2.1 Forest Lepidopteran defoliators

In India, forests are rich with trees that have high commercial value. These trees are habitat to many insects that are defoliators, stem borers, shoot borers, fruit and seed borers. Among these, defoliators are causing one of the major problems in the growing trees. If the trees are timber yielding, defoliation may not kill the trees but cause huge timber loss. The studies conducted by Nair et al. (1985, 1996) described that defoliation leads to an average loss of 44% of the potential volume increment in 4 to 9 year-old teak plantations. A survey conducted on infestation of eastern cottonwood *Populus deltoides* by Singh et al. (2005a) in the nurseries and plantations of six states of north-western India (Uttar Pradesh, Uttarakhand, Haryana, Punjab, Himachal Pradesh and Jammu & Kashmir) led to identification of two major Lepidopteran pests, *viz.*, *Clostera cupreata* and *C. fulgurita*. These pests are responsible for defoliation causing huge economic losses during their outbreaks. In another example, *Ailanthus excelsa*, one of the fast growing multipurpose tree species in India has reportedly
suffered large scale defoliation by various pests including one of the major defoliators, *Atteva fabriciella* Swederus (Lepidoptera: Yponomeutidae) in a number of nurseries and plantations. There are numerous accounts of repeated defoliation, retarding the growth of trees, weakening young plants and drastically reducing seed formation owing to the damage caused to inflorescence (Roychoudhury and Joshi, 2009). Another Lepidopteran, *Euproctis divisa*, was recorded to cause about 15% defoliation to *Albizia procera* in nurseries and plantations at Tropical Forest Research Institute, Jabalpur, Madhya Pradesh (Kalia et al., 1997). In one of the valuable timber trees, Deodar, defoliation caused by *Ectropis deodarae* was reported in Naganalli and Mihani ranges of Theog forest division of Shimla district, Himachal Pradesh (http://www.indiaenvironmentportal.org). The pest had affected about one lakh cedar trees in the region. All these examples are alarming and need special attention for control measures.

Also, there are several reports world wide on insect defoliations. *Lymantria dispar* was such a big challenge in North America by the mid 20th century, resulting in extensive evaluation and introduction of natural enemies from Europe and Asia to control this urban pest.

### 2.2. Microsporidian Pathogens

Microsporidia are obligatory intracellular parasites of animals starting from lower invertebrate to the higher group of mammals. Though a large number of Microsporidian species infects arthropods, especially insects, most animal phyla contain few species that are infected by Microsporidia.

Microsporidia displays a number of characteristics that are unusual for eukaryotic organisms. The amitochondrial nature of Microsporidia led to the hypothesis that Microsporidia should be accommodated in ‘Archezoa’ that is organisms which diverged before the advent of protomitochondria symbiogenesis (Cavalier-Smith 1983). They possess prokaryotic size ribosomes (Weiss, 2001). The genome size of Microsporidia of genus *Encephalitozoon* is less than 3 mb, making them the smallest eukaryotic genome reported to date (Vivares et al., 1996). Earlier molecular phylogenetic studies based on nuclear SSU rDNA
(Vossbrinck et al., 1987), translation elongation factor 1 alpha and 2 (EF-1 α and -2) (Kamaishi et al., 1996a; b) supported an ancient divergence of Microsporidia.

More recently, the view has been challenged by additional protein-based phylogenies. The initial contradicting evidence emerged from α-tubulin analyses, which surprisingly indicated a phylogenetic relationship of Microsporidia and Fungi (Edlind et al., 1996). This novel finding was also supported by other protein based analyses using β-tubulin (Keeling and Doolittle, 1996), DNA dependent RNA polymerase II largest subunit (RPB1) (Hirt et al., 1999), TATA box binding protein (TBP) (Fast et al., 1999), mitochondrial heat shock protein 70 (Hsp70) (Germot et al., 1997; Hirt et al., 1997), and Valyl-tRNA synthetase (ValRS) (Weiss et al., 1999). Hirt et al. (1999) suggested that tubulin trees are sometime distrusted because of apparent long-branch effects and because these proteins are eukaryote specific (and thus cannot be properly outgroup-rooted), the possibility of lateral transfer of tubulin genes was also been possible. Gill and Fast (2006) assessed Microsporidia fungal relationship by analyzing eight genes and the sequenced data of fungi that represented four fungal phyla. The resulted trees placed Microsporidia as a sister to a combined ascomycete +basidiomycete clade.

