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Biology, feeding potential and functional response of *Coccinella septempunctata* L. against *Aphis gossypii* Glover infesting cucumber

Deeksha Kumari, SC Verma and PL Sharma

Abstract
The study on biology, feeding potential and functional response of the coccinellid, *Coccinella septempunctata* L. to *Aphis gossypii* Glover was carried out in the Biological Control Laboratory of Department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India. The study reveals that average incubation period of *C. septempunctata* was 3.20 days. The mean duration of 1st, 2nd, 3rd, 4th larval instar, total larval, pre pupal and pupal period was 2.24, 3.12, 3.32, 4.10, 13. 34, 1.90 and 3.90 days, respectively. Females laid on an average of 189.60 eggs in their life span of 43.84 days. Mean number of aphids consumed per day by 1st, 2nd, 3rd and 4th instars were 15.08, 31.54, 55.94 and 63.10 aphids, respectively. The predator consumed on an average 579.92 aphids during the entire larval stage. The number of aphids consumed increased with advancement of larval instars and increased in prey density. The mean number of aphids consumed by 4th instar of *C. septempunctata* was 23.30 at prey density of 25 and 65.40 aphids at the prey density of 125, respectively. Adults of *C. septempunctata* consumed maximum aphids (78.30) at prey density of 125. Predator's attack rate was lowest (0.075 h^{-1}) for the 1st instar and highest (0.175 h^{-1}) with the adult stage followed by attack rate of 4th instar (0.152 h^{-1}). All the stages as well as adult of *C. septempunctata* followed a Type II functional response. Thus, the 4th instar and adult of *C. septempunctata* could be utilised in biological control of *A. gossipii*.

Keywords: *Aphis gossypii,* biology, coccinellid, functional response, attack rate, handling time

Introduction
The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) is a cosmopolitan, polyphagous species infesting 569 host plants in tropical, subtropical and temperate regions (Sakaki and Sahragard, 2011; Singh *et al*., 2014; Tazerouni *et al*., 2016) [22, 29, 32]. Both nymphs and adults damage the crop by sucking the cell sap, resulting in curling of leaves or appearance of discoloured spots on the foliage. As the aphid become more abundant in juvenile stage, the plants gradually wilts, become yellowish to brownish and ultimately die. Heavy infestation of the aphid, results in reduction of the yield and quality of fruit by contaminating with honeydew which encourages sooty mould that disturbs the normal physiology of the leaves and also act as vector of 76 plant viruses (Escriu *et al*., 2000; Pinto *et al*., 2008; Srinivasulu *et al*., 2010; Bayoumy, 2011) [7, 20, 30, 4]. For the control of *A. gossypii,* many insecticides have been recommended (Mhaske *et al*., 2007; Misra, 2013) [13, 14] but, the indiscriminate use of synthetic pesticides leads to the development of insecticide resistance (Tomquelski *et al*., 2007; Denholm and Devine, 2013; Sarwar *et al*., 2014) [33, 5, 22], pest resurgence, insecticide residue, environment pollution and killing of natural enemies. Cotton aphid, *A. gossypii* developed resistance against many insecticides (Herron and Wilson, 2010; Seyedebrahimi *et al*., 2015; Pan *et al*., 2018) [8, 25, 19] which forces the farmers to increase the dose and/or frequency of pesticide application which further aggravates the problem (Denholm and Devine 2013) [5]. Therefore, there is a need to find out some alternatives of chemical pesticides to protect cucumber from infestation of cotton aphid (Kianpour *et al.*, 2010) [11]. Under such circumstances, biological control can be alternative, ecofriendly and sustainable approach to control this pest (Joshi *et al*., 2010) [9]. A large number of predators of *A. gossypii* have been reported to naturally suppress the aphid populations in different cropping systems belonging to coccinellidae, syrphidae, chrysopidae, and hemerobiidae (Ebert and Cartwright, 1997; Ahmadov and Hasanova, 2016) [6, 1]. Before using a predator in a biological control programme, it is essential to evaluate its predatory efficiency against the target species.

