Chapter 3

MANAGEMENT OF LAMERIN MICROSPORIDIAN INFECTION IN LAMERIN AND OTHER SILKWORM BREEDS

Lamerin a mulberry silkworm breed is a native of North Eastern region of India. Sericulturistists in certain pockets of the region rear this breed. They prepare the eggs of the breed themselves, rear the worms and produce cocoons. The sericulture development agencies in the area in recent years are giving emphesis to introduce productive silkworm breeds as the local breed Lamerin and other are low in silk productivity. However there is a threat that orginatyes from Lamerin breed that it is endemically infected by microsporidia abd it poses a serious threat to sericulture development in the region. The microsporidium infecting the Lamerin breed do not cause high larval mortality but infects other silkworm breeds and may cause crop failure due to the microsporodiosis. In view of this, it is important that the microsporidiosis in Lamerin breed is effectively managed to ensure that it do not spread infection to productive silkworm breeds introduced in the region for development of mulberry sericulture. At the same time it is also important to understand and improve racial and economic characters of the Lamerin breed which is known for high rate of survival not only against microsporidiosis but also to other diseases.

In silkworm, there are no definite curative measures against microsporidiosis. The general disease preventive measures are followed to protect the silkworm from the microsporidiosis (Pebrine). 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. The silkworms hatched out of eggs laid by healthy moths are only used for silkworm rearing and seed production thus eliminating chances of disease occurance by transovarial transmission. Transovum transmission is avoided by surface disinfection of eggs. However, the secondary infection in the larval stage pose serious problem. Disinfection of silkworm rearing environment and adaptation hygienic practices during the rearing is followed to avoid secondary infection. There had been several

attempts to cure the silkworm microsporidiosis resulting from secondary as well as primary infection. There had also been attempts to develop chemotherapy and thermotherapy of disease in silkworm egg and larvaa.

Chemotherapy is an effective way to control the diseases in insects. In the past, chemotherapy was considered as non-realistic way to control silkworm diseases, however in recent years some of the therapeutic drugs / chemicals have proved to be effective. These did not cure the insects microsporodiosis but found promising. Investigations are essential in this direction. Chinese scientists are reported to have identified antimicosporidian drug, which cure silkworm of microsporidiosis resulting from transovarial infection. Analogues of benzimidazole and Benlate (Liu Shi Xian, 1979) Bavistin, Derosal (Baig, 1994), Fumidil - B or fumagillin (Lewis and Lynch, 1970; Hayasaka, 1991; Schmahl and Benini, 1998; Frankenhuyzen *et al*., 2004), methylthiophanate and ethyl thiophanate (Liu, 1987; Hayasaka, 1991) anisomycin (Hayasaka, 1991) have been reported to be effective against different microsporidians. Buquinolate is reported to control microsporidiaosis in Blue crab (Overstreet, 1975). Griyaghey (1976) and Alok Sahay *et al*., (2005) studied the effect of chemotherapic agents on Pebrine in Tassar silkwom, *Antheraea mylitta* and found an effective way to control the microsporidiosis. Several medicinal plants have been screened to control insect disease (Sujatha *et al*., 2005; Singh *et al*., 2005, Jaiswal and Deka, 2005). Girijadevi (2006) tested aqueous extracts of different botanicals against *N. bombycis*. Thymol, an active ingredient of *Ammi copticum* (Ajowan) of family *Umbelliferae* have found effective in controlling *Nosema* disease in honey bees (Rice, 2001). Chemotherapeutic agents are fed to silkworm either through artificial diet or through mulberry sprayed with the antimicrosporidian agent (Iwano and Ishihara, 1981; Hayasaka, 1991; Brooks *et al*., 1978; Frankenhuyzen *et al*., 2004).

Thermotherapy (Thermal treatment) is the temperature treatment of different stages of insect to cure them of diseases such as microsporidiosis.

Thermotherapy was successful in insects which have higher temperature tolerance compared to their microsporidian parasite. In some cases treatment with an elevated temperature has led to successful cure of the disease. The procedure is adopted in cure of microsporidiaosis in European corn borer (Raun, 1961), *Nosema apis* (Cantwell and Shimanuki, 1969) and Spruce budworms (Wilson, 1979). Griyaghey *et al*., (1976) and Sheeba *et al*., (1999) reported thermotherapy of pebrinized silk cocoons and its effect on larval progeny. Kfir and Walter, (1997) and Frankenhuyzen *et al*., (2004) attempted to interrupt the transmission of the *Nosema* parasite from eggs to larvae by treating the egg masses with high temperature. Liu shi Xian (1980) have reported 97-100% cure of transovarially-infected egg of microsporidiosis by treating them at high temperature. Lu Heming *et al*., (1981) reported the inhibition of development of microsporidiosis in silkworm egg treated with hot air of 48-50ºC. Pebrine diseased polyvoltine mulberry and Eri silkworm eggs are treated with hot water with success in China (Howard, 1925; Lu Yuling, 1960; Li Aiqun, 1962). Chilling has been successfully attempted in cure of microsporidiosis in *Plutella xylosetella and Edhazardia aedis* (Undeen *et al*., 1993). Temperature treatment is also reported to reduce transovarial infection while sterilants eliminate possible surface-borne contamination.

Several preventive measures against microsporidiosis are in practice in sericulture. The inspection of mother moth and destruction of eggs laid by moths infected by the microsporidia during the silkworm seed production is the most meticulously followed method to prevent the outbreak of pebrine disease in sericultural areas. The other preventive measures emphasis on elimination of secondary sources of infection. The major sources of secondary infection are the diseased and dead larvae, feces and the gut juice vomited by the diseased silkworm. Alternate hosts also pose problem in management of silkworm microsporodiosis.

The disinfection of silkworm rearing house, silkworm rearing appliances and the silkworm seed production centers is followed to eliminate the spores of

microsporidians which cause the secondary infection. It is coupled with meticulous hygiene practices in silkworm rearing and egg production. Several physical and chemical disinfectants have been screened for their efficacy against the microsporidian spores. Microsporidia are diverse group of organisms and with the exception to the direct effects of sunlight, which quickly kill/inactivate most organisms, the different group of microsporidians respond differently to different environmental stresses (Maddox, 1977; Kava, 1977; Brooks, 1988). Very little information is available on the effect of sunlight or ultraviolet (uv) radiation on the survival of the microsporidian spores (Maddox, 1973). However, it is known that microsporidian spores are susceptible to ultraviolet (uv) radiation. Baribeau and Burkhardt (1970) reported the unpublished work of I. L. Revell where it is observed that *Nosema apis* spores are inactivated in 5 hr by radiation. Subsequently, Wilson (1974) demonstrated that *N. fumiferanae* spores on cherry leaves and artificial diet were inactivated by a 30-W germicidal lamp within 4 and 5hr.The effect of temperature on microsporidian spores is reviewed by Maddox (1973). According to Maddox (1973)the inactivation of microsporidian spores occurs at fairly high temperature, but is dependent on condition and length of exposure.

