DAY TO DAY HISTOPATHOLOGICAL CHANGES IN THE SILKGLAND OF *BOMBYX MORI* L. INOCULATED WITH *BEAUVERIA BASSIANA* (BALS) VUILL

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ABSTRACT

The investigation mainly focused to examine the mutilation caused by the fungal pathogen *Beauveria bassiana* during its growth and development in 5th instar silkworm larvae using histopathological indices. Day to day histopathological analysis of three regions of silkgland i.e., anterior, middle and posterior regions during the development of fungal pathogen in fifth instar silkworm larvae revealed no significant changes in the first two days of the post inoculation. On the third day of the instar mycelia of *Beauveria bassiana* was noticed in the tunica propria the outermost layer of the silk gland and on the 4^{th} an 5^{th} day of inoculation, secretory cells in the glandular layer filled with mycelia. Shrinkage of silkgland tissue and vacuoles was observed on 6^{th} and 7^{th} day of post - inoculation of *Beauveria bassiana*. The histopathological study revealed extensive hyphal growth with a subsequent melanization and shrinkage of the tissue in the silkgland.

Key words: ***Beauveria bassiana*, *Bombyx mori*,** histopathological studies, Silkgland

INTRODUCTION

The major challenge confronted by the silk producing nations all over the world is a high incidence of silkworm diseases. The major diseases affecting mulberry silkworm are flacherie, grasserie, muscardine and pebrine. *Beauveria bassiana* is an aggressive parasite, has been causing great economic loss to sericulturists especially in the favourable season for silkworm rearing. *Beauveria bassiana* infection will affect cocoon production, resulting in substantial economic loss to the sericulture farmers. The progress of the pathogen in the host tissue is often revealed by the gradual changes in the infected tissues like cuticle, midgut, silkgland, fat body etc. In the present investigation an attempt has been made to understand the extent of damage caused to the general architecture of middle region of silkgland silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana.* Pathogens were reported to induce several structural, biochemical and physiological alterations in insects (Bergold, 1963). *Beauveria bassiana* infection results as a consequence of the invasion and growth of a pathogen, which in turn impair the tissue functions. Histopathological studies are desirable as the *Beauveria bassiana* is a intracellular microbe. With this milieu, the study has been carried out to understand day to day changes in the three regions of silkgland of *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana.*

MATERIALS AND METHODS

Pure Mysore × CSR2 silkworm strain was selected for the study. Silkworms are reared by following the methodology suggested by Dandin (2003). Immediately after fourth molt the healthy larvae were selected from the rearing stock and grouped into two sets. Each group consists of 4 replications with 100 larvae for each group. One set of larvae was treated with fungal spore suspension with sub lethal concentration (3.25×10^6 spores/ ml @ 50 ml/100 worms) and another set of larvae were treated with double distilled water and used as control. Silkworms from both the sets of were randomly selected every day, i.e. from 1st day to 7th day of the 5th instar and dissected in physiological saline solution and collected the three parts of silkgland i.e., anterior region, middle region and posterior region for day to day histopathological investigations. Silkgland was washed with saline solution and then fixed in 2% formaldehyde solution for 24 hours and sectioned at 5-6 µm thickness after being embedded in paraffin. They were stained with haematoxylin and eosin solution and viewed under bright field microscope, photographs were taken at a magnification of 100X.

RESULTS AND DISCUSSION

Histopathological alterations have been investigated in the three regions of silkgland of the 5th instar silkworm, infected with fungal pathogen *Beauveria bassiana* with reference to control (Fig I - 1st to 7th day of control and inoculated). No significant histopathological aberrations were noticed in the anterior region of silkgland during early stages of fungal infection i.e., from 1st to 3nd day of post-inoculation with fungal pathogen *Beauveria bassiana* compared to control (Fig I - 1st to 3rd day of control and inoculated). Slight variations were noticed in the cellular arrangement of the anterior region of silkgland due to induction of fungal mycelia on the fourth day of the treated silkworms with reference to control (Fig I - 4th day of control and inoculated). On the 5th day disruption of the three layers of the anterior silkgland and densely stained cells are visible in fungal infected anterior region of silkgland (Fig I- 5th day control and inoculated). Vacuoles were appeared on 6th and 7th day in the tunica intima layer and finally disintegration of the three layers were noticed in the inoculated silkworms compared to control (Fig I- 6th and 7th day of control and inoculated). In control no sign of infection was noticed in the anterior region of silkgland.

