microsporidia are a diverse group of spore-forming obligatory parasitic amitochondrial protozoans that currently include approximately 150 described genera with over 1200 individual species (Patrick and Naomi, 2002). They are eukaryotes with distinct nucleus and nuclear envelope but they do not have centrioles or mitochondria and are considered unique among the eukaryotes because their small sub-unit ribosomal RNA (SSU-rRNA) genes are smaller than those of typical eukaryotes (Vossbrinck et al., 1993). Recent analysis using tabulin, rRNA and Hsp 70 data, support the placement of microsporidia within Fungi (Edlind et al., 1996; Keeling and Doolittle, 1996; Fast et al., 1999; Hirt et al., 1997, 1999; Peer et al., 2000; Keeling and Fast, 2002). These organisms have been shown to be pathogens of a wide range of animal hosts, including insects, fishes and mammals (Cali and Takvorian, 2003; Lom and Nilsen, 2003). Microsporidiosis of silkworm is caused by a highly virulent parasitic microsporidian, *Nosema bombycis* Naegeli. Different strains and species have since been isolated from the infected silkworms and the disease epizootic has become increasingly complex as more number of strains and species are being identified to be infecting silkworm (Ananthalakshmi et al., 1994; Kishore et al., 1994; Samson et al., 1999a, b; Sharma et al., 2003; Singh and Saratchandra, 2003; Nageswara Rao et al., 2004; Shabir Ahmad Bhat and Nataraju, 2004; Selvakumar et al., 2005). All microsporidia are intracellular parasites of great reproductive capacity and are characterized by the possession of thick walled spores containing a polar filament and a sporoplasm.

Microsporidian infection has been reported to have an impact on the economic characters of silkworm. Pebrine infection leads to slower larval growth, thereby prolonging the larval duration and reducing the larval weight (Baig et al., 1988; Baig, 1994). Kudo (1931) reported that heavily infected larvae do not spin cocoons and die, whereas mild infection allows the larvae to spin cocoons. In pebrine infected multivoltine and bivoltine races, inferior cocoon characters have been observed (Noamani et al., 1971; Patil and Geethabai, 1989). Pebrine infected silkworm larvae
spin flimsy and poor quality cocoons (Jameson, 1922; Ghosh, 1944). Silk from the cocoons of pebrine infected larvae is inferior in strength and uniformity of thickness compared to that of healthy larvae (Steinhaus, 1949). Pathogen load also plays a significant role in microsporidian disease incidence. If the host is infected with a very less pathogen load, many of the individuals may survive to adulthood and only few of these adults may be infected. According to Choi et al. (2002), a microsporidian isolated from cabbage white butterfly resulted in death of all the host larvae prior to adult eclosion at a dosage of $1 \times 10^8$ spores/ml whereas, at a lower dosage of $1 \times 10^4$ spores/ml, many of the individuals survived to adulthood and only few of these adults were infected.

The growth of silkworm larvae has been reported to be reduced due to infection with *N. bombycis* (Baig, 1994). In honey bee, *Nosema apis* has been reported to cause severe cytopathology, such as the disintegration and vacuolation of the cytoplasm of the glands (Wang and Moeller, 1971) and decreased RNA synthesis in the midgut cells (Hartwig and Przelecka, 1971) leading to retarded growth.

Transmission of pathogen is a key factor in pathogen-host interactions that can influence the population dynamics of the host (Anderson and May, 1981; Mc Callum et al., 2001). There are several potential pathways by which pathogens are transmitted within a host population – the most common are vertical transmission, i.e. the direct transfer of infection from parent to progeny (Fine, 1975; Becnel and Andreadis, 1999), and horizontal transmission, *i.e.* the transmission of the pathogens from one individual to another of the same generation (Steinhaus and Martignoni, 1970). Microsporidia- the obligate pathogens that are commonly found infecting insects may be transmitted vertically, horizontally or by both means, depending on species-specific microsporidium-host interactions. Vertical transmission may be by one or more of several mechanisms including trans-ovum, trans-ovarial and venereal transmission. Trans-ovarial transmission is known for a wide range of microsporidian species (Becnel and Andreadis, 1999) and is defined as transmission within the egg yolk or embryo. Trans-ovum transmission, a broader term that encompasses transovarial transmission, also includes infection that occurs when hatching neonate larvae feed on egg chorion contaminated with spores (Becnel and Andreadis, 1999). In trans-ovum transmission, the pathogen is transmitted via the egg, either in the embryo or yolk, or adhered to the surface of the egg chorion. The pathogen may actively reproduce and mature in the
embryo or may be occult, continue to develop and reproduce only after embryonation or hatching (Brooks, 1968; Nordin, 1975; Kellen and Lindegren, 1973). Only a few cases of venereal transmission via the male host have been documented and even fewer unequivocally (Kellen and Lindegren, 1971; Toguebaye and Marchand, 1984; Solter, 2006).

Transovarial transmission is common among the microsporidia in both terrestrial and aquatic insect hosts. Little is known about how the pathogens achieve entry into developing eggs but there is evidence for injection of sporoplasts by internally infective (primary) spores (Fries, 1989; Fries et al., 1992; Iwano and Ishihara, 1991a), movement to the ovaries of vegetative forms or primary spores via oenocytes in the haemolymph (Becnel et al., 1989) or possibly movement of vegetative forms across cells (Dunn et al., 2001).

The microsporidian, *Nosema bombycis* has been known for many years as a pathogen causing pebrine disease of the silkworm, *Bombyx mori* L. The transovarial transmission of *N. bombycis* to the silkworm progeny and the destructive effect on the sericulture industry were first clarified by Louis Pasteur (1870). *N. bombycis* is transmitted transovarially in the mulberry pests *viz.* *Spilosoma obliqua* Walker (Lepidoptera: Arctiidae) (Chandra, 1987) and leaf roller, *Diaphania pulverulenta* (Ramegowda and Geethabai, 2005). The percent transovarial transmission differs with different microsporidia. It is highest with *N. bombycis*. The transovarial transmission of *Nosema* sp. NIS-M11 has been demonstrated in silkworm by Han and Watanabe (1988). However, the rate of transmission is lower than that of *N. bombycis* (Iwashita et al., 1990; Fujiwara, 1980). Transmission cycles that include vertical transmission have been observed for Lepidoptera infected with *Nosema* type microsporidia as well as for other microsporidian genera in a variety of insect hosts as mentioned in the following Table.
Microsporidia vertically transmitted in their insect hosts

<table>
<thead>
<tr>
<th>Insect order</th>
<th>Microsporidia</th>
<th>Host</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidoptera</td>
<td>Nosema bombycis</td>
<td>Bombyx mori</td>
<td>Han and Watanabe (1988)</td>
</tr>
<tr>
<td></td>
<td>Nosema bombycis</td>
<td>Spodoptera deparvata</td>
<td>Iwano and Ishihara (1991b)</td>
</tr>
<tr>
<td></td>
<td>Nosema carpocapsae</td>
<td>Cydia pomonella</td>
<td>Siegel et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Nosema fumiferanae</td>
<td>Choristoneura fumiferana</td>
<td>Bauer et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Nosema heliothidis</td>
<td>Helicoverpa zea</td>
<td>Brooks (1968)</td>
</tr>
<tr>
<td></td>
<td>Nosema lymantriae</td>
<td>Lymantria dispar</td>
<td>Novotny and Weiser (1993)</td>
</tr>
<tr>
<td></td>
<td>Nosema marucae</td>
<td>Chilo partellus</td>
<td>Ogwang and Odindo (1993)</td>
</tr>
<tr>
<td></td>
<td>Nosema pyrausta</td>
<td>Ostrinia nubilalis</td>
<td>Kramer (1959a)</td>
</tr>
<tr>
<td></td>
<td>Nosema plodiae</td>
<td>Plodia interpunctella</td>
<td>Kellen and Lindegren (1973)</td>
</tr>
<tr>
<td></td>
<td>Nosema serbica</td>
<td>Lymantria dispar</td>
<td>Pilarska and Vavra (1991)</td>
</tr>
<tr>
<td></td>
<td>Nosema wistmansi</td>
<td>Operophtera brumata</td>
<td>Canning et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Orthosoma operophterae</td>
<td>Operophtera brumata</td>
<td>Canning et al. (1985)</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Nosema scripta</td>
<td>Chrysomela scripta</td>
<td>Bauer and Prankratz (1993)</td>
</tr>
<tr>
<td></td>
<td>Unikaryon sp.</td>
<td>Hypera postica</td>
<td>Solter et al. (1993)</td>
</tr>
<tr>
<td>Diptera</td>
<td>Nosema kingi</td>
<td>Drosophila willistoni</td>
<td>Armstrong (1976)</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Nosema varivestis</td>
<td>Epilachna varivestis</td>
<td>Brooks (1986)</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>Nosema mucidifurax</td>
<td>Mucidifurax raptor</td>
<td>Zchori et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Thelohania solenopsae</td>
<td>Solenopsis invicta</td>
<td>Oi and Williams (2003)</td>
</tr>
<tr>
<td></td>
<td>Vairimorpha invicta</td>
<td>Solenopsis invicta</td>
<td>Briano and Williams (2002)</td>
</tr>
<tr>
<td></td>
<td>Nosema parkeri</td>
<td>Ornithodoros parkeri</td>
<td>Krinsky (1977)</td>
</tr>
<tr>
<td>Orthoptera</td>
<td>Paranoesea locustae</td>
<td>Locustae migratoria migratorioides</td>
<td>Raina et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Pleistophora dixippi</td>
<td>Dixippus morosus</td>
<td>Soldan and Gelbic (1979)</td>
</tr>
</tbody>
</table>


In silkworm, there are no definite curative measures against microsporidiosis. The general disease preventive measures are followed to protect silkworm from the
microsporidiosis. The preventive measures are more of general type involving disinfection of silkworm rearing environment, practice of hygiene and application of prophylactory measures. Maximum precaution is taken to avoid transovarially transmitted infection. Only the disease free layings are used for silkworm rearing and seed production, thus eliminating the chances of disease occurrence by transovarial transmission. Transovum transmission is avoided by surface disinfection of eggs. The other preventive measures emphasize on elimination of secondary sources of infection. The major sources of secondary infection are the diseased and dead larvae, faeces and the gut juices vomited by the diseased larvae. Alternate hosts of different microsporidia especially the lepidopteran pests of mulberry and nearby agricultural crops also pose a serious problem by contaminating the mulberry leaf with the spores carried by them and thus cross infect the silkworm through contaminated mulberry leaf.

Use of certain chemicals fed to silkworm either through artificial diet or through mulberry leaf spray method has been reported to be an effective way to control the microsporidiosis of silkworm (Brooks et al., 1978; Iwano and Ishihara, 1981; Hyasaka, 1991; Frankenhuyzen et al., 2004). As prevention is better than cure, the disinfection of silkworm rearing house, silkworm rearing appliances and the silkworm seed production centers is generally followed to eliminate the microsporidian spores which otherwise may lead to secondary infection. It is coupled with meticulous hygiene practices in silkworm rearing and egg production centers. Various disinfectants viz., Chlorinated lime and hydrochloric acid (Miyajima, 1979b), Formalin (Kagawa, 1980), Asiphore (Venkata Reddy et al., 1990), Chlorine dioxide (Nataraju, 1995; Balavenkatasubbaiah et al., 1999), Kao haite (Balavenkatasubbaiah et al., 2003) and Serichlor (Balavenkatasubbaiah et al., 2006) have been reported to be effective against silkworm pathogens including N. bombycis. In addition to disinfection of silkworm rearing house and appliances, disinfection of mulberry leaves also has been reported to be an efficient method for the prevention and control of microsporidiosis of silkworm (Singh et al., 2007a).

It is observed from the results of Chapter 1 and 2 that five different microsporidia isolated from insect pests of mulberry and some other agricultural crops differ from N. bombycis and also from each other with respect to their infectivity, morphology, ultrastructure, serological affinity, germination response, rate of
sporulation at different temperatures, mode of infection, site of infection, pathogenicity, rate of spread and in terms of their impact on the rearing performance of different productive breeds of silkworm. In the present chapter, the results of investigations on the impact of infection by the isolated microsporidia on the economic characters of silkworm, impact of different pathogen loads on the health status and rearing performance of silkworm, impact of microsporidian infection on the morphology of silkworm larvae and the mode of transmission of the isolated microsporidia are presented and discussed. The study on the effect of disinfection of microsporidian contaminated mulberry leaf on microsporidian disease incidence and rearing performance of silkworm also constitutes the subject matter of the present chapter.

**MATERIALS AND METHODS**

**Impact of infection by isolated microsporidian spores on the economic characters of silkworm:** One popular bivoltine and one multivoltine breed were selected for the study on the impact of microsporidial infection on the economic characters of silkworm. The eggs of the selected bivoltine and multivoltine breed *viz.,* CSR2 and Pure Mysore respectively were received from the germplasm bank of CSR&TI, Mysore for the study. The larvae of the selected breeds were reared under hygienic conditions till the beginning of 3rd instar. The microsporidian spores were inoculated at a concentration of 1×10^7 spores/ml/100 larvae on day zero of 3rd instar. The inoculum was prepared from purified spores of the microsporidia and quantified by standard method using Neubauer haemocytometer as described in earlier chapters. The silkworm larvae were inoculated with different microsporidia by feeding mulberry leaf smeared with one ml inoculum of 1×10^7 spores/ml to 100 larvae. The larvae were allowed to feed on the treated leaves for 24 h to ensure complete consumption of the treated leaves. After 24 h, the larvae were reared as per standard conditions till cocooning. Two controls for each of the breed were maintained for comparison purpose. The first control larvae were treated with mulberry leaves contaminated with 1 ml of 1×10^7 spores/ml of *N. bombycis* to 100 larvae and the second control larvae were treated with mulberry leaves smeared with sterilized distilled water and reared on uncontaminated mulberry leaves. The controls and treatments had three replications of 100 larvae each. The larvae were observed for growth, larval duration and survival. Data with regard to other economic characters such as larval weight,
single cocoon weight, single shell weight and shell ratio was recorded. Data with respect to average filament length was also recorded and the data was analyzed statistically.