Various disinfectants *viz.,* Formalin (Kagawa, 1980), Asiphore (Venkata Reddy *et al.,* 1990), Chlorinated lime and hydrochloric acid (Miyajima, 1979), Chlorine dioxide (Nataraju, 1995; Balavenkatasubbaih *et al*., 1999), Kao haiter (Balavenkatasubbaih *et al*., 2003), Serichlor (Balavenkatasubbaih *et al*., 2006**)**, Calcium chloride (Iwashita and Zhou, 1988; Patil, 1991) have been screened against silkworm pathogens including *N. bombycis*. Chemical disinfectants such as Hilite, Sodium hypochlorite, Bleaching powder and Formalin are used as surface disinfectants of silkworm egg against *N. bombycis* (Baig *et al.,* 1989).

As the response of different microsporidians to physical and chemical agents differ, it is essential to characterize the microsporidians response to physical and chemical agents and utilize the information in management of

microsporidiosis. Hence in the present study efforts were made to screen Lamerin microsporidia for its response to different physical and chemical agents and to develop convenient and efficient preventive/control measures to contain the microsporodiosis disease caused by Lamerin microsporidia in Lamerin breed as well as in other silkworm breeds. Attempt has also been made to develop chemotherapy and thermotherapy against Lamerin microsporidian infection.

MATERIALS AND METHODS

Impact of Lamerin microsporidian infection in Lamerin and other breeds of silkworm: The impact of Lamerin microsporidia on the economic characters of several bivoltine and multivoltine breeds of silkworm was determined by *per os* inoculation of the microsporidian spores in conc. of 1×10^7 spores/ml to 3rd instar silkworm. The eggs of identified bivoltine and multivolotine breeds *viz.*, CSR2, CSR18, CSR19, NB4D2, Lamerin, (Bivoltine) and PM and Nistari (Multivoltine) were received from the germplasm bank of CSRTI, Mysore for the study. The larvae of the selected breeds were reared under hygienic condition till the beginning of 3rd instar and were inoculated *per os* with the purified spores of Lamerin microsporidia. Each breed formed a treatment. The inoculum was prepared from purified spores of the microsporidia and quantified by standard method using haemocytometer. The silkworm larvae were inoculated 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 treated leaves. After 24 h the larvae were continued to be reared on uncontaminated mulberry leaves till cocooning. Two controls for each of the breed were maintained for comparative purpose. The first control larvae were treated with mulberry 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. The controls and treatments had three replications of 100 larvae each.

The larvae were observed for growth, behavior and other economic characters such as larva, cocoon and shell weight, silk ratio and mortality due to microsporidiosis during the rearing and pupal stage, *etc.* recorded and analyzed. The reason for mortality was confirmed by microscopic examination of the dead

larvae and pupa collected during the rearing and cocooning, for the microsporidian spores. All emerged moths were subjected to the microscopic examination and the percent infection was calculated.

Viability response of Lamerin microsporidian spores to physical agents

Efficacy of different factors that could act as physical disinfectants were screened for their effect on the viability of spore stage of Lamerin microsporidia. One ml of stock purified spores were spread in a petridish (9.69 sq. cm surface area) and kept open at 4ºC for 3 days. The spores were released from petridish and further air dried into a fine powder form. 2 mg/dish of dried Lamerin microsporidian spores were taken in petridish/ eppendoff tubes and were subjected to different treatments. The treatments identified were Ultra violet light, Sun light, Hot water and Hot air.

Ultraviolet rays: The microsporidian spores were exposed to Ultra Violet Rays emitted by UV lamp (Philips TL 40W/ 05, Made in Holland). The dried spores in petridish were exposed to UV rays for 30, 60, 300, 600, 900 and 1200 seconds, kept at a distance of 6 inches from the source of UV rays. The viability of the spores was determined by following artificial germination method. The spores that hatched or germinated were presumed as live and the rest of spores that did not hatch or became black were considered as dead. The spores were also subjected to bioassay to determine the infectivity of the inoculum exposed to UV rays. One ml of the inoculum containing 1×10^7 spores / ml was smeared on mulberry leaves and fed to Lamerin breed of silkworm immediately after 2^{nd} moult. A control batch of silkworm fed with spores without exposure to the UV rays was also maintained. The larvae were reared till cocooning and pupae were allowed to emerge and moths were examined for infection. The number of dead larvae, pupae and moth were also examined for infection by the spores exposed to UV rays, the observiations were recorded and analysed.

Sunlight: The microsporidian spores were exposed to Sunlight to determine the effect of sunlight on the viability of spores. The dried spores in petridish were

exposed directly to sunlight (35 ± 5 ^{o}C) for 30, 60, 120 and 180 min. The viability of the spores was determined by following artificial germination method. The spores that hatched were presumed as live and the spores that did not hatch or turned black were considered as dead. The spores were also subjected to bioassay to determine the infectivity of the inoculum exposed to sunlight. One ml of the inoculum containing 1×10^{7} treated spores / ml were smeared on mulberry leaves and fed to the healthy silkworm immediately after 2nd moult. A control batch of silkworm fed with spores without exposure to the sunlight was also maintained. The larvae were reared till cocooning and pupae were allowed to emerge and moths were examined for infection. The dead larvae, pupae and moth were also examined for infection by the spores exposed to sunlight, the observiations were recorded and analysed.

Hot water: The Lamerin microsporidian spores were exposed to hot water of 30, 40, 50, 60, 70, 80, 90 and 100ºC for 1, 3, and 5 min. to determine the effect on viability of hot water on the spores. 1mg of Lamerin microsporidian spores were taken in an eppendoff tube and 0.5ml of hot water was added and kept at that environmental temperature for different duration of time in an incubator.

After the completion of the duration of treatment, 0.5 ml of cold water (4ºC) was added and the suspension was centrifuged at 5000 rpm for 5 min. The pellet of spores were suspended in water and examined for viability and infectivity.

The viability of the spores was determined by observation of germination of spores following the artificial germination method. The spores that hatched were presumed as live and the rest of spores that did not hatch or turned black were considered as dead. The spores were also subjected to bioassay to determine the infectivity of the spore exposed to hot water. One ml of the inoculum containing 1×10^{7} spores were smeared on mulberry leaves and fed to the silkworm immediately after 2nd moult. A control batch of silkworm fed with spores without exposure to the hot water was also maintained. The larvae were reared till cocooning and pupae were allowed to emerge and moths were examined for

infection. The dead larvae, pupae and moth were also examined for infection by the spores exposed to hot water the observiations were recorded and analysed.

Hot air: The Lamerin microsporidian spores were exposed to dry temperature in an incubator (Isuzu incubator; Seisakusho Co. ltd. Japan) to determine the effect of temperature on the viability of spores. The dried spores in petridish were collected and exposed directly to hot air of 30, 40, 50, 60, 70, 80, 90 and 100°C for 1, 5, 30, 60 and 120 minutes in an incubator. The treatments of hot air and duration of exposure of spore formed the treatment. Each treatment and control had five replication.