Biology and predatory potential of different predators against *A. gossypii* on different crops have been studied by many workers in India (Nehare *et al*., 2004; Omkar and Pervez, 2004; Nagamallikadevi *et al*., 2012; Naruka *et al*., 2017; Sharma *et al*., 2017) [17, 18, 15, 16, 27]. However, the information on the biological parameters and feeding potential of *coccinella septempunctata* against *A. gossypii* on cucumber under mid-hill conditions of Himachal Pradesh is lacking. Therefore, the objective of the study was to develop a better strategy for the biological control of *A. gossypii* on cucumber under mid-hill conditions of Himachal Pradesh.

Materials and Methods

Raising of cucumber crop

The cucumber crop was raised in the Experimental Farm of the Department of Entomology by following the recommended package of practices of vegetable crops (Anonymous, 2014) [3]. The variety of the cucumber was *Cucumis sativus* var. *malini*.

Laboratory rearing of Cotton aphid, *A. gossypii*

The cotton aphid, *A. gossypii* was collected from cucumber field and was maintained in the polyhouse throughout the year on cucumber plants. Cucumber leaves having nymphs were brought to laboratory for the further experiments. Before maturity of the old plants new plants were planted to maintain the culture of cotton aphid throughout the year.

Developmental biology of *C. septempunctata*

Developmental biology of the *C. septempunctata* was studied on the cotton aphid, *A. gossypii* on cucumber leaves in culture room fixed at 12:12 h photoperiod, at 25± 1^{0}C and 70± 5 per cent relative humidity. In this experiment, one pair of newly emerged adults were confined in the Petri plates containing wet blotting paper on base. Cucumber leaves (6×6cm) having mixed population of second and third instar of *A. gossypii* were placed on the wet blotting paper in Petri plates. The experiment was carried out with ten replications. The food was changed daily and the Petri plates under observation were examined at 24 h interval for egg laying and moulting of the nymph till all nymphs developed into adults. The duration of development of egg, each nymphal instar, total nymphal duration, pre pupal, pupal and total duration from egg to adult emergence were recorded. After emergence, the adults were identified as male and female under a stereozoom microscope and confined in pairs in Petri plates containing ample food. The pre-oviposition period and fecundity were recorded and then observations were recorded regularly until the first egg was laid which indicated the end of pre-oviposition period. Oviposition period, post- oviposition period, male and female longevity were recorded.

Feeding potential

Feeding potential of different developmental stages of C. *septempunctata* was studied against *A. gossypii*. Newly hatched larvae of the same stage were shifted individually to a single Petri plate having counted number of aphids of equal age. In order to select the aphids of equal age the cucumber leaves were carefully examined under the stereo zoom binocular microscope and the unwanted stages were removed carefully with the help of camel hair brush. The base of the Petri plate was covered with the wet blotting paper. The experiment was carried out in the insect culture room fixed at 12:12 h photoperiod, at 25± 1^{0}C and 70± 5 per cent relative humidity. The data on aphid consumption by the predator was recorded after every 24 h till they enter the next stage and the feed of the predator was changed. The experiment was replicated ten times. The number of aphids consumed per day by each larval instar was calculated. The data obtained was analysed by using one-way analysis of CRD without any transformation through online statistical software OPSTAT (Sheron *et al*., 1998) [28].

Functional response of *C. septempunctata*

Functional response of different stages of *C. septempunctata* to varying densities of *A. gossypii* was studied in Biological Control Laboratory of the Department of Entomology. Five densities (each replicated 10 times) of nymphs of second or third stage of *A. gossypii* were offered to the individuals of different stages of *C. septempunctata* on cucumber leaves in Petri plates separately. The experimental arena consisted of a 6 cm cucumber leaf disk placed in a Petri plate. Prey densities offered were 10, 15, 20, 25 and 30 for first instar; 15, 30, 45, 60 and 75 for second instar; 20, 40, 60, 80 and 100 for third instar; 25, 50, 75, 100 and 125 for fourth instar as well as for adult female of *C. septempunctata*. In order to select the aphids of equal age the cucumber leaves were examined carefully under the microscope and the excess density and unwanted life stages were carefully removed with the help of the camel hair brush. The Petriplates were placed in culture room at 25 ^{0}C, 70± 5 per cent relative humidity and a photoperiod of 12:12 (L:D)h. Observations were made after every 24 h. For studying the functional response of adults only females of *C. septempunctata* were used.

