Int. J. Indust. Entomol. Vol. 6, No. 1, 2003, pp. 1~9

Review

International Journal of *Industrial Entomology*

Microsporidian Disease of the Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

Tribhuwan Singh* and Beera Saratchandra

Central Silk Board, B. T. M. Layout, Madiwala, Bangalore 560 068, India.

(Received 17 November 2002; Accepted 28 February 2003)

The silkworm, *Bombyx mori,* is prone to infection of various pathogenic organisms. Pebrine, one of the deadliest disease of silkworm caused by highly virulent parasitic microsporidian, *Nosema bombycis* has been understood since long. Infections of the disease range from chronic to highly virulent and can result in complete loss to the sericulture industry. Several strains and species of microsporidians have since been isolated from the infected silkworms; the disease is becoming increasingly more and more complex. Epizootiology, development of immunodiagnostic kit, use of chemotherapy and thermotherapy techniques has been addressed for identification and control of the disease. A technique of delayed mother moth examination, which plays a decisive role in the detection of the disease and harvestation of stable cocoon crop, has been described. An attempt has been made to review briefly the literature available on various aspects of the pebrine disease in order to develop efficient model(s) for the prevention and control of the disease and to suggest future avenues of investigation in the field of pebrine disease management.

Key words: *Bombyx mori*, Pebrine disease, Control

Introduction

The microsporidian infection remains a major threat to sericulture industry with its recurrent occurrence. Over seven hundred species of microsporidians are recorded from insects and fishes (Samson *et al.*, 1999a). More than twenty wild insect species are found to have microsporidian spores that can cross-infect silkworms. Pebrine, the spores of microsporidian, *Nosema bombycis*, cause one of the most dreaded diseases of the silkworm, *Bombyx mori.* Pebrine, which determines success or failure of sericulture industry of a nation infects almost all age, stage and breeds of the silkworm by both transovarian and secondary contamination. This is evidenced from the historical fact that the rise and fall of pebrine disease corresponds with the ups and downs of sericulture industry in the silk producing countries of the world (Tatsuke, 1971). Much work has been done by different workers on various aspects of the disease and its causative agent. The earliest research on pebrine was confined especially with the epizootiology and prevention of the disease (Weiser, 1969; Ishihara, 1963; Fujiwara, 1979). Microscopical method of mother moth examination, although widely practiced mainly due to its simplicity, does not assure foolproof solution to the microsporidian detection.

To circumvent this problem, in relatively recent studies, efforts have been made to evolve simple, precise and more accurate method of detection of the disease (Geethabai *et al.*, 1985; Fujiwara, 1993; Baig, *et al.*, 1992; Shi and Jin, 1997), identification of alternate host (Fujiwara, 1993; Samson, 2000), use of chemotherapy and thermo-therapy for the prevention and control of disease (Hayasaka, 1990) besides identification of intermediary stages but with little success. Even though, the researches and fight against the pebrine has been continuing for more than a century, loss due to disease has not been eliminated completely (Patil, 1993). However, historical evidences suggest a significant relationship between success of sericulture industry and the control of the disease. Therefore, for the improvement of sericulture industry and to save it from crop losses caused due to the chronic disease, it is essential to have a foolproof diagnostic and preventive technique.

To review and discuss briefly the recent advances achieved so far on various aspects of the pebrine disease,

*To whom correspondence should be addressed.

Central Silk Board (6th Floor), B. T. M. Layout Madiwala, Bangalore 560 068, India. Tel: +91-080-6688831; Fax: +91-080-6681511; E-mail: tnspriya@yahoo.com

an attempt has been made in this article to present annotated information to develop efficient model (s) for the prevention and control of the chronic disease in the days to come.

Causative organisms

Several historical evidences in various countries of the world show that the outbreak of pebrine disease has greatly influenced the decline of sericulture industry in the past. The damage of crops in Europe in the middle of 19th century was so great and extensive becoming a worldwide scale that the cocoon production declined sharply and the sericulture industry of the world suffered heavily (Tatsuke, 1971). Guerin-Menevillae of France named the pathogen of the disease "Hematozoid" as being microorganism living in haemolymph of the silkworm. De Quadrefages coined the name pebrine because of appearance of pepper-like spots of the diseased larvae. Nageli of Germany gave this pathogen a scientific name *Nosema bombycis* belonging to the family Nosematidae and order Microsporidia. Pasteur in his book "Eiudes Sur La Maladie Des Vers A Sole" called the disease corpuscle disease and made a detailed study on its growth and transmission and discovered that the disease is transmitted through transovarian transmission within the body of the mother moth and suggested the methods of preventing the disease. Balbiani of France stated that the pathogen of pebrine disease belongs to Sporozoa of the phylum Protozoa and order Microsporidia. Stempell of Germany reported the life history of the pathogen of pebrine disease.

