Research Article

Functional response of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) on *Ommatissus lybicus* (Homoptera: Tropiduchidae)

Arif Shah[1]*, Muhammad Qasim Kakar[2], Atta Ullah[2], Abdul Razaq[2], Nikos E. Papanikolaou[3], Toheed Iqbal[4] and Habib Ullah[5]

1. Balochistan Agriculture College Quetta, Pakistan
2. Department of Agriculture (Extension) Balochistan, Pakistan
3. Biodiversity Conservation Laboratory, Department of Environmental Sciences, University of the Aegeen, Mytilene 81100, Greece.
4. Department of Entomology, University of Agriculture, Peshawar, Pakistan
5. Department of Agriculture (Research) Balochistan, Pakistan
Corresponding author's email: arifshahkakar@gmail.com

Abstract

Dubas bug, *Ommatissus lybicus* (Homoptera: Tropiduchidae), is a major insect pest of date palm. Adult and nymph stages suck sap from all green parts and cause direct and indirect damage. Large number of predators feed on it; however, no information is available on their consumption rate and functional response using this prey. We aim to find out consumption rate of *Coccinella septempunctata* with an objective how adult female and fourth nymphal instars of *C. septempunctata* responds to 1st and 3rd instar nymphs of *O. lybicus* at various density levels under controlled condition. Both stages of *C. septempunctata* exhibited a type II functional response. Handling times and attack rates were significantly different between adult female and larval stage indicating that predator responds differentially to prey stage offered at same density. Estimated handling time of *C. septempunctata* adult female and fourth instar (in bracket) at 24 hours were 0.563(0.692) and 1.008(1.492) and estimated attack rate were 0.020 (0.014) and 0.003(0.002) on first and third instar nymphs of prey, respectively, which conclude that adult is more voracious stage than larva. However, further field studies on predator and prey stage specific in the presence of honeydew droplets on leaflets are still needed to quantify biocontrol efficiency of predator.

Key words: Bivoltine; *Coccinella septempunctata*; Dubas bug; honeydew; predation.

Introduction

Dubas bug, *Ommatissus lybicus*is (Homoptera: Tropiduchidae), an important sucking pest of date palm on the Arab peninsula [1]. This monophagous and bivoltine pest is recorded in several countries in the near East and North Africa [2-5]. In Pakistan, it was first recorded in 1999 in district Panjgur of Balochistan [6]. Adult and nymph stages of *O. lybicus* suck the phloem sap from all green parts of date palm and cause direct and indirect damage by puncturing the tissue and secretion of honeydew droplets on leaflets which results in poor photosynthetic activity [1, 7] and may cause yield losses up to 50% in

Iraq [8]. In Pakistan yield losses ranged from 35-80% but susceptible cultivars may fail to produce [6].

Coccinellids, commonly known as ladybirds, feed on a large number of prey and have been getting importance as biocontrol agents [9-11]. Adults and larvae of most species of coccinellids are the potential predators of a variety of prey [11]. *Coccinella septempunctata* L., is one of the potential predators of aphids in India [12].

The functional response of predator is a key factor in the population dynamics of predator–prey systems. It illustrated the rate at which a predator kills its prey at different levels of density [13]. The effectiveness of the predators is directly related to the type of functional response [14, 15] and such information is critical for implementing natural enemies conservation and augmentation of natural biological control program [16]. Type of functional response is of immense practical importance in estimating the bio-efficacy of predatory insect, however ecologists usually face difficulties in determining functional response when the curve lies between Type II and III. Hence suitable analysis that can best determine the functional response is intensely required [17].

In Panjgur, coccinellids spp., *Chrysopa* spp., black ants sp. and spider spp. are commonly found predators in date palm agro-ecosystem where these predators were observed to feed on nymphs and adult stages of dubas bug [6].

Adults of coccinellids, *C. septempunctata*, *C. undecimpunctata* L., *Chilocorus bipustulatus* L. and larvae of *Chrysoperla carnea* Steph. feed upon the nymphs and adults stages of *O. lybicus* [3, 4, 18]. However, no information is available on consumption rate and functional response of any predator who is attacking this pest. The aim of this study was to test the consumption rate and evaluate the functional response of *C. septempunctata* raising the following questions (i) Does the predation rate of adult female and 4th instar larval stages of *C. septempunctata* vary when fed on same density of same or different prey stages? (ii) Do the functional response curves of adult and larva vary when fed on same prey stage?

