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Full Length Research paper

Screening of different insect pests of mulberry and other agricultural crops for microsporidian infection

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In the present study, different insect pests of mulberry and other agricultural crops were collected from mulberry gardens and agricultural crop fields in and around Mysore, Karnataka, India. The collected insects were screened for microsporidian infection and microsporidian spores were isolated from infected specimens, purified and tentatively designated as NIK- 1Pr, NIK- 1Cc, NIK- 1Cpy, NIK- 1So and NIK- 1Dp. The serological affinity test carried out through monoclonal and polyclonal antibodies, showed negative reaction of these microsporidia which indicated that these are niether *Nosema bombycis* nor Lb_{ms}..

Key words: Agricultural crops, mulberry, pests, microsporidia, monoclonal and polyclonal antibodies.

INTRODUCTION

In the silkworm *Bombyx mori* L, the disease caused by the microsporidian infection is called microsporidiosis and it is commonly known as pebrine. It has been reported long back in silkworm and is caused by an endoparasite, *Nosema bombycis*. The disease has become more complex because in addition to the *N. bombycis* several other species/strains of the microsporidians have been isolated from silkworm (Ananthalakashmi et al., 1994; Bhat and Nataraju, 2004; Selvakumar et al., 2005) and from other insects (Kishore et al., 1994; Kawarabata, 2003). Microsporidia have been found to be the common pathogens infecting insects under natural field conditions (Tanada and Kaya, 1993). Over 100 spp. of microsporidia have been described from insects and these include 40 *Nosema* spp. from different insects (Steinhaus, 1949). Infection due to N. bombycis has also been recorded in the insect orders Diptera (45 species), Lepidoptera (25 species), Ephermeroptera (13 species), Hymenoptera (6 species), Trichoptera (3 species), Coleoptera (2 species), Isoptera (2 species), Plecoptera (2 species) and one species in each of the following orders: Anoplura, Hemiptera, Odonata, Siphonoptera and Thysanura (Steinhaus, 1949).

Natural infection with *N. bombycis* has been reported in several lepidopteran insects such as *Pieris rapae* (Pieridae), *Spodoptera deparvata* (Noctuidae), *Spodoptera exigua* (Noctuidae), *Spodoptera litura* (Noctuidae) and *Trichoplusia ni* (Noctuidae). The mulberry pyralid, *Diaphania pulverulentalis* (Pyralidae) is also known to harbour *N. bombycis* (Sharma et al., 2003).

The present study has been undertaken with an aim to screen different insect pests of mulberry and some other agricultural crops for microsporidian infection and to see the cross infectivity of microsporidian infection from these insect pests to the economically beneficial insect, the silkworm *Bombyx mori* L.

MATERIALS AND METHODS

Different insect pests were collected from mulberry gardens and agricultural fields in and around Mysore, Karnataka, India by standard insect collection techniques (Donald et al., 1981). The description of these insect pests of mulberry and agricultural crops is presented in Table 1 and Plate 1. The collected insect pests were homogenized individually in 0.6% K_2CO_3 solution. The smear was prepared and observed for the presence of microsporidian spores

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Table 1. Description of the insect pests screened for microsporidian infection.

Insect pest	Common name	Family	Host plants
Sesamia inferens (Walker)	Pink stem borer	Noctuidae	Maize, Rice, Wheat and Sugarcane.
Phytomyza atricornis (Meigen)	Chrysanthemum leaf Miner	Agromyzidae	Cucumber, Lettuce, Pea, Tomato, Beans, Pepper, Onion, Spinach, Celery and Chrysanthemum.
Pieris rapae (Linnaeus)	Cabbage white Butterfly	Pieridae	Broccoli, Cabbage, Cauliflower and Brussels sprouts.
Eupterote mollifera (Walker)	Moringa hairy Caterpillar	Eupterotidae	Mulberry, Drum stick tree, Cardamom and Cashew.
Catopsilia crocale (Cramer)	Common Emigrant	Pieridae	Red gram, Bitter gourd, Sunflower, *Lantana camara* and *Amaltus*.
Terias hecabe (Linnaeus)	Common grass yellow	Pieridae	Pepper and plants in pea family *viz.*, Indigofera and Yellow pea bush. Also weeds, namely, *Euphorbia geniculata* and *Lantana camara*.
Catopsilia pyranthe (Linnaeus)	Mottled Emigrant	Pieridae	Bittergourd, Red gram and River bean.
Spilosoma obliqua (Walker)	Bihar hairy caterpillar	Arctiidae	Mulberry, Green gram, Soya, Pulses, Sunflower, Jute and Cotton.
Laphygma exigua (Hubner)	Beet armyworm	Noctuidae	Beans, Sugarbeet, Cabbage, Cauliflower, Corn, Tomato, Onion, Turnip and certain weeds , namely, *Parthenium*, *Chenopodium album* and *Amaranthus*.
Colias eurytheme (Boisduval)	Orange sulphur butterfly	Pieridae	Plants in pea family including Alfa alfa and White clover. Also, Milkweeds and Asters.
Diaphania pulverulentalis (Hampson)	Mulberry leaf roller	Pyralidae	Mulberry

at 600 × magnification under Nikon (Type-104) phase contrast microscope. The homogenate was further purified by using percoll. Highly purified microsporidian spores were identified by using monoclonal antibody based Latex agglutination kit (Yakult, Japan) developed for the identification of N. bombycis, Nosema sp. M11 and M12, and polyclonal antibody (Lb_{ms}) developed for the identification of Lemerin breed microsporidian. A drop of purified spores of each microsporidia was mixed with an equal amount of monoclonal antibody based spore specific latex agglutination reagent and also the polyclonal antibody on a clean glass slide with glass rod at room temperature and observed under phase-contrast microscope for spore-latex bead agglutination. Five samples were observed in each category. The observation was recorded as agglutination positive (+) for positive affinity and agglutination negative (-) indicating negative affinity of the microsporidian spores isolated from insect pests of mulberry and agricultural crops with that of the respective specific antibodies.