**Impact of lower pathogen loads on the health status and rearing performance of silkworm:** To study the impact of lower pathogen loads on the health status and rearing performance of silkworm, a popular bivoltine silkworm breed (CSR2) was selected. The eggs of the said breed were received from the germplasm bank of C.S.R. & T.I., Mysore, surface disinfected and then incubated at 25±1°C and 80±5% RH. The hatched larvae were reared till the beginning of third instar following standard silkworm rearing practices under hygienic conditions. The third instar silkworm larvae were inoculated separately with different concentrations of the isolated microsporidian spores. Also, one set of larvae inoculated with different concentrations of *Nosema bombycis* spores was maintained separately for comparison purpose. Four different concentrations of the pathogen inoculum *viz.*, $1 \times 10^2$, $1 \times 10^3$, $1 \times 10^4$ and $1 \times 10^5$ spores/ml were tested to determine their impact on the health status and rearing performance of silkworm. These different concentrations were prepared from purified spores of each isolated microsporidia by serial dilution of the quantified stock inoculum. The quantification was done following the standard method using Neubar haemocytometer (Cantwell, 1970). Each inoculum concentration formed a treatment. For each treatment five replications of 100 larvae were maintained. Different sets of larvae were inoculated separately with different concentrations of each of the microsporidian spores isolated from insect pests of mulberry and other agricultural crops. One ml of specific concentration of specific microsporidian spores was smeared on the ventral surface of mulberry leaf and fed to the silkworm larvae just out of 2nd moult. Also, one normal control batch without any inoculation was maintained for comparison purpose. Observations with regard to larval mortality, pupal mortality, percentage moths infected and total infection percent were recorded. Data with regard to larval weight, larval duration, pupation rate, cocoon weight, shell weight and shell percentage also was recorded. The data was statistically analyzed to arrive at conclusion.

**Impact of infection by the isolated microsporidia on the morphology of the silkworm, *Bombyx mori* L.:** To study the impact of infection by the isolated microsporidia on the larval morphology, CSR2 breed of the silkworm, *Bombyx mori*
L. was selected. The eggs of the said breed were received from the germplasm bank of CSR&TI, Mysore. The larvae were reared under hygienic conditions till the beginning of third instar. Immediately after second moult, the spores of the isolated microsporidia were fed separately to the silkworm larvae (100 larvae/treatment) at a concentration of $1 \times 10^7$ spores/ml by smearing it on the mulberry leaf disc of 100 cm$^2$ surface area. One set of larvae was inoculated with *Nosema bombycis* spores ($1 \times 10^7$ spores/ml) which served as control for comparison purpose. The second feeding was provided with normal leaves to each inoculated batch after 24 hours of microsporidian inoculation and the rearing was continued as per standard methods till the onset of the spinning. From the day of inoculation till the onset of spinning, daily 10 larvae were taken randomly and observations with regard to changes in average larval length, width and weight were recorded. The same observations were recorded daily in healthy control batches also.

**Mode of transmission:** To determine the mode of transmission of the isolated microsporidia, a popular bivoltine silkworm breed (CSR2) was selected and on day zero of fourth instar, larvae of the said breed were per orally inoculated separately with the spores of the isolated microsporidia at a dosage of $1 \times 10^7$ spores/ml. To compare the results, one set of larvae was inoculated with spores of *Nosema bombycis*. The inoculum containing $1 \times 10^7$ spores/ml of the isolated microsporidia or *N. bombycis* was prepared from the stock inoculums by proper quantification using Neubar haemocytometer (Cantwell, 1970). One ml of inoculum ($1 \times 10^7$ spores/ml) of each microsporidia was smeared separately on 100 sq. cms surface area of mulberry leaf disc and fed to 100 larvae immediately after 3$^{rd}$ moult. The larvae were allowed to feed on the contaminated leaves for 24 hours. The second normal feeding was given after 24 hours and the rearing on uncontaminated mulberry leaves was continued till cocooning. After cocoon formation, the cocoons from each treated batch were cut open for sex separation of the pupae. The male and female pupae were kept in separate trays for moth emergence. Yet another set of larvae was reared without inoculation till spinning and moth emergence.

The moths obtained after inoculation of the larvae of CSR2 breed with an inoculum dosage of $1 \times 10^7$ spores/ml of the isolated microsporidia or *Nosema bombycis* were provisionally regarded as infected. The moths obtained from the batches without inoculation were provisionally regarded as healthy. To find out the
mode of transmission of the microsporidia, the male and female moths were allowed to mate in three different combinations \textit{viz.}, Infected female×Infected male (IF×IM); Infected female×Healthy male (IF×HM) and Healthy female×Infected male (HF×IM). After 3 hours of pairing, the male and female moths were depaired and the gravid female moths were allowed to lay eggs for 24 hours on egg sheets. After egg laying, gonads of each male and female moth were dissected out and tested individually for the presence of microsporidian spores and also, the whole body (excluding the gonads) of these moths was subjected to microscopic examination (Phase contrast microscope, Nikon-Type 104) for the presence of microsporidian spores. The egg layings obtained from different treatments were surface sterilized by immersing in 2% formalin solution for 5 minutes at room temperature. The layings were then treated with hydrochloric acid of specific gravity 1.076 at 46.1°C for 5 minutes in a hot water bath to terminate the egg diapause and washed in running tap water to remove the traces of HCl. The layings were incubated for normal embryonic development at 25±1°C temperature and 80±5% RH. During the pinhead stage, the layings were black-boxed to ensure uniform development of embryo and hatching. After two days of black boxing, the layings were exposed to light to stimulate hatching. Fecundity and hatching percent of each laying were recorded. The dead eggs in each laying were also examined for infection by examination of whole wet mount of egg content following its removal from chorion to confirm the infection passing to progeny through infection in gonads. The progeny larvae were reared as per standard methods (Datta, 1992). After I moult, 100 larvae/batch were collected randomly and homogenized individually and the smear was observed under phase contrast microscope for the presence of microsporidian spores and thus, the transmission rate was calculated by the standard formula (Han and Watanabe, 1988) which is as follows;

\[
\text{Transmission rate} = \frac{(A \times B) + (C \times D)}{A + C}
\]

where

- A- Number of dead eggs.
- B- % of dead eggs infected.
- C- Number of larvae hatched.
- D- % of larvae infected.

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Also, the rearing of the remaining progeny larvae was continued to record the mortality, if any, during different larval instars and the pupal stage in the transovarially infected batches. The observations were recorded and analyzed.

**Transmission through surface contaminated layings:** To study the transmission of the microsporidia through surface contaminated layings, disease free layings of CSR2 breed at the blue egg stage were smeared with microsporidian spores (dosage of $1 \times 10^7$ spores/ml) of each microsporidia separately on the egg surface with the help of a camelin brush. The egg layings were air dried, wrapped in tissue paper and subjected to black boxing for uniform hatching. After two days, the layings were exposed to indirect light for hatching. The newly hatched larvae were reared as per standard methods. Mortality due to microsporidian infection in the progeny, if any, was recorded in different larval instars and pupal stage.

**Effect of disinfectants against the spores of the isolated microsporidia and *N. bombycis* through disinfection of mulberry leaves:** To determine the effect of mulberry leaf disinfection on the spores of the isolated microsporidia and *N. bombycis*, two popular disinfectants *viz.*, Decol and Sanitech were selected and the selected disinfectants were used at a concentration of 1.0% and 400 ppm respectively. The said concentrations of the selected disinfectants were fixed on the basis of their palatability to silkworm and for the preparation of Sanitech solution (400 ppm), addition of lime was avoided as it is a feed-repellent. To conduct the test, a popular bivoltine silkworm breed CSR2 was selected. The larvae of the said breed were reared as per standard methods (Datta, 1992) up to the second moult. Mulberry leaf discs were smeared with the spores of the isolated microsporidia and *N. bombycis* separately at an infective concentration of $1 \times 10^7$ spores/ml. The mulberry leaf discs were then air dried and dipped separately in Decol (1.0%) and Sanitech (400 ppm) solutions for 30 minutes and then again air dried and fed to silkworm larvae (100 larvae/replication/treatment) of CSR2 breed on day zero of third instar. The larvae were allowed to feed on the treated leaf discs for 24 hours to ensure the complete consumption of the treated leaf. After 24 hours, the fresh mulberry leaf without any contamination was provided to each treated batch. In one set of experiment, the dead larvae if any, from all the treatments were collected and subjected to microscopic examination to reveal the cause of mortality. Also, on the 13th day of post inoculation, all the survived larvae were homogenized individually in distilled water and the
smears were observed under phase contrast microscope (Nikon, Type-104) to record the total percentage of microsporidian infection. In yet another set of experiment, the treated larvae were reared as per standard methods up to cocooning to determine the effect of mulberry leaf disinfection on the rearing performance of microsporidian inoculated silkworm. The data with regard to larval weight, larval duration, pupation rate, cocoon weight, shell weight and shell percentage was recorded and analysed. For each larval batch fed with the mulberry leaf discs smeared separately with the different microsporidia followed by dipping in Decol and Sanitech solutions, one control batch fed with the mulberry leaf disc smeared with the spores of the concerned microsporidia (1×10^7 spores/ml) without any treatment was also maintained for comparison purpose. Also one normal control batch fed with the mulberry leaf disc dipped in distilled water was maintained for comparison purpose.

RESULTS

Impact of infection by isolated microsporidian spores on the economic characters of silkworm: The results of the studies on the impact of microsporidian infection on economic characters of two silkworm breeds viz., CSR2 and Pure Mysore are presented in Table 3.1 and Figures 3.1 and 3.2. It is observed that the infection by all the five isolated microsporidia had a significant impact on the economic parameters of both the silkworm breeds tested. A comparison with respective healthy control batch indicates that the infection due to the five microsporidia viz., NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp lowered the pupation rate (ERR%), larval weight, single cocoon weight, single shell weight, percent silk content and filament length. The infection also prolonged the larval duration of both the silkworm breeds tested.

Larval weight: The results on the impact of the isolated microsporidia on the weight of 10 larvae on sixth day of final instar of the two silkworm breeds tested viz., CSR2 and Pure Mysore as presented in Table 3.1 show that the microsporidian infection significantly reduced the larval weight of both the breeds tested. The highest reduction in larval weight of CSR2 batches was recorded in the larvae inoculated with the standard microsporidian strain N. bombycis (26.6 g) followed by NIK-1So (30.0 g), NIK-1Cpy (30.3 g), NIK-1Pr (31.1 g), NIK-1Dp (31.8 g) and NIK-1Cc (33.0 g) compared to the normal control batches where a larval weight of 35.7 g was recorded. A similar trend with respect to the impact of microsporidian infection on larval weight
was recorded in Pure Mysore batches also and the highest reduction in larval weight was recorded in silkworm larvae inoculated with *N. bombycis* (18.8 g) followed by NIK-1So (21.1 g), NIK-1Cpy (21.4 g), NIK-1Pr (22.5 g), NIK-1Dp (22.9 g) and NIK-1Cc (23.9 g) compared to the normal control batches where a larval weight of 25.8 g was recorded.

**Larval duration:** In CSR2 batches inoculated separately with the isolated microsporidia and *Nosema bombycis*, the larval duration got increased from 23 to 25 days when compared with the normal control batch. Similarly, the larval duration was prolonged in Pure Mysore batches inoculated separately with the isolated microsporidia and was recorded as 29 days compared to the 28 days recorded in normal control batches.

**Pupation rate (ERR%):** The data as presented in Table 3.1 shows that the pupation rate (ERR%) in CSR2 breed inoculated separately with five different microsporidia was lowered significantly and was recorded to be lowest in NIK-1So inoculated batches (73.0%) followed by NIK-1Cpy (75.0%), NIK-1Pr (79.0%), NIK-1Dp (81.0%) and NIK-1Cc (86.3%) inoculated batches as against 95.7% pupation rate recorded in healthy host population. Also, in Pure Mysore breed inoculated separately with five different microsporidia, the lowest pupation rate was recorded in NIK-1So inoculated batches (79.0%) followed by NIK-1Cpy (80.3%), NIK-1Pr (82.0%), NIK-1Dp (86.0%) and NIK-1Cc (88.3%) which in turn was significantly lower than the normal control batches (95.3%).

When the pupation rate of the batches inoculated with the standard microsporidian strain, *Nosema bombycis* is compared with that of the batches inoculated with the isolated microsporidia, it is observed that in *N. bombycis* inoculated CSR2 batches, the pupation rate was significantly lower (64.7%) compared to the five microsporidia tested. Similarly, in Pure Mysore batches inoculated with *Nosema bombycis* also, pupation rate of only 73.3% was recorded clearly suggesting the five microsporidia isolated from insect pests of mulberry and other agricultural crops are less pathogenic to silkworm than the standard strain, *Nosema bombycis*.

**Single cocoon weight:** In both the silkworm breeds *viz.*, CSR2 and Pure Mysore inoculated separately with different microsporidia, cocoon weight was reduced significantly compared to the healthy control batches. The highest reduction in cocoon
weight of CSR2 batches was recorded in the batches inoculated with *N. bombycis* (1.428 g) followed by NIK-1So (1.470 g), NIK-1Cpy (1.478 g), NIK-1Pr (1.480 g), NIK-1Dp (1.490 g) and NIK-1Cc (1.495 g) compared to the healthy control batches (1.600 g). Similarly, in Pure Mysore batches also, the highest reduction in cocoon weight was recorded in the batches inoculated with *N. bombycis* (0.964 g) followed by NIK-1So (0.996 g), NIK-1Cpy (1.005 g), NIK-1Pr (1.044 g), NIK-1Dp (1.082 g) and NIK-1Cc (1.113 g) compared to the healthy control batches (1.127 g) (Table 3.1).

**Single shell weight:** In the CSR2 batches inoculated separately with the isolated microsporidia and *N. bombycis*, there was a significant reduction in shell weight. The highest reduction in shell weight was recorded in the batches inoculated with *N. bombycis* (0.276 g) followed by NIK-1So (0.292 g), NIK-1Cpy (0.296 g), NIK-1Pr (0.320 g), NIK-1Dp (0.325 g) and NIK-1Cc (0.328 g) compared to the healthy control batches wherein a shell weight of 0.366 g was recorded (Table 3.1). A similar trend with respect to the reduction in shell weight due to microsporidian infection was recorded in Pure Mysore batches also and the highest reduction in shell weight was recorded in the batches inoculated with *N. bombycis* (0.107 g) followed by NIK-1So (0.117 g), NIK-1Cpy (0.122 g), NIK-1Pr (0.130 g), NIK-1Dp (0.137 g) and NIK-1Cc (0.141 g) inoculated batches. In the control Pure Mysore batches, shell weight of 0.154 g was recorded.

