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# EVALUATION OF VARIOUS DIETS FOR MASS REARING OF LADYBIRD BEETLE (*Coccinella septempunctata*) AT PAKISTAN

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## ABSTRACT

A polyphagous predator, *Coccinella septempunctata* L. is a key to develop as biological control agent of aphids on edible crops and vegetables. Larvae, pupae and adults of *C. septempunctata* were fed on natural and artificial diets to determine fecundity, longevity and sex ratio on various ingredients to develop a successful mass rearing technique. Six different diets were used *viz.* D-1 (Natural Diet), D-2 (Protein Hydrolyzate 2 g + casein protein 2.5 g + honey 4 g + dry aphid 2 g + water 100 mL), D-3 (yeast 2 g + agar 1.8 g + honey 6 g + water 100 mL), D-4 (protein hydrolyzate 1 g + honey 4 g + dry aphid 2 g + water 100 mL), D-5 (casein protein 1.5 g + honey 4 g + dry aphid 2 g + water 100 mL), D-6 (honey 10 g + water 100 mL). Results showed that natural diet D-1 produced maximum number of eggs 243.5 while among the artificial diets D-2 and D-4 produced a maximum of 23.76 and 17.89 eggs, respectively. Effect of different diets was tested on longevity of different life stages. Minimum longevity of egg, larval, pupal and adult stages were recorded as D-1 (3.64 days) and D-4 (4.65 days), D-1 (7.32 days) and D-2 (10.87 days), D-1 (8.27 days) and D-2 (11.28 days), D-1 (11.38 days) and D-2 (17.25 days), respectively. Maximum percent emergence of larval, pupal and adult stages was recorded in D-1 (89.45 days) and D-2 (72.14 days), D-1 (91.32 days) and D-2 (83.58 days), D-1 (79.27 days) and D-4 (65.87 days), respectively. D-6 gave maximum adult duration of 81.87 days while there was no significant difference of sex ratio among various diets components.

**Key word**: Seven spotted ladybird beetle, natural diet, artificial diet, mass rearing, biological control.

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## INTRODUCTION

Predator, *Coccinella septempunctata* L. both larvae and adult is a potential bio control agent. It feeds on aphids, thrips, whiteflies, mites, spiders, soft body mealybugs, pollen, honeydews, eggs and larvae of Lepidoptera and Coleoptera (Simmonds *et al.*, 2000; Ragkou *et al.*, 2004; Deligeorgidis *et al.*, 2005). Its larvae undergo 4 instars before pupating, metamorphosing, and giving confinement to adults. It has numerous generations per year while adult hibernates during winter. Aphids are the major damaging pests in wheat crop which is our staple food. This is notorious pest of different vegetables in Punjab (Saleem *et al.*, 2014). Insecticides are not recommended for the pest control of wheat so ladybird beetle are important biological control agent. Coccinellids responds to olfactory cues from their prey and can even distinguish between aphid species on volatiles. The rearing of coccinellids on natural diet is difficult because prey is not available throughout the season. Availability of alternate prey is a most important factor for the preservation of predators in agro-ecosystem. The insufficiency of aphid prey for Coccinellids may prevent its reproduction or initiates its long distance migration to the non-target crop (Bianchi and van der Werf, 2004). Due to excessive uses of chemical in fields caused death of predators and availability or reproduction stopped. Rearing of natural prey is difficult and uneconomical due to continuous non provision of aphids. This difficulty can be solved by rearing the predator on artificial diets components (Talele, 2015). Artificial diets play important role for the mass rearing when natural diets are unavailable, so that continuous and reliable supply of coccinellids can be made for field releases. Artificial diets generally contains protein, free amino acids, lipids, carbohydrates, vitamins and minerals (Talele, 2015) which influence their longevity, fecundity and percent emergence. Food quality has direct impact on the growth, development and reproduction of insects including predators (Mishra and Singh, 2006). In perspective of the biological control the conservative biological control is used in which the beetles are exploited that are already present by avoiding insecticidal spray and second way to rear artificially and make the augmentative releases. The augmentative releases and conservation of predator mostly depends upon some factors to maximize their production in order to integrate as biological control (Rizvi *et al.*, 1994).

## OBJECTIVES

The present study was commenced for obtaining information on the biology of predator by making use of natural prey and artificial diet components. This information would be helpful in mass rearing of predator under laboratory conditions to incorporate into IPM control module during the periods when natural diets are unavailable.

