Pakistan J. Zool., vol. 39(2), pp. 103-107, 2007.

Development of Larval Artificial Diet of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)

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Abstract. - Green lacewing *Chrysoperla carnea* (Stephens), is an important predator, its larvae are considered as generalist beneficials. To enhance the larval efficiency for mass rearing of this predator, an artificial diet was standardized. Three different diets were tested. Larval survival, pupation, emergence percentage as well as adults longevity and egg laying potential were recorded. Maximum larval survival (85.75%), pupation percentage (82.67%) and emergence (66.68%) were recorded on the diet in which ground beef and ground beef liver were added instead of beef powder or beef liver powder. It was concluded that brewer's yeast and vitamin solution effected on fecundity and fertility. Among all diets egg laying was also better in this diet. Artificial larval diet played an important role in whole life cycle of *C. carnea* for mass production and proved better on all above parameters.

Key words: *Chrysoperla carnea*, green lacewing, larval diet, mass rearing of insects.

INTRODUCTION

The importance of predators as pest controlling agents is coming into closer focus as based on modern investigations and experiments. The impact of predacious arthropods in natural communities and agricultural crops is receiving more attention in recent years. The common green lacewing *Chrysoperla carnea* (Stephens) is an important) predator, belonging to the order 'Neuroptera'. Its agricultural importance lies in its carnivorous habits, their larvae are all predators and commonly known as “aphid lions” (Principi and Canard, 1984). *C. carnea* adults feed on honey and pollen and are not themselves predators; they guarantee the continuous supply of new individuals (predatory larvae) in the ecosystem (Medina *et al.*, 2004). Jagadish and Jayaramaiah (2004) studied life cycle and feeding potential of the *C. cornea* on the tobacco aphid, *M. nicotianae*, to determine the potential of the predator for the biological control of the aphid. The predator required 3.10, to.27 and 8.18 days, on average to complete the egg, larval and pupal stages, respectively. The adult male and female lived for 11.50 and 34.25 days, respectively. The larvae of the 1st, 2nd and 3rd instars consumed 20.20, 25.40 and 99.93 aphids in their successive stages. Adane *et al.* (2001) reported feeding potential of first-instar larva of *Chrysoperla carnea* on different prey species in the order of *Aphis craccivora* > *Drosophila melanogaster* > *Corcyra cephalonica.* Based on these studies, *D. melanogaster* appeared to be promising for mass production of the predator.

In the laboratory for mass rearing, no native aphids or other natural hosts could be available through out the year; in this context experiments were conducted to standardize an artificial diet instead of eggs of Angoumois grain moth, to produce their sufficient numbers at a low cost. Development of practical artificial diets for entomophages would greatly reduce cost and enhance the potential for success of augmentative biological control programmes (Yazlovetsky, 1992). Different artificial diets for *C. carnea* have been developed in various localities of Pakistan (Anonymous, 2004-2005). To enhance the larval efficiency for mass rearing in laboratory, different diet combinations were tested in Bio-control laboratory of Nuclear Institute of Agriculture, Tandojam. *C. carnea* larvae were reared on artificial diets of different compositions instead of other conventional host *(Sitotroga cerealella* frozen eggs). The objectives of the study were to standardize the economic mass production of *C. carnea,* and to assess the effect of the diets on

0030-9923/2007/0002-0103 $ 8.00/0

consumption, development, and fecundity of this predator.

MATERIALS AND METHODS

Green lacewings *Chrysoperla carnea* larvae were obtained from adults reared on the eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier). After hatching, newly emerged unfed larvae were placed in plastic tubes, sealed at both ends with the help of impulse sealer and diet was injected with the help of disposable syringe. Various types of diet composition were tested with different ingredients. Presentation of solid ingredients were also used that allowed the predators to recapture their digestive enzymes for further use in their gut, which is a most important aspect of their normal feeding process (Nordlund and Correa, 1995a,b).

