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Development of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), a predator of mealybugs on freeze-dried artificial diet

T. VENKATESAN, S. P. SINGH and S. K. JALALI
Project Directorate of Biological Control
Post Bag No. 2491, H. A. Farm Post, Bellary Road
Bangalore 560 024, Karnataka, India
E-mail: pdblc@kar.nic.in

ABSTRACT: **Development of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) was studied on a freeze-dried artificial diet devoid of insect components. The artificial diet was composed of beef liver (5 g), hen's egg yolk (5g), sucrose (1g), honey (1g), hydrolysed yeast (1g), milk powder (0.5g), brewer's yeast (0.5g), groundnut oil (0.3g), multivitamin (0.04g), vitamin E (0.04g), niphagine (0.004g) and water (16 ml). Biological attributes of the artificial diet reared *C. montrouzieri* were compared with the mealybug, *Maconellicoccus hirsutus* (Green) reared. Mean adult emergence of the predators reared on artificial diet and mealybug reared were 58.0 and 90.0 per cent, respectively. Differences in developmental period, adult weight and female emergence between artificial diet and mealybug reared predators were non-significant. Pre-ovipositional period of the predators reared on artificial diet was significantly longer than the mealybug reared. The mean fecundity of artificial diet reared predator was only 8 eggs/female, which was significantly lower than the mealybug reared (204eggs/female). Artificial diet reared predators laid fertile eggs and the mean viability of the eggs was 93.0 per cent, which was not significantly different from the mealybug reared (94.0 %).**

KEY WORDS: Artificial diet, biological suppression, *Cryptolaemus montrouzieri*, *Maconellicoccus hirsutus*, *Planococcus citri*

The importance of the predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) has been well established because of its role in the biological suppression of mealybugs (Chacko *et al.*, 1978; Mani *et al.*, 1990; Singh, 1996). Generally, the predator is reared on mealybugs in the laboratory. The benefit of rearing parasitoids and predators on an artificial diet has long been recognized (Simmonds, 1944). Few attempts to rear predatory coccinellids on artificial diets have been made (Matsuka *et al.*, 1972; Venkatesan *et al.*, 1998). Studies were thus undertaken to assess the development of *C. montrouzieri* on a beef liver-based freeze-dried artificial diet, and to compare with feeding on *M. hirsutus*. The work on development of artificial diet for the rearing of *C. montrouzieri* is first of its kind in India.

MATERIALS AND METHODS

Stock culture

Cryptolaemus montrouzieri culture was bred on mealybug, *Maconellicoccus hirsutus* infested pumpkin fruits as described by Chacko *et al.* (1978).

Composition and preparation of artificial diet

Among several artificial diets in different combinations tested, freeze-dried beef liver based diet was found to be the promising one (PDBC-ICAR, 2001). The diet was composed of beef liver (5g), hen's egg yolk (5g), milk powder (0.5g), brewer's yeast (0.5g), sucrose (1g), honey (1g), hydrolysed yeast (1g), groundnut oil (0.3g), multivitamin (0.04g), Vitamin E (0.04g), Niphagine (0.004g) and water (16ml). The diet ingredients were accurately weighed using an electronic balance (Sartorius, BP 210 D, Germany) with a precision of 0.01mg. The diet ingredients were blended for 5minutes to form a homogenous semisolid paste, which was subsequently transferred into a glass beaker (25x10cm) and heated in a water bath at 50 °C for 3minutes. Then diet was removed and mixed thoroughly and dried using a Freeze Dry System (Labconco, Labconco Co., England).

Development and rearing of *Cryptolaemus montrouzieri*

Two-day old larvae were kept in a round bottom-opaque plastic container (3x3cm) provided with copper mesh on three sides (mesh size 160micron) and tissue paper at the bottom. Freeze-dried artificial diet was provided once in three days on the tissue paper, moistened cotton swab was also provided on the inside top of the plastic container for water supply to the larva and to maintain the humidity within the container. As a check treatment, the larva was reared in the similar container and provided with ovisac containing eggs and nymphs of the *M. hirsutus* as and when needed till the larva reached pupal stage. During the rearing process, egg period, larval and pupal duration, survival and adult emergence was recorded for *C. montouzieri* reared on artificial diet and mealybug. Newly emerged adults reared on artificial diet and mealy bugs were weighed and kept in pairs in a plastic container (15x10cm). *Cryptolaemus montrouzieri* adults reared on artificial diets were additionally provided with honey (50%) and moist piece of cotton. Further, fecundity, viability of eggs and longevity of adults were recorded. For the adult predators reared on artificial diet, corrugated cardboard strips were provided for egg laying in lieu of mealybug ovisac. Each treatment was replicated 10 times and 20 larvae were taken in each replication. Student's *t* test was used for comparison of artificial diet and mealybug reared *C. montrouzieri*. Entire study was conducted in the laboratory at temperature of 26±1°C and 60±10 per cent relative humidity during 1998-2000 at Project Directorate of Biological Control, Bangalore

