BIOLOGY AND RELEASE OF EXOTIC PREDATOR *CRYPTOLAEMUS MONTROUZIERI* MULSANT ON MEALYBUG *PHENACOCCUS SOLENOPSIS* TINSLEY AT TANDOJAM

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ABSTRACT

Biology of exotic predator, Cryptolaemus montrouzieri Mulsant (Coleoptera: Cocccinellidae) was studied on exotic mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) in controlled laboratory conditions (28±2^{0}C, 65±5 RH% and L: D, 8: 16 h) at IPM laboratory, Tandojam. In laboratory pre-mating, pre-oviposition and oviposition of C. montrouzieri was 5.6±0.08, 9.9±0.19 and 76.4±1.30 days, respectively. The fecundity was 486.9±1.417 per female. The incubation period was 5.3±0.07 days. The oval, cylindrical, pale yellowish eggs were laid by females singly or in groups in ovisacs of the mealybug. After hatching from the eggs the larvae developed through four instars. Mean developmental period for the first instar was 5.9±0.15 days, and the larvae were smooth, pale grey, and gradually increased in size. Mean developmental periods for 2nd, 3rd and 4th instars were 5.0±0.16, 7.0±0.18 and 8.3±0.13 days, respectively, with a total larval development 26.2±0.61 days. The mean pre-pupal and pupal periods were 2.9±0.07 and 8.5±0.2 days, respectively. Total development period from egg to adult was 42.9±0.95 days. Mean longevities for male and female adults were 80.9±0.89 and 84.4±0.9 days, respectively. The copulation period for C. montrouzieri ranged from 5 to 48 minutes. The male mounted and dismounted over the female body and mated repeatedly throughout their life. The predator C. montrouzieri adults survival on different cotton mealybug densities in semi field conditions showed that 1, 2, 3, 4 and 5 pairs lived for 1.86, 2.33, 3.20, 3.80 and 3.47 days, respectively. The mortality of C. montrouzieri was significantly positive correlation 98% with temperature.

Key Words: **Biology, Release, Cryptolaemus Montrouzieri, Phenacoccus Solenopsis, Temperature**

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INTRODUCTION

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is an invasive polyphagous pest on in Pakistan (Abbas *et al*., 2005), in Nigeria (Akintola and Ande, 2008), in China (Wu and Zhang, 2009), Australia (Charleston *et al*., 2010) and in Iran (Moghaddam and Bagheri, 2010). Cotton mealybug is cottony in appearance, small, oval, soft-bodied, sucking insect covered with white mealy wax. It proliferates on field crops, fruits, vegetables and ornamental plants. Cotton mealybug has a broad host range and infested over 194 plants (Vennila *et al*., 2011). It sucks a large amount of cell sap from leaves and stems depriving plants of essential nutrients showing the retarded growth and total drying of the plant (Joshi *et al*., 2010). The cotton mealybug appeared as a major pest of commercial cotton (Tanwar *et al*., 2011) and yield losses due to this pest were estimated upto 50% (Joshi *et al*., 2010). Mealybug caused a reduction in cotton production equal to 1.3 million bales in Pakistan (Abdullah, 2009). *Phenacoccus solenopsis* female is parthenogenetic and can produce between 128 to 812 crawlers (Vennila *et al*., 2010). The potential distribution expanded dramatically, indicating that *P. solenopsis* presents a great economic threat to cotton in Asia and other parts of the world (Wang *et al*., 2010).

Chemical control of cotton mealybug with conventional insecticides is difficult as the pest is covered with the waxy material (Joshi *et al*., 2010). Early-chemical control efforts against *P. solenopsis* proved unsatisfactory in Texas, United States (Fuchs *et al*., 1991). Therefore, biological control of this pest is being tested in different regions of its invasive occurrence. The predator, *C. montrouzieri* is native to Australia and commonly known as 'mealybug destroyer' has been introduced in many countries for biological control of many mealybug species. Adult beetles are 3.8-4.6 mm in length and 2.7-3.3 mm in breath, having black shining elytra with apices reddish yellow. Fore legs in males are reddish yellow and in females completely black. The predator is capable of feeding on a wide host range of mealybugs and has been reported feeding on 45 species including *P. solenopsis* (Gosalwad *et al*., 2009) which support the reproduction and development. However, it has been recorded from another 35 hosts, but these hosts do not support the reproduction and development.

