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Mass Rearing of Mealybug Predator, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) on two Mealybug Species, *Planococcus minor* and *Pseudococcus viburni*

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ABSTRACT

Mealybug predator, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is an effective biocontrol agent against various species of mealybugs. This study was aimed to develop a protocol for mass production of *C. montrouzieri* with the aid of two mealybug host species; *Planococcus minor* and *Pseudococcus viburni*, under laboratory conditions. The predator was reared on *P. minor* and *P. viburni*, using two substrates; local pumpkin (*Cucurbita moschata*) and Malaysian pumpkin (*Cucurbita maxima*). The incubation period of eggs, durations of first instar larvae and pupae of *C. montrouzieri* were significantly different with two host species ($p<0.05$). Production of *C. montrouzieri* was significantly vary ($F_{(1,12)} = 75.32$, $p<0.001$) with the two pumpkin varieties. Production of *C. montrouzieri* was significantly higher with local pumpkins; *C. moschata* ($t_{(14)} = 6.11$; $p<0.05$). The results indicated that, by starting the culture with 20 females and 15 males of *C. montrouzieri*, an average of 300.3 ± 41.8 and 180.4 ± 36.5 *C. montrouzieri* adults can be produced using two pumpkin varieties: *C. moschata* and *C. maxima*, respectively (surface area 1000 cm^2). Production of *C. montrouzieri* was significantly varied with the number of grooves appear on the surface area (1000 cm^2) of the pumpkin variety, *C. maxima* with the two host *P. minor* ($R^2 = 0.9608$, $F_{(1,2)} = 49.06$, $p<0.05$) ($Y = 7.326*X + 41.02$) and *P. viburni* ($R^2 = 0.9470$, $F_{(1,2)} = 35.76$, $p<0.05$) ($Y = 15.14*X - 53.22$). Cost of production for a single *C. montrouzieri*, with the host *P. minor* reared on local pumpkin, *C. moschata* was found to be the lowest. It can be concluded that the selected mealybug species reared on *C. moschata* was more suitable for mass rearing the mealybug predator.

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INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) are soft-bodied insects, having piercing and sucking mouth parts. They feed on the cell sap and cause a considerable damage to a wide range of field and horticultural crops (Venkatesha and Dinesh, 2011). Control of mealybug by using conventional insecticides is difficult as the mealybugs are covered with a waxy material and its cryptic nature (Solangi *et al.*, 2012). The development of resistance against insecticides is a serious problem both under tropical and temperate climates (Ambule *et al.*, 2014). Even though farmers are managing the mealybug infestations in agricultural lands, the mealybug present in non-agricultural lands function as a source of reinfection to the agricultural fields. It is appropriate to manage the mealybug population in non-agricultural lands using a natural and sustainable approach, such as biological control.

Biocontrol approaches are getting more acceptable to the general public with the increasing awareness on ill-effects of insecticides including the pollution of soil, water and air. The effect of insecticides on human and environmental health is a great concern. (Prasanna and Balikai, 2016). Biological control of mealybugs over other approaches has several advantages such as the persistent effect of the bio-control agents, the sustainability of population reduction, and low cost. Moreover, Biological control of pest is considered as one of the fundamental tactics for pest suppression of an effective Integrated Pest Management program (Venkatesha and Dinesh, 2011).

Coccinellid beetles that are found predominantly in agro-ecosystems are largely used as biological control agents of insect pests of different crops by means of augmentation and release (Sahayaraj, 2002). *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is an effective biological control agent of mealybugs (Pak, 2011; Solangi *et al.*, 2012; Saljoqi *et al.*, 2015). The predator was firstly introduced in-to California, USA in 1982 to control *Planococcus citri* (Rashid *et al.*, 2012; Solangi *et al.*, 2012; Kairo *et al.*, 2013). The predatory beetle, *C. montrouzieri*, is commonly known as mealybug destroyer and it has received significant attention from researchers (Solangi *et al.*, 2012; Ambule *et al.*, 2014). Both adult and larvae of *C. montrouzieri* are voracious feeders and showed predatory behaviour on different stages of mealybugs (Solangi *et al.*, 2012; Kairo *et al.*, 2013). *C. montrouzieri* is largely used in indoor situations (i.e. Greenhouses, nurseries) (Ambule *et al.*, 2014). The recommendation for release in open field is 1000 adults/ha and 2-3 adults/m^2 in greenhouses (Abdollahi Ahi *et al.*, 2015).