**Shell percentage:** The infection due to the isolated microsporidia significantly reduced the shell percentage of both the breeds tested. The highest reduction in shell percentage of CSR2 batches inoculated with the different microsporidia was recorded in the batches inoculated with *N. bombycis* (19.3%) followed by NIK-1So (19.8%), NIK-1Cpy (20.0%), NIK-1Pr (21.6%), NIK-1Dp (21.8%) and NIK-1Cc (21.9%) compared to the healthy control batches (22.8%). A similar trend was observed in Pure Mysore batches also and the highest reduction in shell percentage was recorded in *N. bombycis* inoculated batches (11.1%) followed by NIK-1So (11.7%), NIK-1Cpy (12.1%), NIK-1Pr (12.4%), NIK-1Dp (12.6%) and NIK-1Cc (12.7%) compared to the healthy control batches (13.6%) (Table 3.1). The percentage reduction in the shell percentage of the CSR2 and Pure Mysore batches inoculated with the isolated microsporidia and *N. bombycis* compared to that of the healthy control batches is graphically represented in Figures 3.3 and 3.4 respectively.
Filament length: The infection with the isolated microsporidia had a significant impact on the Filament length of both the breeds tested (Table 3.1). The highest decrease in filament length of CSR2 breed was recorded in *N. bombycis* inoculated batches (617.0 m) followed by NIK-1So (635.0 m), NIK-1Cpy (644.0 m), NIK-1Pr (721.0 m), NIK-1Dp (782.0 m) and NIK-1Cc (807.0 m) compared to the filament length of 980.0 m in healthy control batches. Similarly, the highest decrease in filament length of Pure Mysore breed was recorded in the *N. bombycis* inoculated batches (284.0 m) followed by NIK-1So (291.3 m), NIK-1Cpy (325.0 m), NIK-1Pr (369.0 m), NIK-1Dp (371.0 m) and NIK-1Cc (400.7 m) compared to the filament length of 443.0 m in the healthy Pure Mysore batches. The percentage decrease in the filament length of the CSR2 and Pure Mysore batches inoculated with the isolated microsporidia and *N. bombycis* compared to that of the healthy control batches is graphically represented in Figures 3.5 and 3.6 respectively.

Impact of lower pathogen loads on the health status and rearing performance of silkworm: The results on the impact of lower pathogen loads on the health status and rearing performance of the CSR2 breed of the silkworm, *Bombyx mori* L. are presented in Table 3.2. It is clear from the data as presented in the said table that inoculation of the isolated microsporidia to silkworm at the two lowest concentrations viz., $1 \times 10^2$ and $1 \times 10^3$ spores/ml did not cause any larval and pupal mortality whereas at the same two concentrations, larval mortality of 1.0 and 3.0% and pupal mortality of 3.7 and 7.3% respectively was recorded in the batches inoculated with the standard strain *N. bombycis*. Also, in the batches inoculated with the spores of the microsporidia viz., NIK-1Pr, NIK-1Cc and NIK-1Dp at a concentration of $1 \times 10^2$ spores/ml, all the emerged moths were found to be free from microsporidian infection, thereby the total infection percentage was nil. However, in the batches inoculated with the microsporidia viz., NIK-1Cpy, NIK-1So and *N. bombycis* at the same concentration, 8.0, 13.0 and 63.3% of the emerged moths were infected, thereby resulting in a total infection of 8.0, 13.0 and 68.0% respectively. At a concentration of $1 \times 10^3$ spores/ml, the highest total infection percent was recorded in the batches inoculated with the standard microsporidian strain *N. bombycis* (86.0%) followed by NIK-1So (35.0%), NIK-1Cpy (30.0%), NIK-1Pr (28.0%), NIK-1Dp (17.0%) and NIK-1Cc (14.0%). Inoculation of the isolated microsporidia and *N. bombycis* to silkworm at a concentration of $1 \times 10^4$ spores/ml caused a larval mortality of 1.0, 6.7,
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9.7 and 10.7% in NIK-1Pr, NIK-1Cpy, NIK-1So and *N. bombycis* inoculated batches respectively whereas at the same concentration, the larval mortality in NIK-1Cc and NIK-1Dp inoculated batches was nil. A similar trend with regard to pupal mortality also was recorded in the batches inoculated with NIK-1Cc and NIK-1Dp at the same concentration whereas in NIK-1Pr, NIK-1Cpy, NIK-1So and *N. bombycis* inoculated batches, pupal mortality of 1.7, 3.7, 4.7 and 10.0% respectively was recorded at a concentration of $1 \times 10^4$ spores/ml. The highest total infection % at the concentration of $1 \times 10^4$ spores/ml was recorded in *N. bombycis* inoculated batches (89.3%) followed by NIK-1So (50.7%), NIK-1Cpy (45.7%), NIK-1Pr (35.0%), NIK-1Dp (21.7%) and NIK-1Cc (17.3%) inoculated batches. At the highest spore concentration of $1 \times 10^5$ spores/ml, the larval and pupal mortality in case of NIK-1Cc inoculated batches was nil whereas the same in case of the batches inoculated with NIK-1Pr, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* was recorded as 6.7, 16.3, 20.3, 1.0 and 21.7% (larval mortality) and 3.7, 4.3, 6.3, 1.0 and 14.7% (pupal mortality) respectively. The highest total infection percent at the concentration of $1 \times 10^5$ spores/ml was recorded in the batches inoculated with *N. bombycis* (92.0%), followed by NIK-1So (87.0%), NIK-1Cpy (80.3%), NIK-1Pr (44.0%), NIK-1Dp (27.0%) which in turn was followed by NIK-1Cc inoculated batches (21.3%).

The impact of the different pathogen loads on the rearing performance of silkworm as presented in Table 3.2 is mentioned in detail as follows:

**Larval weight:** At a concentration of $1 \times 10^2$ spores/ml, there was not any significant impact on the larval weight in case of the batches inoculated with the isolated microsporidia and it ranged from 38.70 to 39.17 g whereas at the same concentration, there was a significant reduction in larval weight of *N. bombycis* inoculated batches (37.77 g) as against a larval weight of 39.18 g in case of the normal control batches. Similarly, at a concentration of $1 \times 10^3$ spores/ml, the larval weight in case of the batches inoculated with the isolated microsporidia ranged from 38.10 to 38.87 g whereas, the same in case of *N. bombycis* inoculated batches was recorded as 37.17 g. The larval weight ranged from 38.02 to 38.74 g in the batches inoculated with the isolated microsporidia at a concentration of $1 \times 10^4$ spores/ml. The same in *N. bombycis* inoculated batches was recorded as 36.95 g only. At the highest spore concentration ($1 \times 10^5$ spores/ml), there was a significant reduction in the larval weight of the inoculated batches and the lowest larval weight was recorded in the batches
inoculated with *N. bombycis* (36.64 g) followed by NIK-1So (37.74 g), NIK-1Cpy (37.90 g), NIK-1Pr (38.23 g), NIK-1Dp (38.61 g) which in turn was followed by the batches inoculated with NIK-1Cc (38.68 g). In the normal control batches, the same was recorded as 39.18 g.

**Larval duration:** Inoculation of the silkworm larvae with the spores of the isolated microsporidia at the concentrations ranging from $1 \times 10^2$ to $1 \times 10^5$ spores/ml did not cause any effect on the larval duration and the same was recorded as 24 days which was similar to that of the normal control batches. Similarly, in the batches inoculated with the spores of *N. bombycis* at the concentrations of $1 \times 10^2$ and $1 \times 10^3$ spores/ml, there was no effect on the larval duration and the same was recorded as 24 days. However, in case of the batches inoculated with *N. bombycis* spores at the concentrations of $1 \times 10^4$ and $1 \times 10^5$ spores/ml, the larval duration was prolonged by one day and was recorded as 25 days.

**Pupation rate:** Inoculation of silkworm larvae with the spores of the isolated microsporidia at the two lowest concentrations viz., $1 \times 10^2$ and $1 \times 10^3$ spores/ml did not lead to any significant impact on the pupation rate and the same ranged from 92.0 to 95.0% whereas, in case of the batches inoculated with the spores of *N. bombycis*, the pupation rate of 91.0 and 85.3% respectively was recorded at the same two concentrations compared to the normal control batches wherein a pupation rate of 95.7% was recorded. Also, at the inoculum concentration of $1 \times 10^4$ spores/ml, there was no significant impact on the pupation rate of the batches inoculated with NIK-1Pr, NIK-1Cc and NIK-1Dp microsporidia and the pupation rate of 93.0, 94.0 and 94.0% respectively was recorded. On the other hand, at the same concentration, there was a significant reduction in the pupation rate of the batches inoculated with the microsporidia viz., NIK-1Cpy, NIK-1So and *N. bombycis* and the pupation rate of 85.3, 81.3 and 75.0% respectively was recorded. Inoculation of the microsporidia viz., NIK-1Cc and NIK-1Dp to silkworm at the highest concentration ($1 \times 10^5$ spores/ml) also did not lead to any significant impact on the pupation rate and the same was recorded as 94.0 and 93.7% respectively whereas, at the same concentration, the pupation rate was significantly reduced in the batches inoculated with NIK-1Pr, NIK-1Cpy, NIK-1So and *N. bombycis* and was recorded as 85.3, 75.0, 69.0 and 59.3% respectively.
**Single cocoon weight:** The isolated microsporidia did not cause any significant impact on the cocoon weight of the batches inoculated at the two lowest concentrations *viz.*, $1 \times 10^2$ and $1 \times 10^3$ spores/ml and the same ranged from 1.580 to 1.604 g whereas, in the batches inoculated with the spores of the standard strain *N. bombycis* at the same concentrations, the cocoon weight was slightly less and ranged from 1.542 to 1.558 g. In the batches inoculated with the spores of the microsporidia *viz.*, NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp at the concentration of $1 \times 10^4$ spores/ml, cocoon weight of 1.560, 1.572, 1.551, 1.541 and 1.570 g respectively was recorded whereas at the same concentration, the cocoon weight in the *N. bombycis* inoculated batches was recorded as 1.507 g. Inoculation of the spores of the isolated microsporidia and *N. bombycis* to silkworm at a concentration of $1 \times 10^5$ spores/ml significantly reduced the cocoon weight and the lowest cocoon weight was recorded in the batches inoculated with *N. bombycis* (1.487 g) followed by NIK-1So (1.519 g), NIK-1Cpy (1.529 g), NIK-1Pr (1.537 g), NIK-1Dp (1.560 g) which in turn was followed by the batches inoculated with NIK-1Cc (1.565 g) compared to the normal control batches wherein the cocoon weight of 1.607 g was recorded.

**Single shell weight:** At the two lowest concentrations *viz.*, $1 \times 10^2$ and $1 \times 10^3$ spores/ml, there was no significant impact on the shell weight of the batches inoculated with the isolated microsporidia and the same ranged from 0.349 to 0.374 g whereas in the batches inoculated with *N. bombycis*, the shell weight was slightly less and ranged from 0.320 to 0.335 g. In the batches inoculated with the spores of the microsporidia *viz.*, NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp at the concentration of $1 \times 10^4$ spores/ml, shell weight of 0.347, 0.356, 0.342, 0.335 and 0.335 g respectively was recorded whereas at the same concentration, the shell weight in the *N. bombycis* inoculated batches was recorded as 0.302 g only. Inoculation of the spores of the isolated microsporidia and *N. bombycis* to silkworm at a concentration of $1 \times 10^5$ spores/ml significantly reduced the shell weight and the lowest shell weight was recorded in the batches inoculated with *N. bombycis* (0.295 g) followed by NIK-1So (0.326 g), NIK-1Cpy (0.332 g), NIK-1Pr (0.337 g), NIK-1Dp (0.348 g) which in turn was followed by the batches inoculated with NIK-1Cc (0.352 g) compared to the normal control batches wherein the shell weight was recorded as 0.375 g.

**Shell percentage:** In the batches inoculated with the spores of the isolated microsporidia at a concentration of $1 \times 10^2$ spores/ml, there was no impact of
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Microsporidian infection on the shell percentage and the same was recorded as 23.29, 23.31, 22.91, 22.78 and 23.30% respectively in the batches inoculated with NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp whereas in the batches inoculated with *N. bombycis*, there was a significant reduction in shell percentage even at the lowest concentration and the same was recorded as 21.49% as against the shell percentage of 23.32% in the normal control batches. In the batches inoculated with the spores of the isolated microsporidia at a concentration of $1 \times 10^3$ spores/ml, the shell percentage ranged from 22.09 to 22.86% whereas, at the same concentration, the shell percentage in *N. bombycis* inoculated batches was recorded as 20.78% only. Similarly, in the batches inoculated with the spores of the isolated microsporidia at a concentration of $1 \times 10^4$ spores/ml, the shell percentage ranged from 21.74 to 22.65% whereas, at the same concentration, the shell percentage in *N. bombycis* inoculated batches was recorded as 20.03% only. At the highest concentration of $1 \times 10^5$ spores/ml, there was a significant reduction in the shell percentage and the lowest shell percentage was recorded in the batches inoculated with *N. bombycis* (19.82%) followed by NIK-1So (21.47%), NIK-1Cpy (21.74%), NIK-1Pr (21.93%), NIK-1Dp (22.32%) and NIK-1Cc (22.52%).

**Impact of infection by the isolated microsporidia on the morphology of the silkworm, *Bombyx mori* L.:**

**Larval length:** The results with regard to the impact of the infection caused by different isolated microsporidia on the daily increase in larval length of silkworm (CSR2 breed) are presented in Table 3.3. The data indicates that there was no significant impact of infection caused by the isolated microsporidia and *Nosema bombycis* on the progressive increase in the larval length up to 6th day of post inoculation. However, from 7th day of PI onwards, the progressive increase in larval length was significantly reduced in the batches inoculated with NIK-1Cpy (34.50 mm), NIK-1So (34.33 mm) and *Nosema bombycis* (33.33 mm) compared to the healthy control batches wherein the larval length was recorded as 36.00 mm on 7th day PI. In the batches inoculated with the microsporidia NIK-1Pr, NIK-1Cc and NIK-1Dp, there was not any significant reduction in the increase of larval length on 7th day post inoculation which was recorded as 35.33, 35.67 and 35.50 mm respectively. On 8th day PI, the infection with different microsporidia caused significant reduction in the increase in larval length in all the inoculated batches compared to healthy control.
batches and the larval length was recorded as 35.67, 36.00, 35.17, 35.50, 36.00 and 34.33 mm in the batches inoculated with NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *Nosema bombycis* respectively when compared to the 37.00 mm larval length in healthy control batch. On 9th and 10th day PI, the larval length ranged from 36.00 to 37.50 mm in the inoculated batches whereas the same ranged from 38.50 to 39.50 mm in healthy control batches. The same trend with regard to impact of infection by the isolated microsporidia and *N. bombycis* on the daily increase in larval length was recorded on succeeding days which ranged from 36.00 to 37.67 mm, 45.50 to 50.50 mm and 49.00 to 55.00 mm on 11th, 12th and 13th day of post inoculation respectively compared to the healthy control batches wherein the same was recorded as 40.00, 53.33 and 57.67 mm respectively. With progressive infection, the impact of microsporidiosis on larval length was more pronounced and on 14th day PI, the lowest larval length was recorded in the batches inoculated with NIK-1So (56.67 mm) followed by NIK-1Cpy (57.33 mm), NIK-1Pr (58.33 mm) and NIK-1Dp (60.33 mm). The highest larval length was recorded in the batches inoculated with NIK-1Cc (60.50 mm). In *N. bombycis* inoculated batches, the same was recorded as 55.50 mm only compared to the healthy control batches (62.00 mm). One day prior to the spinning i.e. on 15th day PI, the larval length was recorded as 62.67, 64.50, 60.50, 58.50 and 63.50 mm in the batches inoculated with NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp respectively compared to healthy control batches wherein a larval length of 66.17 mm was recorded. In *N. bombycis* inoculated batches, the same was recorded as 56.00 mm. The percentage difference in the larval length of the batches inoculated with different microsporidia compared to that of the healthy control batches one day prior to the onset of spinning is graphically represented in Figure 3.7.

