



Predating Response Of *Chrysoperla Zastrowi Sillemi* (Esben-Peterson) On Raya Aphid *Lipaphis erysimi* (Keltenbach)

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Abstract

The control of insect pests through biological means relies on the ability of natural enemies to prey on pests. This ability, known as the functional response. It plays a crucial role in selecting the appropriate species for biological control programs. In this particular study, the researchers aimed to evaluate the functional response of the third instar grub of *C. zastrowi sillemi*, a species of lacewing belonging to the family Chrysopidae, towards the aphid *Lipaphis erysimi*, which infests Raya plants (*Brassica juncea*). To achieve this, five different densities of aphids were used as Predator: prey ratio (1:50, 1:100, 1:150, 1:200, and 1:250). The results of the study indicated a type II functional curve response, as determined by logistic regression analysis, of *C. zastrowi sillemi* towards *L. erysimi*. The Hollings Type II functional response implies that the rate at which the predator consumes its prey increases with increasing prey density, but eventually reaches a plateau despite further increases in prey density. The aphids' consumption, search rate (a'), and maximum predation rate ($1/Th$) were observed to be lower (19.68, 0.97, and 0.55) in comparison to when grubs were fed on an artificial diet, where they were higher (20.16, 0.20, and 0.59). Nonetheless, the duration required by third instar grubs to handle prey was longer (2.58) when on a natural diet but shorter (2.42) when on an artificial diet.

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

1. Introduction

Brassica juncea (L.) Czern & Coss., commonly known as Rapeseed-Mustard in India, is a member of the Brassicaceae family (AICRPRM, 2018). It has been the focus of ongoing crop improvement efforts, leading to its recognition as a highly nutritious oil seed and a valuable protein source for animal feed (Anonymous 2017.; Banga S S and Labana K S 1984). Within the category of oilseed crops, Indian mustard from the *Brassica* group holds a significant portion of 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, which belongs to the *Brassica* species, is the primary *Rabi* oilseed crop in India. It ranks as the second most crucial oilseed crop in the country, following soybean, and contributes to approximately 20-22% of the total

oilseeds produced in India. India stands as the fourth-largest producer of mustard seed globally, accounting for about 11% of the world's total production (Kumrawat and Yadav 2018). The cultivation of rapeseed and mustard covers an area of 26.21 thousand hectare, yielding 32.10 MT tonnes (Singh and Bansal 2020) with an average production of 1224 kg/ha at the national level (Kaur *et al* 2020) and 1102 kg/ha in the state of Punjab (DACNET, 2009-10).

The mean production of rapeseed mustard in the Indian subcontinent is relatively 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 detrimental impact of *L. erysimi* infestation (Dhillon *et al* 2022). The infestation of this aphid species typically commences in the third week of December and persists until the end of March (Shaila *et al* 2022). Both nymphs and adult aphids feed on the sap of leaves, pods, and shoots during this period, resulting in the curling, blackening, and drying of affected leaves (Pawan K 2022). In cases of severe infestation, sooty mould may develop on pods and leaves, further exacerbating the situation (Athhan *et al.*, 2004). The losses in yield caused by aphids can range from 20 to 50 percent, with extreme conditions leading to potential losses as high as 79 percent (Rao *et al.*, 2013). Rapeseed-mustard (*B. junacea*) serves as an edible oil seed crop, and the utilization of pesticides to control aphid species is not an ecological sin as parse. Nevertheless, research suggests that employing natural enemies could be a viable alternative for managing aphids in integrated pest management and biocontrol initiatives (Mushtaq and Khan 2010). The concept of Biological control, focusing on predator-prey dynamics, is primarily grounded on a community model that consists of distinct trophic levels such as autotrophs, herbivores, and predators. Predators, positioned at the highest trophic level, function as top consumers (Rosenheim *et al* 1999).

The green lacewing *Chrysoperla zastrowi sillemi* (Esben-Peterson) is a highly significant biocontrol agent within the Neuroptera family, specifically the Chrysopidae (Cardenas *et al.*,2020). This species possesses a remarkable ability to tolerate various ecological factors encountered in field conditions, such as pesticide molecules. Additionally, it exhibits a strong capacity for host searching and displays voracious feeding behavior (Tassan *et al.*, 1979). These distinctive characteristics have captured the attention of entomologists, who recognize the potential of this lacewing as an effective predator against various lepidopteron pests, including their eggs and the act of sucking (Ridgway and Jones, 1969). However, it is crucial to understand the functional response of this lacewing species before fully exploiting its predatory capabilities.