Vossbrinck *et al.* (1987) and Undeen and Cockbum (1989) stated that phylogenetically, *N. bombycis* is one of the earliest known eukaryotes because of primitive type of nuclear division, absence of mitochondria, prokaryotic sized ribosomes and ribosomal RNAs. The life cycle of *N. bombycis* includes three stages namely, spore, planont and meront. The mature spore is oval or ovo-cylindrical and measures approximately 3.4 – 3.8 μm in length and 2.0 – 2.3 μm in width with three layered membrane, shining bluish white and exhibiting 'Brownian movement'. The outline is smooth and the spores are heavier than water. 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. Except the spore stage *N. bombycis* can survive only within the host. The spore, when swallowed by the silkworm through contaminated food, germinate 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 and is more than 30 times longer than that of the lengthwise dimension of the spore, on the end of which grows an sporoplasm (Peter *et al.*, 1999). The spore consists of sporoplasm with one or two nuclei and other cell organs with one limiting membrane. The sporoplasm is released into the haemolymph through polar filament and multiplies through fission, comes out of the haemolymph through intra cellular spaces, spreading to every part of the body, lives in various systems, particularly in fat body and muscular tissue, becomes a nucleus and form a spore after multiplication through fission. Spore formation is apansporoblastic, disporous and dimorphic. One type of sporoblast of the long polar tube type turns into single spore with many coils of polar tube. The other type is the sporoblast of the short polar tube, which turns into single spore with few coils of polar tube. Spores of short polar tube hatches directly in the host cell. Secondary sporoplasm reaches other cells of the host and infects. The spore completes its life cycle within 4 days. Complete developmental stages of the pathogen have been studied (Takizawa *et al.*, 1975) and the life cycle has been elucidated in detailed by Kawarabata and Ishihara (1984) and Iwano and Ishihara (1981). The mature spore is unicellular endo-membranous differentiation of its sporoblast (Vavra and Maddox, 1976).

Characteristics of the disease

The disease infects all the ages, stages and breeds of the silkworm. Larvae suffering from pebrine do not show any external symptoms until the disease is far advanced. At advanced stage, larvae become sluggish and show symptoms like poor appetite, retarded growth and development, irregular moulting, appear paler and translucent with wrinkled skin, shrinks in size and becomes flaccid (Jolly, 1986). Pupae are flabby and swollen with lusterless and softened abdomen. Highly infected pupae fail to metamorphose into adult. Irregular moth emergence, clubbed wings, distorted antennae, improper mating, low fecundity, sometimes eggs in lumps, high percentageof unfertilized eggs besides eggs with less gluey substance resulting in their detachment from the egg sheets, easily coming off scales from wings and abdominal area are some of the symptoms of the disease at moth stage.

The incidence of pebrine varies with the variety of silkworm, the developmental stage and the rearing environment. Resistance to pebrine is greater in Chinese breeds, less in Japanese and least in European breeds (Govindan *et al.*, 1988) and that multivoltine breeds are relatively more resistant than bivoltines (Patil and Geethabai, 1989). Young silkworms, newly moulted and starving larvae are susceptible and show high mortality. In India, Nistari and C. Nichi are more resistant silkworm breeds compared to others. Patil and Geethabai (1989) reported among bivol-

tines NB7 was most susceptible followed by NB_4D_2, KA and NB18. Although the disease resistance appears to depend on the genetic constituents of a particular race, nevertheless factors such as pathogen load, inadequate nutrition and the environment in which the insects are reared may also affect resistance (Patil, 1993). In addition, the physical and physiological characteristics of hosts may make the invasion of microsporidians possible (Weiser, 1969). The larvae infected during the 1st and 2nd instars show normal growth up to 3rd instars. Disease symptoms appear during later half of the 4th instars to first half of the 5th instars and die by the time of moulting. Larvae infected during 4th and 5th instars pupate and on emergence lay contaminated eggs. This phenomenon is referred as transovarian transmission. Most of the larvae infected through transovarian transmission show irregular moulting and growth, becomes tiny or under grown and die during the 3rd and 4th instars after discharging spores. If these larvae infected through transovarian transmission are reared with healthy larvae, the spore discharge by infected larvae provides the source of contamination and digestion of spores by healthy silkworms results in spread of the disease. The minimum number of spores required for contamination through per oral infection varies with each instars. Iwano and Ishihara (1981) stated that 1-10 spores are sufficient to cause disease in 2nd instar larvae, while approximately 100 such spores are required in 5th instars for the same symptoms to occur. Transovarian transmission is 100% in case of *N. bombycis* and only 1.2% with *Nosema* sp. M11 (Han and Watanabe, 1988).

The spores of different microsporidia infecting silkworms differ in their morphological characters, some are larger than mature spore and some are long, thin and pear-shaped with different size, shape and luster. Sometimes the conidia of green muscardine and red muscardine bear a striking resemblance to the spore of pebrine disease. Horizontal transmission of pebrine spore is possible through contaminated rearing bed, contaminated mulberry leaf and through contaminated layings (Govindan *et al.*, 1998). Baig *et al.* (1988a) found that spread of disease in rearing trays is also dependent on the density of diseased silkworms.

Physiological stability

Generally, large number of factors *viz.*, temperature, humidity and abiotic components of the substrate influence the survival of microsporidians (Kramer, 1976). The spores belong to the dormant stage of pathogen and possess great resistance, can remain infective after 3 years in the dried body of the female moth, and remain active after being submerged in water for 5 months (Li, 1985). When kept in dark, the spores are reported to remain viable for as long as seven years, but when spores are directly exposed to sunshine (Anonymous, 1980), they remain viable for 6 – 7 hrs and when treated with hot water remain viable for just 5 minutes. Studies conducted on the viability of pebrine spores in soil and compost under tropical conditions showed survival of spores for a maximum period of 225 days in wet soil and a minimum of 135 days in wet compost (Patil, 1993). Srikanta (1986) observed that spores remained infective even after 150 days of refrigeration and after 90 days in moist soil and faeces. He further stated that the viability of spores is lost in 60 days in dry soil and in 5 days when stored at room temperature. Resistance of spores to different disinfectants indicates that it can remain viable for 10 – 30 minutes in a solution of corrosive sublimate, for about 5 hrs in formalin and 10 hrs in chlorinated lime solution (diluted 10,000 times). Bleaching powder containing 1% and 3% active chlorine can render spore inactive in 30 minutes and 10 minutes respectively.

