Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2014; 2 (1): 73-75
Received 06-12-2013
Accepted: 11-12-2013

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Paradigm of vertical transmission of newly isolated microsporidian NIK-5hm in popular multivoltine and bivoltine breeds of silkworm *Bombyx mori* L.,

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ABSTRACT

The microsporidian infection has become increasingly complex today due to different species of microsporidians are being isolated from silkworm and other mulberry insect pests. In India, different microsporidian species *viz.*, NIK-1s, NIK-2r, NIK-4M, NIK-3h and NIK-4hm have been isolated from silkworm. Different microsporidian species differ in their site of infection, rate of multiplication and mode of transmission. Recently a new microspridian strain NIK-5hm was isolated from silkworm which is different from other microsporidian species by havingspore formation inside the haemocytes and their mode of transmission.

In the present study, the paradigm of vertical transmission of NIK-5hm is studied in popular multivoltine and bivoltine breeds and it is observed that the new microsporidian strain NIK-5hm exhibit transovarial transmission both in multivoltine and bivoltine breeds to their progeny, but the rate of transmission significantly varies from standard strain, *Nosema bombycis*in both the breeds. The rate of transmission of NIK-5hm microsporidian to the F1 progeny in Pure Mysore (PM) and CSR2 ranges from 79.33-81.33% and 84.00-86.33%respectively. Where as in case of *Nosema bombycis* the rate of transmission is 100% in both PM and CSR2breeds.

Keywords: Transovarial, NIK-5hm, *Nosema bombycis*, *Bombyx mori* L., progeny.

1. Introduction

Silkworm diseases are the main constraint in successful harvest of quality cocoon in India. The estimated cocoon crop loss due to different silkworm diseases was 27-35% and cocoon yield loss was from 11-15 kg/100 dfls during different seasons [1]. Among different diseases, pebrine is the only disease which transmit both by primary or secondary infection and occurs during all seasons. Several authors reported that the spores of different microsporidian sp. infect different tissues and spore formation occurs in mid-gut epithelium, malpighian tubules, silk glands, fat bodies adipose tissue, gonads and trachea [2, 3, 4, 5]. Pebrine disease causing agent *Nosema bombycis* is a unique pathogen transmitted by way of egg *i.e*., through transovarial transmission and by the ingestion of contaminated mulberry leaf by silkworm. The disease is also transmitted by transovum transmission through the contamination of egg's surface [6]. The transmission may be through the infected ovary (transovarial) or venereal transmission as is observed in some microsporidia such as *Nosema kingi* in drosophilids [7] and *Thelohania* species in mosquitoes[8]. The transovarial transmission of *N. bombycis* is also demonstrated in *Diaphania pulverulentalis* by Ramegowda and[9].

2. Materials and Methods

To determine the mode of transmission of the newly isolated microsporidia, popular multivoltine (Pure Mysore) and bivoltine silkworm (CSR2) breeds were selected and on day zero of fourth instar, larvae of the said breeds were per orally inoculated separately with the spores of the isolated microsporidia at a dosage of 1×10^6 spores/ml. To compare the results, one set of larvae was inoculated with spores of *Nosema bombycis*. The inoculum containing 1×10^6 spores/ml of the isolated microsporidia or *N. bombycis* was prepared from the stock inoculums by proper quantification using Neubauer haemocytometer [10]. One ml of inoculum (1×10^6 spores/ml) of each microsporidia was smeared separately on 100 sq. cm surface area of mulberry leaf disc and fed to 100 larvae immediately after 3rd moult.

The larvae were allowed to feed on the contaminated leaves for 24 hours. The second normal feeding was given after 24 hours and the rearing on uncontaminated mulberry leaves was continued till cocooning. After cocoon formation, the cocoons from each treated batch were cut open for sex separation of the pupae. The male and female pupae were kept in separate trays for moth emergence. Yet another set of larvae was reared without inoculation till spinning and moth emergence.

Moths obtained from the inoculated larvae of different breeds were provisionally regarded as infected and were allowed to pair and lay eggs. Moths obtained from batches without inoculation were provisionally regarded as healthy and were allowed to pair and lay eggs. The pairing of the moths which formed the treatments was as follows,

2.1. In PM with NIK-5hm

T1: Healthy male (HM) × Infected female (IF) moth.
T2: Infected male (IM) × Healthy Female (HF) moth.
T3: Infected male (IM) × Infected female (IF)

2.2. In PM with *Nb*

T4: Healthy male (HM) × Infected female (IF) moth.
T5: Infected male (IM) × Healthy Female (HF) moth.
T6: Infected male (IM) × Infected female (IF)
T7: Healthy male (HM) × Healthy female (HF) (Control)

2.3. In CSR2 with NIK-5hm

T11: Healthy male (HM) × Infected female (IF) moth.
T12: Infected male (IM) × Healthy female (HF) moth.
T13: Infected male (IM) ×Infected female (IF)

2.4. In CSR2 with *Nb*

T14: Healthy male (HM) ×Infected female (IF) moth.
T15: Infected male (IM) × Healthy female (HF) moth.
T16: Infected male (IM) × Infected female (IF) moth.
T17: Healthy male (HM) × Healthy female (HM) (Control).

