Int. J. Indust. Entomol. Vol. 12, No. 1, 2006, pp. 41~43

[Note]

International Journal of *Industrial Entomology*

A Microsporidian Parasite - Lb_{ms} (Protozoa - Microspora) Infecting Lamerin Breed of the Silkworm *Bombyx mori* L. (Lepidoptera - Bombycidae)

Shabir Ahmad Bhat* and B. Nataraju

Silkworm Pathology Laboratory, Central Sericultural Research and Training Institute (CSR&TI), Mysore - 570 008, Karnataka, India.

(Received 6 December 2005; Accepted 2 March 2006)

Lamerin breed of the silkworm *Bombyx mori* L. is the only mulberry silkworm breed which survives with an associated microsporidian (Lb_{ms}) infection from generations. This present comparative investigation has produced information on the influence of Lb_{ms} on the growth and development of the breed. The Lb_{ms} transmits tranovarially as well as perorally at low level. Larvae developed from the eggs laid by female, infected with Lb_{ms} were as normal as larvae developed from the eggs laid by healthy female. In infected tissues early developmental stages and mature spores located intracellularly but did not show hypertrophy of cells of the host. The meronts and sporonts measures 0.46 ± 0.25, 1.11 ± 0.05 μm in length and 0.32 ± 0.05, 0.78 ± 0.10 μm in width respectively. The mature spore measures 4.36 ± 0.06 μm in length and 2.14 ± 0.01 μm in width.

Key words: Microsporidian, Lamerin, Meront, Sporont, Hypertropy

Introduction

Silkworm *B. mori* L. suffers from several diseases caused by microbial organisms such as microsporidiosis, nuclear polyhedrosis, flacherie and muscardine, inflicts an annual crop loss of 27 – 30% (Selvakumar *et al.*, 2002). The microsporodiosis alone causes 5% crop loss during rearing (Sahaf, 2002). Infections of the disease range from chronic to highly virulent and can result in complete loss of sericulture industry. Several strains and species of microsporidians have since been isolated from the infected silkworm, (Govidian *et al.*, 1998). They differ in target tissues, virulence, mode of transmission (Samson, *et al.*, 1999a, b; Nageswara Rao *et al.*, 2004). It is observed that all silkworm breeds are susceptible to the infection no breed are reported to be completely immune. Resistance to microsporodiosis is greater in Chinese breed less in Japanse and least in European breeds (Govindan *et al.*, 1998) and multivoltine breeds are relatively more resistant than bivoltines (Patil and Geethabai, 1989). However Lamerin – a hibernated mulberry silkworm breed survive in-spite of infection by Lb_{ms} for generation without causing much harm (Shabir Ahmad Bhat and Nataraju, 2005). There was no investigation available on the developmental stages of Lb_{ms}, its influence on host, in view of the above the present study was carried out.

Materials and Methods

To study the impact of the Lb_{ms} on the growth and development of the its host, healthy and infected layings were brushed in disinfected rearing trays and rearing rooms separately following standard procedure (Datta, 1992) upto spinning and moth emergence. For the developmental stage study of Lb_{ms} infected tissues were prepared and fixed for 24 hrs in 3% glutaraldehyde in phosphate buffer saline (PBS, pH. 7.4). Samples were post fixed in 1% OsO_4 for 2 h, dehydrated through ascending alcohol series and embedded in araldite. Ultra thin sections were double stained with Uranyl acetate and lead citrate observed under 60KVA (JEOL 100CX) electron microscope.

