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IMMATURE STAGES AND CHAETOTAXY OF *SPODOPTERA LITURA* (F)

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ABSTRACT

This study explores the chaetotaxy, and biology of immature stages of the tobacco cutworm, *Spodoptera litura* (F) which is a major pest of crops. From Himachal Pradesh, apricot (*Prunus armeniaca*), curly dock weed (*Rumex crispus*), apple (*Malus domestica*) and plum (*Prunus salicina*) are reported as its new hosts. It takes 29-39.32 days to complete its lifecycle with six larval instars. The chaetotaxy of final instar and pupal morphology are described and illustrated.

Key words: *Spodoptera litura*, lifecycle, eggs, immature stages, moulting, larva, instar, chaetotaxy, pupa, host plants, *Malus*, *Prunus*, *Rumex*

The genus *Spodoptera* Guenée, 1852 is a relatively derived group in family Noctuidae with about 30 described species (Mitchell et al., 2006; Kergoat et al., 2012). Most species are distributed in tropical and subtropical zones and occur in open-land habitats (Monti et al., 1995). Some species of *Spodoptera* are highly similar in adult morphology, whereas the larvae possess certain valid and useful distinguishable characters (Levy and Habeck, 1976; Zenker et al., 2007). *Spodoptera litura* (F) is a known polyphagous pest and widely distributed throughout tropical and subtropical regions (Gong et al., 2014; Selin-Rani et al., 2016). It occurs on many field crops and vegetables, such as cotton, soybeans and cabbage (Kaur et al., 2016; Gandhi et al., 2016), and move across the fields like an army and hence called as army worm (Ahmad et al., 2007). Due to nocturnal habit, high mobility of adult moths and ability to oviposit on a wide range of host plants, *S. litura* it occurs on a wide range of ecological situations (Chelliah, 1985), and inflicts significant losses to soybean (Choudhary and Srivastava, 2007; Prinithavalli et al., 2014; Sharma et al., 2014), cotton (Gedia et al., 2008) and groundnut (Dhir et al., 1992; Patil et al., 1996). During regular surveys carried out from 2019 to 2022 in different localities of district Kullu in Himachal Pradesh, the leaves of apricot, curly dock weed, apple trees and plum trees have been observed heavily damaged. This study explores the morphological characters of eggs, larval instars and pupae of *S. litura* along with the chaetotaxy of last instar larva.

MATERIALS AND METHODS

Regular surveys were carried out in Sainj valley of District Kullu of Himachal Pradesh (31.77029° N, 77.30515° E), from March, 2019 to November, 2022. To study biology, egg masses were collected from host plants viz., apricot, curly dock weed, apple tree and plum tree and transferred to petridishes with fresh leaves of host plants. After hatching, larvae were placed in insect breeding cages (8″x 8″x 8″) and proper food was provided for further rearing. The final larval instar were transferred to another cage having soil for proper pupation. The pupae thus obtained were collected and kept in small plastic jars covered with muslin cloth. The male and female pupae were sorted out for further studies.

The eggs, different larval instars and pupae were preserved in 70% alcohol for further studies. For larval chaetotaxy, the larval head was detached from the body with utmost precision and the larval body was given a mid-ventral longitudinal cut in order to make skin preparation. Both, head as well body of the larva were potashed and properly dehydrated for further examination. The tactile setae as well as proprioceptors or micro setae present on head and body were observed. The data on pre-oviposition period, oviposition period, fecundity, incubation period, larval period, pupal period and adult longevity of male and females were recorded. The photographs of eggs, larvae along with their feeding pattern, damaged host plants, pupae and adults were taken with digital camera in the field as well as in the laboratory. The nomenclature proposed by Scoble (1992) for eggs; Hinton (1946), Peterson (1962), Stehr (1987) and Huertas-Dionisio (2006) for larval morphology and chaetotaxy; Mosher (1916) and Casagrande (1979) for pupal morphology was followed.

