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Growth and development of ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), on plant and animal based protein diets

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ABSTRACT

Different protein sources from plant and animal origin viz., soybean flour (SF-AD) and soaked soybean (SS-AD), bee pollen (BP-AD), honey bee drone larvae (DL-AD) and frozen aphids (AF-AD) were assessed as an artificial diet of *C. septempunctata* in comparison to their natural diet live aphids. Highest growth index was obtained with SS-AD (4.05) diet almost similar to control (4.0), followed by SF-AD, BP-AD, DL-AD and AF-AD as 3.8, 2.4, 1.98 and 1.94 respectively. Similarly, data showed that success index of the adults fed on an artificial diet containing soybean (SS-AD = 1.03, SF-AD = 0.98) was almost similar to control (1.0), whereas the diets containing bee pollen, drone larvae, and frozen aphids was lower than control as 0.83, 0.84 and 0.78, respectively. Protein concentration was highest in live aphid (5.9 μg/ml) diet followed by SS-AD (5.6), SF-AD (5.2), BP-AD (3.4), AF-AD (2.2) and DL AD (1.3). The results indicated that soybean based diets were more or equivalently suitable to beetles for their growth and development likewise their natural prey and the efficiency of artificial diets was related to its protein concentration.

Introduction

Predatory beetles (Order Coleoptera) have a long history of importance as natural enemies of various phytophagous insect and mite pests. Many coleopteran predators, especially those in the family Coccinellidae (representing the lady beetles, ladybird beetles or ladybugs) have been used with moderate success in managing pest populations throughout the world (Hodek and Honek, 1996; Obrycki and Kring, 1998; Hodek and Evans, 2012). Predatory insects are important not only because they are major biological control agents in IPM program but also because they form important material for behavioral, bio-ecological and screening bioassay studies. Consequently, there is an ever increasing demand for large supplies of these insects in research, agriculture, and industry requires large-scale production of predatory insects. Since their natural food, the host/prey species, is not always available, it becomes necessary to develop alternative feeding media on which the insects can be reared and multiplied. Today, one challenge for greater use of coccinellids is to create cost-effective techniques to rear and stockpile (store) them. Many of the current rearing methods continue to depend on a tri-trophic system of rearing: the host plant, natural prey (herbivorous pest) and predator. This system is labor intensive and not cost effective. Hence there is a need to develop a targeted and specific mass rearing methodology for these very important predators using artificial diets. Use of artificial diets represents a step towards cost-effective rearing of beneficial insects (Cohen, 2004). Developing artificial diet is difficult but once optimized, usually are simple to prepare and easy to handle (Cohen and Smith, 1998). Success or failure of an artificial diet depends on various criteria of considerable effects on different life parameters of coleopteran insect which includes: development time, larval survival, adult emergence, fecundity, and adult life span (Riddick and Chen, 2014; Sun et al., 2017). Predators need a protein rich diet for their growth. Lipid can improve the yield and fecundity of adults of several entomophagous insects (Grenier, 2012). Utilization of an artificial diet, rather than factitious prey/foods or natural prey, would be a further progression in realizing the cost-effective mass production of lady beetles (Riddick, 2009). Purified proteins such as soybean, casein from milk, gluten from wheat and albumin from eggs have been used in artificial diets to meet the protein requirement of insects (Sarwar and Saqib, 2010). Jokar and Zarabi (2014) suggested that the semi-artificial diet was the most accurate diet for mass rearing of a predator.

The seven-spotted ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) was introduced into North America from Europe. *C. septempunctata* is predaceous in its feeding habits. Proteins are therefore a major component of its food. Naturally, they fed upon aphids, thrips, spider mites and various soft-bodied insects and their eggs. In the scarcity of insects, they may also survive on pollen of different flowering plants. To rear *C. septempunctata* on a large scale, it is

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important to incorporate a rich protein source in the artificial diet. Both plants and animals and their products can meet this requirement. In the present study three diverse types of protein sources viz., soybean (flour and soaked seeds), bee pollen, drone larvae and frozen aphids were assessed for their efficiency in artificial diet for mass rearing of *C. septempunctata* in comparison to their natural diet live aphids.

