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# A simplified technique for mass multiplication of *Micromus igorotus* Banks (Neuroptera: Hemerobiidae) on sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner

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**ABSTRACT:** Investigations were made for identifying laboratory host, ovipositional substrate, adult and larval containers for laboratory mass multiplication of *Micromus igorotus* Banks at the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad. Among the different hosts evaluated, *Aphis gossypii* Glover was the most suitable as it recorded the highest feeding potential (733.62 aphids / adult) and fecundity (837.96 eggs), followed by *Ceratovacuna lanigera* Zehntner and *Uroleucon carthami* (Hille Ris Lambers) except in terms of adult longevity which was longer on *C. lanigera*. Among the four different ovipositional substrates provided for egg laying, maximum numbers of eggs were laid on cotton (784.74 eggs / female) and thread (774.42), followed by corrugated brown paper (340.2) and black card sheet (335.8). When black card sheet and corrugated brown paper were provided, 19.32 and 23.93 per cent eggs were laid on extra ovipositional substrates respectively, which were difficult to harvest. For adult rearing, plastic container I [18.75 cm (ht) x 9 cm (dia m)] or container II [25 cm (ht) x 11.25 cm (dia m)] with 25 pairs per container can be used to harvest the maximum number of eggs. For larval rearing, plastic box with ventilated lid [10.00 cm (ht) x 25.50 cm (dia m)] with 75 larvae per container recorded significantly higher per cent pupation (92.89) and adult emergence (83.26).

**KEY WORDS:** Mass multiplication, *Micromus igorotus*

## INTRODUCTION

The sugarcane woolly aphid (SWA), *Ceratovacuna lanigera* Zehntner, has become a major production constraint in Maharashtra (Patil, 2003), Karnataka (Patil *et al.*, 2003) and Tamil Nadu (PDBC 2005). Out of 52 natural enemies recorded (Puttannavar *et al.*, 2005; Joshi and Viraktamath, 2004), 12 have been encountered in Karnataka (Puttannavar *et al.*, 2005). Of these *Dipha aphidivora* (Meyrick), *Micromus igorotus* Banks and syrphids were found to play a predominant role in the suppression of SWA. *Micromus igorotus* has been identified as a potential predator of SWA in India (Lingappa *et al.*, 2004). Scanning through the literature revealed no information on the feeding potential and the mass multiplication of *M. igorotus*. For the mass production of the hemerobiids *viz.*, *Micromus numerosus* Navas, *Micromus angulatus* (Stephens) and *Micromus linearis* (Hagens), cotton has been used as the ovipositional substrate (Sato and Takada, 2004). But, the information on the rearing containers and density of adults is meager. In view of the great potentiality of *M. igorotus*, it became obligatory to develop mass multiplication technology for the utilization of the predator in the sustainable management of SWA.

## MATERIALS AND METHODS

The studies were carried out in the Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Dharwad from September 2003 to June 2005. The nucleus culture of the predator obtained from the field was maintained in laboratory on SWA.

The adult foods evaluated were 10 per cent honey fortified with Proteinex®; sugarcane woolly aphid (*C. lanigera*), cotton aphid (*Aphis gossypii* Glover) and safflower aphid [*Uroleucon carthami* (Hille Ris Lambers)]. A pair of freshly emerged adults was released into a plastic container (12.5 (ht) x 6.25 (dia m) cm) containing treatment foods. Known numbers of aphids were provided every day and unfed aphids were counted before replacement. Adults were transferred carefully using a plastic vial (7.5 cm (ht) x 2.5 cm (dia m)) to fresh containers containing respective food and thread strings hung along inner wall of container neck for every 24 hours. The number of eggs laid on threads was counted at every change till death of adults. Observations on number of eggs laid, number of aphids consumed and the adult longevity were taken and the data were analysed for analysis of variance after transformation.

