of *C. zastrowi sillemi* to *B. brassicae*. The findings indicated that the third instar grub of *C. zastrowi sillemi* displayed a Type II functional response curve in response to the increase in *B. brassicae* from (1:50 to 1:250) predator: prey densities. It was observed that there was a significant ($p<0.05$) reduction or no further more in aphid consumption at higher prey densities possibly due to satiation.
The consumption of *B. brassicae* by larvae or grubs is dependent on their age and the density of prey, as demonstrated by Mushtaq and Khan (2010). Initially, there was an increase in the rate of prey consumption at lower prey densities, eventually reaching satiation with the consumption of *L. erysimi* by third instar grubs. However, further increases in prey density did not result in a higher consumption of prey by the grubs. In the experimental setup, third instar *C. zastrowi sillemi* grubs were provided with either a natural host (*C. cephalonica*) or a semi-synthetic diet. A curvilinear curve was observed, with regression values of $r2=0.992$ and $r2=0.993$, indicating minimal differences between the two diets.
The search rate of third-instar predatory larvae is contingent upon factors such as hunger level, predator and prey density, and the composition of the prey cohort. Third instar larvae displayed the highest search rate at the lowest predator-prey density ratio of 1:50, with a gradual decrease in search rate as predator-prey densities increased from 1:100, 1:150, 1:200, and 1:250 ratios. This decline in search rate may be due to the increased availability of prey for the third instar larvae, leading to reduced energy expenditure during foraging. Additionally, larvae fed artificial diets exhibited greater search efficiency compared to those fed natural diets, possibly due to differences in dietary composition or the influence of artificial diets on foraging behavior. Sultan and Khan (2014) reported a search rate of 0.002 for third-instar predatory larvae of had been recorded for green lacewing fed on mustard aphid complexes in the laboratory conditions.
Natural predators and parasites need a certain amount of time to successfully capture, kill and consume their prey or host. Recent research has shown that the handling time for prey is typically shorter in the third-instar predatory grubs of C. zastrowi sillemi when compared to second-instar grubs. This disparity can be attributed to the advanced muscular and sensory systems of third instar grubs, which enable them to efficiently capture prey and have a larger appetite.

The duration needed for the third instar grub to capture its prey showed a decreasing trend, with measurements dropping from 2.21 to 2.01 and 2.17 to 1.99 as the predator-to-prey density ratio rose from 1:50 to 1:250, prey was offered in natural or artificial diets. This decrease in handling time, from lower prey density to higher prey density, can be attributed primarily to the increased availability of prey or prey density within the 9 cm petriplate, as indicated in Table 1. These findings align with the results obtained by (Hassanpour *et al.*, 2015) observed that the handling time of second-instar predatory grubs was shorter than that of first-instar predatory grubs. Similarly (Mahzoum *et al.*, 2020) reported that the handling time of third instar grubs was shorter than that of first and second instar grubs of *Chrysoperla carnea*. The highest rate of predation was observed when the predator: prey density was at its maximum (1:250), while the lowest rate was observed at a lower predator: prey density (1:50). This difference can be attributed to the scarcity of prey at the lower density, where only 50 individuals were available for consumption. Once these prey were consumed, there were no more individuals left to be consumed. On the other hand, at the highest prey density of 250, there was a greater availability of prey, allowing the predator to continue predating aphids until it reached a state of full appetite satisfaction. Consequently, there was a noticeable increase in the maximum predation rate as the prey densities increased from 1:50 to 1:250, as documented by (Rios-Velasco *et al.*, 2017) in Table 1.

The experiment's current findings provide evidence that the assessment of predating potential is crucial for any pest management program that utilizes bio-agents. The predating potential of a predator can be determined by examining its functional responses, which encompass the search rate, handling time, and predation rate (Memon *et al.*, 2015). By considering these characteristics, one can assess the appropriate dosage of a bio-agent. In the present study, the functional response of *C. zastrowi sillemi* was evaluated by offering it mustard aphid *L. erysimi* (Memon *et al.*, 2015).

The results revealed a Type II functional response curve for third-instar grubs of *C. zastrowi sillemi* (Memon *et al.*, 2015). Similarly, Saljoqi *et al* (2016) found that second and third-instar larvae of *Chrysoperla carnea* exhibited Type II functional response curves when offered *B. brassicae* at various prey densities. By assessing the influence of natural and artificial diets on the third instar grub of *C. zastrowi sillemi*, it was determined that the artificial diet had a more significant effect on the consumption of aphids (*B. brassicae*) when compared to the natural diet. Therefore, based on the findings of the experiment, it can be concluded that the artificial diet played a crucial role in shaping the biology of the predator, leading to the development of predators with heightened abilities to effectively prey on and manage the mustard aphid *B. brassicae*