Material and methods

Plant and insect material

A culture of radish was established in pots (3 gal) in glass house outside Zoology Department, Panjab University, Chandigarh. These plants were used to maintain the culture of aphids *Myzus persicae* Sulz. Aphids were used to feed *C. septempunctata* as a natural diet. The adults of *C. septempunctata* were collected from radish fields, Village - Chappar-chiri, Punjab, India. The adults were kept at a 23–25° temperature and 16:8 hour photoperiod and 50–70% relative humidity. The beetles were reared in a plastic container (470 × 350 × 170 mm) bottom lined with filter paper and top covered with a muslin cloth. Thin apple slices, water, and honey were provided to them inside the container. A plant twig and scrambled paper were also placed inside the box, which provided these beetles extra surface to roam around. The container was observed daily for eggs. The box was changed after two days and replaced with new one. The collected eggs were kept in a small petridish lined with filter paper. These eggs were kept inside incubator till hatching. The newly emerged larvae were fed on aphids for one day and taken for further use.

Protein source: frozen aphids and drone larvae (animal origin)

M. persicae were collected from greenhouse plants and frozen in vials. Drone larvae of *Apis mellifera* L. obtained from honey bee colonies maintained by the Department of Zoology, Panjab University, Chandigarh, India. *Soybean* (*Plant origin*): soybean is one of the richest protein sources of plant origin. Soybean protein was in two different forms as soybean flour and seeds, soaked for 24 h before making the artificial diet. *Bee pollen* (*Plant origin modified by an insect*): as collected from *A. mellifera* colonies, pollen was of mixed type. Pollen was collected from colonies of honey bee by installing pollen trap at the entrance of langstroth bee hive, which were placed in the fields of village Zirakpur, Patiala, India.

Artificial diets

Artificial diets were prepared by following methods described by Tiwari and Bhattacharya (1987). The common components of diet per 100 g of diet were honey, yeast, agar-agar, sorbic acid, methylparaben, sodium ascorbate, 10% formaldehyde, distilled water, and multi-vitamins. Diet 1 contained soybean flour as the test material in addition to components mentioned above. For diets 2, 3, and 4: soaked soybean seeds, bee pollen, and frozen aphids respectively, were used as protein source. Live aphid diet constituted the control. The detailed composition of the four diets is shown in Table 1.

Preparation of artificial diet

For the preparation of artificial diet, the protein sources were: soybean flour, soaked soybean seeds, bee pollen, drone larvae, and aphids frozen. First, the protein source was mixed with half the quantity of distilled water and ground for 1 min to obtain a homogenous mixture. Then other ingredients like yeast powder, methylparaben, sorbic acid, formaldehyde (10%) were added to above mixture and mixed for 2–3 min. In remaining quantity of distilled water boiling of agar-agar was done and after cooling it to 60°, it was added to above mixture and mixed gently for 2 min. Then sodium ascorbate was dissolved in a little amount of water in another beaker. Added sodium ascorbate, vitamin mixture and honey to above diet and blended for 2–3 min. The diet was poured into a plastic container and stored refrigerated for further use.

Table 1
Components incorporated in different artificial diets of *C. septempunctata*. (SF = soybean flour, SS = soaked soybean, DL = drone larvae, BP = bee pollen, AF = frozen aphids and AD = artificial diet).

Composition of artificial diet (100 g)	
Protein source (SF/SS/BP/DL/Af)	14 g
Honey	3 ml
Yeast	3 ml
Sorbic acid	0.31 g
Multi-vitamins	0.31 g
Sodium ascorbate	0.31 g
Methylparaben	0.31 g
10% formaldehyde	0.15 ml
Agar agar	1.5 g
Water (distilled)	77 ml

Experimental methods

One-day-old larvae reared in small containers (2.5 × 2.5 cm) with small holes in a screw cap. The diet placed at the base of the container in slanting position with the help of a spatula. One day old larvae were transferred to plastic containers with the help of camel hair brush. The larvae were reared individually on different diets. Live aphids used as the control diet. Every alternate day larvae transferred to a new container with fresh diet placed in it. For experimentation, there were three replications for each treatment and ten larvae per replication. The whole experiment was repeated twice (n = 360) on two different dates. The observations were taken daily on larvae and pupae alive till the adult emergence. From these observations, various parameters obtained: larval period, pupal period, total developmental period, percentage larval survival, percentage pupal survival and percentage adult emergence. Also, adults who emerged weighed on scale. Growth index and success index calculated and obtained with these observations.

