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Short Communication

Mass Rearing of *Cheilomenes sexmaculata* (Coleoptera :Coccinellidae) on Different Diets Under Laboratory Conditions

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ABSTRACT. *The ladybird beetle, Cheilomenes sexmaculata is a potential biocontrol agent for use in augmentative release programmes. The survival (%) of coccinellid larvae varied significantly among the seven different selected diets (egg yolk, chicken liver, chicken liver with aphids, chicken liver with legume juice, aphids, house fly maggots, fish meal powder) ($p<0.05$). The highest survival was recorded when the larvae were fed on aphids (95.0 ± 3.0%). Larvae did not survive when fed on egg yolk and fish meal powder. The survival of L2 larvae was 72% when fed on chicken liver. The total larval duration significantly varied with different diets ($F=767$ df $=63$; 6 $p<0.05$). The shortest total larval duration was recorded when all larval instars were fed exclusively on aphids (7.60 ± 0.1days). When L2 larvae were fed on chicken liver, 10.86 ± 0.13 days were taken to develop into an adult. The highest growth rate was recorded when all larval instars were fed exclusively on aphids. The longest duration was recorded for L1 larvae fed on aphids and other three instars fed on chicken liver. To test the effect of substrates on the number of egg laid eggs laid during the lifespan, five substrates (plastic boxes, paper pieces, fresh bean leaves, bean cotyledons, sand layer) were also tested. It varied significantly among the five different substrates ($p< 0.05$). The highest number of eggs was laid on the sides of plastic boxes ($n=823.7 \pm 2.2$) while the lowest number of eggs was laid on the sand layer ($n= 80.1 \pm 10.4$). The findings indicate that the replacement of aphid feed by chicken liver for larval instars L2, L3 and L4 is possible with a compromised survival (%) of larvae.*

Keywords*: Biocontrol agent, Ladybird beetle, Larvae, rearing*

INTRODUCTION

Ladybird beetles (Coleoptera: Coccinellidae) are predatory insects with diverse food habits and live in a variety of habitats. Both adults and larvae feed on small soft bodied insects. From Sri Lanka, fifteen coccinellid species belonging to 12 genera in four tribes and three subfamilies have been recorded from the Mid-country (Mayadunnage *et al*., 2007). Adults and larvae of *C. sexmaculata* voraciously feed on a wide range of prey including aphids, coccids, diaspids and aleyrodids etc. (Agarwala and Yasuda, 2000). Nymphs and adults of *Aphis craccivora*(Koch) attack beans and other leguminous crops causing significant damage by feeding on sap of flowers, buds, pods, tender shoots thereby reducing the market

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value (Srivastava and Singh, 1986). At present, Sri Lankan farmers rely mainly on insecticides to control *A. craccivora*. Insecticidal control is not only expensive but also has adverse effects on the environment and also leads to many human health problems. Indiscriminate use of pesticides causes phytotoxicity and destruction of beneficial organisms such as predators, parasitoids, microorganisms and pollinators (Luckman and Metcalf, 1978). Subsequently, Integrated Pest Management has been opted in which biological control is one of the major aspects (Debach and Rosen, 1991). Larvae and adults of *C. sexmaculata* is an effective biocontrol agent and require mass rearing for commercial use. Rearing on natural prey requires the maintenance of host plant and aphid cultures; hence, it is necessary to explore the possibility of mass rearing of *C. sexmaculata* on artificial diet or on alternative hosts.

Food that sustains larval development and oviposition is considered as essential food while the food that serve only as a source of energy which prolong development period leading to poor survival is considered as alternative food (Hodek, 1970). *Cheilomenes sexmaculata* are also known to feed on pollen, nectar and fungal spores (Hemptinne *et al*. 1986). Various formulations of artificial diets have been tested on *H. convergens* adults (Hagen, 1964). The present study examines the possibility of replacing the natural diet: aphids by an alternative diet source thereafter and develop a mass rearing protocol suitable for mass production of the predatory beetles in the laboratory.

METHODOLOGY

The study was conducted in the Entomology Research Laboratory, Department of Agricultural Biology, Faculty of Agriculture, and University of Peradeniya from January to June, 2015. Experiments were conducted under laboratory conditions at 27ºC and 80 % RH.

Development of mass rearing protocol for *C. sexmaculata* under laboratory conditions

Adults of *C. sexmaculata* were collected from bean fields in Dodangolla, Mahakanda and Doluwa areas. The field collected beetles were sexed in the laboratory and pairs were selected for the oviposition.