The growth and multiplication of pathogen in the eggs are influenced by the growth of the host. When the egg diapauses, the growth and multiplication of the pathogen stops simultaneously and when the eggs start growing by incubation, the pathogen also starts growing and multiplying. When the degree of infection is relatively high, the egg often becomes sterile or dead, but when the contamination is of low degree, the egg hatches and the disease develops at larval stage and caused death of larvae at later stages of development.

Alternate host

Most microsporidians prefer to have alternate hosts because of many advantages for them *viz.*, dispersal, transmission and survival. The perpetual incidence of microsporidian infection in silkworms may be due to various sources of secondary contaminations including alternate hosts in and around mulberry garden. In addition to *N. bombycis*, seven other microsporidians belonging to the genera *Nosema*, *Pleistophora*, *Thelohania*, *Vairomorpha* and *Leptomonas* spp. has been isolated from the silk moth (Govindan *et al.*, 1998). They differ in their spore morphology, target tissue and virulence (Table 1) and have been designated as M11, M12 and M14 (*Nosema* sp.), M24, M25, M27 (*Pleistophora* sp.) (Fuziwara, 1984a, b) and M32 (*Thelohania* sp.) (Fujiwara, 1985). *N. bombycis* is also reported to infect *Samia cynthia ricini* and Indian tropical tasar, muga and Chinese tasar silkworms (Talukdar, 1980). *N. bombycis* is also found to infect a number of other insect species like *Telchinia violae*, *Pieris rapae*, *Brachyhinus lingustici*, *Choristoneura fumiferana*, *Bombyx mandarina*, *Glyphodes phyloalis*, *Chilo suppressalis*, *Arctia caja*, *Chilo simplex*, *Pieris rapae*, *P. brassicae etc.*

Table 1. Microsporidians isolated from silk moth (after Fujiwara, 1980, 1984 a, b)

Microsporidian	Spore size (μm)	Site of infection	Virulence
Nosema bombycis	3.8 × 2.2	Systemic	High
Nosema sp. (M11)	3.9 × 1.9	Various tissues	Low
Nosema sp. (M12)	4.2 × 2.7	Various tissues	Low
Nosema sp. (M14)	5.1 × 2.0	Various tissues	High
Pleistophora sp. (M24)	2.7 × 1.6	Mid gut	Low
Pleistophora sp. (M25)	3.2 × 1.8	Mid gut	Low
Pleistophora sp. (M27)	5.4 × 3.0	Various tissues	Low
Thelohania sp. (M32)	3.4 × 1.7	Muscle	Low

(Samson *et al.*, 1999b). The lawn grass cut worm, *Spodoptera depravata* serves as a natural reservoir for the pathogen (Ishihara and Iwano, 1991), which shares the surface specific antigens with *N. bombycis* from *B. mori*. Its infection is systemic and results in transovarial transmission but is less virulent.

Isolation and purification of spores

Isolation, purification and identification of spores from the host are the first step in the study of pebrine disease and its management. To isolate spores, diseased larvae/pupae/moths are homogenized in sterile water using mixer for 1 – 2 minutes. The homogenate is filtered through cotton or fine muslin cloth. The filtrate thus obtained is transferred into centrifuge tube and is centrifuged at 3,000 rpm for 5 minutes. The supernatant is discarded and the sediment thus obtained is mostly consists of spores which can be confirmed with microscopical examination. However, serological and biochemical studies of microsporidians require high degree of purity. Gochnaner and Margetts (1980) described rapid method for concentrating *Nosema* spores based on continuous flow centrifugation method. Another method based on Brownian movement was also reported. Sato and Watanabe (1980) purified spores using sucrose and percol gradient centrifugation and reported that centrifugation using percol at 73,000 g for 30 minutes resulted in 3 bands

a) A sharp band consisting of tissues of silkworms, mulberry leaves bacteria etc.
b) A dim band consisting of mature but inactive spores.
c) Another sharp band consisting of only mature and active spores.

Approaches for pebrine prevention/control

Pebrine has remained a threat to sericulture industry since time immemorial. The disease has become more complex now because of the occurrence of different types of microsporidians infecting the silkworm. Some of them belong to other genera like *Vairomorpha* and *Thelohania* and exhibit differences in their pattern of infection (Samson, 2000). Apparently, the biology of the pathogen has been used as a basis in disease control. The disease is transmitted horizontally by ingestion of spore and vertically by transovarian transmission. This unique characteristic of the disease made difficult to completely eliminate it from the silkworm crops. The earliest method suggested by Pasteur based on selection of pathogen free eggs through careful systematic examination of mother moths for pathogens, after egg laying has been one of the most effective methods even today to avoid the disease in the silkworm crops.