Results and Discussion

The results obtained from the present study are presented

*To whom correspondence should be addressed.
Silkworm Pathology Laboratory, Central Sericultural Research and Training Institute (CSR&TI), Mysore - 570 008, Karnataka, India. Tel: +91-821-2362571; Fax: +91-821-2363757; E-mail:bhat_shabir2003@yahoo.com

Table 1. Influence of Lb_{ms} infection on egg production, hatchability and larval weight

Treatment	No. of egg laid/female	Hatchability	Larval wt. 1st instar (0 - day)	Larval wt. 2nd instar (0 - day)	Larval wt. 3rd instar (0 - day)	Larval wt. 4th instar (0 - day)	Larval wt. 5th insatr (0 - day)	Larval wt. 5th instar (6th - day)
Healthy	338.00 ± 57.58	269.60 ± 52.68	2.90 ± 0.32	34.00 ± 5.16	191.00 ± 7.38	899.00 ± 76.66	4336.00 ± 334.84	25990.00 ± 1205.55
Infected	311.60 ± 29.02	255.40 ± 28.38	2.80 ± 0.42	33.00 ± 4.83	189.00 ± 9.94	889.00 ± 64.54	4132.00 ± 409.28	24960.00 ± 1609.38
t-test	0.92ns	0.53ns	0.60ns	0.45ns	0.51ns	0.32ns	1.22ns	1.62ns

Values are mean ± SD; ns- Non significant.

Table 2. Influence of Lb_{ms} infection on larval duration, pupal period, pupation and adult emergence rate

Treatment	Larval duration in hours (instar wise)					Total	Pupal period (days)	Pupation rate	Emergence rate
	1st	2nd	3rd	4th	5th				
Healthy	96 ± 0	72 ± 0	84 ± 0	108 ± 0	174 ± 0	534 ± 0	11.50 ± 0.53	91.00 ± 2.21	89.30 ± 2.41
Infected	96 ± 0	72 ± 0	84 ± 0	108 ± 0	174 ± 0	534 ± 0	11.70 ± 0.48	84.20 ± 4.05	83.50 ± 3.81
t-test	0ns	0ns	0ns	0ns	0ns	0ns	-0.88ns	4.66**	4.07**

Values are mean ± SD; ns- Non significant; ** significant at 1% level

in Table 1 – 3. The result indicated that there was no significant influence of infection on the growth and development of the host. The numbers of eggs laid by healthy and infected females were 338.00 ± 57.58 and 311.60 ± 29.02 and hatchability were 269.60 ± 52.68 and 255.40 ± 28.38 respectively (Table 1). The freshly hatched larva was dark brown in color and weighed approximately 2.90 ± 0.32 mg and 2.80 ± 0.42 mg in healthy and infected batches respectively (Table 1). As the larva grows the skin color started changing to white at the 5th instar. The 5th instar larva increased 9000 times and 8900 times in healthy and infected batches respectively in body weight from the just hatched larva. The larval duration of infected batch were as many hours as in healthy batch (534 ± 0.00 h) (Table 2). The spinning completed in three days in both the batches and moths emerged out from the cocoons on the 11th day of spining (Table 2). Pupation and emergence rate was found significantly low in infected batch as compared to that of healthy ones. Meronts, sporonts and mature spores could be observed in infected tissues but the whole life cycle could not observed in detail. The meronts and sporonts measures 0.46 ± 0.25, 1.11 ± 0.05 μm in length and 0.32 ± 0.05, 0.78 ± 0.10 μm in width respectively. The mature spore measures 4.36 ± 0.06 μm in length and 2.14 ± 0.01 μm in width (Table 3).

Microsporodiosis is deadliest disease caused by highly virulent parasitic microsporidian, *Nosema bombycis*, infects all ages, stages and breeds of the silkworm and have been observed in different host cell types. It is

Table 3. Measurement of uranyle acetate and lead citrate stained developmental stages of Lb_{ms}

