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Biology on leaf miner, *Phyllocnistis citrella* (Stainton) infesting sweet orange

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Abstract

Investigations on biology of sweet orange leaf miner, *Phyllocnistis citrella* (Stainton) were carried out under laboratory condition at Department of Entomology, College of Agriculture, Junagadh Agricultural University, Junagadh during 2016-17. The average incubation period, hatching percentage, first instar, second instar, third instar, fourth instar, total larval period and pupal period of *P. citrella* were (2.44 ± 0.49), (74.67 ± 3.66), (1.20 ± 0.25), (1.22 ± 0.25), (2.22 ± 0.25), (2.72 ± 0.25), (7.36 ± 0.55) and (11.12 ± 0.78), respectively. The longevity of male and female moth were (4.55 ± 0.51) and (6.44 ± 0.51) days, respectively. The pre-oviposition, oviposition and post-oviposition periods of the insect were (1.18 ± 0.24), (3.22 ± 0.25) and (1.20 ± 0.25) days, respectively. The fecundity was observed to be (54.47 ± 8.89) eggs /female. Sex ratio to male: female was found to be 1: 1.2.

Keywords: Biology, citrus leaf miner, *Phyllocnistis citrella* (Stainton), sweet orange

Introduction

The word "citrus" is derived from the ancient Greek *Kedros* and Latin *Cedrus*. The sweet orange, *Citrus sinensis* is the fruit of the citrus in the family Rutaceae. The fruit of the orange tree can be eaten fresh or processed for its juice or fragrant peel. Among the different citrus trees, sweet orange occupies number one position in production in the world by accounting for about 70 per cent of the total production (Anonymous, 2012) [2]. Among the different citrus fruits, pulp of orange is an excellent source of vitamin C and provides 64 per cent/100 g serving of daily value. In India, so many factors viz., biotic and abiotic are limiting the production of sweet orange per unit area. Among several biotic factors insect pests and diseases caused enormous damage to the particular crop. In India, leaf miner, *Phyllocnistis citrella* (Stainton) (Phyllocnistidae: Lepidoptera) is a major insect pest inflicting severe damage in citrus (Kagzi lime) orchard and now becoming a major pest of citrus as well as sweet orange in Gujarat (Boughdad *et al.*, 1999) [3].

The citrus leaf miner moth lays minute, flattened, transparent eggs on the lower surface of the young leaves near the midrib. Newly hatched larvae mine into the leaf tissue and form galleries. The mature larvae settle down in enlargement of galleries near the leaf margin. By the time, they spin cocoons for pupation and the leaves get twisted or folded over and retarded the growth of plant. Each larva destroys 4 to 100 per cent leaf surface before reaching the pupal stage. The moths rest on the trunks of the trees near trees near the ground. The attacked leaves remain on the plants for long time and the damage gradually spreads from older leaves to fresh ones, which results in direct yield loss.

Materials and Methods

Numbers of sweet orange branches infested with larvae of *P. citrella* were collected from the Horticultural Instructional Farm, College of Agriculture, Junagadh Agricultural University, Junagadh. Further, the branches were wrapped with moist cotton wool on the cut portion and put in the conical flask containing water. Large numbers of such flasks were prepared for mass rearing of citrus leaf miner in the laboratory. Such flasks were kept in iron cage (2.0 m × 1.5 m × 1.0 m) followed by covering of black muslin cloth. The adult moth emerged out from the pupae was collected carefully by opening the iron cage with the help of small plastic vials. Male and female moth was identified with their typical morphological characters and paired. Five citrus seedlings and twenty five pairs of male and female moth of citrus leaf miner were introduced in an iron cage for oviposition. Honey solution (5%) was provided in a fresh sponge twice in a day as a food for adult moths. Egg laid by female moths on the leaves of citrus were marked and numbered. Only one egg /leaf was maintained and rest of the eggs was removed. The eggs were observed every day to know the further development.

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The leaf having pupa was kept in glass petri dish (70 cm × 1.5 cm) containing moist blotting paper till the emergence of moths from pupae. To know the fecundity of 15 numbers of female moth and longevity 25 numbers of adults, freshly emerged moths were paired and transferred in a glass chimney containing citrus seedling. Five per cent honey solution was provided as a food for adult moths.