**Larval width:** The data with respect to the impact of infection by the isolated microsporidia on the daily increase in larval width is presented in Table 3.4. The data indicates that there was no significant impact of infection by the isolated microsporidia on the progressive increase in larval width up to 12th day of post inoculation. However, in the batches inoculated with *N. bombycis*, the increase in larval width got significantly reduced from 7th day of PI onwards and was recorded as 5.50, 6.00, 6.00, 6.17, 6.37 and 7.50 mm against a larval width of 6.67, 7.00, 7.33, 7.33, 7.50 and 8.50 mm in healthy control batches on 7th, 8th, 9th, 10th, 11th and 12th day PI respectively. On 13th day PI, a significant impact of infection by the isolated
microsporidia on the larval width was observed (9.00, 9.00, 8.00, 8.00 and 9.00 mm in the batches inoculated with NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp respectively) as against a larval width of 10.00 mm in healthy control batches. In case of *N. bombycis* inoculated batches, only 7.67 mm larval width was recorded. On 14th day PI, larvae inoculated with the isolated microsporidia attained a larval width of 10.00 mm in all the batches whereas in *N. bombycis* inoculated batches, only 8.33 mm larval width was attained as against a larval width of 11.00 mm in healthy control batches. Before the onset of spinning (15th day PI), the lowest larval width was recorded in NIK-1Cpy and NIK-1So batches (10.00 mm) followed by NIK-1Pr (10.33 mm), NIK-1Dp (10.50 mm) and NIK-1Cc (11.00 mm). In the healthy control batches, the same was recorded as 12.00 mm. In the batches inoculated with the standard strain *N. bombycis*, larval width of 8.50 mm was recorded on 15th day of PI. The percentage difference in the larval width of the microsporidia inoculated batches compared to that of the healthy control batches one day prior to the onset of spinning is graphically represented in Figure 3.8.

**Larval weight:** The results with regard to the impact of infection by the isolated microsporidia on daily increase in larval weight are presented in Table 3.5. From the said table, it is clear that the infection by the isolated microsporidia did not result in any significant impact on the larval weight from the day of inoculation up to 11th day of PI and the larval weight ranged from 0.44 to 9.46 g/10 larvae from the day of inoculation up to 11th day PI. In the healthy control batches, the same ranged from 0.45 to 9.56 g/10 larvae. On the other hand, in case of *N. bombycis* inoculated batches, though there was no significant impact of infection on larval weight upto 6th day of PI, however, from 7th day of PI onwards, the progressive increase in larval weight was slightly reduced and the larval weight was recorded as 7.50, 8.62, 9.00, 9.06 and 9.10 g on 7th, 8th, 9th, 10th and 11th day PI respectively. On 12th day of PI, larval weight of 21.14, 22.04, 20.16, 20.08 and 22.00 g was recorded in the batches inoculated with NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp respectively whereas the same in case of *N. bombycis* inoculated batches was recorded as 18.47 g as against the larval weight (23.94 g) of healthy control batches showing significant reduction in the progressive increase in larval weight in the inoculated batches. The trend continued on 13th and 14th day of PI also where the larval weight ranged from 29.57 to 31.19 g and 33.01 to 34.05 g respectively in the batches inoculated with the
isolated microsporidia. This reduction in larval weight was comparatively more pronounced in *N. bombycis* inoculated batches, where larval weight of 25.17 and 27.02 g on 13th and 14th day of PI respectively was recorded. In healthy control batches, 32.53 and 35.32 g larval weight respectively was recorded which was significantly higher than observed in inoculated batches. One day prior to the onset of spinning (15th day PI), the adverse impact of microsporidian infection on larval weight was further-more pronounced. The lowest larval weight was recorded in the batches inoculated with NIK-1So (34.32 g) followed by NIK-1Cpy (34.54 g), NIK-1Pr (34.94 g), NIK-1Dp (35.31 g) and NIK-1Cc (36.45 g). In case of the batches inoculated with *N. bombycis*, 28.00 g larval weight was recorded as against a larval weight of 38.52 g in the healthy control batches. The percentage reduction in the larval weight of the inoculated batches compared to that of the healthy control batches one day prior to the onset of spinning is graphically represented in Figure 3.9.

**Mode of Transmission:** Microscopic examination of the gonads of the moths from the batches inoculated with the isolated microsporidia revealed infection in NIK-1Pr, NIK-1Cpy, NIK-1So and NIK-1Dp inoculated batches. However, the gonads of the moths from the batches inoculated with the microsporidian NIK-1Cc were devoid of microsporidian infection. Data on transmission of the five isolated microsporidia in silkworm is presented in Table 3.6. The table indicates that dead eggs laid by NIK-1Cc infected female mated with infected male (IF×IM) and also infected female mated with healthy male (IF×HM) were not found infected with the said microsporidian whereas the dead eggs laid by NIK-1Cpy and NIK-1So infected females mated with infected males (IF×IM) and also infected females mated with healthy males (IF×HM) show 100% infection. Infection percentage in the dead eggs laid by NIK-1Pr and NIK-1Dp infected females mated with infected males (IF×IM) and infected females mated with healthy males (IF×HM) ranged from 80.3 to 81.0%. In the standard strain, *N. bombycis*, the infection in dead eggs was 100%. The dead eggs laid by the healthy females mated with infected males (HF×IM) were devoid of infection in all the microsporidian inoculated batches tested. The fecundity and hatching percentage in the combinations viz., IF×IM, IF×HM and HF×IM (in the batches inoculated with the isolated microsporidia and *N. bombycis*) were significantly less and ranged between 367 to 468 and 66.1 to 92.5% respectively compared to the healthy control batches wherein the same was recorded as 512 and
97.0% respectively. The microsporidian NIK-1Pr showed 94.6 and 90.6% transmission and NIK-1Dp showed 82.6 and 80.0% transmission to progeny larvae hatched from the layings obtained after mating infected females with infected males (IF×IM) and infected females with healthy males (IF×HM) respectively. NIK-1Cpy and NIK-1So showed 100% transmission to progeny in the combinations *viz.*, IF×IM and IF×HM which was similar to *Nosema bombycis* (100%). Significantly, the microsporidian NIK-1Cc did not show any transmission to progeny larvae. No infection was observed in the progeny larvae hatched from eggs laid by healthy females mated with infected males (HF×IM) in all the batches indicating that there is no venereal transmission of the isolated microsporidia through male.

**Mortality of transovarially infected progeny populations:**

**IF×IM combinations:** The results as presented in Table 3.7 show that there was no mortality in the progeny batches of NIK-1Cc infected female mated with infected male up to the end of observation period confirming the non-transovarial transmission of the said microsporidian whereas in the progeny batches obtained from the parents infected with NIK-1Cpy, NIK-1So and *N. bombycis*, mortality of 9.3, 14.0 and 20.0% respectively was recorded during I instar, however, the same in case of NIK-1Pr and NIK-1Dp was nil. During II instar, mortality of 9.3, 17.3, 23.3, 9.0 and 24.0% was recorded due to NIK-1Pr, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* respectively. During III instar, the mortality in the transovarially infected progeny batches ranged from 34.0 to 59.3%. During IV instar, mortality of 31.3, 15.3, 3.3 and 31.0% was recorded in NIK-1Pr, NIK-1Cpy, NIK-1So and NIK-1Dp batches respectively. In case of *N. bombycis* infected progeny, all the larvae died before reaching IV instar whereas in case of NIK-1Cpy and NIK-1So infected progenies, all the larvae died before reaching V instar. In case of NIK-1Pr and NIK-1Dp infected progenies, mortality of 20.0 and 26.0% respectively was recorded during V instar. There was no pupation in all the batches under study. Thus, the total mortality in the batches transovarially infected with the isolated microsporidia and *N. bombycis* was 100%.

**IF×HM combinations:** The data as presented in Table 3.8 shows that in the progeny batches of NIK-1Cc infected female mated with healthy male, there was no mortality up to the end of observation period whereas in the progeny batches obtained from the parents infected with NIK-1Cpy, NIK-1So and *N. bombycis*, mortality of 9.3, 12.3
and 20.3% respectively was recorded during I instar, however, the same in case of NIK-1Pr and NIK-1Dp was nil. During II instar, mortality of 8.0, 12.3, 21.3, 6.7 and 23.7% was recorded due to NIK-1Pr, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* respectively. During III instar, the mortality in the transovarially infected progeny batches ranged from 24.7 to 57.7%. During IV instar, mortality of 30.3, 21.0, 8.7 and 28.3% was recorded in NIK-1Pr, NIK-1Cpy, NIK-1So and NIK-1Dp batches respectively. In case of *N. bombycis* infected progeny, all the larvae died before reaching IV instar whereas in case of NIK-1Cpy and NIK-1So infected progenies, all the larvae died before reaching V instar. In case of NIK-1Pr and NIK-1Dp infected progenies, mortality of 33.3 and 40.3% respectively was recorded during V instar. In this moth combination also, there was no pupation in all the batches under study, thus showing a total mortality of 100% within the larval stage.

**Transmission through surface contaminated layings:** The results on the percent mortality observed in the progeny batches obtained from layings externally contaminated with different microsporidia are presented in Table 3.9. No mortality was observed during I instar in any of the microsporidia contaminated batches, whereas in II instar, NIK-1Cpy and NIK-1So contaminated batches showed 9.0 and 9.7% mortality respectively. The same in case of *Nosema bombycis* contaminated batches was recorded as 18.7%. During III instar, mortality of 9.7, 17.0, 19.0, 4.3 and 23.7% was recorded in the batches hatched from the layings externally contaminated with NIK-1Pr, NIK-1Cpy, NIK-1So NIK-1Dp and *N. bombycis* respectively whereas in the batches contaminated with NIK-1Cc, mortality during III instar also was nil. During IV instar, mortality of 22.0, 15.3, 21.0, 21.7, 17.0 and 39.3% was recorded in NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* contaminated batches respectively whereas, the same during V instar was recorded as 23.7, 23.0, 27.3, 29.0, 24.3 and 18.3% respectively. Data also shows that in case of the batches obtained from the layings externally contaminated with *N. bombycis*, all the larvae died before pupation whereas in the batches contaminated with NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp, the metamorphosis of survived larvae into pupae was observed but a mortality of 20.3, 17.7, 13.0, 13.3 and 18.7% respectively was recorded at pupal stage. It is clear from the results as presented in Table 3.9 that the highest total mortality percent was recorded in the batches obtained from the layings...
externally contaminated with *N. bombycis* (100%) followed by NIK-1So (92.7%), NIK-1Cpy (87.3%), NIK-1Pr (75.7%), NIK-1Dp (64.3%) and NIK-1Cc (56.0%).

**Effect of disinfectants against the spores of the isolated microsporidia and *N. bombycis* through disinfection of mulberry leaves:** The results with regard to the effect of two popular disinfectants against the isolated microsporidia and *N. bombycis* through disinfection of mulberry leaves are presented in Table 3.10. No mortality was observed in the larval batches fed with the mulberry leaves smeared separately with the isolated microsporidia and *N. bombycis* followed by dipping in Decol (1.0%) and Sanitech (400 ppm) solutions separately. However, in the inoculated control batches, a mortality of 40.0, 28.0, 70.0, 85.0, 31.7 and 100.0% was recorded due to NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* respectively. In Decol treated NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* batches, total microsporidian infection of 35.3, 18.0, 44.7, 52.7, 28.0 and 62.0% respectively was recorded whereas the same in case of Sanitech treated batches was recorded as 14.7, 7.0, 18.3, 22.3, 10.3 and 33.3% respectively as against the inoculated control batches wherein a total microsporidian infection of 72.7, 53.7, 100.0, 100.0, 65.0 and 100.0% was recorded due to NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* microsporidia respectively. The data as presented in Table 3.10 clearly indicates that among the two disinfectants tested, Sanitech (400 ppm) was comparatively more effective in suppression of the microsporidian disease and the treating of NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* microsporidia smeared mulberry leaf with Sanitech solution resulted in a disease suppression of 79.7, 86.9, 81.7, 77.7, 84.0 and 66.7% respectively when compared to inoculated control batches. Similarly, treating of the mulberry leaf smeared with the microsporidia *viz.*, NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis*, with Decol (1.0%) solution resulted in a disease suppression of 51.3, 66.4, 55.3, 47.3, 56.8 and 38.0% respectively when compared to the inoculated control batches.

The results on the effect of feeding microsporidian smeared leaf dipped in Decol (1.0%) and Sanitech (400 ppm) solutions on the rearing performance of silkworm are presented in Table 3.11.

**Larval weight:** With respect to the two disinfectants tested, the larval weight was comparatively more in the batches fed with the mulberry leaf smeared separately with
the isolated microsporidia followed by dipping in Sanitech (400 ppm) solution and was recorded to be highest in NIK-1Cc treated batches (42.16 g) followed by NIK-1Dp (42.01 g), NIK-1Pr (41.54 g), NIK-1Cpy (40.60 g), NIK-1So (40.42 g) and *N. bombycis* (40.27 g) treated batches whereas, in the batches fed with the mulberry leaf smeared with the isolated microsporidia followed by dipping in Decol (1.0%) solution, the highest larval weight was recorded in NIK-1Cc treated batches (40.84 g) followed by NIK-1Dp (40.66 g), NIK-1Pr (40.35 g), NIK-1Cpy (39.85), NIK-1So (39.78 g) which in turn was followed by the batches treated with *N. bombycis* (39.58 g). However, in the inoculated control batches, the larval weight was significantly less and was recorded as 39.32, 40.11, 38.31, 38.11, 40.00 and 35.24 g in NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp and *N. bombycis* inoculated batches respectively. On the other hand, the larval weight was significantly higher (43.08 g) in the normal control batches compared to that of the disinfectant treated as well as inoculated control batches.

