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Microsporidiosis in silkworms with particular reference to mulberry silkworm (*Bombyx Mori* L.)

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Abstract
Mulberry silkworm, *Bombyx mori* is a poikilotherm, susceptible to several diseases. There are no silkworm races at present, which are deemed as totally resistant to diseases or pests. Mulberry silkworm *Bombyx mori* is affected by a number of diseases caused by viruses, bacteria, fungi and protozoa. These diseases are known to occur in almost all the silkworm rearing areas of the world causing considerable damage to the silkworm cocoon crop. Microsporidiosis of the silkworm, caused by highly virulent parasitic Microsporidian or Nosema bombycis is one of the most serious maladies, which determines the success or failure or sericulture industry in our country. The microsporidiosis of silkworm, commonly known as Pebrine is the earliest known menace to silk industry. Microbiological studies of silkworm were initiated after the outbreak of disease in France in 1845. Several historical evidences in various countries of the world showed that the outbreak of Pebrine disease had greatly influenced the decline of the sericulture industry in the past. This review is intended to present the vast information generated on the status of Microsporidiosis and challenges ahead to work out strategic future plans to prevent the sericulture industry from the losses suffered due to this disease.

Key words: Silkworm, Microsporidiosis, Bombyx mori, Mulberry

1. Introduction
Disease is a condition of abnormality resulting from physical or physiological derangements. It is the result of injury or insult or whatever that goes wrong the insect [1]. Living and non living agents cause diseases in insects and it is the result of interactions between the host and the causal agent. Mulberry silkworm, *Bombyx mori* is a poikilotherm, susceptible to several diseases. There are no silkworm races at present, which are deemed as totally resistant to diseases or pests. Mulberry silkworm, *Bombyx mori* is affected by a number of diseases caused by viruses, bacteria, fungi and protozoa. These diseases are known to occur in almost all the silkworm rearing areas of the world causing considerable damage to the silkworm cocoon crop. Microsporidiosis of the silkworm, caused by highly virulent parasitic Microsporidian or Nosema bombycis, is one of the most serious maladies, which determines the success or failure or sericulture industry in our country. This can be evidenced from the fact that the rise and fall of the microsporiosis diseases corresponds with ups and downs of sericulture industry in silk producing countries of the world [2]. Several strains and species of microsporidia have since been isolated from the infected silkworms, and the diseases is becoming increasingly more and more complex and is also becoming increasing well known in medical arena through the Pathologies they cause in humans with impaired immune system [3, 4]. Epizootiology, development of immunodiagnostic kit, fluorescent antibody technique and use of ideal disinfectant, chemotherapy and thermo-therapy techniques and management strategies have been addressed for identification, destruction, prevention and control of disease causing micro-organisms. Techniques of forced eclosion test and delayed mother moth examination have also been stated to play important roles in the detection of the disease. The microsporidia are spore forming, small, obligate, intracellular living eukaryote infecting both beneficial and non-beneficial insects [5]. More than 40 general and 1200 species of microsporidia have been recorded from insects and fish [6, 7]. Among these, at least 200 belong to the genus Nosema [8] and most Nosema species are parasitic to invertebrates. A majority of these including *N. bombycis* and *N. tyriae* [9], *N. mesnili* [10], *N. algerae* [11], *N. aphis* and *N. trichoplusiae* [12] are pathogenic to various insects. More than twenty wild insect species have been found to have microsporidian spores that can cross infect silkworm. Pebrine, i.e. the spores of microsporidian (*Nosema bombycis*) is one of the most dreaded diseases of the silkworm, it infects almost all ages, stages, breeds and hybrids of the silkworm by both transovarial and per-oral infections. It is highly infectious and difficult to eradicate after the occurrence of infection.