Ovipositional substrates evaluated were thread [15 cm (l) & 0.155 cm (t)], cotton [5 cm (l) x 3 cm (w) & 0.198 mm (t)], corrugated brown paper [20 cm (l) x 3 cm (w) & 0.33 mm (t)] and black card sheet [20 cm (l) x 3 cm (w) & 0.13 mm (t)]. A pair of freshly emerged adults was released into a plastic container with these substrates. Sexing was done based on the size of adult and shape of abdomen (female is larger with bulged abdomen). Thread was hung from the top along the inner side of mouth; corrugated brown paper was loosely folded in a zigzag manner while the black card was placed in a lunar shape (Plate 1). SWA infested leaf bit (7cm) with lower end inserted in glass vial (3.5 cm (l) x 2.5 cm (dia m)) containing water plugged with cotton to maintain leaf turgidity was provided as food. The container mouth was covered with muslin cloth and fastened with rubber band. Adults were carefully transferred to fresh containers containing food and substrates every day. Eggs laid on ovipositional substrates and extra ovipositional substrates were recorded daily till the death of adults. Pre-oviposition, oviposition and adult longevity periods were also recorded. Each treatment was replicated ten times. The percent eggs laid on ovipositional and extra ovipositional substrates were worked out and the data subjected to factorial analysis.

In order to standardize the density of adult predators per ovipositional container and the container volume for maximization of egg laying, two container sizes *viz.*, 18.75 cm (ht) x 9.00 cm (dia m) and 25.00 cm (ht) x 11.25 cm (dia m) and four densities *viz.*, 12, 25, 37 and 50 pairs of adults per plastic container were evaluated with five replications. SWA as food and thread as ovipositional substrate were provided as described earlier. Eggs deposited on threads from different treatments were recorded till death of the adults. The data obtained were subjected to test of significance to discriminate the superiority of treatment.

Similarly for larval rearing, container size and density of larvae per rearing container were evaluated. The containers evaluated were plastic basin 8.75 cm (ht) x 27.50 cm (dia m) covered with muslin cloth and plastic boxes with ventilated lid of three dimensions, *viz.*, 6.25 cm (ht) x 19.00 cm (dia m); 8.00 x 21.00 and 10.00 x 25.50 (Plate 2). Each of the containers with 25, 50, 75 and 100 larvae were evaluated in 9 replications for judging the suitability of container type and size for maximization of adult harvest. Sugarcane leaf bits (15 cm) infested with SWA were provided as food. The larvae were transferred to fresh container with camel hairbrush daily at 09.00 hours. Leaf bits were changed twice daily (09.00 and 16.00 hours). When grubs were about to pupate (5$^{th}$ and 6$^{th}$ day), corrugated brown paper of 15.0 (l) cm x 12.5 (w) cm was provided for pupation to enhance pupation and easy collection of pupae. Number of pupae obtained and adult emerged in each of the treatments were counted

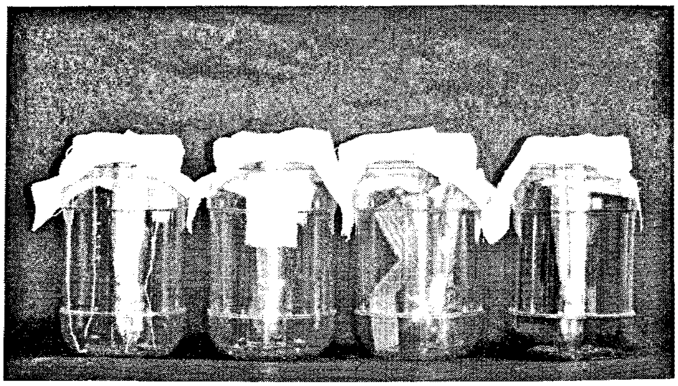

**Plate 1. Adult feeding and oviposition substrates**

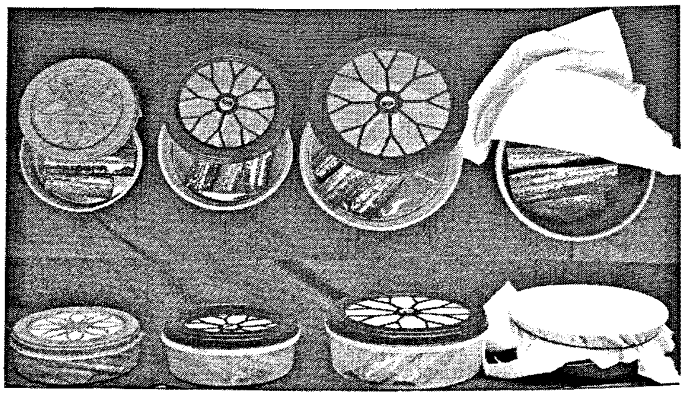

**Plate 2. Containers for larval rearing**

and percentage values were subjected to factorial analysis. Harvest Index (number of adults emerged) was worked out with reference to the number of larvae released per container.