RESULTS AND DISCUSSION

The results reveal that eggs are spherical creamish and laid in masses of 200-300 eggs on the under surface of leaves. The egg mass is approximately 3-6 mm in size and covered with yellowish-brown hairs; eggs are laid one over the other in three layers. The colour changes from yellowish to black few hours prior to hatching. The hatching takes place in the early morning hours in 2 to 6 days. It has been observed that the larvae passes through six instars; The first instar, neonate larvae are brown with black head and distinct black band on the first abdominal segment, 1.3-1.6 mm long, and lasts for about 2-4 days on apple. The 2nd instar are pale green with black head having distinctly visible black hairs on its body and tiny black spot on first thoracic segment; 2.5-3.0 mm long, and lasts for about 3-5 days. The 3rd instar larvae are also pale green, but without distinct hairs. The first and second instar larvae are found scraping the chlorophyll of the leaves and converting the lamina into a papery form. However, after moulting, the third instar larvae start dispersing to feed individually. The third instar larva is 4.0- 4.8 mm long and lasts for about 2-5 days. The 4th instar larvae change their body colour to dark green with three pale yellow longitudinal lines, one medial and other two on lateral sides head to last abdominal segment. A pair of large dorsal black spots on first and eighth abdominal segments, followed by lightly visible, dark crescent shaped spots on both sides of remaining abdominal segments. The length of fourth instar ranges from 10.5-10.8 mm, this lasts for about 3-6 days. The fourth instar larvae change their body colour to dark green in fifth instar, with three pale orange longitudinal stripes, one middorsal and two dorsolateral ones from head to last abdominal segment. A pair of large dorsal black spots on first and eighth abdominal segments, followed by dark crescent shaped spots on both sides of remaining abdominal segments. The spots on first segment are larger than those on eighth segment; length ranges from 12.8-13.4 mm, and lasts for about 5-9 days. The 6th instar is dark greenish-brown with only one mid-dorsal dotted stripe, 30.8-31.2 mm long and lasts for about 2-3 days.

Prior to pupation, the mature larvae curl their body in C-shape and the prepupal period lasts for about 1-2 days. Pupae appear pale yellowish initially, later turn dark reddish-brown in colouration. The male pupae show genital aperture in the form of X shape on 9th abdominal segment on ventral side. Likewise, the female pupae have inverted V shaped genital aperture on 8th abdominal segment. The length of pupa varies from 11.5-13mm and lasts for about 8-12 days. Adult pale ochreous, much suffused with dark brown; abdomen paler. Forewing with some ochreous streaks at base; an angled and oblique subbasal line; a waved and curved antemedial line; orbicular spot oblique, fuscous with black edges; an oblique reniform 'arrow-head' mark; outer half of median nervure and bases of veins Cu_2, Cu_3, M_3, and discocellulars prominently pale black; an indistinct lunulate postmedial line excurved beyond cell; a waved submarginal line with black streaks; cilia with pale streaks. Hindwing opalescent and semi-hyaline white, with dark marginal line. The entire lifecycle is completed in 29-39.32 days.

The first instar larva emerges out from the micropylar region of the egg. After emergence, the larva immediately starts crawling towards the soft leaf of the host plant. The first and second instar larvae are observed scraping the chlorophyll of the leaves and converting the lamina to a papery form. The third, fourth and fifth instar larvae are observed as voracious feeders eating the whole leaf blade and leaving only the midrib intact. Moulting takes place during the daytime i.e., between 12.00 pm to 3.00 pm. It takes about 4-5 hr to moult to the next instar. In the field conditions, the pupation has been noticed in the soil, near the host plant and in the laboratory conditions, it generally takes place along the walls of rearing cages. The final instar becomes motionless one day prior to pupation. It lifts its body from middle and adopts a curved posture to transform into pupa. It takes about 7-8 hr to undergo metamorphosis for pupation. After 8-12 days of pupation, the pupa changes its colouration particularly the abdominal region, turning darker. The wing pattern becomes clearly visible. The eclosion takes place between 11.00 am-2.00 pm. It has been seen that the adults generally rest on the lower part of the host plant near soil surface. After mating, the gravid females start laying eggs in masses on the underside of leaves. The adults show nocturnal behavior and usually get attracted to light. The immature stages are available in the field during April to October. Final instar caterpillar is stout, cylindrical; head orange; dorsal surface dark greenish-brown with only one mid-dorsal dotted stripe; a yellowish-green lateral longitudinal stripe below spiracles; ventral surface pale green in colour. True legs orange; pro-legs pale green. Head sclerotized and projected ventrad (hypognathous) with inverted 'Y' shaped epicranial suture. Lateral ocelli six in number, adjacent to the base of mandibles. Antennae short, three segmented. Mandibles well developed and prominent. Labium with a distinct protruding spinneret. Adfrontal areas well developed with two distinct adfrontal sclerites.

