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RESEARCH

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Dietary assessment across various life stages of seven-spotted lady beetle *Coccinella septempunctata* (Coleoptera: Coccinellidae)

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Abstract

Background *Coccinella septempunctata* (L.) is an important predator in fighting against aphids on edible crops and vegetables. However, to establish a successful mass-rearing technique, it is crucial to determine the optimal diets for larvae, pupae, and adults in terms of fecundity, longevity, and ingredient ratios. The current study aims to learn more about the biology of ladybird beetle using both artificial and natural food components.

Results This study tested six different ratios of natural and artificial diets, ranging from a natural aphid diet to a variety of artificial diets containing protein hydrolyzate, casein protein, honey, dry aphids, yeast, and agar. The results showed that the natural diet (D1) produced from aphids showed approximately 290.2 eggs, while artificial diets D2 and D4 produced 56.5 and 40.2 eggs, respectively. The effect of different diets on the longevity of different life stages was also tested. The minimum longevity of egg, larval, pupal, and adult stages were recorded for D1 and D4. Furthermore, the maximum percentage emergence of larval, pupal, and adult stages was observed for D1 and D2. D6 produced the longest adult duration, with a maximum of 84.60 days. However, there was no significant difference in sex ratio among the various diet components.

Conclusions In conclusion, the results suggest that artificial diets are essential for sustaining *C. septempunctata* cultures in laboratories, especially during periods when natural prey is scarce. The most effective and efficient mass-rearing approach would be to provide both natural and artificial foods simultaneously. These findings may have significant implications for the development of biological control strategies for aphids in agriculture.

Keywords Artificial diet, Mass rearing, Natural diet, Sustainability, Seven-spotted ladybird beetle

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Background

Biological control of seven-spotted ladybird beetle (*Coccinella septempunctata* L.) is an approach adopted, in which beetles are capitalized that already exist by minimizing insecticidal spray and rearing artificially and producing the augmentative releases. Seven-spotted ladybird beetle conservation through augmentative releases rests mostly on maximizing their biological control (Youssif et al., 2021; Yu et al., 2022). Seven-spotted ladybird beetle is a predator that feeds on aphids, thrips, whiteflies, mites, spiders, soft-bodied mealybugs, pollen, honeydews, eggs, and larvae of Lepidoptera and Coleoptera, is a suitable biological control agent (Ragkou et al., 2004; Deligeorgidis et al., 2005; Farooq et al., 2020). Before pupating, metamorphosing, and becoming adults, its larvae go through four instars. It reproduces frequently yearly, with adults hibernating in the colder winter months. The primary harmful pest in the wheat crop-our primary source of food-is aphids. This is a well-known beneficial insect pest of many vegetables in Pakistan (Saleem et al., 2014).

Seven-spotted ladybird beetles are significant biocontrol agents because pesticides are not accepted for the pest control of wheat. *Coccinllids* react to olfactory signals from their aphid prey and are even able to differentiate between different species of aphids on volatile substances. Rearing coccinellids on a natural diet is challenging since prey is not always present. The most significant aspect of the retention of seven-spotted ladybird beetles in an agroecosystem is the presence of alternative prey. The lack of aphid prey for coccinellids may inhibit the development or cause them to migrate far away to a crop that is not their objective (Bianchi et al., 2004; Yu et al., 2022). Due to the unregulated application of insecticides, its native populations in the fields are reduced. Because aphids are never provided in sufficient quantities, raising prey species is challenging and unprofitable. The challenge can be overcome by raising seven-spotted ladybird beetles on artificial food ingredients (Agus, 2007). In the process of facilitating a consistent and stable supply of coccinellids for massive success in the fields, artificial meals are crucial for mass rearing. According to Cohen (2005), artificial diets typically contain protein, free amino acids, lipids, carbohydrates, vitamins, and minerals that affect the longevity, fertility, and percentage of the emergence of the animals. The development, growth, and reproduction of insects, including seven-spotted ladybird beetles are directly influenced by the quality of the food they consume (Dwivedi & Tomer, 2021). The goal of the current study is to learn more about the biology of seven-spotted ladybird beetle using both artificial and natural food components. This knowledge will be useful when seven-spotted ladybird beetles are mass-reared in laboratory settings and added to the IPM control module.

