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Review

Microsporidiosis of silkworm, *Bombyx mori* L. (Lepidoptera- Bombycidae): A review

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Microsporidiosis of silkworm, *Bombyx mori* L. is one of the deadliest diseases caused by intracellular obligatory parasite. Infections of the disease range from chronic to highly virulent and can result in complete loss to the sericulture industry. The disease has become increasingly more and more complex as more number of microsporidian strains / species infecting silkworms are being identified from India and other sericulture practicing countries of the world. These microsporidians differ in pathogenicity, rate of spread, transmission and multiplication. Therefore, an attempt has been made to review the literature on microsporodiosis of the silkworm *B. mori* L.

Key words: *Bombyx mori* L., microsporidia, *Nosema bombycis*, ultra structure.

INTRODUCTION

Microsporidiosis is one of the most deadly diseases in silkworms. Its absence or presence determines the success or failure of the silk industry of a country. The disease is caused by an obligatory endoparasite, *Nosema bombycis.* The name of the pathogen has been derived from two words that is *Nosema* meaning disease and *bombycis* belongs to the scientific name of silkworm. The history of Sericulture speaks volumes of devastating outbreak of microsporidian disease in several European countries in the past (Tatsuke, 1971). Microsporidian disease remained threat to silk industry since time immemorial, because of its unique and recurrent occur-rence and is the only disease transmitted both horizon-tally and vertically. The disease spreads quickly and takes a heavy toll of silkworm and even results in total crop failure when the infection is severe (Steinhaus, 1949). The disease has become more complex now because of the perpetual incidence of different types of microsporidian infection in silkworm, (Sharma et al., 2003). Some of them belong to other genera like *Vairimorpha*, *Pleistophora* and *Thelohania* and exhibit difference in their pattern of infection (Samson et al., 1999a). Despite extensive studies conducted and efforts made, the disease has been frequently causing concern to silkworm growers and Sericulture scientists in India and other sericultural practicing countries of the world, due to its occasional and sporadic outbreak. Therefore, an attempt has been made to review the literature on microsporodiosis of the silkworm, *Bombyx mori* and is presented in this article.

History of the disease

The history of sericulture reveals devastating impact of microsporidiosis in several sericultural countries resulting in severe damage to sericulture industry. Though, the disease is known from very remote times, it attracted the attention of Sericulturists only during the 17th century. The first scientific record of the occurrence of the disease came from European countries in 1809 and the disease wiped off sericulture there. This was followed by another report from France in 1845 where the annual production of cocoons came down from 26,000 tones in 1853 to 4000 tones in 1865 due to epizootics of microsporidiosis and subsequently collapsed the French and Italian silk industry. Later the disease spread to Spain, Syria and Romania (Steinhaus, 1949). In India, the first report of the occurrence of the disease was from Mysore in 1866 followed by an epidemic level outbreak of the disease in Kashmir in 1878 (Baig et al., 1997). It is due to the out break of this disease in 1878 that Kashmir lost its productive indigenous univoltine breed “Kashmir Race” some 131 years back (Kamili and Masoodi, 2000). Prior to this attack, the British managed to export several thousands ounces of disease free seeds of Kashmir

Table 1. Pathogenicity of different microsporidia infecting mulberry silkworm.

Microsporidia isolates	Pathogenicity	Spore form	Spore size (μm)	
			Length	Width
N. bombycis	High	Oval	3.8	2.6
Nosema sp. M11	Low	Oval	3.9	1.9
Nosema sp. M12	Low	Ovo-cylindrical	4.5	2.0
Pleistophora sp	High	Oval	2.7	1.6
NIK-2r	Low	Oval	3.6	2.8
NIK-4m	Low	Oval	5.0	2.1
NIK-5hm	High	Ovo-cylindrical	5.0	3.1
Nosema sp. Lb_{ms}	Low	Ovo-cylindrical	4.36	2.14
Microsporidian sp. S_I	High	Oval	1.73	1.01
NIAP-6p	-	Oval	5.00	2.40
NIAP-7g	-	Oval	4.60	2.50
NIK-5d	-	Oval	3.70	2.70
NIK-1Pr	High	Ovo-cylindrical	5.41	2.85
NIK-1Cc	Low	Oval	4.60	2.77
NIK-1Cpy	High	Oval	4.96	2.85
NIK-1So	High	Ovo-cylindrical	5.26	2.61
NIK-1Dp	Low	Oval	4.27	2.79
Msp	-	Ovo-cylindrical	5.38	2.92

NB: - : Unknown.