## RESULTS AND DISCUSSION

*Micromus igorotus* adults survived significantly for a longer period when they were provided with alternate laboratory hosts (aphids) and laid fertile eggs (Table 1). In contrast, on honey they could not survive beyond 2.5 days and laid no eggs. Sugarcane aphid sustained the adult life for 45 days, followed by cotton aphid (41.2 days) and safflower aphid (35.9 days). The differences in longevity were non-significant between SWA and cotton aphid and were significant between safflower aphid, SWA and cotton aphid. During the survival period, the consumption rate by each adult was highest for cotton aphid (733.62) followed by SWA (461.60) and least for safflower aphid (206.1).

There was significant variation in fecundity when fed with different aphids. The highest fecundity of 837.96 eggs / female was recorded with cotton aphid, followed by SWA (774.44) and least with safflower aphid (309.60).

In terms of adult longevity, feeding potential and fecundity of brown lacewing adults, cotton aphid was found to be the most desirable host, followed by SWA. The highest number of cotton aphids consumed by *M. igorotus* adults may be attributed to the smaller size of these aphids, followed by SWA (medium size). Safflower aphid, being the biggest of the three species studied, met the quantum requirements of the predator by lesser number of aphids. The variation in longevity and fecundity when fed with different aphids may also be attributed to the nutritional status of the aphids. Therefore there is a scope for studying the impact of nutrition on the biological traits of predator. Continuous supply of cotton aphid cannot be ensured, hence SWA was found to be a very ideal host for the mass production of *M. igorotus*.

Maximum and perhaps full compliment of eggs were laid on cotton (784.74) and thread (774.44) followed by corrugated brown paper (340.2) and least on black card sheet (335.8) (Table 2). Cotton and thread proved to be superior substrates for egg laying and were on par with each other. The differences between corrugated brown paper and black card sheet were not adequate to exhibit substrate variability. The adults could lay about 76 - 80 per cent of the eggs on corrugated brown paper and black card sheet which can be easily harvested and the reminder on extra ovipositional substrate. This amounts to loss of about 20 - 24 per cent of eggs and this reduction over generations amplifies to a great extent. High fecundity and high survival are the two crucial issues in mass production of any bioagent.

During our extensive field studies, the hemerobiid predator laid eggs on spider webbing in sugarcane ecosystem (Plate 3). Thread was used to simulate the field situation and it proved to be an ideal substrate for maximum egg collection, easy harvest and minimizing space for handling them in the laboratory. The larval survival from the eggs laid on cotton was low compared to those from cotton. Under this context, use of cotton as a ovipositional substrate is limited. The results suggest that physical requirement for accepting a substrate for oviposition is an important consideration. To exploit the potentiality of egg laying under laboratory condition fully, hanging threads in plastic container along with

**Table 1. Influence of adult food on longevity, feeding potential and fecundity of *Micromus igorotus***

| Adult food | Adult longevity (Days)Mean ± SD | | | Feeding potential (aphids/adult) | Fecundity (Eggs/ female) |
|---|---|---|---|---|---|
| | Male | Female | Mean | Mean ± SD | Mean ± SD |
| 10 % honey fortified with proteinex R | - | - | 2.50$^{d}$± 1.29 | - | - |
| *Ceratovacuna lanigera* Zehntner | 46.68± 5.96 | 49.36± 4.13 | 45.02$^{a}$± 3.47 | 461.60$^{b}$± 43.53 | 774.44$^{ab}$± 189.31 |
| *Aphis gossypii* G. | 35.84± 4.96 | 46.56± 5.34 | 41.20$^{ab}$± 4.94 | 733.62$^{a}$± 97.49 | 837.96$^{a}$ ± 167.24 |
| *Uroleucon carthami* Theo. | 34.30± 8.56 | 37.50± 8.15 | 35.90$^{c}$± 6.58 | 206.10$^{c}$± 53.56 | 309.60$^{c}$± 125.49 |

Means in a column followed by the same alphabet(s) do not differ significantly by DMRT (P = 0.01)