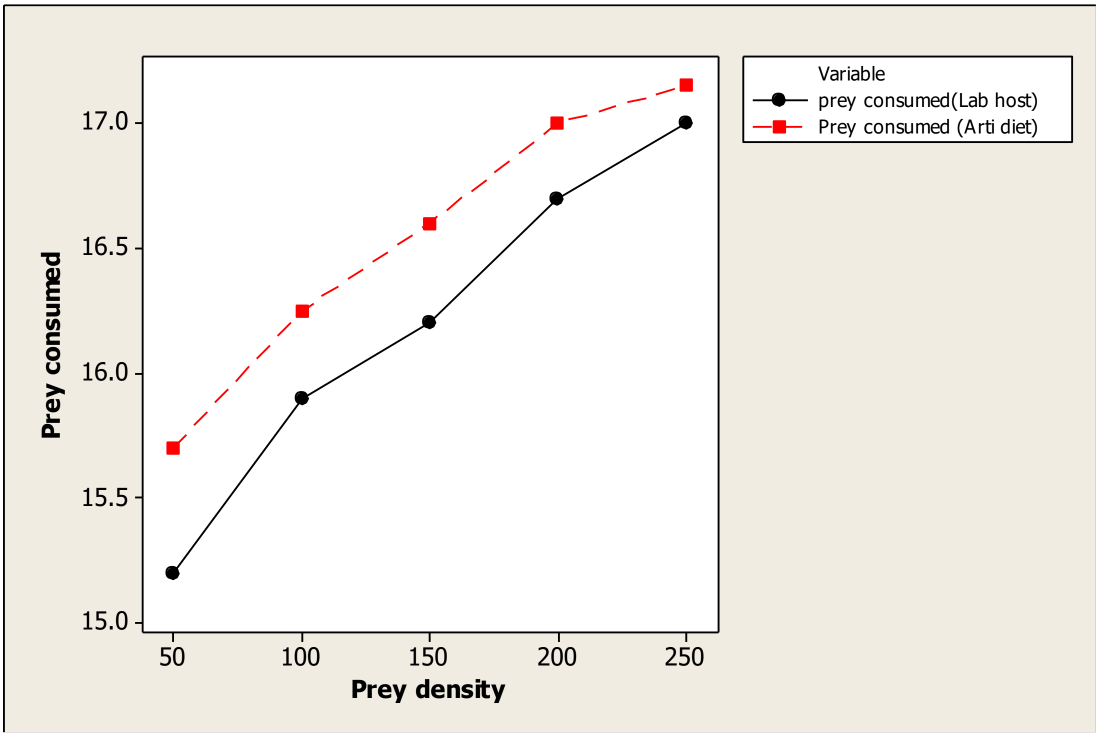

Fig.I : *Functional response of third instar larva reared on laboratory host C. cephalonica and semi- synthetic diet against B. brassicae*

Table No.1 .Functional response of *C. zastrowi sillemi* third instar larva reared on laboratory host and semi-synthetic diet against *B. juncea* aphid *Brevicorynae brassicae*

Treatments (Predator: Prey Density)	Aphids consumed (Laboratory host)	Search rate (a')	Handling time (Th)	Maximum predation rate (1/Th)	Aphids consumed (artificial diets)	Search rate (a')	Handling time (Th)	Maximum predation rate (1/Th)
T1-(1:50)	15.20±0.25[d]	0.362±0.008[a]	2.21±0.13[a]	0.45±0.002[c]	15.70±0.20[c]	0.382±0.002[a]	2.17±0.019[a]	0.459±0.0005[e]
T2-(1:100)	15.90±0.10[c]	0.173±0.001[b]	2.17±0.16[a]	0.45±0.003[c]	16.25±0.15[bc]	0.180±0.003[b]	2.14±0.020[a]	0.467±0.0004[d]
T3-(1:150)	16.20±0.30[bc]	0.114±0.002[c]	2.13±0.01[ab]	0.46±0.004[bc]	16.60±0.33[ab]	0.118±0.0008[c]	2.08±0.002[b]	0.480±0.0021[c]
T4-(1:200)	16.70±0.20[ab]	0.087±0.001[d]	2.06±0.03[bc]	0.48±0.007[ab]	17.00±0.22[ab]	0.089±0.0005[d]	2.02±0.003[c]	0.493±0.0007[b]
T5-(1:250)	17.00±0.13[a]	0.070±0.001[e]	2.01±0.02[c]	0.49±0.005[a]	17.15±0.25[a]	0.073±0.0024[e]	1.99±0.013[d]	0.502±0.0053[a]
Mean±S.E	16.20±0.15	0.1612±0.02	2.11±0.01	0.47±0.002	16.54±0.15	0.1684±0.0233	2.08±0.016	0.4802±0.0035
CD(p=0.05)	(0.62)	(0.01)	(0.04)	(0.01)	(0.78)	(0.01)	(0.04)	(0.01)
r^2	0.992*				0.993*			

4. Conclusion

The research findings indicated that the utilization of an artificial diet, specifically a meridic diet, for feeding third instar grubs has resulted in the successful fulfillment of all the physiological processes of the larvae. Additionally, the introduction of this artificial diet has had a significant impact on the predator's biology, leading to the development of a highly effective natural enemy that can efficiently prey on the mustard aphid *B. brassicae*. The creation of this semi-synthetic diet, referred to as the meridic diet, offers a reliable source of food for the mass multiplication of natural enemies during seasons when natural hosts are scarce. The present findings highlight the crucial role of diet in the functional response of a potent predatory grub, as demonstrated by the type II response curve exhibited by the diet. This curve signifies that the rate of prey consumption by the predator increases as prey density rises, but eventually stabilizes despite further increases in prey density due to satiation.

. 5. Future Prospects:

Artificial diets play a great significant role in the future times as efficient pests management tools, by exploiting all time available artificial dites in mass multiplication of natural enemies. Throughout the seasons the host or prey is not available for the rearing of predatory grub *C. zastrowi sillemi*. In this case 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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