STUDIES ON THE MICROSPORIDIA OF INSECT PESTS OF MULBERRY AND OTHER AGRICULTURAL CROPS

SUMMARY

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SUMMARY

Parasitology is a growing science and in order to control and fight parasitic diseases and infections, a quick and reliable diagnosis of the infection agent is necessary. The insects comprise the largest group of organisms which are vulnerable to various diseases caused by microbes such as viruses, bacteria, fungi and protozoans. Microsporidia are the most diversified fungal entomopathogens that can infect both vertebrate and invertebrate hosts however, most prevalent in insects. The parasite causes a dreaded disease in the host body called microsporidiosis.

Microsporidia are unicellular organisms producing an environmentally resistant spore, characterized by the presence of unique extrusion apparatus housing a highly coiled polar filament and one or more nuclei. Infection occurs when a susceptible insect ingests the environmental spores of a microsporidium. They germinate in the mid gut under the influence of certain ions, alkaline pH and digestive enzymes and infect the epithelial cells and cause chronic infections which can be sometimes lethal. Many species of microsporidia, besides the normal mode of horizontal transmission, are even transmitted vertically from the parents to the progeny.

Furthermore, microsporidia are regarded as efficient regulators of insect populations in nature. Several published reports authenticate this fact and therefore, studies in this track assume greater significance. Microsporidia have been studied for over 160 years, but yet many doubts persist regarding their life cycle and biological features, as well as their taxonomy and phylogeny. Therefore, careful studies are indispensable for the correct identification of new and existing species.

The extensive work has been carried out in several countries on microsporidia infecting the insect species, but it is largely unexplored in India. In India, majority of the studies were carried out on *Nosema bombycis* or other *Nosema* sp. related to silkworm by sericulture personnel. Therefore, in India, there is much scope to study the diversity of microsporidia in the natural insect population. This is the first study on microsporidia being carried out in Lucknow region of Uttar Pradesh. The present study was carried out to gather information about the presence of microsporidia among mulberry and other agricultural insect pests. Therefore, the present study was

carried out to investigate the prevalence of microsporidian infection in insect pests of mulberry and other agricultural crops and to study their morphology, ultrastructure and infectivity to mulberry silkworm, *B. mori* L.

The first chapter of the thesis gives the relevant introduction of the research topic depicting the aims and objectives of the proposed study.

The second chapter includes the review of the literature available from different sources (printed/online).

The third chapter of the thesis gives a brief idea about the distribution and prevalence percentage of microsporidian infection in the insect population commonly inhabiting the mulberry and agricultural fields. There is less literature available about the prevalence of microsporidian parasites in insects. In the present study, a total of 34 different insect species were collected, being categorized into five insect orders *viz.* Lepidoptera, Hymenoptera, Orthoptera, Coleoptera and Hemiptera. The lepidopteran insect species were frequently available in the study sites as compared to other insects. Further, it was observed from literature survey that the lepidopteran and hymenopteran insects are more susceptible to microsporidian infections. Keeping in view the easy availability of the insect species and the intensity of microsporidian infection in the respective host species, four lepidopteran insects (*D. chrysippus, C. florella, M. phedima, S. cynthia ricini*) and one hymenopteran insect (*A. mellifera*) were selected for the detailed investigations. In the present study, among all the insects collected, highest prevalence percentage of microsporidian infection was recorded in *Apis mellifera* (52.33%). Among lepidopteran insect species investigated in the present study maximum prevalence percentage of microsporidian infection was recorded in *D.chrysippus* (33.51%) followed by *S. cynthia ricini* (32.69%), *M. phedima* (25.31%), *C. florella* (24.42%), *C. pyranthe* (13.88%), *D. genutia* (11.11%), *M. leda leda* (6%). Among the orthopteran and coleopteran insects collected during the present investigation, some of the insects were found positive with microsporidian infection but the intensity of the infection was very low. Therefore, these insects were not selected but for the brief study of microsporidia infecting them. From the present study it can be concluded that microsporidian infection was prevalent in insect population of Lucknow region in India. However, the infections often go unnoticed and their role in population dynamics of insects is often not recognized because

microsporidia do not cause dramatic epizootics such as those caused by fungi and viruses.