**International Status**

**2.3. Microsporidia from forest Lepidoptera**

Microsporidia are probably far more common among species of forest Lepidoptera than published reports indicate (Maddox et al., 1998). Many species have been reported from the forestry sectors across the world. The list of reported Microsporidia is furnished in Table 1.
Table 1: List of some prominent Microsporidia reported from forest Lepidoptera

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the Microsporidia</th>
<th>Name of the Host insect</th>
<th>Author/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microsporidia (unidentified)</td>
<td><em>Malacosoma disstria</em></td>
<td>Thomson, 1959</td>
</tr>
<tr>
<td>2</td>
<td><em>Plistophora/Cystosporogenes operophterae</em></td>
<td><em>Operophtera brumata</em></td>
<td>Canning, 1960; Canning and Curry, 2004</td>
</tr>
<tr>
<td>5</td>
<td>Microsporidia (unidentified)</td>
<td><em>Tortrix viridana</em></td>
<td>Franz and Huger, 1971; Lipa, 1976</td>
</tr>
<tr>
<td>6</td>
<td><em>Nosema thomsoni</em></td>
<td><em>Choristoneura conflictana</em></td>
<td>Wilson and Burke, 1971</td>
</tr>
<tr>
<td>7</td>
<td><em>Nosema sp</em> and <em>Thelohania hyphantriae</em></td>
<td><em>Hyphantria cunea</em></td>
<td>Nordin and Maddox, 1974; Weiser and Veber, 1975</td>
</tr>
<tr>
<td>8</td>
<td>Microsporidia (unidentified)</td>
<td><em>Archips cerasivoranus</em></td>
<td>Wilson and Burke, 1978</td>
</tr>
<tr>
<td>9</td>
<td>Microsporidia (unidentified)</td>
<td><em>Operophtera brumata</em></td>
<td>Canning <em>et al.</em>, 1983</td>
</tr>
<tr>
<td>10</td>
<td><em>Nosema sp.</em></td>
<td><em>Samea multiplicaalis</em></td>
<td>Semple and Forno, 1987</td>
</tr>
<tr>
<td>11</td>
<td><em>Orthosomella lambdinae</em></td>
<td><em>Lambdina athasarica</em></td>
<td>Andreadis <em>et al.</em>, 1996</td>
</tr>
<tr>
<td>12</td>
<td><em>Nosema portugal</em></td>
<td><em>Lymantria dispar</em></td>
<td>Maddox <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>13</td>
<td><em>Endoreticulatus sp.</em>, <em>Nosema sp.</em>, <em>Vairimorpha sp.</em></td>
<td><em>Lymantria dispar</em></td>
<td>Solter <em>et al.</em>, 2002</td>
</tr>
</tbody>
</table>
Contd…

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the Microsporidia</th>
<th>Name of the Host insect</th>
<th>Author/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Microsporidia (unidentified)</td>
<td><em>Dioryctria ametella</em></td>
<td>Mihelcic <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>16</td>
<td><em>Vairimorpha</em> sp.</td>
<td><em>Hellula undalis</em></td>
<td>Mewis <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>17</td>
<td><em>Cystosporogenes</em> sp.</td>
<td>a) <em>Lobesia bortana</em>;</td>
<td>a) Kleespies <em>et al.</em>, 2003;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) <em>Choristoneura</em></td>
<td>b) Frankenhuyzen <em>et al.</em>, 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>fumiferana</em></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td><em>Vairimorpha</em> necatrix</td>
<td><em>Pseudalrta</em> unipuncta</td>
<td>Medeiros <em>et al.</em>, 2004</td>
</tr>
</tbody>
</table>
2.4. Microsporidia as a natural enemy