Cryptolaemus montrouzieri is a general predator of mealybugs and scale insects. This predator is adapted to temperatures which exist under tropical conditions. The predator was initially introduced as a classical biological control agent into California in 1882 against *Planococcus citri* Risso where it was unable to survive in sufficient numbers to affect control without augmentation (Luck & Forster, 2003). Since that time, it has been introduced into 50 countries of the world for control of several mealybug species (Olivero *et al*., 2003; Garcia *et al*., 2009). It was introduced into Hawaii, where it fed on at least 17 mealybug species (Leeper, 1976) and to India for control of grapevine mealybug, *Maconellicoccus hirsutus* Green (Babu & Azam, 1988; Srinivasan & Babu, 1989; Mani, 1990). The predator, *C. montrouzieri* was introduced into Cuba for the control of *M. hirsutus* (Aleman *et al*., 2005). Recently, *P. solenopsis* became a target of the classical biological control without a critical evaluation, first time in the world. The predator, *C. montrouzieri* was introduced into Pakistan from California, USA, in December, 2007 for the control of cotton mealybug (Mahmood, 2008). The optimum temperature for development is about 30^0C and 70% RH (Ghafoor, 2011).

Considering the above information about heavy economic losses to cotton crop occurred by this pest in Pakistan. Studies were conducted to describe and quantify the biology of *Cryptolaemus montrouzieri* in the laboratory and quantify the survival under semi-field conditions.

MATERIALS AND METHODS

Mealybug Culture

Active mealybug females were singly collected from pesticide free Natural Enemies Field Reservoir (NEFR) Tandojam. The females were reared in cages sized length, width and height (115x35x45 cm) and maintained in IPM laboratory, Tandojam at controlled conditions 28 ± 2^0C, 65 ± 5 RH% and L: D (8:16 h) on potato *Solanum tuberosum* L. sprouts (Blumberg & Swirski, 1977).

Predator Culture

Cryptolaemus montrouzieri was imported by CABI Central and West Asia Rawalpindi, Pakistan from California, USA in December, 2007 to Multan. From Multan, culture of 50 individuals (30 adults and 20 pupae) was brought to Tandojam on January 21, 2008. Adult beetles were released on potato sprouts infested with mealybug for mass rearing. Water soaked cotton swabs were placed in the cages to maintain the humidity.

Experiment

The study was conducted to determine the biology of exotic predator of mealybug in IPM laboratory of Entomology Section, Agriculture Research Institute, Tandojam, Pakistan during 2008-09. Upon establishment of satisfactory population of *C. montrouzieri*, various biological parameters viz. pre-mating, pre-oviposition, oviposition, fecundity, incubation, larval (1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar), pre-pupal, pupal, total development (egg-adult), mating, adult longevity periods and sex ratio were observed. For this purpose, randomly selected 10 pairs of adult predators of the same age were studied in the plastic jars. Mealybug as a natural food was provided in the plastic jars at the interval of 24 hours. The freshly laid eggs were kept on the cotton leaves in the petridishes (9 cm Ø) half filled with agar medium (8 g/L) to prevent desiccation. The mealybugs were also provided on cotton leaf in the same petridish. Pairs were kept in plastic jars for studies of biological parameters. This experiment was replicated three times.

A semi-field condition (corridor) experiment was conducted at the Institute from August to October 2010. Cotton (cv. NIAB-111) was planted in pots, when plants became 75 days old the experiment was conducted. For experimental purpose number of leaves was maintained as 10 per plant. The plants infested with all stages of *P. solenopsis* were used in the experiment. The plants were infested with 5 prey densities (100, 200, 300, 400 or 500 mealybugs) and were kept in Plexi glass cages sized length, width and height (60x60x75 cm) with main opening hole (15 cm dia.) and two ventilation holes opposite each other (40x40 cm) covered with 80 mesh sieve, one plant was kept per cage.

One day-old *C. montrouzieri* pairs were released into the cages at five densities (1, 2, 3, 4 and 5 pairs) in each cage keeping a constant predator: prey ratio (1: 100) in all cages. The cages were transferred to the cotton field and placed (10 cm) high with their pots dipped in water containing pots to inhibit ants and other insects coming into the cages. The survival of the predator was visually observed and recorded after every one hour throughout the day time. Temperature and relative humidity were recorded with the help of digital thermometer throughout the day.

Data analysis

The experiment was laid down in Randomized Complete Block Design (RCBD) replicated three times. Five adult pairs of *C. montrouzieri* per plant were tested for survival on five mealybug densities. The data was statistically analyzed following the procedures (Gomez & Gomez, 1984). MSTATC computer software was used to carry out statistical analysis (Russel & Eisensmith, 1983). Means were determined and the standard error (SE) calculated. Means were separated following the Duncan Multiple Range Test (DMRT) procedure. The significance of differences among means was compared by using Least Significant Difference (LSD) test at $P < 0.05$ (Steel & Torrie, 1997).