Materials and methods
The study was conducted for the four consecutive generations in spring and summer 2009-10, in district Panjgur (26`59.165°N 064`05.880°E Alt. 3200ft) of Balochistan.

Predator's selection and identification
In order to select the most efficient predator a preliminary study on consumption rate of *C. septempunctata*, on different life forms of dubas bug was carried out in Petri dishes (experiment arena) in summer generation (2nd) in the year 2008. Based on comparatively high consumption rate *C. septempunctata* was selected for the present study.

Frond and cage size selection
Number of fully expanded fronds of Kehraba cultivar with ten (10) middle leaflets; five on each side were selected. Both cut ends (sides) of frond were covered with wet cotton to reduce water losses and keep the turgidity of leaflets during the course of experiment. A single frond was placed in each cages (20x17x3 inch) with a wooden frame and covered by muslin cloth in order to maintain the prey and predator similar to natural/field conditions.

Prey stage selection and culture
Two developmental stages (1st and 3rd instar nymphs) of dubas bug (prey) were chosen. In order to test the predation rate and determine the functional response of both stages of predator on each developmental stage of prey at a time, a number of 1st instar nymphs of dubas bug were collected with aspirator and maintained as stock population. When more than 90% of the stock population of prey reached to 3rd instar, required numbers of 1st instar nymphs were collected from field and offered to the predator. Similarly, stock population of *C.*

septempunctata was maintained on *Medicago sativa* and fed with aphids.

Estimating consumption rate and functional response

Consumption rate and functional response of fourth instar and adult female of *C. septempunctata* were determined by offering the 1st and 3rd instar nymphs of dubas bug at different density levels (1, 5, 10, 20, 40, and 60 bugs per leaflet or a total of 10, 50, 100, 200, 400 and 600 bugs per cage). Each life stage of predator and density level of prey was released randomly into the single cage to each predator stage. Similarly, control cages received corresponding prey density level and developmental stages but no predator. Both stages of predators were starved for 24 hours before introducing into the cages. Exposure time of prey to predator was 24 hours and without replacement of prey. Following 24 hours, mean number of killed/consumed prey were counted in both treated and control cages. The experiment was conducted controlled conditions (temperature 30±2 °C, R.H. 20±2 % and photoperiod 16L:8D) replicated five times.

Data analysis

The logistic regression model [19] was used to determine the shape of the functional response prior to fitting the data for functional response analysis. This model helps distinguishing between Type II and Type III [17]. The relationship between the proportion of prey consumed (N_e / N_0) and number of prey present (N_0) was determined by fitting the data to a polynomial function (equation 1) using statistical software SAS, Version 9.0 (Procedure CATMOD) on personal computer.

$$N_e \big/ N_0 = \frac{\exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)} \quad (1)$$

Where P_o, P_1, P_2, and P_3 were the intercept, linear, quadratic and cubic coefficients, respectively, which were estimated using maximum likelihood method. If $P_1 > 0$ and $P_2 < 0$, the proportion of prey consumed is positively density dependent, thus describing a type III functional response. If $P_1 < 0$, the proportion of prey consumed declines with initial prey density suggesting a type II functional response [19]. Later on, the functional response parameters were estimated using Roger's random predator equation (Rogers 1972). For Type II response the equation will be followed:

$$N_e = N_0\{1 - \exp[a(T_h N_e - T)]\} \quad (2)$$

Where, N_e is number of prey consumed and N_0 is initial prey density, *a* is the attack rate, T is the total time of exposure, and T_h is the handling time. Random predator equation overcomes the problem of prey depletion. Data fitting was performed using nonlinear least square method on SAS Version 9.0 (Procedure NLIN) [19].

For Type III response equation will be followed:

$$N_e = N_o \{1 - \exp[(d + bN_o)((T_h N_e T)/(1+cN_o)]\} \quad (3)$$

where b, c and d are constants.