1.1 History of the Disease
The microsporidiosis of silkworm, commonly known as Pebrine is the earliest known menace to silk industry. Microbiological studies of silkworm were initiated after the outbreak of disease in France in 1845. Several historical evidences in various countries of the world showed that the outbreak of Pebrine disease had greatly influenced the decline of the sericulture industry in the past. The first scientific record of occurrence of the disease came from France in 1845, where the annual cocoon production of cocoons came down from 26,000 tonnes in 1853 to 4000 tonnes in 1865 due to epizootics of microsporidiosis and subsequently collapsed the French and Italian silk industry. Later in the year 1984 disease spread to Spain, Syria and Romania [1]. 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 [13]. It is due to the outbreak of this disease in 1878 that Kashmir lost its productive indigenous univoltine breed "Kashmir Race" some 131 years back [14]. Prior to this attack, the British managed to export several thousand ounces disease free seeds of Kashmir 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 Europe countries. In 1890-1900, the disease swept through Mysore and Madras provinces. Thereafter, the disease reappeared during 1925-1930 in an epizootic form [15]. Disease epidemics were again observed during 1991-1992 in the southern part of the country, which resulted in considerable crop losses and revenue [16]. Since then, the incidence of the disease has been observed intermittently in silkworm crops in the different parts of India. The Pebrine incidence also caused a considerable loss of silkworm seed during1997-1999 in the seed production area of Uttar Pradesh [17]. The prevalence of the disease in India was about 15% in 1983 and declined by 1984 & 1985 and was high in 1990 and reached the peak in 1991-1992. The epidemic in India caused loss of over 200 crores during the period.

The earliest research on Pebrine was confided especially with the epizootiology and prevention of the disease [18-20]. The disease causing microorganism was first observed in the haemolymph of the silk worm and was given the name "Hematozoid" [21]. Quadrefague [22], coined the name Pebrine because of the appearance of pepper-like spots in the diseased larvae. Nageli [23], of Germany stated that the disease is caused by a protozoan parasite and named this pathogen as Nosema bombycis. The noted French microbiologist, Pasteur [24] in his book entitled "Etudes sur la maladie desvers a Soie", called the disease as corpuscle disease" and made a detailed study on its growth and transmission, and discovered that the disease is transmitted through trans-ovarian transmission within the body of the mother moth and suggested methods of preventing the disease. Balbiani [25] said that the pathogen of Pebrine disease belongs to protozoa. Later on in 1909, Stempell published the details of life history of the pathogen of Pebrine [26].

1.2 Symptoms of the Disease

The symptoms of the Pebrine varies depending upon the stage of life cycle of *Bombyx mori* viz., egg, larva, pupa and moth and form an important criteria for identifying the disease. The disease infects all ages, stages, breeds and hybrids of the silkworm. In larvae the detection of the disease by external appearance is difficult initially but as the disease advances the symptoms are manifested. At advanced stage, larvae become sluggish and show symptoms like poor appetite, irregular moulting, retarded growth and development. As the disease advances progresses, the larvae appear pale, dull and translucent with wrinkled skin, shrink in size and become flaccid and the feces have higher moisture content [27, 28]. Due to the chronic nature of the disease, the infected larvae do not die immediately and continue to survive for some time. In some cases there may be appearance of distinct small and large black spots on the skin which are formed due to melanosis of the dead epidermal cells of the skin that become infected. In infected worms the gut and tissues becomes opaque and white pustules appear on the silk glands. Dead worms remain rubbery for longer period and then turn black. Infected pupae are flabby and swollen with lusterless, blackish and softened abdomen and black spots occasionally appear on this region [19]. Highly infected pupae fail to metamorphose into adult. Irregular moth emergence, clubbed wings, distorted antennae, improper mating, low fecundity, and sometimes clumpy egg layings, as well as high percentage of unfertilized and dead eggs, apart from eggs with less gluey substance leading to their detachment from egg sheets, lack of uniformity in egg shape, and easily coming off scales from the wings and abdominal area are some of the symptoms of the disease at the moth stage. The accessory glands of pebrinized moths are also infected and they become incapable of producing sufficient gluey substance this results in production of loose eggs which easily role off the egg sheets.

1.3 Causative agent

By the end of 19th century only *Nosema bombycis* was known as the causative agent of microsporidiosis of silkworm but the advanced molecular, biological and immunological studies of this parasite revealed that there are several other microsporidia belonging to different genera, causing microsporidiosis in silkworm. These spores are different in spore shape, size as well as in pathogenicity (Table-1). Each having different morphological, pathological and antigenic characters; some infecting only midgut cells and others some specific tissues (Table-2). Many of them though infective but have demonstrated low multiplication rate in silkworm [29]. Chitra *et al.* [15] have reported that one of the isolated strains of Nosema bombycis infects only the midgut cells which is less virulent then the normal strain which infects all the tissues of the host.

Table 1 Pathogenicity of different microsporidia infecting Bombyx mori.