C. montrouzieri has been widely used for mealybug control in many countries, eg. USA, Pakistan and India, particularly in greenhouse crops as well as on perennial fruit crops. This predator has proven its effectiveness to manage the mealybug populations under local conditions as well (Gunawardana and Hemachandra, 2017. Unpublished data). *C. montrouzieri* has been mass produced for biological control purposes over 100 years (Al-Khateeb *et al.*, 2012). Mass reared *C. montrouzieri*, has been marketed in more than 40 countries and it is considered as one of the most widely used biocontrol agents (Kairo *et al.*, 2013). The main issue with the augmentative release in pest control is the difficulty in mass producing of biocontrol agents economically (Sahayaraj, 2002). Selection of appropriate host is very important in mass rearing program of a natural enemy (i.e. predators, parasitoids) (Sahayaraj, 2002).

The objective of this study was to develop a mass production protocol for *C. montrouzieri* with the use of two selected mealybugs.

MATERIALS AND METHODS

This study was conducted at Department of Agricultural biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka during the year 2017.

Selection of host mealybug species

Two different mealybug species, *Planococcus minor* and *Pseudococcus viburni* were collected from different hosts around Gannoruwa. The cultures of selected mealybug species were reared on pumpkins under controlled conditions at a constant temperature of 28 ± 2 °C with 55-85% R.H throughout the study period in insect rearing rooms.

Confirmation of identities of mealybug species

Mealybug specimens were prepared as archival-quality slide mounts (Sirisena *et al.*, 2013) and observed the ventral and dorsal characteristics of adult female of each mealybug species using a compound microscope (10x40-60).

Pumpkins as host for mealybug cultures

Local pumpkin (*Cucurbita moschata*) and Malaysian pumpkin (*Cucurbita maxima*) (weighing 1-2 Kg) were used for rearing of host mealybug species. The mealybugs were reared in laboratory using ripe pumpkins, *C. maxima* following the method recommended by Chacko *et al.* (1978) and Singh (1978). Pumpkins were washed with tap water to remove soil and dust particles attached to the surface and pat dry by using tissue papers. Surface sterilization of pumpkins was done by using 70% ethanol to remove possible pathogens. Minor damaged and open spots on the pumpkin surface were covered by hot paraffin wax coating to prevent secondary infections and allowed to dry. Cleaned pumpkins were kept for 48 hours for removal adverse effect of ethanol on mealybugs. Pumpkins were air dried in good ventilated laboratory conditions (26–30 °C, R.H- 55-85%).

Inoculation of pumpkins with mealybugs

Egg masses (ovisacs) (35-40) of each mealybug species were taken from the initial cultures and placed on equally spaced places on dried pumpkin fruits separately, using wet fine haired paint brush. Pumpkins were placed on paper towel, which had been placed on paper plate. Paper towel may help to absorb the honeydew produced by the mealybugs and prevent damp conditions around the fruit. Inoculated pumpkins were examined regularly for any fungal infection, rotting or other issues like infestation of mites. Rotten and infested fruits were discarded.

Introduction of *C. montrouzieri* onto the mealybug developed pumpkins

The adults of *C. montrouzieri* were obtained from meaylbug infested cassava fields around Gannoruwa area, and reared on two mealybug species separately, under laboratory conditions (26–30 °C, R.H- 55-85%) using rearing cages (60 x 50 x 50 cm). Thirty-five (20♀ + 15♂) 20-day old adults of *C. montrouzieri* were introduced onto each fruit after 25-30 days of initial mealybug inoculation. Adults were sexed based on the morphological characters. Ten percent (10%) bee honey solution, smeared on a filter paper was supplied as additional feed for the adult *C. montrouzieri*. Water soaked cotton swabs were placed in rearing cages to maintain the humidity. The larval excrements were cleaned daily. Monthly about 20 new field collected *C. montrouzieri* adults (both males and females) were added to the new production cycles to reduce the incidence of the adverse effect occurring due to inbreeding.