The data on prey consumption by fourth instar and adult female *C. septempunctata* at different prey stage level were subjected to one-way ANOVA and the comparison of means was done using Tukey's HSD test using statistical software, SAS. The number of first and third instar nymphs of dubas bug at different density levels consumed by fourth instar and adult female of *C. septempunctata* in 24 hours were analyzed using two-way ANOVA by using "prey stage" and "prey density" level as independent factor and "prey consumed" as dependent factor using statistical software, SAS.

Results

Consumption Rate (average of four seasons)
C. septempunctata (adult female) vs. Dubas bug first and third instar nymph

Consumption rate of adult female *C. septempunctata* fed on first (F=162.98; P<0.0001; d.f. = 5, 29) and third (F=121.19; P<0.0001; d.f. = 5, 29; Table-1) instars of dubas bug increased significantly with increase in prey density levels. Comparison of means revealed that prey consumption was significant at all the

density levels except at prey densities, 400 and 600 of first instar nymph when adult female was used as predator (Tukey's range = 4.31).

C. septempunctata (grub) vs. Dubas bug first and third instar nymph

Almost similar trend was observed when fourth instar *C. septempunctata* was used as predator at different densities of first and third instars of dubas bug (table 2 and figure. 1). Prey consumption by fourth

instar of *C. septempunctata* increased significantly with increase in densities of first (F=205.82; P<0.0001; d.f. = 5, 29) and third (F=121.93; P<0.0001; d.f. = 5, 29) instar nymphs of dubas bug. Mean values also varied significantly at all the density levels except at prey densities, 400 and 600 of first instar nymph when fourth instar *C. septempunctata* was used as predator (Tukey's range = 4.31).

Table 1. Mean number (±SE) of first and third instar nymphs of *O. lybicus,* consumed (in 24 hours) by adult female *C. septempunctata* at different prey densities (average of four seasons: spring and summer, 2009-10)

Treatment	Dubas bug development stage	
Density /cage	First instar	Third Instar
Control	0.00±00.00 f	0.00±0.00 f
10 bugs	1.45±0.14 e	0.75±0.11 f
50 bugs	7.50±0.27 d	3.95±0.21 e
100 bugs	15.45±0.21 c	8.10±0.35 d
200 bugs	22.00±1.14 b	12.05±0.57 c
400 bugs	27.55±0.62 a	16.70±0.90 b
600 bug	29.40±0.43 a	19.00±0.86 a
F-value	162.98*	121.19*

*Means followed by different letters in the same column are significantly different at P<0.001; Tukey's Range = 4.31)

Table 2. Mean number (±SE) of first and third instar nymphs of *O. lybicus*, consumed (in 24 hours) by fourth instar *C. septempunctata* at different prey densities (average of four seasons: spring and summer, 2009-10)

Treatment	Dubas bug development stage	
Density/cage	First instar	Third Instar
Control	0.00±0.00 e	0.00±0.00 f
10 bugs	1.00±0.07 e	0.60±0.20 f
50 bugs	5.70±0.16 d	3.35±0.29 e
100 bugs	12.60±0.37 c	6.00±0.37 d
200 bugs	19.75±0.63 b	11.35±0.32 c
400 bugs	26.25±0.64 a	16.15±0.40 b
600 bug	28.80±0.85 a	18.25±0.32 a
F-value	205.82*	121.93*

*Means followed by different letters in the same column are significantly different at P<0.001; d.f. = 5, 29; Tukey's Range = 4.31)

Comparative consumption rate of fourth instar and adult female *C. septempunctata*

Significant difference was observed in the predation rate of adult female and fourth instar larva of *C. septempunctata* feeding

on the same density and prey stage with same environmental conditions. Irrespective of prey density, mean predation per 24 hours of adult and grub stages of *C. septempunctata* was recorded as 13.66 and 12.48 prey, respectively (Fig. 3).

Functional response

Parameter estimated for logistic regression of proportion of prey killed (Ne/No) against the number of prey offered (No) for the adult female and 4th instar grub of *C. septempunctata* feeding on first and 3rd instar nymphs of dubas bug displayed type II functional response, i.e. significant linear parameter $P_1 < 0$ (table 3 and fig. 1 and 2).