**Larval duration:** In the batches fed with mulberry leaf smeared with the isolated microsporidia and *N. bombycis* followed by dipping in Decol and Sanitech solutions separately, there was no effect on the larval duration and it was recorded to be same as that of the normal control batches (24 days). On the other hand, in the inoculated control batches of all the isolated microsporidia as well as *N. bombycis*, the larval duration was recorded to be prolonged by one day and was recorded as 25 days.

**Pupation rate:** In the batches fed with the mulberry leaf smeared with the microsporidian spores followed by dipping separately in the two selected disinfectants (Decol and Sanitech), the pupation rate ranged from 94.3 to 95.7% which was similar to that of the normal control batches whereas, in the microsporidia inoculated control batches, there was a significant reduction in pupation rate and it ranged from 10.7 to 67.7% only. Significantly, in the *N. bombycis* inoculated control batches, 100% larval mortality was recorded and as a result, there was no pupation.

**Single cocoon weight:** The cocoon weight was comparatively more in the batches fed with mulberry leaf smeared with the different microsporidia followed by dipping in 400 ppm Sanitech solution and ranged from 1.605 to 1.680 g whereas the same in case of the batches fed with the microsporidian smeared leaf dipped in 1.0% Decol ranged from 1.554 to 1.647 g. In the inoculated control batches, the cocoon weight was significantly less and ranged from 1.532 to 1.595 g. Compared to the disinfectant
treated batches and inoculated control batches, the cocoon weight was higher in the normal control batches (1.735 g).

**Single shell weight:** In the batches fed with mulberry leaf smeared with the different microsporidia followed by dipping in 400 ppm Sanitech solution, the shell weight was comparatively more than that of the batches treated with 1.0% Decol and ranged from 0.337 to 0.373 g. The same in case of the Decol treated batches ranged from 0.319 to 0.358 g. In case of the inoculated control batches, there was a significant reduction in the shell weight and it ranged from 0.305 to 0.331 g only compared to the normal control batches wherein the shell weight of 0.402 g was recorded.

**Shell percentage:** The infection due to the isolated microsporidia though led to the reduction in the shell percentage of the treated batches but this reduction was more pronounced in the inoculated control batches compared to that of the batches treated separately with Decol and Sanitech solutions. Among the two disinfectants tested, the shell percentage was more in the Sanitech treated batches compared to that of the Decol treated batches. In the batches fed with the mulberry leaf smeared separately with the spores of the isolated microsporidia and *N. bombycis* followed by dipping of the leaf in 400 ppm Sanitech solution, the highest shell percentage was recorded in NIK-1Cc inoculated batches (22.23%) followed by NIK-1Dp (22.04%), NIK-1Pr (21.78%), NIK-1Cpy (21.42%), NIK-1So (21.23%) and *N. bombycis* inoculated batches (21.03%). The same trend was recorded in Decol treated batches also and the highest shell percentage was recorded in NIK-1Cc inoculated batches (21.73%) followed by NIK-1Dp (21.59%), NIK-1Pr (21.25%), NIK-1Cpy (21.03%), NIK-1So (20.83%) which in turn was followed by *N. bombycis* inoculated batches (20.56%). In the inoculated control batches, the shell percentage was significantly less and was recorded to be the lowest in the batches inoculated with NIK-1So (19.94 %) followed by NIK-1Cpy (20.06%), NIK-1Pr (20.23%), NIK-1Dp (20.52%) and NIK-1Cc (20.74%) compared to the normal control batches wherein a shell percentage of 23.20% was recorded.

**DISCUSSION**

Mulberry, which is a sole food plant for the silkworm, *Bombyx mori* L. is frequented by a number of insects either for food or a casual visit from nearby agricultural crops. A number of workers have reported that these pests may harbour
different types of microsporidia (Kishore et al., 1994; Sharma et al., 1989, 2003; Singh et al., 2008). Accordingly, in the present study, insect pests collected from mulberry gardens and agricultural crop fields were screened for the presence of microsporidia. The microsporidian infection was recorded in samples of *Pieris rapae*, *Catopsilia crocale*, *Catopsilia pyranthe*, *Spilosoma obliqua* and *Diaphania pulverulentalis* specimens.

A comparison of the infectivity of the isolated microsporidia with that of *N. bombycis* indicates that the isolated microsporidia cause significantly less mortality in larval and pupal stages compared to that caused by *N. bombycis*. The isolated microsporidia differ from *N. bombycis* with respect to infectivity, spore morphology, spore ultra structure, serological affinity, germination response, rate of sporulation at different temperatures, mode of infection, site of infection and pathogenicity. Also, the ability of the isolated microsporidia to spread infection within a healthy colony of silkworm is limited compared to *Nosema bombycis*. The isolated microsporidia are capable of infecting different breeds of silkworm but the susceptibility of different breeds to infection by the isolated microsporidia is less when compared to their susceptibility to infection by *N. bombycis*.

The isolated microsporidia were found to adversely affect the economic characters of silkworm. The survival percentage of the larvae was significantly lowered and the larval duration prolonged. The larval weight, cocoon weight, shell weight and percent silk content in the resultant cocoons was also reduced compared to the healthy control. Kudo (1931) reported that heavily infected larvae of *Bombyx mori* do not spin cocoons and die whereas mild infection allows the larvae to spin cocoons. Noamani et al. (1971) and Patil and Geethabai (1989) also reported inferior cocoon characters in pebrine treated multivoltine and bivoltine races. According to Baig (1994), as the larval weight decreases due to progressive pebrine infection, it results in inferior cocoon characters. Also due to increase in the time duration from the day of inoculation to spinning, the silk glands get infected and due to their impaired function, the cocoons obtained are significantly inferior compared to the cocoons obtained from healthy silkworm larvae. The cocoon characters *viz.*, single cocoon weight, single shell weight and silk ratio are adversely affected. Silk from the cocoons of pebrine infected larvae is inferior in strength and uniformity of thickness to that of healthy larvae (Steinhaus, 1949). Jameson (1922) and Ghosh (1944) have also
reported that pebrine infected silkworms spin flimsy and poor quality cocoons. Similar findings have also been reported in a recent study wherein the impact of a microsporidian (Lbms) isolated from a silkworm breed of North East Indian origin on economic characters of different bivoltine and multivoltine breeds of silkworm has been studied. According to the study, the microsporidian infection significantly lowered the percent survival (ERR%), larval weight, single cocoon weight, shell weight and percent silk content of all the silkworm breeds studied (Shabir Ahmad Bhat and Nataraju, 2005a).

Inoculation of the spores of the isolated microsporidia to third instar silkworm larvae at two lowest concentrations viz., $1 \times 10^2$ and $1 \times 10^3$ spores/ml did not cause any significant impact on the health status and rearing performance of silkworm. At the said spore concentrations, there was no larval or pupal mortality in the batches inoculated with the microsporidia isolated from insect pests of mulberry and agricultural crops. The results are in conformity with an earlier study by Choi et al. (2002) wherein it has been reported that at lower spore dosages, many of the individuals survive to adulthood and only few of these adults are infected. Also, at these two concentrations, there was no significant impact on the larval weight, larval duration, pupation rate, cocoon weight, shell weight and percent silk content of the inoculated batches. However, at the higher concentrations, there was a significant impact on the health status and rearing performance of silkworm. Compared to the isolated microsporidia, the inoculation of the spores of *N. bombycis* to silkworm at the lowest concentration ($1 \times 10^2$ spores/ml) resulted in a significant impact on the health status of silkworm leading to a larval and pupal mortality of 1.0 and 3.7% respectively. Also, 63.3% of the emerged moths were infected and a total infection of 68.0% was recorded. Also, there was a reduction in larval weight, cocoon weight, shell weight and percent silk content. However, at this concentration, there was no impact on the larval duration. The results of the present study, therefore reveal that as the infection of silkworm larvae by the isolated microsporidia at the lower spore dosages does not cause any significant impact on the health status and rearing performance of the host and if this level of infection is detected in a silkworm crop, such cocoons though considered unfit for seed preparation (as some degree of infection is recorded at the moth stage) can be used for commercial purpose to avoid the economic loss.
Infection due to the isolated microsporidia caused significant changes in the larval morphology. The progressive increase in larval length, width and weight were found to be significantly affected in the inoculated batches compared to the healthy control batches. In a few earlier studies also, the growth of silkworm larvae has been reported to be reduced due to infection with \textit{N. bombycis} and also other parasites (Baig, 1994; Nath \textit{et al}., 1990; Rath \textit{et al}.., 2000; Geortz \textit{et al}., 2004). In insects, the initial phase of microsporidian infection is a stimulation of the cell to accumulate endoplasmic reticula around the pathogen, an increase in the number of mitochondria and stimulation of nucleic acid synthesis (Issi, 1986). However, the destruction of the cell begins when the microsporidia go into sporogony leading to the reduction in the number of endoplasmic reticula and an increased vacuolation in the cell (Tanabe, 1971; Liu and Davies, 1972; Issi, 1986). At the terminal stage, only the nucleus and mitochondria remain, with the result, the growth of the host is inhibited. In the present study also, the growth of silkworm larvae (host) was found to be markedly inhibited by microsporidian infection. However, a recent study carried out by Shabir Ahmad Bhat and Nataraju (2007c) on the effect of infection caused by a microsporidian isolated from Lamerin breed of silkworm collected from north eastern state of India shows that it does not cause any perceptible effect on the morphology of silkworm larvae of the infected batches. In yet another study, microsporidian infection has been reported to cause marked ultrastructural changes in the cells of certain glands leading to depletion of ribosomes, disarrangement of endoplasmic reticula, decrease in size of mitochondria, formation of vacuoles in the cytoplasm and thereby, retarded growth (Jurand \textit{et al}., 1967). Brooks (1971) reported that due to microsporidian infection, inflammatory responses develop in some insects and such responses, in severe cases, produce nodules that are infiltrated with haemocytes and melanized leading to derangement of the infected organ. In honey bees also, \textit{Nosema apis} has been reported to cause severe cytopathology, such as the disintegration and vacuolation of the cytoplasm of the glands (Wang and Moeller, 1971) and decreased RNA synthesis in the midgut cells (Hartwig and Przelecka, 1971). Kucera and Weiser (1975) reported that the infection by \textit{Vairimorpha plodiae} in \textit{Barathra brassicae} and \textit{Galleria mellonella} causes enzymatic disturbances. The activity of lactate dehydrogenase isoenzyme increases substantially by the fifth day of infection leading to marked inhibition in the host growth.
Transmission of microsporidian infection is a key factor in pathogen-host interactions that can influence the population dynamics of the host (Anderson and May, 1981; Mc Callum et al., 2001). There are several potential pathways by which pathogens are transmitted within a host population - the most common pathway is the vertical transmission i.e. the direct transfer of infection from parent to progeny (Fine, 1975; Becnel and Andreadis, 1999). Vertical transmission of pathogens may include one or more of several mechanisms including transovum, transovarial (or transovarian) and venereal transfer, and may involve intermediate hosts, sex ratio distorting (e.g., host-feminizing and male sterilization/killing), farming of microorganisms and vectoring of pathogens (Solter, 2006). Vertical transmission via the gametes among microsporidian parasites has been reported by a number of workers (Ishihara and Fujiwara, 1965; Andreadis, 1983; Canning et al., 1985; Becnel et al., 1989; Dunn et al., 1998). However, in certain cases, even if the microsporidian is within the female gamete, vertical transmission does not occur. In a recorded case in the winter moth, Operophthera brumata (Lepidoptera), the embryo within the infected egg was not infected but the larvae subsequently became infected when the microsporidian spores were ingested together with the remains of the yolk as the larvae eat their way through the egg shell (Canning et al., 1985). In the present study also, the larvae hatched from the eggs externally contaminated with the isolated microsporidia and the standard strain, N. bombycis were found to be infected. According to Dunn et al. (1993) and Terry et al. (1997), vertical transmission from generation to generation might maintain the parasite within the host population. In addition to N. bombycis, several other strains and species of microsporidia have been reported to infect silkworm (Ananthalakshmi et al., 1994; Kishore et al., 1994; Sharma et al., 1989, 2003; Shabir Ahmad Bhat and Nataraju, 2004, Singh et al., 2008). It has been observed that some of the microsporidia are not transmitted by transovarial means and others have low rate of transovarial transmission (Fujiwara, 1980, 1985; Han and Watanabe, 1988; Iwashita et al., 1990; Ananthalakshmi et al., 1994; Nageswara Rao et al., 2004; Shabir Ahmad Bhat and Nataraju, 2005b, Singh et al., 2008). The microsporidian strains viz., Nosema bombycis and Nosema sp., NIK-2r transmit infection to progeny to an extent of 100% (Han and Watanabe, 1988; Ananthalakshmi et al., 1994), whereas the microsporidia viz., Vairimorpha sp. NIS-M12, NIK-4m, Microsporidium sp. NIS-M25, Pleistophora sp. NIS-M27 and
Thelohania sp. NIS-M32 do not transmit infection to the progeny (Kawarabata, 2003; Singh and Saratchandra, 2003). In the present study also the microsporidian NIK-1Cc isolated from Catopsilia crocale did not exhibit transovarial transmission in silkworm. However, four microsporidia viz., NIK-1Pr, NIK-1Cpy, NIK-1So and NIK-1Dp isolated from lepidopteran pests showed varying degrees of transovarial transmission to F1 progeny. This finding is significant as it adds one more dimension to the already existing problem of Nosema bombycis, a prominent microsporidian causing pebrine disease in silkworm showing 100% transovarial transmission. Siegel et al. (1986) reported that a high percentage of transovarially infected larvae die. In the present study also, there was significantly higher level of mortality in the transovarially infected larvae. According to Solter (2006), many of the microsporidian species are transmitted from infected females to nearly 100% of offsprings resulting in decreased fecundity and significantly higher mortality in transovarially infected larvae.