The fourth chapter describes the microscopic study of microsporidian spores isolated from insect pests of mulberry and other agricultural crops. The live micosporidian spores were easily detected by their characteristic Brownian movement. The light microscopy revealed that the five different microsporidia, tentatively named M-Dch, M-Cfl, M-Mph, M-Ame, M-Scy were oval in shape with variable sizes. The live microsporidian spores showed translucent properties with high refractivity whereas the germinated spores were easily distinguished from the live spores and observed as black empty spores under phase contrast microscope.

The sizes of the fresh microsporidia were larger than the fixed microsporidia. The size (Length × Width) of the fresh microsporidia, M-Dch, M-Cfl, M-Mph, M-Ame and M-Scy were measured as 4.06±0.12 µm × 2.29±0.08 µm, 4.32±0.11 µm × 2.33±0.06 µm, 5.35±0.09 µm × 3.26±0.11 µm, 4.35±0.10 µm × 2.80±0.07 µm and 3.52±0.12 µm × 2.22±0.10 µm respectively; whereas the size of Nbo spores were measured as 3.27±0.10 µm × 2.09±0.07 µm. Again, it was observed that the mean sizes of the above five microsporidia were smaller than that of the spore size of *N. bombycis*.

Under SEM, the textures of the spore surface were studied. The exospore wall of the M-Dch, M-Cfl, M-Mph and M-Scy spores were smooth whereas the exospores of M-Ame spores were ornamented with ridges and furrows. The meront and spore stages of the microsporidia were clearly distinguished as meronts were rounded in shape and spore stages were oval in shape. Further, since light microscopic techniques have proved inadequate to differentiate between species of similar appearance, ultrastructural methods have become more and more important (Hazard and Oldacre, 1975; Weiser, 1977). For differentiation of microsporidian species, the attention of taxonomists has been increasingly drawn to the surface structures of the spores.

The TEM ultrastructural features of M-Dch, M-Cfl, M-Scy and M-Ame spores confirmed that they belonged to the genus *Nosema* whereas the ultrastructure of M-Mph confirmed that it belonged to the genus *Larssoniella*. The microsporidian species were identified after the descriptions of Weiser (1961) and Maurand (1973) and by

using the additional observations of Maurand (1975) and Larsson (1999). The microsporidia under the genus *Nosema* are characterized by the production of spores with walls consisting of an electron dense exospore and an electron lucent endospore, polar filament coils, and diplokaryotic nuclei (Franzen & Müller, 1999; Huang *et al.*, 2007). The microsporidia *N. bombycis* from *B. mori* has 11-14 coils with the angle of tilt on most anterior to posterior coil is 62° and 61°. M-Dch and M-Cfl spore possessed 11 and 14 polar filament coils respectively. In M-DCh spore, the angle of tilt of the most anterior and most posterior polar filament coil to the spore axis were measured as 42° and 90° whereas in case of M-Cfl spore, the angle of tilt of the most anterior to posterior polar filament coil to the spore axis were measured as 90°. M-Ame spore possessed 21 polar filament coils arranged in two rows with an angle of tilt of 55°. The M-Scy spore contained 12 polar filament coils with an angle of tilt of the most anterior to posterior coil to that of the spore axis being measured as 40°. Further, the exospores and endospore walls of M-Scy spore were measured as 22 nm and 120 nm respectively. The M-Mph spore described in the present investigation possessed a single nucleus and 11 polar tube coils with an angle of tilt of the anterior coil as 25° and therefore, shared ultrastructural characteristics similar to that of the genus *Larssoniella*.

Microsporidian infections in insects are generally chronic, causing pathogenic effect on host and reduce their fecundity and life spans. Microsporidia are highly prevalent in insect population and they are diagnosed under the microscope by their transluscent properties and Brownian movement. However, the shape, size, spore surface and number of polar filament and their angle of tilt differ for each microsporidian species. The SEM study showed that the exospores of M-Dch, M-Cfl, M-Mph and M-Scy were smooth whereas exospores of M-Ame spore was ornamented with ridges and furrows. From the ultrastructure, the microsporidium M-Dch, M-Cfl, M-Ame and M-Scy were identified as genus *Nosema* Naegeli, 1857 whereas the microsporidium M-Mph was identified as genus *Larssoniella*. From the present investigation, it can be concluded that microsporidian parasites belonging to genus *Nosema* are common in insect pests. The microsporidian infection leads to greater economic losses to a country when they infect the insect species like honey bee, silkworm etc. They are considered to be more useful as long-term regulators of

pests and contribute towards the prevention and/or suppression of pest outbreaks. In spite of the role of microsporidia in the natural biocontrol of insect populations, relatively limited informations are available about the diversity of microsporidia. Therefore, the present study provides a much needed input to the information about the morphology and ultrastructure of the microsporidian parasites harbouring in insect populations.