Histopathological studies showed different degrees of histological changes during the development of fungal pathogen in the middle part of silkgland in *Bombyx mori* compared to control (Fig II - 1st to 7th day of control and inoculated). Day to day histopathological analysis of the middle region of the silkgland during the development of fungal pathogen *Beauveria bassiana* in fifth instar silkworm larvae revealed no significant changes in the first two days of the post-inoculation (Fig II- 1st and 2nd day of inoculated). On the 3rd day of post-inoculation, fungal mycelia was noticed on the outermost layer of silkgland i.e tunica propria in the *Beauveria bassiana* infected larvae compared to control (Fig II- 3rd day of inoculated). Histopathological observations on 4th and 5th day of post-inoculation very clearly showed the disruption of glandular cells in the middle layer of the silkgland (Fig II- 4th and 5th day of inoculated). On the 6th and 7th day of the post–inoculated 5th instar silkworm larvae showed complete disintegration and shrinkage of all the three layers of the silkgland compared to healthy larvae (Fig II- 6th and 7th day of control and inoculated). No sign of infection was noticed in the histological sections of silkgland in healthy silkworms (Fig II - 1st to 7th day of control).

The histopathological changes in the posterior silkgland of silkworm treated with fungal pathogen revealed the characteristic histological changes in the treated posterior region of silkgland compared to control (Fig III - 1st to 7th day of control and inoculated). Histopathological assessment revealed, no prominent variations during the early stage of *Beauveria bassiana* infection (Fig III - 1st and 2nd day of control and inoculated) and third day onwards derangement of cellular layers in the posterior part of the silkgland was evident. On the 3rd day fungal mycelia was noticed at the periphery of the basement membrane of the posterior region of the silkgland (Fig III- 3rd day of control and inoculated). Histological observations on 4th and 5th day of post-inoculated 5th instar of the posterior silkgland elucidated the spread of the fungal mycelia in the three layers of the silkgland (Fig III- 4th to 5th day of control and inoculated). On 6th and 7th day extensive damage of the glandular cells and all the cell layers of the posterior gland was evident with reference to healthy silkworm (Fig III - 6th and 7th day of control and inoculated).

Silkgland is a long tubular structure that starts from the labial segment runs near to the caudal region and is morphologically and functionally divided into three parts. A thread like anterior part, a swollen middle part and a tubular crooked and curved posterior part. The anterior parts of the paired ducts unite and open in the final outlet called spinneret. Histologically the silkgland is formed of three layers. The outer tunica propria of uniform thickness, the middle glandular layer and the inner tunica intima of varying thickness. The tunica intima is very thick in the anterior region and is shed at each moult and in other regions it is thin and not shed at each moult. Tunica intima is followed by lumen. Histological arrangement in the anterior region of the silkgland speaks about the poor secretory role of the anterior region. The anterior region is a mere duct line with a thick cuticular intima and does not contribute to the secretion of silk materials. The middle silkgland is ‘S’ shaped with three distinct regions anterior, middle and posterior limbs. It is a storehouse of liquid silk (Kumutha 2009). The posterior region is highly folded and the folds lie in the midst of the dermo-visceral muscles and attached to the tracheal bushes of the region. It is almost uniform in thickness. The terminus of the posterior section ends blindly among the fat bodies in sixth abdominal segment. The posterior silkgland of the normal silkworm has a prominent secretory role and the region secrete the major protein of the silk, namely fibroin. Secretory cells have rich secretory granules and the secretory layer is wide when compared to tunica propria and the tunica intima.

The present histopathological investigations showed a lower degree of aberrations during the development of fungal pathogen *Beauveria bassiana* in the anterior part of silkgland in *Bombyx mori* compared to middle and posterior regions. Lower level of variations may be attributed to the thickness of the tunica intima in the anterior region of silkgland with reference to the middle and posterior region, which may resist the invasion of fungal pathogen and also the anterior silkgland does not have tracheal insertions into the basal lamina. Middle and posterior parts of silkgland are very active regions for the synthesis of silk proteins viz., sericin and fibroin respectively and for this reason there is a need of rich supply of oxygen, provided by tracheal system. The trachea arrives at the basal surface of the cells and their branches penetrate deeply into the cellular cytoplasm and intercellular spaces of the middle and posterior region of silkgland.