Diet preparation

The diet consisted of the following ingredients: ground beef (with 25% tat), ground beef liver, sucrose, hen’s egg, distilled water, honey, brewer’s yeast, acetic acid, to prolong the shelf life of the diet propionate was added, potassium sorbate, streptomycin sulfate, agar, chlortetracycline and vitamin solution were mixed in different quantities (Table I). In diet No.1 agar was used to solidify the diet, than in diet No. 2. Brewer's yeast was added to enhance the fecundity, while in diet No. 3 ground beef and ground beef liver were replaced by beef powder and beef liver powder, less amount of agar, whereas Brewer's yeast and vitamin solution were deleted. A mentioned ingredients were weighed carefully. In diet No.2, the ground beef and ground beef liver was cut in pieces and put in refrigerator for 24 hours, then the mixture of meat, honey water, preservatives (including antibiotics), and brewer's yeast were blended in a food processor prior to addition of the hen egg (put in boiled water for 3-4 minutes). In a 5 liter beaker, 25 m1 of water was heated to about 80-90°C, and 15 gm sucrose and 5.0 ml of acetic acid and antibiotics were added and stirred until the sugar was dissolved. Then 100 gm of blended whole eggs was added and stirred constantly until eggs had gelled but were of a stringy and sticky consistency. All ingredients (Table I) were blended for 6.8 minutes until the entire mixture was of a stringy paste-like consistency. At this point the mixture was a soft solid that was wet to the touch, but it retained any shape given to it. . After that the diet was ready to give larvae in plastic tubes sealed at both ends by impulse sealer; the diet was changed after three days as in the case with *S. cerealella* eggs.

Table I.- Composition of different artificial larval diets of *Chrysoperla carnea.*

Ingredients	Diet 1	Diet 2	Diet 3
Ground beef	100 g	100 g	-
Ground beef liver	100 g	100 g	-
Beef powder	-	-	100 g
Beef liver powder	-	-	100 g
Hen eggs	80 g	100 g	80 g
Sucrose (sugar)	10 g	15 g	10 g
Honey	20 g	25 g	25 g
Brewers yeast	-	12 g	-
Propionate	0.5 g	0.6 g	0.5 g
Potassium sorbate	0.5 g	0.6 g	0.5 g
Streptomycin sulphate	0.1 g	0.1 g	0.1 g
Chlortetracycline	0.8 g	0.1 g	0.1 g
Agar	20 g	-	15 g
Distilled water	20 ml	20 ml	25 ml
Acetic acid	04.0 ml	05.0 ml	05.0 ml
Vitamin solution	05 ml	10 ml	-

Preliminary and accessory experiments

To standardize the diet, several changes were made in formulation. In diet No. 1 first boiled eggs were used and ground beef and ground beef liver were simply used; after preparation, diet was very liquid therefore, in diet No.2, eggs were put in boiled water for 3-4 minutes and also ground beef and beef liver were kept in refrigerator for 24 hours, than used in diet, likewise agar 20 gm used in diet No. 1 to solidify the diet but larvae disliked that diet also disliked the diet No.3, in which beef powder and beef liver powder were used. These changes were made because to represent important improvements that will have bearing on the success of future research.

Quality assessment

Prior to the development of promising formulations of artificial diet, studies were made on three parameters that are absolutely essential for successful commercial based mass rearing, production of continuous generations on diet, continuous increase in colony size and to enhance the ability to kill and feed upon natural prey in the field. Side by side the observations were also recorded for different parameters on the comparisons of natural host *S. cerealella* eggs and artificial diet rearing. The data collected on above parameters, were subjected to analysis of variance and the entire treatment means were compared using Duncan's Multiple Range Test (Gomez and Gomez, 1984) with the help of MSTAT computer soft-ware.

RESULTS AND DISCUSSION

Among all three artificial diet standards checked, diet No.2 showed good results for successful commercial based mass rearing of green lacewings (Table II). As a semisolid diet, the diet presented no leakage problems and was packaged in plastic tubes both the ends sealed with impulse sealer; no any type of problem was seemed in any stage. The larvae fed readily on the diet and showed activity of good quality predator and appeared to use the same type of feeding behaviour or activity that they fed on natural diet. Highest larval survival was observed (85.75) on diet No.2, followed by (66.00) on diet No. 1. Maximum pupation (73.50) showed in diet No. 2, while minimum (11.50) in diet No.3. Emergence was the highest (65.00) in diet No.2, followed by (36.50) in diet No.1, and the lowest (11.50) in diet-No. 3.

Table II.- Effect of artificial larval diet on *Chrysoperla carnea.*

Diet	Larval survival	Pupation	Emergence	Fecundity
1.	66.00 b	51.33 b	28.67 b	101.70 b
2.	85.75 a	82.67 a	66.68 a	288.31 a
3.	35.00 c	25.01 c	15.67 c	50.67 c
L.S.D.= 0.050	9.041	14.28	9.301	26.24

Means followed by the same letters are not significantly different from each other (P>0.05) using LSD test.