Growth index and success index

Growth index (Pant, 1956) and success index (Prasad and Bhattacharya, 1975) were computed as

$$\text{Growth index (G. I.)} = \frac{N}{Av}$$

where,

N = percent adult emergence
Av = average development period (days)

For success index, various indices were calculated as:

$$\text{Larval period index (L. P. I.)} = \frac{\text{Larval period (days) in control}}{\text{Larval period (days) in treatment}}$$

$$\text{Pupal period index (P. P. I.)} = \frac{\text{Pupal period (days) in control}}{\text{Pupal period (days) in treatment}}$$

$$\text{Pupation index (P. I.)} = \frac{\text{Percent pupation in treatment}}{\text{Percent pupation in control}}$$

$$\text{Adult emergence index (A. E. I.)} = \frac{\text{Percent adult emergence in treatment}}{\text{Percent adult emergence in control}}$$

$$\text{Success index} = \frac{\text{L. P. I.} + \text{P. P. I.} + \text{P. I.} + \text{A. E. I.}}{4}$$

Protein estimation of artificial diets

Protein Estimation Teaching Kit (X-Pert™, Product Code: HTBC005, HiMedia Laboratories™) was bought for this purpose. Immediately after preparation of little amount of artificial diets were frozen in the deep freezer. For protein extraction, frozen diets were homogenized in 50 mmol L^{-1} sodium phosphate buffer (pH 6.8) (Hung and Kao, 2004; Comparot et al., 2002) to make a crude extract. Centrifugation of extracts at 17,600*g* for 20 min was performed, and the supernatants were used for determination of protein by the method of Bradford (1976). OD was obtained with Digital Photo Colorimeter (Electronic India, Model 312). Three replicates were obtained from each diet. Replicates were combined and analyzed statistically.

Statistical analysis

Data on development period were analyzed by ANOVA and means were compared using Student's *t*-Test. Data were analyzed by all replicates combined and also by different dates combined, separately (JMP SAS, 2005).

Results

Effect of four different artificial diets (soybean flour, soaked soybean seeds, bee pollen, drone larvae and frozen aphids) and natural prey (live aphids) as control, on the development of *C. septempunctata*, was studied. The effect of these artificial diets on *C. septempunctata* would help to ascertain the suitability of artificial diet for its mass rearing under laboratory conditions.

Developmental parameters

One-day-old larvae of *C. septempunctata* were released on different diets and also with live aphids (control). The results obtained showed that in both the soybean based diets (SF-AD and SS-AD) first, second and fourth instar larval duration was significantly less than those fed upon live aphids (Table 2). In the case of bee pollen (BP-AD), drone larvae (DL-AD) and aphids frozen (AF-AD) diets larval duration of second, third and fourth instars was significantly less than control. However, total larval period among all the diets was significantly less than control (Table 2). In the case of the pupal period, SF-AD and SS-AD showed the non-significant difference with control whereas BP-AD and AF-AD showed a significant increase in this period than control. However, total developmental period among all the diets showed a non-significant difference than control (Table 2). Both the soybean based diets, SF-AD and SS-AD, showed non-significant differences in percentage larval and pupal survival and adult emergence in comparison to control; however, they were significantly less on BP-AD, DL-AD and AF-AD diets (Table 3). Weights of adults emerged did not show any significant difference among soybean based diets and live aphid diets. However, they were significantly less on bee pollen, drone larvae and aphid frozen diets in comparison to control (Table 3).

Growth and success indices

Table 4 indicates calculated data of growth and success indices of different diets compared to control. Highest growth index was obtained with SS-AD diet followed by control, SF-AD, BP-AD, DL-AD, and AF-AD. Similarly, data showed that success index of the adults fed on an artificial diet containing soybean was almost similar to control, whereas the diets containing bee pollen, drone larvae, and frozen aphids was lower than control.

Protein estimation

Quantification of protein concentration for all the artificial diets and natural prey (aphid) by Bradford assay. Quantification data reveals that maximum protein concentration was present in aphid (control) (5.9 µg/ml) diet which was non-significantly different with protein concentrations of SS-AD (5.6 µg/ml) and SF-AD (5.2 µg/ml). However, DL-AD (1.2 µg/ml), BP-AD (3.4 µg/ml) and AF-AD (2.2 µg/ml) showed significantly fewer values than control.