The investigation mainly focused to examine the mutilation caused to the middle region of silkgland by the fungal pathogen *Beauveria bassiana* during its growth and development in 5th instar silkworm larvae using histopathological indices. No significant histopathological aberrations were noticed in the first two days of the post inoculation. Similar findings were recorded by Sohaf *et al* (1993), they observed the hyphal growth after 48 hours of inoculation of *Beauveria bassiana* in silkgland of silkworm. On the 3rd day of post-inoculation, fungal mycelia was noticed on the outermost layer of silkgland i.e., on tunica propria in the *Beauveria bassiana* infected larvae compared to control. It is well supported by several workers. Brancalhao (2009) detected multiple nucleopolyhedrosis virus (BmMNPV) infection after 72 hours of post-inoculation in the cells of the middle and posterior silkgland. Rahman and Gopinathan (2004) noticed the infection in the middle region of silkgland of silkworm at 72 hours of post-inoculation with recombinant virus and no infections in the anterior silkgland. On 4th and 5th day of post-inoculation histopathological observations very clearly showed the disruption of glandular cells in the middle layer of the silkgland. This may be due to the growth of mycelium into the silkgland cells and tissue was filled with a mycelial mat which absorbs the nutrients from silkgland tissue. Further, fungal mycelia secrete certain enzymes (protease, lipase and carbohydrase), toxins and secondary metabolites like beauvericin during its growth and development in the host tissue. These substances released by the fungal pathogen may lead to poor secretory activity in infected glandular cells in the middle layer of the silkgland which is a storehouse of silk. Similar studies were conducted by Jhansi Lakshmi (2003). She observed the network of the mycelia all over the silkgland infected with *Beauveria bassiana* in silkworm *Bombyx mori*. 6th and 7th day of the post–inoculated 5th instar silkworm showed complete disintegration and shrinkage of all the three layers of the silkgland compared to healthy larvae. It may be due to mechanical damage caused by the fungal pathogen during its growth and development and also toxins released by the pathogen which may rupture the secretory cells in glandular layer leading to burst of silkgland. Secondly, the pathogen may absorb the nutrients and water from the host for its growth and development, which may lead to dehydration. It is very clearly evident from the investigation that silkworm larvae infected with fungal pathogen

Beauveria bassiana caused irreparable architectural changes in the middle region of silkgland, as a consequence there is reduction of silk output. The gradual histopathological changes causing damage and disintegration of the posterior silkgland due to *Beauveria bassiana* infection may be attributed to the massive proliferation of mycelia into the host tissue, which exerts mechanical force to penetrate and the penetrated mycelia draw the nourishment for its growth and development. Another rationale for the extensive damage of the posterior region of silkgland is that, it is well attached to the tracheal bushes of the region, as the tracheal system as an infection-spreading organ.

The observations are well supported by several workers. Akai *et al* (1993) reported the absence of the tracheal system in the anterior silkgland. Brancalhão *et al* (2009) examined the cytopathological changes in anterior, middle and posterior regions of silkgland of *Bombyx mori* under light and transmission electron microscope infected with multiple nucleopolyhedrovirus (BmMNPV). They detected infection was only at 72 h of post-inoculation in cells of the middle and posterior regions of silkgland. No sign of infection was found in the anterior silkgland. They stated that the histopathology showed early infection in regions surrounding tracheal insertions, demonstrating that trachea is an infection-spreading organ in the insect body. Trachea penetrates the middle and posterior silkgland basal lamina, considered a barrier to viruses, facilitating the penetration of budded virus. The anterior silkgland does not have tracheal insertions into the basal lamina, which reduces budded virus infection. The role of the tracheal system as an infection-spreading organ was described previously for larval nucleopolyhedrovirus infection (Rahman and Gopinathan 2004, Torquato *et al* 2006 and Pereira *et al* 2008).

It is well supported by Rahman and Gopinathan (2004), Torquato *et al* (2006) and Pereira *et al* (2008). Charnley (1984) stated that proliferation of blastophores and/or hyphal bodies in the haemolynph may be a prelude to early tissue invasion and extensive growth of mycelia in the haemolymph and penetration of host tissues will disrupt host physiology, cause stress reactions. Zacharuk, (1973) expressed that there is a considerable circumstantial evidence from Deuteromycete pathogens for the involvement of fungal toxins in host death.