Harvest of the predatory beetles

After about 28 ± 1 day from introduction of predators, newly emerged beetles were collected using an aspirator. Rearing cages were examined daily for adult emergence and emerged adults were collected. Total adult mortality was determined by counting the dead adults in the rearing cage. The sex ratio was calculated for each treatment from the emerged adults. After emerging adult beetles, the rearing system was kept for 20 days to facilitate the mating of newly emerged beetles.

Temporary store of recovered adult beetles

The *C. montrouzieri* adults were released in to 1 L flask immediately after collected with an aspirator from the mass rearing cages and fed with 10% honey solution. Corrugated paper stripes were kept inside the flask for facilitating the movement of individual beetles. In certain situations, temporary storage is necessary until the field releases are get arranged.

Removal of deteriorated pumpkin fruits

In the case of deterioration of pumpkin substrate within the period of larval development of *C. montrouzieri*, all larval instars, pupae and adults were transferred on to newly infested pumpkin of the same mealybug species. Larval stages were collected with the aid of fine haired paint brush and adult beetles were collected with an aspirator.

Cost analysis for mass rearing of *C. montrouzieri*

Cost of production (COP) was worked out separately for all treatments. Various parameters like; insectary facilities, equipment required for production, capital investment were considered to determine the COP.

Development of *C. montrouzieri* on two mealybug species

Developments of *C. montrouzieri* on two mealybug species were recorded separately on daily basis with the aid of microscope. The egg, larval and pupal developmental periods and adult emergence on two host mealybug species were recorded. The colour and shape of egg, larvae and pupae were observed. The size of the eggs and larvae of *C. montrouzieri* were measured under microscope.

Oviposition preference of *C. montrouzieri* towards two mealybug species

Adult female beetles were confined to plastic petri dishes (diameter 16 cm, height 4.5 cm) after premating and pre-oviposition period to examine oviposition on two mealybug species, *P. minor* and *P. viburni*, separately (n=18). Twelve egg masses from each mealybug species were placed in one replicate. Number of eggs laid after 24 h were counted by using a binocular microscope (Vinutha, 2011).

Data analysis

The data were analyzed using two-way ANOVA, followed by determining the significant differences between means by Student's t-test at $p<0.05$ using Minitab statistical software (version 18). Regression analysis was done by using Graphpad Prism 8 software.

RESULTS AND DISCUSSION

(a) Developmental duration for *C. montrouzieri* reared on two mealybug species; *P. minor* and *P. viburni*

The period between the egg laying and the egg hatching was considered the developmental period of *C. montrouzieri* eggs. Moulting of *C. montrouzieri* individuals were confirmed by the presence of casted-off skin (exuviae) of larva. The duration between formations of pupa to adult emergence was considered the pupal period. The host insect species, *P. minor* and *P. viburni* significantly affected on the development of several life stages of *C. montrouzieri* as observed with development durations. The incubation period of eggs ($t_{(40)} = 6.09$; $p<0.05$), durations of first instar larva ($t_{(40)} = 7.42$; $p<0.05$) and pupa ($t_{(28)} = 2.42$; $p<0.05$) were significantly different with the host species; *P. minor* and *P. viburni*. Incubation period and pupal period were longer when *C. montrouzieri* was on *P. viburni;* however, first instar larva grew faster when on *P. viburni.* Adult of *C. montrouzieri* spent nearly one day in the pupal case after emergence. Total developmental period of *C. montrouzieri* (eggs to adults) on two mealybug species; *P. minor* and *P. viburni* was significantly different ($t_{(28)} = 2.74$; $p<0.05$). The development duration of all larval instars and other life stages are given in Table 1.