RESULTS AND DISCUSSION

Our preliminary investigation with the rearing of *Cryptolaemus montrouzieri* using semisolid diet revealed that the young larva was found getting stuck to the diet and also the diet was sticking on the mouthparts of the larva. Hence, freeze-dried artificial diet was formulated and it was readily accepted by young larva. Further the advantage of the freeze dried diet is that it can be stored relatively for 3-4 months whereas semi-solid diet has to be heated up daily before feeding the larva and cannot be stored for more than a week. The observations recorded on the growth and quality parameters *viz.* egg, larval and pupal durations, survival and adult emergence, adult weight, fecundity, egg viability and longevity of the *Cryptolaemus montrouzieri* reared on artificial diet and mealybug are presented in Table 1. There was no significant difference in egg, larval, pre-pupal and pupal durations between artificial diet and mealybug reared *C. montrouzieri*. Frequent cannibalism was observed when the larvae were reared in mass using the artificial diet and hence, the larvae were reared singly. The survival and adult emergence of the *C. montrouzieri* reared on artificial diet were 62.0 and 58.0 per cent, respectively. However, these parameters were significantly lower than that of mealybug reared namely, 92.0 and 90.0 per cent, respectively ($P<0.01$). Differences in performance of the predator reared on artificial diet and natural insect may be related to the nutritional quality of the prey and prey acceptance (De Clercq *et al.*, 1998; Venkatesan *et al.*, 2000).

The female emergence in artificial diet reared predator was 48.1 per cent, which was not significantly different from that of mealybug reared. Adult weight of the *C. montrouzieri* reared on artificial diet and mealybug was 8.0±0.316mg and 13.0±0.447mg, respectively and the difference was non-significant. It was observed that the adult beetles reared on the artificial diet mated normally as in the case of mealybug reared predators. But the pre-oviposition period of the artificial diet reared predator was 18days, which was significantly more than that of mealybug reared (8.8days). However, for the first time the predators were found to lay eggs during our study on the cardboard strips in the absence of mealybug ovisac. The mean fecundity of the mealybug reared predator was 204eggs/female, which was significantly higher than that of artificial diet reared predator (8eggs/female) ($P<0.01$) in the absence of mealybug ovisac. Further, to test the influence of the mealybug on the fecundity, a batch of artificial diet reared beetles were reared on mealybugs and the fecundity of the predator was increased to 45eggs/female. Merlin *et al.* (1996) found that contact chemical cues perceived by females of *C. montrouzieri* when probing the wax filaments produced by its prey *P. citri* with their mouth parts were the signals inducing the search for oviposition sites. It was also found that the females delayed oviposition and withheld mature eggs in their lateral oviducts in the absence of wax filaments.

The predators survived on artificial diet and mealybug for 53 days and 59.6 days, respectively and the difference between these two was non-significant. Eggs laid by *C. montrouzieri* reared on artificial diet were highly viable with no significant difference in viability of eggs of predator reared on artificial diet (93.0 %) and mealy bug (94.0 %). It is evident from the findings

Table 1. Comparisons of biology of *C. montrouzieri* reared on artificial diet and *M. hirsutus*

Growth attributes	*Cryptolaemus* reared on artificial diet	*Cryptolaemus* reared on *M. hirsutus*	Student's *t* test ($P<0.05$)
Egg period (days)	4.6±0.399 [a]	4.6±0.399 [a]	NS
Larval period (days)	16.0±0.316 [b]	14.0±0.316 [a]	4.47**
Pre-pupal period (days)	2.0 [a]	2.0 [a]	NS
Pupal period (days)	8.0±0.316 [a]	8.0±0.316 [a]	NS
Survival (%)	62.0±1.14 [b]	92.0±0.707 [a]	22.36**
Adult emergence (%)	58.0±1.14 [b]	90.0±1.14 [a]	19.85**
Female emergence (%)	48.1±0.617 [a]	48.0±0.627 [a]	NS
Adult weight (mg)	8.0±0.316 [a]	13.0±0.447 [a]	NS
Pre-oviposition period (days)	18.0±1.14 [b]	8.8±0.509 [a]	7.69**
Viability of eggs (%)	93.0±1.049 [b]	94.0±1.049 [a]	0.67**
Longevity (days)	53.0±2.70 [b]	59.6±2.87 [a]	1.67**

Means accompanied by same letter are not significantly different.

that the artificial diet was a promising one as it consists of cheap diet ingredients devoid of insect components and method described for the preparation of the artificial diet is simple. The diet evolved can be further enriched/modified to improve the fecundity of the predator, which would help in their mass production without depending on the host insects and host plants.

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