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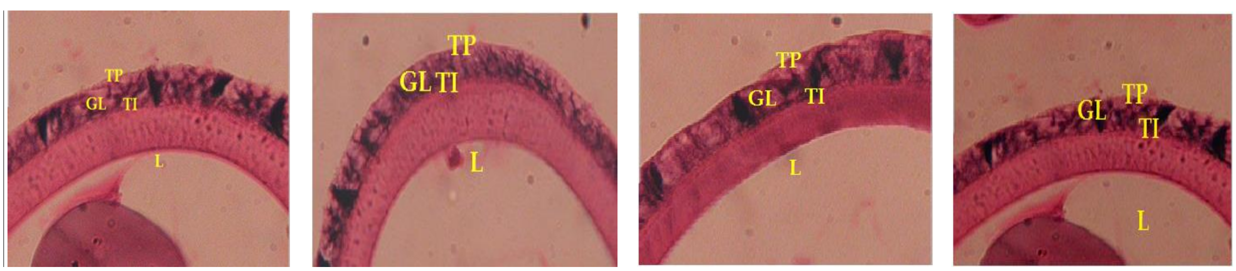
Figure - I Day to day histopathological changes in the transverse section of anterior region of silkgland of 5th instar silkworm *Bombyx mori* infected with fungal pathogen *Beauveria bassiana*



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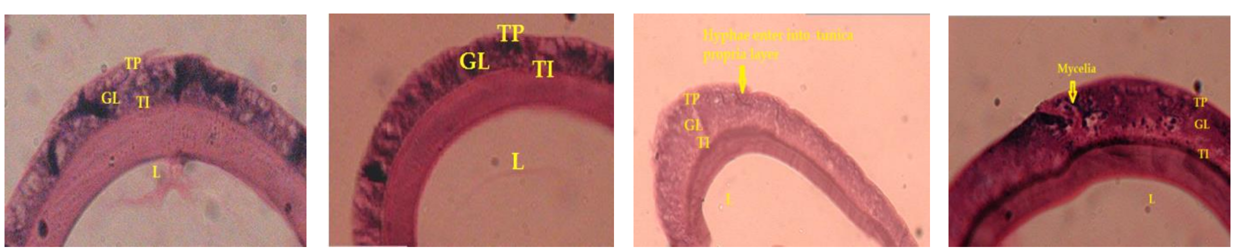

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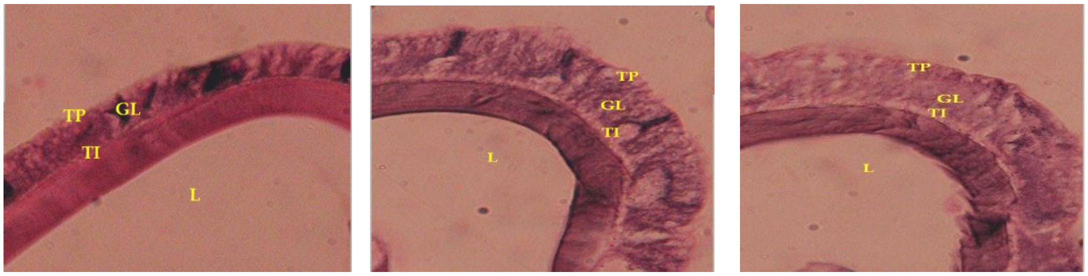