Race to Europe after microsporidiosis attacked the European silk industry in 1809 to revive sericulture there. However, in the absence of any research back-up and scientific management, Kashmir could not maintain this race, though some blood of the race might be available in European countries.

Prevalence of the disease in field

The prevalence of the microsporidiosis in India was about 15% in 1983 (Nataraju and Dandin, 2006) . In 1991 - 1992 an outbreak of microsporidiosis was witnessed in southern sericultural states of India causing a loss of over 200 crores. In Jammu and Kashmir the disease inflicts an annual loss of 5% (Sahaf, 2002). However with the advancement of silkworm disease management, the disease level in the field has again come down to 15 - 20%. The incidence of microsporidiosis in Kashmir Division ranged between 18.98, 13.46 and 9.00% in south, north and central zone of Kashmir, respectively (Ganie et al., 2008) . Normally microsporidiosis occurrence in India is of low intensity during summer and high in winter season in tropics due to the increase in the lepidopteron insects during winter and rainy seasons. In temperate sericultural areas like Jammu and Kashmir, the incidence of microsporidiosis is high in summer and low in spring.

The incidence of microsporidian disease varies with the race of silkworms, the developmental stages and the rearing environment. Tolerance to the microsporidian disease is greater in Chinese breeds, less in Japanese and least in European breeds. Recently in India a silkworm breed namely Lamerin is reported to be comparatively tolerant to the microsporidian diseases as compared to other bivoltine and multivoltine breeds of the silkworm (Bhat, 2007). Although the disease tolerance appear to depend on the genetic constituents of a particular breed, nevertheless factors such as pathogenic load, inadequate nutrition and the environment in which the silkworms are reared may also affect tolerance (Singh and Saratchandra, 2003).

Causative agent

By the end of 19^{th} century, *N. bombycis* alone was known as causative agent of microsporidiosis in silkworm but present research work has revealed that there are several other microsporidia belonging to different genera, causing microsporidiosis in silkworm (Table 1). These spores are different in spore shape, size as well as in pathogenicity. Many of them, though infective but have demonstrated low multiplication rate in silkworm (Bhat and Nataraju, 2006). Many of them live, in the - gut as harmless commensals, however the NIK-4m was highly pathogenic to the silkworm and causes acute disease by cyst formation on the gut surface (Ananthlashmi et al., 1994). *N. bombycis* and NIK-2r are equally virulent while NIK-3h is comparatively lower in its pathogenicity (Fujiwara, 1993). The microsporidian spores (NIK-5hm)

Table 2. Taxonomic position of *Nosema bombycis*.

Phylum	:	Microspora
Class	:	Microsporea
Order	:	Microsporida
Sub-order	:	Apanosporoblastina
Family	:	*Nosematidae*
Genus	:	*Nosema*
Species	:	*Bombycis*

isolated from haemocytes of the silkworm are highly pathogenic while microsporidia Lb_{ms} isolated from Lamerin breed of the silkworm was found low in pathogenicity (Selvakumar et al., 2005; Bhat and Nataraju, 2006) respectively. The microsporidia isolated from butterflies cause low pathogenicity to silkworm when inoculated through feed compared to *N. bombycis* (Kishore et al., 1994; Ifat, 2008). Chitra et al. (1975) have reported that one of the isolated strains of *N. bombycis* infects only the midgut cells which is less virulent then the normal strain which infects all tissues of the host.

Classification of microsporidia

The taxonomy of microsporidia has undergone several revisions since its original description in the middle of the 19^{th} century, when Naegeli considered *Nosema* to be yeast like fungus. The concept of Protista or protozoan was in its infancy, so it was common to pigeonhole microbial organism into animal, plants or sometimes fungi depending on their characteristics. Nageli considered *Nosema* to be the member of the schizomycetes fungi although classification at that time did not reflect the true diversity of microbial life and schizomycetes were considered a grab bag of yeasts and bacteria. After further study, Balbiani, in 1882 created a new group “microsporidia” for *Nosema*. Microsporidia are unusual group of eukaryotic, obligate intracellular parasites causing microsporidiosis in insects and is the earliest known disease. As the real diversity of microbial eukaryotes begin to dawn on biologists, more complex hierarchal classification schemes were developed based on certain common features of morphology. One of the cell types identified and classified together were spore-forming parasites collectively called Sporozoa. This group contains what we call as apicomplexa, myxosporidia, actinomyxidia, haplosporidia, microsporidia and a handful of individuals of genera within sporozoa. The microsporidia were considered to be most closely related to a variety of other parasites at different times but were most often believed to be a kin to myxosporidia and actinomyxidia with microsporidia collectively called the Cnidosporidia (Kudo, 1918).