Proper monitoring and testing of seed crops at every successive stages of progress of the crop is also to be ensured to produce pebrine free seed cocoons for commercial seed production. Destruction of infected crops as soon as infection is noticed is another important step towards pebrine disease management. Since the disease is seed borne, the surface sterilization of eggs immediately after egg laying and also during pin-head stage of incubation should be followed to prevent the disease occurring from surface contamination (Singh *et al.*, 1993). Several reports documented the efficiency of thermal treatment of silkworm eggs in minimizing pebrine infection (Bedniakova and Vereiskava, 1958; Fujiwara and Kagawa, 1984; Hayasaka, 1990). The maximum lowering of infection rate was reported when eggs were heated during the first two days of their development to 44°C. Krishnaswami *et al.* (1971) reported that heat treatment of eggs within 3 days of laying results in significant reduction in pebrine disease. Thermal treatment was also combined with hydrochlorization to achieve dual objectives of elimination of pebrine and termination of diapause (Austrurov *et al.*, 1969). Liu *et al.* (1971) reported remarkable success in reducing pebrine infection after treatment at 47°C for 10 – 20 minutes. Chowdhary (1967) suggested exposure of cocoons to high temperature (33.8°C) at the time of pupation for 16 hrs a day at 55 – 65% humidity tends to reduce infection in the resulting eggs. Sheeba *et al.* (1999) reported that thermotherapy of 7 days old pebrinized cocoons at 36°C for 16 hrs tends to reduce pebrine infection significantly without affecting the growth and development of larvae.

Hot water treatment to reduce the incidence of pebrine was also attempted by several workers. Ovanesyan and Lobzhanidze (1960) and Austrurov *et al.* (1969) attempted hot water treatment of pebrinized eggs and reported sharp decrease in the degree of infection. Smyk (1959) expressed varying success with hot water treatment. Fujiwara and Kagawa (1984) reported that the parasites in

non-diapausing eggs are more sensitive to hot water (46°C for 4 minutes) treatment and there is no harmful effect of the treatment on the normal development of the silkworm embryos. However, these methods are not effective enough to eliminate infection completely. Of the several therapeutic drugs, Benomyl, Nosematol, Bavistin and Thiophanate have been identified as antimicrosporidian agents to control *N. bombycis* infections (Chandra and Sahakundu, 1983 and Alenkseenork, 1986). Though these fungicides have proved experimentally effective in reducing the multiplication of spores but further studies has clearly showed that Nosematol, Benomyl and Bavistin cannot eliminate transovarian transmission. *N. bombycis* is made to be inactive by hilite (Potassium dichloro isocyanurate) at 0.001, 0.01 and 0.1% concentration (Iwano and Ishihara, 1981). Baig *et al.* (1988b) studied comparative efficacy of four disinfectants *viz.*, hilite, sodium hypochlorite, bleaching powder and formalin in four concentrations (0.5%, 1%, 1.5% and 2%) as surface sterilents against the spread of pebrine disease in a colony of silkworm hatched from surface contaminated layings and reported that all the tested concentrations were effective in preventing the spread of the disease and were also effective in inactivating spores of *N. bombycis* when exposed to 5, 10, 20 and 30 minutes respectively. Kagawa (1980) studied the efficacy of formalin as disinfectant against pebrine and reported increased death rate of spores with increase in formalin concentration and temperature. Hitoshi and Ishihara (1981) tested nine kinds of chemicals as inhibitory agent against the hatch of *N. bombycis* including hilite, which showed high degree of inhibitory effect on the spores. However, chemical control of pebrine disease attempted so far by several workers was found to have limited success. Therefore, development of better and more reliable diagnostic methods to detect pebrine during seed production and silkworm rearing has always remained one of the important and valid strategies to ward the disease from silkworm crops. Recently recommended delayed mother moth test is a significant step in the area of pebrine disease diagnosis by microscopic test. In this method, the moths after oviposition are preserved alive at room temperature for a period of 3 – 4 days before subjecting for microscopic test. This allows improved sporulation of the pathogen facilitating easy and more accurate detection of the disease (Samson, 2000). It has been reported that the rate of multiplication of *N. bombycis* increases substantially with the age of moths and cephalothoracic region had the highest spore concentration, especially around the wing and wing muscles (Sasidharan *et al.*, 1994) (Table 2) and therefore, testing of silk moths 3 – 4 days after oviposition would be more effective method to detect pebrine with better accuracy. An improved testing method has also been recommended for better detection at egg stage. A sample of egg is incubated at a moderately higher temperature of 32 ± 1°C for 48 hrs to enhance sporulation of *N. bombycis*. Testing of such eggs therefore enhances the chances of detection of the disease. In these lines, even diagnostic techniques based on the principles of immunology were also attempted in several countries including India for detection of pathogen and spore identification, but with only limited success (Baig *et al.*, 1992).