The microsporidian infected adults of European corn borer, P. nubilalis (Zimmack and Brindley, 1957) and Spruce budworm, Choristoneura fumiferana (Thomson, 1958) have been reported to lay fewer eggs than the normal females. Talukdar (1960, 1962) working with Philosoma ricini observed that the fecundity of the infected moths was only 62 as against 324 in healthy females. Verber and Jassic (1961) observed reduction in fecundity of Hyphantria cunea infected by N. bombycis. They also reported reduced fecundity of females of Bombyx mori infected with N. bombycis. Jolly and Sen (1972) observed reduction in egg laying capacity of the females of Antheraea mylitta infected with a Nosema sp. The reasons for the decreased fecundity have been given by different workers as follows: Fecundity is dependent on the reserves of nutritious substances accumulated in the larvae before pupation, as the silk moths do not consume any food (Verber and Jassic, 1961). The microsporidia mainly develop in the fat bodies of host wherein many metabolic processes occur (Wyatt, 1980) and the destruction/depletion of the nutritive reserves due to the infection and multiplication of microsporidia may result in less fecund females (Armstrong and Bass, 1986). Continuous multiplication and deriving of nutrients by the parasite from the host results in retarded growth which is evidenced by depletion of host cytoplasmic components. This has a direct effect on the fecundity, development of embryo and subsequently on hatching (Jyothi and Patil,
In the present study also, a significant decrease in fecundity of microsporidian inoculated batches (IF×IM and IF×HM combinations) was recorded which is in confirmation with a recent report by Jyothi and Patil (2008) wherein fecundity has been found to be significantly reduced in the batches where both male and female moths were pebrine infected and crossed (IF×IM) and also where pebrine infected female moths were crossed with uninfected male moths (IF×UM).

Transovarial transmission of *Nosema bombycis* has been reported in several insects in addition to *Bombyx mori* viz., European cabbage worm, *Pieris brassicae* L. (Blunk, 1952), imported cabbage worm, *Pieris rapae* L. (Tanada, 1955), European corn borer, *P. nubilalis* (Zimmack et al., 1954; Kramer, 1959b), spruce bud worm, *Choristoneura fumiferana* Clem. (Thomson, 1958), fall web worm, *Hyphantria cunea* (Weiser, 1957), alfalfa snout beetle, *Brachyrhinus linguistics* L. (Weiser, 1958) and mulberry leaf roller, *Diaphania pulverulentalis* (Ramegowda and Geethabai, 2005). Venereal transmission also has been observed in ceratin microsporidia such as *Nosema kingi* in drosophilids (Armstrong, 1976) and *Thelohania* species in mosquitoes (Kellen et al., 1965). Venereal transmission of a microsporidian in the Indian meal moth, *Plodia interpunctella* has been reported by Kellen and Lindegren (1971). Also, a few cases of venereal transmission have been documented by Solter (2006). However, in the present study, the isolated microsporidia did not exhibit any venereal transmission showing that though the male moth carries infection, it does not have any role in vertical transmission of the pathogen via sperm into the progeny eggs. Patil et al. (2002) also reported that male moth does not play any significant role in disease transmission to progeny but the infected male moth may transfer the spores along with the seminal fluid to the bursa copulatrix of the female moth during mating and the same is not transferred to the progeny.

The disinfection of silkworm rearing house, silkworm rearing appliances and the silkworm seed production centers is generally followed to eliminate the microsporidian spores which otherwise may lead to secondary infection. Chemical disinfectants such as Hilite, Sodium hypochlorite, Bleaching powder and Formalin have been reported to be effective against the spores of *N. bombycis* (Baig et al., 1989). Iwashita and Zhou (1988) and Patil (1991) also have reported the efficacy of Calcium chloride against *N. bombycis*. Nataraju (1995) and Balavenktasubbaiah et al. (1999) have reported that Chlorine dioxide is an effective disinfectant against the
silkworm pathogens including *N. bombycis*. In a recent study by Singh et al. (2007a), disinfection of mulberry leaf in addition to the disinfection of rearing house and appliances also has been reported to be effective against the spores of *N. bombycis*. Hence, in the present study also, disinfection of the microsporidian spore smeared mulberry leaf with two popular disinfectants *viz.*, Decol (1.0%) and Sanitech (400 ppm) was carried out to test their efficacy against the spores of the isolated microsporidia as well as *N. bombycis*. Both the disinfectants were found to be effective against the spores of the isolated microsporidia and *N. bombycis*. In the batches treated with the two disinfectants, there was no mortality due to microsporidiosis whereas in the inoculated control batches, mortality due to microsporidiosis was recorded which ranged from 28.0 to 100.0%. Among the two disinfectants tested, Sanitech (400 ppm) was comparatively more effective in microsporidian disease suppression and led to the disease suppression percentage ranging from 66.7 to 86.9% whereas Decol (1.0%) led to a disease suppression ranging from 38.0 to 66.4%. The two disinfectants also ensured better rearing performance of the microsporidia treated batches compared to the inoculated control batches. The rearing parameters *viz.*, larval weight, pupation rate, cocoon weight, shell weight and shell percentage were recorded to be better in the batches fed with the mulberry leaf smeared with the spores of the different microsporidia followed by dipping separately in Sanitech (400 ppm) and Decol (1.0%) solutions compared to the inoculated control batches. However, the disease suppression due to the treatment of microsporidian contaminated mulberry leaf with the said disinfectants was not 100.0%, therefore, the only available option is that the insect pests of mulberry and other agricultural crops should be strictly kept under check as they carry microsporidian spores cross infective to silkworm which may not be eliminated to cent percent level by the use of the disinfectants which are commonly used in Sericulture industry.

Present study, therefore, establishes that a number of microsporidian strains are harboured by wild lepidopterans and can cause cross infection in silkworm. This also explains sudden and sporadic outbreak of pebrine disease from time to time in sericultural areas in India. The study concludes that out of five different microsporidia isolated from insect pests collected from mulberry gardens and other agricultural fields, four microsporidia showed considerable infectivity and transovarial
transmission in silkworm. These microsporidia, therefore, constitute a potential threat of gaining entry into silkworm rearing and perpetuate the infection despite routine care taken in mother moth examination and sanitations. To keep this disease under check, sericulture industry has to keep in place a robust system of mother moth examination in seed production centers. Present study also underlines the need for detailed studies on the population dynamics of various insect pests of mulberry and other agricultural crops and outbreak of microsporidian disease in silkworm.
Table 3.1: Impact of microsporidian infection on the economic characters of silkworm

<table>
<thead>
<tr>
<th>Silkworm breed</th>
<th>Microsporidian isolates</th>
<th>Larval weight (g)</th>
<th>Larval duration (Days: Hours)</th>
<th>Pupation rate %</th>
<th>Cocoon weight (g)</th>
<th>Shell weight (g)</th>
<th>SR %</th>
<th>Filament length (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSR2</td>
<td>NIK-1Pr</td>
<td>31.1 ± 0.04</td>
<td>25:00</td>
<td>79.0 (62.7) ± 1.00</td>
<td>1.480 ± 0.00</td>
<td>0.320 ± 0.00</td>
<td>21.6 ± 0.26</td>
<td>721.0 ± 1.53</td>
</tr>
<tr>
<td></td>
<td>NIK-1Cc</td>
<td>33.0 ± 1.00</td>
<td>25:00</td>
<td>86.3 (68.2) ± 1.53</td>
<td>1.495 ± 0.004</td>
<td>0.328 ± 0.002</td>
<td>21.9 ± 0.13</td>
<td>807.0 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>NIK-1Cpy</td>
<td>30.3 ± 0.12</td>
<td>25:00</td>
<td>75.0 (60.0) ± 1.00</td>
<td>1.478 ± 0.002</td>
<td>0.296 ± 0.002</td>
<td>20.0 ± 0.08</td>
<td>644.0 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>NIK-1So</td>
<td>30.0 ± 0.04</td>
<td>25:00</td>
<td>73.0 (58.7) ± 1.00</td>
<td>1.470 ± 0.002</td>
<td>0.292 ± 0.002</td>
<td>19.8 ± 0.13</td>
<td>635.0 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>NIK-1Dp</td>
<td>31.8 ± 0.08</td>
<td>25:00</td>
<td>81.0 (64.1) ± 1.00</td>
<td>1.490 ± 0.002</td>
<td>0.325 ± 0.002</td>
<td>21.8 ± 0.12</td>
<td>782.0 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>Nosema bombycis</td>
<td>26.6 ± 0.06</td>
<td>25:00</td>
<td>64.7 (53.5) ± 1.53</td>
<td>1.428 ± 0.001</td>
<td>0.276 ± 0.002</td>
<td>19.3 ± 0.12</td>
<td>617.0 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>Normal Control</td>
<td>35.7 ± 0.39</td>
<td>23:00</td>
<td>95.7 (78.0) ± 0.58</td>
<td>1.600 ± 0.050</td>
<td>0.366 ± 0.002</td>
<td>22.8 ± 0.69</td>
<td>980.0 ± 2.00</td>
</tr>
<tr>
<td>Pure Mysore</td>
<td>NIK-1Pr</td>
<td>22.5 ± 0.15</td>
<td>29:00</td>
<td>82.0 (64.9) ± 2.00</td>
<td>1.044 ± 0.004</td>
<td>0.130 ± 0.002</td>
<td>12.4 ± 0.15</td>
<td>369.0 ± 1.70</td>
</tr>
<tr>
<td></td>
<td>NIK-1Cc</td>
<td>23.9 ± 0.03</td>
<td>29:00</td>
<td>88.3 (70.0) ± 0.58</td>
<td>1.113 ± 0.003</td>
<td>0.141 ± 0.002</td>
<td>12.7 ± 0.13</td>
<td>400.7 ± 3.06</td>
</tr>
<tr>
<td></td>
<td>NIK-1Cpy</td>
<td>21.4 ± 0.01</td>
<td>29:00</td>
<td>80.3 (63.6) ± 1.53</td>
<td>1.005 ± 0.003</td>
<td>0.122 ± 0.001</td>
<td>12.1 ± 0.03</td>
<td>325.0 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>NIK-1So</td>
<td>21.1 ± 0.03</td>
<td>29:00</td>
<td>79.0 (62.7) ± 2.00</td>
<td>0.996 ± 0.002</td>
<td>0.117 ± 0.001</td>
<td>11.7 ± 0.04</td>
<td>291.3 ± 2.52</td>
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<tr>
<td></td>
<td>NIK-1Dp</td>
<td>22.9 ± 0.02</td>
<td>29:00</td>
<td>86.0 (68.0) ± 2.00</td>
<td>1.082 ± 0.003</td>
<td>0.137 ± 0.002</td>
<td>12.6 ± 0.11</td>
<td>371.0 ± 2.00</td>
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<tr>
<td></td>
<td>Nosema bombycis</td>
<td>18.8 ± 0.06</td>
<td>29:00</td>
<td>73.3 (58.9) ± 1.15</td>
<td>0.964 ± 0.003</td>
<td>0.107 ± 0.002</td>
<td>11.1 ± 0.22</td>
<td>284.0 ± 6.00</td>
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<tr>
<td></td>
<td>Normal Control</td>
<td>25.8 ± 0.27</td>
<td>28:00</td>
<td>95.3 (77.5) ± 0.58</td>
<td>1.127 ± 0.006</td>
<td>0.154 ± 0.002</td>
<td>13.6 ± 0.11</td>
<td>443.0 ± 2.20</td>
</tr>
</tbody>
</table>

Values are mean ± SD and the values in parenthesis are angular transformed

A CD at 5 % 0.19 - (0.7) 0.008 0.001 0.14 6.08
B CD at 5 % 0.36 - (1.2) 0.016 0.002 0.27 11.38
A×B S. E. ± 0.17 - 0.59 0.008 0.001 0.13 5.55
CD at 5 % 0.51 - (1.7) 0.020 0.003 0.38 16.09
Table 3.2: Impact of lower pathogen loads on the health status and rearing performance of the silkworm, *Bombyx mori* L.