There are several reports of Microsporidia found in European *Lymantria dispar* populations (Maddox et al., 1999; Pilarska et al. 1998; Solter et al., 2000; Weiser, 1957; Zelinskaya, 1980), indicating that Microsporidia are important components of the natural enemy complex of this insect in Europe. *Nosema lymantriae* from *Lymantria dispar* was not found to be infective to *E. chrysorrhoea* when it was inoculated in the laboratory (Weiser, 1957). According to Canning (1982) *Nosema pyrausta* is restricted to a single host, the European corn borer, and has low pathogenicity. Hence it is not considered pathogenic enough to be used as a microbial pesticide, but, is an important factor in regulating natural populations. She estimated that *Nosema locustae* when multiplied in *Melanoplus bivittatus* yields $3.9 \times 10^9$ spores per grasshopper and was enough to treat more than one hectare of rangeland. *Vairimorpha necatrix* when multiplied in *Heliothes zea*, yields $1.67 \times 10^9$ spores per larva. Briano and Williams (1997) evaluated a Microsporidium *Thelohania solenopsae* as a potential biocontrol agent by studying their virulence to imported fire ants (Solenopsis richteri) in a laboratory in USA. The study concluded that the Microsporidium *T. solenopsae* affected the mortality rate and shortened the longevity of colonies of *S. richteri* reared under laboratory conditions. These laboratory findings are consistent with results of field work promoted by Briano et al., 1995a, 1995b. Though *T. solenopsae* was the first microorganism evaluated in South America as a potential biocontrol agent, detailed study is required to establish *T. solenopsae* as a biological control agent of the imported fire ants in the United States. Mihelcic et al. (2003) had emphasised on utility of Microsporidia isolated from *Dioryctria amatella* as a biological control agent as the prevalence of the pathogen encountered by the author was upto 51% of the larvae collected from the York Co., SC population. Campbell et al. (2007) suggested that the Microsporidium *Nosema fumiferanae*, isolated from Spruce budworm *Choristoneura fumiferana* might play a crucial role in regulating spruce budworm population.

2.5. Characterization of Microsporidia
Morphology and Ultrastructure

The studies on the structure of Microsporidian spores (Huger, 1960; Lom and Vavra, 1961) revealed their main components: sporoplasm, polar filament, polaroplast, polar cap, posterior vacuole and spore shell. On the basis of spore ultrastructure, Sato *et al.* (1982) determined that the *Nosema* M12 was not really *Nosema*, but a species of *Vairimorpha*. Kleespies *et al.* (2003) studied the ultrastructure of the Microsporidia, *Cystosporogenes sp.* isolated from *Lobesia botrana*. Most of the parts share the general characteristics of Microsporidia except the straight anterior (manubrial) part of the polar filament which is enclosed by a remarkable binary polaroplast. The outer cup-like part of the polaroplast is composed of a densely packed system of lamellae that extends further into the spore, finally surrounding more than half of the nucleus. The inner part of the polaroplast consists of a more closely arranged system of lamellae. In the mature spore, the isofilar polar filaments range from 7-8. These coils are arranged in a single row along the inside periphery of the spore. Canning and Curry (2004) had described the ultrastructure of the Microsporidia *Cystosporogenes operophterae* isolated from *Operophtera brumata*. According to the study the spores are elongate, ovoid with a distinctly weavy outline involving both the 25-40 nm exospore and the endospore. The endospore is usually distorted and probably measures no more than 125 nm, thinning to 25 nm over the anchoring disc. The nucleus is elongate, and occupying a third of the length of the cytoplasmic structure. Number of polar tubule is 10-12. Larsson (2005) had established the significance of electron microscopy for identification of Microsporidia in the recent era by studying two *Nosema* species *Nosema apis* and *Nosema bombi*. He expressed that electron microscopic studies are ignored in recent times because detailed cytological investigation is time consuming.