Data analysis of functional response

The data generated on functional response were analysed using the predation data for 24h. A logistic regression between proportion of prey consumed and prey density offered were fitted to determine the shape (e.g., type II or type III) of functional response:

$$N_e/N_0 = \frac{\exp\,(p_0 + p_1N_0 + p_2N_0{}^2 + p_3N_0{}^3)}{1+\exp\,(p_0 + p_1N_0 + p_2N_0{}^2 + p_3N_0{}^3)}$$

Where N_e is the number of prey eaten; N_0 is the initial number of preys, p_0 is intercept, p_1 is linear coefficient, p_2 is quadratic coefficient and p_3 is cubic coefficient. Significant negative and positive linear coefficients (i.e. p_1) from the regression indicate type II or type III functional response, respectively (Juliano, 2001) [10]. After determining the functional response the random predator equation of Rogers (1972) [21] was used to describe the functional responses. The form of the equation is as follows: $N_e = N_0$ [1- exp ($aT_hN_e – aT$)].Where N_e is the number of prey eaten per predator, N_0 is the prey density offered, T is the duration of the experiment (24h), T_h is the handling time i.e. time required by the predator to pursue, kill and digest the prey and a is the predation coefficient or predators attack rate. The value a/ T_h indicates the effectiveness of predator which was calculated by dividing a by T_h and maximum predation rate K = T/ T_h was also calculated.

Results and Discussion

Developmental biology of *C. septempunctata*

Data contained in Table 1 reveal that the average incubation period of *C. septempunctata* was 3.20 days. Suhail *et al*. (1999) [31] recorded that the incubation period of *C.*

septempunctata was of 3.53 days which was nearly close to the present study. The mean duration of 1st, 2nd, 3rd and 4th larval instar was 2.24, 3.12, 3.32 and 4.10 days, respectively. Total larval, pre pupal and pupal period was 13.34, 1.90 and 3.90 days, respectively. The present findings agree with the findings of Suhail *et al*. (1999) [31] who observed that the total larval and pupal period of *C. septempunctata* was 11.58 and 3.64 days, respectively. Similarly, Ali *et al*. (2017) [2] recorded that larval period of *C. septempunctata* preying on *A. gossypii* was 13.33 days and pupal period was 6.33 days. In the present investigation, time taken from egg to adult emergence was 22.66 days which was nearly same as reported by Sattar *et al*. (2008) [24] where egg to adult emergence period was 25.6 days. The male longevity of the adult beetles of *C. septempunctata* was 35.80 days. Females laid an average of 189.60 eggs in their life span of 43.84 days. Pre oviposition, oviposition and post oviposition periods were 11.38, 21.54 and 8.78 days, respectively (Table 1). In a similar study Sharma *et al*. (2013) [26] recorded that male and female longevity of adult beetles of *C. septempunctata* was 37.71 and 45.29 days, respectively at 25 ^{0}C temperature on *A. fabae*. Slight difference in findings of both the studies may be due to the different aphid species offered. Ali *et al*. (2017) [2] observed that on an average adult beetles of *C. septempunctata* survived for 33 days on cotton aphid.

Table 1: Duration of developmental stages (day's ± SE) of *C. septempunctata* reared on *A. gossypii*

Development Stage	*C. septempunctata* (days ± SE)
Incubation period	3.20 ± 0.18
1st instar	2.24 ± 0.05
2nd instar	3.12 ± 0.22
3rd instar	3.32 ± 0.23
4th instar	4.10 ± 0.31
Total larval period	13.34± 0.28
Pre-pupa	1.90 ± 0.11
Pupa	3.90 ± 0.12
Egg-adult emergence	22.66 ± 0.25
Female longevity	43.84 ± 3.41
Male longevity	35.80 ± 1.03
Pre oviposition period	11.38 ± 0.92
Oviposition period	21.54 ± 1.12
Post oviposition period	8.78 ± 0.90
Fecundity (Eggs/female)	189.60 ± 7.46

Predatory potential of *C. septempunctata* against *A. gossypii*

Predatory potential of *C. septempunctata* against *A. gossypii* was studied by assessing the feeding potential of different developmental stages and functional response of the *C. septempunctata* to varying densities of cotton aphid.

