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Use of Alternative Foods in the Rearing of Aphidophagous Ladybird Beetle, *Cheilomenes sexmaculatus* (Fabricius) (Coleoptera: Coccinellidae)

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ABSTRACT

Aphidophagous ladybird beetle, *Cheilomenes sexmaculatus* (Fabricius) developed and reproduced in captivity when offered alternative foods like drone honeybee powder, ant eggs or a combination of ant eggs and aphids (2:1). However, female ladybird beetles showed longer pre-reproductive delay and laid less number of viable eggs by feeding on alternative foods in comparison to those females which fed on living aphids. Adults fed on drone honeybee powder oviposited more number of eggs and such adults lived longer than those fed on ant eggs or a combination of ant eggs and aphids.

KEY WORDS : Ladybird beetles, *Cheilomenes sexmaculatus*, alternative foods

Aphidophagous ladybird beetles are important agents in the natural control of aphids (Hodek, 1973). Success in the control of aphid pests in greenhouses through use of biocontrol agents which include ladybird beetles have been reported (Begon *et al.*, 1986). Some of the potential ladybird beetle species are therefore being extensively studied in respect of their biological and ecological properties prior to their field release (Smirnoff, 1958; Smith, 1965; Niijima *et al.*, 1986). *Cheilomenes sexmaculatus* (Fabricius) is one such species. This ladybird beetle species is a common predator of aphids and also scale insects in Far East and South East Asia (Hukusima and Kouyama, 1974). In view of common occurrence of this ladybird beetle species in association with a wide range of aphid species (Agarwala and Ghosh, 1988), attempts are being made in recent times to augment the rearing of this ladybird beetle on alternative foods in order to exploit its biocontrol potential under natural conditions (Patnaik and Sahu, 1980; Niijima *et al.*, 1986). In our efforts to continue the search for suitable alternative foods in the rearing of *C. sexmaculatus*, eggs of an ant species, *Oecophylla smaragdina* (Fabricius), drone honeybee powder prepared as suggested by Matsuka and Niijima (1985) using drones of *Apis mellifera* (L) and soyabean food were tried in captivity. The results obtained are presented and discussed in this paper.

MATERIALS and METHODS

Individual egg batch, grub or adult of *C. sexmaculata* was obtained from the stock culture which were maintained in the laboratory during the period of this study at ambient temperature by offering live aphids, *Aphis craccivora* Koch. A batch of eggs, a recently hatched grub or an adult was individually placed in a pair of 5 cm diameter Petri dishes. Adequate quantity of food by weight, aphid or alternative food, was provided on a piece of tissue paper to each of the experimental larva or adult. The food was replaced by fresh supply at an interval of 24 h when observation was recorded. A piece of filter paper soaked with water was used as a source of moisture in the Petri dish. Experiments were performed at 20° C ± 2°C and 12h light: dark photoperiod in an environment chamber and run through three successive generations.

Preparation of Alternative Foods

i. **Ant eggs (AE):**
Fresh supplies of eggs of *O. smaragdina*, commonly available in the local market as

fish-bait, were periodically procured and stored in a deep freeze at -5^0. Eggs of ants, about 3-5 mm long, were used as such in the experiment.

ii. **Ant eggs + Aphids (AE+A) :**
Ant eggs, as foresaid, were crushed in a porcelain bowl and mixed with fresh aphids (*A.craccivora*) in 2 : 1 proportion by fresh weight. The mixed food was heated at 50^{0}C for 30 minutes to remove excess water and then used in small flakes. We found that this preparation made a satisfactory recipe for beetles.

iii. **Drone Honeybee Powder (DHBP):**
A sample quantity (250 g) of this food was obtained form the Institute of Honeybee Research, Tamagawa University, Japan for the present study. This food was prepared following the method described by Matsuka and Niijima (1985).

iv. **Soyabean Food (SF) :**
Each 150 g of this food was made from the following constituents : Soyabean powder (commercial) 23.24 g, sucrose powder 23.24 g, yeast powder 19.45 g, casein powder 15.56 g, natural honey 7.78 g, agar 7.78 g, ascorbic asid 1.94 g, vitamin B-complex 0.78 g prepared in about 50 ml of distilled water.