Life cycle

Development of *Nosema* spp. was studied by Fujiwara (1980, 1984) and Iwano and Ishihara (1988, 1991) in silkworm and other Lepidopteran species. Canning (1990) had described the complex and diverse types of sporogony observed in different Microsporidian genera. Sprague *et al.* (1992) had introduced
a classification system based on whether species is diplokaryotic at some point of
time in the life cycle (dihaplophasea) or uninucleate throughout its life cycle
(haplophasea). The dihaplophasea are further separated into those in which the
diplokaryon is formed through meiosis (Meiodihaplophasida) and those in which
the diplokaryon is formed through nuclear dissociation (dissociodihaplophasida).
Iwano and Kurtti (1995) had observed dimorphic spores of *Nosema furnacalis*
when it was cultured in *Helicoverpa zea* cell line. Sprague and Becnel (1999)
defined that the term schizont to be restricted to haplophasic (uninucleate)
individuals whereas, the meront is diplokaryotic (two closely appressed nuclei).
Kleespies *et al.* (2003) had studied the life cycle of Microsporidia
*Cystosporogenes* sp. isolated from European grape vine moth *Lobesia botrana.*
He had observed that the earliest detectable stage is uninucleate schizont. A life
cycle study had been carried out on *Cystosporogenes operophterae* isolated from
*Operphtera brumata* by Canning and Curry (2004) and they found that the
division of meront and sporont were chiefly by binary fission. Hyliš *et al.* (2006)
had studied the life cycle of *Nosema chrysorrhoea* isolated from Browntail moth
*Euproctis chrysorrhoea* that consisted of primary and secondary developmental
cycles, which differ in time and tissue specificity in the host organisms and the
type of spores produced.

**Phylogeny**

Baker *et al.* (1994) established the phylogenetic relationship between
*Vairimorpha* and *Nosema* species using rRNA sequence data. Kleespies *et al.*
(2003) performed phylogenetic analysis on *Cystosporogenes* sp. isolated from
European grape vine moth *Lobesia botrana,* based on the SSU rDNA gene. The
PCR product was cloned and sequenced. The result indicated that the species was
similar to the species re-described by Lipa (1981) from the same host
*Pleistophora legeri.* With the similarities of small subunit ribosomal DNA (SSU-
rDNA), Baker *et al.* (1995) established the phylogenetic relationship of several
Microsporidian genera. Pieniazek *et al.* (1996) had analysed the SSUrRNA
consensus sequence of *Nosema bombycis* isolated from *Bombyx mori* and
*Spodoptera exigua* and concluded that both the isolates are identical; also they
tallied the SSUrRNA sequences with *Nosema trichoplusiae* deposited by them in gene bank. The sequence was 1233 base long and the GC content was calculated to be 34.1%. These values are close to those found for SSUrRNAs of other *Nosema* species available in the gene bank database. On the basis of the study the authors proposed that *N. trichoplusiae* should be considered as synonym of *N. bombycis*. If sequence differences between *N. trichoplusiae* and *N. bombycis* were to be found in other region of the RNA cluster, then *N. trichoplusiae* should be treated as a subspecies of *N. bombycis*. Vossbrinck et al. (2005) had conducted a comparative analysis of 125 species of Microsporidia that had shown that groups and clades were formed largely based on habitat and host. Wang et al. (2006) had sequenced SSUrDNA for a Microsporidium *Nosema antheraeae* isolated from Chinese Oak Silkworm *Antheraea pernyi* and compared with 35 other species of Microsporidia by phylogenetic study. The result showed that the species had a close relationship with *Nosema bombycis*. Ku et al. (2007) had isolated a Microsporidium species from *Plutella xylostella* designated as PX2 and compared with another isolate of *P. xylostella* PX1 and with *Nosema spodopterae* and *Nosema bombycis*. Phylogenetic analysis of the LSUrRNA and SSUrRNA gene sequences and the sequences of the alpha-tubulin, beta-tubuin and RPB1 genes found that PX1 was closer to *N. spodopterae* and *N. bombycis* than PX2. Comparison of the identities of PX2 with other three rRNA domain showed a high divergence in the sequences of the rRNA spacer region (ITS and IGS). Hence, the authors indicated that PX2, if not PX1, might represent a new species of *Nosema*.