After the exposure period, the Lamerin microsporidian spores were suspended in 1 ml of sterilized water. The viability of the spores was determined by observation on their germination following the artificial germination method described else where in the thesis. The spores that hatched were presumed as live and the rest of spores were considered as dead. The spores were also subjected to bioassay to determine the infectivity of the inoculums exposed to dry temperature. One ml of the inoculum containing 1×10^7 spores/ml were smeared on mulberry leaves and fed to the silkworm immediately after 2^{nd} moult. A control batch of silkworm fed with spores without exposure to hot air was also maintained. Each treatment and control had 100 larvae. The larvae were reared till cocooning and pupae were allowed to emerge and moths were examined for infection. The dead larvae, pupae and moth were also examined for infection and recorded the observations.

Viability response of Lamerin microsporidian spores to chemical disinfectants

Disinfection ability of Chemical disinfectants such as Chlorine dioxide (20,000ppm ClO_2) from M/s Sericare, Divn. of Ashchem Agrotech (Pvt Ltd. Bangalore), Formaldehyde (37% HCHO from M/s Hindustan Organic Chemicals Ltd. India), Chlorinated lime (30% Chlorine from DCM Sriram Consolidated Ltd., India), Phenol (from Glaxo Laboratories (India) Ltd. Bombay) and Potassium

permanganate ($KMnO_4$ from Glaxo Laboratories (India) Ltd. Bombay were determined by suspending the spores in the chemical disinfectants. The purified spores were suspended in different concentrations of the chemical disinfectants for a period of 5 to 30 min and diluted, pelleted by centrifugation and washed by repeated centrifugation to remove the traces of chemical disinfectant. The final pellet was suspended in distilled water and examined for viability and infectivity. The different concentrations of different disinfectants forming the treatment are listed in table 8.

The following method was adopted for preparation of different concentration of different disinfectants.

Chlorine dioxide of 50, 100, 200, 300, 400 and 500 ppm in water was prepared from commercially available stabilized chlorine dioxide (Sanitech) containing 20,000 ppm. The stabilized chlorine dioxide was activated by adding 0.25g of activator in 2.5ml of Sanitech solution. To the activated Sanitech solution, 97.5 ml of water was added to obtain 100 ml of 500 ppm Chlorine dioxide solution. The 500ppm solution was serially diluted with distilled water to get the required chlorine dioxide solution of concentration 400, 300, 200, 100 and 50 ppm.

Formalin of 0.5, 1, 1.5 and 2.0% concentrations were prepared from commercial formaldehyde (37% formaldehyde) by using the formula N_1V_1 / N_2V_2 where N_1 is the original concentration, V_1 is the original volume, N_2 is the required concentration and V_2 is the required volume.

Bleaching powder of 0.5, 1.0, 1.5 and 2.0% in water was prepared from Bleaching powder containing 30% Chlorine by mixing 0.5, 1, 1.5 and 2g of Bleaching powder in 100 ml sterilized distilled water respectively.

Phenol solutions of 1, 3, 5, 7 and 9% concentrations were prepared by mixing 0.1, 0.3, 0.5, 0.7 and 0.9 ml of commercial phenol with 9.9, 9.7, 9.5, 9.3 and 9.1 sterilized distilled water respectively.

Potassium permanganate solution of 1, 3 and 5% potassium permanganate were prepared by dissolving 1, 3 and 5g of KMnO4 in 100 ml sterilized distilled water respectively.

***In vitro* tests:** The viability of the spores was determined by observation on the germination of the spores following artificial germination method mentioned elsewhere in the chapter. The spores that hatched were presumed as live and the rest of spores that did not hatch or turned black were considered as dead. Each treatment had three replications. The observation were recorded and analyzed.

***In vivo* tests:** The treated spores were subjected to bioassay to determine the infectivity of the spore exposed to different chemical disinfectants. One ml of the inoculum containing 1×10^{7} treated spores / ml were smeared on mulberry leaves and fed to the 100 silkworm immediately after 2^{nd} moult. A control batch of silkworm fed with spores without exposure to any chemical disinfectants was also maintained. Each treatment had three replications of 100 larvae. The larvae were reared till cocooning and pupae were allowed to emerge and moths were examined for infection. The dead larvae, pupae and moth were also examined for infection by the spores exposed to different chemical disinfectants. The observations were recorded and analyzed.

Therapy of microsporidiosis

In order to develop management practices against the microsporidiosis, thermotherapy and chemotherapy were attempted. Lamerin microsporidia infected silkworm eggs were exposed with high temperature (thermotherapy) and disease larvae were fed with different chemical/drugs and botanicals (chemotherapy) to determine the efficacy as theauptic agents.

Thermotherapy: Silkworm eggs laid by Lamerin microsporidia infected moths of Lamerin breed were subjected to different hot air treatment in an incubator. The eggs laid by infected moths were surface sterilized with 2% formalin for 5min, treated them with hydrochloric acid of specific gravity 1.075 at 46.1ºC for 5 min. These eggs were kept at an incubation temperature of 25±1 ºC and humidity of 80±5% RH for 24h, and then incubated at different treatment temperatures (40, 45 and 50^{o} C) for 5 , 10 and 15 min and constant relative humidity of 80%. The eggs after the treatment duration were incubated at standard incubation environmental conditions (25±1^{o}C, 80±5% RH). A group of eggs derived from the same infected mother moths treated with hydrochloric acid and were incubated at a temperature of 25±1^{o}C, and relative humidity of 80± 5% % RH formed the control. The larvae hatched from the treated eggs and the controls were reared up to spinning following standard procedure. The data on percent dead eggs, hatchability, larval weight, larval and pupal mortality due to Lamerin microsporidia, reduction in mortality over control, single cocoon weight, single shell weight and SR% were recorded and analyzed statistically. The moths were examined and observation on infection and mortality was recorded.

Chemotherapy

Chemotherapy was attempted using different chemical/ drugs and botanicals.

Chemical based: Based on the literature available several anti - protozoan drugs /chemicals such as benzimidazole derivatives *viz.*, Metronidazole (M/s R.P.L. Pharmaceuticals Pvt. Ltd., New Delhi, India), Albendazole, (M/s Cipla Ltd. by

Meditab Specialties Pvt. Ltd., Goa, India), Tinidazole, (M/s Kopran Pvt. Ltd. Khalapur), Ornidazole, (M/s Vapicare Pharma Pvt. Ltd. India), Mebendazole, (M/s Cipla Ltd. by Meditab Specialties Pvt. Ltd., Goa, India), Satinidazole, (From M/s Nicholas Piramal India Pvt. Ltd. Auragabad, India), Quine derivatives *viz.*, Primiquine (M/s Ipca Laboratories Ltd. Mumbai), Sapriquine (M/s Fulford India, limited, Pathampur, Distt. Dhar, M.P.), Chloroquine phosphate (M/s Rhone-Poulenc Pvt. Ltd. Auragabad, India), Polaramine (From M/s Fulford India, limited, Pathampur, Distt. Dhar, M.P.), one veterinary anti-protozoan drug - Berenil (M/s Intervet India Pvt. Ltd. Pune) and one antibiotics Sparquine (M/S Aresto Pharmaceuticals Pvt. Ltd., Daman) were selected for the screening for antimicrosporidian activity against Lamerin microsporidian spores.

