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A.R. Narasimha Nayaka
Central Sericultural Research and Training Institute, Mysore-570008, India.
Mail: nayaka_1965@rediffmail.com

S.D. Sharma
Central Sericultural Research and Training Institute, Mysore-570008, India
Mail: sdsharmacsb@gmail.com

Correspondence:
A.R. Narasimha Nayaka
Central Sericultural Research and Training Institute, Mysore-570008, India
Email: nayaka_1965@rediffmail.com

Dynamics of total haemocyte count (THC) in popular bivoltine and multivoltine breeds of mulberry silkworm *Bombyx mori* L., during the progressive infection of newly isolated microsporidian NIK-5hm

A. R. Narasimha Nayaka* S.D.Sharma

ABSTRACT

The silkworm, *Bombyx mori* L. is a delicate and sensitive lepidopteron insect, which has been domesticated for silk production since time immemorial. Due to continuous domestication, silkworm becomes susceptible to various diseases. During the course of infection the cellular defense mechanism in silkworm is mediated by different types of haemocytes. In the present study, popular bivoltine and multivoltine breeds were selected to carry out experiment to understand the day to day changes in total haemocyte count (THC) in silkworm larvae inoculated with newly isolated microsporidian NIK-5hm. During the course of progressive infection of NIK-5hm, significant enhancement of total haemocyte count (THC) was observed till eighth day. Later gradual reduction of the haemocyte population was noticed in the inoculated batches of both the breeds.

Keywords: NIK-5hm, *Bombyx mori* L., Total haemocyte count (THC).

1. Introduction

Insects stimulate a complex defensive response during the course of invasion of foreign body. Insects exhibit both cellular and humoral immune responses in addition to metabolic alterations that are effective against various pathogens like bacteria, fungi, protozoa *etc*., Humoral reaction involves slow synthesis of anti-bacterial, anti-viral and anti-misrosporidian principles and requires several hours for full expression. Cellular response is direct interaction between circulatory haemocytes invading non-self-material is immediate through phagocytosis, nodule formation and encapsulation. In Insects, different types of haemocyte are observed in the haemolymph [1,2]. Haemocytes are a complex of several types of cells that circulate within the haemolymph, but are sometime attached loosely to other tissues or are enmeshed within them. Among them, there are well defined types of haemocytes in most of the insects (prohaemocytes, plasmatocytes and granulocytes) and one or more other types present in some other insects (coagulocytes, spherulocytes, adipocytes and oenocytoids). The blood cells in the silkworm, *Bombyx mori* L. classified into six types *viz*., prohaemocytes, plasmatocytes, granulocytes, spherulocytes, imaginalspherulocytes (observed only at adult stage, but occasionally in pupa on the day before emergence) and oenocytes [3]. It was reported that five types of haemocytes *viz*., prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytes in silkworm, *Bombyx mori* L. [4,5,6].

2. Materials and Methods

Susceptible and resistant silkworm breeds (CSR2 and Pure Mysore) were collected from Germplasm bank of Central Sericultural Research and Training Institute, Mysore, Karnataka, India, for this study. The silkworm larvae were brushed and reared in the laboratory under optimum conditions according to [7]. Immediately after third moult *i.e.* on the first day of the fourth instar, the larvae were inoculated, through *per-os* administration of spore suspension (1.00×10^6 spores/ ml @ 1.00 ml/100 worms). From zero hours onwards of inoculation, haemolymph was collected every alternative day by clipping third pair of abdominal legs and drained into pre-chilled eppendof tubes. The haemolymph was used for the enumeration of total haemocyte count by using phase contrast microscope.

Total haemocyte count (THC) was enumerated in the haemolymph of inoculated and control batches along with standard *Nosema bombycis* using haemocytometer following standard method [8]. THC per mm^3 of haemolymph was estimated according to the formula suggested by [9]. The percent difference of THC with respective control and inoculated batches due to the infection of NIK-5hm and *Nosema bombycis* presented in Fig.1.

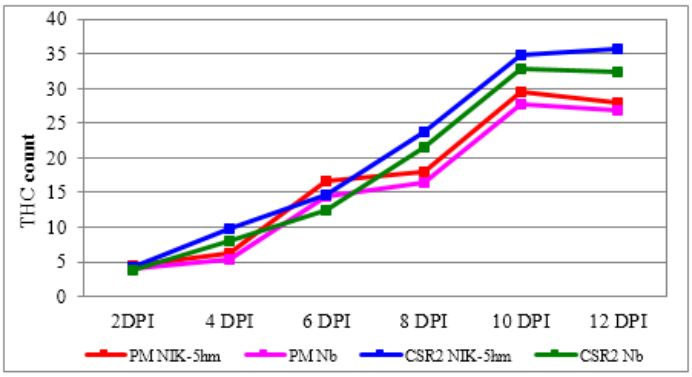

Fig 1: Percent difference of THC in haemolymph during the course of NIK-5hm and *Nb* infection in the CSR2 and PM breeds of silkworm, *Bombyx mori* L.

