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Comparative Histology of the Larval Digestive System of Two Genera of Noctuidae (Lepidoptera): *Agrotis and Spodoptera*

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ABSTRACT

Noctuid moths and caterpillars act as very serious pests in causing damage to crops throughout the world. Owing to their wide distribution and polyphagy, these pests attack different parts of the plant causing huge economic losses. In this study, the morphology of the gut canal is described and illustrated for the fifth instars of *Agrotis ipsilon* and *Spodoptera litura* based on light microscopic images. For this study histological preparation was made from gut wall tissues of two genera. This histological preparation was done by section preparation of gut tissue with ultramicrotome which is followed by staining of tissues with haematoxylene – eosin (Delafieled) method. Particular attention was given to the gut histology of two genera *Agrotis ipsilon* and *Spodoptera litura*. The digestive system of species in these two genera was similar, but some differences in morphology and cytology were reported between the two genera. The histology of these pests are also compared with same species feeding on another host to unveil the effect of host plant on gut structure. The knowledge gained through this study will clarify the biology of these pests.

***Key words*:** Lepidoptera, Noctuidae, Gut, Histology, Larvae

Noctudae is a family under the super family Noctuidea of order lepidoptera. Noctuidae is also the second largest family of Noctuoidea. This family comprises more than 35,000 known species placed in 29 sub families and 4,200 genera. The word Noctuidae is derived from the name of the type genus Noctua, which is the Latin name for little owl. So, this group is also known as owlet moth, commonly called as worms, as cutworms, leafworms, armyworms etc. These larvae are serious pests of many plant crops. Cut worm and armyworm these two names are used because of the larval behavior of this group. Moths of this family have prominent eyes and robust structures. These moths fly during night and are very much attracted to bright light. These moths are terrestrial and have enormous importance in food web and ecosystem. Many species of this group are considered as an agricultural problem around the world. Many species have larvae that live in soil and many have larvae that feed upon leaves. Majority of Larvae of this group is polyphagous and herbivorous. Their efficiency as pest can be visualized by the fact that almost every cultivated plants believed to have at least one pest belonging to this family. Their attack can be found on any part of the plant such as the shoot, foliage, root or fruits [1]. Their larvae destroy crops, gardens and orchards every year. All around the world species including *Helicoverpa armigera* Hübner, 1808. *Spodoptera litura*, *Agrotis ipsilon* Hufnagel, 1766. *Helliothis virescens* Fabricius, 1777. are major pests of staple crops such as sorghum, maize, potato and millet. In India 218 genera, 1431 species [2-4] of Noctudae are reported. These pests are believed to have potentially high economic impact in terms of direct damage as this cause wide damage to a variety of agricultural, medicinal as well as forest plants. Mostly two types are categories in terms of type of damage one is climbing type affect on leaves another group is soil pest affect on stems. Leaf eating pests like *Spodoptera litura* affect both young and matured leaves and soil pests like *Agrotis ipsilon* feed upon both stems from underground and stems above the ground. In these cases i.e., for climber and soil pests young instars do not harm most but fourth instars destroy large area of crop field. Some noctuidae pests like tobacco bud worm also feed not only upon foliages but also upon reproductive parts of plants and fruits. Another species Corn earworm feeds upon blossoms, leaves and fruits also. Insects belonging to genus *Spodoptera* are well known pests in agricultural sector attacking a wide range of crops, primarily paddy and tobacco and secondarily tomato, ground nut, castor, soya bean etc. Similarly, another Noctuid pest, *Agrotis ipsilon* owing to its polyphagous nature and wide distribution is reported to affect almost 100 crops such as, Wheat, Corn, Soyabean, vegetables etc. [5]. *Helicoverpa armigera*, another pests belonging to the same family is known to

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affect mango, cotton, castor, gram opium etc. plantation throughout India [6]. These pests are widespread throughout India affecting a variety of crops. If we consider economic damages by these noctuidae pests regarding tobacco in that case loss is huge. For an example The Old-World bollworm or *Helicoverpa armigera* produces agricultural losses in every year that exceed 2 billion US dollar. Additionally, the variegated cutworm or *Peridroma saucia* is also one of the most damaging pests to vegetables.

