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Effect of different artificial diets on some biological traits of adult green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory conditions

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Abstract
The present study was aimed to find out the effect of different diets on some biological parameters of the adult predator, *Chrysoperla carnea* and to introduce an economic artificial diet for its mass-rearing. The *C. carnea* was reared for four successive generations in laboratory conditions on baker's yeast, honey, and sucrose (YHS) (2: 1: 1). The effect of seven different diets including VAYH, AK + HY, WMHY, WMH, YS + SC, YVYH, and YMH was studied on some biological parameters of the fifth generation. All experiments were done under 25 ± 1^{0}C, 65±5% RH and 16:8 h (L:D) conditions. Data were analyzed using a completely randomized design (CRD) with 15 replicates. Results revealed that the YVYH diet influenced the biological traits of the predator more positively. The mean of female longevity, male longevity, oviposition period, post oviposition period and fecundity calculated 43.53, 34.86, 26.26, 10.46 days and 618.07 (eggs), respectively.

Keywords: *Chrysoperla carnea,* biological parameters, artificial diets, adult green lacewing

1. Introduction

The resistance of pests to pesticides and particularly toxic effects of pesticides on the environment and non-target organisms has proved the need for the use of other strategies to control pests including biocontrol agents [1]. Compatibility between biocontrol agent and other strategies to control pests and the advantages of common controlling methods such as chemical control has highlighted the importance and considerable role of biological methods in the integrated pest management (IPM) [2].

The common green lacewing (*Chrysoperla carnea*) is a generalist predator in most agricultural systems. This species is considered as suitable biocontrol agent due to its wide geographical distribution, compatibility with various agricultural ecosystems, high ability to foraging prey, resistance against pesticides, high predation rate and easy mass rearing under laboratory conditions [3]. Finding effective natural enemies to use in biocontrol programs needs to recognize their desirable characteristics and these characteristics are specified through laboratory, greenhouse and field examinations [4]. One of the important characteristics of natural enemies in biological programs is their reproductive attributes [5]. One of the important factors for the improvement of natural enemies is adults' diet. Therefore, many artificial diets have been evaluated for the rearing of adult lacewings not only due to their cost effectiveness and easy preparation but also their positive effects on the biological parameters of lacewings against their natural diets [6]. The effects of different diet on biological parameters of this predator have been studied by different researchers. Female adults of green lacewings require to free amino acids to achieve the maximum fecundity [7]. Predators usually obtain their food requirements by the environment from the pollen and extracts of flowers [8, 9]. Finney [10] reported that when common green lacewing fed only on honey, eggs laid per female and oviposition period was reduced. Therefore, predators were fed with Citrus mealybug honeydew. Other researchers used semi-artificial and artificial diet to feed common green lacewing to increase the fecundity of female adults and reduce the costs of mass rearing of this predator [11, 12]. Rousset [13] reported improved fecundity by adding enzyme hydrolyzed protein of yeast to the diet of female adults. McEwen and Kidd [11] studied the effect of various components of the conventional artificial diet (sugar, yeast, and distilled water) on fecundity and longevity of adults. According to their results, the longevity of the adults fed only sugar solution was more significant to other food treatments. The presence of yeast in adults' diet is necessary for egg production, but when only the yeast was used in their food treatment, adults produced a few eggs. The use of diet with yeast and sugar is recommended to produce more eggs. Milevoj [14] reared *C. carnea* adults by an artificial diet containing milk, egg, fruit extract and baker's yeast and reported that the mentioned regime had favorable effects on the fecundity of female adults. Ribeiro and Freitas [5] achieved success in the mass rearing of *Chrysoperla externa* (Hagen) by food regime containing honey, yeast, and pollen. Adane and Gautam [15] examined the effect of a different mixture of honey, pollen, and yeast on the fecundity of common green lacewing and showed that the diet improved the oviposition period in females. Ulhaq *et al*. [16] examined the effect of several diets on the biological characteristics of green lacewing *C. carnea* and showed that regime containing egg yolks, milk and honey had the highest effect on increasing longevity and reproductive attributes and decreasing larval and pupal period compared with other regimes. Usman *et al*. [17] examined six diets containing the different mixture of honey, milk, sugar, egg yolk, egg whites and casein on fecundity and longevity of *C. carnea*. Finally, the diet containing egg yolk, milk, and 25% honey solution showed significantly better results than the others. Faez *et al*. [18] examined the effect of different diets on longevity and fecundity of *C. carnea* adults and concluded that food containing yeast and sucrose had a greater effect on fecundity and longevity compared with flower nectar and water. Nawaz *et al*. [19] used vitamin E as a supplement in the diet of adult green lacewing *C. carnea* and increased their longevity and fecundity. They reported that food supplements increase oviposition period. Suitable food regimes at a lower cost can be useful for utilization of this biocontrol agent to control different pests and finding of the present study will improve mass rearing of *C. carnea* in IPM programs. Therefore the present study was conducted to evaluate the effect of different artificial diets on some biological traits of adult green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory conditions.

