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Life cycle of South American tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) in Nepal

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Abstract

In order to develop integrated management strategy against recently introduced invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) in Nepal, life cycle and few biological parameters were studied in laboratory conditions (27 ± 2 °C temperature and 75 percent relative humidity) at Entomology Division, NARC during August to September, 2017. The average developmental period from egg to adult of *T. absoluta* was found 18.37 days. Average egg, larval and pupal periods was recorded 3, 8.26, and 7.11 days respectively. Adult longevity was found 13.47 days irrespective of sex. Length and breadth of egg was found 0.43 mm and 0.21 mm, whereas, 4.88 mm and 1.45 mm of pupa. Length of first, second, third and fourth instar larvae were recorded 0.63 mm, 1.59 mm, 3.86 mm and 7.19 mm, respectively. The head capsule width was found 0.15 mm, 0.27 mm and 0.41 mm of first, second and third larval moults.

Keywords: *Tuta absoluta*, life cycle, developmental stages, NARC, head capsule.

1. Introduction

South American tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is invasive insect pest of tomato (*Lycopersicon esculentum*, Miller) [1]. Larvae of this insect can damage 80-100 percent of cultivated tomato plants in newly invaded areas, if control measures are not applied [1]. Larvae of this insect pest feed on leaves, apical stems and fruits of tomato plants. This insect pest had been recorded in Nepal for the first time in May 2016 from Kathmandu and subsequently spread into tomato fields of adjoining districts of Kathmandu valley [2]. Recently this insect has been reported from tomato growing areas of 23 districts out of 77 districts in Nepal [3]. This pest has been recorded mainly from mid hills and plain areas of Nepal.

Tuta absoluta is multivoltine r-selected lepidoptera [4]. Average developmental period depends upon temperature 76.3 days at 14 °C, 39.8 days at 19.7 °C and 23.8 days at 27.1 °C [6]. Temperature thresholds for egg, larva and pupa were 6.9 ± 0.5 °C, 7.6 ± 0.1 °C and 9.2 ± 1.0 °C respectively; overall, the threshold for egg - larva - adult was 8.1 ± 0.2 °C [6]. The optimum temperature for *T. absoluta* development was found 19 – 23 °C with 52 percent survival rate from egg to adult [5]. Development period decreases with increase in temperature above 23°C and adult longevity was found 40 days at 10 °C whereas, 16 days in 19°C [5].

T. absoluta is a devastating pest of tomato and very difficult to manage with a single management technique. Integrated pest management need to be practiced for proper management of *T. absoluta* [2, 3]. Knowledge on life cycle and biology of insect pest is necessary in designing an effective integrated pest management strategy. Some study on life cycle of *T. absoluta* had been done in United Kingdom and South America but such study in Nepal and similar climatic conditions are lacking. Considering this fact life cycle and some biological parameters of the *T. absoluta* was studied in laboratory conditions of Entomology Division, NARC. This paper highlights major findings on life cycle of *T. absoluta* with duration of different developmental stages along with measurements of their sizes.

2. Materials and methods

2.1 Source of *T. absoluta* and Maintenance of insect culture

Source of insect culture of *T. absoluta* was obtained from commercial tomato growing farms within Kathmandu valley. Larvae collected from infested fields were reared into adults for maintaining insect culture in Entomology Division of NARC. Adults without sexing were reared in rearing cages made of insect proof net in wooden frames with dimensions of 47cm X 47cm X 77cm of length, breadth and height. Pesticide free fresh shoots of tomato plants along

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with leaves were provided for egg laying. 30 - 35cm long three tomato shoots were bundled with cotton ball and wrapped with aluminum foil in lower portion of stem. These shoots were dipped into water in conical flask of 1000 ml capacity to keep fresh. Cotton swabs soaked with 10 percent honey were kept on petri-plates in insect rearing cages as adult food. The tomato shoots were changed daily and eggs were collected along tomato shoots. These old shoots along with eggs were kept in separate cages according to collection date. After hatching of eggs fresh tomato shoot along with leaves were provided regularly for larval food and habitat. Pupae were collected from these cages and kept in transparent plastic boxes of 250 ml capacity covering with black muslin cloth. Separate boxes were maintained for pupae collected in different dates. After emergence of adults, were transferred to rearing cages for egg laying. Continuous cycles of *T. absoluta* were maintained throughout the year for regular supply of various stages of insect needed for scientific studies.