Figure 1 and 2 illustrated that functional response curves and predation percentage of both developmental stages of *C. septempunctata* was significantly high feeding on 1st than 3rd instar nymphs of dubas bug. Similarly, irrespective of prey stage the overall functional response curve of adult was higher than the grub, whereas at higher prey density the prey consumed by both developmental stages was at par (fig. 3). Two-way ANOVA revealed a significant main effect of "prey stage" (F= 314.79; P<0.0001; d.f. = 1) and "prey density" (F= 278.93; P<0.0001; d.f. = 5) when adult female *C. septempunctata* was searching. The interaction between "prey stage" and "prey density" was also found to be significant (F= 20.87; P<0.001; d.f. = 5). Similarly, main effect of "prey stage" (F= 584.64; P<0.0001; d.f. = 1) and "prey density" (F= 321.23; P<0.0001; d.f. = 5) was also significant when fourth instar *C. septempunctata* was searching. The interaction between "prey stage" and "prey density" was also found to be significant (F= 37.71; P<0.001; d.f. = 5).
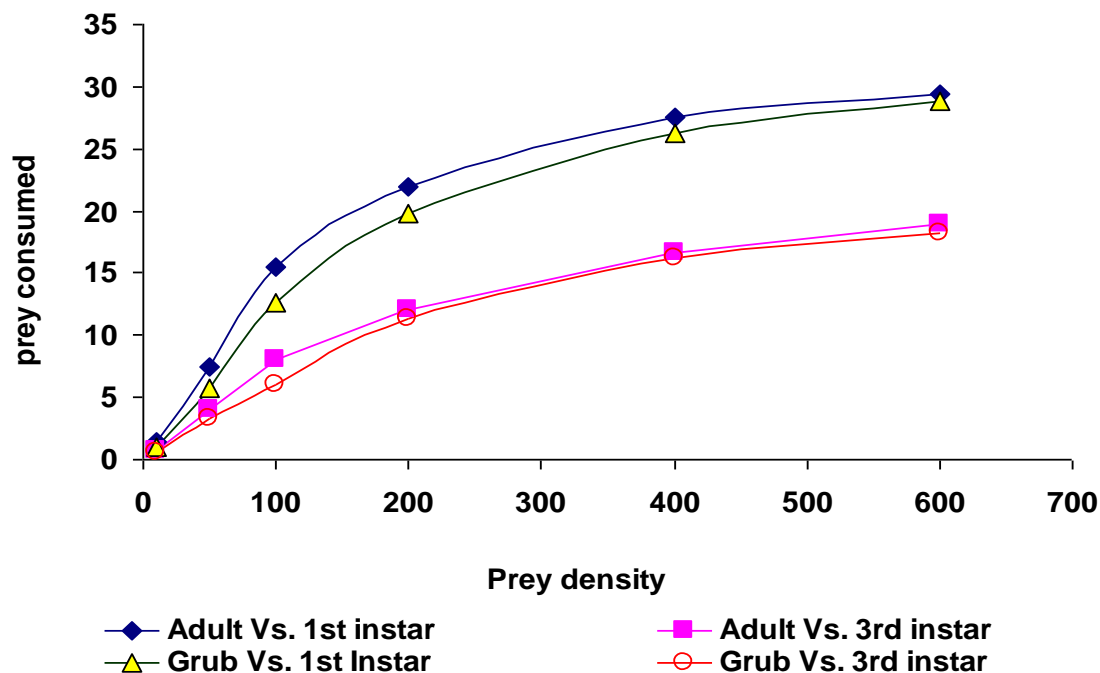