Stage	Size (μm)		
	Length ± SD	Width ± SD	Length/Width
Meront	0.46 ± 0.25	0.32 ± 0.05	1.41 ± 0.58
Sporont	1.11 ± 0.05	0.78 ± 0.10	1.43 ± 0.25
Mature spore	4.36 ± 0.06	2.14 ± 0.01	2.05 ± 0.03

observed that all silkworm breeds are susceptible to the infection of *N. bombycis* and no breed has been found tolerant. Larvae suffering from the microsporidian infection do not show any external symptoms until the disease is far advanced. At the advanced stage, the larvae become sluggish and show symptoms like poor appetite, retarded growth, irregular molting, appear paler and translucent with wrinkled skin and the larval duration extended 6 – 12 hrs when infected in early stage (1st and 2nd instar) and dies by the 4th or 5th instar (Baig, 1994). The larvae infected during 4th and 5th instar reach the adult stage and the infection increase geometrically in the host tissues. *N. bombycis* cause 100% mortality by 3rd instar when transmitted vertically (Hane and Watanbe, 1988). The results of the present study showed that infected larvae showed normal growth up to spinning and did not exhibit visible sign of infection externally were active. The Lamerin breed is the definitive host for the Lb_{ms}. The Lb_{ms} caused inapperent infection which does not have significant

effect on the growth and development of the breed. However significant differences were observed in the pupation and emergence rate in infected batch. The development of the Lb_{ms} occurred in direct contact with the cytoplasm, but did not show hypertrophy of cells, indicating that it appears to be different from the *N. bombycis*, but at the same time many characters are overlapping with genus *Nosema* Nageli, 1857. Hence to this purpose this is new species. Indeed, the taxonomic position of the Lb_{ms} to be clarified with further studies, especially its ribosomal - RNA analysis.

References

Baig, M. (1994) Studies on *Nosema bombycis* N.- A pathogen of silkworm *Bombyx mori* L. Ph. D. Thesis, University of Mysore, Mysore.

Datta, R. K. (1992) Guidelines for bivoltine rearing. Bulletein of Central Silk Board, Bangalore.

Govindan, R., T. K. Narayanaswamy and M. C. Devaiah (1998) Principles of silkworm pathology, Seri. Scientific Publisher, Bangalore, India, pp.420.

Han, M. S. and H. Watanabe (1988) Transovarial transmission of two microsoridia in the silkworm, *Bombyx mori*, and disease occurrence in the progeny population. *J. Invertebr. Pathol.* **51**. 41-45.

Nageswara, Rao. M. Muthulaskshmi. S. Kanginakudra and J. Nagraju (2004) Phylogenetic relationships of three microsporidian isolates from silkworm, *Bombyx mori. J. Invertebrt. Pathol.* **3**, 87-95.

Patil, C. S. and M. Geethabai (1989) Studies on the susceptibility of silkworm races to Pebrinr spore. *J. Appl. Entomal.* **108**, 421-423.

Sahaf, K. A. (2002) Incidance of protozoan disease (pebrine) of silkworm, *Bombyx mori* L. in Jammu and Kashmir. *J. Ent. Res.* **26**, 305-307.

Samson, M. V., P. C. Santha, R. N. Singh and T. O. Sasidharan (1999a) A new microsporidian infecting *Bombyx mori* L. *Indian Silk.* **37**, 10-12.

Samson, M. V., P. C.Santha, R. N. Singh and T. O. Sasidharan (1999b) Microsporidian spore isolated from *Pieris* sp. *Indian Silk.* **38**, 5-8.

Selvakumar, T., B. Nataraju, M. Balavenkatasubbaiah, V. Shivaprasad, M. Baig, Virendra Kumar, S. D. Sharama, V. Thaigarajan and R. K. Datta (2002) A report on the prevelance of silkworm diseases and estimated crop loss. Proceedings of national confrence on stratigies for sericulture research and development 16 - 18 Nov. 2000, pp. 354-357. CSR and TI (CSB), Mysore.

Shabir Ahmad Bhat and B. Nataraju (2005) A report on the impact of microsporidian parasiteon Lamerin breed of the Silkworm *Bombyx mori* L. in India. *Int. J. Indust. Entomol.* **2**, 143-145.