N. bombycis and closely related spores were diagnosed with the use of antibody-sensitized latex agglutination

Table 2. Sporulation rate of *Nosema bombycis* in different tissues after emergence of moths of silkworm (source: Sasidharan *et al.*, 1994)

Body parts	Race	Quantity of spores on different days after emergence ($\times 10^7$/g wt of tissue)				
		0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Whole moth	PM	4.39	4.50	5.67	21.90	25.50
	NB18	5.92	6.34	12.40	22.00	28.70
Cephalothorax	PM	8.20	10.50	9.40	35.80	44.00
	NB18	7.10	10.20	14.70	38.40	40.10
Abdomen	PM	1.49	2.60	5.50	14.90	21.60
	NB18	5.02	3.80	7.94	14.00	20.30
Wing	PM	6.30	8.50	11.00	25.00	31.30
	NB18	8.61	12.80	24.45	28.60	34.60
Gut	PM	9.62	10.81	10.60	24.60	22.60
	NB18	8.11	8.94	12.40	20.60	21.20
Fat body	PM	0.19	10.15	0.10	0.20	0.20
	NB18	1.34	2.17	2.10	1.77	2.41

technique (Hayasaka and Ayuzawa, 1987), slide agglutination technique (Li, 1985; Baig *et al.*, 1992), the use of ELISA procedures (Kawarbata and Hayasaka, 1987), fluorescent antibody technique (Sato *et al.*, 1981; Sato *et al.*, 1982), serological technique (Grobov and Rodionova, 1985) and SPA coagglutination test (Mei and Jin, 1988) etc. Development of monoclonal antibody techniques, which has very high specificity and stability, has played great role in the study of classification and identification of specific microsporidians (Chen *et al.*, 1989; Carlos *et al.*, 1996). Ke *et al.* (1990) raised monoclonal antibodies against *N. bombycis* spores and applied them to identify pebrine and other closely related microsporidian spores infecting silkworms using the ELISA procedure. Shi and Jin (1997) reported that agglutination test using N5 McAb (hybridoma cell lines secreting monoclonal antibody) sensitized latex particle is a very practical technique for the diagnosis of pebrine disease. A simple dipstick immuno assay method was also tried later, for diagnosis of pebrine, which was also unsuccessful in the field. A simple negative staining procedure (Geethabai *et al.*, 1985) and an immunoperoxidase staining procedure (Han and Watanabe, 1987; Kawarabata and Hayasaka, 1987) have been developed for the clarity during examination of spores. Sironmani (1997) has developed a Western blot method to identify the microsporidian infection and observed that immunological reaction with *N. bombycis* infected silkworm larvae and eggs showed the presence of 17 kDa polypeptide, which is specific to infection. He further reported that 17 kDa polypeptide can be used as a virulent marker for the identification of microsporidian infection. DNA based probes have also been developed for identification of *N. bombycis* (Malone and McIvor, 1995). A new technique based on identification of intermediary stages has also been suggested for diagnosis of pebrine (Santha *et al.*, 2001). Though these tests are simple and sensitive, unless standard methods are evolved for their effective field applicability, they cannot create any impact on pebrine disease diagnosis in the field. To maintain the quality of silkworm eggs, several attempts have been made to improve the sampling procedure from time to time (Kurisu, 1986; Kurisu *et al.*, 1985; Fujiwara, 1993). Methods have been developed for detection of pebrine spores in soil/dust in rearing and grainage houses, on mulberry leaves, egg-shells/unhatched eggs, in litter etc. The sample size for examination of faecal matter to detect presence of pebrine has been described recently by Patil *et al.* (2001) (Table 3). As it is not possible to examine all emerging moths in the commercial grainages, Fujiwara (1993) have suggested 20% sampling method and reported probability of detection of pebrine disease (Table 4).

Table 3. Faecal pellet sample size for 100 dfls (after Patil *et al.*, 2001)

Age of larvae		No. of trays per instar (4.5 dia)	Sample size	
Instar	Day		No. of samples of 10 gm each	Total weight of faecal matter (gm)
I	2	2	1	10
II	2	2	1	10
III	2	4	2	20
IV	2	8	2	20
V	2	18	4	40
V	4	25	6	60
V	6	30	8	80

Table 4. Probability of detection of pebrine in 20% sampling method (source: Fujiwara, 1993)

No. of egg cards	Population	Sample	Probability	
			Non detectable	Detectable
20	400	80	0.6400	0.3600
30	600	120	0.5120	0.4880
40	800	160	0.4096	0.5904
50	1000	200	0.3277	0.6723
60	1200	240	0.2621	0.7379
80	1600	320	0.1678	0.8322
100	2000	400	0.1074	0.8926
150	3000	600	0.0352	0.9648
200	4000	800	0.0115	0.9885
250	5000	1000	0.0380	0.9620
300	6000	1200	0.0012	0.9988
500	10000	2000	0.0000	1.0000

Rate of pebrine infection = 0.5% in female moths.

Destruction of disease-causing microorganisms at various levels is general method of preventing and controlling the disease. Surface sterilization of disease free layings, maintenance of strict sanitation, hygienic rearing, frequent and careful examination of stock, disinfections of rearing rooms and appliances, removal of dead and infected larvae to be adopted strictly to get-rid-off the disease. Exposing all the contaminated materials and equipments to direct sunlight, disinfections with 2% formaline solution or 5% bleaching powder solution is the most effective and simple eradication method of the disease. However, the pathogen killing action of the disinfectants is influenced by several factors such as temperature, humidity, concentration of disinfectants and duration of treatment (Kagawa, 1980). Recently, a new disinfectant chlorine dioxide has been considered as an ideal disinfectant for all the types of rearing/grainage houses. In combination with

slaked lime, it is effective 2.5 times stronger than chlorine and 2 times stronger than sodium-hypochloride. It is least corrosive and non-hazardous. When no single technique is sufficient to check the disease in field, it becomes obligatory to choose a multi-pronged approach. However, the techniques only help in detecting the disease and the only way out is to destroy the diseased silkworm crops, which causes loss and efforts are to be made at all levels for the prevention of the disease.

