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Biology of the Mealybug, *Phenacoccus solenopsis* Tinsley Infesting Bt Cotton

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A B S T R A C T

The biology of the mealybug *Phenacoccus solenopsis* Tinsley studied on *Bt* cotton under laboratory conditions at Department of Entomology, N. M. College of Agriculture, Navsari Agricultural University, Navsari during September to November 2010 with the average room temperature of 28.37 ± 1.51°C and relative humidity of 60.63 ± 2.40 per cent. The incubation period of the eggs ranged from 30 to 56 minutes with an average of 42.04 ± 7.81 minutes. The duration of first, second and third instar nymphs was 6.40 ± 1.10, 8.45 ± 0.82 and 5.70 ± 1.81 days in male and 7.00 ± 0.92, 7.85 ± 1.04 and 6.45 ± 1.15 days in female, respectively. The quiescence period of fourth instar in male (cocoon) was 5.35 ± 1.18 days. The total nymphal period of male and female nymphs was 25.90 ± 2.57 days and 21.30 ± 2.23 days, respectively. Average pre-oviposition, oviposition and post-oviposition periods was 5.90 ± 0.80, 17.60 ± 4.03 and 9.45 ± 1.11 days, respectively. The female oviposited on an average 328.70 ± 120.07 eggs in groups within the cottony mass (ovisac) during its oviposition period. Average longevity of male was 3.85 ± 0.88 days while of female as 32.95 ± 4.56 days. Female adult lived longer than male adult. The sex ratio of male: female was found to be 1: 3.68 as observed while rearing in the laboratory and it was 1: 3.16 as observed from the field collected populations indicating preponderance of female over male. The total life cycle of males of *P. solenopsis* occupied on an average 29.75 ± 2.77 days ranging from 25 to 34 days in male and 54.25± 4.95 days ranging from 45 to 60 days in female.

Introduction

Cotton is a major fibre crop of global significance. It is also called as "white gold" and its importance as a multipurpose crop that supply five basic products *i.e.* lint, oil, seed meal, hulls and linters. India stands first in the world cotton area and it contributes 21 per cent of global cotton produce. Gujarat has emerged as India's number one cotton producing state and it contributing about 32.82 per cent to the national cotton production from 23.81 per cent of total cotton area with corresponding increase in productivity from 317 to 635 kg/ha (Anon., 2011). Cotton scenario in India is now dominated by *Bt* cotton covering more than 90 per cent area (Barik, 2010). More than 1300 *Bt* hybrids comprising of six different events are commercialized in India (Kranthi, 2010). Change in pest dynamics has become more common in recent times in rapidly changing cropping systems and environment with the dominance of *Bt* cotton across India. Worldwide, cotton ecosystem harbored as many as 1326 insect species (Hargreaves, 1948). In India, 162 insect species have been reported in cotton ecosystem (Dhaliwal *et al.*, 2004). In the *Bt* era, cotton pests especially the bollworms have drastically reduced but

sucking pests especially leaf hopper, aphid, whitefly, thrips and mealybug attained the status of pests of great concern after the introduction of *Bt* cotton in India. The mealybug, *Phenacoccus solenopsis* Tinsley was hitherto not familiar or its identity was not established which caused significant economic damage in Pakistan and India (Muhammad, 2007; Jhala *et al.*, 2008; Nagrare *et al.*, 2009). The mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has a wide geographical distribution with its origin in Central America (Williams and Granara de Willink, 1992). *P. solenopsis* described as a serious and invasive pest of cotton in Pakistan and India (Hodgson *et al.*, 2008).