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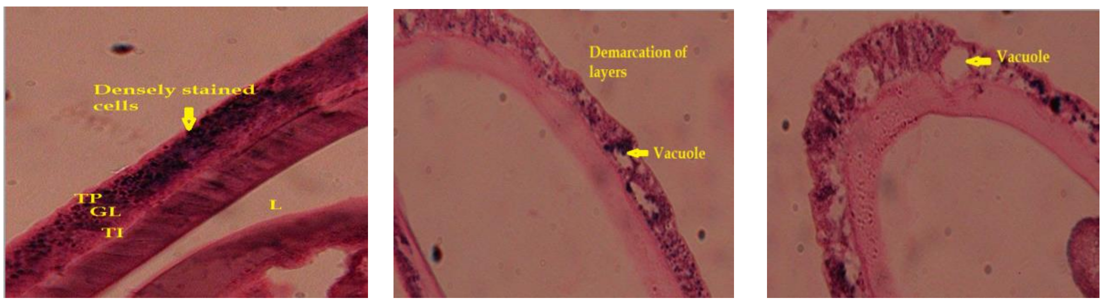
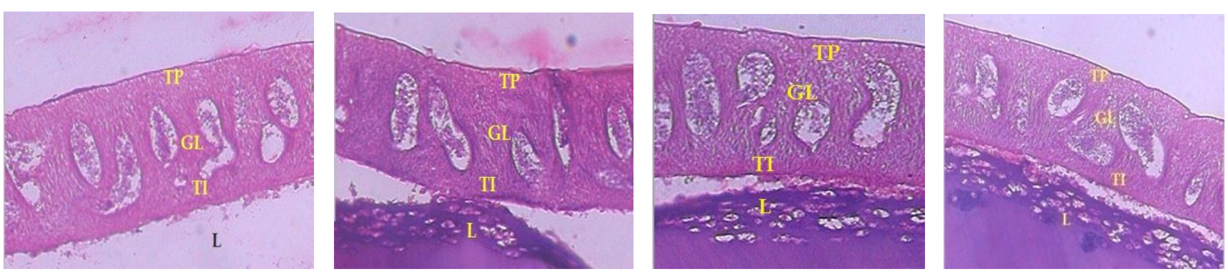


5th day Inoculated 6th day Inoculated 7th day Inoculated

Figure - II Day to day histopathological changes in the transverse section of middle region of silkgland of 5th instar silkworm *Bombyx mori* infected with fungal pathogen *Beauveria bassiana*



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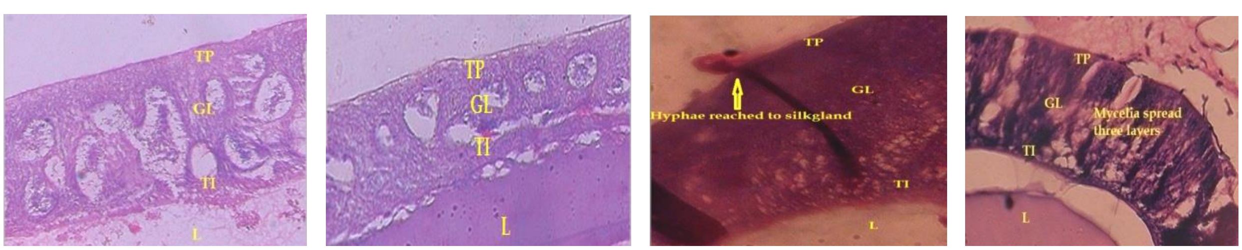

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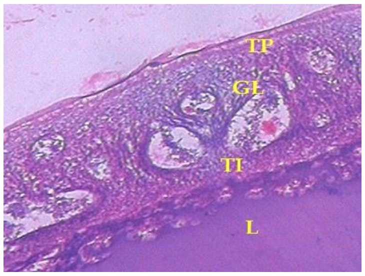

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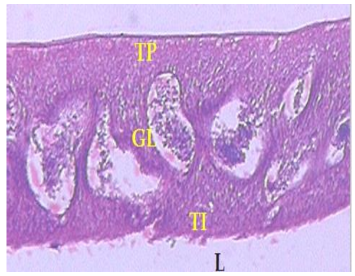

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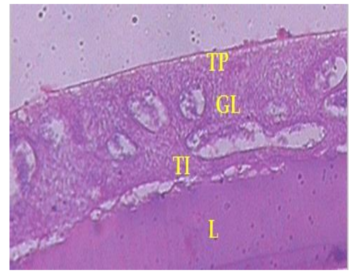
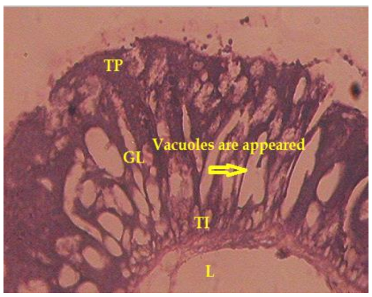
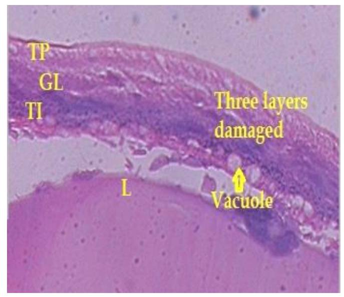