The functional response of a predator refers to its ability to capture and consume prey at varying prey densities, thereby influencing the population dynamics within a predator-prey system in an ecosystem. Key components of the functional response include the searching rate (a), handling time (T_h), and maximum predation rate ($1/T_h$). This information allows entomologists to estimate the number of prey consumed per predator per unit of time and determine the maximum prey capacity per day, aiding in the development of effective pest management strategies (Mahzoum *et al* 2020; Parvez and Omarkar, 2005; Khan and Mir, 2008; Hassel *et al* 1976; Holling 1959).

The green lacewing grub, known for its predatory nature, demonstrates remarkable skills in locating prey, efficient dispersal, quick prey handling, and high predation rates on aphid pests (Holling 1961). Therefore, investigating the functional response is crucial for comprehending the dynamics of predator-prey interactions, unveiling evolutionary connections, and aiding in the biological control of agricultural pests (Khan 2009). The primary aim of this research was to assess the predatory capabilities of *C. zastrowi sillemi* in targeting the mustard aphid *L. erysimi* by analyzing its functional response.

2. Materials and methods

Research was conducted at Dr. G S Kalkat Laboratory, Biocontrol Unit, Punjab Agricultural University, Ludhiana, Punjab, India to investigate the functional response of *C. zastrowi sillemi* reared on best semi-synthetic (Diet B) and laboratory host *C. cephalonica* (Diet E). The study was carried out under laboratory conditions with five treatments of aphid densities 1:50, 1:100, 1:150, 1:200, and 1:250 (Predator: Prey ratio), each replicated ten times. The grubs were individually kept in vials, focusing on the mustard aphid complex of *L. erysimi* (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. The rearing of *C. cephalonica* followed the methodology outlined by Sharma *et al* (2016). Larvae of *C. cephalonica* were fed on large grains of white sorghum, which were milled into pieces and heat sterilized at 100 °C for 30 minutes. *Streptomycin sulfate* was added to the sorghum to prevent bacterial infection. Rearing boxes made of medium-density fiberboard were filled with the prepared sorghum for the study.

The rearing boxes were loaded with *C. cephalonica* eggs at a rate of 0.5 cc (equivalent to 8000 eggs; based on the conversion of 1.0 cc = 16,000 eggs; Jalali and Singh 1989) per box. The egg volume was determined using a measuring cylinder. Following the loading process, the boxes were sealed with perforated lids containing iron mesh (20 meshes) on both the exterior and interior surfaces. These boxes were placed on iron racks (measuring 90 cm in length, 45 cm in breadth, and 180 cm in height) within a rearing laboratory maintained at temperatures of 27 ±2°C and relative humidity of 70±5 percent. The emerging moths from these boxes were gathered daily and transferred to specialized oviposition cages (35 × 25 × 18 cm Rescholar Equipment, India). The eggs were manually collected and sifted through 30 mesh sieves to eliminate moth scales. Subsequently, the eggs underwent further screening with a 40-mesh sieve to remove any dust particles. The embryonic development of the eggs was arrested by exposing them to freezing temperatures, after which they were utilized for the cultivation of *C. zastrowi sillemi*.

2.2 Formulation of grubs/larval semi-synthetic diet (Diet B)

Ingredients	Quantity
<i>Corcyra</i> 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

The Biocontrol unit in Dr. G S Kalkat's Laboratory developed a semi-synthetic diet by combining various nutrient compositions. Specifically, the diet was tailored for the larvae of *C. zastrowi sillemi*. This particular diet for the grubs was an adaptation of the diet originally suggested by Sattar *et al* (2007) for the purpose of rearing *C. carnea*.

In order to improve the larval life parameter and enhance efficiency in experimental rearing and mass production, precise measurements were taken for all ingredients used in various diet combinations. The diet was prepared by mixing ingredients such as sucrose, preservatives (*streptomycin sulfate* and chlortetracycline), salt mixture, and Brewers' yeast in water, followed by blending in a food processor before incorporating the hen's egg. Subsequently, vitamin solution, agar, honey, acetic acid and lyophilized powder of *Corcyra* eggs were added to the mixture and thoroughly mixed by stirring. The egg was introduced after boiling to prevent any unpleasant odor caused by raw egg, which larvae dislike. The ingredients were then blended for 6-8 minutes until a stringy paste-like consistency was achieved. The resulting mixture was soft, wet, and malleable, yet retained its shape. Once prepared, the diet was ready to be fed to the larvae.