Cranium is sclerotized, dark brown; epicranial suture very short, one third length of lateral adfrontal suture (Fig. 1). Epicranial suture and lateral adfrontal areas well marked. Epicranial notch deep, forming two lateral rounded hemispheres. Integument smooth, with few shallow wrinkles. Frontoclypeus long and broad. Stemmatal area well differentiated from rest of cranium, with six stemmata. Surface smooth with 16 pairs of long primary setae including on mouthparts and no secondary setae. Out of which, 14 pairs of long tactile setae comprising of two pairs of adfrontal setae (AF1 and AF2), three pairs of anterior setae (A1, A2 and A3), two pairs of ocellar setae (O1 and O2), one pair of lateral setae (L1), two pairs of posterior-dorsal setae (P1 and P2), one pair of frontal setae (F1), one pair of clypeal setae (C1) and two pairs of minute proprioreceptor setae on vertex (V1 and V2). Mandibles having two pairs of primary setae (MD1 and MD2) below well-developed anteclypeus. Adfrontal group (AF): Adfrontal setae (AF1 and AF2) clubbed and aligned to parallel with the adfrontal suture. AF2 is close to the junction of epicranial and adfrontal sutures. Anterior group (A): A1 and A2 filiform, nearly vertically aligned; A3 clubbed. A1 and A2 of same length, A3 slightly longer than A1 and A2. A2 more distant from A3 than A1. A3 next to stemmata 3. Clypeal group (C): C1 clubbed, located laterally near adfrontal suture. Frontal group (F): F1 clubbed, nearly aligned with C1. Lateral group (L): L1 clubbed, located at middle of lateral head capsule, close to stemma 1. Posteriodorsal group (P): P1 and P2 clubbed. P2 much further away from epicranial suture than P1. P1 slightly longer and P2 more ventral than P1. Ocellar group (O): Ocellar setae (O1 and O2) clubbed. O1 located close to stemma 5. O2 located inside the semicircle delimited by stemmata 1-6 and close to stemma 3. O1 larger than O2. Proprioreceptor group (V): V1 and V2 clubbed. Mandibular group (MD): MD1 and MD2 clubbed and vertically aligned. MD1 shorter than MD2. Stemmata 1-6 arranged in a semicircle at the lateral sides of head capsule. Thorax is three segmented with three pairs of four segmented true legs. Fourth segment having a single curved claw with distinct arolium. Prothorax with one pair of spiracles. Whitish or pale white middorsal dotted stripe and two white dorsolateral stripes present on prothoracic shield.

Chaetotaxy of thoracic segments (Fig. 2) reveal the following- Prothorax (T1): Prothoracic shield much chitinized, bearing four pairs of setae, XD1, XD2, D1 and D2, all filiform. XD1 and XD2 present on anterior margin and longer than D1 and D2. D1 located close to posterior margin of the shield and the middorsal stripe, more dorsal and posterior than D2. D2 distributed near posterior lateral margin of the shield, nearly of same length as D1. Three lateral setae (L1, L2 and L3) present, L1 located anterior to the spiracle. L1 longer and more dorsal than L2 and L3. L2 and L3 of same length. Mesothorax and Metathorax (T2 and T3): Each having seven pairs of setae, D1, D2, L1, L2, L3, SV1 and V1. D1 and D2 almost of equal length. L2 shortest, L1 and L3 nearly equal in length. SV1 posterior and ventral to L3 and larger than L3. V1 very short and close to midventral line. D1, D2, L1, L2 and L3 filiform, SV1 and V1 clubbed.

Abdomen is ten segmented with five pairs of fleshy, non-segmented pro-legs on 3rd, 4th, 5th, 6th and last segments. A pair of rounded spiracles present on lateral sides of each segment (A1-A8); smaller in size than prothoracic spiracles. Chaetotaxy (Figs. 3-5) reveals the following- Abdominal segment I and II (A1 and A2): All filiform. D1 anterior and dorsal to D2, slightly longer than D2. SD1 typically above the spiracle, nearly as long as D2. L1, L2 and L3 of equal length, L1 aligned vertically to the spiracle, whereas L2 and L3 located posteriorly to L1. SV1, MV1 and V1 present only on A2. SV1 and MV1 present on A1. Abdominal segment III-VI (A3-A6): All filiform. D1, D2, SD1, L1 and L2 arranged in a similar pattern as on A1 and A2, but slightly lower in position. SV1 located near the surface of prolegs. V1 and MV1 absent. Abdominal segment VII and VIII (A7 and A8): All setae filiform. D1 slightly shorter than previous ones present on A1-A6. SD1 dorsal to the spiracle. L1 and L2 aligned ventrally to the spiracle. SV1, SV2 and SV3 present only on A7, absent on A8. Abdominal segment IX (A9): D1 and SV1 absent on A9. SD1 located anteriorly, L1 and L2 nearly in vertical alignment. All setae filiform. Abdominal segment X (A10): All setae filiform. Anal shield subtriangular, bearing four pairs of setae, D1, D2, SD1, and SD2. D1 much longer and more anterior than D2. SD2 anterior and ventral to SD1. V1 and V2 positioned near midventral line on the inner side of anal proleg. Crochets: Crochets on abdominal prolegs uniordinal mesoseries.