After mating, female moths were allowed to lay eggs on egg sheets. The layings were prepared from individual mother moths. The male and female moths were homogenized separately after egg laying and the wet mount were examined for the spore of microsporidia and the observation were recorded. The laying laid by moth confirming to the treatment requirements were picked for further study. The progeny larvae were reared as per standard methods [11]. After I moult, 100 larvae/batch were collected randomly and homogenized individually and the smear was observed under phase contrast microscope for the presence of microsporidian spores and thus, the transmission rate was calculated by the standard formula [12] which is as follows;

$$\text{Transmission rate} = \frac{(A \times B) + (C \times D)}{A + C}$$

Where A- Number of dead eggs.
B- % of dead eggs infected.
C- Number of larvae hatched.
D- % of larvae infected.

3. Results and Discussion

The result of studies on the vertical transmission of NIK-5hm microsporidian in two silkworm breeds *viz.*, PM and CSR2 is presented in Fig 1and 2. The results were compared with rate of transmission of *N. bombycis* for both the breeds. It is observed that larvae hatched from the eggs obtained from infected male × healthy female (IM×HF) in respect of both NIK-5hm microsporidian and *N. bombycis*, in both silkworm breeds (T2, T5, T12 and T15) and healthy male × healthy female (HM × HF) *viz.*, T7 and T17 did not reveal infection in the progeny of both the breeds, while in progeny larvae hatched from eggs obtained from healthy male × infected female (HM × IF) *viz.*, T1, T4, T11 and T14 as well as infected male × infected female (IM × IF) *viz.*, T3, T6, T13 and T16 revealed infection.

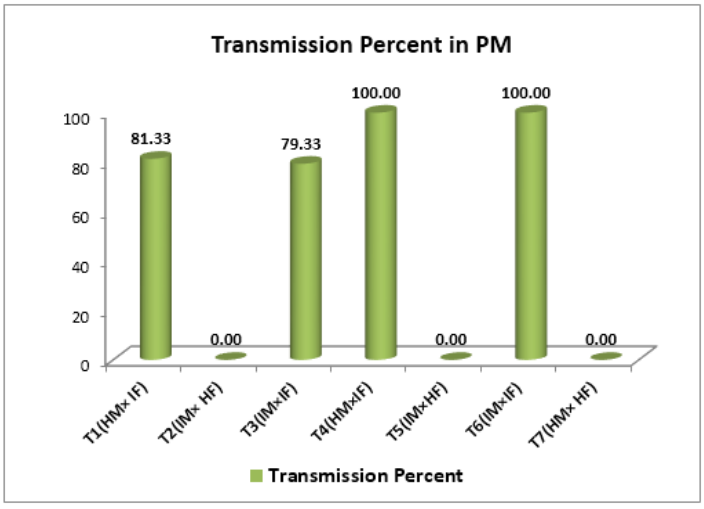

Fig 1: Comparison of rate of transmission in PM

The F1 progeny larval examination of NIK-5hm microsporidian infected male x infected female (IM×IF) *viz.*, T3, and T13 revealed infection in all the breeds. The rate of transmission in PM and CSR2 breeds were ranged from 79.33±0.00 -81.33±1.53 and 84.00±4.58-86.33±3.79respectively. The F1 progeny larval examination of *N. bombycis* infected male × infected female (IM ×

IF) *viz.*, T6, and T16 revealed infection and the rate of transmission was 100% in both the breeds.

The progeny larval examination of NIK-5hm microsporidian and *N. bombycis* infected male × healthy female (IM×HF) *viz*., T2, T5, T12, and T15 did not reveal infection in the progeny and the rate of transmission was nil with respect to both the breeds tested. The progeny of the control group (HM × HF) *viz.*, T7 and T17 of all the two breeds with no inoculation with any of the microsporidians (NIK-5hm microsporidian and *N. bombycis*) did not reveal infection and the rate of transmission was nil.

No transmission of either NIK-5hm microsporidian or *N. bombycis* to the progeny occurred in the healthy female × infected male (HF×IM) crosses, indicating that there was no venereal pathway for either microsporidian. Only the female moth transmits the infection and male moth does not transmit to next progeny.

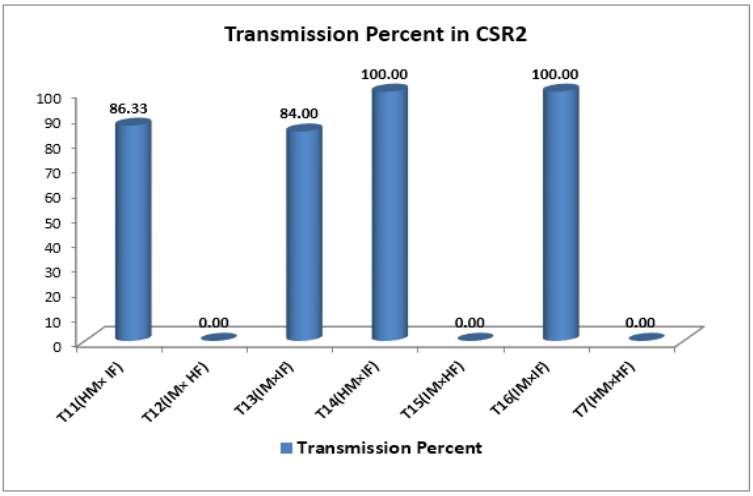

Fig 2: Comparison of rate of transmission in CSR2

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