RESULTS AND DISCUSSION

Oval cylindrical white pale yellowish eggs (capsule shaped) were laid by females singly or in groups in ovisacs of mealybug *P. solenopsis*. The mean incubation period was 5.3±0.07 days. After hatching, *C. montrouzieri* larva developed through four instars. The larva is smooth, pale grey and gradually increased in size. Waxy filaments appeared on the body of larva after 24 hours. Development periods of first, second, third and fourth instars were 5.9±0.15, 5.0±0.16, 7.0±0.18 and 8.3±0.13 days, respectively. Total mean larval period was 26.2 ± 0.61 days. Disturbed larvae exude a yellow or red fluid for defense from the dorsal surface of body. Cannibalism was recorded among the larvae of predator in case of scarcity of the food. Similarly, development period of pre-pupa and pupa was 2.9±0.07 and 8.5±0.2 days, respectively. Pupae were attached to a substrate like host plant leaves and covered with waxy filaments. Total developmental period from egg to adult was 42.9±0.95 days. The adult longevity of male was 80.9±0.89 and female 84.4±0.95 days (Table I).

Table I ***Developmental durations for* Cryptolaemus montrouzieri *Mulsant (Coleoptera: Coccinellidae) reared on cotton mealybug in laboratory***

Biological parameters		Mean ± SE (days)
Incubation period		5.3 ± 0.07
Larval period		
1st instar		5.9 ± 0.15
2nd instar		5.0 ± 0.16
3rd instar		7.0 ± 0.18
4th instar		8.3 ± 0.13
Total		**26.2 ± 0.61**
Prepupal period		2.9 ± 0.07
Pupal period		8.5 ± 0.20
Total development period (egg-adult)		42.9 ± 0.95
Adult longevity	Male	80.9 ± 0.89
	Female	84.4 ± 0.95

The predator larvae and adults were voracious feeders of *P. solenopsis* all stages and can play important role in classical biological control of this pest. Similar results for incubation, larvae, pupa and pre-pupa, pre-mating, pre-oviposition, oviposition, adult longevity, male and female sex ratio were recorded (Bhat *et al*., 1983; Satyanarayanamoorthy and Narayana, 1986; Balakrishnan *et al*., 1987; Mani & Thontadaraya, 1987; Mani, 1988; Gautam, 1996; Baskaran *et al*., 1999; Mali & Kurtadikar, 2008; Gosalwad *et al*., 2009; Fand *et al*., 2010; Kaur *et al*., 2010; Ghafoor *et al*., 2011). Waxy filaments on prey prompted to coccinellid for foraging and oviposition (Merlin *et al*., 1996; Dixon, 2000).

Adults of *C. montrouzieri* spent one day in the pupal case after emergence. Pre-mating, pre-oviposition and oviposition periods were 5.6±0.08, 9.9±0.19 and 76.4±1.13 days, respectively. The fecundity was 486.9±1.41 per female (Table II). The copulation of predator was recorded from 5 to 48 minutes. Male mounted and dismounted over female and mated repeatedly throughout courtship. The eggs per female were 486.9±1.41. The male and female sex ratio was observed as 1:1.

Table II ***Mating, oviposition and fecundity of* Cryptolaemus montrouzieri *Mulsant (Coleoptera: Coccinellidae) in laboratory***

Life stages	Mean±SE (days)
Pre-mating	5.6±0.08
Pre-oviposition	9.9±0.19
Oviposition	76.4±1.30
Fecundity	486.9±1.41

The predator, *C. montrouzieri* adult survival on different cotton mealybug densities in semi field conditions (corridor) showed that one pair lived for 1 to 4 days with mean of 1.86 days, 2 pairs for 1 to 4 days with mean of 2.33 days, 3 pairs for 1 to 7 days with mean of 3.20 days, 4 pairs for 1 to 7 days with mean of 3.80 days and 5 pairs for 1 to 6 days with mean of 3.47 days. However, the minimum longevity was recorded as 1.33 days in 2 and 5 pairs of the predators (Table III). The copulation of *C. montrouzieri* was also observed in semi-field condition.

Statistical analysis for *C. montrouzieri* survival on different densities of cotton mealybug in semi field conditions showed significant differences at 5% probability level. The mortality of *C. montrouzieri* was significantly positive correlation (98%) with temperature (Fig. 1).