Table 1. Developmental duration for *C. montrouzieri* reared on mealybug species *P. minor* and *P. viburni* (n=15)

	Duration of development (days) (Mean ± SE)	
Stage/ period	*Planococcus minor*	*Pseudococcus viburni*
Incubation period	4.39 ± 0.10 [a]	5.30 ± 0.10 [b]
Larval period		
L1	5.34 ± 0.10 [a]	4.26 ± 0.10 [b]
L2	2.66 ± 0.18 [a]	3.06 ± 0.18 [a]
L3	3.06 ± 0.24 [a]	3.21 ± 0.15 [a]
L4	4.20 ± 0.14 [a]	4.53 ± 0.21 [a]
Total Larval period	15.2 ± 0.22 [a]	14.86 ± 0.29 [b]
Pupal period	9.73 ± 0.52 [a]	11.80 ± 0.67 [b]
Total developmental period (egg-adult)	29.26 ± 0.61 [a]	32.06 ± 0.81 [b]

*means accompanied by the same letter within a row are not significantly different

The general morphology of immature *C. montrouzieri* was observed and no clear differences of morphological characteristics were observed. The freshly laid eggs (0.71 ± 0.01 mm) were pale yellowish, white, smooth and cylindrical, both ends smoothly rounded. The first instar larva (1.21 ± 0.01 mm) was tiny grub, pale greyish with white lines across the body along intra segmental regions. Second instar larvae (4.46 ± 0.16 mm) was oval in shape and greyish in colour and the larvae was flat and slightly convex dorsally and wax strands could be observed. The third instar larvae (5.92 ± 0.10 mm) was similar to the general appearance of second instar larva except in size. In the third instar larva the wax strands were little larger than that of the 2nd instar. The 4th instar larvae (8.08 ± 0.09 mm) was similar in general appearance to the 3rd instar larvae, excluding larger in size and wax strands are little longer than 3rd instar. The pupa (8.03 ± 0.12 mm) was fully covered dorsally with the white waxy filaments and the larval exuvium. The newly emerged adults were soft, reddish yellow in colour. Males (3.82 ± 0.04 mm) were smaller in size than females (4.01 ± 0.03 mm) and the first pair of legs in male beetles was brown and the other two pair being black, whereas in the female, all the three pairs were black in colour. The last abdominal segment of male beetle was round, while in female, it was pointed. Similar morphological parameters were also reported by Mali and Jeevan (2008) and Siddhapara *et al.* (2013).

(b) Oviposition of newly emerged adults of *C. montrouzieri* on two mealybug species; *P. minor* and *P. viburni*

The *C. montrouzieri* female preferred egg sacs of mealybugs for oviposition. The number of eggs laid by the newly emerged, mated adult *C. montrouzieri* on two mealybug species; *P. minor* and *P. viburni* was significantly different ($p>0.05$). The average numbers of eggs laid by the newly emerged adult *C. montrouzieri* on *P. minor* and *P. viburni* were 8.17 ± 1.39 and 7.94 ± 1.24 eggs, respectively. The total number of egg clusters per female ranged from 2 to 28 and 1 to 17 by the newly emerged mated adults on *P. minor* and *P. viburni*, respectively under controlled conditions of 28 ± 2 °C and 55-85 % R.H.

(c) Comparative yield/ production of *C. montrouzieri* on two mealybug species; *P. minor* and *P. viburni* on two pumpkin varieties

Pumpkins had been successfully used as host materials to mass rearing of mealybug species like *Pseudococcus comstocki* (Meyerdirk and Newell, 1979), *Planococcus citri* (Chandler *et al.,* 1980). Comparative yield/ production of *C. montrouzieri* were assessed based on the four treatments (Table 3).