2.2 Life cycle study of *T. absoluta*

Life cycle of *T. absoluta* was studied in laboratory conditions of Entomology Division, NARC. The life cycle was studied during August to September 2017, in tomato leaves. Temperature of laboratory during experimental period was maintained at 27 ± 2 °C, whereas, average relative humidity was maintained at 75 percent. Leaves along with fresh eggs were kept in glass petri-plates of nine cm diameter. Leaves were kept above slightly moistened filter paper spread on petri-plates in order to protect leaves from drying. After hatching eggs, fresh tomato leaves were provided and larvae were transferred to fresh leaves from old leaves every day. Single larva was kept in each petri-plate and observation from 47 such petri-plates were recorded, which completed life cycle. Larval exuviae were observed to determine moulting during transfer of larvae to fresh tomato leaves. Whole process was performed under stereo-microscope (Bestscope BS-3040T). Pupae were left intact in petri plates until adult emerged. After emergence, adults were transferred to transparent plastic boxes of 250 ml capacity with black muslin cloth cover. One pair of adult sexed on the basis of abdomen described by Andrew *et al.* 2013^[5] was kept in each plastic box. 10 percent honey solution was given as adult food by spreading few drops on muslin cover. Longevity recorded from these adults and boxes were discarded when both adults died.

During life cycle study, time required for the first instar larva to enter fully into tomato leaf was also recorded with digital camera (Bestscope, BUC5C-500C, 5MP) attached to stereo-microscope (Bestscope BS-3040T). The digital camera was connected to laptop computer to observe whole leaf entering process of larva.

2.3 Sizes of developmental stages of *T. absoluta*

Sizes of developmental stages of *T. absoluta* were measured with help of ocular micrometer. Measurements were done with help of ocular micrometer in stereo-microscope (Bestscope, BS-3040T). Ocular micrometer was calibrated with stage micrometer of one millimeter size (ERMA INC. Japan) at observed level of magnification. Length and breadth of egg and pupa, length of larvae, length and wing span of adult moth were measured. Head capsule widths of moults were also measured.

All observed data were entered in excel sheet and analyzed using Genstat Discovery edition 4. Mean values \pm SEM (standard error of mean) along with range values was used in results.

3. Results and Discussion

3.1 Life cycle of *T. absoluta*

Life cycle of *T. absoluta* consists of four developmental stages viz. egg, larva, pupa and adult. Adults of *T. absoluta* generally laid eggs on tomato leaves both on dorsal and ventral surfaces in laboratory conditions. Eggs were creamy white in color which turns into light yellow color before hatching. There were four larval instars. The first instar was creamy white in color which turns into greenish color in process of development. The larvae after third moult turned into pinkish color and pupae were green in color which later changes into brown color. Pupation generally took in leaf or filter paper in laboratory.

Mean duration of various developmental stages of *T. absoluta* at 27 ± 2 °C temperature and 75 percent average relative humidity is given in Table 1. Result showed that after being laid eggs hatched in three days of time period. Average larval period in present study was found 8.26 days ranging between 8 - 9 days. Average period of larval instars were found 2.5 days, 1.79 days, 1.5 days and 2.68 days for the first, second, third and fourth instars, respectively. The average pupal period was found 7.11 days which ranged between 6 - 9 days in laboratory conditions. The average developmental period from egg to adult emergence was found 18.37 days ranging between 17 - 21 days. Barrientos *et al.*^[6] found the developmental cycle of *T. absoluta* depended on environmental conditions: average developmental time was found 76.3 days at 14 °C, 39.8 days at 19.7 °C and 23.8 days at 27.1 °C temperature. Present study found shorter developmental period than study conducted by Barrientos. A study conducted in UK, showed development from egg to adult took 53 days in 13 °C, 37 days in 19°C and 23 days at 25 °C temperature, 65 percent relative humidity and 16:8 hours of light and dark regime. In both studies conducted by Barrientos^[6] and Cuthbertson^[5] revealed lower developmental period from egg to adult in higher temperatures. In present study the developmental period was shorter, as average temperature is 27 °C, which is higher than study conducted in UK. The relative humidity was also higher (75 percent) and photoperiod was not controlled.

Average adult longevity of *T. absoluta* was found 13.47 days which ranged between 7 - 21 days irrespective of sex. Total life span of adult moths was found 72 days at 13 °C and 35 days at 23°C in UK^[5]. In our present study adult life span was found quite shorter than study conducted in UK. However, Polat *et al.*^[7] recorded adult longevity as 3.37 – 6.14 days in males and 7.7 – 9.75 in females in natural conditions, which is shorter duration than our present finding. Estay^[8] found adult longevity ranged between 10 - 15 days for female and 6 - 7 days for male, which was similar to our present finding of 13.47 days irrespective of sex.

Table 1: Mean duration of various developmental stages of *T. absoluta* in laboratory conditions.