2. Material and Methods

2.1 *Sitotroga cerealella* culture

Barley grains were disinfected by Phostoxin gassing tablets (contain aluminum phosphide, which reacts with normal atmospheric moisture to liberate phosphine (hydrogen phosphide) gas) and then was poured into the containers (size = 10 × 20 × 30 cm) and was sterilized with boiling water for 3 minutes and then air drying until their waste are completely eliminated. In order to eliminate the stored grain mites and other potential store pests, the grains of barley were kept 24 hours in the oven at the temperature of 65-67° C. Freshly laid eggs of *S. cerealella* (1-1.5 gr) were added to the per kilogram of barley. After mixing and adding the eggs of grain moth to the rearing containers, barely containers were kept in a growth chamber of Plant Protection Department of Maragheh University (25 ± 1 ° C, 65± 5% RH and 16:8 h (L: D)). The emerging young larvae of *S. cerealella* were fed on grains and adults were collected after 30 days by aspirator in containers with a fine polyester mesh (mesh size = 50). The freshly collected eggs were used for the experiments and the eggs were frozen for 24 hours at -4 ° C in a freezer and have been used as prey for common green lacewing larvae.

2.2 Rearing of common green lacewing

Hundred common green lacewing eggs have been collected from the farms of Maragheh University and transferred to the insectarium of Plant Protection Department of Maragheh University. In order to homogenize the population of this predator physiologically, collected predators were reared under laboratory condition for four consecutive generations. To enhance the genetic base of the stock culture, the main culture of laboratory population was mixed with the feral population. To avoid cannibalism, rearing of predator larvae was done separately inside the Makkarti bottles (size = volume 35 cc) and adults insects were kept in a Polyvinyl chloride (PVC) plastic cylinder type rearing containers (20*16 cm) which its inner surface was covered with blue cardboard and it’s both ends were covered with fine polyester mesh (mesh size=50). Adults in the pre-oviposition, oviposition and post-oviposition periods were fed using an artificial diet consisting yeast + honey + distilled water (2:1:1). The mentioned diet was put on the blue radiography tapes (5×12 cm) to feeding adult insects. Distilled water was provided using pies of saturated sponges in the upper surface of rearing containers. Rearing containers of green lacewing were placed inside of a growth chamber (25 ± 1^0C, 65± 5% RH and 16:8 (L:D)) and were monitored daily [18, 11].

2.3 Treatments

Regimes used in this study were as follows:

Standard diet (control): Mixture of 2: 1: 1 yeast, honey, sucrose (YHS)

1) Mixture of 1: 1: 1: 1 solution of vitamins, amino acids, yeast, honey (VAYH)
2) Mixture of 1: 1: 1 extracts of *A. kuehniella* (Zeller) eggs, honey, yeast (AK + HY)
3) Mixture of 1: 1: 1: 1 egg whites (albumen), milk, honey, yeast (WMHY)
4) Mixture of 1: 1: 1 egg whites, milk, honey (WMH)
5) Mixture of 1: 1: 1 egg yolk, sucrose, extract of *S. cerealella* (YS + SC)
6) Mixture of 1: 1: 1: 1 egg yolk, soluble vitamins, yeast, honey (YVYH)
7) Mixture of 1: 1: 1 egg yolk, milk, honey (YMH)

For extraction of eggs (grain moth and Mediterranean flour moth), 0.01 gm of the mentioned eggs have been used per 1 ml of distilled water. The eggs were homogenized by utilizing a homogenizer (11,000 rpm) for 3 minutes. The resulting mixtures were kept in a fixed position for one hour. Obtained supernatant solutions were isolated using a sampler and were used as egg extract in experiments.