A burning problem in the field of microsporidiosis is the increasing number of different microsporidians that are being encountered in silkworm crops (Fuziwara, 1980, 1993). These microsporidians have shown to exhibit varying degree of virulence and many of them, though infective and pathogenic, have demonstrated low multiplication rate in the silkworm. Some of them have not shown vertical transmission in the host. However, as of today, there has been no specific testing procedure to discriminate these microsporidians in the field to take appropriate action while preparing disease free silkworm seed. If pebrine is to be controlled effectively, a system has to be evolved where either a seed cocoon grower or a seed producer is not put in hardship due to occurrence of the disease. This is possible only when such losses are compensated. Simultaneously, rigid steps have to be taken to check the disease at all levels.

Future Research

- Development of better, rapid, systematic and feasible procedure for early detection of pebrine disease both during seed production and silkworm rearing.
- Development of specific procedures for discriminating virulence and non-virulence microsporidians and studies on cross-infectivity of different microsporidians infecting silkworms.
- Studies on biochemistry of both the parasite and the host to identify the potential target organ for chemical agents in the control of the disease.
- Studies on molecular aspects of disease resistance in silkworms and mechanism of gene action.
- Development of more effective and reliable method of identification of intermediary stages of the pebrine disease.
- Development of pebrine resistant season and region specific breeds/hybrids of silkworm for commercial use.
- Epidemiological studies of microsporidians in natural epizootics.
- Electron microscopy, biochemical and serological studies involving biology, host parasite interactions, taxonomy etc. to develop effective method of detection/forecasting of disease out breaks and in curtailing cocoon crop loss mainly by developing immunodiagnostic kits.

References

Alenkseenork, A. (1986) Nosematol for control of nosematosis of bees and pebrine of silkworm. *Veterinariya, USSR* **10**, 45-47.

Anonymous (1980) Textbook of Tropical Sericulture. Japan Overseas Cooperation Volunteers, Tokyo, Japan, pp. 551-556.

Austrurov, B. L., E. L. Baburashvli, T. A. Bedniakova, V. N. Vereiskaia, V. I. Labzhanidze and T. T. Ovanesyan (1969) Thermal intravital disinfections of eggs with simultaneous of the embryonic diapause as a new method of control of silkworm pebrine disease. *Zvakad Nauk, SSSR, Seric. Biol.* **6**, 811-818.

Baig, M., R. K. Data, B. Nataraju, M. V. Samson and V. Sivaprasad (1992) Protein-A linked antisera test for the detection of *Nosema bombycis* spores. *J. Invertebr. Pathol.* **60**, 310-313.

Baig, M., M. V. Samson, T. O. Sasidharan, S. D. Sharma, M. Balavenkatasubbaiah and M. S. Jolly (1988a) Study on the spread of pebrine after introduction of transovarially infected worms in a colony of silkworm, *Bombyx mori* L. *Sericologia* **28**, 75-80.

Baig, M., M. V. Samson, S. D. Sharma, M. Balvenkatasubbiah, T. O. Sasidharan and M. S. Jolly (1988b) Effect of certain disinfectants as surface sterilants against pebrine in surface contaminated layings. *Sericologia* **28**, 81-87.

Bedniakova, T. A. and V. N. Vereiskava (1958) The disinfection action of high temperature on eggs of mulberry silkworm (*Bombyx mori* L.) infected with pebrine (*Nosema bombycis*) at different stages of the diapausal cycle of development. *Doklady Akad Nauk USSR, Biol. Sci. Sect.* **122**, 760-763.

Carlos, A., P. Didier and C. Jean (1996) Recovery and characterization of a replicas complex in rotavirus infected cells by using a monoclonal antibody against NSP2. *J. Virol.* **70**, 985-991.

Chandra, A. K. and A. K. Sahakundu (1993) The effect of drug on pebrine infection in *Bombyx mori* L. *Indian J. Seric.* **21 & 22**, 67-69.

Chen, J., J. Teng, C. Hu and M. Michael (1989) Production of monoclonal antibodies to densovirus of silkworm (*Bombyx mori*) and their application in diagnosis. *Chinese J. Virol.* **5**, 77-81.

Chowdhary, S. N. (1967) The silkworm and its culture. Mysore Printing and Publishing House, Mysore, India, pp. 76.

Fujiwara, T. (1979) Infectivity and pathogenecity of *Nosema bombycis* to larvae of the silkworm. *J. Seric. Sci. Jpn.* **48**, 376-380.

Fujiwara, T. (1980) Three microsporidians (*Nosema* spp.) from the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **49**, 229-236.

Fujiwara, T. (1984a) A *Pleistophora* like microsporidian isolated from the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **53**, 398-402.

Fujiwara, T. (1984b) *Thelohania* sp. (Microsporidia Thelohanidae) isolated from the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **53**, 459-460.

Fujiwara, T. (1985) Microsporidia from silk moths in egg production sericulture. *J. Seric. Sci. Jpn.* **54**, 108-111.

Fujiwara, T. (1993) Comprehensive report on the silkworm disease control. A report on "*Bivoltine Sericulture Technology Development in India*" submitted to Central Silk Board, Bangalore, India, pp. 110.