Table 2. Different treatments evaluated using mass rearing protocol

Treatment	Description
T 1	*Planococcus minor* on local pumpkin (*Cucurbita moschata*)
T 2	*Planococcus minor* on Malaysian pumpkins (*Cucurbita maxima*)
T 3	*Pseudococcus viburni* on local pumpkin (*Cucurbita moschata*)
T 4	*Pseudococcus viburni* on Malaysian pumpkins (*Cucurbita maxima*)

The pumpkin varieties had a significant effect ($F_{1,12)}$ = 75.32, $p<0.001$) on production of *C. montrouzieri*, but not the host insect species: *P. minor* or *P. viburni.* However, the interaction between the host insect and the host substrate was significant ($F_{(1,12)}$ = 16.21, $p<0.05$). This may due to the differences in host mealybug quality which reared on different pumpkin varieties. Production of *C. montrouzieri* was significantly higher with local pumpkins; *Cucurbita moschata* ($t_{(14)}$ = 6.11; $p<0.05$). The average of 300.3 ± 41.8 *C. montrouzieri* adults were obtained with the use of local pumpkin variety, *C. moschata* (surface area 1000 cm^2). The average of 180.4 ± 36.5 *C. montrouzieri* adults were obtained with the use of Malaysian pumpkin variety, *C. maxima* (surface area 1000 cm^2). Male and female sex ratio was observed as 1:1 in all treatments. However, Pak (2011) has reported that, the total yield of 246 ± 15 adults *C. montrouzieri* could be obtained from an initial release of ten predatory beetles on 25 old colony of mealybug, raised by releasing 50 ovipositing females of *P. citri* on pumpkin. Present findings are not in agreement with Chacko, *et al.* (1978) and Singh (1978) who reported a yield of 100 to 200 adult *C. montrouzieri* beetles per ovipositing cage having culture of *Planococcus* sp. irrespective of its host. This difference may be due to the variation in rearing conditions or due to difference in host mealybug species used. During the production cycle average adult mortality were 12.06%, 15.88%, 20.16% and 15.32% for treatment 1, 2, 3 and 4, respectively.

Regression analysis was used to test the production of *C. montrouzieri* using *P. minor* and *P. viburni* on two pumpkin varieties with reference to the surface area. The results indicated that the *C. montrouzieri* production significantly varied with the surface area of the pumpkin variety, *C. moschata* and with the host mealybug species, *P. minor* (R^2= 0.9901, $F_{(1,2)}$ = 199.6, p<0.05) (Y = 0.3415*X - 6.854) (Figure 1a). The *C. montrouzieri* production was not significantly varied with the surface area of the pumpkin variety, *C. moschata* and with the host mealybug species, *P. viburni* (p>0.05) (Y = 0.1387*X + 74.96) (Figure 1b). Further *C. montrouzieri* production was significantly varied with the surface area of the pumpkin variety, *C. maxima* and with the host mealybug species, *P. minor* (R^2= 0.9424, $F_{(1,2)}$ = 32.74, p<0.05) (Y = 0.2127*X - 56.90) (Figure 1c). And also, the *C. montrouzieri* production was not significantly varied with the surface area of the pumpkin variety, *C. maxima* and with the host mealybug species, *P. viburni* (p>0.05) (Y = 0.2723*X - 65.84) (Figure 1d). This may be due to the nature of colonization of mealybug species on pumpkin's surfaces. It was observed that *P. viburni* show more scattered growth nature on pumpkin varieties compare to that of *P. minor* which was shown more even growth on the pumpkin surfaces. Thus, significant production of *C. montrouzieri* may be observed with the *P. minor* with the increasing surface area of two pumpkin varieties.

