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Research Article

EFFECT OF *ASPERGILLUS FUMIGATUS* INFECTION ON THE SILKGLAND OF *BOMBYX MORI* L.

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ABSTRACT

In the present study we evaluated that effect of *Aspergillus fumigates* infection on the silk gland of *Bombyx mori*. The fifth instar *Bombyx mori* larvae was used and inoculated with *Aspergillus fumigates* inoculum of conidia spore suspension by sprayed on mulberry leafs and fed to silkworms. Mulberry leaves sprayed with distilled water served as control. A pair of silkgland was removed from infected and control group larvae at 72 and 96 hours of post infection and washed with distilled water. The wet weight of the silk gland was measured on an electronic balance, the wet weight of the silk gland was decreased on the 72 and 96 hours of infection when compared with control group. The salivary glands and transverse section of middle silk gland did not exhibit any signs of fungal infection but the cocoon weight was significantly declined following infection over uninfected larvae.

INTRODUCTION

Silkworm is the domesticated and economical important insect, which produces luxuriant silk thread in the form of cocoon by consuming mulberry leaves during larval period (Rahmathulla, 2012). Silk worm good invertebrate animal model that are killed by pathogenic bacteria and fungi when they are injected into haemolymph. There are several advantages to using silkworm as an infection model, such as low cost, no ethical problems and large body size (Chikara Kaito and Kazuhisa Sekimizu, 2007). Aspergillosis disease is most common fungal disease caused by *Aspergillus* in *Bombyx mori* and cause major economic loss in sericulture in dustry. The genus Aspergillus includes several hundred fungal species, among these *A. fumigatus, A. flavus, A. oryzae and A.niger* are responsible for Aspergillosis in silkworm. Low temperature and high humidity conditions required for their growth of silkworm these conditions also favorable for the growth of Aspergillus, which are causing high risk for the occurrence of Aspergillosis disease (Shobha *et al*., 2016). Silk gland is an exocrine gland it secrete large amount of silk protein. There is one pair of silk gland located at the two lateral sides under the alimentary canal, produce threads of silky material to form the cocoon and are mainly composed of three parts, the anterior, the middle, and the posterior silk glands. During fifth instar the silkglands are reach maximum size due to the accumulation of silk protein and degenerates completely during larval-pupal transformation through the process of autophagy and apoptosis. The present study we evaluated that pathogenicity of *A. fumigatus on* the silk gland of *Bombyx mori* at72 h and 96h after infection.

Rearing of Bombyx mori larvae

Fifth instar day sixth larvae of CSR-4 bivoltine breeds ware reared on fresh mulberry leaves. The silkworms are maintained on mulberry leaves at a temperature of 27ºC and relative humidity of 75%. The life span of the silkworm under these conditions was 30-32 days (Harinathareddy and Venkatappa, 2016).

Experimental design

In the present study 5th instar silkworm (*Bombyx mori* L.) larvae were used and divided into two groups, each group consisting of 30 larvae. One group was maintained as control (normal and healthy ones) and the other group was infected with the *A. fumigatus* by sprayed on mulberry leafs and fed to silkworms. Strict hygiene was maintained and beds clearing were followed strictly.

Inoculation of silkworm larvae

The fungal culture *A. fumigatus* isolated from the silkworm rearing house. Fungal samples were maintained on Potato Dextrose Agar Medium. Fungal spore suspension was prepared by adding 2 ml of sterile distilled water to freshly (7 days) grown slants of the above cultures (Shobha *et al*.,

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2016).Aspergillus inoculam of condia spore suspension was inoculated (1.0×10^6 to 1.0×10^7 CFU/mL) by sprayed on mulberry leafs and fed to silkworms. Mulberry leaves sprayed with distilled water served as control.

Measurement of silk gland weight

Silkworms were sacrificed from infected and control groups at 72 hr and 96 hr of infection. A pair of silk gland was removed from *Bombyx mori* by making cut opened longitudinally along the mid dorsal line and wash with distilled water. The wet weight of the silk gland was measured using a balance just after removal.