The new semisolid diet supported continuous production of *C. carnea* that were large, aggressive predators. Performance of artificial diet was although not generally superior or equal to that on the factitious host *S. cerealella* eggs at larval stage, yet it proved nearer to natural diet at pupal and adult stages (Table III). Bartlett (1984) observed that no reduction in population numbers in early generations referred to as a "winnowing" phenomenon widely observed when insects are first subjected to feeding on artificial diet. The ration behind developing a semisolid diet was the realization that most predaceous arthropods consume the semisolid diet instead of their prey (Cohen, 1995).

Table III.- Development of *Chrysoperla carnea* on natural and artificial treatments.

Life stage (gm)	Natural host	Artificial diet	F-value	P value
Larvae weight	0.008±0.000	0.004±0.000	40.69	0.0014
Pupal weight	0.007±0.000	0.005±0.000	3.76	0.1101
Adult weight	0.009±0.000	0.008±0.0003	22.27	0.0052

In Integrated Pest Management strategy, biological control has proved effective for the management of insect pests (Olkowski *et al.*, 1992). Larvae of green lacewings, *Chrysoperla carnea* (Stephens), are important predators, so an artificial diet and a feeding method were sought for rearing *C. carnea,* because this insect is useful as a predator of sucking *pests* (Bellows, 2001). Ridgway and Jones (1968), Sengonea and Henze (1992) demonstrated that *Heliothis* species can be controlled on cotton by inundative releases of *C. carnea* eggs and larvae. Like our observations, certain earlier workers also used Gt. different diets for rearing of this important predator. Adane *et al.* (2002) examined the effects of different combinations of 50% honey solution, castor pollens and yeast on the longevity, fecundity, reproductive age and other reproductive attributes of the predatory insect, *C. carnea.* The adult food supplements significantly influenced oviposition period, post-oviposition period and fecundity, while having no significant effects on pre-oviposition period and longevity of *C. carnea.* The highest number of eggs/female (1245.2) were laid when adults were supplemented with Baker's yeast granules + 50% honey followed by Baker's yeast granules + castor pollen + 50% honey (1069.2) and castor, pollen + 50% honey (450). The oviposition ranged from 6.14 to 27.11 eggs/day when fed with different adult food supplements. The productive age of female was observed to reach up-to 8, 9, 8 and 4 weeks when fed with baker's yeast granules + castor pollen + 50% honey, baker's yeast granules + 50% honey, castor pollen+50% honey and 50% honey, respectively. Zhang *et al.* (2004) studied development; survival rate and oviposition of *C. septempunctata* (*Chrysopa pallens*) reared by artificial diets; brewers' yeast dry powder (brewer's yeast:sugar = 10:8) freeze-dried powder of pupae of Chinese tussah silkworm (dried powder:honey:sugar = 5:2:2), and pupae of *Trichogramma* and *Aphis craccivora.* All larvae of *C. septempunctata* reared by brewer's yeast or freeze-dried powder of Chinese tussah silkworm died at first instar. The survival of larvae and pupae of *C. septempunctata* reared on the pupae of *Trichogramma* on artificial eggs were similar to those reared by rice moth eggs. They were significantly longer than those reared in *A. craccivora*, and could complete the whole generation, but adults, reared by rice moth eggs, lacked fecundity.

At present the insect is being reared in the laboratory on *Sitotroga cerealella* eggs. However, the use of an artificial diet would eliminate the need for rearing and would make mass production for release purposes much more reasonable. Earlier, Smith (1992) observed that larvae will feed from pieces of absorbent cotton which are saturated with sugar water. A semisolid artificial diet is described for rearing larvae of green lacewing, because an artificial-based rearing system would be useful on economic point of view for mass rearing. These artificial solid diets can accommodate nearer to than other natural diets as shown in the larval survival, pupation and emergence fed on artificial diet for chrysopids and other predaceous arthropods that use external digestion (Penny, 2000). Extensive work on the use of exterritorial digestion by *Chrysoperla* spp. and other socalled liquid feeders sheds light on the actual feeding mechanism, digestion of solid contents of prey before they are ingested. Therefore, that provision of a solid diet would improve performance in *Chrysoperla* spp. reared on artificial diet (Cohen, 1995). Diet No.2 showed successful results in good quality. Prior to the development of promising formulations of artificial diet, these three standards were checked for successful commercial-based mass rearing. Production of continuous generations on artificial diet (with no resource to real prey), continuous increase in colony size, and to enhance the ability of a predator to kill and feed on natural prey (Cohen, 1992), could be achieved by manipulating the results of the current studies.

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(Received 20 February 2006, revised 15 September 2006)