Figure 1. Functional response of adult female and fourth instar *C. septempunctata* (obtained from the predicted values of prey consumed by PROC-NLIN procedure of SAS), preying upon 1st and 3rd instar nymphs of dubas bug offered at different density levels (average of four seasons, 2009-10)
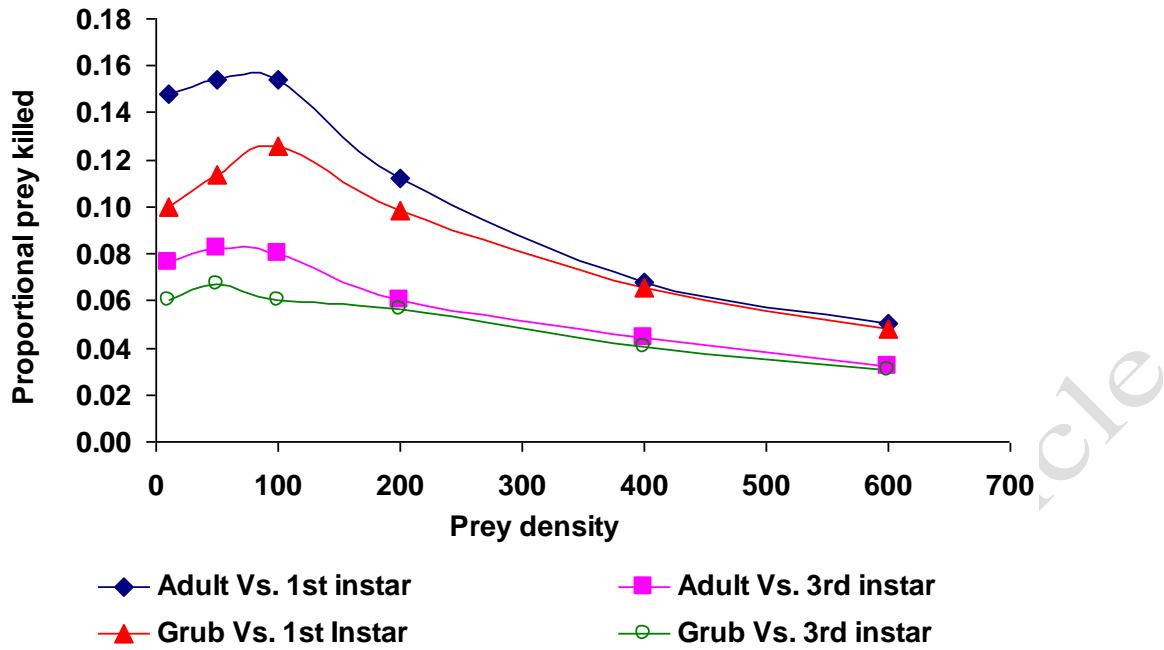
Figure 2. **Functional response of *C. septempunctata* adult and grub preying upon 1st and 3rd instar nymphs of dubas bug offered at different density levels (average of four seasons, 2009-10)**
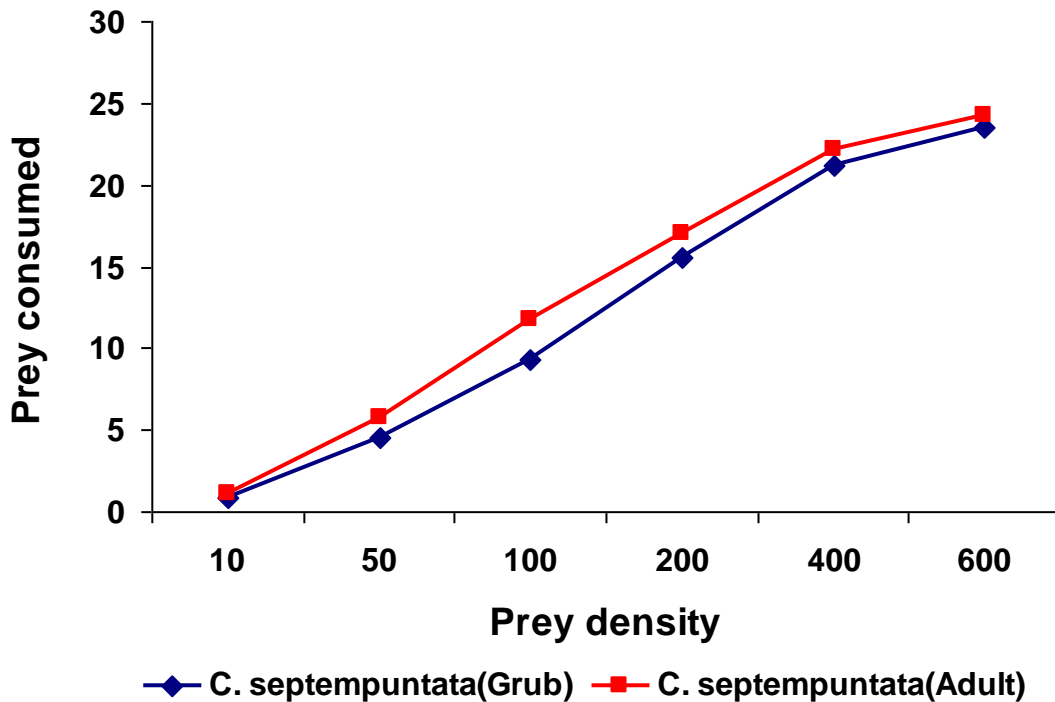