Pupa (Figs. 6-8): Adecticous and obtect; soft and pale yellowish immediately after pupation, becoming shiny greenish-brown after a few hours. Dark brown and hardened after four to five days. Body Elongate, cylindrical. Head round, vertex extending towards posterior region along prothorax and antenna. Thorax with partly visible legs. Abdomen tapering, edges of all segments well marked; punctuation marks on anterior

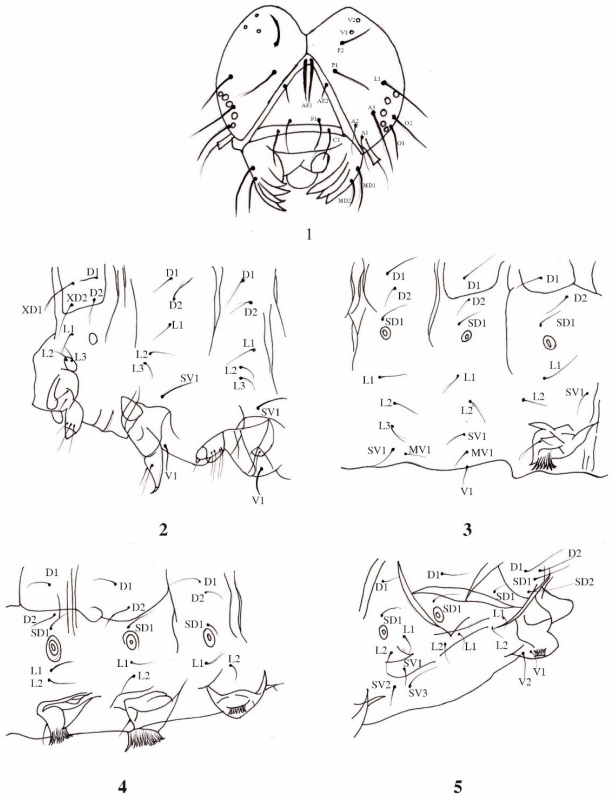

Fig. 1-5. *Spodoptera litura*: 1. Cephalic capsule of final instar (frontal view); 2-5. Thorax and abdomen of final instar (lateral view)

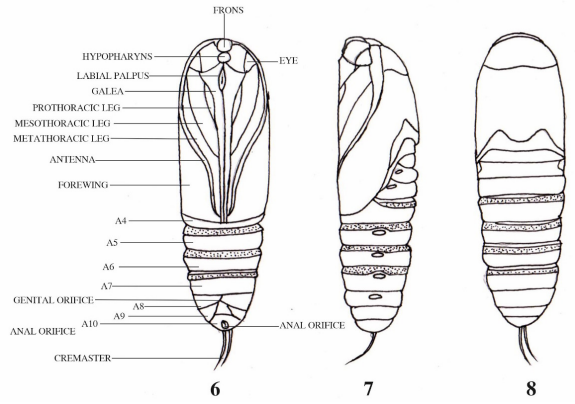

Fig. 6-8. *Spodoptera litura*: Pupae: 6. Ventral view, 7. Lateral view, 8. Dorsal view

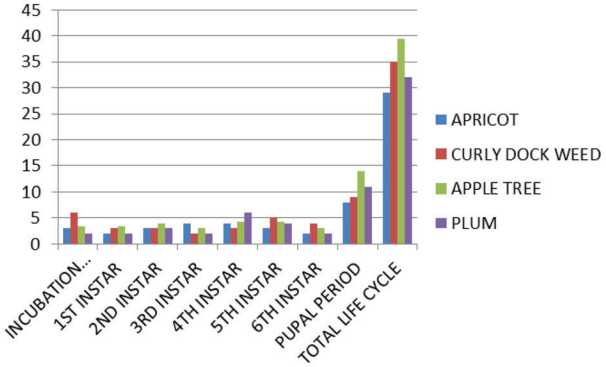

Fig. 9. Life stages of *S. litura* on host plants

third portion of segments 4-7; A9 and A10 equal in length; A10 with forked cremaster. Male pupae having genital aperture on 9th abdominal segment and female pupae with genital aperture on 8th abdominal segment on ventral sides.