Discussion

A good artificial diet is nutritionally comparable to a natural diet, and the present study demonstrated that *Coccinella septempunctata* larvae had similar developmental parameters in SS-AD and SF-AD diets when compared with control (live aphids).

Larval period among all the diets showed reduction as compared to control. The pupal period in BP-AD and AF-AD revealed an increase than the control diet. However, total developmental period among all the diets showed a non-significant difference than control. Both the soybean based diets, SF-AD and SS-AD, showed a similar percentage of larval and pupal survival and adult emergence in comparison to control; however, a significant reduction was noticed on BP-AD, DL-AD, and AF-AD diets. Weights of adults emerged had similar values on soybean based diets and live aphid diets. However, they were significantly less on bee pollen, drone larvae and aphid frozen diets in comparison to control.

Growth and success indices indicated that soybean soaked artificial diet showed even better results than live aphid control. The results indicated that the efficiency of artificial diets was related to its protein concentration. The increase in weight of adults (Fig. 1A) and both indices (growth and success) were significantly correlated with the protein concentration in the artificial diet ($P < 0.001$). Higher the concentration, more suitable was the diet for beetles.

Soybean is considered to be a very economical and valuable agricultural commodity, and India ranks fifth in the world in the area under cultivation and production after USA, Brazil, China, and Argentina. Soybean seeds are one of the richest sources of protein. The high protein content of soybean is responsible for its use as artificial diet

Table 2
Duration (days) of larval instars, mean larval period (days), mean pupal period (days), mean total developmental period (days) and average adult weight (mg) of *C. septempunctata* receiving different diets (SF = soybean flour, SS = soaked soybean, DL = drone larvae, BP = bee pollen, AF = frozen aphids and AD = artificial diet) (n = 360).

Treatments	1st instar	2nd instar	3rd instar	4th instar	Larval period (A)	Pupal period (B)	Total developmental period (A + B)
SF-AD	2.8 ± 0.1b*	2.9 ± 0.2c**	2.6 ± 0.1a	3.8 ± 0.1a	14.2 ± 0.3b	7.9 ± 0.2b	22.8 ± 0.4a
SS-AD	2.6 ± 0.1b	3.1 ± 0.1c	2.3 ± 0.1a	3.7 ± 0.1a	13.7 ± 0.3b	7.6 ± 0.2b	22.2 ± 0.3a
DL-AD	3.1 ± 0.1a	3.0 ± 0.2b	1.4 ± 0.1b	1.6 ± 0.3c	9.8 ± 0.5c	10.7 ± 0.5a	21.9 ± 0.5b
BP-AD	3.5 ± 0.1a	4.0 ± 0.2b	1.8 ± 0.2b	1.9 ± 0.3c	12.6 ± 0.4b	10.4 ± 0.6a	23.2 ± 2.5a
AF-AD	3.4 ± 0.2a	3.4 ± 0.2bc	1.5 ± 0.1b	1.9 ± 0.4c	11.7 ± 0.6c	10.5 ± 0.6a	22.3 ± 1.6ab
Aphid (control)	3.1 ± 0.1a	4.6 ± 0.2a	2.6 ± 0.1a	4.6 ± 0.2b	15.0 ± 0.2a	7.5 ± 0.2b	23.3 ± 0.3a
F (df) P	5.5 (5, 475), 0.0015	17.1 (5, 475), < 0.0001	29.9 (5, 459), < 0.0001	67.1 (5, 445), < 0.0001	37.6 (5, 398), < 0.0001	28.1 (5, 392), < 0.0001	2.8 (5, 392), 0.0451

Student's t LSD test, α = 0.05.
* Means in the same column followed by different letters are significantly different.
** Mean values are followed by ± standard error of the mean.

Table 3
Average percentage of larval, pupal and adult emergence of *C. septempunctata* receiving different diets (SF = soybean flour, SS = soaked soybean, DL = drone larvae, BP = bee pollen, AF = frozen aphids and AD = artificial diet).