The fifth chapter describes the studies on the infectivity of the microsporidian spores isolated from insect pests of mulberry and other agricultural crops to the silkworm *Bombyx mori* L. In this chapter, the infectivity of the microsporidian isolates M-Dch, M-Cfl, M-Mph, M-Ame, M-Scy and *N. bombycis* (Nbo) were checked against mulberry silkworm *B. mori*. The larval mortality due to microsporidian infection was not recorded in the silkworm batches inoculated with M-Ame and M-Scy spores where as the dead larvae in the silkworm batches inoculated with M-Dch, M-Cfl, M-Mph and *N. bombycis* (Nbo) were found positive for the infection.

At 16 days post inoculation, the LC_{50} value of M-Dch, M-Cfl and M-Mph spores against larval mortality of *B. mori* were calculated as 3.2×10^7 spores/ml, 2.69×10^8 spores/ml and 9.67×10^7 spores/ml respectively whereas the LC_{50} value of *N. bombycis* (Nbo) against larval mortality of *B. mori* was calculated as 1.1×10^6 spores/ml. The results indicate that among the isolated microsporidia, M-Cfl was more virulent to silkworm *B. mori* followed by M-Dch and M-Mph spores but were found to be less virulent than *N. bombycis* as the highest larval mortality was recorded in silkworm batches inoculated with *N. bombycis* spores.

Studies on the impact of infection on larval characteristics showed that there was no significant reduction in larval length of silkworm in the batches inoculated with microsporidia M-Ame and M-Scy. It was also observed that these microsporidia were not pathogenic to the silkworm *B. mori* L. In the present investigation, the infection due to the isolated microsporidia M-Dch, M-Cfl and M-Mph caused significant changes in the larval morphology. The progressive increases in larval length, width and weight were found to be significantly affected in the inoculated batches as compared to the healthy control batches. Again, it was observed that these microsporidia significantly adversely affected the cocoon characters *viz.* cocoon

weight, shell weight, shell ratio % and filament length but to a lesser extent as compared to the effect of *N. bombycis* inoculated batches of silkworm.

The present study, therefore, establishes that a number of microsporidian strains are harboured by many insects and these can also cause cross infection in silkworm. This may also explain the sudden and sporadic outbreaks of pebrine disease from time-to-time in the sericultural areas in India. The study concludes that out of five different microsporidia isolated from the insect pests collected from mulberry gardens and other agricultural fields, three microsporidia showed considerable infectivity to the silkworm *B. mori* L. These microsporidia, therefore, constitute a potential threat of gaining entry into silkworm rearing and perpetuating the infection despite routine care taken in mother moth examination and sanitations.

The present study revealed that the five microsporidia (M-Dch, M-Cfl, M-Mph, M-Scy and M-Ame) isolated from the insect pests of mulberry and some other agricultural crops were cross infective to silkworm and possessed the characteristic features resembling umpteen microsporidia on one hand, but differed from the standard microsporidian strain *N. bombycis* infecting the silkworm, *B. mori* L. on the other hand in terms of their ultrastructural features. The microsporidia M-Dch, M-Cfl and M-Mph showed infectivity to the mulberry silkworm, *B. mori* L. through oral portals.

The present study underlines a detailed study on the prevalence, morphology and ultrastructure of the microsporidian infections in various insect pests of mulberry and other agricultural crops which may also contribute to the outbreak of microsporidian disease in silkworm. It can be concluded that the insect pests associated with mulberry and other agricultural crops harbor a number of microsporidian strains that are also cross-infective to the silkworm. However, these microsporidia are less pathogenic to silkworm and are not transmitted by transovarial means but constitute a potential threat to the silkworm rearing through contaminated mulberry leaf.