GENERAL INTRODUCTION

Silk, the queen of fabrics is graceful in lustre and unmatched in comfort and warmth. The first known silk fabric is about 5000 years old (Kuhn, 1988; Nagaraju *et al.*, 2001) Archeological and bibliographical evidences indicate that the sericulture was practiced in China by about 2500 BC. The history of sericulture speaks of sericulture in 12th century BC along the bank of the river Hwang Ho in China and its spread to the neighbouring countries by smuggling of the mulberry seeds and silkworm eggs.

Today, the sericulture has grown into an agro-based rural cottage industry par excellence with agricultural and industrial superstructure. It is a labour-intensive activity involving mulberry cultivation, silkworm seed production, silkworm rearing, silk reeling and other post cocoon processes. It is also remarkable for its low investment, low gestation period and quick and high returns. It fits well into the socio-economic fabric of most developing countries. In India, it has proved as an effective tool for socio-economic development of the rural population. The cultivation of mulberry and the rearing of silkworms are entirely an agricultural activity, whereas unwinding of silken thread from cocoons and the weaving of the fabric form the industrial superstructure (Kuhn, 1988). These two are interdependent for their economic viability and stability.

Sericulture has emerged as an economically viable modern industry and has spread to over 60 countries. In India, mulberry sericulture was confined to the states like Jammu and Kashmir, West Bengal, Karnataka, Andhra Pradesh and Tamil Nadu. However, in the recent past, sericulture has been introduced to several other states. As per the available information, mulberry is being cultivated in 1,71,956 hectares of land (Anon, 2004). This is evident from the production figure of 14,620MT of raw silk. India earns foreign exchange of Rs. 2879.56 crores. At present India also imports about 7000-8000 MT of raw silk and 2000-3000 MT of silk fibre (Dandin, 2006) to meet the domestic requirement.

Silk is produced by over 1,10,000 species of the insects belonging to the insect order Lepidoptera. Over 30,000 species are the spiders and the silk

produced by them are not of commercial grade. Besides, the members of several other insect orders also produce silk. However, the bulk of world natural silk is derived from the domesticated Lepidopterous insect, the mulberry silkworm *Bombyx mori* L. belonging to Phylum: Arthropoda, Class: Insecta, Subclass: Pterygota, Order: Lepidoptera, Sub-order: Heteroneura, Family: *Bombycidae*, Genus: *Bombyx*, Species: *mori*.

China and India occupy the top two positions in mulberry raw silk production. India also has the unique distinction of being the only country in the world bestowed by nature with all the four known species of the silkworms *i.e.*, *Bombyx mori* (Mulberry), *Antheraea mylitta* (Tassar), *Philosamia ricini* (Eri), and *Antheraea assamensis* (Muga) for commercial exploitation. India enjoys the monopoly in the production of golden yellow colored muga silk from Assam.

DISEASES IN SILKWORM

Mulberry silkworm, *Bombyx mori*, L. is domesticated and reared in colonial form for the past several centuries under controlled environmental, nutritional and germ free conditions. The domestication has resulted in loss of some of the ancestral characters including the resistance to diseases. In view of this, today we find silkworm suffers from several diseases, which are broadly categorized into infectious and non-infectious diseases. Microbes cause infectious diseases in silkworm and form major factors that greatly contribute to the crop failure and instability in cocoon production. Arthropods, toxic chemicals and physiological ailments cause non-infectious diseases in silkworm. The lists of infectious and non-infectious diseases of the silkworm are presented in Table 1 and 2. The infectious diseases such as Virosis (Nuclear polyhedrosis, Cytoplasmic polyhedrosis, Infectious flacherie and Densonucleosis), Mycosis (White, Green, Yellow and Red muscardine and Aspergillosis), Bacterimia (Bacterial disease of digestive organ, Septicemia and Toxicosis) and Microsporidiosis (Pebrine) are common in silkworm. The annual cocoon crop loss in India due to these diseases was estimated to be over 30-40% (Janakiraman, 1961; Chitra *et al*., 1975;

Hanumappa, 1986). However with the advancement of silkworm disease management technology, the disease level has come down to 15-20%. Recently Selvakumar *et al.,* (2002) reported the point prevalence level of diseases in silkworm as 2.87, 5.81 and 3.81% during winter, summer and rainy seasons respectively in Karnataka. Based on the rate of spread of the disease and the point prevalence in silkworm colony, Selvakumar *et al.,* (2002) estimated the cocoon crop loss as 11.486, 14.864 and 14.500 kg/100 dfls during winter, summer and rainy seasons respectively in India.

