

Original Research Article

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Microscopical Observations on the Microsporidians Isolated from Insect Pests

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ABSTRACT

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etc.

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Morphological observations on the microsporidian spores from *Catopsilia crocale*, *Catopsilia pyranthe*, *Diaphania pulverulentalis*, *Pieris rapae* and *Spilosoma oblique* were recorded in the present paper. The spores were provisionally designated as NIK-1Cc, NIK-1Cpy, NIK-1Dp, NIK-1Pr, NIK-1So. Wherein NIK represents “National Institute Karnataka” and Cc, Cpy, Dp, Pr and So represent the first letters of the generic and species name of the insect pest from which these microsporidians were isolated. The spore shape of NIK-1Cc, NIK-1Cpy, NIK-1Dp were oval and NIK-1Pr, NIK-1So were ovo-cylindrical. However the spores of standard strain *Nosema bombycis* was oval in shape also. The scanning electron microphotographs of all microsporidian including *N. bombycis* indicated that the surface of spores was smooth in texture.

Introduction

Microsporidia are found infecting nearly all insects orders but over half of the susceptible insect hosts occur in two orders, Lepidoptera and Diptera.

Most of the entomopathogenic microsporidia occur in genus *Nosema*, more than 150 described species found in 12 orders of insects (Becnel and Andreadis, 1999). Different microsporidian isolates have been reported to be isolated from silkworm *Bombyx mori* in India (Bhat and Nataraju,

2004; Selvakumar *et al.*, 2005; Bhat *et al.*, 2009a).

The early descriptions of microsporidian were mainly based on spore morphology and lack ultra structural details but in the recent identification it was necessary to use at least a minimum of ultrastructural characters (Larson, 1988).

We report herein, the morphological characteristic features of five different microsporidians isolated from insect pests of mulberry and agricultural crops.

Materials and Methods

Microsporidian isolates

Eleven (11) different insect pests of mulberry and agricultural crops were collected separately from mulberry garden and agricultural fields. The insects were homogenized and the smear was examined under microscope for microsporidian infection. Only five out of eleven insects were found infected with microsporidian infection which were collected and purified by the method described by Sato and Watanabe (1980). The standard strain *Nosema bombycis* were collected from the Silkworm Pathology Laboratory, Central Sericultural Research and Training Institute Mysore for comparison. The micro-photographs were taken using phase contrast microscope Nikon (Type - 104) at 600 x magnifications for determination of spore shape.

The purified spores were air dried at room temperature for electron microscopic studies. The samples were transferred onto double stick cellophane tape pasted on copper stubs used for mounting specimen for scanning electron microscopy. The mounted stubs were

coated with about 20 nm gold in sputter coater (EMS-550) and viewed under JEOL-100 CX-II electron microscope fitted with an ASID-4D scanning attachment (Tokyo, Japan 20 KV). The spores were observed and photographed to study the spore shape, surface and compared with that of *N. bombycis*.

Results and Discussion

The microsporidian spores from *Pieris rapae*, *Catopsilia crocale*, *Catopsilia pyranthe*, *Spilosoma obliqua* and *Diaphania pulverulentalis* were ovocylindrical, oval, oval, ovocylindrical and oval in shape respectively whereas the spore of the standard strain *N. bombycis* was oval (Table 1; Plate 1). The scanning electron micrographs indicated that the surface of the spores of all the five microsporidia were smooth in texture which is same as that of *N. bombycis* spores (Plate 2). The wet mounts of the microsporidian spores viz., NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp exhibited characteristic Brownian movement and high refractive index as is exhibited by the *N. bombycis*.

Table.1 Screening of different insect pests of mulberry and other agricultural crops for

Insect pest	Family	Host plant	Microsporidian isolates	Shape
<i>Pieris rapae</i>	<i>Pieridae</i>	Cabbage, Cauliflower etc,	NIK-1Pr	Ovo-cylindrical
<i>Catopsilia crocale</i>	<i>Pieridae</i>	Red gram, Bitter gourd etc,	NIK-1Cc	Oval
<i>Catopsilia pyranthe</i>	<i>Pieridae</i>	Red gran, River bean etc	NIK-1Cpy	Oval
<i>Spilosoma obliqua</i>	<i>Arctiidae</i>	Mulberry, Green gram etc	NIK-1So	Ovo-cylindrical
<i>Diaphania pulverulentalis</i>	<i>Pyralidae</i>	Mulberry	NIK-1Dp	Oval

Plate.1 Light microscopic observation on different microsporidians isolated from insect and compared with that of *Nosema bombycis* (600x)