Microsporidian isolate	Pathogenicity	Spore form	Spore size (mm)	
			Length	Breadth
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
Nosema sp. Lb_{ms}	Low	Ovo-cylindrical	4.36	2.14
Pleistophora	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
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
NIAP-6p	-	Oval	5.0	2.40
NIAP-7g	-	Oval	4.60	2.50
Microsporidian sp. S1	High	Oval	1.73	1.01
Msp	-	Ovo-cylindrical	5.38	2.92

Table 2: Site of infection of different microsporidians in silkworm.

Microsporidian	Host tissues					
	Gut epithelium	Malpighian tube	Muscle	Fat body	Silk gland	Gonad
N. bombycis NIS-001	+	+	+	+	+	+
Nosema sp. NIS-M11	-	+	+	+	+	-
Vairimorpha sp. NIS-M12	-	+	+	+	+	-
Nosema sp. NIS-M14	-	+	+	+	+	-
Microsporidian NIS-M25	-	+	+	+	+	-
Plistophora sp. NIS-M27	+	-	-	-	-	-
Thelohania sp. NIS-M32	-	+	-	-	-	-
Nosema sp. NIK-is	+	+	+	+	+	+
Nosema sp. NIK-2r	+	+	+	+	+	+
Nosema sp. NIK-3h	-	+	+	-	+	-*
Vairimorpha sp. NIK-4m	+*	-	-	-	-	-

(-*): Cyst formation on the surface of gut. (+*): Formation of spores only in heavily infected larvae. (-): No infection. (+): Infection.

Table 3: Microsporidian diversities.

Characteristics	Diversity
Spore size	1µm (Enterocytozoon bieneusi) 40 µm (Bacillidium filiferum
Spore shape	Generally ovoid, also spherical, rod-shaped, or crescent-shaped
Exospores	Thickness : 10nm ˜200nm
	Appendages: absence or presence of tails, fibres,
Polar Filament	Isofilar filament* and anisofilar or heterofilar filament*;
Polar tube (PT)*	Length:0.1 µm ˜ 0.25 µm; diameter: 50 µm 500 µm;
Nucleus configuration	Monokaryon or Diplokaryon
Host-parasite interface	Parasite is indirect contact of the parasite plasmalemma with the host cell cytoplasm (Nosema bombycis) or parasite is isolated from host cell cytoplasm by parasitophorous vacuole (PV)
Life cycle and host involved	Simple life cycle and only host involved: Extremely complex life cycle and two host involved
Sporulation sequence	One sporulation sequences such as Endoeticulatus fidelis;
Spore types in one life cycle	One spore type and two or more spore types as a result of two or more sporulation sequences
Reproduction mode	Asexual: merogony and sporogony Sexual: karyogamy,gametogensis, and plasmogamy
Number of sporoblast	Two sporoblasts (bisporous) and many sporoblasts (polysporous)
Genome size	2.3 Mbp (Encephalitozoon intestinalis)˜ 19.5 Mbp (Glugea atherinae)

*Isofilar filament, the polar filament with the same thickness along its entire length;
*Anisofilaror heterofilar filament with different thickness along its entire length, often with the anterior part thicker. * Polar type (PT), after microsporidia germinate, the external form of "polar filament" is called "polar tube"

Table 4: The Different Major Geographical Reference Nosema Bombycis Strains In The World.

Isolate	Isolation	Original host	Identification
Nosema bombycis NIS-001	Before 1918, Honshou, Japan	Bombyx mori larva	Spore morphology and biological assays,, ELISA,ssur RNA
N. bombycis	1985, Gunma, Japan	Neptis Sappho, adult	Fluorescent antibody technique
N. bombycis NB-Prc-SES-H7901	1979, Tokyo Japan	Pieris rapae, adult	Spore morphology and biological assays, fluorescent antibody technique
N. bombycis Sd-U-IW 8401	1984, Kanagawa, Japan	Spodoptera depravata, adult	Spore morphology and biological assays, latex adhesion test
N. bombycis Y9101	1990, Kumamoto Japan	Spodoptera exigua, larva	Spore morphology and biological assays, Latex adhesion test, ssu-rRNA
N. bombycis Pr-S-19	1990, Okinawa, Japan	Pieris rapae, adult	Latex adhesion test
Nosema sp. NIS-402	1974, Fukushima	B. mori female Japan	Spore morphology and biological assays, ELISA, ssu-rRNA
Nosema sp. NIS-408	1974, Gunma, Japan	B. mori female	Spore morphology and biological assays, ELISA, ssu-rRNA
N. bombycis	Ca. 1990,Sichuan,	B. mori	Nosema bombycis surface antigen