**Table 2. Mean number of eggs laid on various oviposition substrates and adult longevity**

| Particulars | Number of eggs laid | | | |
|---|---|---|---|---|
| | Cotton | Thread | Corrugated brown paper | Black card sheet |
| Ovipositional substrates** 232.23[a] (100) | 784.74± 189.31[ab] (100) | 774.44± 155.56[c] (76.07) | 340.20± 75.40[c] (80.68) | 335.80± |
| Extra ovipositional substrates | | | | |
| 1. Box | - | - | 55.00 (12.29) | 34.40 (8.26) |
| 2. Cotton | - | - | 31.00(6.94) | 23.40(5.62) |
| 3. Leaf | - | - | 20.00(4.47) | 12.20(2.95) |
| 4. Cloth | - | - | 1.00(0.23) | 10.40(2.49) |
| Total | | 602.05 | 447.25 | 416.20 |
| Pre oviposition period (Days*) | | 4.5 ± 0.51 | | |
| Adult longevity (Days*) | | | | |
| Male | 27.90 ± 7.23 | 28.40 ± 6.18 | 34.60 ± 9.23 | 29.00 ± 5.70 |
| Female | 35.90 ± 12.57 | 35.80 ± 11.78 | 45.60 ± 2.70 | 41.00 ± 2.90 |

Figures in parentheses are per cent of total eggs laid; * Mean ± SD; ** Means in a row followed by the same alphabet(s) do not differ significantly by DMRT (P = 0.01)

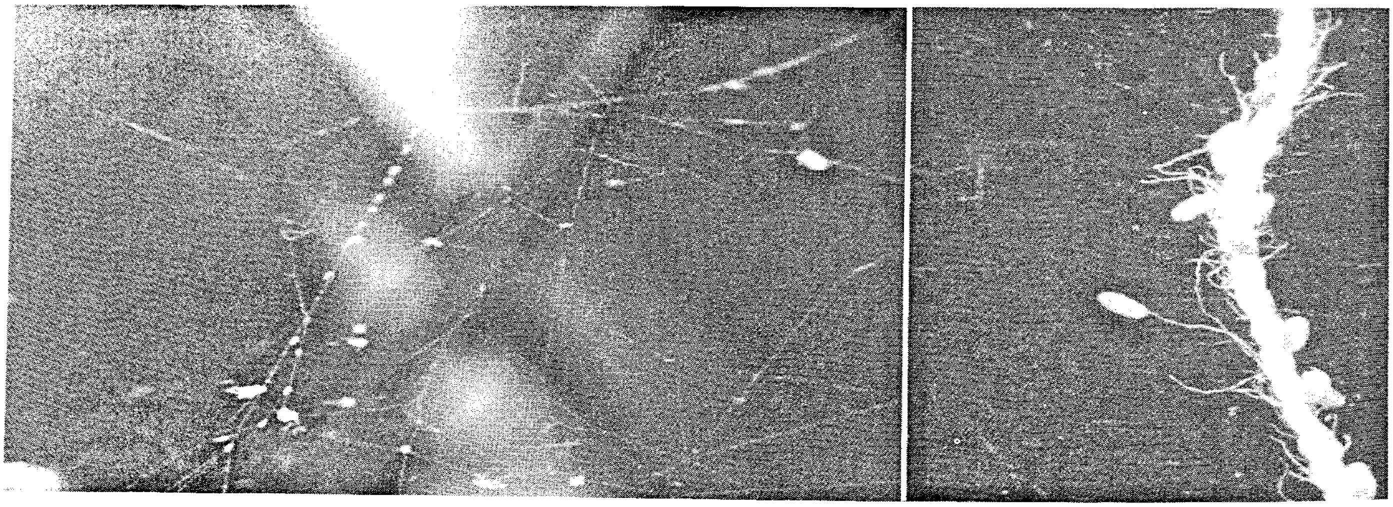

**Plate 3. Eggs laid on spider web (field) and thread (lab)**

SWA as food was most ideal at least cost, easy availability and good larval recovery.

Significantly highest and on par number of eggs per female was harvested at a density of 12 pairs per container (74.76) and 25 pairs per container (74.23) (Fig. 1). The egg laying efficiency declined with increase in density and was significantly least at 50 pairs. These results indicate negative effect of crowding beyond 25 pairs for a given volume of oviposition container.