Treatment	Larval survival (%)	Pupal survival (%)	Adult emergence (%)	Adult weight (mg)
SF-AD	86.7 ± 4.2ab*	100.0 ± 0.0a**	86.7 ± 4.2a	28.3 ± 2.7a
SS-AD	90.0 ± 4.5a	100.0 ± 0.0a	90.0 ± 4.5a	25.1 ± 2.5a
DL-AD	53.3 ± 12.3b	65.3 ± 15.3b	43.3 ± 12.0b	17.4 ± 0.5b
BP-AD	73.3 ± 6.7bc	78.6 ± 7.9b	56.7 ± 6.2b	20.7 ± 0.6b
AF-AD	63.3 ± 8.0c	66.1 ± 3.9b	43.3 ± 8.0b	15.3 ± 3.5b
Aphid (control)	93.3 ± 4.2a	100.0 ± 0.0a	93.3 ± 4.2a	25.5 ± 1.6a
F (df) P	6.1 (5, 30), 0.0039	6.3 (5, 30), 0.0035	12.6 (5, 30), < 0.0001	61.2 (5, 249), < 0.0001

Student's t LSD test, α = 0.05.
* Means in the same column followed by different letters are significantly different.
** Mean values are followed by ± standard error of the mean.

Table 4
Success index and growth index of *C. septempunctata* receiving different diets.

Treatment	Success index (SI)	Growth index (GI)
SF AD	0.98	3.80
SS AD	1.03	4.05
DL AD	0.84	1.98
BP AD	0.83	2.44
AFAD	0.78	1.94
Aphid (control)	1.00	4.00

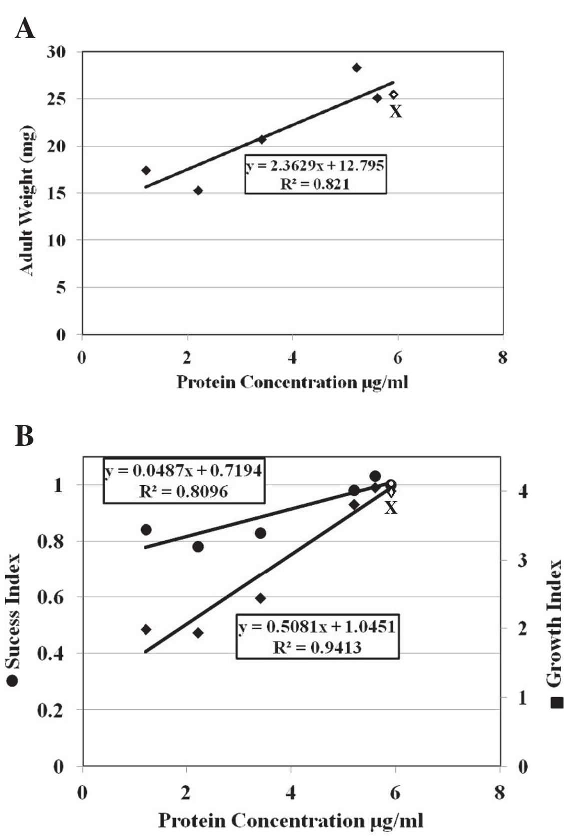

Fig. 1. Mean adults weight (A) and growth and success indices (B) reared on five different artificial diets showing protein concentration levels of each diet. X = variable on natural diet.

component for insects. Also, it is very cost effective as compared to other protein sources. Shaver and Raulston (1971) also proposed that there was a reduction in rearing cost when larvae of tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) were fed soybean meal and flour instead of casein in the wheat germ diet. In agreement with the present study, Smith and Circle (1972) also reported that soybean flour is richer in both protein and fat as compared to other beans and is relatively low in carbohydrates. Also, Singh (1999) confirmed that soybean diet was more nutritive as larvae of *Helicoverpa armigera* (Hubner) consumed less amount of diets and at the same time showed better developmental period. These results are also supported by Kamioka et al. (1971) when larvae of the silkworm *Bombyx mori* L., were reared on four kinds of artificial diets having different amounts of soybean meal and sugar showed with the increase in the dietary soybean meal, the silk production increased. Nettles (1986) found an increase of 4.2- to 5.7-fold by the addition of defatted toasted soy flour on yields of *Eucelatoria bryani* Sabrosky adults. In another study, Sarwar and Saqib (2010) observed and suggested that if artificial diet (agar, yeast, honey, protein hydrolyzate and alfalfa flour) and aphids *Lipaphis erysimi* (Kalt), were given simultaneously, development of *C. septempunctata* larvae became faster, and could be reared more in number. They have also reported the better percentage larval survival on this artificial diet as compared to aphid diet. Wang et al. (2013) when reared legume pod borer *Maruca vitrata* (Fabricius) on artificial diets containing soybean powder and wheat-germ found better developmental time over its natural food source. Silva et al. (2009) evaluated the suitability of 1-day frozen eggs of *Anagasta kuehniella* in combination with artificial diet when fed to *Eriopis connexa* Germar, a ladybird beetle. Artificial diets contained honey, yeast, soybean crumbs, wheat germ, $FeSO_4$, ascorbic acid, propionic acid, sorbic acid, nipagin, milk, cat food, water in different proportions.