Efficiency of pests can be linked to their gut structure and function. Insect gut function and morphology are dependent on many factors including feeding behavior, food source, development stages and taxonomic position [7]. All insect gut histology follows the same basic plan. The fore and hind gut originates from embryonic ectoderm and is lined with cuticle [8]. The mid gut originate from embryonic endoderm [9]. Fore gut is designed for short term food storage and transport of food to mid gut [10]. In the mid gut food is digested by enzymes and nutrients are absorbed through the inner columnar epithelium of mid gut lining [11]. Hind gut is divided into rectum, ileum and pylorus. In the hind gut water and salts are absorbed. Feces pass from the body through anus [12].

Excretory organ or malpighian tubules attach at the junction of the mid gut and hind gut [13-14]. The digestion process and organ of digestion shows great variation due to alteration of food consumption. Physiological, biochemical and behavioral process altogether play role in adaptation to food of an insect [15]. The structure and length of the gut is associated with diet. If the insect is fond of protein in its diet, then the length of the gut must be short. In many insects at the joining of fore and mid gut there is a storage organ known as crop. Preliminary digestion sometimes takes place in crop. Mid gut is endodermal in origin and largest among the three i.e., fore, mid and hind gut. Mid gut does not have any chitinous lining. Mid gut is foremost site of digestion and releases major digestive enzymes for insects [16-17]. Mid gut of insects have three types of histological cells; columnar, goblet and regenerative cells [18]. These cells have variation in functions in different insect species [19-20]. Especially lepidopteran larvae have goblet cells in their hind gut [21-22]. The last part of alimentary canal which is hind gut starts with ileum followed by colon and rectum and ends with anus exteriorly. Hind gut wall is lined with thin layered permeable cuticle which prevents loss of useful food substances [23]. The excretory organ of insects is present between mid and hind gut. In most larval lepidopteran the malphigian tubules become closely attached with the rectal pads so that two water absorbing system are placed in series. In lepidopteran malphigian tubules are composed of two layers of cells. In this article, the histology of the gut of two polyphagous Noctuid species, namely *Spodoptera litura* and *Agrotis ipsilon* have been studied. In West Bengal, there abundance has been well studied in Coochbehar district having serious damages to tobacco plantations. Study on the structure of insect gut helps in gaining fundamental knowledge about the biology and their control.

MATERIALS AND METHODS

Larval collection

Tobacco plants leaves containing *S. litura* larvae were collected from Golokganj village near Dharala river of Mathavanga sub division of District Cooch Behar (26.2752°N, 89.2041°E) during December 2018. Larvae were maintained in perforated plastic pouches for maximum 48 hours in normal temperature. Larvae were removed from the foliage and head capsule measurements were taken using a dissecting microscope equipped with a calibrated micrometer in the objective lens. Larvae with head capsule width between 0.96 to 1.5 mm, which correspond to third to fifth instar larvae [24] were transferred to sterile petri dishes for 10 to 12 hours starvation period. Larvae were then placed in 0.25% aqueous NaCl followed by three 15 min rinses in tap water.

Histological preparations

The heads and tails were removed at the head capsule and eighth proleg respectively and then the gut was pulled from the hemocoel into fresh fixative using fine forceps. For histological study, the entire gut was cut into 3 pieces, fore mid and hind respectively. These 3 parts were fixed in 4% formalin solution for 7 days. After 7 days these gut tissues were placed in liquid paraffin within a porcelin cup and paraffin blocks were prepared then. These paraffin blocks were sectioned in ultramicrotome at 5 to 7 µm thickness for ribbon preparation. Paraffin ribbons containing tissue sections were prepared and placed within the Mayer's egg albumin coated glass slides and then placed on a hot plate to fix the tissue on the slide. The tissues on slides were washed in 50% alcohol and transferred to 70% alcohol. This step was followed by gradual dehydration as usual. After dehydration tissues were cleared in xylene. Then the sections were stained with haematoxylene – eosin (Delafieled) method [25]. Slides were mounted with DPX. After staining the tissue sections observations were made and microphotography was done.

RESULTS AND DISCUSSION

Fore gut of Agrotis ipsilon

Epithelium of the oesophagus is composed of cuboidal cells with large nuclei. A musculature is present surround the epithelium. Epithelium is longitudinally folded. This musculature includes a thick layer of longitudinal muscle (Fig 1). Lysis cells (LC) are followed by Peritrophic membrane (PM). Peritrophic membrane is surrounded by destroyed epithelium (DE), destroyed muscle (DM), epithelium (E) and muscle (M).