The occurrence, severity, and invasive nature of mealybug on cotton were experienced in cotton ecosystem of Gujarat during the 2004-05, 2005-06, and 2006-07 crop seasons. The identity of the species involved documented as *P. solenopsis* by Jhala and Bharpoda (2008a) and Jhala *et al.*, (2008). Widespread infestation of *P. solenopsis* and economic damage to Bt cotton across Gujarat led urgent need to formulate strategies for its management. The present study investigates the biology of *P. solenopsis* under laboratory conditions at Department of Agricultural Entomology, Navsari Agricultural University, Navsari

Materials and Methods

Rearing techniques

Studies on biology of *P. solenopsis* were carried out in the laboratory at Department of Entomology, N. M. College of Agriculture, Navsari Agricultural University, Navsari during September to November 2010 at the average room temperature of 28.37 ± 1.51°C and relative humidity of 60.63 ± 2.40 per cent. The nucleus culture of *P. solenopsis* maintained on *Bt* cotton at Main Cotton Research Station, Surat under polyhouse condition and it utilized for the study. For the purpose, twigs of cotton plants infested with reproducing females of *P. solenopsis* brought to the laboratory. Cotton leaves with petioles were collected from terminal portion of the plants (*cv.* VICH-5 *Bt*) grown in net house without insecticidal spray and free from mealybug infestation. Collected leaves were washed with tap water and shade dried and used as food source. For the study of reproduction of *P. solenopsis*, newly emerged 20 pairs of male and female adults were collected and confined in glass jar containing one month old cotton seedlings raised in plastic cup covered with muslin cloth in the laboratory at room temperature.

On settlement of female on twig, it started to lay egg mass or ovisac. Crawlers emerged out from the egg mass or ovisac laid on twig or leaves were transferred on the fresh leaves. For the purpose, newly emerged crawlers from ovisac or egg mass were placed individually on cotton (*cv.* VICH-5 *Bt*) leaf having long petiole with the help of fine camel hair brush. The petiole of the cotton leaves dipped in vial filled with water and wrapped with thin parafilm roll/tape to keep the leaves turgid for longer period. Such cotton leaves placed in glass petri plates. Crawlers, when settled to feed on leaves were observed daily in the morning till they attained adult stage for studying various aspects of biology. Transfer of immature stages allowed to fresh leaves by placing previous leaf over fresh one and ensuring its migration from older to fresh leaf.

Developmental stage

The eggs examined under stereo-binocular microscope for studying their colour, shape and size. For the purpose, gravid adult females from the potted plant were picked up

individually and placed on the leaf of the cotton plant with the help of fine camel hair brush. The leaf petiole of the cotton leaves was dipped in vial filled with water and wrapped with parafilm to keep the leaves turgid for longer period.

Such leaves along with freshly laid egg masses or ovisacs were examined critically under microscope. The eggs within the ovisac and egg mass were counted.

The duration of eggs was noted in minutes from date of laying egg mass/ovisac to successful emergence of crawlers by observing critically for first one and half hour since egg laying. Each freshly emerged crawler (first instar crawler) was transferred to fresh cotton leaf kept in petri plate. Total number of eggs within single ovisac/ egg mass recorded.

For duration of different nymphal instars, freshly hatched 20 crawlers/nymphs were marked individually and observed daily under microscope in the morning hours to study the moulting. The moulting was confirmed by the presence of exuivum on the leaf or attached on the posterior end of the body of second instar nymph. The shape, size and colour of each nymphal instar were critically observed.

The measurement of length and breadth was recorded under the microscope with the help of stage and ocular micrometers.

The duration of different nymphal stages was recorded during each moulting and later separated as duration of individual instars of male and female nymphs based on final confirmation of sex as male nymphs formed additional instar called cocoon (IV instar).

The adults emerged out after the last moults in the laboratory were observed under microscope to study their shape, size and colour. Total life cycle of female and male was calculated from the egg stage to the death of adult.

Oviposition, fecundity and longevity periods

Freshly emerged adults were paired and reared separately on leaves of cotton plant to study pre-oviposition, oviposition and post-oviposition periods. A period between date of emergence of the female and date of starting of laying egg mass or ovisac was considered as pre-oviposition period, whereas the period between dates of starting of laying egg mass and date of ceasing of laying egg mass was considered as oviposition period and the period between ceasing of laying egg mass and death of female adult was considered as post-oviposition period.