The drugs were screened *invivo* for their toxicity and the non-toxic chemical / drugs were screened for efficacy by *in vitro* and *in vivo* screening for anti-microsporidian activity.

***In vivo* toxicity studies:** The drugs were screened for their toxicity to silkworm by feeding the drug at 1% concentration in water. The drug at 1% concentration was smeared on to mulberry and fed to silkworms immediately after 2^{nd} moult once on day 1, 2 and 3. The larvae were observed for symptoms and mortality due to the drugs for 7 days, recorded and the non toxic drugs / chemicals, to the silkworm were short listed. The non toxic drugs/chemicals were selected for further study and screened *in vitro* and *in vivo* for anti- microsporidians activity.

***In vitro* screening:** The nine non toxic drugs / chemicals *viz.*, Metronidazole, Albendazole, Tinidazole, Ornidazole, Mebendazole, Satranidazole, Primaquine, Sapraquine, Chloroquine, were selected and screened *in vitro* for anti microsporidian activity. The microsporidian spores (1×10^7 sp/ ml) were suspended in one ml of different chemical / drugs of concentrations *viz.*, 0.25, 0.50 and 1.00% for 30 min. The spores were sedimented and the sediment containing the treated spores was washed 4 times in sterilized distilled water by repeated centrifugation. The spores were tested for viability by visual and artificial

germination test as described elsewhere in the thesis. The observations were recorded.

***In vivo* screening**: Second instar disease free silkworm of Lamerin and CSR2 breeds inoculated with Lamerin microsporidia were used for *in vivo* screening of drugs/chemicals against the Lamerin microsporidia. 100 silkworms of these breeds were inoculated by *per os* with Lamerin microsporidian spores of 1 $\times 10^7$spores/ml immediately after 1st moult. The breeds were then fed on mulberry leaf treated with the drugs/chemicals in 0.25, 0.50 and 1.00 % concentrations. The drugs / chemicals in identified concentrations were smeared on the surface of mulberry (1 ml / 50 sq. cms) and fed to 100 third instar silkworm on alternate days up to spinning. A control group of larvae inoculated with Lamerin microsporidian spores of concentration 1 $\times 10^7$spores/ml were reared on normal leaves for comparative purpose. Observations were recorded for mortality due to microsporidiosis and its impact on economic characters.

Botanical based: To develop eco and user friendly approach in management of microsporidiosis in Lamerin and other silkworm breeds, Six botanicals, identified based on the literature (Thomas, 1998), were screened for anti-microsporidian activity The identified botanicals were the seeds of *Ammi copticum* (Ajowan) of family *Umbelliferae, Citrus paradesi* (Grape seed) of family *Rutaceae,* Pedicle (Clove) of *Myrtus caryophyllus* of family *Myrataceae*; Leaves of *Phyllanthus niruri* (Phyllanthus) of family-*Euphorbiaceae;* leaves of *Lawsonia alba* (Mehandi) of family *Lythraceae* and rhizome of *Curcuma longa* (Turmeric) of family *Zingiberaceae*. The plant materials were either powdered or homogenized in distilled water and made into solution of 1, 2 and 3% concentrations in distilled water. The botanical were screened *invivo* for toxicity and the non-toxic botanicals were screened for efficacy by *in vitro* and *in vivo* screening for their anti-microsporidian activity.

***In vivo* toxicity studies:** The aqueous extracts of the botanicals were screened for toxicity to silkworm. The extracts of 3% concentration was smeared on to

mulberry leaf and fed to silkworm after 2nd moult once on day 1, 2 and 3. The larvae were observed for symptoms and mortality due to the toxicity for 7 days. The non-toxic botanicals were selected for further study and screened *in vitro* and *in vivo* for anti- microsporidians activity.

***In vitro* screening:** The aqueous extracts of non toxic botanicals were screened *in vitro* for their anti microsporidians activity. The microsporidian spores (1×10^7 sp/ ml) were suspended in one ml of different concentrations *viz.,* 1, 2 and 3% of botanicals in water for 30 min. the spores were sedimented and the sediment containing the treated spores were washed 4 times in sterilized distilled water by repeated centrifugation. The spores were tested for viability of spore by visual and germination test. The botanicals showing anti- Lamerin microsporidia activity were short listed.

***In vivo* screening:** Second instar silkworm of Lamerin and CSR2 breeds inoculated with Lamerin microsporidia were used for *in vivo* screening of botanical extracts against the Lamerin microsporidia. 100 silkworm of these breeds were inoculated *per os* with Lamerin microsporidian spores of 1×10^7spores/ml concentration immediately after 1st moult. The breeds were then fed with botanical extract of 1, 2 and 3% concentration. The extracts was smeared on the surface of mulberry (1 ml / 50 sq. cms) and fed to 100 third instar silkworm on alternate days upto spining. A control group of larvae inoculated with Lamerin microsporidian spores of 1×10^7spores/ml were reared on normal leaves for comparative purpose. The dead larvae were collected and examined microscopically to determine the cause of death. Observations were recorded for mortality due to microsporidiosis and its impact on economic characters. The data were statistically analyzed.

RESULTS

Impact of Lamerin microsporidian infection in Lamerin and other breeds of silkworm: The results of the studies on the impact of Lamerin microsporidia and *N. bombycis* in silkworm on Lamerin and other breeds of silkworm are presented

in Table 1. It is observed that the infection by Lamerin microsporidia resulted in significant impact on the economic parameters of all the silkworm breeds studied *viz.*, CSR2, CSR18, CSR19, NB4D2, Lamerin, Pure Mysore (PM) and Nistari. A comparison with the respective healthy control indicate that the infection with Lamerin microsporidia significantly lowered the percent survival (ERR %), larval weight, single cocoon weight, shell weight and percent silk content of all the silkworm breeds studied with the exception of breed Nistari. In this breed the cocoon weight and shell weight though reduced but not significantly. The survival percent after Lamerin microsporidian infection was lowest in breed CSR2 (80.40%) followed by CSR19 (82.20%), CSR18 (82.60%), NB4D2 (84.40%), Nistari (85.00%) and PM (85.80%). However, the maximum survival of 89.20% was recorded in Lamerin breed as against the healthy host survival of 96.20%. Though high survival was observed in all the breeds the moths emerged from different breeds were found infected. Out of the total moths emerged in different breeds, 40.00% to 49.5% were infected. The healthy stock of different breeds recorded survival ranging from 94.20% to 96.20% and none of the moths emerged were infected.