3. Results and Discussion

The THC in haemolymph during the progressive infection of NIK-5hm and *Nosema bombycis* in CSR2 breed of silkworms is presented in the Table 1. As the larval age progressed from IV to V instar, the THC increased in both the breeds. In CSR2 control, the THC was 5550 mm^3 on 0 day and as the larval age advanced, the THC count increased from 5550 to 10917 mm^3 on 12DPI. In case of NIK-5hm inoculated batches, the THC increased from 5550 to 7022 mm^3 on 12 DPI. Results indicated that during the progressive infection of NIK-5hm, the THC increase was relatively less when compared to respective controls. The percent of increase in THC decreased from 4.21 to 35.68 on 2 to 12 DPIwhen compare to control batches. Similar trend was observed in THC in haemolymph during the progressive infection of *Nosema bombycis* in CSR2 breed. In case of *Nosema bombycis* inoculated batches, the THC increased from 5550 to 7377mm^3 on 12 DPI. Results also indicated that during the progressive infection of *Nosema bombycis*, the percent increase was less in inoculated batches when compared to respective controls. The percent increase in THC count in *Nosema bombycis* infected batches decreased was 3.82 to 32.43 on 2- 12 DPI. Which is relatively less when compare to percent increase in THC over NIK-5hm inoculated batches.

Table 1: Total heamocyte count (THC) in haemolymph during the course of NIK-5hm and *Nosema bombycis* infection in the CSR2 breed of silkworm, *Bombyx mori* L.

DPI	Total heamocyte count in NIK-5hm infected haemolymphNo.of Cells/mm3			Total heamocyte count in *Nb* infected haemolymphNo.of Cells/mm3		
	CSR2 Control	CSR2 inoculated	% of Difference	CSR2 Control	CSR2 inoculated	% of Difference
0	5,550	5,550	0.00	5,550	5,550	0.00
2	7,442	7,128	4.21	7,442	7,158	3.82
4	8,617	7,775	9.77	8,617	7,920	8.08
6	9,649	8,225	14.76	9,649	8,442	12.51
8	10,708	8,166	23.75	10,708	8,400	21.56
10	11,824	7,710	34.80	11,824	7,943	32.83
12	10,917	7,022	35.68	10,917	7,377	32.43

In PM breed,THC in haemolymph during the progressive infection of NIK-5hm and *Nosema bombycis* is presented in Table 2. As the larval age progressed from IV to V instar, the THC increased in both the breeds. In PM control, the THC was 7342 mm^3 on 0 day and as the larval age advanced, the THC count increased from 7342 to 11550 mm^3 on 12DPI. In case of NIK-5hm inoculated batches, the THC increased from 7342 to 8308 mm^3 on 12 DPI. Results indicated that during the progressive infection of NIK-5hm, the THC increase was relatively less when compared to respective controls. The percent of increase in THC was comparatively less in NIK-5hm infected batches when compare to the controls. Similar trend was observed in THC in haemolymph during the progressive infection of *Nosema bombycis* in PM breed. In case of *Nosema bombycis* inoculated batches, the THC increased from 7342 to 8438 mm^3 on 12 DPI. Results indicated that during the progressive infection of *Nosema bombycis* the percent increase was less in inoculated batches when compared to respective controls.

Table 2: Total heamocyte count (THC) in haemolymph during the course of NIK-5hm and *bombycis* infection in the PMbreed of silkworm, *Bombyx mori* L.

DPI	Total heamocyte count in NIK-5hm infected haemolymphNo.of Cells/mm3			Total heamocyte count in *Nb* infected haemolymphNo.of Cells/mm3		
	PM Control	PM inoculated	% of Difference	PM Control	PM inoculated	% of Difference
0	7,342	7,342	0.00	7,342	7,342	0.00
2	8,488	8,102	3.55	8,488	8,150	3.99
4	9,258	8,688	6.16	9,258	8,754	5.45
6	9,900	8,258	16.58	9,900	8,472	14.43
8	11,030	9,038	18.06	11,030	9,209	16.51
10	12,067	8,492	29.63	12,067	8,732	27.64
12	11,550	8,308	28.07	11,550	8,438	26.94

The observation of THC level in both susceptible and resistant breeds is in response of the insect host in terms of the THC level during the progressive infection, point to the possibility of a correlation between the susceptible and resistance nature of the breedsto the disease and THC. The results also indicate that the low rate of increase in THC level in NIK-5hm inoculated batches over *Nosema bombycis*in both susceptible and resistant breeds during the progressive infection shows that, NIK-5hm is more virulent than *Nosema bombycis*.

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