The arrangement of eggs is found in groups having 2 to 3 layers. Waterhouse and Norris (1987), Hill (1975) and Ahmad et al. (2013) also reported 3-4 consecutive layers of eggs in a single batch hatching generally in 2-3 days. The observation about egg lying on underside of leaves is in conformity with Thomas et al. (1969), as they also recorded the egg lying of *S. litura* on under surface of tobacco, castor and banana leaves. The abundant female hair silk (modified scales) are found covering egg masses forming a 'net' appearance on egg surfaces. The previous studies of Peterson (1964), Skudlik et al. (2005), Zenker et al. (2007), Korycinska (2012) and Rolim et al. (2013) also reported such coverings. Similarly, Rao et al. (1993) and Ramaiah and Maheshwari (2018) also observed the egg mass covering with the scales from the tip of abdomen of females in *S. litura* (Fabricius, 1775). Cardona et al. (2007) also observed rounded eggs laid in masses by *S. litura* (Fabricius, 1775) as in the present study. Latha et al. (2014) reported that the larvae of *S. litura* (Fabricius, 1775) undergo five moults and six larval instars as observed in the present study. But, Cardona et al. (2007), Amin et al. (2011) and Deepak et al. (2020) about five instars. As far as feeding is concerned, the early instar larvae feed on soft leaf tissue and older ones with stronger biting mouthparts even feed on veins and leaf ribs. The mature larvae usually feed at night and remain in soil during day time at the base of host plants. Similar findings were reported by Gupta et al. (2015) on mango tree. In present study, the total duration of life cycle on apricot, curly dock weed, apple and plum trees is 29, 35, 39.32 and 32 days, respectively. Narvekar et al. (2018) reported its total lifecycle of 37 days on cassava. They also remarked that lifecycle on castor, cowpea, mulberry, okra, sweet popato, taro and groundnut is 28.33, 32, 34.33, 32.67, 30.67, 31.67, 31.33 days, respectively; on castor by Ashok and Pavithran (2021) showed 35.08+6.68 days. The total developmental period is 32.67 days on germinating seeds of soybean and 43.72 days on linseed (Sharma, 1994). Arvinthraju et al. (2022) observed the total lifecycle on cassava as 40.0+2.83 days.

Certain larval characters, such as setal pinacula, chaetotaxy and crochets are considered as valid and useful characters in lepidopteran taxonomic utility (Kitching 1984; Miller 1991; Deml and Dettner 2002; Hasenfuss and Kristensen, 2003; Vegliante and Zilli, 2004; Beck, 2009; Mutanen et al., 2009; Cardoso et al., 2017; Xue and Hua, 2018). In chaetotaxy, the setae are usually categorized in three categories i.e., primary, sub-primary and secondary setae (Hinton, 1946; Peterson, 1962; Stehr 1987). The primary setae of the first instar larvae are considered as the basis of homology of setae in mature larvae (Dyar, 1905; Fracker, 1915). Zenker et al. (2007) studied and illustrated the chaetotaxy of last instar of *Spodoptera cosmioides* Walker. Hu et al. (2021) examined the head and body chaetotaxy of the first instar larva of *Spodoptera depravata* (Butler). Chamundeswari et al. (2021) described the morphological characters and chaetotaxy of thoracic and abdominal segments (particularly of 3rd abdominal segment) and arrangement of crochets on ventral prolegs in four species of pod borers namely spotted pod borer, *Maruca vitrata* (Geyer); tobacco caterpillar, *S. litura* (Fabricius); blue butterfly, *Lampides boeticus* (L) and pea pod borer, *Etiella zinckenella* (Trieitscke). While providing standard guidance for identification of four species of *Spodoptera* i.e., *S. frugiperda* (Smith), *S. eridania* Stoll, *S. littoralis* (Boisduval) and *S. litura*, EPPO (2015) described larval morphology and pupal morphology of *S. exigua* (Hübner). During present study, it has been observed that the larvae of *S. litura* are eruciform and each larva bears three pairs of thoracic legs and five pairs of prolegs on A3-A6 and A10 (anal prolegs). The crochets of prolegs are arranged in uniordinal mesal penellipse and protected by membranous stripes. The same type of characterization was reported in previous studies of Forbes (1954), Kristensen 1998) and Hu et al. (2021).

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AUTHOR CONTRIBUTION STATEMENT

K S A: Compiled and analyzed data; K Y: Insect collection, rearing, photography, chaetotaxy and compilation.

CONFLICT OF INTEREST

No conflict of interest.

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