### 2.6. Pathogenic potential of Microsporidia

#### Survival of Microsporidia

According to literature, Microsporidia from terrestrial insects will survive for several years at 2 to 5°C in sterile water suspensions. In sterile water suspensions at 2 to 5°C, Oshima (1964) maintained viable spores of *Nosema bombycis* for 10 years and Revell (1960) stored viable spores of *Nosema apis* for 7 years. If the water suspension contains organic debris leading to microbial growth, the spores will not survive for more than a few days (Brooks, 1980; White, 1919) in the water suspension. Some Microsporidia from aquatic insects
will not survive storage at lower temperatures (Undeen et al., 1993). Other than water suspension, it is also documented that some species of Microsporidia seasonally persist in the environment of their hosts in infected cadavers (White, 1919; Brooks, 1988; Fuxa and Brooks, 1979) and the cadaver unquestionably provides protection from ultraviolet radiation. It is not clear whether protection against any other environmental factors result from overwintering in infected cadavers.

**Infectivity**

Microsporidian infectivity is largely strain dependent and also greatly influenced by eco-climatic conditions and host characteristics. Most of the effects of high temperature on Microsporidia were conducted in an attempt to eliminate Microsporidian infections from insect colonies. Infected insect hosts as well as extracorporeal spores have been involved in these studies (Baribeau and Burkhardt, 1970; Benjakova and Verejskajs, 1958; Hartwig, 1970; Vandemeer and Gochnauer, 1969). Maddox (1977) observed that dry *V. necatrix* spores survived for three weeks at 40°C but survived for only five hours at 50°C and 30 minutes at 60°C. Kaya (1977) found that *V. necatrix* spores survived for 144 hours at 35°C, suggesting that the moisture provided by the bean leaf on which the spores were placed reduced the effect of the higher temperature. Li and Fayer (2006) studied the effect of exposure of three Microsporidian spores *Encephalitozoon intestinalis*, *E. hellem* and *E. cuniculi* to the temperature extremes and chemical disinfectants. The spores were exposed to 50, 60, 70, 80 and 100°C for different durations and to 70% ethanol and sodium hypochlorite diluted to 1:50,000, 1:10,000 and 1:1,000 with deionized water for the duration of 10, 30, 60 and 120 min. The study revealed three different tolerance levels for different spores with 12.1 to 100% noninfectivity for temperature treatments, 100 noninfectivity in ethanol treatments and 98.2 to 100% noninfectivity in sodium hypochlorite treatments. Fenoy et al. (2009) evaluated resistance of *Nosema ceranae* (parasite of honey bee) by exposing it to a temperature of 60°C for 1, 2, 4 or 6 h, 35°C for 2 h and to the same temperature for one month and by autoclaving the spore. Effect of desiccation was also tested for the same spore by
using various processes. The “autoclaving” resulted in nearly 96.6% mortality of the spore (rests of the spores were extruded). Spores treated with two other temperatures caused mortality ranging from 5.9-7.9%. Zhengyong et al. (2010) studied the effect of chlorine dioxide (ClO₂) to the infectivity of *Nosema bombycis* by treating the spores with four different concentrations, viz., 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L for 30 min. Results showed variation in the infectivity from 0 to 6.67% depending upon the rearing techniques.