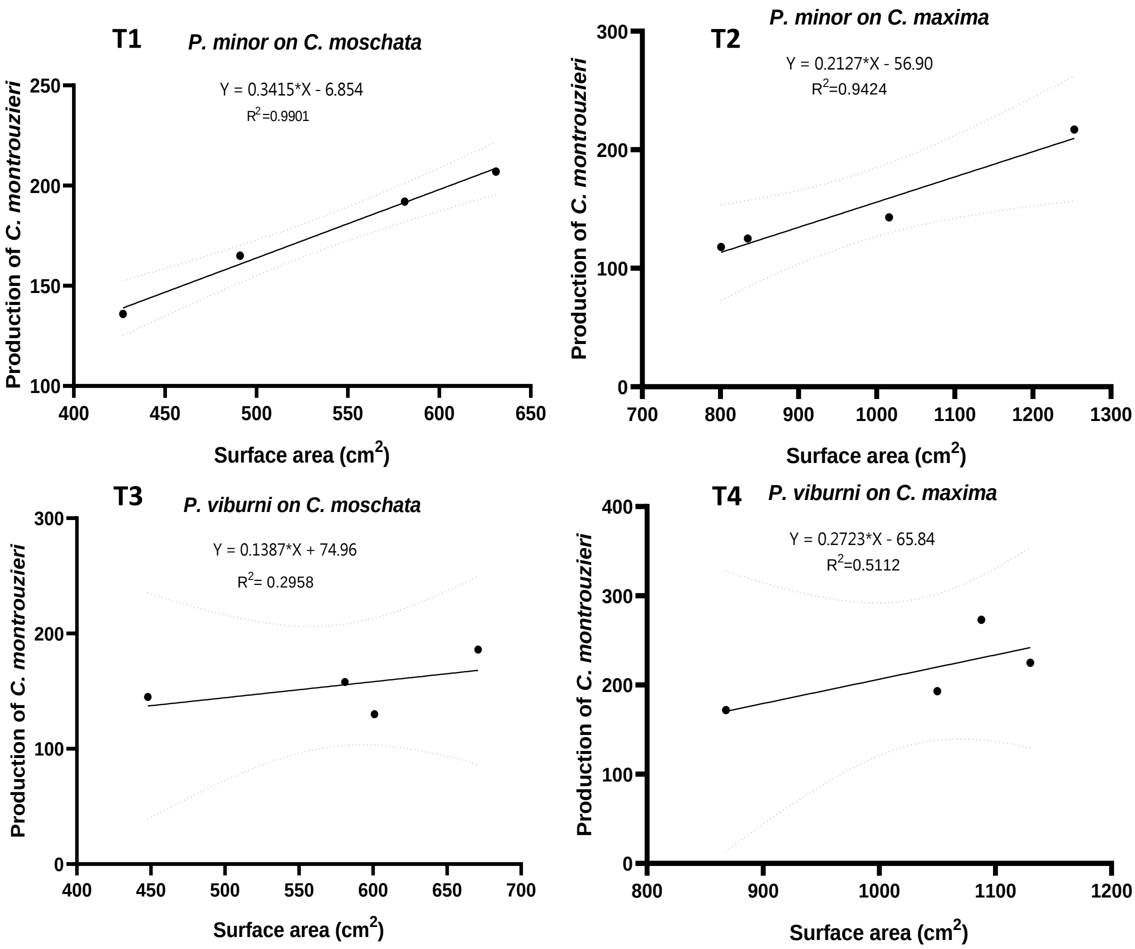

Figure 1. Relationship of *C. montrouzieri* production/ yield with the surface area of pumpkins which reared *P. minor* and *P. viburni*.

The production of *C. montrouzieri* was also tested with the number of grooves appear on the surface of the Malaysian pumpkin; *C. maxima*. The results of the regression indicated that the *C. montrouzieri* production was significantly varied with the number of grooves appear on the surface of the Malaysian pumpkin variety, *C. maxima* with both the host mealybug species; *P. minor* (R^2= 0.9608, $F_{(1,2)}$ = 49.06, p<0.05) (Y = 7.326*X + 41.02) and *P. viburni* (R^2= 0.9470, $F_{(1,2)}$ = 35.76, p<0.05) (Y = 15.14*X – 53.22) (1000 cm^2 surface area) (Figure 2).