Figure 3. Comparative functional response of *C. septempunctata* adult and grub at different densities of dubas bug (irrespective of life stages)

Handling time and search rate
Prey stage significantly affected the handling time and it increased with increasing prey size. Adult female *C.* *septempunctata* spent shortest time, followed by fourth instar feeding on 1st instar nymphs of dubas bug. Similarly search rate of predator decreased when

prey stage developed. Regardless of prey stage overall search rate of fourth instar *C.*

septempunctata was shorter than adult (table 4).

Table 3. Functional response model based on analysis of maximum likelihood estimates of parameters (±SE) using proportional prey killed as function of initial prey densities by *C. septempunctata* (average of four seasons: spring and summer 2009-10)

Predator stage	Prey stage	Intercept (P_0)	Linear (P_1)	Quadratic (P_2)	Cubic (P_3)	Best Fitted model
Adult Female	1st Instar	-1.655 ±0.25	-0.004 ±0.053	-0.000081 ±0. 000001	0.000000096 ±0.000000001	Type II
	3rd Instar	-2.529 ±0.33	-0.004 ±0.003	0.000062 ±0. 000001	0.000000046 ±0.000000002	Type II
Fourth Instar	1st Instar	-1.868 ±0.263	-0.004 ±0.004	-0.000025 ±0. 000001	0.000000023 ±0.000000001	Type II
	3rd Instar	-3.037 ±0.413	-0.002 ±0.005	0.000045 ±0. 00001	0.000000014 ±0.000000001	Type II

Table 4. Estimated attack rate (a') and handling time (T_h) of *C. septempunctata* on two life stages and seven densities of dubas bug, *O. lybicus* (average of four seasons; spring and summer 2009-10)

Predator Stage	Dubas stage	Parameter	Estimated	SE	t value	Pr(>|t|)
(Adult female)	1st Instar	a'	0.02	0.002	8.621	0.00
		Th	0.563	0.024	23.020	0.00
	3rd Instar	a'	0.003	0.0001	8.050	0.00
		Th	1.008	0.121	8.300	0.00
	Average	a'	0.011	0.001	8.335	0.00
		Th	0.785	0.027	15.66	0.00
(4h instar grub)	1st Instar	a'	0.014	0.001	9.917	0.00
		Th	0.692	0.029	23.840	0.00
	3rd Instar	a'	0.002	0.0001	6.563	0.00
		Th	1.492	0.227	6.571	0.00
	Average	a'	0.008	0.0001	8.240	0.00
		Th	1.092	0.128	15.205	0.00

a'(cm ^{2} /hour), T_h (days or hours/prey) , Predation time for predators was 24 hours

Discussion

Developmental stage of dubas bug significantly influenced the consumption rate of both stages of *C. septempunctata*. This may be due to difference in body size and mobility of and 3rd instar nymphs of dubas bug. Similar results were indicated by [20], who found that prey size and hunger level of predators affected the predation ratio. Density level of prey significantly affected the predation rate of 4th instar grub and adult of C.

septempunctata. Predation rate increased with increasing prey density, while percentage of predation was inversely proportional to prey density level. The more predation at higher density could be due to more chance for predator for searching and killing of prey than lower prey density. Generally these investigations are in line with [21-23]. At high prey density predation frequency of ladybirds decrease with predation time however, it was not observed at low prey

density [22]. *C. septempunctata* consumed more than 50% of prey during the first three hours exposed for 24 hours [24]. In other study, [25] reported that 4th instar grub of seven spotted beetle consumed 2.8 and 2.55 aphids (*Lipaphis erysimi* Kalt.) under laboratory and field conditions offered at density level of 10 aphids, respectively. It is assumed that this difference may be on account of difference in prey species, experimental arena, prevailing environmental condition during experiment and difference in geographical provenance of *C. septempunctata* species.