Mid gut of Agrotis ipsilon

In the mid gut lysis cells are well arranged and condensed, arrangement of destroyed muscle, destroyed epithelium and peritrophic membrane are more or less similar with that of fore gut. Mid gut is composed of 3 types of cell layers, one is circular cell layers on the outside, in the middle euglenoid cells and in the inner side there is a flat cell layer (Fig 3).

Hind gut of Agrotis ipsilon

In the hind gut, the lysis cell becomes more circular, peritrophic membrane beneath lysis cell are degenerating. Other musculatures are almost similar with that of fore and mid gut of *Agrotisipsilon.* This also consists of two layers of structures at the outer side circular testes lobe like structures andat the middle longitudinal cells. There are patches of musculature at the inner side of the middle longitudinal cells (Fig 5).

Fore gut of Spodoptera litura

Epithelium of the oesophagus is composed of cuboidal cells with spherical nuclei. There is chitinous intima medial to the epithelium, and mass of muscles encircle the outer part of the epithelium. The longitudinally invaginated epithelium is connected to the surrounding muscles only at some portions. This musculature consists of thick layer of circular muscles. Through the bands of circular muscles there are dialator muscles in certain part of esophagus. Thin-walled crop is with chitinous intima surrounded by squamous epithelium and thin layer of circular muscle (Fig 2).

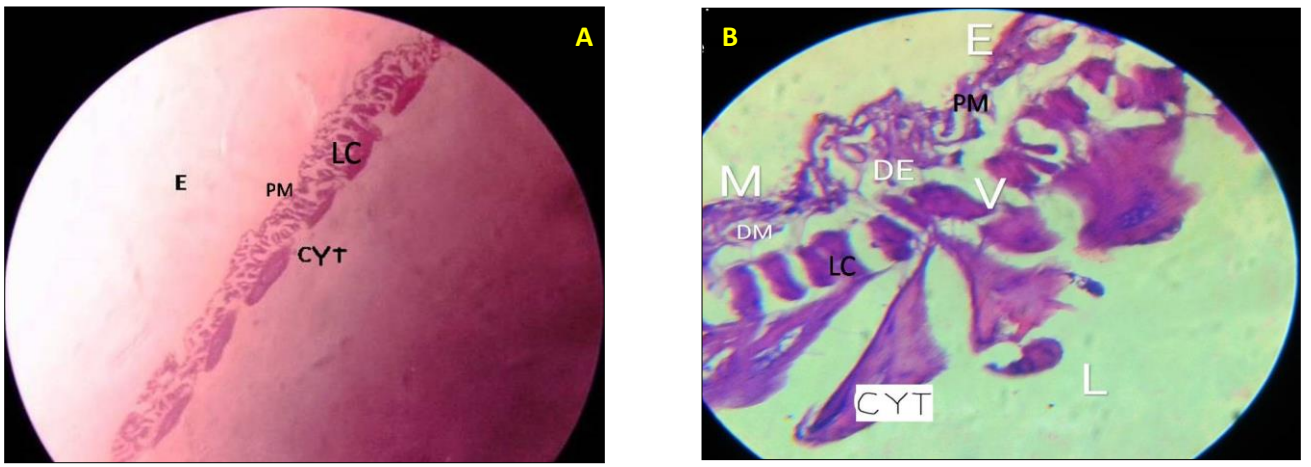

Fig 1 Sectional view of foregut of *Agrotisipsilon*at a. 10X magnification, b. 40X magnification
[E: Epithelium, CYT: Cytoplasmic mass, M: Muscle, DM: Degenerated muscle, DE: Destroyed epithelium, V: Vesicle, L: Lumen, LC: lysis cell, PM: Peritrophic membrane

