Detection, Morphology and Molecular Characterization of Microsporidia, *Nosema* in Invertebrate(s)

SUMMARY

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SUMMARY

The host-parasite environment system is exceptionally dynamic and in nature there are different aspects of the parasitism phenomenon. Microsporidian parasites represent itself as an important model for studying host-parasite interaction. These are enigmatic parasites having a wide host range of invertebrates and vertebrates with a diminutive dimensions (1to 40µm). These include 144 genera and approximately1200 species with more than 200 species that has been reported belonged to genus *Nosema.* These diversified fungal pathogens have a unique extrusion apparatus to cause infection in hosts.

The microsporidian parasites are considered as an important regulator of insect population, hyperparasite of many helminthes, also reports were there on microsporidia causing opportunistic infections in immune-compromised hosts and microsporidiosis in humans. Though microsporidians have been studied for over 160 years but there are many aspects including its life cycle, taxonomic position as well as morphological features that are unclear and require proper investigation.

Extensive work has been carried out on Microsporidia worldwide but in India there is very scanty report on it. Therefore, it is required to study the diversity of microsporidia in different invertebrate and vertebrate hosts and identifying them morphologically and by molecular tools. Therefore, the present thesis has been designed to investigate microsporidia of genus *Nosema* morphologically and by molecular tools in different invertebrate hosts.

The first chapter of the thesis gave description on Microsporidia, its origin, spore morphology, life cycle, and occurrence of microsporidia in invertebrates and method of diagnosis by microscopy and molecular tools and techniques in context of genus *Nosema* of microsporidia alongwith the aim and objective relevant to the theme of the proposed topic.

The second chapter includes the Review of Literature highlighting parasite identification, prevalence, morphological description, molecular characterization, and impact of microsporidian infection on their hosts, factors affecting microsporidian

growth and development as well as microsporidian parasite interaction with its hosts considering the aims and objective of the proposed thesis.

The third chapter gave a brief description of microsporidia detected in different invertebrate hosts by Light and Scanning Electron Microscope and compared it with genus *Nosema* of microsporidia in invertebrate(s). Microsporidia has been found to be hyperparasitic in Cestode parasite, *Moniezia* sp. Oval and elliptical spores were observed. Spore length was 5.21±0.62μm length and 2.62±0.31μm width with a mean spore count of 4.47×10^{6}spores/ml. Both spore proliferation stage (merogony) and spore maturation stage (sporogony) were found simultaneously in the Giemsa stained smear but transition of merogonial stage to sporogony could not be traced out. All Cestode parasites collected were infected with microsporidia. Scanning Electron Microscope revealed oval shape of the spore having length 7.35μm and width 3.42μm. The above result of microsporidian parasite in cestode is the first report on detection of hyperparasitic microsporidia in cestode from Lucknow region of Uttar Pradesh, India. Also, microsporidia has been found to be hyperparasitic in Nematode parasites. All nematode parasites were found to be infected. The spore size measured was 4.32±0.32μm in length and 1.57±0.26μm in width having uniform oval shape with a mean spore count of 6.11×10^{6}spores/ml. In Giemsa stained smears diplokaryotic meronts were observed that is the characteristic of microsporidian spores. Though exactly the transition of merogony to sporogony could not be traced but mature spore with its surrounding wall was easily recognizable. Application of Scanning Electron Microscope illustrated intracellular spore development inside the epithelial tissue of nematode parasite (*Ascaridia* sp.) and spore size was 3.49μm in length and 3.14μm in width. The present finding is the first report of hyperparasitism of microsporidian parasites in nematode *Ascaridia* sp. that parasitize fowl intestine from Lucknow (UP), India.

In Silkworms (Arthropods: Lepidoptera), had 28.5% microsporidia infection. Shape and size was oval with 2.94μm±0.28μm length and 1.71±0.31μm width and the mean spore counts observed were 1.76×10^{6} spores/ml. The Giemsa staining illustrated both merogony and sporogony stages of microsporidian spore life cycle. Under

Scanning Electron Microscope oval shape of the spore was confirmed and the size of the fully grown mature spore was 5.05µm in length and 2.35µm in width. Large xenoma of size 9.09µm was observed in the gut epithelium of silkworm larvae that appeared to have numerous spores inside it. Spore development was going inside xenoma. In bollworms (*Helicoverpa* sp.) 56% of microsporidian infection was found. Additionally, the shape of the spores were oval and had size of 3.2±0.2µm length and 1.58±0.28µm width with a mean spore count of 3.58×10^{6} spores/ml. Merogony was easily noticeable as diplokaryotic meronts in Giemsa stained slides undergoing binary fission to form tetranulceate meronts. Under Scanning Microscope the merogonial stage was observed in the midgut epithelial tissue of insect larvae. Mature spore with a protrusion of an anchoring disk was observed. The size observed had 3.29µm length and 1.99µm width of a mature spore.