Among the two sizes of plastic containers evaluated, the number of eggs laid by each female was higher in plastic container II (62.88) as against in plastic

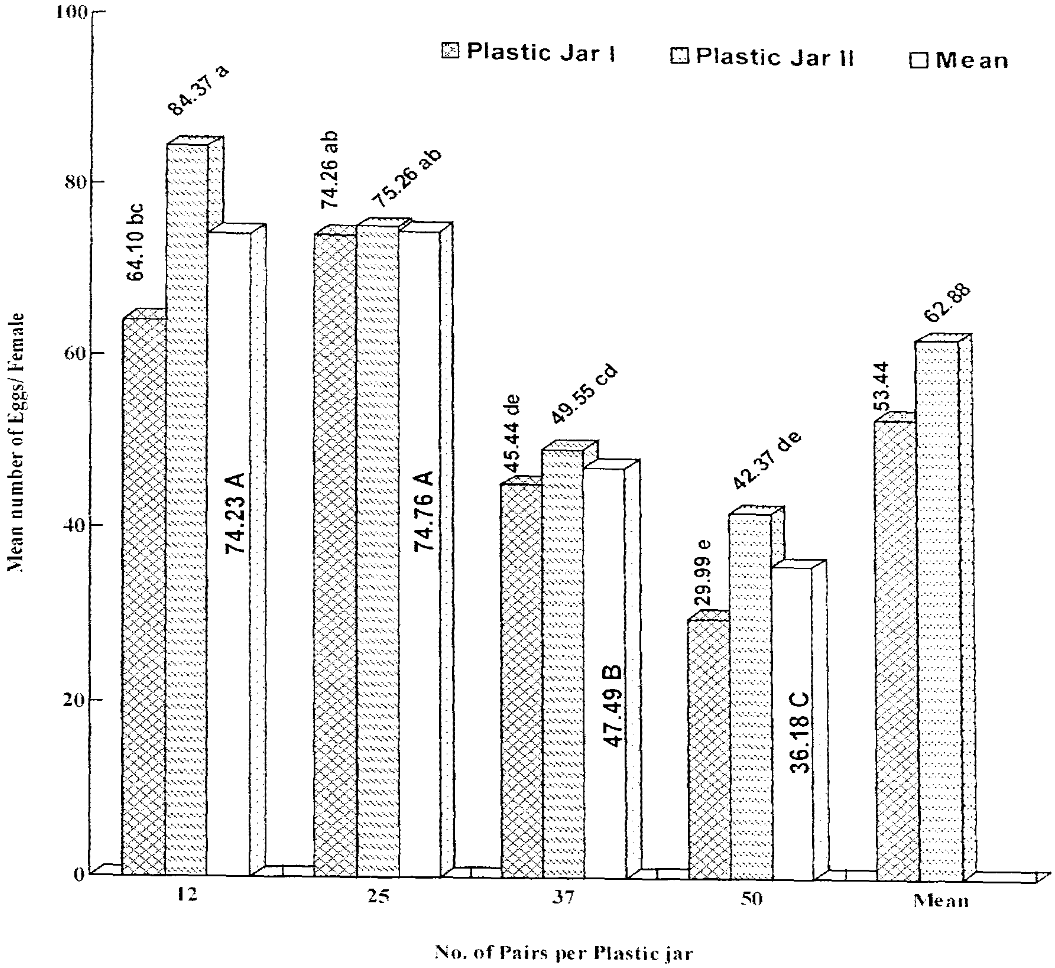


**Fig 1. Evaluation of containers and density of adults for maximization of oviposition**

container I (53.44). However, the predator density influenced the effect of container sizes on egg laying. The difference between the two container sizes was statistically non-significant at densities higher than 12 pair per container. From these results it is inferred that releasing 25 pairs per container using either container I or II is ideal to harvest maximum number of eggs.

Irrespective of the containers, survival of larvae and successful transformation to pupae (78.41) was significantly highest at density 75 larvae /box, over other three densities, which however, were on par with each other (Table 3). Similarly ventilated box III proved to be the best container to yield highest percentage pupation (88.39), followed by box II. The plastic tray and box I appeared to be poor rearing containers as revealed from lower pupation percentage. Thus, for higher pupal harvest, box III [10.00 cm (ht) x 25.50 cm (diam)] with 75 larvae was appropriate.

Significantly higher percent adult emergence (77.43) was recorded in box III, regardless of larval density, followed by box II (61.02). Box I and plastic tray performing at par, recorded significantly lowest percent adult emergence (Table 3). Larval densities of 75, 100 and 50 per box did not differ in terms of percent adult emergence, however, at lowest larval density, adult emergence was least. Though 75 larvae / box proved to be the best with significantly higher pupation, similar effect was not exhibited on adult emergence. Increase in adult emergence was not commensurate with increase in pupation percentage. These observations suggest that pupal mortality and / or healthy adult emergence was affected at higher densities.