Feeding potential of *C. septempunctata*

The number of the aphids consumed increased at each successive stages of the predator. The larva of the predator consumed on an average of 33.80, 98.70, 185.70 and 258.72 aphids during 1st, 2nd, 3rd and 4th instars, respectively (Table 2). Mean number of aphids consumed per day by 1st, 2nd, 3rd and 4th instars was 15.08, 31.54, 55.94 and 63.10 aphids/day, respectively. The predator consumed on an average 579.92 aphids during the entire larval stage. Female and male beetles consumed 3376.94 and 2457.84 aphids in their entire life span and the average number of aphids consumed per day by each female or each male beetle was 77.02 or 68.66 aphids, respectively. The results recorded in the present study were supported by the study of Suhail *et al*. (1999) [31] who recorded that 1st, 2nd, 3rd and 4th in stars of *C. septempunctata* consumed on an average 15.23, 36.40, 42.23 and 47.15 individuals of *A. gossypii*/day, respectively. Ali *et al*. (2017) [2] recorded that at temperature of 18 ^{0}C and 65 per cent relative humidity 1st, 2nd, 3rd and 4th instar of *C. septempunctata* consumed 55.33, 102.66, 172.00 and 315.00 aphids, respectively. Slight differences in the results of the present study and those of Ali *et al*. (2017) [2] may be due to the stage of the aphids offered to the predator and the temperature conditions provided during the experimental period. The present results also agree with the findings of Sattar *et al*. (2008) [24] who recorded that the average number of the *A. gossypii* consumed by an adult female of *C. septempunctata* per day was 77.80.

Table 2: Feeding potential of different developmental stages of *C. septempuncata* against *A. gossypii*

Stage	Number of aphids Consumed		Number of aphids consumed/ day	
	Mean ± SE	Range	Mean ± SE	Range
1st instar	33.80 ± 3.70	25-45	15.08 ± 0.47	13-17
2nd instar	98.70 ± 4.10	88-108	31.54 ± 0.83	28-34
3rd instar	185.70 ± 7.75	155-198	55.94 ± 1.57	50-61
4th instar	258.72 ± 10.76	235-287	63.10 ± 4.14	58-78
1st- 4th instar	579.92 ± 12.33	538-604	43.48 ± 3.65	36-52
Adult female	3376.94 ± 58.56	3156-3490	77.02 ± 1.52	73-83
Adult male	2457.84 ± 21.07	2375-2490	68.66 ± 1.36	65-73

Functional response of *C. septempunctata*

The data presented in Table 3 reveal that cubic polynomial fit between prey density offered (N) and proportion of prey consumed (Na/N) by all the stages of the predator resulted significant negative linear coefficients (1st instar: -0.05; 2nd instar: -0.02; 3rd instar: -0.008; 4th instar: -0.005 and adult: -0.002) confirming a Type II functional response for all the developmental stages of the predator. Previously many workers have reported a Type II functional response in coccinellids (Xue *et al*. 2011; Kumar *et al*. 2014; Sharma *et al*. 2017) [34, 12, 27] to their prey. A predator with a Type II functional response has an inverse density-dependent action against its prey.