Egg Stage

Leaves containing eggs were separated out by examining under microscope and used for further study. Citrus leaves with leaf miner eggs so obtained were maintained in a petridish (10 cm diameter) for incubation. Daily twenty leaves from the day of oviposition to egg hatching were dissected to determine the incubation period and per cent hatching.

Larval Stage

On hatching, a leaf miner larva was allowed to feed individually inside the leaves in a petridish (10 cm diameter). Every day, mines were dissected out to see the different stages of the larvae. The dissection of leaves/mines was made up to the pupal stage. The period between egg hatching and pupation was observed as larval period.

Pupal Stage

The pupal period of the pest was studied by observing the same larvae for pupation inside the leaves/mines. This was maintained and the observation was made till the adult emergence. The period between formations of pupae till the adult emergence was noted as pupal period.

Adult Longevity

The adults emerged from the pupa were introduced individually in to a glass tube to study the fecundity and longevity. Fresh citrus leaves were provided in to glass tube for food and oviposition. The leaves were changed after 24 hours and the older leaves confined to another glass tube for emergence of progenies. Observations on fecundity and longevity of adults were recorded.

Results and Discussion:

Egg

The freshly laid oval shaped eggs of *P. citrella* were transparent, minute which looks like a small droplet of water. The eggs were laid singly on the lower or upper surface of the leaf lamina (Fig. 1). Similar morphological characters of *P. citrella* eggs were observed by Rahman *et al.* (2003) [7] and Sarada *et al.* (2014) [8].

Incubation period

The results incorporated in Table 1 revealed that the incubation period of *P. citrella* was varied from 2.0 to 3.0 days with average of 2.44 ± 0.49 days at average temperature of 20.77 ± 2.73 and average relative humidity of 49.66 ± 9.55 per cent. Jadhav (2015) [4] reported the incubation period (2.55 ± 0.62 days) of citrus leaf miner.

Hatching percentage

It can be seen from the Table 2 that, hatching percentage of eggs of *P. citrella* was varied from 70 to 80 per cent (av. $74.67 \pm 3.66\%$) with an average temperature of 20.75 ± 2.09 °C and an average relative humidity of 46.60 ± 11.11 per cent. The more or less the same results i.e. 72 percentage hatching was observed by Jadhav (2015) [4].

Larva

From the present investigations, it was found that the larval stages of *P. citrella* were passed through four different instars. The description of each instars are given as under.

First instar

The newly hatched first instar larva of *P. citrella* was flat in shape with triangular head and pale greenish yellow in colour with shining glossy appearance when removed from the gallery. Thoracic segments of larva were broader than the abdominal segments. The first instar larva was apodous with smooth body (Fig. 1). Larva enters into the leaf tissue on upper or lower surface of the leaf and feeds on the epidermis and causes mines. At the time of moulting, larva becomes quite sluggish and it stops feeding. The head is gradually protruded out and the old cuticle is pushed back due to constant contraction and relaxation produced by the body movement. The exuviae remain as dark brown specks in the galleries. The reports of Rahman *et al.* (2003) [7] and Kernasa *et al.* (2008) [5] are almost similar to present findings. The larval period of first instar citrus leaf miner (Table 1) was ranged from 1.0 to 1.5 days (av. 1.20 ± 0.25 days) and it was confirmed as per the report of Abo *et al.*, (2006) [1], who found 1.79 ± 0.41 days of first instar larval period.

Second instar

The newly moulted second instar larva of *P. citrella* was flat in shape with triangular head and pale greenish yellow in colour with glossy appearance when removed from the gallery. They were similar to the first instar larva in general appearance and morphological characters except in body size (Fig. 1). From the Table 1, it can be seen that the second instar larval period of citrus leaf miner was ranged from 1.00 to 1.50 days (av. 1.22 ± 0.25 days) and it was in the conformity with the results obtained by Abo *et al.*, (2006) [1] who reported 1.25 ± 0.44 days of second instar larval period.

Third instar

The freshly moulted third instar larva of *P. citrella* was eruciform, flat in shape with pale yellowish in colour and a pair of small antennae present on triangular head. The larva possesses disc shaped mandibles which were specially modified for sap feeding. Three pairs of thoracic legs were present. Thoracic segments were broader than abdominal segments. The size of third instar was larger than the previous instar (Fig. 1). The third instar larval period of *P. citrella* (Table 1) was ranged from 2.00 to 2.50 days (av. 2.22 ± 0.25 days). Abo *et al.*, (2006) [1] reported that the third larval period was 1.91 ± 0.28 days.