Methods

Methodology

Experimental site and culture maintenance

Six treatments and three repeats made up the CRD (Complete Randomized Design) experiment, which was carried out at the Entomological Research Institute of the Ayyub Agricultural Research Institute in Faisalabad, Agricultural Entomology and Pest Control Laboratory, and Conservation Tillage Pest and Disease Monitoring Institute (GPS: E125.398 N43.815, No.JLP-BCC-21-31-CC-01) of the Jilin Agricultural University. To collect eggs and larvae, newly emerging adults of *C. septempunctata* were taken from Brassicae and wheat fields and released in pairs inside plastic Petri dishes (6 cm × 2 cm), one pair inside each petri dish, at 25 °C, 75% R.H., and 10 D: 14 L hours.

Treatment preparations

Seven-spotted ladybird beetle adults were fed various artificial diet component types to help identify the best nutrient combinations for artificial rearing. Petri plates were lined with filter paper to allow adults to lay eggs for 3–4 days. The filter paper was cut into pieces to remove eggs for larval emergence. The camel hair brush was used to collect the eggs on the container's surface and the Petri dishes. Daily observations of the hatching were recorded. Larvae began to hatch from the eggs after about 4–5 days. To prevent cannibalism, each developing larva was swiftly removed with a fine brush. Several artificial food components were administered to the larvae using a cotton swab. Data on various life stages were collected and analyzed.

Statistical analysis

For each of the diets listed, the fecundity (number of eggs), egg incubation time (days), sex ratio (number of male and female), and larval, pupal, and adult periods (days) were calculated. The statistical analysis was performed using Statistics 8.1 and Mstat-C software at a significance level of 0.05. The analysis was made using ANOVA to compare the means of different treatments. After the ANOVA, the LSD (Least Significant Difference) test was employed to identify significant differences between individual treatment means. Additionally, error means were calculated to assess the variability within each treatment group. To visually represent the data, graphical figures were created using Microsoft Office Excel 2016.

Treatments

(a) Natural Diet

D_1 = Natural Diet (*Rhopalosiphum padi/SchizAphis graminum*) 120 aphid/24 h for each pair

(b) Artificial Diet

D_2 = Protein Hydrolyzate (4 g) + Casein Protein (3.5 g) + Honey (8 g) + Dry aphid (4 g) + Water (100 ml)

D_3 = Yeast (3 g) + Agar (2.5 g) + Honey (5.5 g) + Water (100 ml)

D_4 = Protein Hydrolyzate (2.5 g) + Honey (4.5 g) + Dry aphid (3 g) + Water (100 ml)
D_5 = Casein Protein (1.5 g) + Honey (3 g) + Dry aphid (2 g) + Water (100 ml)
D_6 = Honey (14 g) + Water (100 ml)

As a natural diet, aphids were harvested straight from the field. An artificial diet's components were all bought in the market. A mixing kettle filled with boiling water was used to thoroughly combine all the materials for the preparation of fake diets, which were subsequently cooled at 30–35 °C.

Results

Natural diets (D1) of aphids produced approximately 290.2 eggs while whereas D-2, D-4, D-5, and D-3 produced the least-56.5, 45.6, 30.8, 19.4, and 0.0 eggs, respectively (Fig. 1). Because D-6 lacks the protein components required for fecundity, no eggs were formed. Because aphids are a natural meal for beetles and meet their nutritional needs, reproduction was highest when eating them. The beetles continued to receive nutrition from artificial meals. The sex ratio in various diets did not differ significantly from one another. The sex ratios in diets D-1, D-2, D-4, D-3, and D-5 were 70.2:62.4, 62.5:55.4, 55.6:48.5, 57.7:52.6, and 47.9:42.5, respectively (Fig. 2). In every artificial diet component, there was a higher proportion of females than males. The computation of weight for the two sexes demonstrates that the weight of women was consistently higher on all types of diets (Fig. 3). On a natural diet of 14.66 and 11.05 mg, respectively, the maximum weights of males and females were discovered. Among the artificial diets, D-2 had the highest female and male weights, with 8.97 and 7.01 mg, correspondingly. Recorded hatching times were 4.34 days for D-1, 3.87 days for D-2, 5.45 days for D-3, 4.68 days for D-4, and 6.37 days for D-5. In D-1, D-2, D-3, D-4, and D-5, the larval periods were 7.30, 10.6, 13.0, 15.6, and 17.0 days, respectively (Table 1). In D-1, D-2, D-3, D-4, and D-5, the pupal stage averaged 7.76, 9.03, 12.5, 15.7, and 16.8 days, respectively. The adult duration was also determined to be 12.40, 15.7, 17.8, 21.4, 26.7, and 84.60 days in D-1, D-2, D-3, D-4, D-5, and D-6, respectively, for various diets (Table 1). The percentage of each stage that emerged from the egg was 90.3, 74.4, 66.4, 64.9, and 55.0% in D-1, D-2, D-4, D-3, and D-5, respectively. While in D-1, D-2, D-4, D-3, and D-5, respectively, adult emergence from the pupal stage was 85.1, 70.9, 68.4, 61.8, and 64.7% (Table 2). The study found that a natural diet was superior to artificial diets because it enhanced the fecundity rate of the biocontrol agent, producing more eggs (290.2), had a shorter life cycle (31.80 days), and allowed for the maximal emergence of all life stages (85.3–93.4%). Although a natural diet is the most