Stages	Duration (Days)*	Duration range (Days)
Egg	3.00 \pm 0.000	3
Larva first instar	2.50 \pm 0.080	2 - 3
Larva second instar	1.79 \pm 0.092	1 - 3
Larva third instar	1.50 \pm 0.104	1 - 3
Larva fourth instar	2.68 \pm 0.116	1 - 4
Total larval period	8.26 \pm 0.085	8 - 9
Pupa	7.11 \pm 0.179	6 - 9
Adult	13.47 \pm 0.594	7 - 21

*Values presented as Mean \pm SEM (Standard Error of Mean)

The egg hatching percentage was found 75 in present study. Cuthbertson *et al.* [5] reported hatching percentage of *T. absoluta* egg was above 80 at 23 °C temperature and hatching percentage declined above 23 °C temperature. In our study the egg hatching percentage was found 75, which was lower than above findings as average temperature was higher. The time required for the first instar larva to fully tunnel into leaf tissue after hatching was found 54 minutes in present study. Study conducted in UK found the first instar larva was exposed approximately for 82 minutes in leaf surface before completely entering into leaf tissue [5]. This first instar larval wandering time before tunneling into leaf tissue is very important from management perspective, as exposed larvae are vulnerable to chemical insecticides, biopesticides and predators [5]. The biopesticide, *Bacillus thuringiensis* has shown capacity to kill *T. absoluta* larvae in laboratory conditions [9]. The success of *Bacillus thuringiensis* is dependent on contact or ingestion of lethal dose which is only possible, when larvae are free living in exposed condition: once larvae tunnel into leaf tissue, it is difficult to get contact with *Bacillus thuringiensis*.

3.2 Sizes of developmental stages of *T. absoluta*

Mean size of various developmental stages of *T. absoluta* reared at 27±2 °C temperature and 75 percent average relative humidity in Entomology Division is given in Table 2. The length and breadth of egg was found 0.43 mm and 0.21 mm, respectively. Length of first, second, third and fourth instar larvae were measured 0.63 mm, 1.59 mm, 3.86 mm and 7.19 mm, respectively. Mean size of pupa was recorded 4.88 mm long and 1.45 mm wide. Adult moths were 5.49 mm long irrespective of sex in average ranging from 5.3 - 6.2 mm. Average wing span of adults were observed 9.6 mm which ranged between 9 - 10 mm. Arno and Gabarra also mentioned the adult wingspan reaching 10 mm and eggs are 3.5 mm long [10].

Table 2: Mean size of various developmental stages of *T. absoluta* reared in laboratory conditions.

Stages	Measurement (mm)*	Range (mm)
Egg length	0.43±0.005	0.41 - 0.45
Egg breadth	0.21±0.003	0.20 - 0.22
Length of first instar larva	0.63 ±0.007	0.57 - 0.65
Length of second instar larva	1.59±0.019	1.43 - 1.63
Length of third instar larva	3.86±0.041	3.70 - 4.05
Length of fourth instar larva	7.19±0.069	7.00 - 7.50
Length of pupa	4.88±0.008	4.67 - 5.20
Breadth of pupa	1.45±0.001	1.45 - 1.33
Adult length	5.49±0.093	5.30 - 6.20
Adult wing span	9.60±0.163	9.00 - 10.00

*Values presented as Mean±SEM (Standard Error of Mean)

3.3 Head capsule width of larval instar

Average width of head capsule of different larval moult of *T. absoluta* reared at 27±2 °C temperature and 75 percent average relative humidity in Entomology Division is given in Table 3. The head capsule was measured from the larval exuviae, which is generally found on leaf surface or entry-hole made by larval feeding. Head capsule width of the first instar larva after moult was measured 0.15 mm, second and third instar larvae were measured 0.27 mm and 0.41 mm, respectively. Head capsule size and instar statistics are necessary to understand basic phenomena associated with larval stage of insect [11]. The effect of any insecticide varies according to larval instar [12]. This fundamental entomological information is important for proper timing of insecticide

application as it depends upon the dominance of particular larval stage in field conditions. Instar information is also necessary in proper application of entomopathogens for effective management of insect pest.

Table 3: Mean head capsule width of various larval instars of *T. absoluta* reared in laboratory conditions.

Stages	Measurement (mm)*	Range (mm)
First moult	0.15±0.002	0.14 - 0.16
Second moult	0.27 ±0.003	0.25 - 0.29
Third moult	0.41 ±0.003	0.39 - 0.43

*Values presented as Mean±SEM (Standard Error of Mean)

4. Conclusion

Life cycle of *T. absoluta* was studied in Entomology Division in laboratory conditions during 2017 in Nepal. The average developmental period from egg to adult of *T. absoluta* was found 18.37 days, ranging 17-21 days. The egg, larval and pupal periods were recorded 3 days, 8.26 days and 7.11 days, respectively. Adult longevity was found 13.47 days ranging between 7-21 days irrespective of sex. Length and breadth of egg was found 0.43 mm and 0.21 mm, whereas, 4.88 mm and 1.45 mm of pupa. Length of first, second, third and fourth instar larvae were recorded 0.63 mm, 1.59 mm, 3.86 mm and 7.19 mm, respectively. The head capsule width of first, second and third moults were found 0.15 mm, 0.27 mm and 0.41 mm. These finding on life cycle and biological parameters will help in understanding development of *T. absoluta* and formulation of integrated pest management strategy against it.

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