Fourth instar

The fourth instar larva of *P. citrella* was cylindrical in shape and pale yellowish in colour. Thoracic legs were well developed than the previous instar. The larva was similar to the third instar in general appearance and morphological characters except in body size (Fig. 1). The fourth instar larva curls the edges of mined leaf over its body and spins a white cocoon and pupate inside the gallery. Data depicted in Table 1 revealed that the fourth instar larval period of citrus leaf miner was ranged from 2.50 to 3.00 days (av. 2.72 ± 0.25 days).

Total larval period

The data on total larval period of *P. citrella* (Table 1) revealed that it was varied from 6.50 to 8.50 days (av. 7.36 ± 0.55 days) when reared at average temperature of 20.77 ± 2.73 °C and relative humidity of 49.66 ± 9.55 per cent. Jadhav (2015) [4] reported total development period of larva was 7.25 ± 0.40 days.

Pupa

Freshly formed pupa of *P. citrella* was cylindrical in shape and yellowish brown in colour. After some period colour changed to brownish with smooth, soft and shining surface

and provided with sharp spine on its head. Pupa was obctect type. Wings, legs and other appendages and firmly fixed to the body (Fig. 1). It is observed that the pupal period of citrus leaf miner was varied from 10.0 to 12.0 days (av. 11.12 ± 0.78 days) [Table 1]. Jadhav (2015) ^[4] reported the average pupal period was found 9.80 ± 0.74 days. While, Kernasa *et al.* (2008) ^[5] was reported 8.04 ± 0.92 days of pupal period.

Adult

The adult moth of *P. citrella* was tiny and silvery in colour. The head was conspicuous with prominent dark brown compound eyes and head possessed a pair of antennae. They possess three pairs of thoracic legs. The prothoracic and mesothoracic legs were more or less similar in length while, metahoracic legs were much longer than prothoracic and mesothoracic legs. Adult has two pair of wings. Both the pairs of wings were fringed with minute hairs. Forewings were whitish in colour and had brown stripes and prominent black spot near the apical margin while, the hind wings were white in colour (Fig. 1). The male is distinguished by the presence of brush like tuft of scales on the last abdominal segment when observed under the microscope but it was absent in female moth. Female moth was comparatively bigger in size than male. The present observations are almost similar to the reports of Rahman *et al.* (2003) ^[7] and Kernasa *et al.* (2008) ^[5]. The adult period female of citrus leaf miner was varied from 6 to 7 days (av. 6.44 ± 0.51 days) and adult period male citrus leaf miner was varied from 4 to 5 days (av. 4.55 ± 0.51 days) [Table 1]. Kernasa *et al.* (2008) ^[5] reported male and female adults were 2.59 ± 0.93 and 4.04 ± 1.26 days respectively. According to Maryam and Shiva (2017) ^[6], both females and males were lived longer at 20°C (9.31 and 7.38 days, respectively). The present finding is more or less in conformity with the reports of earlier worker.

Pre-oviposition, oviposition and post-oviposition periods

During the study of pre-oviposition, oviposition and post-oviposition periods, the given temperature and relative humidity was 20.77 ± 2.73 and 49.66 ± 9.55 per cent, respectively. The data presented in (Table 3) indicated that the pre-oviposition period of *P. citrella* was ranged from 1.0 to 1.5 days (av. 1.18 ± 0.24 days). The oviposition period Table 3 of *P. citrella* was varied from 3.0 to 3.5 days (av. 3.22 ± 0.25 days). While, the post-oviposition period Table 3 of *P. citrella* was varied from 1.0 to 1.5 days (av. 1.20 ± 0.25 days). Jadhav (2015) ^[4] reported the average pre-oviposition, oviposition and post-oviposition period were 1.20 ± 0.24 , 3.35 ± 0.45 and 1.5 ± 0.44 days, respectively.