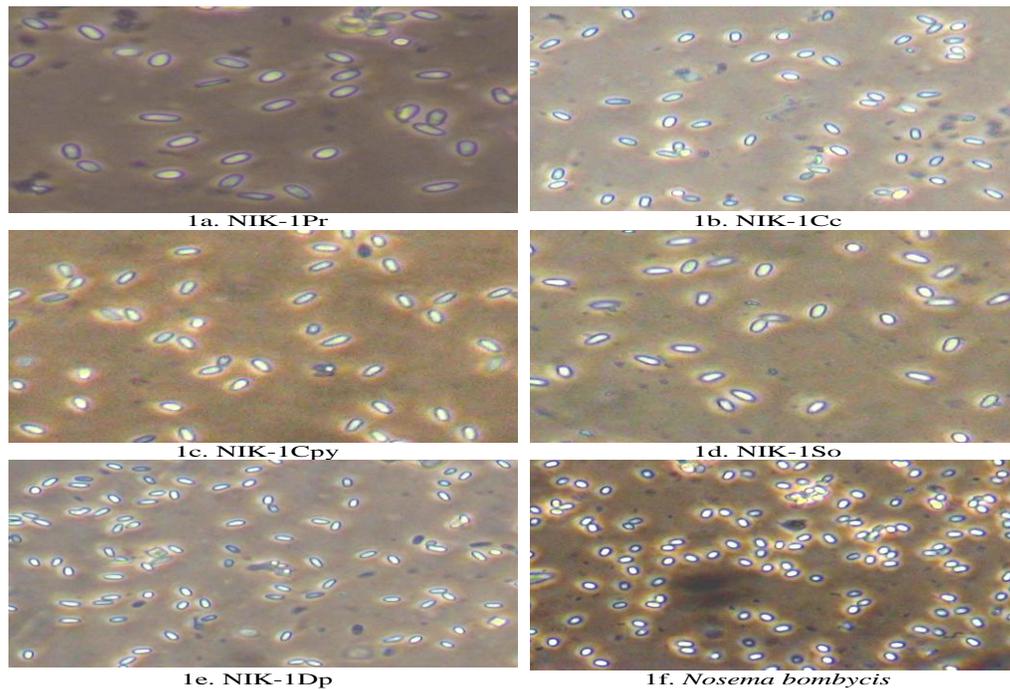
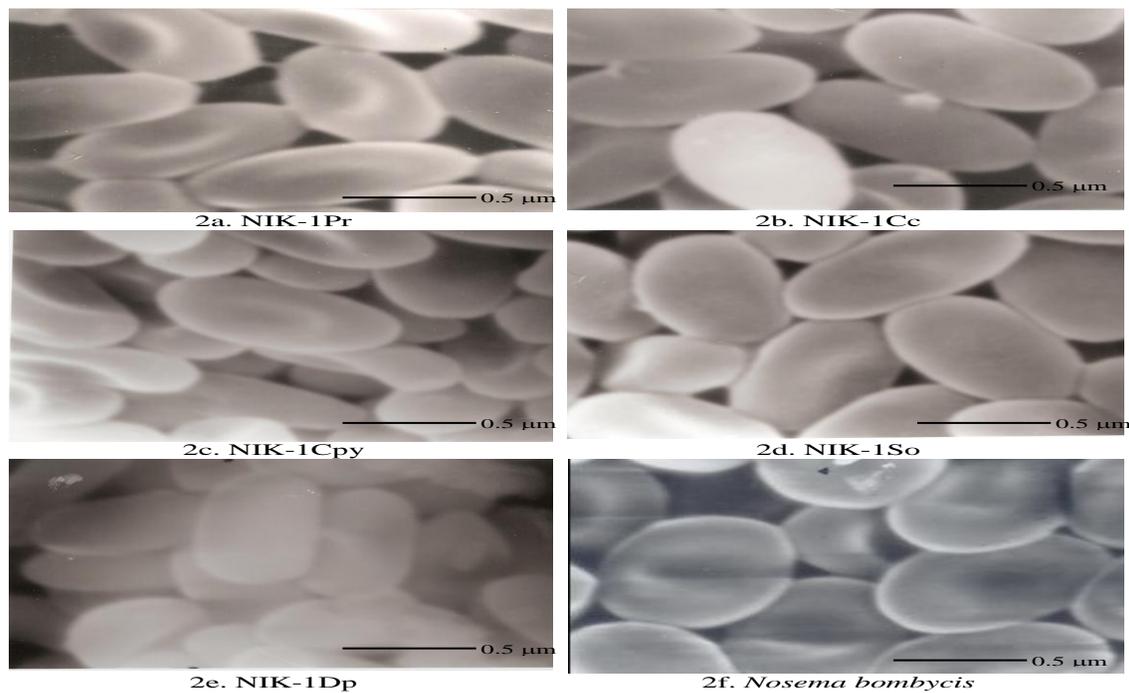


Plate.2 Scanning electron micrograph of microsporidians isolated from insect pests and compared with that of *N. bombycis* (35700x)



The morphological observations carried out under the present study are of considerable practical as well as academic value in the field of silkworm diseases. This preliminary study shows that they possess all the characteristic features of typical microsporidian. The spores of these microsporidians were oval or ovocylindrical in shape. In addition to *N. bombycis*, several other microsporidia infect silkworm which differ in spore shape, texture (Kawarabata, 2003; Sasidharan *et al.*, 2003; Singh and Saratchandra, 2003; Bhat *et al.*, 2009b). Sprague (1982) stated that most microsporidian spores are in the size range of 3-6 / 2-4 μm , but some are over 20 μm in length. The size range of microsporidian spores vary greatly within a single species (*Stempellia*, *Pleistophora*) or there may be two distinct sizes (dimorphic) as in *Vairimorpha*, *Burenella*, *Parathelohania*, *Amblyospora* (Issi, 1986). The surface sculpturing of certain spores, when observed with the scanning electron microscope, are distinct and characteristic and may also serve in classifying the microsporidia (Flower and Reeves, 1975b). The spores of *Nosema* sp. C01 isolated from *Pieris rapae* in Korea have been reported to be spherical in shape and the surface sculpturing is crumpled (Choi *et al.*, 2002). The study thus, reveals that the said microsporidians isolated from different insect pests of mulberry and other agricultural crops are different from each other and also from standard strain *N. bombycis*. Indeed the taxonomic position of these microsporidians to be clarified in further studies.

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