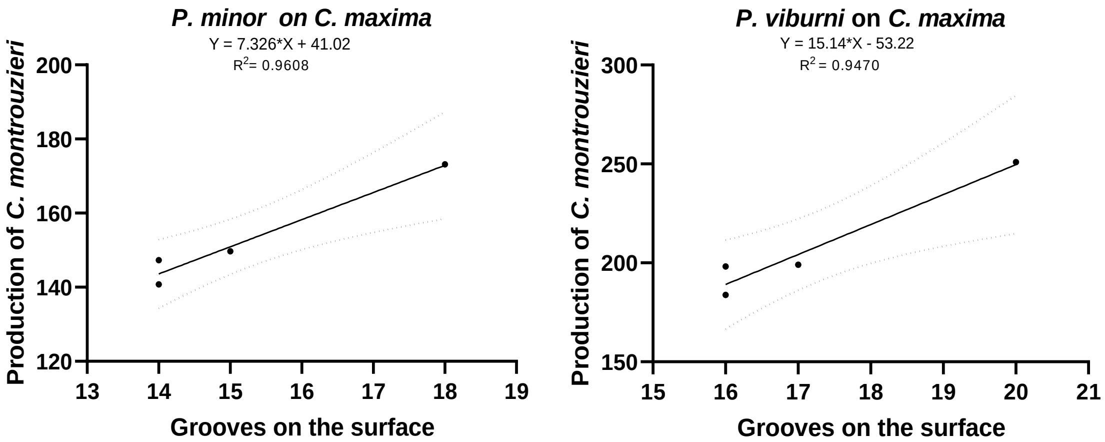

Figure 2. Relationship of *C. montrouzieri* production with the no. of grooves present on Malaysian pumpkin; *Cucurbita maxima* for two mealybug species (1000 cm^2 surface area).

(d) Cost of production related to *C. montrouzieri* mass rearing

Average variable cost for producing one beetle were Rs. 6.02, Rs. 11.37, Rs. 7.30 and Rs. 8.56 for treatment 1, 2, 3 and 4, respectively. Average fixed cost for producing one beetle were Rs. 18.09, Rs. 38.89, Rs. 21.08 and Rs. 28.55 for treatment 1, 2, 3 and 4, respectively. Average total cost for producing one beetle were Rs. 24.11, Rs. 50.62, Rs. 29.10 and Rs. 37.11 for treatment 1, 2, 3 and 4, respectively. The cost of production for a single predatory beetle *C. montrouzieri*, with mealybug species *P. minor* reared on local pumpkin; *C. moschata* was found to be the lowest (Rs. 24.11). The cost of production for a single predatory beetle *C. montrouzieri*, with mealybug species *P. minor* reared on Malaysian pumpkin; *C. maxima* was found to be the highest (Rs. 50.62) (Figure 3).

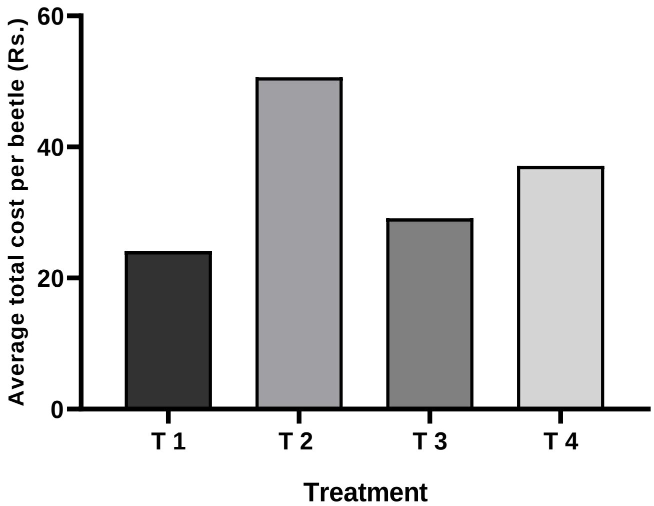

Figure 3. Average total cost associate for a *C. montrouzieri* mass rearing for different treatments. See Table 2 for treatment details.