	China		protein P30.4 (AF245278)
N. bombycs and N. bombycis CGS,MG	Guangzhou, China	B. mori	Spore morphology and biological assays, latex adhesion test
N. bombycis	Dongtai, China	B. mori	Spore morphology and biological assays, ssu-rRNA
N. bombycis ISC-ZJ (used in this study)	Zhenjiang, China	B. mori, larva	Spore morphology and biological assays, ssu-rRNA
N. bombycis	2000, China	Spodoptera litura	ssu-rRNA
N. bombycis	China	B. mori	ssu-rRNA
N. bombycis	India	Antheraea mylitta	ssu-rRNA
N. bombycis	Northern India	B. mori	ssu-rRNA
N. bombycis	Southern India	B. mori	ssu-rRNA
N. bombycis NIK-2r	Mysore, India	B. mori adult	Spore morphology and biological assays, ssu-rRNA
N. bombycis NIK-1s	Mysore, India	B. mori adult	ISSR-PCR and ssu-rRNA
N. bombycis	Before 1954, Europe	B. mori	Spore morphology and biological assays, fluorescent antibody technique
N. trichoplusiae Synonym: N. bombycis	1959, Hawaii, U.S.A	Trichoplusiae ni, larva	Spore morphology and biological assays, ssur

1.4 Biological features of microsporidia

On the basis of morphological and molecular features Undeen and Cockburn and Voss brinck *et al.* stated that Nosema bombycis is one of the earliest known primitive eukaryotes because of primitive type nuclear division [30, 31]. Microsporidia have a number of important, unique features; no typical Golgi apparatus, which is the accumulation of small and opaque vesicles enclosed by single membrane comparing to the classical Golgi apparatus composed of stacked lamellar cisternae; no peroxisome and hydrogenosome; mitochondrial remnants called "mitosomes" instead of typical mitochondria; ribosomes of prokaryotic type. Further, the microsporidian spore is equipped with a unique infection apparatus in the living world (polar filament, polaroplast, posterior vacuole). Upon the appropriate stimulus, the polar filament everts from the spore and expels the infectious sporoplasm into the host cell. In fact the polar filament is the definitive characteristic of microsporidia [32, 33]. While these common features, the microsporidia exhibit a large diversity in many aspects. The most obvious diversity is their great morphologic and cytologic differences which are summarized in Table-3. Nevertheless new microsporidian species are still being described and sometimes new genera are created to accommodate them [34, 35].

1.5 Geographical reference

Since Nageli named the first described microsporidian *Nosema bombycis*, it has been the reference species for the genus Nosema in which about 150 species have been described from at least 12 insect orders [36]. The "*Nosema bombycis* Nageli 1857" belongs to the Nosema genus (Nageli, 1857, Nosematidae family, Microsporidia order [25], Microsporea class and Microsporidia Balbiani, 1882 phylum [37]. The type host is *Bombyx mori* but numerous other Lepidoptera are susceptible, for example, five Lepidopteran insects. *Pieris rapae* (Pieridae), *Spodoptera diparvata* (Noctuidae), *Spodoptera exigua*, *Spodoptera litura* and *Trichoplusia ni*, which are known to be natural hosts of *Nosema bombycis* isolates in Eastern Asia [38]. These susceptible hosts can be a wild reservoir for *N. bombycis* and increase the possibility of horizontal transmission in sericulture.

So far, there are at least 20 different geographical major reference stains of *N. bombycis* in the world [38-40] (Table-3). In recent years, previously undescribed microsporidia pathogenic to *B. mori* have been isolated from sericultural farms. Some of them have been identified as the type *N. bombycis*, as *N. bombycis* different variants or transferred to other genus based on their molecular phylogeny and life cycles studies [40-43]. In fact many of the early classifications of microsporidia based on life cycle features, spore size, shape, and ultrastructure of spores including number of polar tube coils and host-parasite relationship resulted in the unnecessary creation of new Nosema species, With the application of combining molecular evidences with biological features, many of Nosema species have been transferred to other genus, for example, *N. corneae* to *Vittaforma corneae* [44], *N. connori* to *Brachiola connari* [45] and *N. algerae* to *Brachiola algerae* [46], *N. grylli* to *Paranosema grylli* [34] and *N. locustae* to *Antonospora locustae* [47].