In Honey bees (*Apis mellifera*) the shape of spore was ovo-cylindrical and the size were 5.65±0.6µm in length and width 2.40±0.11µm with a mean spore count of 10.85×10^{6} spores/ml. Giemsa staining represented a clear image of a mature spore (red arrows) that can be recognized as dark blue nuclei with a covering of unstained wall. In Scanning electron microscope the external morphology of the spore surface appeared sculptured and ornamented and was oval shaped with a spore dimension of 4.10×2.12µm. Heavy microsporidian spore infection was observed in the abdomen of honey bee. Apansporoblastic development, diplokaryotic meronts, tetranucleate meront stage, wall thickening of mature spore, long polar tubule, extensive merogony, oval shaped spore, size range, ornamented and furrowed spore wall demonstrated that the micropsoridian parasite belonged to the *Nosema* strain of microsporidia. In carpenter bees (*Xylocopa* sp.) 46.6% microsporidian infection was present. Shape observed was oval to elliptical and size measured was 5.88±0.23µm in length and 3.22±0.55µm in width with a mean spore count of 7.85×10^{6}spores/ml. Sporogenesis could not be traced out in Giemsa stained smears. An ultrastructure of mature spore had a size range of 7.99×5.40µm and was found to be oval in shape. Thus, the light and electron microscopy investigation considered the microsporidian parasite in Carpenter bees (*Xylocopa* sp.) to be placed in genus *Nosema* based on its morphological details.

Flour beetle (*Tribolium* sp.) revealed 100% microsporidian infection. The size measured was 7.58±1.63µm in length and 3.6±1.06µm in width. The mean spore count was 13.22×10^{6}spores/ml.Various merogonial stages and spore maturation as well as differentiation was determined by Giemsa staining. The Scanning Electron Microscopy determined spores of variable shape and size. Maximum spore had size range of more than 10µm. The microsporidia morphology and life cycle in Flour beetle resembled genus *Nosema* of microsporidia.

The fourth chapter confirmed the genus *Nosema* of microsporidia by molecular applications (PCR). CTAB method was followed for DNA extraction of microsporidian infected samples. DNA Quantification was done by using lambda DNA marker in Agarose gel as well as spectrophometer reading $O.D_{260/280}$ nm. *Nosema ceranae* primer has been used for Polymerase Chain Reaction. Sequence of 152 bps generated in Honey bees, Carpenter bees and Flour beetle were determined. On blasting the amplified sequence of the microsporidian infected Honey bee revealed 98% homology with *Nosema ceranae* with an accession numbers XR002966746.1, KM001610.1,U26533.1,KU937105.1,GU131058.1,GU131057.1 and *Nosema* sp. with an accession number MG745975.1 and also few *Nosema ceranae* sequences with accession number KC680652.1, KM001616.1 showed 97% homology with query sequence. Similarly, blasting of microsporidian infected amplified product of Carpenter bee sequence revealed 97% homology with *Nosema* sp. and *Nosema ceranae* with accession numbers MG745952.1, KC680652.1, KC680641.1, U26533.1, XR002966746.1, KC680632.1, KU937105.1, JN872266.1, KM001617.1 and 87% identity (99% query cover) with *Nosema bombi* (Acc No. AY007383.1). Microsporidia infected flour beetle generated sequence revealed 96% homology with *Nosema ceranae* sequences with accession numbers LT548993.1, LT548999.1, JN872262.1, MG745975.1, KM001610.1, KC680651.1, KM001618.1 and *Nosema whitei* (Acc No. AY305323.1).

The fifth chapter dealt with the significance and future prospects of the presented research study on microsporidia and also focussed on the significance of

light microscopy findings for studying morphology and life cycle of microsporidia referring genus *Nosema* into a three dimensional comprehensible ultrastructure picture of intracellular host-parasite interaction by Scanning Electron Microscope alongwith molecular study that sounds essential for identifying and characterising Microsporidia.