Fecundity

Data presented in Table 3 revealed that the egg laying capacity of female moth was varied from 40 to 76 eggs with an average of 54.47 ± 8.89 eggs /female at an average room temperature of $20.77 \pm 2.73^{\circ}$ C and an average relative humidity of 49.66 ± 9.55 per cent. Jadhav (2015) ^[4] reported the average fecundity of female was 52.40 eggs.

Longevity

The data presented in Table 1 showed that the longevity of male moth of *P. citrella* was varied from 4.0 to 5.0 days with an average of 4.55 ± 0.51 days, while the longevity of female moth was ranged from 6.0 to 7.0 days with an average of 6.44 ± 0.51 days at an average temperature of $20.77 \pm 2.73^{\circ}$ C and an average relative humidity of 49.66 ± 9.55 percent. Thus, the result on adult longevity clearly indicated that the life span of female moth was longer than male moth. Abo *et al.*, (2006) ^[1] reported the adult longevity lasted without any feeding 1.7 ± 0.5 days for males, 2.3 ± 0.59 days for females. While, Jadhav (2015) ^[4] reported the longevity of male and female was 5.6 ± 0.99 and 7.0 ± 0.77 days, respectively.

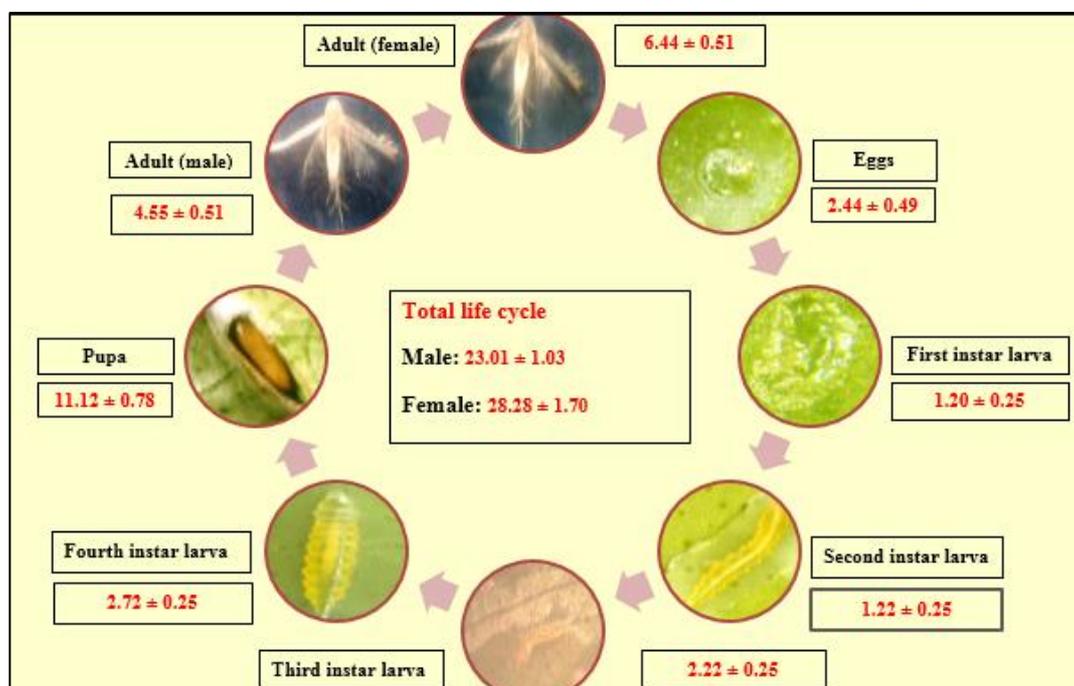


Fig 1: Life cycle of leaf miner, *P. citrella* [Days (Av. \pm SD)]

Sex ratio

During the present study, dead adults of *P. citrella* obtained from the stock culture were dried, pinned and maintained in the laboratory to study the sex ratio. It can be seen from the

data presented in Table 4 that, out of 80 adults observed, 37 were males and 43 were females indicating preponderance of female population over male. Thus, the sex ratio of male: female was found to be 1: 1.2. The sex ratio of *P. citrella* for

the offspring was 1:1.2, 1:1.4 and 1:1.3 male: female at 20, 27 and 35 °C, respectively (Maryam and Shiva, 2017) [6]. While, According to Jadhav (2015) [4], the sex ratio of *P. citrella* was 1:1.2 (male: female).