## MATERIALS AND METHODS

The experiment was conducted at Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad under CRD (Complete Randomized Design) with six treatments and three replications. Collections of adult beetles

were also made from Arid Zone Research Institute Bhakkar and University of Agriculture Faisalabad. To obtain eggs and larvae, the newly emerged adults of *C. septumpunctata* were collected from Brassica and wheat fields and released in pairs inside the plastic Petri dishes (6 cm x 2 cm), one pair inside each petri dish at 25±2°C, 75±5 % R.H. and 10 D: 14 L hours. Female are always larger and heavier in weight than male. To determine suitable and appropriate nutrients combinations for the artificial rearing, adult predators were fed with different types of artificial diet components. Paper was placed inside the petri dishes in order to allow adults to lay eggs for 3-4 days. The papers containing the eggs were collected and cut with scissors. The eggs on the surface of container and petri dishes were collected with camel hair brush. The eggs collected from cards and containers were counted. Observations regarding the hatching were recorded on daily basis. After 4-5 days larvae started emerging from the eggs. Each emerging larvae was removed with camel brush instantly to avoid cannibalism. The larvae were fed with different artificial diet components with the help of cotton swab. The fecundity, incubation period of eggs, sex ratio, larval, pupaland adult periods were determined for each diet mentioned below. Means of all treatments were used compute one way ANOVA, LSD and error means by using Statistix 8.1 and Mstat-C @ 0.05. All the graphical representation was made by using Microsoft Office Excel-2010. The treatments were mentioned below

$D_1$ = Natural eiet (*Rhopalosiphum padi/Schizaphis graminum*) 100 aphid/24 hrs for each pair

$D_2$ = Protein hydrolyzate (2 g) + Casein protein (2.5 g) + Honey (4 g)+ Dry aphid (2 g) + Water (100 mL)

$D_3$ = Yeast (2 g) +agar (1.8 g) + honey (6 g) + water (100 mL)

$D_4$ = Protein hydrolyzate (1 g) + honey (4 g) + dry aphid (2 g) + water (100 mL)

$D_5$= Casein protein (1.5 g) + Honey (4 g) + Dry aphid (2 g) + water (100 mL)

$D_6$ = Honey (10 g) +water (100 mL)

Aphids were collected directly from field as natural diet. All the ingredients for artificial diet were purchased from the market. To prepare artificial diets, all the ingredients were mixed thoroughly in mixing kettle by using hot water and then cooled at 30-35 °C.

**RESULTS**

The results from the current study revealed that on D-1 (natural diet/ aphids) beetle gave maximum eggs 243.5/female while other diets produced fewer no of eggs as D-2, D-4, D-5 and D-3 with 23.76, 17.89, 14.12 and 2.54 eggs, respectively during its life cycle (figure 1). No eggs were produced in D-6 because there is no protein ingredients included which is necessary for the fecundity. On the natural diet aphids there was maximum fecundity because it meets beetles nutritional requirements. While artificial diets were still under nutritious for the beetles. There was no significant difference in the sex ratio in different diets after study. Sex ratio of females and males in diets D-1, D-2, D-3, D-4 and D-5 was 56.2 and43.8, 60.4 and 39.6, 55.5 and 44.5, 53.4 and 46.6, 58.4 and 41.6, respectively (figure 2). Female population was always higher than male in all the artificial diet components. Weight of male and female sexes was calculated (figure 3), which shows that weight of females was always high on all types of diets. Maximum weight of female and male was 12.36 mg and 9.15 mg, respectively on natural diet while. 10.52 mg and 7.26 mg on artificial diets, respectively. Duration and percent emergence of all the stages egg, larvae, pupae and adult was computed as shown in the Table 1.

Figure 1: Fecundity ratio of predator on different diets.

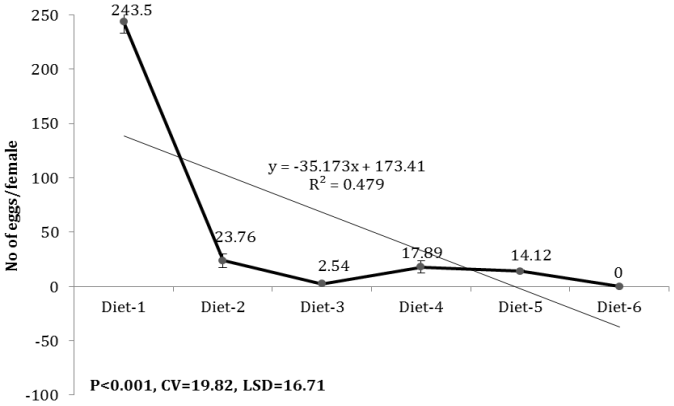



Figure 2: Sex ratio of predator on different diets.