**Pathogenicity to hosts**

Microsporidia in general produce chronic infections manifested by slow development and reproductive anomalies in several insect species. Thomson (1958) reported that larvae and pupae of the spruce budworm, *Choristoneura fumiferana*, had retarded development when they were infected by *Perezia fumiferanae*. Even though the naturally infected specimens of this insect had light initial infections, some larvae took an average of about 7 days longer for development compared to the healthy larvae and the development period of infected pupae was increased by 0.7 days. Kellen and Lindegren (1974) studied comparative virulence of *N. plodiae* and *N. heterosporum* in the Indian meal moth *Plodia interpunctella* and described that *N. plodiae* prolonged larval development and there were no relationship between the dose and adult emergence in the case of *N. heterosporum*. The cause was attributed to the possible relatively low incidence of infection in the surviving adults. Siegel et al. (2001) experimentally inoculated *N. carpocapsae* spores to the neonate and 3rd instar (1 week old) larvae of *Cydia Pomonella*, to check the differences in mortality depending upon the stage of inoculum. No adults were emerged in the former experiment, but the latter one resulted in emergence of two adults. In another study (Goertz et al., 2004), the impact of two *Nosema* isolates from gypsy moth *Lymantria dispar* was determined. The authors concluded that the isolates caused high mortality independent of spore dosage administered. The latency period was 7 to 8 days for both the isolates and was similar to other *Nosema* species (Onstad and Maddox, 1988). In this experiment, authors also observed the prolonged larval development among the inoculated larvae than the control. Mewis et al. (2003)
had carried out a bioassay study with laboratory maintained *Hellula undalis* larvae by inoculating them with *Vairimorpha* spores isolated from wild *H. undalis* insects. The study resulted in about 80% mortality in larval and pupal stage.

**Multiplication aspects**

Multiplication of the Microsporidian spores was studied by several workers in different time periods. Fowler and Reeves (1975) had studied invivo propagation of Microsporidia, *Nosema necatrix*, in the insects *Trichoplasia ni* and *Heliothis zea* that is dependent on temperature. Lai and Canning (1983) reported that the replication of *Nosema algerae* is affected by dose of inoculum, the instar of *Pieris brassicae* larvae inoculated and the pretreatment of the insects at 20°C when the spores were injected into haemocoel of the larvae. Pilley et al. (1978) studied large scale production of *Nosema eurytremae* in *Pieris brassicae*. Similarly, the influence of temperature on development of parasite/ host system *Edhazardia aedis* (Microsporidia) and *Aedes aegypti* was investigated by Becnel and Undeen (1992). Lai and Canning (1983) and Whitlock and Brown (1991) reported that multiplication of Microsporidia and spore production is dependent on age of the host. The spore replication related with spore dosage was investigated by Hostounsky and Weiser (1972), Teetor and Kramer (1976) and Lai and Canning, (1983). Abe and Fujiwara (1979) studied the mode of multiplication of *Pleistophora* sp., in the midgut epithelium of the silkworm.

The effect of temperature on the development of *Nosema algerae* was investigated and successful germination and development was documented at temperature ranging from 24-38 °C (Smith et al., 1982; Trammer et al., 1999; Undeen, 1975). Undeen (1975) indicated that *N. algerae* had the ability to infect mammalian cells at 37 °C but did not undergo the formation of spores. Infection was not observed when *N. algerae* was inoculated at 38 °C (Smith et al., 1982; Undeen, 1975); but Trammer et al. (1999) reported successful growth at 38 °C in cultured human muscle fibroblasts. Lowman (2000) observed that *N. algerae* formed a functional spore by 72h post inoculation when it was cultured at 29 °C, but, at elevated temperatures *i.e.* at 36 °C, 36.5 °C and 37 °C the Microsporidia undergo only one or two proliferative divisions and at 37 °C some degenerate or
hibernate by 72h. Martín-Hernández et al. (2009) determined the effect of temperature on the biotic potential of honey bee Microsporidia. The authors estimated the percentage of infection in honey bee at 3 different temperatures, viz., 25°C, 33°C and 37°C, when they were experimentally infected with two different Noema species, N. apis and N. ceranae. It was observed that the spore counts for both Microsporidia at 37°C were always the lowest and the highest performances were observed by the Microsporidia maintained at 33°C.