RESULT AND DISCUSSION

Measurement of silk gland weight

Silkworms were dissected and a pair of silk gland (20 pairs) was collected and the weight of the each silk gland was recorded in grams. There was no major changes were observed in the silk gland of infected group when compared to healthy larvae. The wet weight of the silk gland in infected group was lower when compared with control group (Table 1).

Table 1 Measurement of silk gland weight of the control and infected larvae.

Fifth instar silkworm larvae	Control group silk gland weight in grams (Mean values)	Infected group silk gland weight in grams (Mean values)
After 72 h of infection	1.42	1.35
After 96 h of infection	1.58	1.21

The salivary gland cells did not exhibit any signs of fungal infection (Fig 1a) but silk gland weight and cocoon weight was significantly declined following infection over uninfected larvae. Silk gland assimilates the mulberry protein in the course of larval development and grows considerable size and weight to provide protein material during the spinning of the cocoon. The process of protein assimilation and growth of silk gland depend mainly on the physiological conditions of the silkworm during larval growth (Harinatha Reddy and Venkatappa, 2016). This suggests that *A. fumigatus* interferes with the normal physiology of the silkworm and affects the weight of the silk gland possibly by reducing the assimilation of proteins.

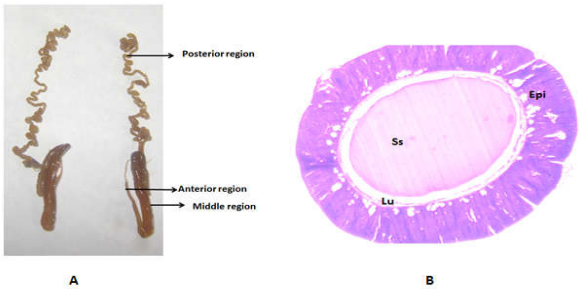

Figure 1 (a). A pair of silk gland form infected silkworm larvae (b). Transverse section of middle silk gland of the silkworm larvae after 96 h of fungal infection; Lumen (Lu); Epithelial cells (Epi); Secretary substance (Ss).

Silk gland is larval specific tissue of lepidopteran insects begins to degenerate shortly before pupation (Terashima *et al*., 2000). Each gland is basically a tube made of glandular epithelium with two rows of cells surrounding the lumen (Mondal *et al*., 2007), and divided anatomically and physiologically into three distinct regions the anterior, middle and posterior divisions. The anterior silk gland is aduct consisting of a single cell layer of approximately 300 substantially large, polyploidy cells which have branched nuclei and are lined with a thick cuticular intima at the internal surface. During the prepupal period, anterior silk gland cells die in a rapid and synchronous manner in response to the pulse of ecdysone (Terashima, 2000; Kakei *et al*., 2000; Iga *et al*., 2007).

In microscopic observation of the middle silk gland, the cavity of the silk gland of *B. mori* was filled up with liquid like secretary substances (Fig 1b). At the spinning stage no liquid-like silk protein materials were seen in the inner cavity of the silk gland. The silk gland of *Bombyx mori* degenerates during larval-pupal metamorphosis via programmed cell death and the steroid hormone ecdysone triggers this cell death process (Terashima *et al*., 2000; Chinzei, 1975).The silk fiber protein is synthesized by silk gland cells and stored in the lumen of the silk glands. Subsequently, it is converted into silk fibers. When the silkworms secrete the liquid silk during the spinning, it passes through the anterior gland and expelled out through the spinneret opening (Shimizu, 2000).

Mortality

The percentage of mortality was increased gradually after inoculation with *A. fumigatus*. In the infected group 60% of mortality was observed after 96 hr of infection. There is no death cases reported in the control larvae feed with distilled water.

CONCLUSION

Aspergillosis disease is most common fungal disease caused by *Aspergillus* in *Bombyx mori* and cause major economic loss in sericulture industry. Infection of *A. fumigatus* interferes with the normal physiology of the silkworm and affects the weight of the silk gland possibly by reducing the assimilation of proteins. The silk glands did not exhibit any signs and symptoms of fungal infection indicated that silk glands are resistant to *A. fumigatus* infection.

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