7th Day Control



5th day Inoculated



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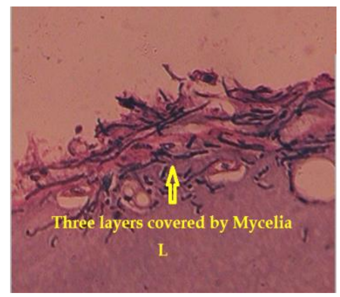
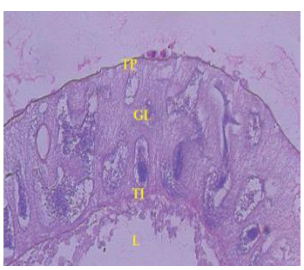


7th day Inoculated

Figure - III Day to day histopathological changes in the transverse section of posterior region of silkgland of 5th instar silkworm *Bombyx mori* infected with fungal pathogen *Beauveria bassiana*



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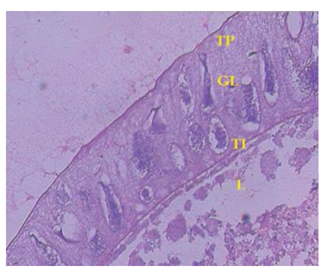

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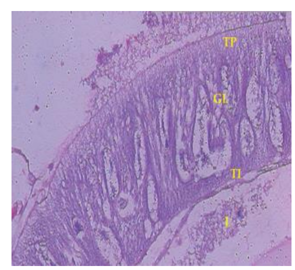
3rd day Control



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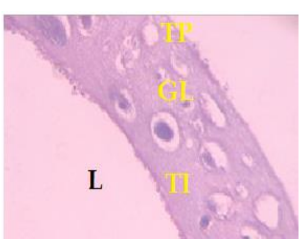

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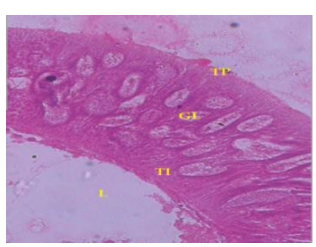

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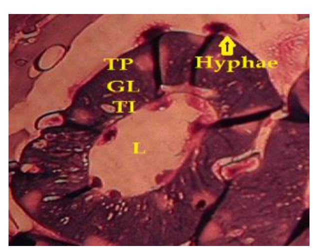
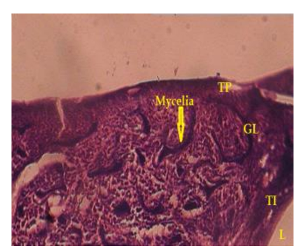


3rd day Inoculated



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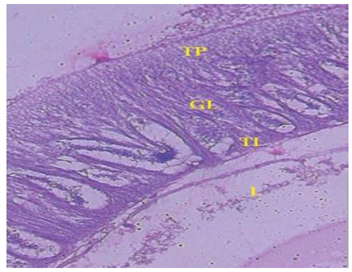

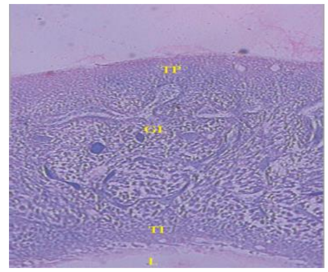

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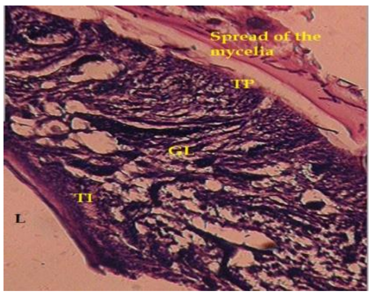

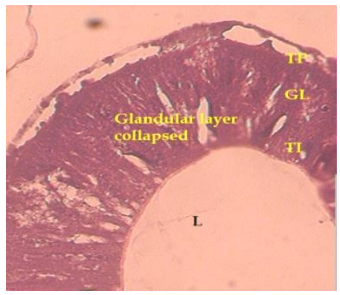

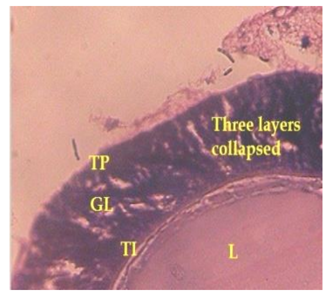

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TP- Tunica Propria GL-Glandular Layer TI – Tunica Intima L- Lumen