2.3 Methodology of rearing aphids

In order to rear aphids, *Brassica juncea* var. PBR 91 was sown in earthen pots and placed in an open field setting during the cropping season. All agronomic practices were conducted without employing any plant protection measures. Subsequent to the occurrence of natural aphid infestation, the population of aphids was maintained on the potted plants to acquire a maximal number of aphid colonies for experimental purposes in laboratory conditions.

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

The functional response of third instar larvae of *C. zastrowi sillemi* reared on both laboratory host *Corcyra* eggs (Diet E) and best semi-synthetic (Diet B) against *L. erysimi* on *B. juncea* var. Raya was studied in the laboratory. The third instar larvae taken from the culture reared on *C. cephalonica* eggs and semi-synthetic diet B were starved for 12 hours, before the start 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 1:50, 1:100, 1:150, 1:200 and 1:250 for feeding. The number of each prey consumed by the predatory larvae was recorded by counting the live prey after 24 hours. This trend was followed until the completion of the experiment.

2.5 Statistical analysis

The functional response of predatory larvae *C. zastrowi sillemi*, which were raised on semi-synthetic diets and laboratory host *C. cephalonica*, was assessed in relation to varying prey densities of *L. erysimi*. This assessment was conducted using Holling's disk equation, as outlined in Holling's seminal work from 1959. The application of Holling's disk equation allowed for the measurement and description of the Type II functional response exhibited by the third instars of *C. zastrowi sillemi* towards *L. erysimi*. Confidence interval limits (at 95% confidence level) and asymptotic standard errors were employed as indicators to discern differences in searching rates, handling time, and maximum predation rate.

The expression of the functional response of predatory larvae, which were raised on a semi-synthetic diet and laboratory host, towards varying prey densities can be determined by fitting the data to the Hollings equation.

$$Na = a' TN / (1 + a' ThN) \dots\dots\dots (1)$$

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 spend identifying, pursuing, killing, consuming and digesting prey).

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

$$a' = 1/P \ln [N1 / (N1 - N2)] \dots\dots\dots (2)$$

Where, a = Search rate

ln= Natural logarithm

P = number of predators used

N1= density of prey

N2 = number of prey consumed.

The results of the functional response assessment involving third instar grubs in relation to *L. erysimi* were analyzed through regression analysis using the Statistical Package for Social Science (SPSS, IBM version 25 software).

3. Results and Discussion

The daily consumption of aphids (*L. erysimi*) by third instar grubs ranged from 15.40 to 17.30 and 15.90 to 17.60 when reared on a natural diet 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 0.36 to 0.07 and 0.38 to 0.07, as the predator-to-prey densities increased from 1:50 to 1:250 when 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 2.09 and 2.14 to 1.89 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 0.451 to 0.478 and 0.465 to 0.528, as the predator-to-prey densities increased from 1:50 to 1:250 (Table 1), respectively.