The single cocoon weight was significantly lower in Lamerin breed silkworm infected with Lamerin microsporidia. The cocoon weight in infected batches 1.046g while the healthy cocoon weighed 1.482g. In other breeds the difference in cocoon weight between the treatment and respective control group was low compared to the Lamerin breed. The single cocoon weight of CSR2 was 1.632g as against 1.666g in the respective control. In CSR18, CSR19, NB4D2, Pure Mysore and Nistari values in respect of single cocoon weight were 1.594g and 1.638g; 1.588g and 1.630g; 1.666g and 1.648g; 1.016g and 1.036g and 0.888g and 0.912g respectively. Similar trend were noticed for shell weight and percent silk content also. The shell weight in the treated and respective control of CSR2, CSR18, CSR19, NB4D2, Lamerin, Pure Mysore and Nistari were 0.374 and 0.390g; 0.368 and 0.390g, 0.374 and 0.384g, 0.364 and 0.376g, 0.132 and 0.196g, 0.124 and 0.134g, 0.140g and 0.144g respectively. While the percent silk

content were 22.91 and 23.41%, 23.05 and 23.81, 23.34 and 23.54, 21.80 and 22.81%, 12.45 and 13.28%, 12.02 and 12.94% and 13.28 and 15.79% respectively.

A comparative study of Lamerin microsporidia and *Nosema bombycis* infections in above stated breeds indicate that the *N. bombycis* is more virulent than Lamerin microsporidia. The survival percent of Lamerin breed infected with *N. bombycis* was 55.60% which was high than the other breeds. In CSR2, CSR18, CSR19, NB4D2, Pure Mysore and Nistari the survival percent were 25.60, 21.80 24.40, 27.40, 42.80 and 36.40% respectively. All moths (100%) emerged from different breeds were found infected with *N. bombycis*. However, the impact of *Nosema* infection on other parameters did not vary much among different breeds including Lamerin. There was significantly higher reduction in larval weight, single cocoon weight, shell weight and percent silk content due to *Nosema* infection compared to Lamerin microsporidian infection in the breeds. The single cocoon weight, shell weight and percent silk content in the *Nosema bombycis* infected CSR group *viz.*, CSR2, CSR18, and CSR19 ranged from 1.588g to 1.632g, 0.374g to 0.368g and 22.91% to 23.34% while in their respective control it was 1.630g to 1.666g, 0.384g to 0.390g and 23.41% to 23.81%. In case of NB4D2, *Nosema* infected batch the single cocoon weight, shell weight and silk percent was 1.416g 0.274g and 19.35% while in their respective control, it was 1.648g, 0.376g and 22.81% respectively. In case of Lamerin, PM and Nistari breeds infected with *N. bombycis*, the single cocoon weight, Shell weight and silk content percent were 1.482g, 1.016g and 0.888g; 0.132g, 0.124g and 0.140g, and 12.45%, 12.02% and 13.28% respectively while in the respective healthy control it was 1.482g, 1.036g and 0.912g; 0.196g, 0.134g and 0.144g and 12.45%, 12.02% and 13.28% respectively.

Viability response of Lamerin microsporidian spores to physical agents

The response of Lamerin microspodia to the sporocidal activity of few physical agents on their germicidal activity against Lamerin microsporidia is presented in Table 2 -7.

Ultraviolet rays: The response of Lamerin microsporidia to ultraviolet rays is presented in table 2. The spores of Lamerin microsporidia exposed to UV rays for 1200 seconds, resulted in 100% kill of the spores. The exposure of spores for shorter period has resulted in lower percent of spores killed. It ranged from 18.40 % (60 sec) to 94.27% (900 sec) spores killed. The *per os* inoculation of spores, exposed for 1200 seconds did not cause larval infection / mortality. The *per os* inoculation of spores, exposed for shorter period (60-900 sec) caused infection in silkworm and it was lower with longer period of exposure. It ranged from 0.4% (900 sec) to 10.0% (30 sec). The spores that were not exposed to UV rays (Control) caused infection to an extent of 10.6%.

Sunlight: The response of Lamerin microsporidium to sunlight is presented in table 3. All spores of Lamerin microsporidian exposed to sunlight (35 ± 5^{o}C) upto 180 min were not killed. Only 15.0% spores were killed when the spores were exposed to sunlight for 180 min. 30 min exposure of spores to sunlight resulted in killing of 2.00% of spores. 60 and 120 min exposure of spores resulted in killing of spores to the extent of 6.30% and 12.2%. The *per os* inoculation of spores exposed for 180 min caused larval mortality of 8.4% while the unexposed spores (Control) caused a mortality of 10.6%. The spores exposed for a period of 30, 60 and 120 min also caused mortality ranging from 10.40 to 9.68%.

Hot water: The response of microsporidium to hot water is presented in table 4. The spores of Lamerin microsporidia exposed to hot water of 30-40^{o}C temperature for 1-5 min did not result in the mortality of spores. A temperature of 50, 60, 70, 80, 90 and 100^{o}C for 1, 3 and 5 min resulted in mortality of spores as is indicated by the germination test and visual observation. The mortality percentage varied with temperature and duration of exposure. The percent of spores killed varied from 2.9–100 % at temperatures 50^{o}C and above. The spores

suspended in hot water of temperature 90 and 100ºC for 3-5 minutes were killed (Table 4). *Per os* inoculation of spores suspended at 90 and 100 ºC for 3-5 min. did not cause infection in Lamerin breed of silkworm (Table 5). While spores suspended at temperature of 50ºC and above for 1-5 min and upto 90ºC for 1min resulted in infection of silkworm. The infection ranged from 1.67% (90ºC for 1min) to 9.67% (50ºC for 1min).

Hot air: The response of Lamerin microsporidium to hot air is presented in table 6 and 7. It is observed that the spores exposed to hot environmental temperature of 30ºC for 1 to 120 min, 40 and 50ºC for 1- 30 min. did not result in spore killing. Spores exposed to 60 -70 ºC for 1 min did not result in spore killing. The exposure of spores to 90 and 100ºC for 120 min resulted in 100% spores killing. At lower temperature and different duration of exposure, the mortality of spores ranged from 2.60 - 99.00%. *Per os* inoculation of spores exposed to temperature of 100 ºC for 60min 90 and 100 ºC for 120 minutes did not cause infection in silkworm. However, *per os* inoculation of spores exposed for 1-120 min to the temperature of 40 - 90 ºC resulted in infection of silkworm ranging from 1.33 (80 ºC for 120 min) to 9.67% (40ºC for 1min).

Chemical disinfectants

The response of Lamerin microsporidium to chemical disinfectants are presented in table 8 - 9. All the chemical disinfectants except Potassium permanganate were sporicidal to spores of Lamerin microsporidian. Potassium permanganate of 1-5% concentration did not kill the spores as indicated by germination test and bio assay.