<table>
<thead>
<tr>
<th>Microsporidian isolates</th>
<th>Spore concentration (spores/ml)</th>
<th>Mortality due to infection (%)</th>
<th>Moths infected (%)</th>
<th>Total infection (%)</th>
<th>Larval weight (g)</th>
<th>Larval duration (D:H)</th>
<th>Pupation rate (%)</th>
<th>Single cocoon weight (g)</th>
<th>Single shell weight (g)</th>
<th>SR %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Larva</td>
<td>Pupa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIK-1Pr</td>
<td>1×10^2</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>39.17</td>
<td>24.00</td>
<td>95.0(77.1)</td>
<td>1.602</td>
<td>0.373</td>
<td>23.29</td>
</tr>
<tr>
<td></td>
<td>1×10^3</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>28.0(31.9)</td>
<td>38.66</td>
<td>24.00</td>
<td>93.0(74.6)</td>
<td>1.587</td>
<td>0.357</td>
<td>22.48</td>
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<tr>
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<td>1×10^4</td>
<td>1.0(5.7)</td>
<td>1.7(7.5)</td>
<td>32.3(34.6)</td>
<td>38.54</td>
<td>24.00</td>
<td>93.0(74.6)</td>
<td>1.560</td>
<td>0.347</td>
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<td>1×10^5</td>
<td>6.7(15.0)</td>
<td>3.7(11.1)</td>
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<td>38.23</td>
<td>24.00</td>
<td>85.3(67.4)</td>
<td>1.537</td>
<td>0.337</td>
<td>21.93</td>
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<td>NIK-1Cc</td>
<td>1×10^2</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>39.17</td>
<td>24.00</td>
<td>95.0(77.1)</td>
<td>1.604</td>
<td>0.374</td>
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<td>0.0(0.0)</td>
<td>14.0(21.9)</td>
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<td>24.00</td>
<td>94.0(75.8)</td>
<td>1.591</td>
<td>0.364</td>
<td>22.86</td>
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<td>1×10^4</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>17.3(24.6)</td>
<td>38.74</td>
<td>24.00</td>
<td>94.0(75.8)</td>
<td>1.572</td>
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<td></td>
<td>1×10^5</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>21.3(27.5)</td>
<td>38.68</td>
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<td>94.0(75.8)</td>
<td>1.565</td>
<td>0.352</td>
<td>22.52</td>
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<tr>
<td>NIK-1Cpy</td>
<td>1×10^2</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>8.0(16.4)</td>
<td>38.80</td>
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<td>94.3(76.2)</td>
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<td>0.365</td>
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<td>0.0(0.0)</td>
<td>30.0(33.2)</td>
<td>38.30</td>
<td>24.00</td>
<td>92.0(73.6)</td>
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<td>0.350</td>
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<td>3.7(11.1)</td>
<td>35.3(36.4)</td>
<td>38.21</td>
<td>24.00</td>
<td>85.3(67.4)</td>
<td>1.551</td>
<td>0.342</td>
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<td>16.3(23.8)</td>
<td>4.3(11.9)</td>
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<td>37.90</td>
<td>24.00</td>
<td>75.0(60.0)</td>
<td>1.529</td>
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<td>21.74</td>
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<tr>
<td>NIK-1So</td>
<td>1×10^2</td>
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<td>0.0(0.0)</td>
<td>13.0(21.1)</td>
<td>38.70</td>
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<tr>
<td></td>
<td>1×10^3</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>35.0(36.3)</td>
<td>38.10</td>
<td>24.00</td>
<td>92.0(73.6)</td>
<td>1.580</td>
<td>0.349</td>
<td>22.09</td>
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<tr>
<td></td>
<td>1×10^4</td>
<td>9.7(18.1)</td>
<td>4.7(12.5)</td>
<td>36.3(37.0)</td>
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<td>24.00</td>
<td>81.3(64.4)</td>
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<td>20.3(26.8)</td>
<td>6.3(14.5)</td>
<td>60.3(50.9)</td>
<td>37.74</td>
<td>24.00</td>
<td>69.0(56.2)</td>
<td>1.519</td>
<td>0.326</td>
<td>21.47</td>
</tr>
<tr>
<td>Microsporidian isolates</td>
<td>Spore concentration (spores/ml)</td>
<td>Mortality due to infection (%)</td>
<td>Moths infected (%)</td>
<td>Total infection (%)</td>
<td>Larval weight (g)</td>
<td>Larval duration (D:H)</td>
<td>Pupation rate (%)</td>
<td>Single cocoon weight (g)</td>
<td>Single shell weight (g)</td>
<td>SR %</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------------------------</td>
<td>-------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>--------------------------</td>
<td>------------------------</td>
<td>------</td>
</tr>
<tr>
<td>NIK-1Dp</td>
<td>$1 \times 10^2$</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>39.17</td>
<td>24.00</td>
<td>95.0(77.1)</td>
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<td>0.0(0.0)</td>
<td>17.0(24.3)</td>
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<td>94.0(75.8)</td>
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<td>0.0(0.0)</td>
<td>21.7(27.7)</td>
<td>38.71</td>
<td>24.00</td>
<td>94.0(75.8)</td>
<td>1.570</td>
<td>0.355</td>
<td>22.6</td>
</tr>
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<td>$1 \times 10^5$</td>
<td>1.0(5.7)</td>
<td>1.0(5.7)</td>
<td>25.0(30.0)</td>
<td>38.61</td>
<td>24.00</td>
<td>93.7(75.5)</td>
<td>1.560</td>
<td>0.348</td>
<td>22.32</td>
</tr>
<tr>
<td>N. bombycis</td>
<td>$1 \times 10^2$</td>
<td>1.0(5.7)</td>
<td>3.7(11.1)</td>
<td>63.3(52.7)</td>
<td>37.77</td>
<td>24.00</td>
<td>91.0(72.5)</td>
<td>1.558</td>
<td>0.335</td>
<td>21.49</td>
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<td>$1 \times 10^3$</td>
<td>3.0(9.9)</td>
<td>7.3(15.7)</td>
<td>75.7(60.5)</td>
<td>37.17</td>
<td>24.00</td>
<td>85.3(67.4)</td>
<td>1.542</td>
<td>0.320</td>
<td>20.78</td>
</tr>
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<td>$1 \times 10^4$</td>
<td>10.7(19.1)</td>
<td>10.0(18.4)</td>
<td>68.7(55.9)</td>
<td>36.95</td>
<td>25.00</td>
<td>75.0(60.0)</td>
<td>1.507</td>
<td>0.302</td>
<td>20.03</td>
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<td>$1 \times 10^5$</td>
<td>21.7(27.7)</td>
<td>14.7(22.5)</td>
<td>55.7(48.3)</td>
<td>36.64</td>
<td>25.00</td>
<td>59.3(50.3)</td>
<td>1.487</td>
<td>0.295</td>
<td>19.82</td>
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<td>Normal Control</td>
<td>-</td>
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<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>39.18</td>
<td>24.00</td>
<td>95.7(78.0)</td>
<td>1.607</td>
<td>0.375</td>
<td>23.32</td>
</tr>
<tr>
<td>A</td>
<td>CD at 5 %</td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(0.5)</td>
<td>(0.5)</td>
<td>0.02</td>
<td>--</td>
<td>(0.7)</td>
<td>0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>B</td>
<td>CD at 5 %</td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td>(0.5)</td>
<td>0.02</td>
<td>--</td>
<td>(0.7)</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>A x B</td>
<td>S. E. ±</td>
<td>0.23</td>
<td>0.27</td>
<td>0.39</td>
<td>0.43</td>
<td>0.015</td>
<td>--</td>
<td>0.57</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>CD at 5 %</td>
<td>(0.7)</td>
<td>(0.8)</td>
<td>(1.1)</td>
<td>(1.2)</td>
<td>0.04</td>
<td>--</td>
<td>(1.6)</td>
<td>0.003</td>
<td>0.0010</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Values in parenthesis are angular transformed
Table 3.3: Impact of infection by the isolated microsporidia on the progressive increase in larval length of CSR2 breed of the silkworm, *Bombyx mori* L

<table>
<thead>
<tr>
<th>Microsporidian isolates</th>
<th>Larval length (mm) Days post Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>NIK-1Pr</td>
<td>13.50 ± 0.55</td>
</tr>
<tr>
<td>NIK-1Cc</td>
<td>13.50 ± 0.55</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
<td>13.50 ± 0.55</td>
</tr>
<tr>
<td>NIK-1So</td>
<td>13.33 ± 0.52</td>
</tr>
<tr>
<td>NIK-1Dp</td>
<td>13.50 ± 0.55</td>
</tr>
<tr>
<td><em>Nosema bombycis</em></td>
<td>13.33 ± 0.52</td>
</tr>
<tr>
<td>Healthy control</td>
<td>13.67 ± 0.52</td>
</tr>
<tr>
<td>S. E. ±</td>
<td>0.22 ± 0.22</td>
</tr>
</tbody>
</table>

Values are mean ± SD
Table 3.4: Impact of infection by the isolated microsporidia on the progressive increase in larval width of CSR2 breed of the silkworm, *Bombyx mori* L.

<table>
<thead>
<tr>
<th>Microsporidian isolates</th>
<th>Larval width (mm) Days post inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>NIK-1Pr</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>NIK-1Cc</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>NIK-1So</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>NIK-1Dp</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>Nosema bombycis</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>Healthy control</td>
<td>3.00 ± 0.00</td>
</tr>
</tbody>
</table>

S. E. ± -- 0.20 -- -- -- -- -- 0.22 0.17 0.12 0.14 0.14 0.17 0.08 0.11 0.18
CD at 5% -- -- -- -- -- -- -- 0.62 0.48 0.33 0.39 0.40 0.48 0.23 0.32 0.53
CD at 1% -- -- -- -- -- -- -- 0.83 0.64 0.44 0.53 0.54 0.64 0.30 0.43 0.71

*Values are mean ± SD*
Table 3.5: Impact of infection by the isolated microsporidia on the progressive increase in larval weight of CSR2 breed of the silkworm, *Bombyx mori* L.

<table>
<thead>
<tr>
<th>Microsporidian isolates</th>
<th>Weight (g/10 larvae) Days post inoculation</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIK-1Pr</td>
<td></td>
<td>0.44</td>
<td>1.07</td>
<td>1.66</td>
<td>2.06</td>
<td>2.08</td>
<td>2.09</td>
<td>5.12</td>
<td>7.62</td>
<td>8.96</td>
<td>9.13</td>
<td>9.15</td>
<td>9.19</td>
<td>21.14</td>
<td>30.08</td>
<td>33.51</td>
<td>34.94</td>
</tr>
<tr>
<td></td>
<td>S.E. ±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<td>±</td>
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<td>±</td>
</tr>
<tr>
<td>NIK-1Cc</td>
<td></td>
<td>0.45</td>
<td>1.07</td>
<td>1.66</td>
<td>2.06</td>
<td>2.08</td>
<td>2.09</td>
<td>5.12</td>
<td>7.64</td>
<td>9.02</td>
<td>9.44</td>
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</table>

Values are mean ± SD
Table 3.6: Assay on transovarial and venereal transmission of the isolated microsporidia in silkworm

<table>
<thead>
<tr>
<th>Microsporidian isolates</th>
<th>Moths paired</th>
<th>Fecundity (No.)</th>
<th>No. of dead eggs</th>
<th>Infected dead eggs (%)</th>
<th>No. of larvae hatched</th>
<th>Hatching %</th>
<th>Larvae infected (%)</th>
<th>Transmission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIK-1Pr</td>
<td>IF×IM</td>
<td>435.0±6.5</td>
<td>18.0±1.5</td>
<td>81.0(64.1)±1.7</td>
<td>370.0±5.0</td>
<td>85.1(77.4)±0.1</td>
<td>95.3(77.4)±0.2</td>
<td>94.6(76.5)±0.1</td>
</tr>
<tr>
<td></td>
<td>IF×HM</td>
<td>436.0±7.0</td>
<td>18.0±6.6</td>
<td>80.3(63.6)±1.5</td>
<td>372.0±5.6</td>
<td>85.3(67.4)±0.2</td>
<td>91.2(72.7)±0.1</td>
<td>90.6(72.1)±0.0</td>
</tr>
<tr>
<td></td>
<td>HF×IM</td>
<td>468.0±5.6</td>
<td>11.0±1.2</td>
<td>0.0(0.0)±0.0</td>
<td>432.0±6.0</td>
<td>92.3(73.8)±0.3</td>
<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
</tr>
<tr>
<td>NIK-1Cc</td>
<td>IF×IM</td>
<td>455.0±3.0</td>
<td>11.0±1.2</td>
<td>0.0(0.0)±0.0</td>
<td>407.0±1.5</td>
<td>89.4(71.0)±0.3</td>
<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
</tr>
<tr>
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<td>IF×HM</td>
<td>458.0±3.5</td>
<td>11.0±1.0</td>
<td>0.0(0.0)±0.0</td>
<td>410.0±2.5</td>
<td>89.5(71.1)±0.1</td>
<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
</tr>
<tr>
<td></td>
<td>HF×IM</td>
<td>468.0±3.1</td>
<td>10.0±0.6</td>
<td>0.0(0.0)±0.0</td>
<td>433.0±1.5</td>
<td>92.5(74.1)±0.3</td>
<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
<td>IF×IM</td>
<td>427.0±2.5</td>
<td>26.0±2.5</td>
<td>100.0(90.0)±0.0</td>
<td>357.0±2.6</td>
<td>83.5(66.0)±0.2</td>
<td>100.0(90.0)±0.0</td>
<td>100.0(90.0)±0.0</td>
</tr>
<tr>
<td></td>
<td>IF×HM</td>
<td>430.0±2.1</td>
<td>22.0±2.0</td>
<td>100.0(90.0)±0.0</td>
<td>360.0±2.1</td>
<td>83.7(66.2)±0.1</td>
<td>100.0(90.0)±0.0</td>
<td>100.0(90.0)±0.0</td>
</tr>
<tr>
<td></td>
<td>HF×IM</td>
<td>463.0±2.5</td>
<td>12.0±1.0</td>
<td>0.0(0.0)±0.0</td>
<td>424.0±3.1</td>
<td>91.7(73.2)±0.2</td>
<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
</tr>
<tr>
<td>NIK-1So</td>
<td>IF×IM</td>
<td>423.0±1.5</td>
<td>29.0±2.1</td>
<td>100.0(90.0)±0.0</td>
<td>350.0±1.5</td>
<td>82.6(65.3)±0.1</td>
<td>100.0(90.0)±0.0</td>
<td>100.0(90.0)±0.0</td>
</tr>
<tr>
<td></td>
<td>IF×HM</td>
<td>428.0±0.6</td>
<td>23.0±1.5</td>
<td>100.0(90.0)±0.0</td>
<td>355.0±0.6</td>
<td>82.9(65.5)±0.0</td>
<td>100.0(90.0)±0.0</td>
<td>100.0(90.0)±0.0</td>
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<td></td>
<td>HF×IM</td>
<td>462.0±0.6</td>
<td>13.0±0.6</td>
<td>0.0(0.0)±0.0</td>
<td>422.0±0.6</td>
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<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
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<tr>
<td>NIK-1Dp</td>
<td>IF×IM</td>
<td>442.0±2.5</td>
<td>18.0±6.6</td>
<td>80.3(63.6)±1.5</td>
<td>385.0±2.5</td>
<td>87.1(68.9)±0.1</td>
<td>82.7(65.4)±3.1</td>
<td>82.6(65.3)±0.1</td>
</tr>
<tr>
<td></td>
<td>IF×HM</td>
<td>445.0±2.0</td>
<td>17.0±6.6</td>
<td>80.3(63.6)±0.6</td>
<td>389.0±1.5</td>
<td>87.3(69.1)±0.1</td>
<td>80.0(63.4)±4.0</td>
<td>80.0(63.4)±0.1</td>
</tr>
<tr>
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<td>HF×IM</td>
<td>468.0±1.5</td>
<td>10.0±0.6</td>
<td>0.0(0.0)±0.0</td>
<td>433.0±1.0</td>
<td>92.5(74.1)±0.1</td>
<td>0.0(0.0)±0.0</td>
<td>0.0(0.0)±0.0</td>
</tr>
<tr>
<td>Nosema bombycis</td>
<td>IF×IM</td>
<td>367.0±2.1</td>
<td>43.0±1.2</td>
<td>100.0(90.0)±0.0</td>
<td>243.0±2.0</td>
<td>66.1(54.3)±0.2</td>
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<td>IF×HM</td>
<td>370.0±1.5</td>
<td>40.0±1.5</td>
<td>100.0(90.0)±0.0</td>
<td>249.0±1.0</td>
<td>67.4(55.2)±0.3</td>
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<tr>
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<td>HF×IM</td>
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<td>377.0±3.0</td>
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</table>

Values are mean ± SD and the values in parenthesis are angular transformed.
Table 3.7: Assay on mortality of transovarially infected progeny populations (IF×IM) combinations

<table>
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<tr>
<th>Microsporidian isolates</th>
<th>Larval mortality %</th>
<th>Total mortality %</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>I instar</td>
<td>II instar</td>
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<tr>
<td>NIK-1Pr</td>
<td>0.0(0.0) ± 0.0</td>
<td>9.3(17.7) ± 1.2</td>
</tr>
<tr>
<td>NIK-1Cc</td>
<td>0.0(0.0) ± 0.0</td>
<td>0.0(0.0) ± 0.0</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
<td>9.3(17.7) ± 2.5</td>
<td>17.3(24.5) ± 1.2</td>
</tr>
<tr>
<td>NIK-1So</td>
<td>14.0(21.9) ± 2.0</td>
<td>23.3(28.8) ± 4.2</td>
</tr>
<tr>
<td>NIK-1Dp</td>
<td>0.0(0.0) ± 0.0</td>
<td>9.0(17.4) ± 1.0</td>
</tr>
<tr>
<td><em>Nosema bombycis</em></td>
<td>20.0(26.5) ± 0.0</td>
<td>24.0(29.3) ± 1.0</td>
</tr>
<tr>
<td>CD at 5 %</td>
<td>(2.2)</td>
<td>(2.4)</td>
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</table>