To determine the fecundity of female, soon after pairing and mating, adult females were observed daily. Since the female laid eggs in cottony sac located at posterior end of its abdomen, the ovisac was collected daily and counted the number of eggs present in each ovisac laid during different days of oviposition. Based on this, average fecundity of the female was worked out.

The records of deaths of males and females were maintained separately and the longevity was calculated from the date of emergence to the death of adult.

Sex ratio

For determine the sex ratio, freshly emerged third instar nymphs were collected from laboratory culture and observed for the emergence of male and female. The actual sex ratio was worked out by counting males and females after successful emergence of adults. Similarly, sex ratio from field collected population was also determined.

Results and Discussion

Egg

The gravid female of *P. solenopsis* laid whitish yellow eggs within the white cottony ovisac which initially remained underneath to body of the mother and later female moved leaving the ovisac on the leaf surface. The eggs were whitish yellow, semi-transparent, oval to oblong in shape and loosely remained inside the white thread like cottony mass (Plate 1). The present appearance and colour of eggs was more or less similar with the reports of Nikam *et al.,* (2010) and Kamariya and Patel (2011). The data on measurement of the eggs (Table 2) revealed that length and breadth of eggs varied from 0.30 to 0.38 mm and 0.17 to 0.22 mm with an average of 0.35 ± 0.02 mm and 0.20 ± 0.01 mm, respectively. The results on size of eggs are also in accordance with the findings of Dhawan and Saini (2009) and Nikam *et al.,* (2010). Table 1 indicates incubation period of eggs of *P. solenopsis* was very short and it varied from 30 to 56 minutes with an average of 42.05 ± 7.81 minutes. The incubation period as reported to be 30 to 45 minutes (Aheer *et al.,* 2009).

Nymph

The female of *P. solenopsis* moulted three times, whereas male moulted four times to attain maturity. Similar observations reported by Nikam *et al.,* (2010) and Vennila *et al.,* (2010). Newly emerged crawlers were light yellow in colour (Plate 2), light red eyes, three pairs of legs and a pair of seven segmented filiform antennae as observed under microscope. The colour of nymphs appeared to grayish yellow within two to three days after hatching. They were found to crawl out of thread like pouches to leaf surface within a day. The crawler was very active, fast moving and observed to settle down on lower surface of the leaf near the midrib or petiole of the leaf. According to Akintola and Ande (2008), first instar nymphs was oblong in shape and yellow in colour devoid of mealy scale cover on body, which is in close proximity with present findings. The body length of first instar nymphs measured from 0.39 to 0.43 mm with an average of 0.41 ± 0.01 mm and breadth from 0.11 to 0.17 mm with an average of 0.13 ± 0.01 mm (Table 2). The size of first instar nymph was more or less in accordance with the reports of Dhawan and Saini (2009) and Nikam *et al.,* (2010). Duration of first instar nymph of male was 5 to 8 days with an average of 6.40 ± 1.10 days whereas in case of female first instar nymph was 6 to 9 days with an average of 7.00 ± 0.92 days (Table 1). The present finding is not in accordance with the reports of Dhawan and Saini (2009) who reported the average duration of first instar nymph of female and male as 4.6 ± 1.10 and 4.8 ± 1.10 days.

The second instar nymphs were larger than first instar nymphs, whitish yellow in colour, oblong in shape and carried the exuvium at the posterior end of the abdomen when freshly formed. Later they secreted waxy material on their body and white mealy covering initiated to appear and the body margins showed rudimentary waxy filaments (Plate 3). The length of second instar nymphs ranged from 0.69 to 0.81 mm with an average of 0.75 ± 0.03 mm and breadth from 0.31 to 0.37 mm with an average of 0.34± 0.01(Tables 1 and 2). The present findings on size are quite closer to the report of Nikam *et al.,* (2010) reported it to be 0.66 to 0.87 mm in length and 0.28 to 0.40 mm in breadth. The duration of second instar male nymphs ranged from 7 to 10 days with an average of 8.45 ± 0.82 days whereas of female nymphs ranged from 7 to 10 days with an average of 7.85 ± 1.04 days (Tables 1 and 2). The present finding on duration is slightly differed from the reports of Aheer *et al.,* (2009) who

reported it to be 4 to 9 days in male and 3 to 4 days in female on cotton. This variation in duration might be due to surrounding environment and host nutrition.