Among various diseases, the viral diseases *viz.,* Nuclear polyhedrosis, Cytoplasmic polyhedrosis, Infectious flacherie and Densonucleosis cause extensive damage to silk cocoon crop throughout the year. The bacterial diseases *viz.,* bacterial septicemia, bacterial toxicosis and bacterial gastro-enteric diseases are collectively known as Flacherie. They are more common in hot and humid seasons. The corpses of dead larvae becomes soft and rotten. The fungal diseases, commonly known as muscardine, are caused by the parasitisation of larva, pupa and moth by fungi such as *Beauveria bassiana, Aspergillus flavus etc.* Muscardine are of many kinds and are classified based on the color of the conidia formed on the dead larvae and white muscardine is most common. The microsporidiosis (Pebrine) is yet another disease in silkworm caused by a microsporidia *Nosema bombycis* and it is the most dangerous of all diseases with the potential to eliminate the whole sericulture industry, if it is not managed effectively. It is the most destructive of all diseases in silkworm as it is transmitted from infected mother to the offspring by transovarial transmission of the pathogen as well as by secondary *per os* infection (Han and Watanabe, 1988; Baig, 1994; Ananthalkshmi *et al.,* 1994).

MICROSPORIDIOSIS (PEBRINE) IN SILKWORM

The microsporidiosis in silkworm, commonly known as Pebrine, is the earliest known menace in the silk industry. It is the most disastrous and destructive disease of silkworm and the history of sericulture speaks volumes on the

devastating outbreak of Pebrine disease in several sericulture countries resulting in near elimination of sericulture in some of those countries (Tatsuke, 1971). The first record of the disease came from France in 1845. The annual production of silk cocoons, which amounted to 26,000 tons in 1853, has come down to 4000 tons in 1865 due to the epizootic of pebrine (Mozziconacci, 1921). The disease, which created havoc in France, later spread to Italy, Spain, Syria and Romania (Steinhaus, 1949; 1956).

In India, the first report of the occurrence of the disease was in 1866 in Mysore. There was an epidemic level of out break of the disease in Kashmir in 1878 and in Mysore and Madras provinces in 1890-1900. The prevalence of the disease in India was about 15% in 1983 and declined by 1984 and 1985 (Baig, 1994; Nataraju, 2006). However, during 1991-1992, it reached peak epizootic level and caused heavy loss to the industry. The incidence was as high as 83% during 1991 (Baig *et al.*, 1997). The epidemic caused a loss of over 200 crores during the period. In Jammu and Kashmir, the disease inflicts an annual loss of 5% (Sahaf, 2002).

Pebrine disease is unique in being transmitted by way of egg. Louis Pasteur made extensive study of the disease and established that the disease is caused by a pathogen and the pathogen is transmitted by way of egg as well as by the ingestion of contaminated food by silkworms. Microsporidia are characterized by having infectious spores with unique an organelles involved in their invasion of a host. Louis Pasteur developed a practical method of screening for infectious spores and elimination of infected eggs and adults and for the supply of healthy eggs to sericulturists. This approach has saved the sericulture industry all over the world (Steinhaus, 1956).

N. bombycis is an intracellular parasite, belonging to the Phylum: Microspora; Class: Microsporea; Order: Microsporidia; Sub-order: Apanasoporoblastina; Family: Nosematidae; Genus: *Nosema;* Species: *Bombycis* (Sprague, 1977; 1982). It infects silkworm by per oral mode as well by transovarial