Table 3: Parameters of polynomial regression between proportions of prey consumed (Na/N) and initial prey density (N) of *C. septempunctata*

Predator stage	Parameter	Coefficient Mean ± SE	t- value	P
1st instar	P0	1.05 ± 0.13	8.23	< 0.001
	P1	-0.05 ± 0.02	-2.73	0.023
	P2	0.003 ± 0.0009	2.67	0.025
	P3	-0.00005 ± 1.8E-05	-2.66	0.026
2nd instar	P0	1.11 ± 0.03	33.05	< 0.001
	P1	-0.02 ± 0.003	-7.63	< 0.001
	P2	0.0004 ± 7E-05	5.46	< 0.001

	P3	-0.000003 ± 4.5E-07	-6.02	< 0.001
3rd instar	P0	1.04 ± 0.03	30.92	< 0.001
	P1	-0.008 ± 0.002	-4.15	0.0024
	P2	0.0001 ± 0.00001	3.56	< 0.001
	P3	-0.0000009 ± 1.9E-07	-4.68	0.0012
4th instar	P0	1.04 ± 0.02	67.85	< 0.001
	P1	-0.005 ± 0.0006	-9.12	< 0.001
	P2	0.00005±8.4E-06	5.71	< 0.001
	P3	-0.0000003 ± 3.9E-08	-7.61	< 0.001
Adult	P0	0.99 ± 0.01	98.81	< 0.001
	P1	-0.002 ± 0.0004	-4.84	< 0.001
	P2	0.00002 ± 6.2E-06	3.49	< 0.001
	P3	-0.0000002 ± 4.2E-08	-4.50	0.0014

P0= Constant, P1= Linear coefficient, P2= Quadratic coefficient, P3= Cubic coefficient

Data presented in Table 4 reveal that the number of aphids consumed by 1st instar of *C. septempunctata* was maximum at the prey density of 30 which was statistically on par with the prey density of 25. But, the proportion of prey consumed was maximum at the lowest prey density (73.00%) and proportion of prey consumed decreased with increasing prey density. The proportion of prey consumed was minimum at the prey density of 30 (48.30%).

Table 4: Functional response of different instars and adult of *C. septempunctata* to different densities of *A. gossypii*

Instars/Adult	Prey density	Mean number of aphids consumed (Mean ± SE)	Proportion of aphids consumed (%)
1st instar	10	7.30 ± 0.15^d	73.00
	15	9.80 ± 0.13^c	65.30
	20	12.90 ± 0.28^b	64.50
	25	14.30 ± 0.15^a	57.20
	30	14.50 ± 0.17^a	48.30
	CD (p= 0.05)	0.53	
2nd instar	15	12.90 ± 0.10^d	86.00
	30	21.00 ± 0.26^c	70.00
	45	29.90 ± 0.31^b	66.44
	60	33.00 ± 0.26^a	55.00
	75	33.10 ± 0.23^a	44.13
	CD (p= 0.05)	0.70	
3rd instar	20	18.60 ± 0.16^d	93.00
	40	33.60 ± 0.31^c	84.00
	60	50.00 ± 0.33^b	83.33
	80	56.10 ± 0.23^a	70.12
	100	56.30 ± 0.26^a	56.30
	CD (p= 0.05)	0.76	
4th instar	25	23.30 ± 0.21^d	93.20
	50	41.50 ± 0.34^c	83.00
	75	59.70 ± 0.30^b	79.60
	100	65.30 ± 0.26^a	65.30
	125	65.40 ± 0.22^a	52.32
	CD (p= 0.05)	0.77	
Adult female	25	23.90 ± 0.10^d	95.60
	50	46.50 ± 0.16^c	93.00
	75	65.70 ± 0.26^b	87.60
	100	78.10 ± 0.28^a	78.10
	125	78.30 ± 0.21^a	62.64
	CD(p=0.05)	0.61	