The third instar nymphs were bigger in size than the earlier instars. Pair of antennae and smoky brownish legs was clearly visible with naked eyes whereas the eyes were covered with mealy secretion and not clearly visible. The secretion of waxy material on body surface intensified in 3rd instar and whole body appeared with milky white cover except at the either side of dorsal region of metathorasic and few abdominal segments along the mid dorsal line of the body. The wax deposition on the body surface appeared as waxy filaments (Plate 4).

The male and female nymphs were difficult to distinguish in this stage, however, later in this instar; the male being started to shrunken its body and waxy covering on body being loosen. The present finding is in concurrence with the results of Nikam *et al.,* (2010) and Vennila *et al.,* (2010) who observed male cocoon after third molt and reported that sex differentiation could be difficult in third instar and in disagreement with the report of Hodgson *et al.,* (2008) who observed the male cocoon after second moult.

This variation might be due to different ecological situations where variants may occur. The body length of third instar nymphs ranged from 1.00 to 1.30 mm with an average of 1.11 ± 0.05 mm and breadth from 0.48 to 0.55 mm with an average of 0.53 ± 0.02 mm. The present finding is in contrast with Nikam *et al.,* (2010) who reported the size of third instar female nymph as 1.99 to 2.07mm in length and 1.02 to 1.14 mm in width (Table 2). The duration of third instar male nymph ranged from 3 to 8 days with an average of 5.70 ± 1.81 days and of female nymph from 5 to 9 days with an average of 6.45 ± 1.15 days (Table 1). The present findings of duration is more or less in conformity with the findings of Nikam *et al.,* (2010) who reported the duration of third instar nymphs as 4 to 6 days with an average of 4.80 ± 0.65 days.

The third instar nymph of mealybug observed to cease feeding, stopped movement and shrunken its body prior to moulting. The whole body surface covered completely with white waxy covering except for legs unlike in third instar where body remained uncovered at dorso-posterior part. The mealybug in this instar remained sluggish and appeared encased in loose waxy white covering called cocoon from which only the male adults emerged (Plate 5).

The data on measurement of cocoons revealed that the length ranged from 1.34 to 1.42 mm with an average of 1.38 ± 0.02 mm and breadth from 0.47 to 0.57 mm with an average of 0.54 ± 0.03 mm (Table 2). Aheer *et al.,* (2009) recorded it to be 1.80 mm in length and 0.50 mm in width which is more or less in conformity to present findings.

Duration of male cocoon was varied from 4 to 7 days with an average of 5.35 ± 1.18 days (Table 1). Nikam *et al.,* (2010) and Vennila *et al.,* (2010) reported that male nymph passed through the fourth instar and the duration of which varied from 6 to 7 (av. 6.40 ± 0.48) days and 5 to 7 (av. 5.5 ± 0.5) days, respectively which is in close proximity with the present findings.

The total nymphal period of male and female varied from 21 to 29 days with an average of 25.90 ± 2.57 days and 18 to 25 with an average of 21.30 ± 2.23 days, respectively (Tables 1 and 2). Thus, there was longer developmental period of males in nymphal stages compared to females due to an additional moulting and quiescence period in male.