2.4 Data Analysis

After rearing four successive generations in laboratory conditions, adults of the fifth generation were reared according to Gharekhani *et al.* [20] in plastic cups (5×10 cm) covered with 50 mesh on mentioned treatments (seven diets). Feeding effect of this treatment on some biological parameters of adult insects such as female longevity, male longevity, pre-oviposition period, oviposition period, post-oviposition period, fecundity, fertility, sex ratio and percentage of egg hatchability were evaluated. All experiments were conducted in 15 replicates in a growth chamber (25 ± 1^0C, 65± 5% RH and photoperiod 16:8 h (L: D)). Means were compared using LSD test ($P < 0.01$) using the mean procedure in SAS software [21].

3. Results and Discussion
3.1 Green lacewing longevity
The means of adult longevity (Mean±SE) are presented in Table 1. The results of the data analysis showed that effect of food regimes on the longevity of males and females showed the significant difference for various parameters recorded (P <0.01). The average of female longevity in YHS, VAYH, AK + HY, WMHY, WMH, YS + SC, YVYH, and YMH were 40.86, 36.80, 36.20, 40.13, 38.60, 39.40, 43.53 and 40.93 (days) (F=11/81, df=7, *P*<0/0001), and the means of male longevity were 30.46, 27.13, 29.26, 29.53, 28.86, 28.46, 34.86, and 30.33 (days) respectively (F=18/76, df=7, *P*<0/0001). The mean comparison showed that males and females fed by the YVYH diet had significantly more longevity (Fig 1 and 2). The results of the present research on the effect of egg yolk on the longevity of the adult green lacewing have partial agreements with those of Ulhaq *et al*. [16].

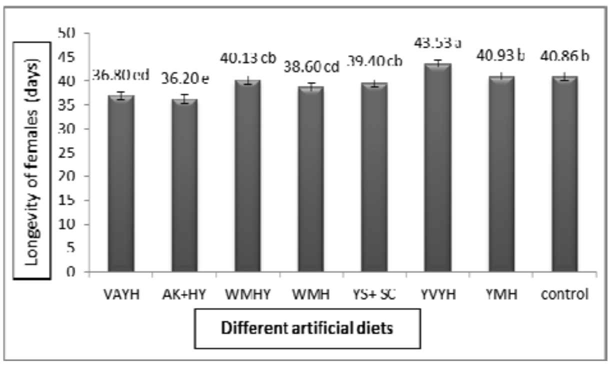

Fig 1: Comparison of average longevity of *C. carnea* female due to feeding on different diets.

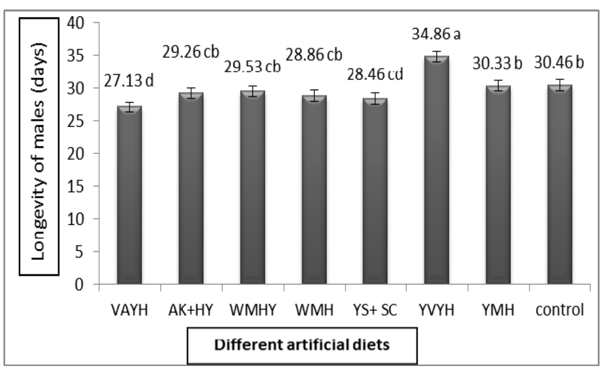

Fig 2: Comparison of average longevity of *C. carnea* male due to feeding on different diets.

3.2 Fecundity
The average rate of eggs laid per female was observed in YHS, VAYH, AK + HY, WMHY, WMH, YS + SC, YVYH, and YMH were 427.73, 112.60, 190.27, 474.00, 226.20, 256.40, 618.07, and 525.27 (eggs) respectively (F=39/86, df=7, *P*<0/0001). The average numbers of eggs laid per female (mean fecundity) in the treatments were significantly different from each other (Fig. 3). The maximum average of fecundity rate was observed in the adult females fed on YVYH diet. The results of present study on the effect of yeast and honey on fecundity of male and female adults were consistent with results of Ulhaq *et al*. [16], Tesfaye and Gautam [22] and Nawaz *et al*. [19].

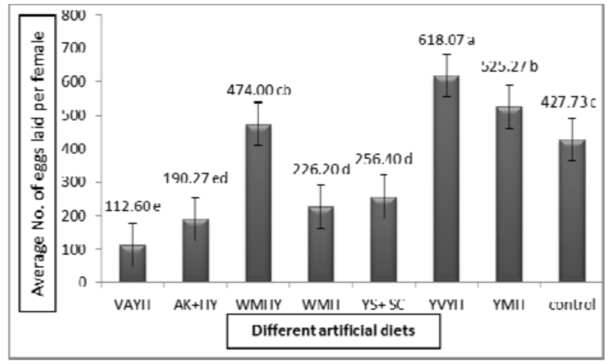

Fig 3: Comparison of average fecundity of *C. carnea* due to feeding on different diets.