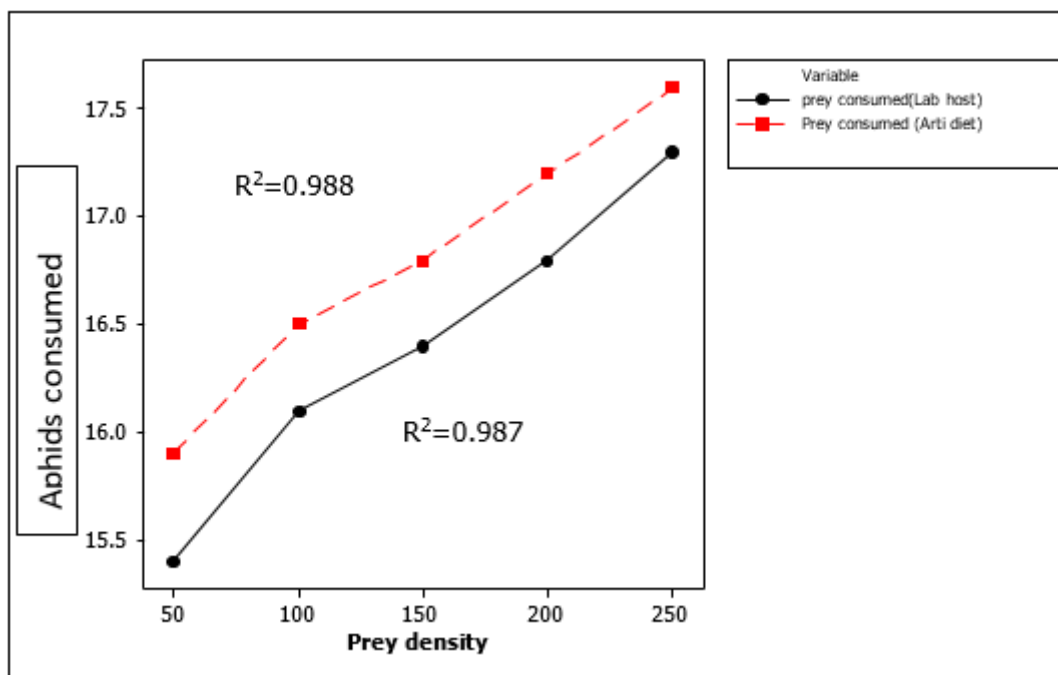


Figure.1. Functional response of *C. zastrowi sillemi* (third instar grub) against *L. erysimi* fed on lab host *C. cephalonica* and best semi- synthetic diet

The graph in Figure (1) illustrates the functional response of the third instar grub of *C. zastrowi sillemi* to *L. erysimi*. 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 *L. erysimi* from (1:50 to 1:250) predator: prey density. It was observed that there was a significant ($p < 0.05$) decrease in aphid consumption at higher prey densities, possibly due to satiation.

The consumption of prey 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 $r^2 = 0.987$ and $r^2 = 0.988$, 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 to 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 green lacewing.

Natural predators and parasites require a certain amount of time to capture, kill, and consume their prey or host. Research indicates that the handling time for prey was generally shorter in the third instar predatory grub compared to the second instar grub of *C. zastrowi sillemi*. This difference could be attributed to the well-developed muscular and sensory systems of the third instar grub, allowing for more efficient prey capture and a larger appetite.

The duration required for the third instar grub to handle its prey exhibited a declining pattern, with values decreasing from 2.21 to 2.09 and 2.14 to 1.89 as the predator: prey densities increased from 1:50 to 1:250, when the prey was provided in the form of natural and artificial diets, respectively. 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), who 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.

Table 1. Functional response of *C. zastrowi sillemi* third instar larva reared on laboratory host and semi-synthetic diet against *B. juncea* var. PBR-91 aphid complex of *L. erysimi* during (pooled 2017 and 2018)

Treatments (Predator: PreyDensity)	Aphids consumed (Laboratory host)	Search rate (a ⁻¹)	Handling time (Th)	Maximum predation rate (1/Th)	Aphids consumed (Artificial diets)	Search rate (a ⁻¹)	Handling time (Th)	Maximum predation rate (1/Th)
T1-(1:50)	15.40±0.29 ^c	0.368±0.008 ^a	2.21±0.013 ^a	0.451±0.002 ^c	15.90±0.24 ^c	0.382±0.007 ^a	2.14±0.02 ^a	0.465±0.004 ^d
T2-(1:100)	16.10±0.10 ^{bc}	0.175±0.001 ^b	2.18±0.016 ^a	0.456±0.003 ^c	16.50±0.27 ^{bc}	0.180±0.003 ^b	2.09±0.02 ^{ab}	0.477±0.005 ^{cd}
T3-(1:150)	16.40±0.36 ^b	0.115±0.002 ^c	2.16±0.019 ^{ab}	0.463±0.004 ^{bc}	16.80±0.33 ^{ab}	0.118±0.002 ^c	2.03±0.01 ^b	0.490±0.004 ^c
T4-(1:200)	16.80±0.25 ^{ab}	0.087±0.001 ^d	2.11±0.030 ^{bc}	0.472±0.007 ^{ab}	17.20±0.12 ^{ab}	0.089±0.0006 ^d	1.96±0.01 ^c	0.508±0.004 ^b
T5-(1:250)	17.30±0.25 ^a	0.071±0.001 ^e	2.09±0.023 ^c	0.478±0.005 ^a	17.60±0.29 ^a	0.073±0.001 ^e	1.89±0.02 ^d	0.528±0.006 ^a
Mean±S.E	16.40±0.17	0.163±0.22	2.154±0.127	0.464±0.002	16.80±0.16	0.168±0.02	2.02±0.02	0.494±0.005
CD(p=0.05)	(0.79)	(0.01)	(0.06)	(0.01)	(0.78)	(0.01)	(0.06)	(0.02)

Significant at 0.05 level of probability

The experiment's current findings provide evidence that the assessment of predating potential is crucial for any pest management program that utilizes bio-agents (Memon *et al.*, 2015). 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 second-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 densities (Saljoqi *et al.*, 2016). 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 (*L. erysimi*) 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 *L. erysimi*.

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 *L. erysimi*. 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 dities 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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