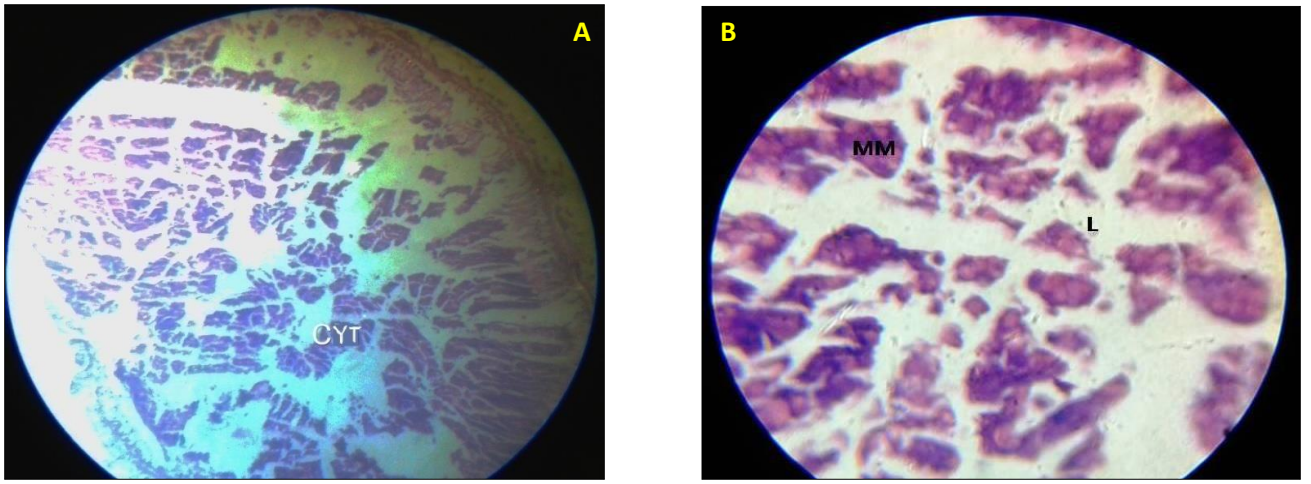

Fig 2 Sectional view of foregut of *Spodoptera litura*at a. 10X magnification, b. 40X magnification [CYT: Cytoplasmic mass, MM: Muscle mass, L: Lumen]

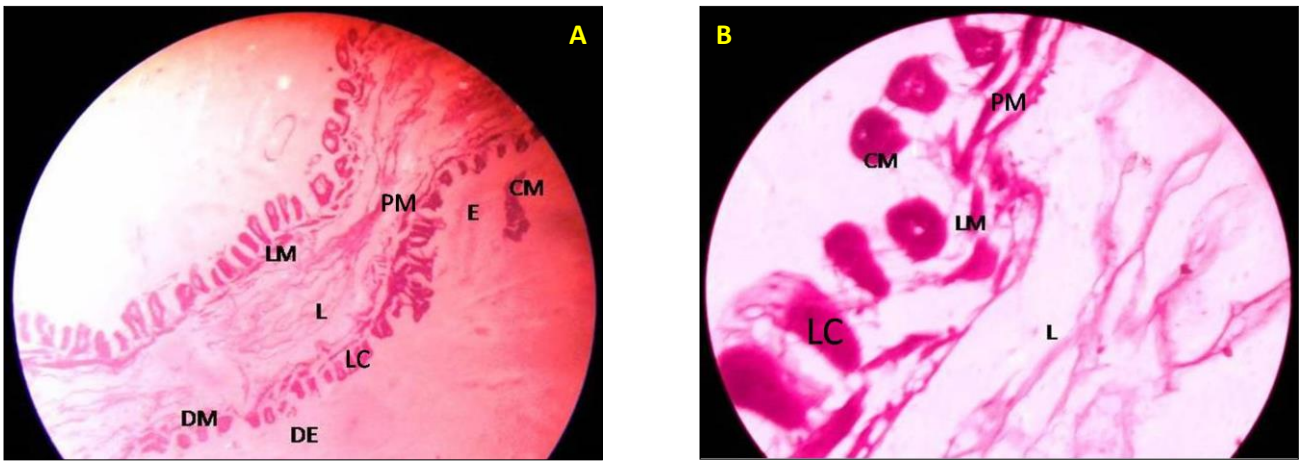

Fig 3 Sectional view of midgut of *Agrotisipsilon*at a. 10X magnification, b. 40X magnification
[E: Epithelium, CM: Circular muscle, DM: Degenerated muscle, DE: Destroyed epithelium, LM: Longitudinal muscle, L: Lumen], LC: lysis cell, PM: Peritrophic membrane