Protein concentration pattern indicated the direct relation of concentration of protein in the artificial diet with its efficiency. Hence, more the protein concentration more successful was the diet for predator culture. Similarly, Achiano and Giliomee (2006) performed artificial rearing of *Carcinops pumilio* (Erichson), and they compared artificial diet with a natural diet *Drosophila melanogaster*, and artificial diet was composed of protein rich food additives (PRO-PLEX). The egg production, developmental time, weights of 1st, 2nd instar larvae were strongly correlated with protein concentration. Blanco et al. (2009) reared *Heliothis virescens* on an artificial diet consisting of two main components: NutriSoy flour (2.15%) and wheat germ (2.26%), in different proportions. It was observed that reduction in the protein of ≥5.5% had a negative effect on reproduction and larval growth.

Similar to our results, Farag et al. (2011) also found that bee pollen was the least suitable diet for *Coccinella undecimpunctata* L. larvae, among seven different combinations of diets tested. The highest percentage larval survival, adult emergence and egg production was shown by royal jelly + bee honey mixed with aphids diet. Maurice et al. (2011) reared *Coccinella transversalis* Fab. on eight different species of aphids and four alternative foods such as mealy bug, pollen, sugar syrup and honey. They observed that among alternative foods, only honey and mealy bugs provided satisfactory development of larvae as compared to pollen and sugar syrup. Also, Henderson et al. (1992) developed three insect-based diets: bee brood, wasp brood and pupae of light brown apple moth, *Epiphyas postvittana*, with various additives (natural wheat germ, reground Difco TC yeastolate, yeast extract powder, Vitamin C and Royal jelly) for *Chilocorus bipustulatus* L. and *Chilocorus cacti* L. They found that wasp brood diet gave a greater yield of adults (40%) *C. bipustulatus*. However, *C. cacti* showed the best yield when fed on a diet of wasp brood (75%) followed by *E. postvittana* (55%) and bee brood (35%).

Silva et al. (2013) assessed the development and reproduction of ashy gray lady beetle *Olla v-nigrum* Mulsant, fed *Anagasta kuehniella* eggs supplemented with an artificial diet. The artificial diet consisted of yeast, honey, ascorbic acid, and water. The durations of instars I, II, III and IV and the durations of the larval, prepupal, pupa and larva to adult stages were 2.4, 1.8, 2.0, 5.8, 12.1, 1.0, 4.0 and 17.1 days, respectively. The viabilities of the larval, prepupal and pupal stages of this predator were 46.3, 90.0 and 100%, respectively. Chen et al. (1980) investigated the influence of diet on the feeding and fecundity of *C. septempunctata*. The coccinellid was reared either on aphids or a 5:1 (w/w) mixture of pig liver homogenate and honey. On the artificial diet, the pre-oviposition period was longer, and the percentage of ovipositing females and the number of eggs laid were lower than on the aphid diet.

In conclusion, Soaked soybean diet had highest protein concentration among all four diets viz. soaked soybean, soybean flour, bee pollen and frozen aphids. *C. septempunctata* showed positive response with soaked soybean diet and soybean flour diets. Protein concentration values were directly proportional to the efficiency of artificial diets for *C. septempunctata* larvae. These diets are recommended for possible implementation in mass rearing of these predators. This research while conclusive needs further evaluation on effects of soybean based diets on fecundity and predatory quality of *C. spetumpunctata* adults obtained under laboratory.

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