Laboratory experiments

i. **Development**
First instar larva, within 12h of its emergence, (n=10, each for different food) was weighed in a microbalance to the accuracy of 0.1 mg and kept in a pair of 5 cm diameter Petri dishes. It was offered either an alternative food or live aphid, by weight, in the following order : I instar - 1 mg, II instar - 4 mg, III instar 10 mg, and IV instar - 15 mg. The food was replaced by fresh supply at 24 h interval. Observations were made at 12 h interval in respect of duration of development of different instar larvae, pupa and emergence of the adult. Mortality, if any, in the experiment was recorded. A full grown larva preparing for pupation was weighed again. Adults, within 12 h of emergence, were sexed under the microscope using the characters suggested by Majerus and Kearns (1989) and weighed after anesthetizing them with ether for 30 seconds.

ii. **Reproduction and longevity of adults**
A pair of adults, within 24 h of their emergence, was offered with adequate quantity of either an alternative food or living aphids in a pair of clean dry 5 cm diameter Petri dishes. A corrugated piece of paper, as a means of oviposition site, was provided to each pair of adults. Observations were made at an interval of 12 h for the laying of first batch of eggs. Thereafter, observation was continued at an interval of 24 h until the last batch of eggs was laid. These adults were continued to be kept in separate Petri dishes and observed for the duration of their post-reproductive period until each of both the sexes died. This was repeated 10 times using each of the aforesaid alternative foods and aphids.

iii. **Hatching success of eggs**
A batch of eggs, within 12 h of laying, was removed to a pair of 5 cm diameter Petri dishes. Number of eggs hatched successfully was recorded during observations at an interval of 12 h. Unhatched eggs, if any, were observed under the microscope for any sign of embryogenesis.

The results obtained from these studies were analysed through computer programmes, namely MACSTAT I and II and MULTISTAT and compared for any significant difference by Duncan's Multiple Range Test (DMRT) at 5% probability.

RESULTS AND DISCUSSION

Duration of larval, pupal and total preadult development was longest by feeding DHBP and shortest by feeding on AE+A. None of the larvae developed beyond second instar stage when offered SF. Mean duration of development of a larva, pupa and preadult stages (total) was significantly higher by feeding on DHBP or AE in comparison to those which developed by feeding on live aphids but not so by feeding on AE+A (Table 1). Highest mortality was recorded in larvae which were offered DHBP (Table 1). Only 25% of these larvae reached adulthood. Adult emergence was 42.18% in case of larvae which developed by feeding on AE and 53.85 % by feeding on AE+A. These did not differ significantly from 55.50% of adults which emerged successfully when fed on live aphids.

A first instar grub provided with aphids showed a mean fresh weight of 0.53 ± 0.002 mg within 12 h of hatching. A full grown fourth instar larva, when stopped feeding, was significantly heavier by feeding AE than other alternative foods or aphids offered in this study (Table 2). Net gain in the fresh weight of larvae during its life and of adults at emergence which developed by feeding on AE or DHBP were significantly different from each other and with those that were fed on aphids. However, the larvae which were fed on AE+A did not show significant difference when compared with those that fed on aphids alone (Table 2).

Oviposition by an adult female ladybird beetle usually follows pre- oviposition delay of several days depending on the quality of habitat experienced by larvae and delay was shortest when adults were offered aphids but longer in adults which were offered either of the alternative foods. DHBP or AE+A fed-adult females showed significant difference when compared to adults fed on aphids, but in case of AE-fed adults the difference was not significant (Table 3). Analyses of results among the adults fed on alternative foods did not show significant differences.

Adult females fed aphids showed significantly longer oviposition period compared to those fed on alternative foods (Table 3).

Table 1. Mean duration of larval instars (L 1-4), pupa and total development of *C.sexmaculatus* obtained by feeding AE, DHBP, AE+A and their separate comparison with results obtained by feeding aphids (*A.craccivora*)

Treatment (Food)	Duration of development in days (mean ± SE)			Average mortality (%)
	L1-4	Pupa	Total	
Aphid	80 ± 0.67 (a)	4.2 ± 0.20 (a)	12.7 ± 0.77 (a)	44.50 (a)
AE	12.4 ± 0.52 (b)	5.9 ± 0.31 (b)	18.1 ± 0.69 (b)	57.82 (a)
AE+A	8.4 ± 0.53 (a)	4.1 ± 0.29 (a)	12.4 ± 0.49 (a)	46.14 (a)
DHBP	17.0 ± 0.47 (c)	7.2 ± 0.24 (c)	23.8 ± 0.44 (c)	75.00 (b)

Figures in a column followed by the same letter in parentheses do not differ significantly at <0.05 level of probability : DMRT

Table 2. Mean value of fresh weight of a fully grown larva (L4), and an adult at emergence (within 12-h) and net gain in fresh weight of a larva in its life (NGL) of *C.sexmaculatus* when reared on aphid, AE, AE+A or DHBP

Treatment (Food)	Fresh weight of larva and adult (mg)		
	L4	NGL	Adult
Aphid	9.57 ± 0.45 (a)	9.52 ± 0.45 (a)	8.04 ± 0.43 (a)
AE	8.10 ± 0.45 (b)	8.05 ± 0.45 (b)	6.80 ± 0.41 (b)
AE+A	11.90 ± 0.60 (a)	9.70 ± 0.51 (c)	9.70 ± 0.51 (c)
DHBP	9.48 ± 0.48 (a)	9.27 ± 0.43 (a)	8.40 ± C.46 (a)

Figures in a column followed by the same letter in parentheses do not differ significantly at <0.05 level of probability : DMRT

Table 3. Mean duration of pre-oviposition delay (POD), oviposition period, total number of eggs laid, hatching success of eggs and longevity of adult (AL) male and female of *C.sexmaculatus* when fed on DHBP, AE or AE+A and their separate comparison with those fed on live aphids, *A.craccivora*.