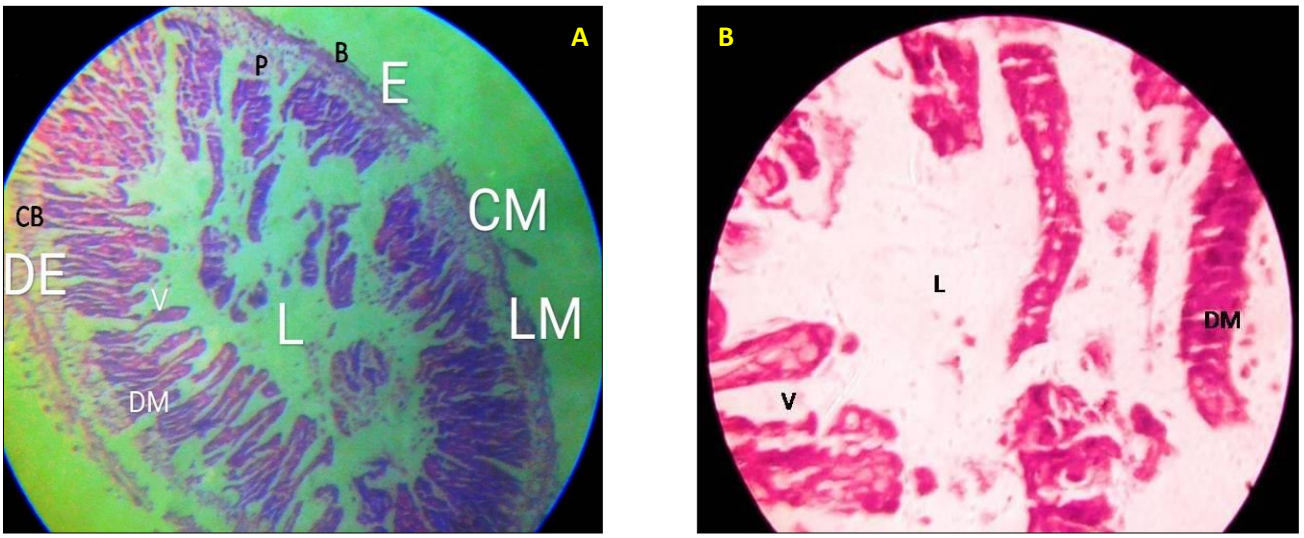

Fig 4 Sectional view of midgut of *Spodoptera litura*at a. 10X magnification, b. 40X magnification
[E: Epithelium, CM: Circular muscle, DM: Degenerated muscle, DE: Destroyed epithelium, LM: Longitudinal muscle, L: Lumen, V: Vesicle, P: papillary crypt, CB: ciliated border; B: basement membrane]

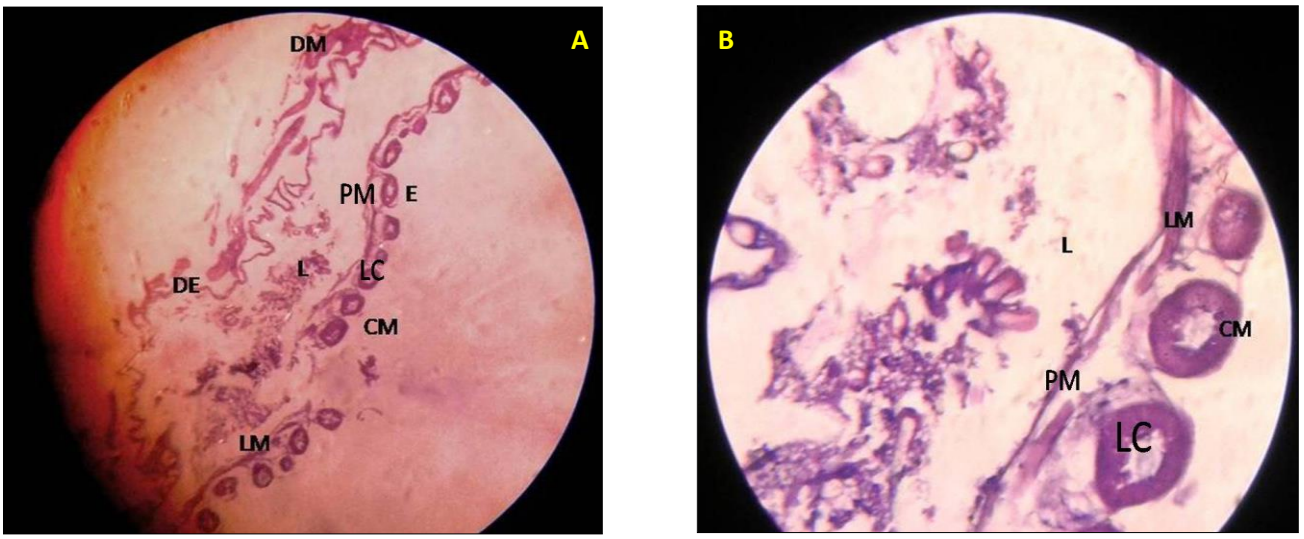

Fig 5 Sectional view of hindgut of *Agrotisipsilon*at a. 10X magnification, b. 40X magnification
[E: Epithelium, CM: Circular muscle, DM: Degenerated muscle, DE: Destroyed epithelium, LM: Longitudinal muscle, L: Lumen], LC: lysis cell, PM: Peritrophic membrane