All major reference *N. bombycis* strains have their species essential features; all stages are diplokarytic and in direct contact with host cell cytoplasm; it has two sporulation sequences and produce primary and environmental spores sequencially; it can be transmitted horizontally and transovarially; it infects nearly all tissues of *Bombyx mori* (systemic infection) except the external cuticle, the spire filament of trachea, the inner wall of the foregut and hindgut, the cutinized parts like mouthparts [36, 48].

1.6 Life cycle of nosema bombycis

The intracellular cycles of *N. bombycis* include the proliferative merogonic stage (merogony) and sporogonic stage (sponogony). The mature spore is oval or ovo-cylindrical and measures approximately 3.4-3.8 µm in length and 2.0 – 2.3 µm width, with three-layered membrane (inner, middle and outer). The spores are highly refractive, and shine bluish white under microscope exhibiting 'Brownian movement'. The outline is smooth and the spores are heavier than water. The resistant form of the disease is spore and it remains either in an infected tissue of the body or discharged through excreta by leaving infected host tissue. *N. bombycis* infects silkworm both horizontally by ingestion of spores and transmitted to progeny by the vertical means [49]. The spore, when swallowed by the silkworm through contaminated food,

germinates under alkaline conditions inside the gut of host with the help of digestive juice and produces a long polar filament measuring 500 µm in length and 0.5 µm in width [Fig.-1], and it is more than 30 times longer than that of the lengthwise dimension of the spore, on the end of which grows a sporoplasm [50]. The sporoplasm has one or two nuclei and other cell organs and possesses limited membrane. The polar filaments are short our and anchor firmly to the gut wall by penetrating the epithelial cells of the alimentary canal. The sporoplasm emerging from the spore invades the cytoplasm of the host. The force that propels the everting of filament and drives the sporoplasm through the polar tube appears to be osmotic. Subsequently the polar filament gets digested in the alimentary tract. The two nuclei of the sporoplasm unite to form a uninucleate planont. The planont measures 0.5-1.5 µm and is formed in 1-2 days. The planont is sub shell, performs amoeboid movement and reproduces by binary fission. The planont which initially infects the gut later passes through the gut wall and invades the various tissues. Once the planont penetrates the host cell, it transforms in to a sedentary form and becomes localized. The stage is known as meront. Meront is an intracellular stage and has a definite cell wall which absorbs nutrients from host cell. The meront is formed in 2-3 days after infection. It reproduces by binary fission, multiple budding. When cytoplasm of the host cell is exhausted, meronts are arranged in parallel rows. The meront after massive proliferation fills up the host cells and when nutrients are depleted, sporulation takes place. The spore completes its life cycle within 4 days. Complete developmental stages of the pathogen have been studied and elucidated in detail (Takiwaza *et al.* 1975) [51]. The mature spore is unicellular endomembranous differentiation of its sporoblast [52]. These authors designated the sporoblasts as phase-I sporoblasts and Phase-II sporoblasts. The phase-I sporoblasts are characterized by the presence of a dark staining spherical body [53].

Nosema bombycis completes its relatively simple life cycle with two sporulation sequences both with diplokaryotic sporont and disporoblastic sporogony [54-58]. The primary (early) sporulation sequence produces a thin-walled binucleate spore called "primary spore, internal spore or FC spore" (few coils of polar tube) with 4 coils of short polar filament and a large posterior vacuole. The primary spore can germinate quickly after formation in the infected cells and serve to disseminate infection within *Bombyx mori* (auto infection) and they are responsible for transovarial transmission because of the infection of the gonads. In cultured insect cells or in vivo, the primary spores are present at 48 h after infestation [55, 58]. The second sporulation sequence produces a binucleate spore with thicker spore wall and 10 to 13coils of long polar filament in one row arrangement [57-59]. They are detected 72 h after infestation of the insect cell cultures [60]. These external or environmental spores are involved in the infestation of new hosts and function to infect the new host in horizontal transmission.