Preparation of egg laying cages

Polymate Plastic boxes were used as egg laying cages. A 2.5 cm sand layer was placed at the bottom of the box. Water mixed with (100:1) hydroponic solution was added to the sand layer and mixed properly. Bean seeds were placed on the sand bed for germination. Ventilation pores were made on the lid of plastic box. Two days after seeding, aphids were introduced into the box. After 4 days of placing the bean seeds, field collected mated pairs of *C. sexmaculata* were introduced into one box. Three paper pieces were placed in the box to facilitate egg laying by *C. sexmaculata* introduced into the container. The numbers of eggs laid were counted daily.

Preparation of rearing cages

Paper on which eggs were laid was collected for separation of each egg. Egg masses glued onto the papers were removed and put into water. Eggs in an egg mass were separated using a small paint brush. A layer of wetted coir dust was placed in small plastic cups and a few holes were made for aeration. Subsequently, bean seeds were placed on the coir dust layer.

After 2 days of germination of bean seeds, aphids were placed on the germinated cotyledons. Eggs were placed individually in the plastic cup and covered using a polythene sheet. The durations of the larval stage were recorded.

Rearing of Pupae

Soon after pupation, each pupae was placed in a Petri dish that had ventilation holes on the lid. A wet cotton plug was placed on the Petri dish to avoid desiccation. Adults were sexed. Durations of pupal stage were recorded. Newly emerged pairs were transferred for separate rearing.

Survival and Development of *C. sexmaculata* Larvae on natural and formulated diets

Adults of *C. sexmaculata* that were obtained from laboratory cultures were sexed and confined in pairs into Petri dishes (15 cm diameter) with aphids for oviposition. Egg masses were separated and incubated. After the eggs hatched, the young larvae that emerged individually were transferred into the Petri dishes (15 cm diameter). L1 larvae were fed only with *Aphis craccivora*. L2 larvae were used for the experimentation. The survival rate and development duration of larvae were recorded. Each treatment was replicated 15 times.

Larvae were provided with seven different diets. Diet 1 (D1) consisted of chicken egg yolk kept in Petri dishes as small droplets. Fresh feed was supplied to the larvae daily. Diet 2 (D2) was prepared by boiling 10 g of chicken liver at 60 ^{0}C for 10 minutes in 100 ml of water. Boiled chicken liver was ground to a paste using a motar and pestle. The diet was given to larvae in the same manner as in D1. Diet 3 (D3) consisted of boiled chicken liver as in D2 ground to a paste while adding 60 adult aphids. Diet 4 (D4) was boiled chicken liver as in D2 and ground to a paste by adding legume juice (5 ml). Bean leaves were used to extract 5 ml of legume juice. Diet 5 (D5) was the natural diet: aphids. Diet 6 (D6) was prepared by grinding house fly maggots using a motor and a pestle. As diet 7 (D7), fish meal powder was used in dried form. The survival rates of *C. sexmaculata* larvae and total development durations of larvae were recorded for each diet. Each treatment was replicated 15 times. Data were analyzed using one-way ANOVA, followed by LSD mean separation using Minitab Statistical software (Version 14).

Development durations of *C. sexmaculata* larvae and survival on different feeding plan on a formulated diets

Adults of *C. sexmaculata*that obtained from laboratory cultures were sexed and placed in pairs in Petri dishes (15 cm diameter) with aphids for oviposition. The collected egg masses were separated and incubated. After the eggs hatched, immature larvae were transferred individually into Petri dishes (15 cm diameter). Three diets were used to feed the larvae. Diet 1 (D1) consisted only of live aphids. Diet 2 (D2) was prepared by boiling 10 g of chicken liver (10g) in 60 ^{0}C hot water for 10 minutes and ground by using the motor and pestle. Diet 3 (D3) was prepared by boiling 10 g of chicken liver in water heated to 60 ^{0}C for 10 minutes and ground using a motor and pestle until a paste was formed while adding 60 adult aphids. The three different diets were administered as nine treatments (Table 1).

Table 1. **Feeding plan for *C. sexmaculata* larvae with aphids and cooked chicken liver.**

Treatments	L1	L2	L3	L4
1	D2	D2	D2	D2
2	D1	D2	D2	D2
3	D1	D1	D2	D2
4	D1	D1	D1	D2
5	D3	D3	D3	D3
6	D1	D3	D3	D3
7	D1	D1	D3	D3
8	D1	D1	D1	D3
9	D1	D1	D1	D1

The survival rate *C. sexmaculata* larvae and the total development durations of larvae were recorded. Each treatment was replicated 15 times. Data were analyzed using one-way ANOVA, followed by LSD mean separation using Minitab statistical software.

Most suitable egg laying substrate for *C. sexamculata* under laboratory conditions

Cheilomenes sexmaculata, females laid eggs on the walls of the plastic petri dishes. Five different substrates, plastic boxes, paper pieces, fresh bean leaves, bean cotyledons and sand layer were tested to find an optimum substrate for egg laying. Three pieces of papers and bean leaves were inserted into each experimental setting. The number of eggs laid / day / female beetle and the total egg number / substrate were recorded. The experiment was replicated 15 times.