Plate.1 Egg of *P. solenopsis*

Plate.2 First instar nymph of *P. solenopsis*

Plate.3 Second instar nymph of *P. solenopsis*

Plate.4 Third instar nymph of *P. solenopsis*

Plate.5 Pupa (white cocoon)

Plate.6 Male adult of *P. solenopsis*

Plate.7 Adult female of *P. solenopsis* (dorsal and ventral view)

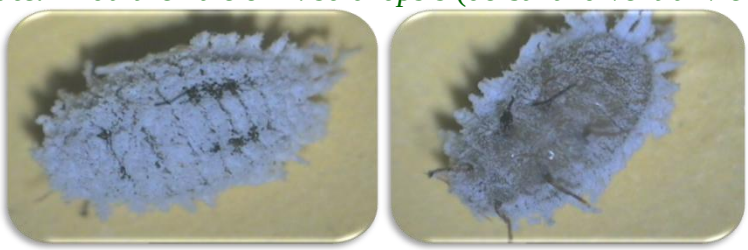

Table.1 Details of entire life cycle from egg to death of *P. solenopsis*

Stage	No. of observations	Range (days)		Mean (days)
		Min	Max	
Egg (Minutes)	50	30	56	42.04 ± 7.81
Male nymph				
1st instar	20	5	8	6.40 ± 1.10
2nd instar	20	7	10	8.45 ± 0.82
3rdinstar	20	3	8	5.70 ± 1.81
4th instar (Cocoon)	20	4	7	5.35 ± 1.18
Total nymphal period	20	21	29	25.90 ± 2.57
Female nymph				
1st instar	20	6	9	7.00 ± 0.92
2nd instar	20	7	10	7.85 ± 1.04
3rd instar	20	5	9	6.45 ± 1.15
Total nymphal period	20	18	25	21.30 ± 2.23
Adult longevity				
Male	20	3	5	3.85 ± 0.88
Female	20	26	39	32.95 ± 4.56
Pre-oviposition	20	5	7	5.90 ± 0.80
Oviposition	20	12	23	17.60 ± 4.03
Post-oviposition	20	8	11	9.45 ± 1.11
Fecundity (Eggs/ovisac)	20	126	532	328.70 ± 120.07
Sex ratio (male:female)				
Laboratory	262	1:2.08	1:4.56	1:3.68
Field	491	1:2.76	1:3.78	1:3.16
Total life cycle				
Male	20	25	34	29.75 ± 2.77
Female	20	45	60	54.25 ± 4.95
Temperature (°C)	91	24.7	30.7	28.37 ± 1.51
Humidity (%)	91	57.6	72.0	60.63 ± 2.40

Table.2 Measurement of different stages of *P. solenopsis*

Stages	No. of observations	Length (mm)			Breadth (mm)		
		Min.	Max.	Av. ± SD	Min.	Max.	Av. ± SD
Egg	30	0.30	0.38	0.35 ± 0.02	0.17	0.22	0.20 ± 0.01
Nymph							
I instar	30	0.39	0.43	0.41 ± 0.01	0.11	0.17	0.13 ± 0.01
II instar	30	0.69	0.81	0.75 ± 0.03	0.31	0.37	0.34 ± 0.01
III instar	30	1.00	1.17	1.11 ± 0.05	0.48	0.55	0.53 ± 0.02
IV instar (cocoon)	30	1.34	1.42	1.38 ± 0.02	0.47	0.57	0.54 ± 0.03
Adult							
Male	30	1.10	1.30	1.17 ± 0.06	2.15	2.42	2.33 ± 0.07
Female (newly emerged)	30	1.92	2.80	2.53 ± 0.27	0.70	1.00	0.92 ± 0.07
Female (egg laying time)	30	3.10	4.45	3.86 ± 0.48	1.25	1.98	1.66 ± 0.28

Adult

Male adult of *P. solenopsis* was slender, delicate, smoky white in colour due to waxy powder all over the body. It possessed single pair of very delicate 10 segmented long and slender antennae, three pairs of brownish legs, two pairs of terminal filaments and one pair of well-developed mesothoracic wings. The abdominal region was pale yellow (Plate 6). The second pair of wings was modified as hamulohalters. Males were smaller than females. The data of measurement of adult males (Table 2) revealed that length of body varied from 1.10 to 1.30 mm with an average of 1.17 ± 0.06 mm and breadth with expanded wings varied from 2.15 to 2.42 mm with an average of 2.33 ± 0.07 mm. The present findings on appearance and size are more or less in conjunction with the observations of Dhawan and Saini (2009) and Nikam *et al.*, (2010).