RESULTS AND DISCUSSION

Out of eleven insect pests screened for the microsporidian infection, only five were found infected (Table 2). The microsporidia isolated from these mulberry and agricultural pests were tentatively designated as NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So and NIK-1Dp wherein NIK represents “National Institute Karnataka” and Pr, Cc, Cpy, So and Dp represent the first letters of the generic and species name of the insect pests, namely, *P. rapae*, *Catopsilia crocale*, *Catopsilia pyranthe*, *Spilosoma obliqua* and *D. pulverulentalis* respectively from which these microsporidia were isolated. Microsporidia have been isolated from different agricultural and mulberry pests, namely, Spodoptera litura, Spodoptera exigua, Helicoverpa armigera, Plutella xylostella, P. rapae Crucivora and Pieris conidia sordida and Spilosoma obliqua (Singh et al., 2008). Several species of microsporidia also have been reported from the spruce budworm, Choristoneura fumiferana (Kees Van Frankenhuyzen et al., 2004).

The results of the affinity test are presented in Table 3. The spores of all the isolated microsporidia did not react positively with the monoclonal antibody based agglutination kit of N. bombycis and Nosema strains,

Plate 1. Insect pests of mulberry and other agricultural crops.

Table 2. Screening of different insect pests of mulberry and some other agricultural crops for microsporidian infection.

Name of the insect	Place of collection	No. of insects screened	No. of insects infected	Microsp-oridian infection	Infection %
Sesamia inferens (Lepidoptera: Noctuidae)	Gadige Srirangapatna Mallavali Maddur	105	0	-	Nil
Phytomyza atricornis (Diptera: Agromyzidae)	G B Sargur Pandavpura	113	0	-	Nil

Table 2. Contd.

Pieris rapae (Lepidoptera: Pieridae)	Dharmapura H D Kote Pandavpura C.S.R & T.I, Mysore	90	18	+	20.00
Eupterote mollifera (Lepidoptera: Eupterotidae)	Dharmapura Pandavpura Mallavali C.S.R & T.I, Mysore	107	0	-	Nil
Catopsilia crocale (Lepidoptera: Pieridae)	H D Kote Pandavpura C.S.R & T.I, Mysore	125	23	+	18.40
Terias hecabe (Lepidoptera: Pieridae	G B Sargur Pandavpura Bogadi C.S.R & T.I, Mysore	155	0	-	Nil
Catopsilia pyranthe (Lepidoptera: Pieridae	H D Kote Dharmapura C.S.R & T.I, Mysore	75	19	+	25.33
Spilosoma obliqua (Lepidoptera: Arctiidae)	C.S.R & T.I, Mysore Bogadi G B Sargur H D Kote Maddur	82	21	+	25.60
Laphygma exigua (Lepidoptera: Noctuidae)	Pandavpura Mallavali Dharmapura Bogadi	145	0	-	Nil
Colias eurytheme (Lepidoptera: Pieridae	H D Kote Dharmapura C.S.R & T.I, Mysore	162	0	-	Nil
Diaphania pulverulentalis (Lepidoptera: Pyralidae)	C.S.R & T.I, Mysore Bogadi G B Sargur	88	17	+	19.31

- :Absence of microsporidian infection; +: presence of microsporidian infection.

namely, M_{11} and M_{12} indicating that the microsporidia isolated from insect pests of mulberry and some other agricultural crops are serologically different from them. The isolated microsporidia also showed a negative reaction towards the polyclonal antibody of Lb_{ms} indicating their serological difference from the Lamerin microsporidian. The screening of different insect pests thus resulted in the isolation of five different microsporidians which are also serologically different from N. bombycis, Nosema spp. M_{11}, M_{12} and $Lb_{ms.}$ The results, thus, indicate that the microsporidians isolated from the insect pests of mulberry and other agricultural crops are different from each other, N. bombycis and also from $Lb_{ms.}$ Such microsporidia thus constitute a potential

Table 3. Serological affinity of the microsporidia isolated from insect pests and their comparison with serological affinity of *Nosema bombycis.*

Microsporidian isolates	Antibodies			
	Nosema bombycis **(Monoclonal)**	**M11 (Monoclonal)**	**M12 (Monoclonal)**	**Lbms (Polyclonal)**
NIK-1Pr	-	-	-	-
NIK-1Cc	-	-	-	-
NIK-1Cpy	-	-	-	-
NIK-1So	-	-	-	-
NIK-1Dp	-	-	-	-
Nosema bombycis	+	-	-	-

-: Negative reaction; +: positive reaction.

threat of gaining access to silkworm rearing through contaminated mulberry leaf and perpetuate infection despite routine care taken in mother moth examination and sanitation thereby may have an adverse impact on the sericulture industry. Hence, such pests should be kept under check in order to prevent the cross infectivity of microsporidian disease.

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