Transmission dynamics

Transmission is a key factor in pathogen-host interaction that can influence the population dynamics of the host (Anderson and May, 1981; McCallum et al., 2001). There are several potential pathways by which pathogens are transmitted within a host population; the most common are vertical transmission, the direct transfer of infection from parent to progeny (Fine, 1975; Becnel and Andreadis, 1999) and horizontal transmission, the transmission of the pathogens from one individual to another of the same generation (Steinhaus and Martignoni, 1970). Microsporidia are horizontally transmitted when a susceptible host ingests spores that are released into the environment in the feces of infected individuals or via decomposed cadavers (Becnel and Andreadis, 1999). Spores of enterogastric Microsporidia are released in the feces of the host throughout the larval stage (Weiser, 1961) and are also found in the meconium of newly eclosed adults that survive larval infection (Inglis et al., 2003). It can result in second-order density dependent regulation (Dwyer, 1994). Horizontal transmission experiments, based on exposure of uninfected L. dispar larvae to infected L. dispar larvae, demonstrated that the Microsporidia were far more host specific than the direct feeding experiments suggested (Solter and Maddox, 1998a). According to Solter (2006) pathogen may be vertically transmitted by one or more of several mechanisms including transovum, transovarial (or transovarian) and venereal transfer, and may involve intermediate hosts, sex ratio distortion (eg. host-feminizing and male sterilization or killing), and vectoring the pathogens. Transovum transmission is a broad term for transmission of a microorganism via the egg, either in the embryo or yolk, or adhered to the surface of the egg chorion.
Transovarial transmission specifically refers to transovam transmission in which the pathogen is transmitted to the host embryo by invasion of the egg or yolk via the ovarioles of the infected female host (Brooks, 1968; Nordin, 1975; Kellen and Lindergen, 1973). Only a few cases of venereal transmission via the male host have been documented and even fewer unequivocally (Solter, 2006).

Cross-infectivity

The crossinfectivity study is a primary step towards predicting the ecological range (host range in the field) of the species. Weiser (1957) tested crossinfectivity of *Nosema Lymantriae* isolated from *Lymantria dispar* to the closely related species *Euproctis chrysorrhoea*. Smirnoff (1968) reported that *Thelohania pristiphorae*, a Microsporidium initially isolated from larch sawfly *Pristiphora erichsonni*, showed positive result when it was experimentally inoculated to *Malacosoma disstria* and *M. americanum* in the laboratory. Solter et al. (1997) experimentally inoculated five biotype of Microsporidia isolated from *Lymantria dispar* from the field population of Europe to the forest Lepidoptera that were native to the Northeastern United States. Among them some of the inoculated hosts were refractory to the Microsporidium tested, some species showed atypical infections and rests of them were heavily infected and produced environmental spores. Solter et al. (2000) isolated three different Microsporidian species, *viz.*, *Nosema* sp., *Vairimorpha* sp. and *Endoreticulatus* sp. from *Lymantria dispar* from three sites in Central and Western Bulgaria. Apart from that, 11 isolates of Microsporidia were recovered from 1494 species of Lepidoptera from four sites of the Central and Western Bulgaria including three above mentioned sites of which, 3 isolates produced atypical infections in *L. dispar* and one *Nosema* isolate from a noctuid host produced heavy infection. Hyliš et al. (2006) tested cross infectivity of *N. chrysorrhoeae* isolated from *Euproctis chrysorrhoea* to other Lepidoptera larvae *viz.*, *Lymantria dispar*, *Mamestra brassicae* and *Spodoptera littoralis* by inoculating them with the spores. All the tested laboratory hosts were found to be refractory; *i.e.* no parasite stages were found in these hosts when dissected from 7-12 dpi. Campbell et al.
(2007) multiplied *N. fumiferanae* in *Choristoneura occidentalis* as a substitute of *C. fumiferana* to conduct his set of experiments.

### 2.7. Microsporidia as a microbial insecticide

Worldwide, various ecofriendly approaches are being considered among which microsporidia are a group of potential candidates which can be exploited as biological control agents. Spores of *Vairimorpha necatrix* and *Endoreticulatus schubergi* were formulated with various ajuvants in an effort to protect spores from ultraviolet radiation (Brooks, 1980). The survival of *V. necatrix* spores exposed to sunlight greatly increased when UV protectants were included in formulations (Kaya, 1977); but *V. necatrix* persistence was affected more by spore dosage than by UV protectants (Fuxa and Brooks, 1978). Canning (1982) suggested that *Vairimorpha necatrix* could be used as microbial pesticide for a short term control. *Nosema (Paranosema) locustae* Canning is now a registered microbial pest control agent which can suppress grasshoppers and mormon crickets. The spores of this microorganism ingested by the target pest develop primarily in the insects’ fat cells. In doing so the microorganism competes with the host for the energy reserves and as a result the host becomes weak and eventually dies. The end-use product, ‘Nolo bait Biological insecticide’, is a commercial class insecticide product that contains *Nosema locustae* as the active ingredient. The end use of the product exists as a bait formulation (Evaluation report, Health Canada, 2010).