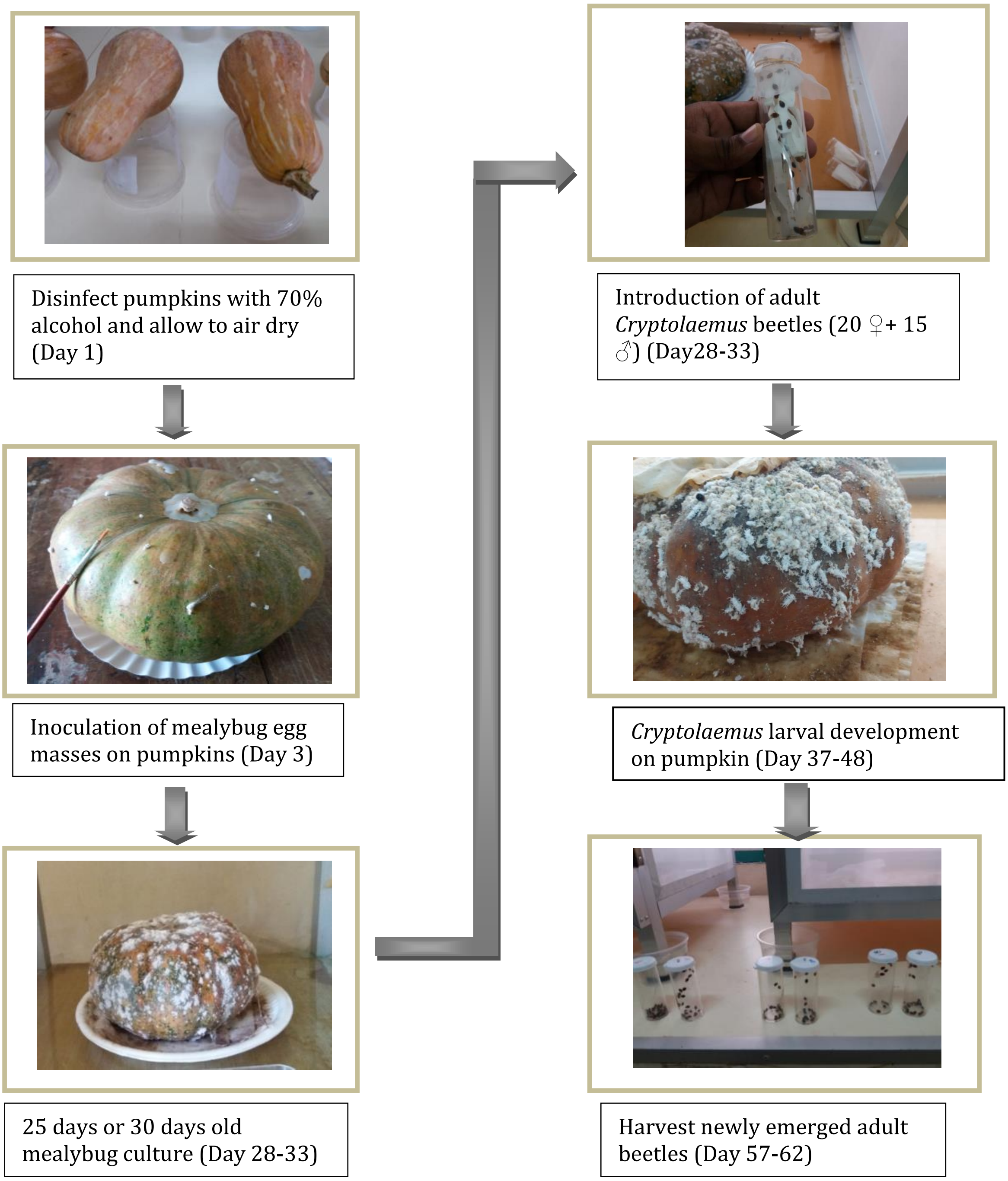

Figure 4. Chronological steps in the mass rearing of *C. montrouzieri* on pumpkin.

CONCLUSIONS

The present findings showed that the mealybug predator, *C. montrouzieri* can be easily mass reared on mealybug species; *P. minor* and *P. viburni* cultured on pumpkins. Production of *C. montrouzieri* was significantly higher with the protocol by the use of local pumpkins; *Cucurbita moschata*. The average of 300.3 ± 41.8 *C. montrouzieri* adults could be obtained with the use of local pumpkin variety, *C. moschata* (surface area 1000 cm^2). It was found that the cost of production for a single predatory beetle *C. montrouzieri*, with mealybug species *P. minor* reared on local pumpkin; *C. moschata* was found to be lowest (Rs. 24.11).

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