Lamerin microsporidian spores suspended for 20 min in Chlorine dioxide solution of 400 ppm and higher concentration resulted in killing of all the spores suspended. At lower concentration and shorter suspension period, all the spores were not killed. The *per os* inoculation of spores exposed to 400ppm for 20 and 30min and 500ppm for 5–30min Chlorine dioxde did not cause infection in silkworm. At lower concentration and the exposure period of 5 –30 min the

Lamerin microsporidia caused an infection ranging from 1.66 to 10.00% (Table 9).

Bleaching powder of 30 % chlorine content at 0.5 % concentration with exposure period of 5–30 min was not sporicidal to spores of Lamerin microsporidia. At higher concentration of 1, 1.5, and 2.00%, bleaching powder was sporicidal and killed all the spores suspended in it for 10 -30 minutes. *Per os* inoculation of spores suspended in 0.5% and 1% bleaching powder for 5 and 10 min, 1.5% for 5 min. caused infection in silkworm ranging from 0.67 to 10.67%. While the spores suspended in 1% bleaching powder for 20 -30 min, 1.5% for 10-30 min. and 2% for 5-30 min did not cause infection of silkworm after *per os* inoculation indicating the germicidal action of bleaching powder on Lamerin microsporidia (Table 9).

One percent formalin was sporocidal to Lamerin microsporidian spores when exposed for 20 min or longer period. 0.5% Formalin with exposure period of 5min at 1% Formalin with exposure period of 5min did not cause 100 % killing of spores as indicated by *in vitro* germination test. At higher concentration of 1% for 10 – 30 min and 2% for 5 –30min exposure, period formalin was sporicidal killed all the spores exposed. In such cases, 100 % mortality of spores was observed as is indicated in *in vitro* spore germination test. *Per os* inoculation of spores exposed to formalin of 1% for 20-30 min and 1.5-2% for 5 to 30 min did not cause infection in silkworm while spores exposed to lower concentration of 0.5% for 5 –30min and 1% for 5 – 10 min lead to the infection ranging from 0.33 - 9.67% in silkworm.

Phenol was 100% sporicidal to Lamerin microsporidian at 9% conc. and exposure period of 10-30min. It was also 100% sporicidal at 7% conc. exposure period of 30min. At lower concentration of 1-5 and exposure period 5-30min and at 7%conc. and exposure period of 5-20min it was not sporicidal. Per os inoculation of spores exposed to phenol of conc. of 9% for 20-30min did not cause infection in silkworm and was found 100% effective (Table 9).

Thermotherapy

The observations made on the effect of thermal treatment of eggs infected with Lamerin microsporidia is presented in Table 10. It is observed that thermal treatments of infected eggs. However the mortality due to Lamerin microsporidians infection in the progeny. This is also reflected in the percentage of hatching of eggs. In the infected control laying the percent of hatching was 84.30% while in the treatments involving temperature of 40 and 45°C, it ranged from 90.70 – 92.90%. The percent of reduction in mortality due to the microsporidian infection in different treatments was lower and it ranged from 66.9 – 100% with 100% reduction in mortality due to Lamerin microsporidian infection in larvae that were obtained from the treatment of eggs at 50°C for 5min but 50°C for 5min lowered the hatching of eggs. However the treatment of eggs at 45°C for 5 – 15min did not affect hatching but reduced the mortality of the larvae by 92.10 – 96.30%. The percent of infection in moth stage was also lowered from 45.3 % in control to 10.00% (50 – 5min) 17.0 % (40°C for 5min). In treatment 45°C- 5 - 15min the infection at moth ranged from 10. 7- 11.1%.

Chemotherapy

Chemical based: The observations on the efficacy of chemicals and drugs on the microsporidiosis caused by Lamerin microsporidia in Lamerin breed of silkworm is presented in Table 11- 23. Among the drugs screened**,** Palramine and Berenil were found toxic to the silkworm and were not considered for screening for their anti-microsporidian activity. Nine drugs *viz.,* Metronidazole, Albendazole, Tinidazole, Ornidazole, Mebendazole, Satranidazole, Primaquine, Sapraquine and Chloroquine, were found non toxic to silkworm.

In vitro screening of benzimidazole and quine derivative drugs/chemicals indicated the efficacy of Albendazole, Mebendazole, Ornidazole, Cloroquine, Primiquine at 1% and Sparquine at 0.25-1%. Metronidazole, Tinidizole and Satinidizole were not found effective at 0.25 – 1% (Table 11).

The result of *in vivo* screening of drugs and chemicals for anti-microsporidia activity in Lamerin and CSR2 breeds indicated that among the benzimidazole derivatives, Albendazole (1.00%) and Mebendazole (0.25-1.00%) are effective in reduction of larval mortality due to microsporidiosis to an extent of 100% (Table 12 and 13). The other chemicals at concentrations ranging from 0.25 to 1.00% reduced the mortality due to microsporidiosis caused by Lamerin microsporidian to the extent ranging from 78.56% to 96.46% in Lamerin breed and 73.15-95.09% in CSR2 breed. At 1% concentration Orinidazole is effective in reducing the mortality by 96.46% in Lamerin and 92.68% in CSR2 breed. It was followed by Metronidazole at 1.00% (92.92 and 87.78 %) in Lamerin and CSR2 breed respectively and Satranidazole at 1.00% (92.92% and 90.27%).

The percent of infected moths was significantly low in all treatments. In the inoculated control it was 59.38% and it was reduced 22.44-30.04% in Lamerin (Table 14) and from 64.59% to 13.98-31.01% in CSR2 (Table 15). This has resulted in reduction in infection from 49.41-62.60% in Lamerin moth and 51. 98-78.35 of moths of CSR2 breeds.

Among the quine derivatives (Table 16 and 17) Sapraquine at 0.25% to 1.00% concentration is effective in reduction of larval mortality to an extent of 100% in both the breeds *viz.*, Lamerin and CSR2. The other quine derivatives (chloroquine and primaquine) were effective in reducing the larval mortality. It ranged from 82.20-96.46% in Lamerin breed and 85.36- 95.00% in CSR2 at 1% concentration. The treatment also lowered the percent infection in moth (Table 18 and 19) from 59.38 – 23.72% in Lamerin and 67.51-15.74% in CSR2 breed. This has resulted in reduction of percentage of infected moths by 51.97-60.05% in Lamerin and 66.67-76.68% in CSR2 breeds. All the benzimidazole derivatives improved the survival percent from 84.67 to 94.33-98.00% in Lamerin and from 80.00 to 91.33 – 95.00% in CSR2 breed (Table 20 ans 21). The quine derivatives also improved the survival percent from 84.67 to 95.33-98.00% in Lamerin and from 80.00 to 93.33-97.33% in CSR2 breed (Table 22 and 23). The economic

characters such as larval weight, single cocoon weight, shell weight and silk content were not affected by the treatment with the benzimidazole and quine derivative in Lamerin and CSR2 breeds (Table 20 – 23).