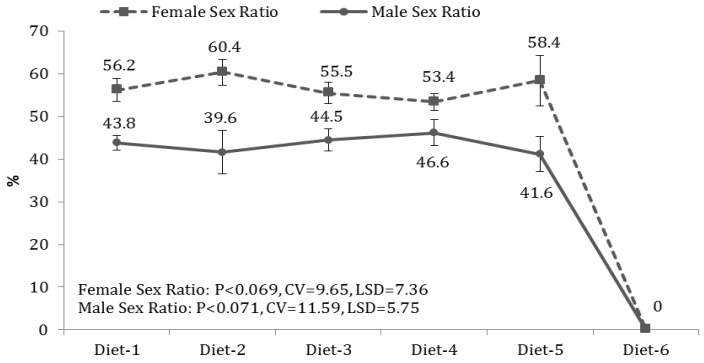
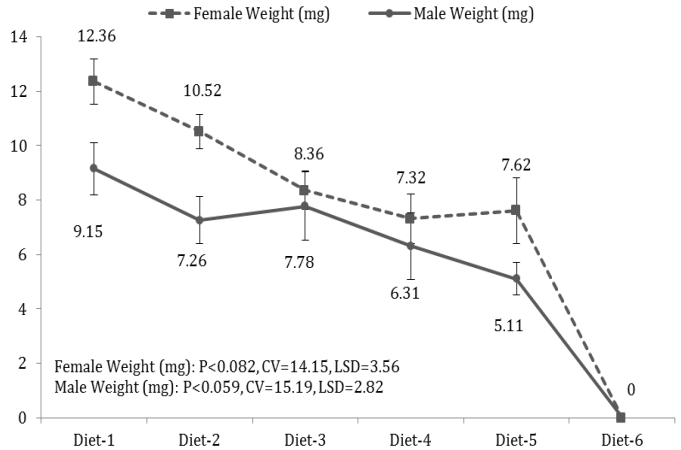



Figure 3: Weight of male: female on different diets (mg).



| Diets | LONGEVITY OF DIFFERENT LIFE STAGES (DAYS) | | | | Total life span |
|---|---|---|---|---|---|
| | Egg | Larvae | Pupae | Adult | |
| Diet-1 | 3.64±0.3742 | 7.32±0.5831 | 8.27±0.7781 | 11.38±0.5031 | 30.61 |
| Diet-2 | 5.31±0.4734 | 10.87±0.5099 | 11.28±0.7071 | 17.15±0.5099 | 44.61 |
| Diet-3 | 6.23±0.3742 | 15.25±0.4821 | 16.89±0.9148 | 37.14±1.2083 | 75.51 |
| Diet-4 | 4.65±0.2691 | 13.52±0.5131 | 13.81±0.5089 | 25.29±0.5831 | 57.27 |
| Diet-5 | 5.14±0.5742 | 14.81±0.6831 | 14.57±0.6782 | 28.19±0.9831 | 62.71 |
| Diet-6 | - | - | - | 81.87±0.8602 | 81.87 |
| CV (%) | 7.24 | 17.8 | 16.7 | 22.1 | |
| P. Value | P<0.0041 | P<0.0345 | P<0.0568 | P<0.0601 | |
| EMERGENCE OF DIFFERENT LIFE STAGES (%) | | | | | |
| Diet-1 | - | 89.45±1.9419 | 91.32±1.6432 | 79.27±2.4495 | - |
| Diet-2 | - | 72.14±3.1402 | 83.58±2.3191 | 65.54±1.4697 | - |
| Diet-3 | - | 68.25±2.5852 | 70.63±3.6981 | 60.21±2.1254 | - |
| Diet-4 | - | 69.68±1.5438 | 74.21±2.3784 | 65.87±4.2261 | - |
| Diet-5 | - | 53.27±1.7436 | 65.85±5.3784 | 61.54±3.2261 | - |
| Diet-6 | - | - | - | - | - |
| CV (%) | - | 14.2 | 19.2 | 10.5 | |
| P. Value | | P<0.0005 | P<0.0163 | P<0.0481 | |

Table 1: Longevity and emergence of different life stages of ladybird beetle.