Molecular studies of microsporidia drastically changed traditional taxonomy. Several genes including those for mitochondrial Hsp-70, alpha and beta tubulins and translation elongation factors suggesting a close affinity of microsporidia with fungi and are considered as highly specialized and reduced organisms. Modern taxonomists follow the system developed by Sprague (1982) (Table 2).

Characteristics of the disease

The disease infects all stages and breeds of the silkworm and is characterized by certain typical symptoms. The symptoms and intensity of infection vary depending on the stage of silkworm development and the tissues. Heavy concentration of spores can be seen through the cuticle of some lightly pigmented silkworms as white yellow cysts viewed against a black background. These visible signs might be accompanied by swelling caused by hypertrophy of infected cells at the site of infection. Less obvious signs can be often found by skillful observer with a sufficient experience. However sometimes spores can occur in large number that appear to entirely fill the host without sign of infection. Dissection of these hosts will often reveal sites of infection visible to naked eye. Excessive mortality or reduced longitivity may be the first indication of the microsporodiosis in an insect colony. Some microsporidian stages in microsporidia life cycle are benign and produce a few spores that only through microscopic examination will reveal an infection. Some of these pass infection from hosts to their offspring in which potent infections and heavy mortality is displayed. When the degree of infection is high, eggs often becomes sterile or dead but when the contamination is of low degree, the eggs hatch and the disease develops at larval stage and causes death of larvae at latter stages of the development of the host. Reports are available on wide spectrum effects of this parasite on insect tissues, reproductive potential and fertility (Bansal et al., 1997). The spore concentration was found to be much higher in gonads followed by fat body, gut and malpighian tubules in sericigenious species viz., *Antheraea mylitta*, *Antheraea assamensis* and *B. mori* (Bansal et al., 1997).

Disease lesion in various tissues

Observations of microsporidia are usually conducted by examing living organisms for visible signs of infection or by microscopically screening samples of macerated tissues for spores (Baigh, 1994). Sometimes the prevalence of microsporidia can be quite high exerting a strong suppressive effect on the host population. Most often, infection rates are low in natural population, necessitating the examination of a large number of individuals to detect it and even larger number in order to estimate the incidence of infection (Bhat, 2007).

N. bombycis derives nourishment from the host cells

but do not disrupt the basal membrane and microvilli immediately. Thus, the infected host survives for longer durations possibly for the perpetuation of the pathogen. This shows that the parasite lives in close association with the host during the early stages of its development and extensive destruction of the host cell organelle takes place only in the later stages of the development of parasite. Ultra structure studies of infected silk gland have shown marked structural disorganization in the host accompanied by reduced number of free ribisomes, mitochondria and endoplasmic reticulum (Joythi et al., 2005). Due to the decrease in vital cell organelle, silk synthesis gets generally affected in microsporidian infected larvae. When the muscle cells are affected, the tissues show necrotic cavities that result in the sluggish movement of the larvae and their shrunken appearance. Meronts invade blood cells such as granulocytes, leucocytes and plasma cells that become enlarged and the spores released by them make the haemolymph turbid. Infected hypodermal cells get encircled by granulocytes forming cysts and new dermal cells cover these cysts. These cysts form black pepper like spots on the integument of the infected larvae. Infected hypodermal cells become enlarged and vacuolated and get blackened due to the formation of melanin (Ganga, 2003).

Source of microsporidian infection

The diseased and dead silkworm larvae are the source of secondary contamination (Samson et al., 1999a and b). The microsporidian disease spread in silkworm rearing at faster rate. Silkworm gets also infected on consumption of microsporidian contaminated mulberry leaf infested by lepidopteran and other agricultural pests (Kishore et al., 1994) . The spores liberated from the above sources settle along with the dust on the mulberry leaf forming the source of secondary contamination in the rearing bed. Improper and careless disposal of silkworm litter and infected larvae and use of undecomposed silkworm bed refuse in mulberry fields may form potential source of contamination. Also, if the layings are incubated in a contaminated room without proper disinfection, it could lead to the surface contamination of eggs and during the process of hatching, the healthy larvae gets infected (Singh et al., 2007). The moth scale and the urine are also other possible sources of surface contamination.