Table III ***Mean survival (days) of C. montrouzieri on mealybug in semi field conditions (35-37.5oC and 65-70% RH).***

Predator Pair(s)	Mealybugs					Mean (days)
	100	200	300	400	500	
1	1.67^{fg}	1.67^{fg}	1.67^{fg}	1.3^{g}	3.0^{cdefg}	1.867^{b}
2	1.3^{g}	2.67^{defg}	3.3^{cdef}	2.0^{efg}	2.3^{defg}	2.33^{b}
3	2.3^{defg}	1.67^{fg}	3.0^{cdefg}	3.0^{cdefg}	6.0^{ab}	3.2^{a}
4	2.3^{defg}	3.67^{cde}	1.67^{fg}	4.67^{bc}	6.67^{a}	3.80^{a}
5	1.3^{g}	3.0^{cdefg}	3.3^{cdef}	5.67^{ab}	4.0^{cd}	3.47^{a}

Different letters in each column indicate significant difference at 5% probability level.
LSD 5% = 1.57
SE = 0.55

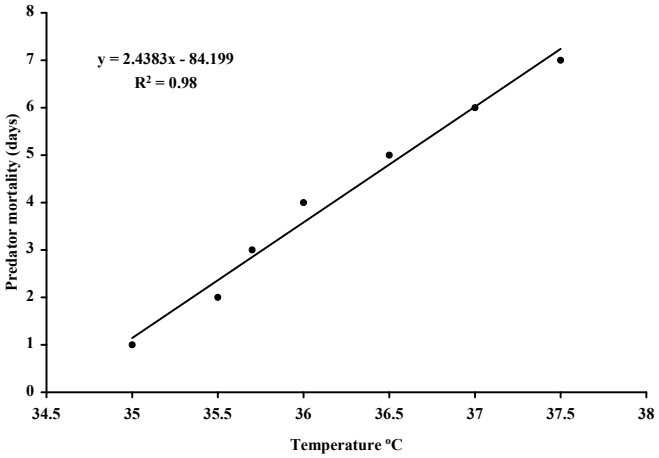

Fig. 1. Relationship of *C. montrouzieri* mortality with temperature in field.

The predator, *C. montrouzieri* was continuously released from March, 2008 to August, 2010 in field on cotton, abutilon and China rose infested with *P. solenopsis*, but after inundative and inoculative releases, adult beetles or larvae were not observed during that period. After that, second experiment was conducted under semi field conditions. In this semi field condition the predator, *C. montrouzieri* survived from 1 to 7 days on different cotton mealybug densities. The maximum mortality of beetles was observed during mid day when the temperature was high. The predator, *C. montrouzieri* was first time observed in cotton field against *P. solenopsis* in Australia (Charleston *et al*., 2010). *Cryptolaemus montrouzieri* is adapted to temperatures which exist, under tropical conditions. The optimum temperature for development is about 30^{0}C (Babu & Azam, 1987). Developmental time was profoundly affected by cool temperature, could not complete development between 10 to 17^{0}C (Babu & Azam, 1987; Hennekam *et al*., 1987). Panis & Brun (1971) and Codling (1977) reported that a

minimum temperature of 21^{0}C was needed for the predator to feed and lay eggs. Predator was unable to persist and effectively control target mealybugs below 20^{0}C and *C. montrouzieri* populations often died out during the winter in temperate countries (Panis & Brun, 1971; Codling, 1977; Carrero, 1980; Oncuer & Koldas, 1981; Copland, 1983; Hennekam *et al*., 1987; Orlinskii & Izhevskii, 1987). Adults of *C. montrouzieri* are active under sunny condition and unproductive above 33^{o}C and become sluggish below 16^{o}C. Larvae showed similar behaviour related to temperature range like adults and peak activity was observed around 28^{o}C (Hussey & Scopes, 1985). However, Bartlett (1974) was able to select cold tolerant biotypes of *C. montrouzieri* which might persist in colder climates. It was not clear how widely such biotypes have been used in practical biological control.

This was preliminary study and *C. montrouzieri* was not used before in cotton agro-ecosystem against *P. solenopsis* in any part of the world. After successful development in laboratory, the predator was first time released in cotton crop during 2008 but unfortunately could not survive in the field. All adults and larvae died due to high temperature. Larvae were chased by ants. Similarly, the *C. montrouzieri* was attended by Argentine ant, *L. humile* in France (Panis, 1981) and against the pink sugarcane mealybug in Australia (Carver *et al*., 2007).

CONCLUSION

It is concluded that *C. montrouzieri* successfully completed development on cotton mealybug, *P. solenopsis* and proved ideal biocontrol agent in laboratory. However, in semifield conditions survival of exotic predator was severely affected by temperature. There is need to acclimatize the population of *C. montrouzieri* for cold tolerance as has been done previously. This is a voracious feeder and very effective predator of mealybugs. Further studies are needed on its intraguild relationships under field conditions.

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