3.3 Fertility
The average number of fertility per female was obtained in YHS, YAYH, AK+HY, WMHY, WMH, YS+SC, YVYH, and YMH were 340.40, 65.33, 132.20, 342.27, 131.20, 172.93, 517.93, and 400.53 (eggs) respectively (F=57/15, df=7, *P*<0/0001). The average rate of fertility per female in mentioned treatments had the significant difference from each other (Fig. 4). The highest rate of fertility was observed in the female adults fed by YVYH diet. The results of this study on the effect of yeast on fertility of female adults were similar to the results of Nawaz *et al*. [19] and McEven and Kidd [11].

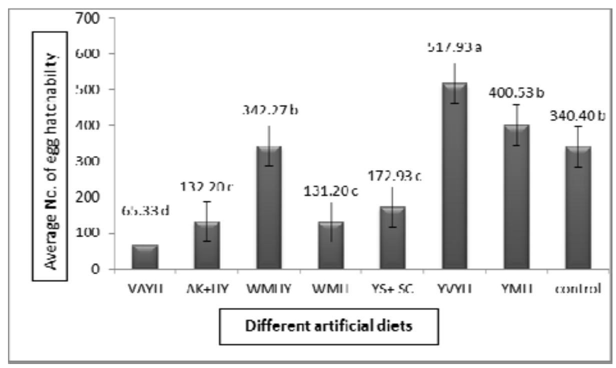

Fig 4: Comparison of average fertility of *C. carnea* due to feeding on different diets.

3.4 Percentage of egg hatchability
The effect of food diets on egg hatchability of female adults showed significant differences on food treatments (Fig. 5) (F=136/09, df=7, P<0/0001). The highest percentage of egg hatchability was observed in YVYH food treatment (86.33%) that was significant to other treatments (comparison of 99% CI).

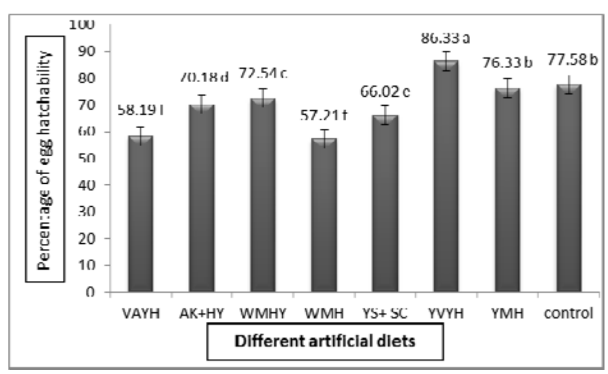

Fig 5: Comparison of average percent hatchability of eggs laid by *C. carnea* due to feeding on different diets.

3.5 Pre-oviposition period, oviposition period and post-oviposition period
The results indicated that the effect of diets on pre-oviposition, oviposition and post-oviposition periods were significantly different at the probability level of 1% (P <0.01) (Fig. 6). The average of pre-oviposition period in YHS, YAYH, AK+HY, WMHY, WHM, YS+SC, YVYH, and YMH were 8.53, 13.20, 10.73, 10.33, 13.26, 9.93, 6.80, and 10.06 (days) respectively (F=23/00, df=7, *P*<0/0001). The minimum pre-oviposition period (6.80 days) was recorded for females fed on YVYH diet that was significant to other treatments (p<0.01). The highest average of oviposition period in the female adults fed on YVYH diet was (26.26 days) that was significantly different to other treatments at the probability level of 1% (Fig. 7). The average of oviposition period in treatment such as YHS, VAYH, AK+HY, WMHY, WHM, YC+SC, YVYH, and YMH were 24.46, 19.66, 19.80, 21.93, 21.53, 21.06, 26.26, and 22.20 (days) respectively (F=31/14, df=7, *P*<0/0001) (Table 1). The average of post-oviposition period in treatments such as YHS, YAYH, AK+HY, WMHY, WMH, YS+SC, YVYH, and YMH were 7.86, 3.93, 5.66, 7.86, 3.80, 8.40, 10.46, and 8.66 (days) respectively (F=23/49, df=7, *P*<0/0001). The female adults fed on YVYH diet showed highest post-oviposition period (10.46 days) that was significantly different from other treatments at the probability level of 1% (comparison of 99% CI) (Fig. 8). The results of this study on the effect of vitamin solution, egg yolk, and yeast on pre-oviposition period, oviposition period and post-oviposition period were similar to the results of Nawaz *et al.* [19], Usman *et al.* [17] and Ulhaq *et al.* [16].