RESULTS AND DISCUSSION

Development of mass rearing protocol for *C. sexmaculata*under the laboratory condition

Polymate plastic boxes containing bean cotyledons were found to be suited for rearing of coccinellid adults. Larval rearing cups were also suitable for rearing.

Table 2. Duration of the Life cycle of *C. sexmaculata*when fed on. *craccivora*within an experimental setting (140 cm^2)

Instar /Stage of *C. sexmaculata*	Duration of development (d)± S.E (n=25)
L1	1.85 ± 0.03
L2	1.92 ± 0.03
L3	2.00 ± 0.03
L4	1.78 ± 0.03
Pre-pupa	1.85 ± 0.03
Pupa	3.07 ± 0.05

Survival and Development of *C. sexmaculata* Larvae on natural and formulated Diets

The survival percentage of larvae was significantly different among the treatments ($p<0.05$). The highest survival percentage was recorded when larvae were fed only on aphids (93.33 ± 2.35 %). The lowest survival percentage was recorded when larvae were reared only on chicken liver diet (10.00 ± 2.35 %). Survival percentage of larvae increased with the addition of aphids into the diets. Diet D_7 appears to be a suitable replacement for aphids with chicken liver while maintaining a 78.88% survival of larvae. The total larval duration varied significantly with different treatments ($p<0.05$). The highest development rate was recorded when all larval instars were fed exclusively on aphids and when L1, L2, and L3 instars were fed exclusively on aphids and L4 stage on D3. The highest larval duration was observed when L1 larvae were fed on aphids and other three instars on chicken liver.

Table 3. The total larval duration and survival percentages of *C. sexmaculata* when reared on aphids (D1) chicken liver (D2), and their combination (D3)

Treatment	Larval Duration ± SE (Days)	Survival % ± SE
T1	11.6 ± 0.5^{a}	10.0 ± 2.4^{a}
T2	12.2 ± 0.1^{a}	13.3 ± 2.4^{a}
T3	10.8 ± 0.1^{ab}	33.3 ± 1.7^{b}
T4	9.9 ± 0.1^{b}	58.9 ± 2.0^{c}
T5	8.8 ± 0.1^{c}	67.8 ± 1.5^{d}
T6	8.3 ± 0.2^{c}	67.8 ± 1.5^{d}
T7	8.0 ± 0.2^{cd}	78.9 ± 1.1^{e}
T8	7.3 ± 0.2^{d}	85.6 ± 1.8^{ef}

Means with different superscripts in the same column are significantly different ($p<0.05$)

Survival and development of *C.sexmaculata* larvae on natural and formulated diets

The percentage of survival was 72% when L2 larvae were fed on chicken liver. Replacement of aphids by chicken liver for L2, L3 and L4 was possible with a compromise of survival percentage of larvae. The total larval duration significantly varied with different treatments ($p<0.05$). The fastest growth rate was recorded when all larval instars were fed exclusively on aphids. Total larval development duration was nearly one week. When the L2 larvae were provided with chicken liver, 10.86 ± 0.13 days were taken to become a pupa.

Table 4. The total larval durations and survival percentages of *C. sexmaculata*when reared on natural and alternative diet sources

Treatment	Larval Duration ±SE (Days)	Survival %± SE
D1	0.86 ± 0.13[a]	00.00 ± 0.0[a]
D2	10.86 ± 0.13[b]	72.00 ± 0.2[b]
D3	9.00 ± 0.10[c]	32.00 ± 0.1[c]
D4	12.73 ± 0.11[d]	26.00 ± 0.2[cd]
D5	7.60 ± 0.13[e]	95.00 ± 3.0[e]
D6	1.87 ± 0.17[f]	20.00 ± 0.1[f]
D7	0.60 ± 0.13[fg]	0.00 ± 0.0[fg]

Means with different superscripts in the same column are significantly different ($p<0.05$)

This study demonstrates that mass rearing of different coccinellid species on artificial diets is possible. Adults of *Coccinella septempunctata* L. and *Coccinella transversoguttata* Faldermann increased in body weight but failed to produce eggs when fed on weevil larvae (Richards and Evans 1998). Pollen is an unsuitable source of food because pollen grains become clumpy and adheres to larval cuticle. Subsequently, larvae become desiccated and die. This has been observed in *Coleomegilla maculata* (Michaud and Grant 2005). Allen (1985) reported that ox liver is a nutritionally adequate diet for predator development but no eggs were laid. It is evident that the artificial diet containing chicken liver, yeast and sucrose support the growth of *C. sexmaculata* (Hussein *et al.*, 1986). According to the present findings, the most suitable diet of the predatory larvae is the natural diet of aphids but chicken liver can be used to replace the natural diet to some extent for L3 and L4 instars of larvae.