Adult *C. septempunctata* consumed significantly more dubas bug than 4th instar grub when offered at the same density, stage and under the same environmental condition. This was probably due to comparatively poor visual perception of 4th instar larvae of *C. septempunctata* than adult, which detect the prey at distance of about 1.0 cm and 0.7 cm, respectively [26]. *Coccinella septempunctata* finds the first prey (patch) only under light conditions (at a distance of 7 mm) and upon coming in contact under dark conditions [27]. Generally, present results are in consonance with the findings of [28] who found that larvae of *C. septempunctata* spent longer time in finding a prey and adapted to search for aggregated prey. Our findings is in close agreement with those of [29], who claimed that adult *C. septempunctata* had a lesser prey handling time with high attack rate than the fourth instars. Food consumption of *C. septempuctata* grub increased with development of instars [30], while, [21] reported that *C. septempunctata* grub feeding rate was two fold greater than those of adult feeding on aphid species. This variation may be due to feeding behavior /preference of predator which may change under different environmental variables [31].

Present study showed that both developmental stages of *C. septempunctata*, illustrated type II functional response feeding on 1st and 3rd instar nymphs of dubas bug. Predators displayed such type of functional response consumed higher number of prey with high prey availability, but the rate of predation/consumption decreased with increasing prey density and plateau in functional response curve indicated predator saturation. Similar type of response (FR II) has been reported for *C. septempunctata* preyed on: *Lipaphis erysimi* (Kaltenbach) [32], green apple aphid *Aphis pomi* De Geer [33], *Aphis glycines* [34].

Predators demonstrate similar functional response curve cannot be deemed to respond in the same way in term of handling time and search rate [12]. Present results showed that handling time of both stages of *C. septempunctata* varied significantly and it increased with prey (dubas) developmental stage, which indicates that predator have different abilities to response against different prey stage/size. Being the first study no reference is available to compare the result of present study with earlier work. However, generally these results are in line with [35] who found that handling time includes time it takes to: chase, kill, subdue and eat/digest a prey item, once the prey is located. While this study disagree with [36] who reported that handling time of *Exochomus nigromaculatus* decrease with developmental stage of *H. pruni*. This discrepancy may be due to dissimilarity in prey and predator: species, size, development stage, degree of predator hunger and most importantly some environmental variables.

Search rate (a) and handling time (Th) of both stages of *C. septempunctata* was significantly high preying on 1st instar nymphs of dubas bug compared to 3rd instar nymphs; indicated comparatively more mobility of 3rd instar than 1st instar dubas bug. Other possibly reasons may be due to variation in prey distribution [28], effect of temperature on predation and search rate of predator [37, 38]. While higher handling time of both stages on 3rd

instar nymphs showed that both stages of predator need more time to grasp 3rd instar than 1st instar.

In comparison, adult had significantly shorter handling time and higher search rate compared to grub, they take less time to process the food and take comparatively more time to locate the prey (may be due to mobility), which showed that *C. septempunctata* adult is more efficient in terms of food processing.

Based on present result of consumption rate, functional response, handling time and attack rate between two stages of predator that varied significantly we can conclude that though both development stages of *C. septempunctata* illustrated type II functional response but adult is the most efficient than grub. Functional response of predators can be affected by different biotic and a-biotic factors. However, field studies on predator and prey stage, sex specific, temperature and host plant in the presence of honeydew droplets on leaflets are still needed to quantify biocontrol efficiency of predator.

Authors' contribution

Conceived and designed the experiments: A Shah, MQ Kakar & T Iqbal, Performed the experiments: A Shah & H Ullah, Analyzed the data: NE Papanikolaou & A Shah, Contributed reagents/ materials/ analysis tools: A Shah, A Ullah & A Razaq, Wrote the paper: A Shah.

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