### National Status

Microsporidia are undoubtedly prevalent in the tropical forest ecosystem but relatively few studies have been conducted in India, among which most of the studies were carried out on *Nosema bombycis*, which is a very serious pathogen of the mulberry silkworm, *Bombyx mori* L.. Different authors have reported the site of infection of *Nosema bombycis* Nägeli in different Lepidopteran hosts (Jolly and Sen, 1972; Talukdar, 1980). Three different *Nosema* spp. designated as NIK-2r, NIK-3h, NIK-4m, isolated from *Bombyx mori*, were reported by Ananthalakshmi *et al.*, 1994a. Another report was published
(Samson et al., 1999) on isolation of new Microsporidia from a butterfly Pieris sp. Site of infection and developmental stages of N. bombycis in silkworm were studied by Ananthalakshmi et al., 1994b. Bhat and Nataraju (2004) reported a new Microsporidium from Lamerin breed of the silkworm B. mori. Detailed ultrastructural and immunological study was carried out to compare the strain with N. bombycis. The infection and developmental stages of N. bombycis was studied in Antheraea eucalypti cell line by Balavenkatasubbaiah et al., 2009. For the first time a Microsporidian parasite was recorded from the teak defoliator Hyblaea puera by our research team (Sasidharan et al., 2008) and was identified as Nosema sp. after partial sequencing of the SSUrDNA (Remadevi et al., 2010). Satheeshkumar and Ananthan (2004) undertook ultrastructural (TEM and SEM) study to identify a Microsporidian specimen, Enterocytozoon bieneusi isolated from the stool sample of HIV positive patient. Hatakeyama et al. (2000) used SSU-rRNA sequencing to study the systematic position of Microsporidia isolated from Antheraea mylitta. From the study it was established that the Microsporidians were very close to Nosema bombycis, but differed widely from Vairimorpha, Encephalitozoon, Pleistophora. Rao et al. (2005) studied characterization and phylogenetic relationship among Microsporidia infecting silkworm, B. mori by using ISSR and SSU-rRNA sequence analysis. Also the genetic diversity and phylogenetic relationships among Microsporidia infecting the mulberry silkworm in India was studied by random amplification of polymorphic DNA (Rao et al., 2007). Johny (2002) reported a Microsporidian pathogen from the field population of the tobacco caterpillar, Spodoptera litura Fabricius from Tamilnadu, and later characterized the isolate on the basis of development, life cycle and molecular studies (Johny et al., 2006).

The experiment on survivability of N. locustae spores stored at different temperatures and exposed to different weather conditions was carried out by Rai et al. (2000). Reddy et al. (1989) studied the effect of disinfectant, Asiphor, on Nosema bombycis.

Spore replication rate of N. bombycis was studied in silkworm, Bombyx mori by Sasidharan et al. (1994). Role of temperature on multiplication and sporulation of N. bombycis was reproted by Singh et al. (2005b). Nanu et al.

The spread of pebrine after the introduction of transovarially infected worms in a colony of silkworm *B. mori* was studied by Baig *et al.* (1988). Transovarial transmission of *N. locustae* in the migratory locust, *Locusta migratoria migratorioides* was studied by Raina *et al.* (1995). Rate of spread by 5 different strains of *Nosema* viz., NIK-1Pr, NIK-1Cc, NIK-1Cpy, Nlk-1So, NIK-1Dp were studied in silkworm by Bashir and Sharma (2008).

A field trial of *N. locustae* was carried out to control paddy grasshopper *Hieoglyphus* spp. in Vidarbha region of India (Rai *et al.*, 2003).