Fujiwara, T. and T. Kagawa (1984) Control of *Nosema bombycis* parasitizing silkworm eggs by treatment with hydrochloric acid or exposure to various temperature. *J. Seric. Sci. Jpn.* **53**, 394-398.

Geethabai, M., C. S. Patil and A. R. Kasturi Bai (1985) A new method for easy detection of pebrine spores. *Sericologia* **25**, 297-300.

Gochnaner and Margetts (1980) A microsporidian disease in the silkworm, *Bombyx mori* L. *Sericologia* **33**, 201-210.

Govindan, R., T. K. Narayanaswamy and M. C. Devaiah (1998) *Principles of Silkworm Pathology.* Seri Scientific Publishers, Bangalore, India, pp. 420.

Grobov, O. F. and Z. E. Rodinova (1985) Serological diagnosis of microsporidiosis in useful insects. *Trudy Vsesoyuznogo Institute Eksperimental noi Vetrinarii* **62**, 112-116.

Han, M. S. and H. Watanabe (1987) Immunoperoxidase-staining method for discrimination of microsporidian spores in the pebrine infection of silkworm mother moths. *J. Seric. Sci. Jpn.* **56**, 431-435.

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

Hayasaka, S. (1990) Inhibitory effect of high temperature on the development of *Nosema bombycis* in the silkworm, *Bombyx mori*. *Acta Seric. Entomol.* **3**, 59-65.

Hayasaka, S. and C. Ayuzawa (1987) Diagnosis of microsporidians, *Nosema bombycis* and closely related species by antibody-sensitized latex. *J. Seric. Sci. Jpn.* **56**, 169-170.

Hitoshi, I. and R. Ishihara (1981) Inhibitory effect of several chemicals against the hatch of *Nosema bombycis* spores. *J. Seric. Sci. Jpn.* **50**, 276-281.

Ishihara, R. (1963) Effect of injection of *Nosema bombycis* Nageli on pupal development of the silkworm, *Bombyx mori* L. *J. Insect Pathol.* **5**, 131-140.

Ishihara, R. and H. Iwano (1991) The lawn grass cutworm, *Spodoptera depravata* (Butter) as a natural reservoir of *Nosema bombycis* Nageli. *J. Seric. Sci. Jpn.* **60**, 236-337.

Iwano, H. and R. Ishihara (1981) Inhibition effect of several chemicals against the hatch of *Nosema bombycis* spores. *J. Seric. Sci. Jpn.* **50**, 276-281.

Jolly, M. S. (1986) Pebrine and its control. Central Silk Board, Bangalore, India.

Kagawa, T. (1980) The efficacy of formalin as disinfectant of *Nosema bombycis* spores. *J. Seric. Sci. Jpn.* **49**, 218-222.

Kawarabata, T. and R. Ishihara (1984) Infection and development of *Nosema bombycis* in cell lines of *Antheraea eucalypti*. *J. Invertebr. Pathol.* **44**, 52-62.

Kawarabata, T. and S. Hayasaka (1987) An enzyme-linked immunosorbent assay to detect alkali-soluble spore surface antigens of strains of *Nosema bombycis* (Microspora: Nosematidae). *J. Invetebr. Pathol.* **50**, 118-123.

Ke, Z., W. Zie, Z. Wang, Q. Long and Z. Py (1990) A monoclonal antibody of *Nosema bombycis* and its use for identification of microsporidian spores. *J. Invertebr. Pathol.* **56**, 395-400.

Kramer, J. P. (1976) The extra corporeal ecology of Microsporidia; in *Comparative Pathobiology,* Bulla L. A. and T. C. Cheng (eds.), vol. I., pp. 127-135, Plenum Press, Publishing Corporation, New York.

Krishnaswami, S., M. A. Khan and P. Mediratta (1971) *Silk Industry in Soviet Union.* Report of Technical Delegation, Central Silk Board, India, pp. 72.

Kurisu, K. (1986) Simplified calculation of the operating characteristics in the pebrine inspection of the grouping mother method. *J. Seric. Sci. Jpn.* **55**, 351-352.

Kurisu, K., T. Nakao, M. Dohi and M. Hamazaki (1985) Studies on the pebrine inspection of the mother moth in the silkworm, *Bombyx mori* for commercial eggs. III. Practical application of sampling inspection table. *Bull. Fac. Text. Sci., Kyoto Univ.* **11**, 19-30.

Li, D. (1985) Studies on the serological diagnosis of the pebrine of silkworm, *Bombyx mori*, by slide agglutination test. *Sci. Seric.* **11**, 99-102.

Liu, S. X., D. Z. Zhu, W. Y. Zheng, Y. C. Huo, Y. N. Wu and J. Y. Liang (1971) A summery of the techniques for the control of *Nosema bombycis* Nageli in *Bombyx mori* with instant acid treatment at high temperature. *Guangdong Agric. Sci.* **3**, 20-21.

Malone, L. A. and C. A. McIvor (1995) DNA probes for two microsporidia, *Nosema bombycis* and *Nosema costelytrae*. *J. Invertebr. Pathol.*, **65**, 269-273.

Mei, L. and W. Jin (1998) Study on fast distinguishing *Nosema bombycis* by SPA co-agglutination. *Sci. Seric.* **14**, 110-111.