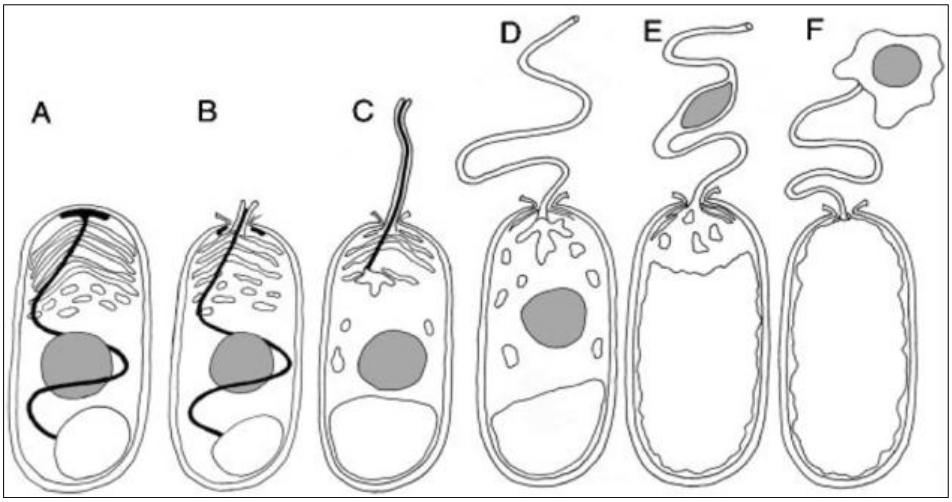

Fig. 1: Model of spore germination. [83]. (A) Dormant spore, showing polar filament (black), nucleus (grey), polaroplastand posterior vacuole. (B) Polaroplastand posterior vacuole swells, anchoring disk ruptures, and polar filament begins to emerge and evert. (C) Polar filament continues to evert. (D) Once the polar tube is fully everted, the sporoplasm is forced into (E) through the polar tube. (F) Sporoplasm emerges from the polar tube bound by new membrane.

1.7 Transmission and spread of disease

Pasteur (1870) reported the transmission of the Pebrine disease from infected parents to the offspring in the form corpuscles. *Nosema bombycis* transmits the infection both vertically by transferring the infection directly from parent to offspring and horizontally by ingestion of the spores. Transovarial transmission (primary infection) of pathogen results from the infected eggs laid by the infected mother moth. Transovarial transmission occurs when the Pebrine pathogen infects the 4th and 5th instar larvae, and then invades the epithelial cells of the ovaries from where the parasites are transferred to oogonia, oocytes and nutritive cells. Parasitism of oocytes results in the death of eggs. The result of embryonic infection differs according to the stage when invasion of embryo occur. If the infection occurs during the formation of embryo, then no embryonic development follows and the eggs die. It is only when the embryo has reached the stage of reversion of blastokinesis. The Pebrine pathogen enters the digestive tract of the embryos with the absorbed yolk nutrients, so that the ist instar larva that hatch out are embryonically infected. Han and Watanabe 1988 [49] studied the transovarial transmission of two microsporidia in

Bombyx mori and the examination of dead eggs and newly hatched larva showed that Nosema bombycis is transmitted transovarially by all infected mother moths. The percent transovarial transmission of the disease differs with different microsporidians being highest in Nosema bombycis. The rate of transmission of N. sp. NIS-M11 is 1.2% [49, 61, 62]. The rate of transmission of N. sp. NIK-3h is 1.80% while the standard strain Nosema bombycis and NIK-2r transmit to an extent of 100%. The transovarial transmission rate of Lb_{ms} is 64.53 % [63] while NIK-4m does not transmit infection by transovarial means [64].

The transovarial transmission of Varimorpha sp. Nis-M12, microsporidium sp. *Nis-M25*, *Pleistophora sp Nis-M27* and Thelhonia sp. *Nis-M32* has not be reported in silkworm. The larvae infected through transovarial transmission show irregular moulting and growth, die during 3rd and 4th instar. If these larvae are reared with healthy silkworm, the spore discharges by the infected larvae provide the source of contamination and digestion of these spores result in the spread of disease. The minimum numbers of spores required for the spread of disease through per-oral infection varies with each instar, Iwano and Ishira [65] stated that 1-10 spores are sufficient enough to cause disease in 2nd instant larvae while 100 of such spores are required in 5th instar for the same symptoms to occur. Horizontal transmission of the Pebrine spore is possible through contaminated rearing bed, mulberry leaf and layings [66]. Baig *et al.* [67] reported that the spread of disease in rearing trays is also dependent on the density of diseased silkworms. Growth and multiplication of pathogen are also influenced by the growth of its host. When egg enters into diapause, the growth and multiplication of pathogen stops simultaneously and when egg starts to grow, the pathogen will also start to grow and multiply.