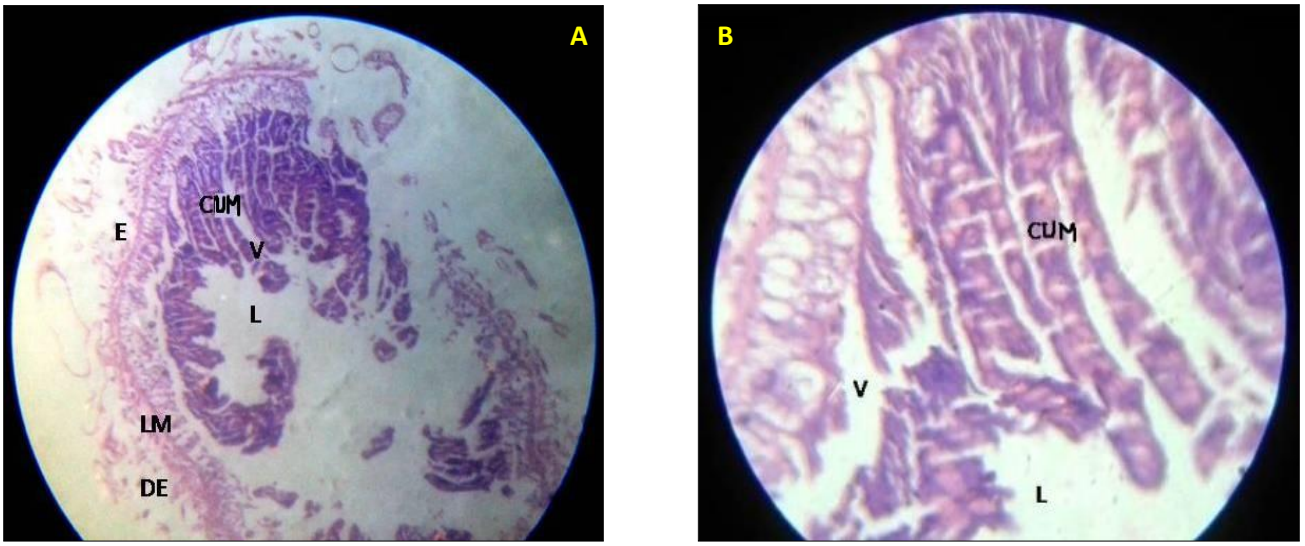

Fig 6 Sectional view of hindgut of *Spodoptera litura*at a. 10X magnification, b. 40X magnification
[E: Epithelium, CUM: Cuboidal muscle, DM: Degenerated muscle, DE: Destroyed epithelium, LM: Longitudinal muscle, L: Lumen, V: Vesicle]

Mid gut of Spodoptera litura

There is a series of small knob like gastric caecae at the junction of fore and mid gut and these are evenly distributed. These caecae are lined by low columnar epithelium cells with small nuclei. Mid gut of *Spodoptera* is consisting of three main parts, peritrophic membrane, mucosa and musculature. The peritrophic membrane is chitinous in nature. The mucosa consists of mid gut epithelium and regenerative cells. Regenerative cells have closely packed small spherical nuclei. These cells are also located basally among the mid gut epithelium. Two types of cells are present in mid gut and these are simple columnar and goblet cells. Columnar cells and goblet cells are also of two types. Type 1 has small spherical nucleus and type 2 has large nucleus. Type 3 is with nucleus at center and type 4 has large and densely packed nucleus. Types of secretion in the mid gut is both merocrine and holocrine. Two types of musculature is seen in mid gut; inner circular and outer longitudinal muscles (Fig 4).

Hind gut of Spodoptera litura

The posterior part of intestine is made up of low columnar epithelium cells possessed of small round nuclei. Intima is chitinous and starts at the posterior intestinal ring and end at the anus. Pyloric chamber is surrounded by squamous epithelium. Pylorus is subdivided into anterior, middle and posterior parts. Anterior part has thick squamous epithelium with large cuboidal cells and thickest musculature. In middle part squamous is interrupted by cuboidal cells. Posterior pylorus is with small lumen and has regular cuboidal epithelium (Fig 6).

