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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.

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

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.

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,