Egg laying substrate for *C. sexamculata*

The highest number of eggs per female was laid on the walls of plastic box (823.7 ± 2.2 / entire female lifespan) and the lowest on the sand layer (80.1 ± 10.4 / entire female life span). Thus best egg laying substrate for *C. semaculata*, is the wall of the rearing cages or boxes.

Table 5. Total number of eggs laid by *C. sexmaculata* on different substrates during the lifespan of female adult beetle

Substrate	No. of laid eggs ± SE
On wall	823.73 ± 11.34 [a]
On paper	746.87 ± 21.63 [ab]
On bean leaves	429.80 ± 16.79 [c]
On cotyledons	532.40 ± 8.72 [cd]
On sand	80.07 ± 40.43 [e]

Means with different superscripts in the same column are significantly different ($p<0.05$)

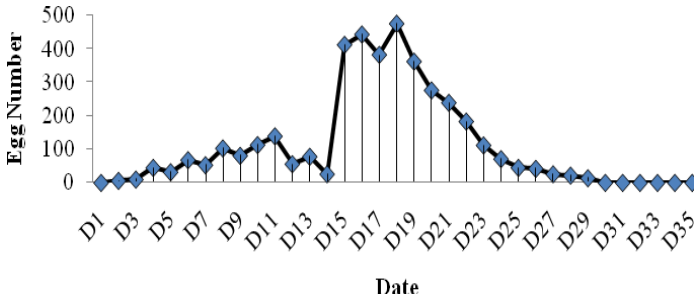

Fig 1. Egg laying pattern during entire lifespan of *C. sexmaculata* when fed on *Aphis craccivora* under the laboratory conditions at 27 ºC and 80% RH.

CONCLUSIONS

Replacement of aphids by chicken liver for L2, L3 and L4 was possible with a compromise of survival percentage. A wall of the rearing cages was the preferred substrate for oviposition by *C. sexmaculata* under the laboratory conditions.

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REFERENCES

Agarwala, B.K. and Yasuda, H. (2000). Competitive ability of ladybird predators of aphids: A review of *Cheilomenes sexmaculata* (Fabr.) (Coleoptera: Coccinellidae) with a worldwide checklist of preys. J. Aphidology. *14*, 1 - 20.

Allen, C.C. (1985). Simple methods of rearing the insect predators *Geocoris punctipe* (Heteroptera: Lygaeidae) on meat diet, J. Econ. Entomol. *78*, 1173 - 1175.

Debach, P. and Rosen, D. (1991). Biological control by natural enemies. McMillan com. New York, London. *5*, 97 - 112.

Hagen, K.S. (1964). Nutrition of entomophagous insects and their hosts, 356-36l. *In* P. DeBach [ed.], Biological Control of Insect Pests and Weeds. Chapman and Hall, London.

Hemptinne, J.L. and Desprets, A. (1986). Pollen as a spring food for *Adalia bipunctata*. In: Hodek I, (ed) Ecology of Aphidophaga, Academia, Prague. 29–35.

Hodek, I. (1970). Coccinellids and the modern pest management. Biosci. *20*, 543 - 552.

Hussien, M.Y., Tan, H.S. and Rizvi, M.Z. (1986). Biology, Nutritional Requirement and Predation Efficiency of *Menochilus sexmaculatus* Fab.Coleoptera: Coccinellidae, Proceedings of 2nd International Conference of Plant protection in Tropics. 380–389.

Luckman, W.H. and Metcalf, R.L. (1978). The pest management concept. Introduction to Insect Pest Management. John Willey & Sons, New York.

Mayadunnage, S., Wijayagunasekara, H.N.P., Hemachandra, K.S. and Nugaliyadde, L. (2007). Predatory Coccinelids (Coleoptera:Coccinellidae) of vegetable Insect pests: A survey in Mid-country of Sri Lanka. Trop. Agric. Res. *19*, 69 - 77.

Michaud, J.P. and Grant, A.K. (2005). Suitability of pollen sources for development and reproduction of *Coleomegilla maculata* (Coleoptera: Coccinellidae) under stimulated drought conditions. Biol. Control. *32*, 363 - 370.

Richards, D.R. and Evans, E.W. (1998). Reproductive responses of aphidophagous lady beetles (Coleoptera: Coccinellidae) to non-aphid diets: An example from alfalfa. Annual of Entomology Society of America. *91*, 632 - 640.

Srivastava, K.M.N. and. Singh, L.N. (1986). A review of the pest complex of Kharif pulses in U.P. PANS*28(3),* 333 - 355.