**Table 3. Influence of container size and density of larvae on per cent pupation, per cent adult emergence and harvest index**

| Density / Container | 25 | | 50 | | 75 | | 100 | | Mean | | Harvest Index |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Per cent | | | | | | | | | | |
| | Pupation | Adult emergence | Pupation | Adult emergence | Pupation | Adult emergence | Pupation | Adult emergence | Pupation | Adult emergence | |
| Ventilated lid box -I | 69.78$^{efg}$ (17.44) | 45.78$^{f}$ (11.44) | 68.00$^{efg}$ (34.00)$^{f}$ | 46.00 (23.00) | 71.11$^{efg}$ (53.33) | 46.81$^{f}$ (35.11) | 62.11$^{g}$ (62.11) | 45.56$^{f}$ (45.56) | 67.75$^{C}$ (41.72) | 46.04$^{C}$ (28.78) | 0.46$^{C}$ |
| Ventilated lid box- II | 82.22$^{bcd}$ (20.56) | 66.22$^{cd}$ (16.56) | 73.56$^{def}$ (36.78) | 59.33$^{de}$ (29.67) | 76.74$^{cde}$ (57.56) | 61.63$^{de}$ (46.22) | 74.78$^{cdef}$ (74.78) | 56.89$^{de}$ (56.89) | 76.82$^{B}$ (47.42) | 61.02$^{B}$ (37.33) | 0.61$^{B}$ |
| Ventilated lid box- III | 87.11$^{ab}$ (21.78) | 72.44$^{bc}$ (18.11) | 84.67$^{abc}$ (42.33) | 76.22$^{ab}$ (38.11) | 92.89$^{a}$ (69.67) | 83.26$^{a}$ (62.44) | 88.89$^{ab}$ (88.89) | 77.78$^{ab}$ (77.78) | 88.39$^{A}$ (55.67) | 77.43$^{A}$ (49.11) | 0.77$^{A}$ |
| Plastic tray | 49.33$^{h}$ (12.33) | 24.44$^{g}$ (6.11) | 64.89$^{fg}$ (32.44) | 54.44$^{ef}$ (27.22) | 72.89$^{def}$ (54.67) | 55.41$^{ef}$ (41.56) | 69.33$^{efg}$ (69.33) | 60.33$^{de}$ (60.33) | 64.11$^{C}$ (42.19) | 48.66$^{C}$ (33.81) | 0.49$^{C}$ |
| Mean | 72.11$^{B}$ (18.03) | 52.22$^{B}$ (13.06) | 72.78$^{B}$ (36.39) | 59.00$^{A}$ (29.50) | 78.41$^{A}$ (58.81) | 61.78$^{A}$ (46.33) | 73.78$^{B}$ (73.78) | 60.14$^{A}$ (60.14) | | | |
| Harvest Index | 0.52$^{B}$ | 0.59$^{A}$ | 0.62$^{A}$ | 0.60$^{A}$ | | | | | | | |

Figures in parentheses are the number of pupae formed adults emerged

Harvest index was significantly highest in box III over others. The harvest index remained the same at densities 50, 75, and 100 and was the lowest at 25, therefore, any density over 25 is suitable (Table 3). However, as the numbers of adults obtained were in proportion to the number of larvae released, 100 per box III was considered as optimum number for highest productivity, lower spacial and labour requirement. With increase in the size of the ventilated lid boxes, there was a linear increase in the percent pupation, adult emergence and harvest index, providing scope for further investigations on the higher dimensions of ventilated lid boxes for larval rearing in the mass multiplication of *M. igorotus*. The poor performance of plastic tray may be due to improper ventilation compared to the ventilated lid boxes and possibly loss of energy leading to death in search of food, which was scattered in plastic tray. During the study no cannibalism was observed in adult, first and second larval stages, however, it was evident in third instar under scarcity of food.

From the studies, it is concluded that *A. gossypii* is the most desirable adult food followed by *C. lanigera* with longest adult longevity, feeding potential and fecundity. Hanging of thread strings emerged as the most ideal ovipositional substrate. An adult density of 25 pairs per container resulted in maximum egg yield per female. Ventilated lid box III with a density of 100 larvae can be deployed for the mass multiplication of the predator on sugarcane woolly aphid.

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