transmission. The disease is also transmitted by transovum transmission through the external contamination of eggs by spores (Masera, 1938). In addition to *N. bombycis* several other microsporidia belonging to genera *Nosema, Pleistophora*, *Thelohania* and *Leptomonas* were reported to cause infection in silkworms and other insects (Tanaka *et al.*, 1972; Abe, 1979; Fujiwara, 1980; 1984a,b 1985; Sharma *et al.*, 1989; Fang *et al.*, 1991; Bauer and Pankratz. 1993; Ananthalakashmi *et al.*, 1994; Kishore *et al.*, 1994; Baig, 1994; Sharma *et al.*, 1989; 2003; Govindan *et al.*, 1998; Samson *et al.*, 1999a, b; Hatakeyama, *et al.*, 2000; Oumouna, *et al.*, 2000; Hayasaka *et al.*, 2002; Choi, *et al.*, 2002; Sokolova and Lange, 2002; Singh and Saratchandra, 2003; Tsai *et al.*, 2003; Dash and Nayak, 2003; Nageswara Rao *et al.*, 2004; Canning *et al.*, 2004; Goertz *et al.*, 2004; Matos and Azevedo 2004; Wang *et al.*, 2005; Ovcharenko and Wita, 2005; Johny *et al.*, 2005; Selvakumar *et al.*,2005; Mohanan *et al.*, 2005; David *et al.*, 2005; Yaman *et al.*, 2005; Nataraju *et al.*, 2005; Hylis *et al.*, 2006). Among them, the most common ones are *Nosema* sp (NIS–001, NIS-M11, NIS-M14, and NIK-2r, and NIK-3 h.), *Vairimorpha* sp (NIS-M12 and NIK-4m), *Microsporidium* sp. (NIS-M25), *Pleistophora* sp. (NIS-M27), *Thelohania* sp (NIS-M32) and *Leptomonas* sp. These microsporidians differ in their spore morphology, virulence, tissue infected and nature of transmission. The environmental spore of these microsporidians is oval, cylindrical or ovocylindrical and either uninucleate or binucleate (Kawarabata, 2003). The *Nosema* sp. NIS-11, *Vairimorpha* sp. NIS-12 and *Microsporidium* sp. NIS-M14 also form uninucleate octospores in the muscle and adipose tissue but all the environmental spores recovered are binucleate. The environmental spores of *N. bombycis*, NIS-001 is oval in shape and medium in size while *Nosema* sp. NIS-M14 produces larger environmental spores than NIS-001. In *Pleistophora*, NIS-M27, *Microsporidium* NIS-25 and *Thelohania NIS*-M32, the spores are oval and uninucleate (Kawarabata, 2003).

Nosema sp. NIS-001, *N.* sp. NIS-M11, *Vairimorpha* sp. NIS-M12, *Nosema* sp. NIS M-14 and *Microsporidium* sp. NIS-M25 infection in silkworm larvae are systemic and severe. They form environmental spores in all the tissues and organs

such as gut epithelium, muscles, fat body, silk gland, malphigian tubules *etc.* However, *N. bombycis* alone form environmental spores in the midgut epithelium. *N. bombycis* NIS-001 is also highly gonadotropic while *Nosema* sp. NIS-M11 is moderately gonadotropic. The other microsporidians show only initial development of environmental spores in the midgut epithelium (Kawarabata, 2003). *Pleistophora* sp. do not form primary spores. They infect only the midgut epithelium of silkworm and form uninucleate environmental spores. (Tanaka *et al.*, 1972 ; Becnel and Andreadis, 1999). *Thelohania* sp. form uninucleate octospore only in the larval muscles (Fujiwara, 1984a). *N. bombycis*, NIS-001, *Vairimorpha* sp. NIS M-12 and *Pleistophora* sp. NIS-M27 are highly infectious having high infection rate and mortality of larvae. *Nosema* sp. NIS-M14 and *Microsporidium* sp. NIS-M25 are moderately infectious while *Thelohania* sp. NIS-M32 is low in its infectivity. The pathogenicity of *N. bombycis*, NIS-001 and *Vairimorpha* sp. NIS M-12 are high and usually larvae die before pupation if the infection is prior to the 4^{th} larval instar. The other microsporidians with moderate or low infectivity viz., *Nosema* sp. NIS-M11, *Nosema* sp. NIS-M14, *Microsporidium* sp. NIS-25 and *Thelohania* sp. NIS- M32 cause low level of mortality in the larval stages (Kawarabata, 2003; Singh and Sarachandra, 2003).

The percent transovarial transmission differs with different microsporidians. It is highest with *N. bombycis*. The transovarial transmission of *Nosema sp*. NIS-M11 has been demonstrated (Han and Watanabe, 1988). However, the rate of transmission of *Nosema sp*. NIS-M11 is lower than that of *N. bombycis* (Fujiwara, 1980; Iwashita *et al.,* 1990). The rate of transmission of *Nosema* sp. NIK-3h is reported to be low. It transmits the infection to the progeny by only 1.80 %, while the standard strain *N. bombycis* and NIK-2r transmit to an extent of 100 % (Fujiwara, 1993; Ananthalakashmi *et al.,* 1994). There are no reports of transovarial transmission by *Vairimorpha* sp. NIS-M12, NIK-4m and Microsporidium sp. (Kawarabata, 2003).

Different silkworm breeds differ in their susceptibility to the microbial infections including *Nosema* sp. Such differences are genetically determined and have been studied extensively in silkworm with reference to infection by viruses (Tanada and Kaya, 1993; Nataraju *et al.,* 2005). In silkworms, large difference exists among various breeds in their susceptibility to infections by *N. bombycis*. (Chinnaswamy and Devaiah, 1984). Silkworm races such as Pure Mysore, Nistari and C Nichi have high survival ability than other silkworm breeds (Devaiah, 1975; Devaiah *et al.,* 1975; Patil and Geetabai, 1989). The high survival of Pure Mysore breed has been investigated and it is attributed to the regenerative capacity of midgut of the breed to recover from the infection (Fujiwara, 1993). However, Liu (1984) reported that a silkworm breed Baipidan is resistant to *N. bombycis*. Wild silkworm, *Antheraea pernyi* and *Platysamia cecropia* show comparatively higher resistance to microsporidia than others (Weiser, 1969). In honeybee also, the resistance to infection by *Nosema apis* is attributed to the heterosis and to the polygenic system (Sidorov *et al.,* 1975).