Botanical based: The results of screening of botanicals for anti microspporridian activity against the Lamerin microsporidia are presented in tables 24-30. All the botanicals screened *viz.,* seed of *Ammi copticum* (Ajowan),seed of *Citrus paradesi* (Grape seed), pedicel of *Myrtus caryophyllus* (Clove), leaves of *Phyllanthus niruri* (Phyllanthus) leaves of *Lawsonia alba* (Mehandi) and rhizome of *Curcuma longa* (Turmeric) did not show any toxic effect in silkworm at 3% concentration and were considered as non toxic botanicals to silkworm.

The *in vitro* screening of botanicals indicate that the aqueous extracts of seeds of *Ammi copticum* (Ajowan) at 1-3% and *Citrus paradesi* (Grape seed) and pedicel of *Myrtus caryophyllus* (Clove) at 3%, and *Phyllanthus nuriri* at 2-3% concentration are effective against Lamerin microsporidian (Table 24).

The *in vivo* screening of the botanicals to Lamerin and CSR2 breeds confirmed the observation of *in vitro* screening. *C. paradesi* (Grape seeds) at 0.25-1% and *A. copticum* (Ajowan) at 2.00-3.00 concentration in Lamerin were effective in reduction of mortality due to Lamerin microsporidiosis to an extent of 100% (Table 25). In CSR2 breed the seeds of *C. paradesi*, *A. copticum* at 1.00-3.00 concentration were effective against Lamerin microsporidian to an extent of 100% (Table 26). The percent of moth infected was also low in these treatments (Table 27 and 28). The percent moth infected in infected control was 63.82% in Lamerin breed (Table 27) and it lowered the percent infection to 13.51-18.65% by *A. copticum*, 15.58-21.02% by grape seeds at 1.00-3.00% concentration. The leaves of *P. nuriri* at 3% concentration and the pedicel of *M. caryophyllus* at 2 and 3% concentration lowered the infection level to 18.16, 18.49 and 18.49% respectively. The overall reduction in microsporidian infection in moth stage due to the treatment with *A. copticum* seeds and *C. prardesi* seeds ranged from 70.77-78.83% and 67.06-75.58% respectively. In CSR2 breed the (Table 28) the seeds

of *A. copticum* and *C. paradesi* at 1.00-3.00% concentration reduced the larval mortality to an extent of 100%. However in the moth stage infection was observed to an extent of ranging from 10.37-12.88% and 10.07-12.96% respectively. 1% extract of *M. caryophyllus* which also reduced the mortality in larval and pupal stage to an extent of 100%, 19.02% infection in moth stage was observed. The infection level in moth stage was reduced by seeds of *A. copticum, C. paradesi* and *M. caryophyllus* from 62.86% to 79.51-80.65%, 79.38-82.56% and 69.74 respectively. The treatment of silkworm with the extracts of botanicals in general has improved the survival from 86.67% in Lamerin to 95.33-98.67% and from 82.67% in CSR2 to 94.33-99.33% (Table 29 and 30).

DISCUSSION

A comparison of infectivity and transmission of Lamerin microsporidia with that of *Nosema bombycis* indicate that Lamerin microsporidia have low rate of infectivity, transmission and mortality in the larval and pupal stage. The ability of the Lamerin microsporidia to spread the infection within a colony is also low. The Lamerin microsporidia infects different breeds of silkworm and its infectivity and transmission is similar to that caused in Lamerin breed. Its impact on the economic characters is significant. The survival percentage of larvae is lowered, infection is transmitted to the progeny, the cocoon weight, shell weight and silk content is reduced. While the Lamerin breed is not of great economic value in terms of quality and quantity of silk produced, the ability of the breed to survive infection is of great interest. The breed not only has high tolerance to microsporidia isolated from Lamerin breed, it is also comparatively more tolerant to *N. bombycis*. The high percent of survival of Lamerin breed to both microsporidia *viz.*, isolate infection from Lamerin breed or *N. bombycis* confirms that the breed is comparatively more tolrent to microsporidian infection. It is possible that the Lamerin breed could serves as reservoir for the pathogen, Lamerin microsporidia and may be to *N. bombycis* if the microsporidia in Lamerin breed is left unchecked, it could derail the introduction of high yielding

silkworm breeds for the development of sericulture in the area as the source of pathogen is available in the form of Lamerin breed reared and nurtured by the traditional farmers who prepare the laying of Lamerin breed by themselves. In view of this, the understanding and management of the disease in Lamerin breed is of practical importance in the process of introduction and improvement of mulberry sericulture in the area. The understanding will also be useful in management of microsporidiosis in general.

The charcaterisation of Lamerin microsporidian spores for viability response to physical and chemical agents is important in management the disease. It is observed that the Lamerin microsporidian spore are sensitive to physical and chemical agents such as ultra violet rays, sunlight, hot water and hot air. These agents destroy the germs and some of them are in use in sericulture (Baribeau and Burkhardt, 1970; Maddox, 1973). The exposure of Lamerin microsporidian spores to ultraviolet rays for 1200 seconds kills the spore and such spores do not cause infection. Similar observations have been made on the germicide action of UV rays on various pathogens (Maddox, 1973). Contrary to it, high percent of the spore survival after exposure to sunlight continuously for 3 h, clearly indicate that sunlight is not an efficient sporicidel against the microsporidia. Hot water and hot air are other physical agents employed and both are effective at significantly high temperature. Exposure for 5 min to hot water of 100ºC and hot air of 100ºC for a period of 2h is observed essential to kill the spore stage of Lamerin microsporidia. Among the physical agents, Uv rays, hot water and hot air are dependable to kill the spores but there are constraints with regards to their application and their use as a general disinfectant. It is also important to examine the ability of these agents to penetration the dust and debris to reach the germs to kill them.

Among the chemical disinfectants in use in sericulture, 400 ppm Chlorine dioxide, 1% bleaching powder (30% chlorine) and 1% formalin are effective sproricides against Lamerin microsporidia. Phenol and Potassium permanganate were found ineffective even at high concentration of 5% in water. This suggests the

existing practice of use of Chlorine dioxide, bleaching powder or formalin as disinfectant in sericulture is good enough for the disinfection of silkworm rearing house and appliances.