1.8 Pathogen stability

A large number of factors viz, temperature, humidity an abiotic components of the substrate influence the survival of microsporidians [68]. The spores of Nosema are extremely resistant to environmental factors. It can survive for over 7 years under dark conditions and for a period of one year under conditions suitable for silkworm (26^0C and 60-70% relative humidity. The spores belonging to the dormant stage of pathogen and possessing great resistance can remain infective after 3 years in the dried body of the female moth, and become active after being submerged in water for 5 months [69]. The spores can survive for 6-7 hours when exposed to direct sunlight and 5 minutes in hot water. Studies conducted to reveal the variability of spores in soil and compost under tropical conditions have shown the survival of spores for a maximum period of 225 days in wet soil and a minimum of 135 days in wet compost [70]. Srikanta observed that spores remained infective even after 150 days of refrigeration and after 90 days in moist soil and faeces [71]. It also retains its infectivity in liquid manure for more than 3 weeks. He further stated that the viability of spores is lost in 60 days in dry soil and in 5 days when they are stored at room temperature. The resistance of spores to different disinfectants indicates that they can remain viable for 10-30 minutes in the solution (diluted 10,000 times). Bleaching powder containing 1% and 3% active chlorine can render spore inactive in 30 minutes and 10 minutes, respectively. When the degree of infection is relatively high, the egg often becomes sterile or dead, but when the contaminations are of low degree, the egg hatches and the disease develops at the larval stage and causes death of larvae at later stages of development.

1.9 Sources and stage of contamination

Transovarially infected seeds are primary source of contamination. Diseased larvae and wild insects affected by the diseases, excreta and faeces, urine of ripe larvae and moths, discarded egg shells, pupal skin and scales, epicuticle, cocoon shell, contaminated rearing and grainage building, appliances and mulberry leaf fed to the silkworm harboured by the infected insects etc, also contribute to the spread of disease. Larvae infected during 4th and 5th instar pupate and on emergence lay contaminated eggs. This phenomenon is known as 'transovarian transmission'. The contamination occurring from transovarially infected larvae is termed as the first stage of contamination. When the infected silkworm larvae are reared with the healthy larvae, the spore discharge by the infected larvae provides the source of contamination and the digestion of spores by healthy larvae result in a spread of disease. This state of contamination is known as second stage of contamination.

1.10 Host resistance and susceptibility

The incidence of Pebrine varies with the variety of silkworm, the development stage and the rearing environment. Different silkworm breeds differ in their susceptibility to microsporidian infections such differences are genetically determined and have been studied extensively in silkworm with reference to infection by viruses [72]. Large differences exist among various silkworm breeds in their susceptibility to infections by Nosema, bombycis [73]. Meanwhile, resistance to Pebrine is greater in the Chinese breeds but less in the Japanese and the least in European breeds [66]. The multivoltine breeds are relatively more resistant than bivoltines [74]. Young silkworms, newly moulted and starving larvae are susceptible and show high mortality. In India silkworm races such as Pure Mysore, Nistari and C.Nichi have high survival ability than other silkworm races [74-77]. The high survival of pure mysore breed have been investigated and it is attributed to the regenerative capacity of their midgut to recover from infection [78]. Patil and Geethabai reported that among the bivoltine breeds, NB7 is the most susceptible and is followed by NB4D2, KA and NB18 [74]. A silkworm race Baipidan is reported to be resistant to *N. bombycis* [79]. Although the disease resistance appears to depend on the genetic constituents of a particular breed, factors such as pathogen load, in adequate nutrition and the environment in which the insects are reared may also affect their resistance. In addition, the physical and physiological characteristics of the host make the invasion of microsporidians possible [20, 80].

1.11 Future strategies

Lack of accurate disease diagnosis in sericulture causes severe spread of several virulent diseases including microsporidiosis leading to crop losses of 30-40%. Earlier, microsporidia were identified based on their morphological characteristics using microscopy being an age old, inexpensive and common practice in use from the times immemorial, However, new strains of microsporidia occur in more complex forms which makes their identification

difficult since, microsporidian spores are visible only after 3–8 days of onset of infection. Hence, there is a dire need to employ the latest biotechnological approaches of PCR-Based DNA Marker technology which should enable the early detection of microsporidians of silkworms and help sericulture researchers and farmers for effective disease management in sericulture to save the crop losses due to such virulent pathogens for sustainable sericulture.

2. References

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