

**BEAUVERIA BASSIANA (HYPHOMYCETES: MONILIALES)  
INFECTION DURING ECDYSIS OF SILKWORM *BOMBYX  
MORI* (LEPIDOPTERA: BOMBYCIDAE)**

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[Chandrasekharan, K. & Nataraju, B. 2011. *Beauveria Bassiana* (Hyphomycetes: Moniliales) Infection During Ecdysis Of Silkworm *Bombyx Mori* (Lepidoptera: Bombycidae). Munis Entomology & Zoology, 6 (1): 312-316]

**ABSTRACT:** Mulberry silkworm, *Bombyx mori* is the most commercially exploited insect which is domesticated worldwide for the production of silk cocoons. Domestication and constant rearing for the past several thousands of years made the silkworm highly susceptible to different pathogens. Different micro-organisms such as viruses, bacteria, fungi and microsporidia cause infectious diseases in silkworm. White muscardine caused by the fungal pathogen *Beauveria bassiana* is one of the most common and devastating diseases of silkworm. All the life stages of silkworm are susceptible to *B. bassiana* and its infection to silkworm during ecdysis (moulting) is studied and discussed in the present paper. The silkworms were inoculated with the pathogen at  $1 \times 10^5$  conidia/ml dose at different durations (18, 12 and 6 hours) before and after moult. Inoculation was also done during the moult. Mortality was 100% when the inoculation was done 12 hours before and 6 hours after moulting. Mortality was significantly less when the larvae were inoculated during moulting or 6 hours before moult. The present findings indicate the inability of *B. bassiana* to invade the silkworm during its moulting period. Skin casting and related processes during ecdysis may be preventing the fungus to enter inside the haemocoel of silkworm to cause infection and mortality.

**KEY WORDS:** *Beauveria bassiana*, silkworm, *Bombyx mori*, moulting.

Among the diseases of silkworm, white muscardine caused by *B. bassiana* inflicts heavy economic loss to the sericulturists in India. The climatic condition in the tropics is congenial for survival, infection and spread of this disease. All the life stages of the silkworm, viz. egg, larvae, cocoon, pupae and moth are found susceptible to the fungal pathogen, *Beauveria bassiana* (Chandrasekharan, 2008) The degree of susceptibility varied based on the age of individual stages as well as the dosage of the pathogen.

All the larval instars are susceptible to *B. bassiana* and the susceptibility varied among the instars. *B. bassiana* infects cutaneously and when the silkworm grows the integument becomes comparatively more resistant as far as the fungal pathogen is concerned. The integument of insects by itself is a strong barrier for many microbes such as viruses, bacteria and protozoa. It is not only a water proof physical barrier but also contains chemicals that inhibit the growth and penetration of micro organisms (David, 1967). The waxy epicuticular layer of silkworm integument contains free medium chain saturated fatty acids, presumably caprylic, capric and linoleic acids that inhibit the invasion of the fungi. Higher amount of free medium chain fatty acids is observed in the cuticle of silkworm strains resistant to Aspergillois (Koidsumi, 1957). Lipids in the epicuticle of silkworm, *Bombyx mori* inhibit the invasion of *B. bassiana* and *Paecilomyces fumosa-roseus*. The living integument and exuviae of resistant Chinese silkworm race had a greater anti-fungal action than that from susceptible Japanese strain (Koidsumi & Wada, 1955).

Fifth instar larvae are reported to be more susceptible than other instar larvae (Steinhaus, 1949; Reddy, 1978). Studies of Mukerji (1919) indicated that moulting larvae were more susceptible than other stages of instar. Susceptibility varies according to different stage of development of silkworm. Within the same instar, newly ecdysed larvae are more susceptible than those approaching the moult. In fifth instar, the infection decreases as the silkworm grows but ripe larvae and early pupa again shows increased susceptibility.

The most common route of host invasion is through the external integument, although infection through the digestive tract is possible (Gabriel, 1959). Yanagita (1987) studied on the oral infection of silkworm with *B. bassiana* and Yanagita & Iwashita (1987) revealed the histology of silkworm inoculated orally with the fungus. Usually the conidia attach to the cuticle, germinate and penetrate the cuticle (Boucias et al., 1988; Lefebvre, 1934). The surface ultra structure of *B. bassiana* infecting silkworm was studied by Vineet Kumar et al. (1994) and the infection process such as germination, penetration, and invasion was also studied further by them (Vineet Kumar et al., 1999). Once in haemocoel, the mycelium ramifies throughout the host, forming yeast like hyphal bodies often referred to as blastospores (Masera, 1952).

## MATERIALS AND METHODS

Infection of *B. bassiana* during ecdysis was studied during all the four moults of CSR2 × CSR4 silkworm hybrid larvae. *B. bassiana* conidia were harvested from the culture plates by using sterilized loop and suspended in sterilized distilled water which formed the stock inoculum. The concentration of conidia in the stock inoculum was determined using Neubauer haemocytometer. The required conidial suspensions i.e.  $1 \times 10^5$  conidia/ml was prepared by diluting the original stock suspension in sterilized distilled water. The silkworms were inoculated 18, 12 and 6 hours before moult (HBM) and 18, 12 and 6 hours after moult (HAM). Inoculation was also done during the moult. Hundred larvae were kept in each replication and three replications for each treatment were maintained. Mortality due to white muscardine was recorded daily and the progressive mortality was calculated.