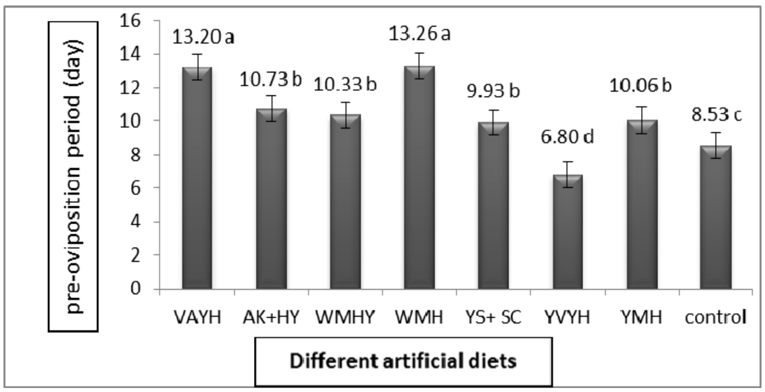

Fig 6: Comparison of average pre-oviposition period of *C. carnea* due to feeding on different diets.

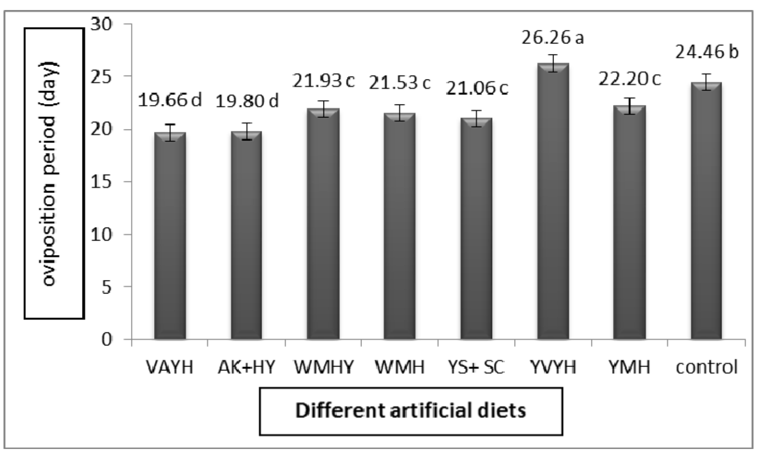

Fig 7: Comparison of average oviposition period of *C. carnea* due to feeding on different diets.

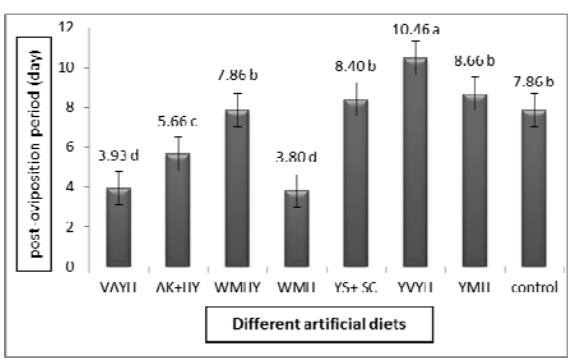

Fig 8: Comparison of average post-oviposition period of *C. carnea* due to feeding on different diets.

3.6 Sex ratio
When adults emerged, male and female predators were segregated in the fifth generation and the sex ratio has been specified in the mentioned food treatments (Table 1). Female and male adults can be specified through the size of body and shape of the abdomen. Females have a body with bigger size than males and their abdomen is large and fusiform, but males have small and cylindrical abdomen.

Table 1: Effect of different artificial diets on some biological parameters (Mean±SE) of *C. carnea* under laboratory conditions.