Hatching period in D-1 (3.64 days), D-4 (4.65 days), D-5 (5.14 days), D-2 (5.31 days) and D-3 (6.23 days) was recorded. Larval periods were 7.32, 10.87, 13.52, 14.81, and 15.25 days in D-1, D-2, D-4, D-5 and D-3, respectively while duration of pupal stage was 8.27, 11.28, 13.61, 14.57 days and 16.89 in D-1, D-2, D-4, D-5 and D-3, respectively. Similarly the adult duration in different diets was found to be 11.38, 17.25, 25.29, 28.19, 37.14 and 81.87 days in D-1, D-2, D-4, D-5, D-3 and D-6, respectively. Percent emergence of each stage was calculated during the study as in table 1 which depicted that larval emergence from egg stage was 89.45, 72.14, 69.68, 68.25 and 53.27 % in D-1, D-2, D-4, D-3 and D-5, respectively. Pupa formation from larval stage was 91.32, 83.58, 74.21, 70.63 and 65.85 % in D-1, D-2, D-4, D-3 and D-5, respectively while adult emergence from pupal stage was 79.27, 65.87, 65.54, 61.54 and 60.21 % in D-1, D-4, D-2, D-5 and D-3, respectively. In the current study natural diet proved better as compared to the artificial diets as it increased the fecundity rate of bio control agent having more no of eggs (243.5), with short life cycle of 30.61 days and maximum emergence of all life stages up to 79.27-91.32 %. Although natural diet proved to best but it is not available throughout the year so during the scarcity periods of artificial diets we must emphasize on the artificial diet nutrients. Among the artificial diets D-2 and D-4 are much better as they produced 23.76 and 17.89 eggs having shorter life cycles of 44.61 and 57.27 days, respectively. D-2 and D-4 gave maximum percent emergence 72.14, 83.58, 65.54 and 69.68, 74.21, 65.87 in larval, pupal and adult stage, respectively. D-6 was very effective to prolong the life of the adult ladybird beetle.

Natural diet enhanced the egg laying while alternative diets produced fewer no of eggs. Fecundity, longevity and emergence always vary with varying prey species.

**DISCUSSION**

The predator development depends upon the prey which they consumed (Evans, 2000). Evans *et al.* (1999) fed coccinalids with variety of prey and foods. Female produced very few eggs on alternative foods. They only served to maintain the predator population. These findings are quite similar to the findings of Sarwar and Saqib (2010) who reported that *C. septempunctata* produced 255 (natural diet), 11.0 and 18.0 eggs (artificial diets). Incubation periods was 3.4 (natural diet), 4.6 and 4.6 days (artificial diets) with larval, pupal duration of 13.0, 4.0 (natural diet) 0.00, 0.00 and 17.0, 6.20 days (artificial diets), respectively. While Agus *et al.* (2013) disproved our results who reported 323 eggs per female on artificial diet. Larval stage durations on various prey species were found 8.1 days when fed on *D. noxia* and *R. padi* (Michaud, 2005), 10.82 days on *C. atlantica*, (Nava and Parra, 2005) 6.3-7.2, 8.1-8.4 and 9.7-8.5 on *D. noxia*, *A. Pisum*and and *M. Persicae*, respectively (Michaud, 2000). Srivastava (2003) reported 14.2 and 9.91 days larval development periods. The life cycle of beetle ranges from 19-24 days depending upon the prey species of aphids. Total minimum life span on D-1 was 30.61 days on natural diet. Rest of artificial diets showed longevity of predator increased from 44.61-81-87 days. In contrast to Sarwar and Saqib (2010) who reported minimum 20 days longevity on natural diet. This difference is due to different prey specie consumed by the predator. Honey and water solution (D-6) exhibited longest longevity of 81.87 days. When female predator was given sugar solution it showed longest longevity of 71.3 days as compared to other treatments (Agus *et al.*, 2013). Artificial diets never allow the predator to complete development of life stages rather they prolonged all the developmental stages of this predator. Berkvens *et al.* (2007) and Ragkou *et al.* (2004) suggested that additional nutrients such as amino acid and mineral salts are needed to generalize the feeding of predator in order to complete the development by using different prey and pollen grains. In our studies female weight was always higher than

male. This feature is common for Coccinellidae (Sighinolfi *et al.*, 2008; Silva *et al.*, 2009). This difference may be due to the physiological process regulated by nutrients stored in the female body (Zanuncio *et al.*, 2002).

**CONCLUSION**

Study demonstrated that aphid population as a natural prey proved to be excellent with shorter life cycle. Among the artificial diets only protein based diets were effective having shorter life cycle as compared to other artificial diets. If the natural and artificial diets are given simultaneously, mass rearing would be more successful and faster. Artificial diets increased the longevity of this predator. Artificial diets are very helpful to main the culture of ladybird beetle in the laboratories during the dearth periods of natural prey.

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