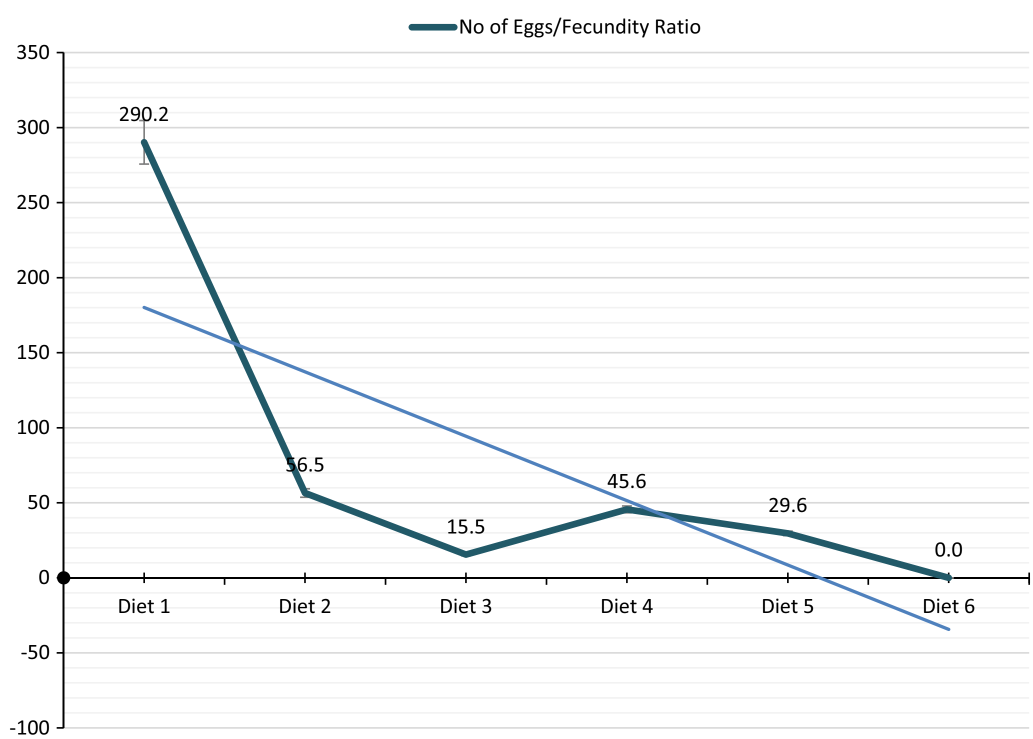

Fig. 1 Number of eggs during the life cycle

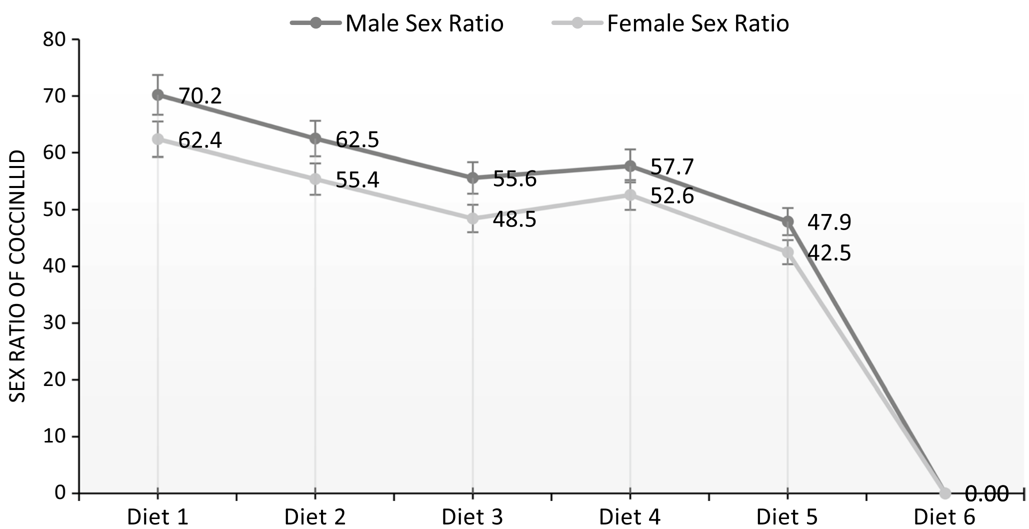

Fig. 2 The sex ratio of females and males in diets

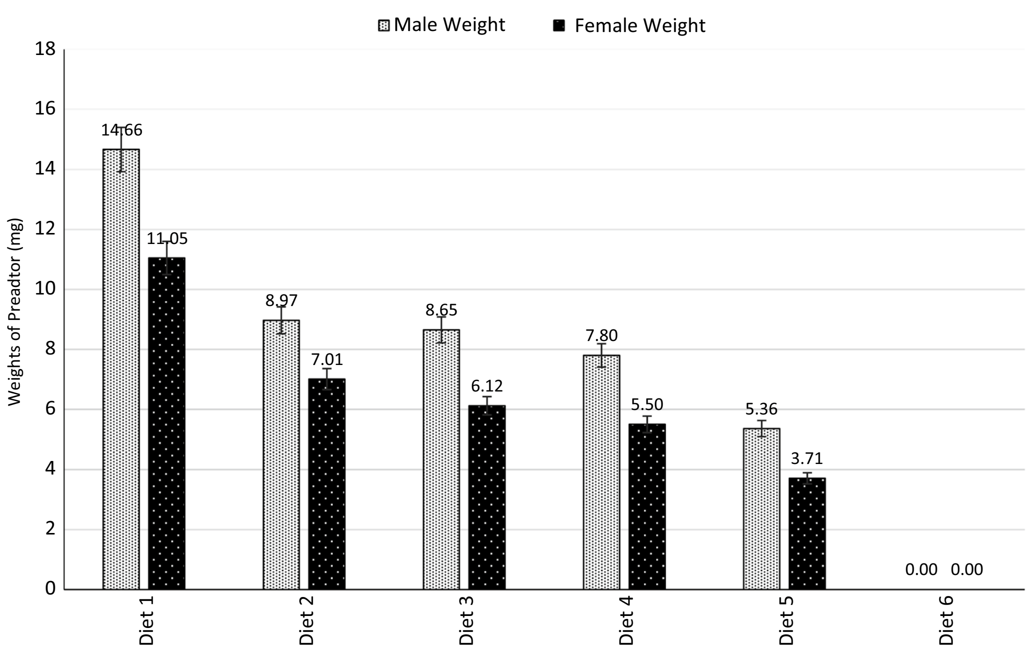

Fig. 3 The sex ratio of females and males in diets

Table 1 Duration and percent emergence of all the stages of ladybird beetle

Life stages (days)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	CV (%)	*P* value
Egg	4.34 ± 0.22	3.87 ± 0.32	5.45 ± 0.55	4.68 ± 0.4	6.37 ± 0.57	–	8.02	0.003
Larvae	7.30 ± 0.43	10.6 ± 0.57	13.0 ± 0.67	15.6 ± 0.48	17.0 ± 0.74	–	18.01	0.01
Pupae	7.76 ± 0.53	9.03 ± 0.34	12.5 ± 0.67	15.7 ± 0.72	16.8 ± 0.80	–	18.78	0.01
Adult	12.40 ± 0.64	15.7 ± 0.46	17.8 ± 0.47	21.4 ± 0.96	26.7 ± 0.75	84.60 ± 0.53	23.1	0.05
Total life span	31.80	39.23	48.76	57.36	66.84	84.60		

Table 2 The emergence of different life stages of seven-spotted ladybird beetle (%)

Life stages (days)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet6	CV (%)	*P* value
Egg	–	–	–	–	–	–	–	–
Larvae	90.3 ± 0.43	74.4 ± 0.57	64.9 ± 0.67	66.4 ± 0.48	55.0 ± 0.74	–	18.01	0.01
Pupae	93.4 ± 0.53	80.3 ± 0.34	65.6 ± 0.67	72.7 ± 0.72	60.8 ± 0.80	–	18.78	0.01
Adult	85.1 ± 0.64	70.9 ± 0.46	61.8 ± 0.47	68.4 ± 0.96	64.7 ± 0.75	–	23.1	0.05

effective, it is not always available; hence during times of scarcity, we must highlight the nutrients in manufactured diets. The artificial diets D-2 and D-3 perform the best since they generated 22.3 and 26.9 eggs, respectively, with shorter life cycles of 39.23 and 48.76 days. The adult ladybird beetle's lifespan was significantly increased by D-6.