One *N. bombycis* carrier when introduced during zero day of 2^{nd} instar in a batch of hundred silkworms, results in 41.00% disease outbreak by the time larvae comes to spinning stage (Bhat, 2007). Baigh (1994) also reported that the spread of microsporidian disease in rearing bed is also dependent on the density of the diseased silkworms. In an earlier study, Ishihara and Fujiwara (1965) reported that the change of the epizootic pattern corresponded to the change in number of larvae excreting spores of *N. bombycis*. Contamination with microsporidia occurs in any time in the larval stage. Low infection of the pathogen tends to pass unnoticed in one generation and gradually build up over generations. The disease may pass unnoticed due to the partial infection of the eggs also and the death of the infected larvae during early instars may also escape. The silkworm larvae infected during early stages of 1^{st} and 2^{nd} instar die upto 5^{th} instar, but if infection occurs during 4^{th} or 5^{th} instar, the larvae manages to survive and form cocoons but the silk from the cocoons of infected larvae is usually much inferior.

Mode and rate of transmission

The transmission of silkworm microsporidia depends primarily on host parasite relationships. *N. bombycis* infects silkworm both horizontally by ingestion of spores and transmitted to progeny by the vertical means (Han and Watanabe, 1988). The larvae infected through transovarian transmission show irregular moulting and growth, die during 3^{rd} and 4^{th} instars. If these tranovarially infected larvae are reared with a healthy colony of silkworms, the spores discharged by infected larvae provide the source of contamination and digestion of these spores by healthy larvae results in the spread of disease. The minimum number of spores required for contamination through per oral infection varies with each instar. Iwano and Ishira (1991) stated that 1 - 10 spores are required to cause disease in 2^{nd} instars larvae while approximately 100 such spores are required in 5^{th} instar for the same symptoms to occur. Among the isolated microsporidia from silkworms, the rate of transovarial transmission was 100% with *N. bombycis* and it was only 1.8% with *Nosema* sp. NIS-M11 (Han and Watanabe, 1988). The transovarial transmission of *Vairimorpha* sp. NIS-M12, *Microsporidium* sp.NIS-M25, *Pleistophora* sp. NIS-M27, and *Thelohania* sp. NIS-M32 has not been documented in silkworm. The transovarial transmission rate of NIK-3h was hardly 1.8%, while NIK-4m does not transmit infection by transovarial means (Baigh, 1994). The tranovarial transmission rate of Lb_{ms} is 64.53% (Bhat and Nataraju, 2005). Two microsprodia strains, *viz.,* strain -1 and strain- 2, showed low infectivity compared to *N. bombycis* which produced 97.2% infection by the 10^{th} day of inoculation while strain-1 produced only 67.6% and strain-2 produced 47.2%infection. With regard to the transovarial transmission, strain-1 caused almost 100% while strain-2 showed 62.5% transmission (Sasidharanet al., 2003).

Factors governing the transmission rate

The ability of pathogen to persist and flourish with-in a host population is dependent upon the efficiency with

which it is transmitted from one host to another. There are many factors that affect the transmissibility of insect pathogens in a host population: (1) Transmission route factors; (2) Pathogen factors; (3) Host factor; (4) Environmental.

Survival of microsporidian spores

The microsporidia alternate between two forms, the actively feeding vegetative stages (meronts, sporonts, sporoblasts) and the dormant spore. The dormant spore is easily recognizable and is the only stage that can remain viable outside the host cell and its survival in the environment is influenced by several factors. Microsporidian disease show more prevalence in the rainy and cooler seasons probably due to the persistence and survival of the pathogen in the environment for longer period and congenial condition for pathogen multiplication leading to easy spread (Baigh, 1994). Studies as conducted on the viability of the microsporidian spores in soil and compost under tropical conditions have revealed survival of spores up to 225 days in wet soil and 135 days in wet compost (Singh and Daniel, 2007). Survival of spores depends on the kind of material in which they are found, in addition to the moisture content of the material. The growth and multiplication of microsporidia in the eggs are influenced by the growth of host. When the egg diapauses, the growth and multiplication of the microsporidia stops simultaneously and when the eggs start growing by incubation, the microsporidia also start growing and multiplying (Singh and Saratchandra, 2003).

Future research

Earlier, the identification of microsporidia was based on the morphological characters but the occurrences of different microsporidia strains in the field make the disease more complex. So development of specific procedure for early detection of the microsporidian disease and discrimination of virulent and non-virulent species/ strains of the pathogen.

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