Biological parameters	Standard diet YHS	VAYH	AK+HY	WMHY	WMH	YS+ SC	YVYH	YMH
Fecundity (eggs)	[c] 6.09±427.73	[e] 8.34±112.60	[ed] 23.85±190.27	[cb] 35.62±474.00	[d] 17.69±226.20	[d] 51.92±256.40	[a] 36.68±618.07	[b] 16.66±525.27
Fertility (eggs)	[b] 4.39±340.40	[d] 4.70±65.33	[c] 15.87±132.20	[b] 25.98±342.27	[c] 10.15±131.20	[c] 35.42±172.93	[a] 31.92±517.93	[b] 12.79±400.53
Egg Hatchability (%)	[b] 0.30±77.58	[f] 0.50±58.19	[d] 0.46±70.18	[c] 0.39±72.54	[f] 0.43±57.21	[e] 0.27±66.02	[a] 0.19±86.33	[b] 0.20±76.33
Pre-oviposition (day)	[c] 0.19±8.53	[a] 1.32±13.20	[b] 0.70±10.73	[b] 0.43±10.33	[a] 0.34±13.26	[b] 0.61±9.93	[d] 0.47±6.80	[b] 0.30±10,06
Oviposition period (day)	[b] 0.23±24.46	[d] 1.36±19.66	[d] 0.32±19.80	[c] 0.31±21.93	[c] 0.43±21.53	[c] 0.68±21.06	[a] 0.49±26.26	[b] 0.53±22.20
Post-oviposition (day)	[b] 0.21±7.86	[d] 0.46±3.93	[c] 0.41±5.66	[b] 0.64±7.86	[d] 0.27±3.80	[b] 0.64±8.40	[a] 0.49±10.46	[b] 0.58±8.66
Female longevity (day)	[b] 0.49±40.86	[ed] 1.43±36.80	[e] 0.77±36.20	[cb] 071±40.13	[cd] 0.65±38,60	[cb] 0.65±39,40	[a] 0.67±43,53	[b] 0.83±40,93
Male longevity (day)	[b] 0.29±30.46	[d] 0.44±27.13	[cb] 0.48±29.26	[cb] 0.57±29.53	[cb] 0.60±28,86	[cd] 0.46±28,46	[a] 0.73±34,86	[b] 0.49±30,33
Sex ratio (Female: Male)	1.1 : 1	1.04 : 1	1.15 : 1	0.90 : 1	0.82 : 1	1.05 : 1	1.09 : 1	1.15 : 1

Based on LSD Test, means followed by the same letter in each row are not significantly different from each other at 1% (comparison of 99% CI).

Rearing conditions such as temperature, relative humidity, photoperiod and quality of diets are important factors that affect the developmental time, longevity and oviposition period of adults [7, 10, 14, 16, 17, 19]. Artificial diets high in protein (amino acid), lipids, carbohydrates, mineral, and vitamins affect oviposition period of adult common green lacewing and increase their longevity and fecundity. Rousset [13], Principi and Canard [8] and Ulhaq *et al*. [16] who reported that egg yolk in the food treatment increased the quality of diet. They explained that the egg yolk contain cholesterol, protein, and carbohydrates and had desirable effects on the biological parameters of *C. carnea*. McEwen and Kidd [11] studied the effect of different artificial diets on fecundity and longevity of adult common green lacewing; they revealed that adults only fed by sugar have the highest longevity compared to the insects fed by yeast and sugar. However, yeast is important for egg production, but results showed that the eggs laid per female decreased if only used yeast alone in the artificial diets. Tesfaye and Gautam [22] examined the effect of different diets such as honey, castor pollen, and yeast on longevity and fecundity of adults *C. carnea*. These researchers showed that food diet affects the oviposition period and post-oviposition period and fecundity of this predator, the pre-oviposition period and longevity of adults were not affected. Adult common green lacewing in the natural environment requires nectar and pollen; therefore, by providing their requirements in the agricultural ecosystem, the population of this predator can be established and increased in the environment [23]. For example, Cardoso and Lazzari [24] reported that for increasing the population of *C. carnea* in the agricultural ecosystems, artificial diets can be used in their habitats. They showed that by spraying artificial diets in agricultural ecosystems, adults of *C. carnea* can mate and lay eggs and control the pests better.

4. Conclusion

According to present results, using mixture 1:1:1:1 of an artificial diet containing egg yolk, vitamin solution, yeast, and honey can increase longevity and reproductive attributes of *C. carnea* and this is probably due to the higher amounts of protein (amino acids), lipids, carbohydrates, minerals, vitamins and cholesterol in this diet. It is expected that mentioned diet has desirable effects on biological parameters and efficiency of this predator for pest control.

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