Values are mean ± SD and the values in parenthesis are angular transformed.
Table 3.8: Assay on mortality of transovarially infected progeny populations (IF×HM) combinations

<table>
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<tr>
<th>Microsporidian isolates</th>
<th>Larval mortality %</th>
<th>Total mortality %</th>
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<tbody>
<tr>
<td></td>
<td>I instar</td>
<td>II instar</td>
</tr>
<tr>
<td>NIK-1Pr</td>
<td>0.0(0.0) ± 0.0</td>
<td>8.0(16.4) ± 0.0</td>
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<tr>
<td>NIK-1Cc</td>
<td>0.0(0.0) ± 0.0</td>
<td>0.0(0.0) ± 0.0</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
<td>9.3(17.7) ± 0.6</td>
<td>12.3(20.5) ± 0.6</td>
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<td>NIK-1So</td>
<td>12.3(20.5) ± 0.6</td>
<td>21.3(27.4) ± 0.6</td>
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<tr>
<td>NIK-1Dp</td>
<td>0.0(0.0) ± 0.0</td>
<td>6.7(15.0) ± 0.6</td>
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<tr>
<td>Nosema bombycis</td>
<td>20.3(26.7) ± 0.6</td>
<td>23.7(29.1) ± 0.6</td>
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<tr>
<td>CD at 5 %</td>
<td>(0.6)</td>
<td>(0.7)</td>
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Values are mean ± SD and the values in parenthesis are angular transformed.
Table 3.9: Percent mortality in the progeny populations hatched from eggs externally contaminated with the isolated microsporidia

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<th>Microsporidian isolates</th>
<th>I instar</th>
<th>II instar</th>
<th>III instar</th>
<th>IV instar</th>
<th>V instar</th>
<th>Pupal mortality%</th>
<th>Total mortality %</th>
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<td>0.0 (0.0) ± 0.0</td>
<td>0.0 (0.0) ± 0.0</td>
<td>9.7 (18.1) ± 0.6</td>
<td>22.0 (27.9) ± 0.0</td>
<td>23.7 (29.1) ± 0.6</td>
<td>20.3 (26.7) ± 0.6</td>
<td>75.7 (60.4) ± 1.5</td>
</tr>
<tr>
<td>NIK-1Cc</td>
<td>0.0 (0.0) ± 0.0</td>
<td>0.0 (0.0) ± 0.0</td>
<td>0.0 (0.0) ± 0.0</td>
<td>15.3 (23.0) ± 0.6</td>
<td>23.0 (28.6) ± 0.0</td>
<td>17.7 (24.8) ± 0.6</td>
<td>56.0 (48.4) ± 0.0</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
<td>0.0 (0.0) ± 0.0</td>
<td>9.0 (17.4) ± 0.0</td>
<td>17.0 (24.3) ± 0.0</td>
<td>21.0 (27.2) ± 0.0</td>
<td>27.3 (31.5) ± 0.6</td>
<td>13.0 (21.1) ± 0.0</td>
<td>87.3 (69.1) ± 0.6</td>
</tr>
<tr>
<td>NIK-1So</td>
<td>0.0 (0.0) ± 0.0</td>
<td>9.7 (18.1) ± 0.6</td>
<td>19.0 (25.8) ± 1.0</td>
<td>21.7 (27.7) ± 0.6</td>
<td>29.0 (32.5) ± 1.0</td>
<td>13.3 (21.4) ± 0.6</td>
<td>92.7 (74.3) ± 2.1</td>
</tr>
<tr>
<td>NIK-1Dp</td>
<td>0.0 (0.0) ± 0.0</td>
<td>0.0 (0.0) ± 0.0</td>
<td>4.3 (11.9) ± 0.6</td>
<td>17.0 (24.3) ± 0.0</td>
<td>24.3 (29.5) ± 0.6</td>
<td>18.7 (25.6) ± 0.6</td>
<td>64.3 (53.3) ± 1.2</td>
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<td>Nosema bombycis</td>
<td>0.0 (0.0) ± 0.0</td>
<td>18.7 (25.6) ± 1.2</td>
<td>23.7 (29.1) ± 0.6</td>
<td>39.3 (38.8) ± 0.6</td>
<td>18.3 (25.3) ± 1.2</td>
<td>--</td>
<td>100.0 (90.0) ± 0.0</td>
</tr>
<tr>
<td>CD at 5 %</td>
<td>--</td>
<td>(0.7)</td>
<td>(0.9)</td>
<td>(0.5)</td>
<td>(0.9)</td>
<td>(0.6)</td>
<td>(1.9)</td>
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Values are mean ± SD and the values in parenthesis are angular transformed.
Table 3.10: Effect of mulberry leaf disinfection on microsporidian disease incidence

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<tr>
<th>Microsporidian isolates</th>
<th>Treatment (Leaf dipping)</th>
<th>Concentration</th>
<th>Mortality due to infection (%)</th>
<th>Total infection (%)</th>
<th>Disease suppression % over inoculated control</th>
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<tr>
<td></td>
<td>Decol</td>
<td>1.0 %</td>
<td>0.0 (0.0)</td>
<td>35.3 (36.4)</td>
<td>51.3 (45.7)</td>
</tr>
<tr>
<td></td>
<td>Sanitech</td>
<td>400 ppm</td>
<td>0.0 (0.0)</td>
<td>14.7 (22.5)</td>
<td>79.7 (63.2)</td>
</tr>
<tr>
<td></td>
<td>Inoculated control</td>
<td></td>
<td>40.0 (39.2)</td>
<td>72.7 (58.5)</td>
<td>--</td>
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<tr>
<td>NIK-1Pr</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Decol</td>
<td>1.0 %</td>
<td>0.0 (0.0)</td>
<td>18.0 (25.1)</td>
<td>66.4 (54.5)</td>
</tr>
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<td>0.0 (0.0)</td>
<td>7.0 (15.3)</td>
<td>86.9 (68.8)</td>
</tr>
<tr>
<td></td>
<td>Inoculated control</td>
<td></td>
<td>28.0 (31.9)</td>
<td>53.7 (47.1)</td>
<td>--</td>
</tr>
<tr>
<td>NIK-1Cc</td>
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</tr>
<tr>
<td></td>
<td>Decol</td>
<td>1.0 %</td>
<td>0.0 (0.0)</td>
<td>44.7 (41.9)</td>
<td>55.3 (48.0)</td>
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<td>81.7 (64.6)</td>
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<td>Inoculated control</td>
<td></td>
<td>70.0 (56.8)</td>
<td>100.0 (90.0)</td>
<td>--</td>
</tr>
<tr>
<td>NIK-1Cpy</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decol</td>
<td>1.0 %</td>
<td>0.0 (0.0)</td>
<td>52.7 (46.5)</td>
<td>47.3 (43.4)</td>
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<tr>
<td></td>
<td>Sanitech</td>
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<td>22.3 (28.2)</td>
<td>77.7 (61.8)</td>
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<tr>
<td></td>
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<td>85.0 (67.2)</td>
<td>100.0 (90.0)</td>
<td>--</td>
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<tr>
<td>NIK-1So</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decol</td>
<td>1.0 %</td>
<td>0.0 (0.0)</td>
<td>28.0 (31.9)</td>
<td>56.8 (48.9)</td>
</tr>
<tr>
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<td>84.0 (66.4)</td>
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<tr>
<td></td>
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<td></td>
<td>31.7 (34.3)</td>
<td>65.0 (53.7)</td>
<td>--</td>
</tr>
<tr>
<td>N. bombycis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decol</td>
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<td>0.0 (0.0)</td>
<td>62.0 (51.9)</td>
<td>38.0 (38.0)</td>
</tr>
<tr>
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<td>Sanitech</td>
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<td>0.0 (0.0)</td>
<td>33.3 (35.2)</td>
<td>66.7 (54.7)</td>
</tr>
<tr>
<td></td>
<td>Inoculated control</td>
<td></td>
<td>100.0 (90.0)</td>
<td>100.0 (90.0)</td>
<td>--</td>
</tr>
<tr>
<td>Normal control</td>
<td>Distilled water</td>
<td></td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>CD at 5 %</td>
<td></td>
<td>(0.5)</td>
<td>(0.9)</td>
<td>(0.9)</td>
</tr>
<tr>
<td></td>
<td>CD at 5 %</td>
<td></td>
<td>(0.4)</td>
<td>(0.7)</td>
<td>(0.8)</td>
</tr>
<tr>
<td></td>
<td>S.E ±</td>
<td></td>
<td>0.33</td>
<td>0.61</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>CD at 5 %</td>
<td></td>
<td>(0.9)</td>
<td>(1.7)</td>
<td>(1.9)</td>
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</table>

Values in parenthesis are angular transformed
Table 3.11: Effect of disinfection of microsporidian smeared mulberry leaf on rearing performance of silkworm

<table>
<thead>
<tr>
<th>Microsporidian isolates</th>
<th>Treatment (Leaf dipping)</th>
<th>Concentration</th>
<th>Larval weight (g)</th>
<th>Larval duration (D:H)</th>
<th>ERR %</th>
<th>Single cocoon weight (g)</th>
<th>Single shell weight (g)</th>
<th>SR %</th>
</tr>
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<tbody>
<tr>
<td>NIK-1Pr</td>
<td>Decol</td>
<td>1.0 %</td>
<td>40.35</td>
<td>24.00</td>
<td>95.3  (77.5)</td>
<td>1.614</td>
<td>0.343</td>
<td>21.25</td>
</tr>
<tr>
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<td>Sanitech</td>
<td>400 ppm</td>
<td>41.54</td>
<td>24.00</td>
<td>95.7  (78.0)</td>
<td>1.655</td>
<td>0.360</td>
<td>21.78</td>
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<tr>
<td></td>
<td>Inoculated control</td>
<td>-</td>
<td>39.32</td>
<td>25.00</td>
<td>55.7  (48.3)</td>
<td>1.567</td>
<td>0.317</td>
<td>20.23</td>
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<tr>
<td>NIK-1Cc</td>
<td>Decol</td>
<td>1.0 %</td>
<td>40.84</td>
<td>24.00</td>
<td>95.3  (77.5)</td>
<td>1.647</td>
<td>0.358</td>
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<td>42.16</td>
<td>24.00</td>
<td>95.7  (78.0)</td>
<td>1.680</td>
<td>0.373</td>
<td>22.23</td>
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<td>Inoculated control</td>
<td>-</td>
<td>40.11</td>
<td>25.00</td>
<td>67.7  (55.4)</td>
<td>1.595</td>
<td>0.331</td>
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<tr>
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<td>Decol</td>
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<td>39.85</td>
<td>24.00</td>
<td>95.0  (77.1)</td>
<td>1.587</td>
<td>0.334</td>
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<td>40.60</td>
<td>24.00</td>
<td>95.3  (77.5)</td>
<td>1.636</td>
<td>0.350</td>
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<td>-</td>
<td>38.31</td>
<td>25.00</td>
<td>25.7  (30.5)</td>
<td>1.545</td>
<td>0.310</td>
<td>20.06</td>
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<tr>
<td>NIK-1So</td>
<td>Decol</td>
<td>1.0 %</td>
<td>39.78</td>
<td>24.00</td>
<td>94.7  (76.7)</td>
<td>1.575</td>
<td>0.328</td>
<td>20.83</td>
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<td>40.42</td>
<td>24.00</td>
<td>95.3  (77.5)</td>
<td>1.624</td>
<td>0.345</td>
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<td>-</td>
<td>38.11</td>
<td>25.00</td>
<td>10.7  (19.1)</td>
<td>1.532</td>
<td>0.305</td>
<td>19.94</td>
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<td>NIK-1Dp</td>
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<td>40.66</td>
<td>24.00</td>
<td>95.3  (77.5)</td>
<td>1.640</td>
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<td>25.00</td>
<td>64.0  (53.1)</td>
<td>1.585</td>
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<td>N. bombycis</td>
<td>Decol</td>
<td>1.0 %</td>
<td>39.58</td>
<td>24.00</td>
<td>94.3  (76.2)</td>
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<td>40.27</td>
<td>24.00</td>
<td>95.3  (77.5)</td>
<td>1.605</td>
<td>0.337</td>
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<tr>
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<td>-</td>
<td>35.24</td>
<td>25.00</td>
<td>0.0   (0.0)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Normal control</td>
<td>Distilled water</td>
<td>-</td>
<td>43.08</td>
<td>24.00</td>
<td>95.7  (78.0)</td>
<td>1.735</td>
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<tr>
<td>A</td>
<td>CD at 5 %</td>
<td>-</td>
<td>0.04</td>
<td>--</td>
<td>(0.8)</td>
<td>0.002</td>
<td>0.001</td>
<td>0.05</td>
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<td>B</td>
<td>CD at 5 %</td>
<td>-</td>
<td>0.04</td>
<td>--</td>
<td>(0.7)</td>
<td>0.002</td>
<td>0.001</td>
<td>0.04</td>
</tr>
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<td>A x B</td>
<td>SE ±</td>
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<td>--</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.03</td>
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<tr>
<td>CD at 5 %</td>
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<td>--</td>
<td>(1.7)</td>
<td>0.005</td>
<td>0.002</td>
<td>0.002</td>
<td>0.09</td>
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</table>

Values in parenthesis are angular transformed
Fig. 3.1: Impact of infection by the isolated microsporidia on the economic characters of CSR2 breed of the silkworm, *Bombyx mori* L.
Fig. 3.2: Impact of infection by the isolated microsporidia on the economic characters of Pure Mysore race of the silkworm, *Bombyx mori* L.
Fig. 3.3: Percentage reduction in SR% of CSR2 batches inoculated with different microsporidia compared to that of the healthy control.

Fig. 3.4: Percentage reduction in SR% of Pure Mysore batches inoculated with different microsporidia compared to that of the healthy control.
Fig. 3.5: Percentage decrease in Filament length of CSR2 batches inoculated with different microsporidia compared to that of the healthy control

Fig. 3.6: Percentage decrease in Filament length of Pure Mysore batches inoculated with different microsporidia compared to that of the healthy control
Fig. 3.7: Percentage difference in larval length of microsporidian inoculated batches compared to that of the healthy control (One day prior to the onset of spinning)

Fig. 3.8: Percentage difference in larval width of microsporidian inoculated batches compared to that of the healthy control (One day prior to the onset of spinning)
Fig. 3.9: Percentage reduction in larval weight of microsporidian inoculated batches compared to that of the healthy control (One day prior to the onset of spinning)