Ovanesyan, T. T. and V. I. Lobzhanidze (1960) First results of experiments on the thermal disinfection of pebrinous silkworm eggs by a brief immersion in hot water. *Tr. Inst. Mortl, Zhivotnykh Akad. Nauk, SSSR* **21**, 184-215.

Patil, C. S. (1993) Pebrine a microsporidian disease in the silkworm, *Bombyx mori* L. *Sericologia* **33**, 201-210.

Patil, C. S., N. B. Jyothi and C. M. S. Dass (2001) Silkworm faecal pellet examination as diagnostic method for detecting pebrine. *Indian Silk* **39**, 11-12.

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

Peter, A., F. Sadatulla and M. C. Devaiah (1999) The viral, bacterial and protozoan disease of the silkworm, *Bombyx mori* L.; in *Advances in Mulberry Sericulture*, Devaiah, M. C., K. C. Narayanaswamy and V. G. Maribashetty (eds.), pp. 378-457, CVG Publications, Bangalore, India.

Samson, M. V. (2000) Cocoon production and silkworm protection. National Conference on Strategies for Sericulture Research & Development, 16-18 Nov., CSR&TI, Mysore, India. pp. 38-48.

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.

Santha, P. C., T. O. Sasidharan, R. N. Singh, A. G. K. Daniel and T. M. Veeraiah (2001) Identification of intermediary stages of *Nosema bombycis* for diagnosis of pebrine - a new approach. *Indian Silk* **40**, 13-14.

Sasidharan, T. O., R. N. Singh, M. V. Samson, A. Manjula, P. C. Santha and Chandrashekhariah (1994) Spore replication rate of *Nosema bombycis* (Microsporidia: Nosematidae) in the silkworm *Bombyx mori* L. in relation to the pupal development and age of moths. *Insect Sci. Appl.* **15**, 427-431.

Sato, R. and H. Watanabe (1980) Purification of mature microsporidians spores by iso-density equilibrium centrifugation. *J. Seric. Sci. Jpn.* **49**, 512-516.

Sato, R., M. Kobayashi and H. Watanabe (1981) Internal ultra structure of spores of microsporidians isolated from the silkworm, *Bombyx mori* L. *J. Invertebr. Pathol.* **40**, 260-265.

Sato, R., M. Kobayashi, H. Watanabe and T. Fujiwara (1982) Serological discrimination of several kinds of microsporidian spores isolated from the silkworm, *Bombyx mori* L. by an indirect fluorescent antibody technique. *J. Seric. Sci. Jpn.* **50**, 180-184.

Sheeba, R., M. C. Devaiah, K. P. Chinaswamy and R. Govindan (1999) Thermotherapy of pebrinized cocoons and its effect on larval progeny; in *Proc. Natl. Semi. Trop. Seric.* Govindan, R., K. P. Chinnaswamy, N. K. Krishnaprasad and D. N. R. Reddy (eds.), Vol. II., pp. 266-272, UAS and Swiss Agency for Development and Cooperation, Bangalore, India.

Shi, L. and P. Jin (1997) Study on the differential diagnosis of *Nosema bombycis* of the silkworm, *Bombyx mori* by monoclonal antibody-sensitized latex. *Sericologia* **37**, 1-6.

Singh, R. N., T. Singh and P. R. Yadav (1993) Containing pebrine problem in sericulture. *Indian Silk* **30**, 43-44.

Sironmani, A. (1997) Detection of *Nosema bombycis* infection in the silkworm, *Bombyx mori* by Western blot analysis. *Sericologia* **39**, 209-216.

Smyk, D. (1959) Methodes physiques de lutte contre *Nosema bombycis* dans les graines du ver a soie du murier. *Revue du Ver a Soie*. **11**, 155-164.

Srikanta, H. K. (1986) Studies on the cross infectivity and viability of *Nosema bombycis* (Microsporidia: Nosematidae). M. Sc. Thesis UAS Bangalore, India.

Takizawa, H., E. Vivier and A. Pettprez (1975) Recherches cytochimiques sur la microsporidie *Nosema bombycis* an cours de son development chez lever a soil (*Bombyx mori*). *J. Protozool.* **22**, 359-368.

Talukdar, J. N. (1980) Prevalence of transovarian infection of microsporidian parasite infecting muga silkworm, *Antheraea assamensis*. *J. Invertebr. Pathol.* **36**, 273-275.

Tatsuke, K. (1971) Pebrine disease of silkworm a technical report, Overseas Technical Cooperation Agency, Tokyo, Japan, pp. 1-20.

Undeen, A. H. and A. F. Cockburn (1989) The extraction of DNA from microsporidian spores. *J. Invertebr. Pathol.* **54**, 132-133.

Vavra, J. and J. V. Maddox (1976) Methods in microsporidiology; in *Comparative Pathobiology*, Bulla, L. A. and T. C. Cheng (eds.), vol. I., pp. 107-121, Plenum Publishing Corporation, New York.

Vossbrink, C. B., J. V. Maddox, S. Friedman, B. A. Debrunner-Vossbrink and C. R. Woese (1987) Ribosomal RNA sequence suggests Microsporidia are extremely ancient eukariotes. *Nature* **326**, 411-414.

Weiser, J. (1969) Immunity of insects to protozoa; in *Insects Immunity to Parasitic Animals*. Jackson, C. J., R. Herman and I. Singer (eds.), pp. 129-147. Appleton Century Crofts, New York.