Treat ment (Food)	POD (days)	Oviposition period (days)	No. of eggs	Hatching success of eggs (%)	AL (days)	
					Female	Male
Aphid	14.75 ± 1.60a	43.75 ± 7.22a	255.0 ± 71.01a	41.71 ± 3.61ac	75.0 ± 6.65a	66.75 ± 7.35a
AE	13.62 ± 3.44a,b	7.75 ± 3.58c	21.37 ± 6.16c	21.61 ± 6.72b	41.25 ± 3.25c	36.25 ± 2.83b
AE+A	18.4 ± 0.87a	10.72 ± 2.11c	73.4 ± 9.82 a	31.84 ± 2.50a,b	46.80 ± 2.48c	38.60 ± 3.14b
DHBP	9.56 ± 2.54b	67.5 ± 24.6b	815.6 ± 211.60b	52.0 ± 5.07c	98.0 ± 12.76b	80.0 ± 11.34a

Figures in a column followed by the same letter in parenthesis do not differ significantly at <0.05 level of probability : DMRT

Among the alternative foods, females fed on DHBP showed significantly longer oviposition period in comparison to those females which fed on AE or AE +A but the difference was not significant when compared among AE and AE+A - fed females. Adult females feeding on DHBP, AE or AE+A laid significantly less number of eggs compared to those females which fed on aphids (Table 3). Among the females fed on alternative foods, DHBP-fed females oviposited significantly higher number of eggs compared to AE - fed females but the difference was not significant in comparison to those females which fed on AE or AE+A but the difference was not significant when compared to eggs of those adults which fed on DHBP (Table 3). AE or AE+A - fed adults showed significantly shorter longevity compared to those that fed on aphids. However, no significant difference occurred between DHBP and aphid-fed adults, both males and females (Table 3).

Mean oviposition period showed positive and significant correlation with the mean number of eggs laid when adults of *C.sexmaculatus* were offered aphid, DHBP, AE or a AE+A foods ($r=0.998$; $P<0.001$). Regression analysis showed linear relationship between the two factors ($y=91.79+12.35\ x$).

Results suggest that a combination of AE+A was almost equal in nutritional value to aphids in terms of duration of development, number of adults which emerged successfully after development, fresh weight attained by a fourth instar grub, net gain in fresh weight of larve in its life and of adults at emergence. The same did not hold good in respect of ladybird beetles which developed by feeding on AE or DHBP. Soyabean food proved to be almost non- starter.

Results on reproduction and adult longevity suggest that adult beetles of *C. sexmaculata* when fed on DHBP, AE or AE+A, showed significantly reduced level of performance in respect of pre- oviposition delay, oviposition period, number of eggs laid, their hatching success and longevity of adults compared to those that fed on aphids. It is obvious that natural food provides the best value for any cost-effective performance of an organism, however, it is remarkable that the DHBP, AE and AE+A foods induced some level of oviposition response.

Though heavier larvae and adults were produced by feeding AE, this food seems to be deficient in some vital ingredients not yet known, which were possibly responsible for the weak ovipositional response in an adult female. Niijima *et al.* (1986) found that the nutritional value of DHBP is almost equal to aphids and considered the former an effective substitutional diet for many aphidophagous Coccinellidae including *C. sexmaculatus*. But *Coccinella septempunctata bruckii* Mulsant, a native Japanese ladybird beetle, laid fewer eggs and showed a longer duration of pre-oviposition delay by feeding DHBP. Remarkably the same subspecies could not be successfully reared in Finland on an artificial diet (Hamalainen and Markkula, 1972). This implies that adequate nutritional requirement alone possibly is not enough for a ladybird beetle to cause oviposition. Balanced artificial diets used in many studies either failed to cause complete development (Smith, 1965; Hukusima and Takeda, 1975) and/or evoke adequate ovipositional response (Smirnoff, 1958; Kariluoto *et al.*, 1976). Some other factors like gustatory and mechanical properties may also influence the response of oviposition provided the nutritional supply is adequate. Inadequate ovipositional response of the Indian population of *C. sexmaculata* compared to Japanese population when fed on DHBP could be another example of similar situation.

Results suggest that a combination of AE+A holds promise for use in short term culture of aphidophagous ladybird beetles. DHBP is useful in captive culture of ladybeetle on a limited scale and for the maintenance of stock of adults in particular.

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