The adult female was wingless, oblong in shape and light to dark yellow in colour ventrally. Dorsally the whole body was well segmented, covered with waxy deposition except at the posterior abdominal region where blackish stripes on either side of mid dorsal line were visible. The deposition of waxy material along the margin of the body was so intense that waxy filament clearly visible on outer margin. It was well distinguished segmented, apterous soft bodied insect (Plate 7). It was also possessed a pair of brownish, short, filiform eight segmented antennae and three pairs of brownish red legs. Earlier, several workers had observed the similar kind of features (Arve, 2009; Dhawan and Saini, 2009; Nikam *et al.*, 2010). There was a considerable difference in size of freshly emerged female adult and female adult which underwent post embryonic development as reflected in the measurement of size of newly emerged female and gravid female just prior to egg laying. The newly emerged female adult varied from 1.92 to 2.80 mm with an average of 2.53 ± 0.27 mm in length and 0.70 to 1.00 mm with an average of 0.92 ± 0.07 mm in breadth. Whereas, at the time of egg laying the length varied from 3.10 to 4.45 mm with an average of 3.86 ± 0.48 mm and the breadth varied from 1.25 to 1.98 mm with an average of 1.66 ± 0.28 mm (Table 2). Dhawan and Saini (2009) recorded the length of adult female varied from 4.1 to 4.7 (av. 4.4 ± 0.24) mm and width from 2.8 to 3.0 (av. 2.9 ± 0.1) mm, which is more or less closer to present findings.

Pre-oviposition, oviposition and post-oviposition periods

The data presented in table 1 revealed that pre-oviposition, oviposition and post-oviposition periods varied from 5 to 7 (av. 5.90 ± 0.80), 12 to 23 (av. 17.60 ± 4.03) and 8 to 11 (av. 9.45 ± 1.11) days, respectively. According to Dhawan and Saini (2009), the pre-oviposition, oviposition and post-oviposition period ranged from 3 to 5 (av. 4.4 ± 0.4), 8 to 9 (av. 8.2 ± 0.8) and 2 to 3 (av. 2.6 ± 0.6) days, respectively. Whereas, Nikam *et al.*, (2010) reported it to be 8 to 9 days (av. 8.56 ± 0.61), 16 to 18 days (av. 16.73 ± 0.57) and 9 to 10 days (av. 9.33 ± 0.47), respectively. The variation might be due to different conditions of rearing and nutrition.

Fecundity, Longevity and Sex ratio

The fecundity of each female was recorded by counting the eggs within each ovisac laid during different periods of oviposition by each female. The results obtained are presented in table 1. The data revealed that the fecundity ranged from 126 to 532 with an average of 328.70 ± 120.07 eggs per female during its entire life cycle. Aheer *et al.*, (2009) reported it to be 98 to 239 (av.160) eggs per female, whereas Nikam *et al.*, (2010)

reported it to be 400 to 700 with an average of 572 ± 102 eggs per female.

The data presented in table 1 showed the longevity of males varied from 3 to 5 (av. 3.85 ± 0.88) days and that of females from 26 to 39 (av. 32.95 ± 4.56) days. Thus, males lived shorter than females. According to Akintola and Ande (2008) longevity of female was 37 days. Vennila *et al.,* (2010) observed it to be ranged from 1 to 2 (av. 1.5 ± 0.1) days in males and 36 to 51 (av. 42.4 ± 5.7) days in case of females.

The sex ratio of male: female of *P. solenopsis* was 1: 3.68 as observed while rearing in the laboratory and it was 1:3.16 as observed from the field collected populations (Table 1). Thus, males were more abundant in the fields as compared to that emerged from laboratory culture. According to Nikam *et al.,* (2010), it was 1:4.69 in laboratory reared adults.

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