Total life period

The total life period (from egg to the death of adult moth) of male moth of *P. citrella* was varied from 20.50 to 24.50 days

(av. 23.01 ± 1.03 days). While, it was varied from 25 to 31 days (av. 28.28 ± 1.70 days) in case of female when reared at an average temperature of 20.77 ± 2.73 °C and an average relative humidity of 49.66 ± 9.55 per cent Table 1. Jadhav (2015) [4] reported the total life cycle from egg to adult stage was completed within 24.90 ± 1.04 and 26.45 ± 1.55 days in male and female, respectively.

Table 1: Period of different stages of citrus leaf miner, *P. citrella*

S. No.	Particulars	Period (Days)			
		Min.	Max.	Av. \pm S.D.	
1.	Egg period	2.0	3.0	2.44 \pm 0.49	
2.	Larval period	1 st instar	1.00	1.50	1.20 \pm 0.25
		2 nd instar	1.00	1.50	1.22 \pm 0.25
		3 rd instar	2.00	2.50	2.22 \pm 0.25
		4 th instar	2.50	3.00	2.72 \pm 0.25
3.	Total larval period	6.50	8.50	7.36 \pm 0.55	
4.	Pupal period	10.0	12.0	11.12 \pm 0.78	
5.	Adult period	Male	4.0	5.0	4.55 \pm 0.51
		Female	6.0	7.0	6.44 \pm 0.51
6.	Total life period	Male	20.50	24.50	23.01 \pm 1.03
		Female	25.0	31.0	28.28 \pm 1.70
7.	Temperature (°C)	16.05	25.02	20.77 \pm 2.73	
8.	Relative humidity (%)	33.5	70.0	49.66 \pm 9.55	

Table 2: Hatching percent of eggs of citrus leaf miner, *P. citrella*

Period of study	Average temperature (°C)	Average Relative Humidity	Number of eggs Observed	Number of egg hatched	Hatching Percentage
5 th Jan., 2017 to 7 th Feb., 2017	20.75 \pm 2.09	46.60 \pm 11.11	20	15	75
			25	19	76
			20	15	75
			25	18	72
			30	24	80
			20	14	70
			20	14	70
			20	16	80
			25	18	72
			30	23	76
Min.					70
Max.					80
Av. \pm S.D.					74.67 \pm 3.66

Table 3: Pre-oviposition, oviposition, post-oviposition period and fecundity of *P. citrella*

Sr. No.	Particulars	Min.	Max.	Av. \pm S.D.
1.	Pre-oviposition period (days)	1.0	1.5	1.18 \pm 0.24
2.	Oviposition period (days)	3.0	3.5	3.22 \pm 0.25
3.	post-oviposition period (days)	1.0	1.5	1.20 \pm 0.25
4.	Fecundity	40	76	54.47 \pm 8.89
5.	Temperature (°C)	16.05	25.02	20.77 \pm 2.73
6.	Relative humidity (%)	33.5	70.00	49.66 \pm 9.55

Table 4: Sex ratio of *P. citrella*

Sr. No	Total number of adults observed	Number of		Sex ratio
		Male	Female	Male: female
1.	14	7	7	1:1.0
2.	20	9	11	1:1.2
3.	20	10	10	1:1.0
4.	12	5	7	1:1.4
5.	14	6	8	1:1.3
Total	80	37	43	1:1.2

Conclusion

The average incubation period, hatching percentage, first instar, second instar, third instar, fourth instar, total larval period and pupal period of *P. citrella* were (2.44 \pm 0.49), (74.67 \pm 3.66), (1.20 \pm 0.25), (1.22 \pm 0.25), (2.22 \pm 0.25),

(2.72 \pm 0.25), (7.36 \pm 0.55) and (11.12 \pm 0.78), respectively. The longevity of male and female moth were (4.55 \pm 0.51) and (6.44 \pm 0.51) days, respectively. The pre-oviposition, oviposition and post-oviposition periods of the insect were (1.18 \pm 0.24), (3.22 \pm 0.25) and (1.20 \pm 0.25) days, respectively. The fecundity was observed to be (54.47 \pm 8.89) eggs /female. Sex ratio to male: female was found to be 1: 1.2.

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