Investigations on efficacy of thermotherapy have given interesting results. Thermal treatment (45ºC, 10-15 min.) of Lamerin microsporidia infected eggs resulted in the increase of hatching percentage from 84.30% to 88.00-92.90%. It also reduced the larval mortality due to Lamerin microsporidian infection by 92.12-96.20%. The percent of infected moth was also lowered from 45.30% to 10.70-12.1%. Similar observations of thermal treatment of silkworm egg at 47^{o}C for 10-20 min have been made by Liu *et al.,* (1971). Reduction in infection level of *Nosema* in silkworm pupa due to thermal treatment is reported by Choudary (1967) and Sheeba *et al.,* (1999). Thermal therapy of pupa has been found to be effective in the silkworm against infectious flacherie and cytoplasmic polyhedrosis (Inoue 1977; Savithri, 2006). Thermotherapy is also known to adversely affect protozoa infecting the insects (Weiser, 1961). Allen and Brunson (1947) reduced *Nosema* incidence in the potato tuber moth by incubating eggs at 47^{o}C for 20min while a 30min exposure to 43.3^{o}C was required to eliminate *Nosema pyraustae* from a European corn borer colony (Raun, 1961). Eggs as well as pupae infected with microsporidians, exposed to high temperature resulted in marked reduction, but not complete eradication of the pathogen. It may be due to the resistance of the spore stage of the microsporidia to temperature treatment and susceptibility of vegetative stages of the pathogen to high temperature. The egg treatment may result in killing of vegetative stages of the pathogen and survival of the spore stage. The thermal susceptibility of the vegetative stages of the pathogen has been confirmed in the case of microsporidia infecting insect cell lines (Ishihara, 1968; Undeen, 1975; Wilson and Sohi, 1977). The management of microsporidiosis in silkworm through Chemotheraptic approach has not given good success till date. However, it is an effective way to control the diseases in insects. Analogues of benzimidazole (Colbourn, *et al.*, 1994; Schmahl and Benini, 1998), Benlate, Bavistin, Derosal (Baig, 1994), Fumidil - B or Fumagillin (Lewis

and Lynch, 1971; Hayasaka, 1991; Frankenhuyzen *et al*., 2004), methylthiophanate and ethyl thiophanate (Liu, 1987; Hayasaka, 1991) and Anisomycin (Hayasaka, 1991) have been found to be effective against different microsporidians. Buquinolate is reported to control microsporidiaosis in Blue crab (Overstreet, 1975). Griyaghey (1976) and Alok Sahay *et al*., (2005) studied the effect of chemotherapy on Pebrine in Tassar silkworm, *Antheraea mylitta* D. and found an effective way to control the microsporidiosis. In the present study benzimidazole derivatives *viz.,* Albendazole and Mebendazole were found effective anti-sporozoan drugs. At 0.25-1% concentration the drugs could inhibit the development of the disease to an extent of 100%. Different benzimidazole derivitaves were tested *invivo* against *Glugae anomala* parasiting the connective tissues of sticks *Gasterosteus aculeatus* (Schimaha and Benini 1998). In *N. bombycis* clumping of chromatin in the nuclei, inhibition of spindle formation and also malformation of spores after exposure to albendazole has been shown by transmission electron microscopy (Haque *et al.,* 1993). In addition an enlargement of their nuclei and disruption of the nuclear membrane was also observed. An antimicrosporidial activity has also been demonstrated for another benzimidazole compounds, benomyl (Hsiao and Hsiao, 1973). The authors states that the mode of action of albendazole and the related benzimidazole derivitives, is to a large extent the prevention of microtubule assembly which in the case of susceptible microsporidian species will inhibit the formation of intranuclear spindle, the only known case microtubule formation in microsporidians. The distorted and leached cytoplasm observed in the merogonic and sporogonic stages of *Glugea anomala* after medication was also reported for *Encephalitozoon cuniculi* as an effect following albendazole treatment (Colbourn *et al.,* 1994). The authors argue that this effect in *E. cunicuculi,* and also the paucity of ribosomes, is likely to result from the loss of cytoplasm from disrupted merogonic and sporogonic stages rather than prevention of ribosomal synthesis, since ribosomes were abundant in other samples which had other damage. All the three quinine derivatives *viz.*, Chloroquine, Primiquine, and Sapraquine were also found effective in reduction of

mortality to an extent of 96.46%. In view of the high cost of drugs/chemicals and their hazardous consequenceces, now a days use of biodegradable materials like fresh plant extracts have been on the top priority for the control of diseases in plants (Jesper and Ward, 1993) and animals (Kumar *et al.*, 1999). Use of botanical for the control / suppression of microsporidiosis disease in mulberry silkworm, *B. mori* L. are scanty. Nathan *et al.*, (2005) reported that feeding *Azidirachta* exctracts along with leaves of food plants to *Spodoptrera litura* resulted in reduction of microsporidiosis disease as well as reduction in the ingestion and digestion of food. Kalaivani *et al.*, (2003) reported that neem at a concentration of 2, 4 and 6% reduced the virulence of pebrine spores during 4^{th} and 5^{th} instar. He also reported that higher doses of neem affects the endocrine system and kills the pebrine spores. Girijadevi (2006) reported that aqueous extracts of som botanical like neem, turmeric, tulsi and garlic kills 10 – 26% spores of *N. bombycis*. In present study it is also observed that the seeds of *A.copticum* of family *Umbelliferae, C. paradisi* of family *Rutaceae* are effective in reduction of mortality due to Lamerin microsporidia to an extent of 100%. Thymol the active ingredient of seeds of *A. copticum* is reported to be effective in control of microsporidians disease in *Apis mellifera*, the Honey bee (Rice, 2001). Thymol, (3-Hydroxy-p-cymcnc), a phenol compound is a constituent of essential oil derived from Thymc, Sage and many other plant species. Thymol, is also made synthetically and in pure form, thymol is colorless crystal with a pleasant, yet strong odour. Thymol is reported to be an anti-microbial agent and acts directly on the spore, penetrate the spore coat and disrupt the plasma membrane. This action prevents the germination of spore and subsequently the disease (Rice, 2001). Grape fruit seeds of *C. paradisi* are known to contain numerous polyphenolic compounds such as *Aquercetin, Hesperidin, Rutin,* Apigenin and Campherol. It is rich in vitamin C and potassium, folate, iron, calcium, and other minerals. It is also high in fiber and low in calories, and contains bioflavonoids and other plant chemicals that are known to protect against cancer and heart disease. Grapefruit seeds are well known as an anti-fungal agent in that their consumption kills many

different types of parasites and assists the body in producing beneficial bacteria. A biologically active natural ingredient found in the seeds kills *Streptococcus, Staphylococcus, Salmonella, Esturia coli, Candida, Herpes*, Influenza parasites and fungi. It is also used to control traveler's diarrhoea and is used commonly as an antibiotic, anti-fungal., anti-protozoan, antiviral, antiseptic and disinfectant. The indication in the present study that it is effective against microsporidiosis caused by Lamerin microsporidia and it is most encouraging not only in the management of microsporidiosis but also in the management of several other diseases in silkworm. It is expected to function against different bacteria, fungus and viruses infecting silkworm.

The investigations on the management of microsporidiosis caused by Lamerin microsporidia have given vital information which could go long way in management of microsporidiosis in silkworm caused by *N. bombycis*. Drugs such as Albendazole and Mebendazole and botanicals *viz., A. copticum* and *C.paradise* are potent in control of microsporidiosis. Apart from the general management practices with the use of disinfectants such as bleaching powder and chlorine dioxide, chemicals and botanicals such as seeds of *A.copticum,* and *C. paradise* could be used as an important component in integrated management of microsporidiosis in silkworm.