Discussion

Natural diets fostered egg laying of ladybird beetles while artificial diets resulted decrease in egg laying. The development of the seven-spotted ladybird beetle is influenced by the prey they are eating (Ebrahimifar et al., 2020; Omkar et al. 2006; Evans, 2004). Ladybird beetles were fed a variety of prey and meals by Rasekh and Riddick (2021) and Evans (2004). On other diets, females deposited very few eggs which were necessary to keep the ladybird beetle population stable. These results are quite comparable to those of Sarwar and Saqib (2010) and Yu et al. (2022), who reported that C. septempunctata produced 255 (natural diet), 11.0, and 18.0 eggs (artificial diets). The length of the larval and pupal stages, respectively, is 13.0, 4.0, 0.00, and 17.0, 6.20 days (for natural diets), artificial diets, and incubation periods of 3.4, 4.6, and 4.6 days. Agus et al. (2013) agreed with our findings, which showed that on an artificial diet, females produced 323 eggs on average. Larval stage lengths on several prey species were discovered to be 8.2 days when fed on *D. noxia* and *R. padi* (Michaud, 2005), 10.82 days on *C. atlantica* (Navas & Parra, 2005), and 6.3–7.2, 8.1–8.4, 9.7–8.5, and 9.7–8.5 on *D. noxia*, *A. pisum*, and *M. persicae*, respectively (Michaud, 2000). Larval developmental durations of 14.2 and 9.91 days were observed by Omkar et al. (2013) and Youssif et al. (2021), respectively. Depending on the variety of prey, beetle life cycles can last anywhere between 19 and 24 days. On a natural diet, the minimum life expectancy on D-1 was 30.61 days. Longevity results from other artificial diets ranged from 44.61 to 81–87 days. Unlike Sarwar and Saqib (2010), who claimed that a natural diet could extend longevity by at least 20 days. This discrepancy results from the seven-spotted ladybird beetle consumption of various prey species. The longest longevity was shown by the honey and water solution (D-6), which lasted 81.87 days. The sugar solution administered to the female ladybird beetle resulted in the longest lifetime of 71.3 days compared to other treatments (Agus et al., 2013; Youssif et al., 2021). Artificial diets prevent the ladybird beetle from completing any of its life stages and instead lengthen each stage of growth. According to Berkvens et al. (2008) and Ragkou et al. (2004), extra nutrients like amino acids and mineral salts are required to generalize the ladybird beetle feeding to finish the development using various prey items and pollen grains. Female weight was consistently greater than male weight in our trials. This characteristic is typical of Coccinellidae (Kato et al., 1999; Sighinolfi et al., 2008; Silva et al., 2009). The physiological mechanism controlled by nutrients stored in the female body may be the cause of this discrepancy (Farooq et al., 2020; Zanuncio et al., 2002). The study demonstrated that the implementation of a natural diet resulted in superior performance compared to artificial diets. Specifically, the natural diet significantly enhanced the fecundity rate of the biocontrol agent, providing compelling evidence for its efficacy as a means of promoting reproductive success. These findings underscore the importance of considering dietary factors when designing strategies for optimizing biocontrol agent performance in ecological management approaches.

Conclusions

According to the study, aphids are great natural prey because they have short life cycles and large populations. Only protein-based diets outperformed other artificial diets in terms of effectiveness, and they also had a shorter life span. Mass upbringing would be more effective and quicker if natural and artificial foods were administered simultaneously. The results suggested that artificial diets are essential for sustaining C. septempunctata cultures in laboratories, especially during periods when natural prey is scarce. The most effective and efficient mass-rearing approach would be to provide both natural and artificial foods simultaneously. These findings may have significant implications for the development of biological control strategies for aphids in agriculture. These findings underscore the importance of considering dietary factors when designing strategies for optimizing biocontrol agent performance in ecological management approaches.

Abbreviations

CRD Complete randomized design
LSD Least significant difference
IPM Integrated pest management

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Author contributions

SA (Writing-Original Draft, Writing-Review and Editing, Data Curation, Introduction, Results and References), MA (Data Analysis, Discussions), AA (Data collection), XF (Statistical Analysis and Review), AR (References), MS (Review), WQ (Review), XH (Review), CRZ (Supervision, Methodology, Funding, Resources and Writing-Review and Editing). "All authors have read and approved the manuscript".

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Availability of data and materials

All data analyzed in this study is provided in the article and data can be provided on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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