## RESULTS AND DISCUSSION

Some insects are susceptible to diseases only at certain stages of the life cycle. In the case of fungal infections, the reports are varied. Certain fungi infect all stages of host insects, but in some cases the egg and pupal stages are more resistant than the larval and adult stages. The larval stages may show maturation immunity. The critical period in the development of the larvae to infectious diseases is shortly after moulting. When its' newly regenerated gut epithelium and newly formed integument are more susceptible to the fungal penetration (Muller Kogler, 1967). The amino acid composition in the larval surfaces varies among instars and with the time after moulting. Amines and peptides are also present but do not inhibit the germination of fungi. The fungal cell wall components are also reported to activate immune response in silkworm (Bidochka & Hajek, 1998).

Studies on the infection during the moulting period of larvae indicated some interesting results. Just prior to and just after moulting the larvae succumbed heavily to *B. bassiana* but during moulting the larvae were found less infected. The progressive mortality due to white muscardine is provided in Table 1. 100% mortality was recorded when the larvae were inoculated more than 12 hours

before moulting and more than 6 hours after moulting. The mortality after inoculating *B. bassiana* during moulting time was 1.00, 9.33, 30.33 and 39.33% in the fourth, third, second and first moults respectively. Inoculation during 6 hours before the third and fourth moult resulted in significantly less mortality of 60.67 and 31.33%, respectively (Figures 1a -1d).

Our results are in contrary to the studies of Mukerji (1919) who indicated moulting larvae as more susceptible than other stages of instar. He also reported that the susceptibility varied according to different stages of the silkworm. Within the same instar, newly ecdysed larvae are more susceptible than those approaching the moult. In fifth instar, the infection decreases as the silkworm grows but ripe larvae and early pupae again show increased susceptibility. The present finding indicates the inability of the fungus to invade the integument during moulting as it would be in the process of skin casting. The germinated conidia on the outer old skin may be inactivated during the process of ecdysis and ultimately they might be pushed off from the vicinity of silkworm skin. During moulting, the multiplication and development of inclusion bodies of NPV or CPV viruses were nearly inhibited until after moulting. Early instars silkworms are comparatively more susceptible to infection by microbial pathogen and it decreased with larval ageing from first to fourth instars (Aruga & Watanabe, 1964; Kobara et. al., 1967).

#### ACKNOWLEDGEMENTS

The first author wishes to express his gratitude to the Director, Central Sericultural Research and Training Institute, Mysore for providing laboratory facilities. The authors are also thankful to the colleagues in Silkworm Pathology section of the Institute.

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Table 1. Influence of moulting of silkworm on *B. bassiana* infection.

Time of Inoculation	Percent mortality due to white muscardine during different moults (Mean $\pm$ SD)			
	I Moult	II Moult	III Moult	IV Moult
18 HBM	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)
12 HBM	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)
6 HBM	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	60.67 $\pm$ 2.52 (51.16)	31.33 $\pm$ 2.52 (34.04)
Moulting	39.33 $\pm$ 4.51 (38.82)	30.33 $\pm$ 1.53 (33.42)	9.33 $\pm$ 1.53 (17.75)	1.00 $\pm$ 1.00 (4.62)
6 HAM	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)
12 HAM	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)
18 HAM	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)	100.00 $\pm$ 0.00 (90.00)
SE $\pm$	0.816	0.29	0.65	1.38
CD at 5%	1.751	0.63	1.39	2.95

HBM: Hours before moulting; HAM: Hours after moulting; Figures in parenthesis are angular transformed values.

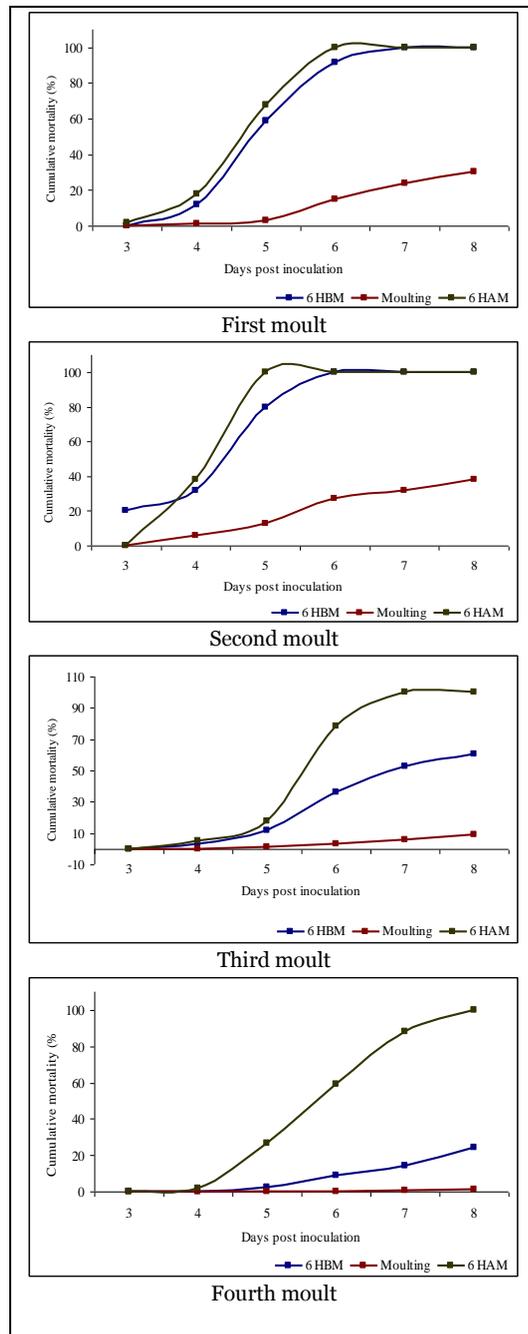


Figure 1. Progressive mortality due to *B. bassiana* infection during the moults of silkworm.