Management of microsporidiosis (Pebrine) in sericulture is among the most important activity. The production of pebrine disease free eggs and elimination of the pathogen in silkworm rearing environment is the most practical and effective method adopted meticulously at present. Several physical and chemical disinfectants have been identified and adapted in sericulture to ensure exclusion of the pathogen (Nataraju *et al.,* 2002; Virender Kumar *et al.,* 2002; Balavenkatasubbiah *et al.,* 2003; Singh *et al.,* 2005a and b; Balavenkatasubbiah *et al.,* 2006; Nataraju *et al.,* 2005). In the past, therapy was considered as a non-realistic approach to control silkworm diseases such as pebrine. However in recent years some of the therapeutic drugs / chemicals have been proved to be effective in cure of diseases in silkworm and other insects (Baig, 1994; Alok Sahay, 2005).

MICROSPORIDIOSIS IN LAMERIN BREED

Lamerin, a local silkworm breed, is confined to Manipur state located in northeastern part of India. Manipur (Fig. 1) is endowed with very rich flora and

fauna. The high hilly terrain, forests, hills, deep forestation, high rainfall, high humidity, moderate climate in plains, valleys and cold at high altitudes are the main ecological components. The state admits a temperature range of 0 - 38.5^{o}C and average rainfall varying from 933 – 2593 mm and relative humidity ranging from 20 - 100%. The state is located between 23.83 and 25.58^{o}N latitude and 93.05 and 94.78^{o}E longitude with 22.365 sq. km area. Manipur is an important state with conducive environment and potential for sericulture. Traditional pockets of sericulture villages like Leimaram, Leimapokpam, Tera Khongshangbi, Awangjiri *etc.* are still active. The use of silk in the state is associated since the period of Khamba Thoibi. In the field of silk weaving Utlou is famous for its silk sarees and other items such silk Phanek, Enaphee *etc.* At present Manipur practices Mulberry, Eri and Tasar sericulture. The government is planning to introduce mulberry sericulture in a big way.

Lamerin is a hibernating tetramoulter mulberry silkworm breed and larvae are plain, small and slender. They form loose, elongated oval orange yellow color cocoons with one end open. The larvae weigh about 2 – 2.6 g and complete the larval period in 22-24 days. The average cocoon and shell weight were 1.47 and 0.192 g respectively. About 11 - 13% silk could be obtained from the cocoon. The silk filament length ranges from 193 – 212 meters and the denier from 1.9-2.00.The moths are comparatively small and lay 311-338 numbers of hibernating eggs.

The breed suffers from all major diseases of silkworm such as nuclear polyhedrosis, flacherie, muscardine and microsporidiosis. Microsporidiosis in silkworm generally leads to mortality and in most cases do not come to spinning, if the infection is in the early instars or got infection by transovarial transmission. However there were unconfirmed and unpublished reports that a microsporidian strain is associated with the Lamerin breed for past several generations without being eliminated. The breed is observed to survive infection and form cocoon. The larva metamorphoses into pupa and the moth. The moth lays infected eggs and the larvae hatched carries infection. It was not clear whether the breed possesses high

level of resistance to the microsporidian infection or the microsporidian itself is low in virulence to Lamerin breed of the silkworm. There was no published information on any of the above aspects with reference to Lamerin breed. In view of the importance, the microsporidiosis commands, in sericulture the understanding of role of the breed and the pathogen in survival of the host gains importance especially in the management aspect of the disease. Hence, the study was proposed which aim at isolation and Characterisation of the microsporidian infecting the Lamerin breed and understanding its pathogenicity to the silkworm. It also aims at development of management practices against the microsporidiosis caused by the Lamerin microsporidia.

The above objectives were achieved through studies under the following heads and presented in three chapters.

Chapter 1. Isolation and Characterisation of microsporidian infecting Lamerin breed of the silkworm, *Bombyx mori* L.

Chapter 2. Pathogenicity of Lamerin microsporidian to Lamerin and popular multivoltine and bivoltine silkworm breeds.

Chapter 3. Management of Lamerin microsporidian infection in Lamerin and other silkworm breeds.