The number of aphids consumed by the 2nd instar of *C. septempunctata* at prey densities of 15, 30, 45, 60 and 75 was 12.90, 21.00, 29.90, 33.00 and 33.10, respectively. The number of aphids consumed at the prey density of 75 were statistically on par with the prey density of 60. The maximum proportion of the aphids were consumed at the prey density of 15 (86.00%) and minimum proportion was consumed at prey density of 75 (44.13%). The 3rd instar of *C. septempunctata* consumed 18.60, 33.60, 50.00, 56.10 and 56.30 aphids on the prey densities of 20, 40, 60, 80 and 100, respectively. The number of aphids consumed at the prey density of 100 was statistically on par with the prey density of 80. The proportion of aphids consumed was maximum (93.00%) at the lowest prey density and minimum (56.30) at the highest prey density. The proportion of the aphids consumed were maximum at the prey density of 25 (93.20%) and minimum at the prey density of 125 (52.32%) (Table 4). The mean number of aphids consumed by 4th instar of *C. septempunctata* was 23.30, 41.50, 59.70, 65.30 and 65.40 aphids at the prey densities of 25, 50, 75, 100 and 125, respectively. The number of aphids consumed at the prey density of 100 were statistically on par with the prey density of 80. Prey densities offered to adults of *C. septempunctata* were 25, 50, 75, 100 and 125 and mean number of aphids consumed at these prey densities was 23.90, 46.50, 65.70, 78.10 and 78.30, respectively (Table 4). The number of aphids consumed at the prey density of 125 was statistically on par with the prey density of 100. The proportion of aphids consumed was highest at the prey density of 25 (96.00%) and lowest at the prey density of 125 (62.64%).

In the present findings, the proportion of prey consumed at higher density decreased which may be due to satiation of the predator at increased number of prey. These findings were in agreement with the findings of Pervez and Omkar (2005) [18] who found that there was a decline in the consumption rate of *C. septempunctata* at higher prey densities. The present study (Table 4) reveal that the number of the aphids consumed increased with the increase in prey densities for all the developmental stages of the predator and when satiation was attained by the predator there was no significant increase in the number of aphids consumed and there was no effect of prey density (Omkar and Pervez, 2005) [18]. The proportion of the aphids consumed was maximum at the lowest density and minimum at the highest prey density.

The predation efficiency of *C. septempunctata* against *A. gossypii* increased with the advancement of the developmental stages of the predator which is numerically illustrated by parameters of attack rate (a) and handling time (T_h) (Table 9). Predator's attack rate was lowest (0.075 h^{-1}) for the 1st instar and highest (0.175 h^{-1}) with the adult stage followed by attack rate of 4th instar (0.152 h^{-1}). Handling time of the adult beetle (0.205 h^{-1}) was statistically on par with the 4th instar grub (0.262 h^{-1}) and was shorter than rest of the stages. The present study predicts that the 1st, 2nd, 3rd, 4th instar and adult of *C. septempunctata* could predate a maximum of 26.70, 46.20, 80.27, 91.60 and 117.07 aphids in a 24 h period. Functional response parameters indicated that the adult and 4th instar grubs were the effective stages of *C. septempunctata* against *A. gossypii*. In the present investigations, the attack rate of the predator increased and handling time decreased with the gradual increase in stage/ age of predator. Similar to the present investigations, Sharma *et al*. (2017) [27] also observed that the attack rate of *Harmonia dimidiata* increased with the advancement of stages against *A. gossypii* and handling time shortened with the increase in stages of the predator.

Table 5: Parameters of functional response of *C. septempunctata* to *A. gossypii* over 24 h period

Predator stage	Parameter				
	a ± SE	T_h ± SE	a/T_h	K	R^2
1st instar	0.075 ± 0.004^e	0.903 ± 0.053^d	0.09	26.70	0.952
2nd instar	0.109 ± 0.003^d	0.521 ± 0.012^c	0.21	46.20	0.984
3rd instar	0.141 ± 0.006^c	0.281 ± 0.008^b	0.47	80.27	0.989
4th instar	0.152 ± 0.008^b	0.262 ± 0.007^a	0.58	91.60	0.991
Adult	0.175 ± 0.001^a	0.205 ± 0.008^a	0.85	117.07	0.995
CD (p= 0.05)	0.014	0.071			

a= Coefficient of attack rate, T_h= Handling time, K= maximum theoretical predation rate, R^2= Coefficient of determination

Conclusion

Coccinella septempunctata can be utilized as a component of integrated pest management programmes for the management of cotton aphid, *A. gossypii* in cucumber under mid-hill conditions of Himachal Pradesh.

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