From the transverse section of gut histology of *Spodoptera litura*, Fabricius, 1775. feeding tobacco leaves, it is observed that the lumen of the foregut is surrounded by closely attached cytoplasmic mass which is followed by a layer of degenerating epithelium and degenerating muscle layer [26-27]. There is a space between cytoplasmic mass and epithelium in case of fore, mid and hind gut [28-30]. There is a cytoplasmic mass forming near degenerating epithelium in case of foregut. Some vesicles are seen in between the forming cytoplasmic mass in case of mid gut of *Heliothis zea* [31-32].

From the transverse section of gut histology of *Agrotis ipsilon*, it is observed that in fore gut the epithelium is followed by muscle layer which is followed by rectangular or cuboidal cells and triangular cytoplasmic mass which can be relate to gut histology of *Bombyx mori* [33-34].

In mid gut there is a gap between epithelial layer and cytoplasmic mass. The rectangular cells are degenerating. This gap becomes larger in case of hind gut and new cells are present in cytoplasm. The cells are circular and there is a space in the centre of the cell which is followed by lumen which is comparable to *Ostrina nubilalis* and *Cecropia* gut [35-36].

While comparing the results with other studies it was noted that, the fore gut and hind gut histology of *Agrotis ipsilon* fed with potato and Castor bean leaves have no remarkable difference with those *Agrotis ipsilon* fed with Tobacco leaves [37]. But in case of hind gut there are some differences. In hind gut histology, in both the larvae, lysis cell is present, but lysis cell is less condensed along with the presence of a peritrophic membrane beneath the lysis cell in case of larvae fed with potato leaves. Also, the basement membrane is near the lysis cell in larvae fed with potato leaves whereas in larvae fed with tobacco leaves it is placed apart [37]. Comparison with larvae feeding on castor bean leaves, it was noted that the Lumen, Circular muscle and epithelium cells of the hindgut are more or less similar along with the position of the basement membrane. However, the absence of goblet cells and microvilli in our study marks it different from larvae fed with castor bean leaves [38]. Such variations in gut histology might be due to the dissimilarity of feeding preferences of the studied larvae.

In *Agrotis ipsilon* gut, the presence of several strong circular muscle at the junction of buccal cavity and pharynx marks similarity with *Erionota torus* Evans 1941, *Euploea core* and Cramer, 1780 gut [39]. In all the three species, there is dorsoventral and dorsolateral dialetor muscle near the oesophagus. The musculature of the cephalic region of foregut in *Agrotis ipsilon* is similar to many lepidopteran larvae [40]. The crop wall is made of inner longitudinal muscle and outer circular muscle, this structure store large amount of food when needed. Mid gut is the largest organ located from the third thoracic segment to the sixth abdominal segment. The wall consists of strong longitudinal and circular muscle involved in peristalsis. The arrangement of the longitudinal muscle of the mid gut is external to the circular muscle. There are distinct pair of longitudinal muscle bands running along the mid line of the dorsal and ventral area of the gut one pair on each side. A transparent membrane lines beneath the epithelium along the entire mid gut, this is known as the peritrophic membrane. Hind gut consists of three regions: the pylorus, the ileum and rectum. The musculature of the mid gut terminates at the junction immediately before the confluence of mid gut and pylorus. The gut musculature of *Agrotis ipsilon* bears huge similarity to that of *Manduca sexta* Linnaeus, 1763 [41].

Similar comparison of *Spodoptera litura* larvae fed with caster bean leaves did not show any significant differences in fore and hind gut histology with the gut of larvae fed with tobacco leaves. But significant differences were noted in case of mid gut. The circular muscle, longitudinal muscle, destroyed epithelium and vacuoles are almost similar. But in case of larvae reared with caster bean leaves there are endocuticle, epicuticle, cytoplasmic granules and hypodermis present in the mid gut wall, however, no such structures were noted in the midgut of larvae reared with tobacco leaves in our study. We have also noted an absence of peritrophic membrane unlike other studies where this structure was noted [42].

CONCLUSION

This study presents the complete details of the histology of two important Noctuid pests of India. The knowledge gained through this study will be helpful in understanding the biology of these pests.

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