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Histopathological studies of newly isolated microsporidian NIK-5hm infecting tissues of Silkworm, *Bombyx mori* L.

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Abstract
Ultrastructure of Gut of Silkworm, *Bombyx mori* L. infected with newly isolated microsporidian NIK-5hm shows hypertrophy and structural disorganization in the infected tissue. From 0 to 6 DPI, there were no clear histopathological symptoms. On 8 DPI, histo-pathological symptoms were initiated and few midgut goblet and columnar cells developed abnormality and became shrunken. On 10 DPI, The cells were less stained throughout the progressive infection. On 12 DPI multi-layered and undifferentiated regenerative *nidi* cells were found. The infected cells were continuously pushed off into the midgut lumen due to newly developed immature cells of regenerative *nidi* cells. The number of regenerative *nidi* cells were more in this tolerant breed (PM) compared to susceptible breed (CSR2). From 12 DPI, the damaged goblet cells were continuously replaced by the newly formed regenerative *nidi* cells.

Keywords: *Bombyx mori*, hyperthropy, NIK-5hm, disorganization, gut lumen

1. Introduction
Microsporidian disease of silkworm is caused by different strains of microsporidia, which produces atypical morphological symptoms in diseased larvae, spreads in silkworm population at an alarming rate and causes severe crop loss. Microsporidia are reported to cause infection in various susceptible tissues [1, 2]. The intensity of microsporidian infection varies with reference to breed and tissues [3]. Earlier *Nosema Bombycis* known to be more virulent but recently newly isolated microsporidia NIK-5hm also a virulent strain causes economic injury to the silkworm *Bombyx mori* and transmits the diseases both horizontally and vertically. However no information was available on the infection of newly isolated microsporidian (NIK-5hm) on resistant (PM) and susceptible (CSR2) silkworm breeds thus the present study was undertaken.

2. Materials and Methods
One susceptible breed (CSR2) and one resistant (PM) silkworm breeds were selected for the present study. These breeds were brushed enmass and reared following the standard rearing method up to III instar. Immediately after III moult, one hundred newly ecdysed 4th instar larvae were *per orally* inoculated with an infective dose by smearing on mulberry leaf @ 1ml/100 sq. cm leaf/100 larvae. The control batches were also maintained without any inoculation. The larvae were reared up to 12 DPI and collected the samples. To study histopathological changes microtome technique was used. This technique involves number of steps *viz.,* collection of samples, fixation, dehydration and clearing of samples, embedding of sample in wax and block making, cutting of blocks in to section by microtome, downgrade hydration of sections, staining and upgrade dehydration of sections, clearing of section and finally mounting of section as mentioned below.
From both inoculated and control batches of CSR2 and PM, larvae were dissected individually and midgut tissue was taken very carefully to avoid damage to the tissue from every alternative days 0-12 DPI (0,2,4,6,8,10 and 12) and the hisptopathological work was carried out as per the standard methodology described by Laren Lacy (1997) [4].

3. Result and Discussion
The comparative histopathological changes in the midgut tissue during the progressive infection of NIK-5hm, *Nosema bombycis* batches shows marked structural disorganization of tissue in both susceptible (CSR2) and resistant (PM) breeds. Where in case of control batches midgut shows healthy midgut epithelium, columnar and goblet cells without any abnormal changes.
In the silkworm, epithelium of larval midgut is the sole target of microsporidian infection, After infection by NIK-5hm and *Nosema bombycis* microsporidia, epithelial cells of the midgut shows various cytopathic changes characteristic to respective microspordia, and general outcome of the infected cells is either to be discharged into midgut lumen. These processes of infection and resulting cytopathic events are altered depending upon the different physiological status of the midgut epithelium and the physiology of the midgut epithelium changes markedly [5]. In the present study, histopathological changes in the midgut of susceptible breed (CSR2) indicated that though there was infection in the midgut and there were no clear histopathological symptoms from 1 to 6 DPI. On 8 DPI onwards histopathological symptoms were manifested and midgut goblet and columnar cells developed abnormality and became shrunken and discharged into the lumen. From 10 to 12 DPI which was an advanced stage of infection, more number of infected cells (goblet and columnar cells) were discharged into the gut lumen and only few regenerative *nidi* cells developed into new goblet and columnar cells which leads to physiological damage of the silkworm [6]. When LD_{50} dose fed to silkworm, it was observed that scattered matured spores of both NIK-5hm and *Nosema bombycis* microsporidia in the infected tissue and also the gut lumen, but spore intensity were more in case of NIK-5hm infected tissue (Fig 1).

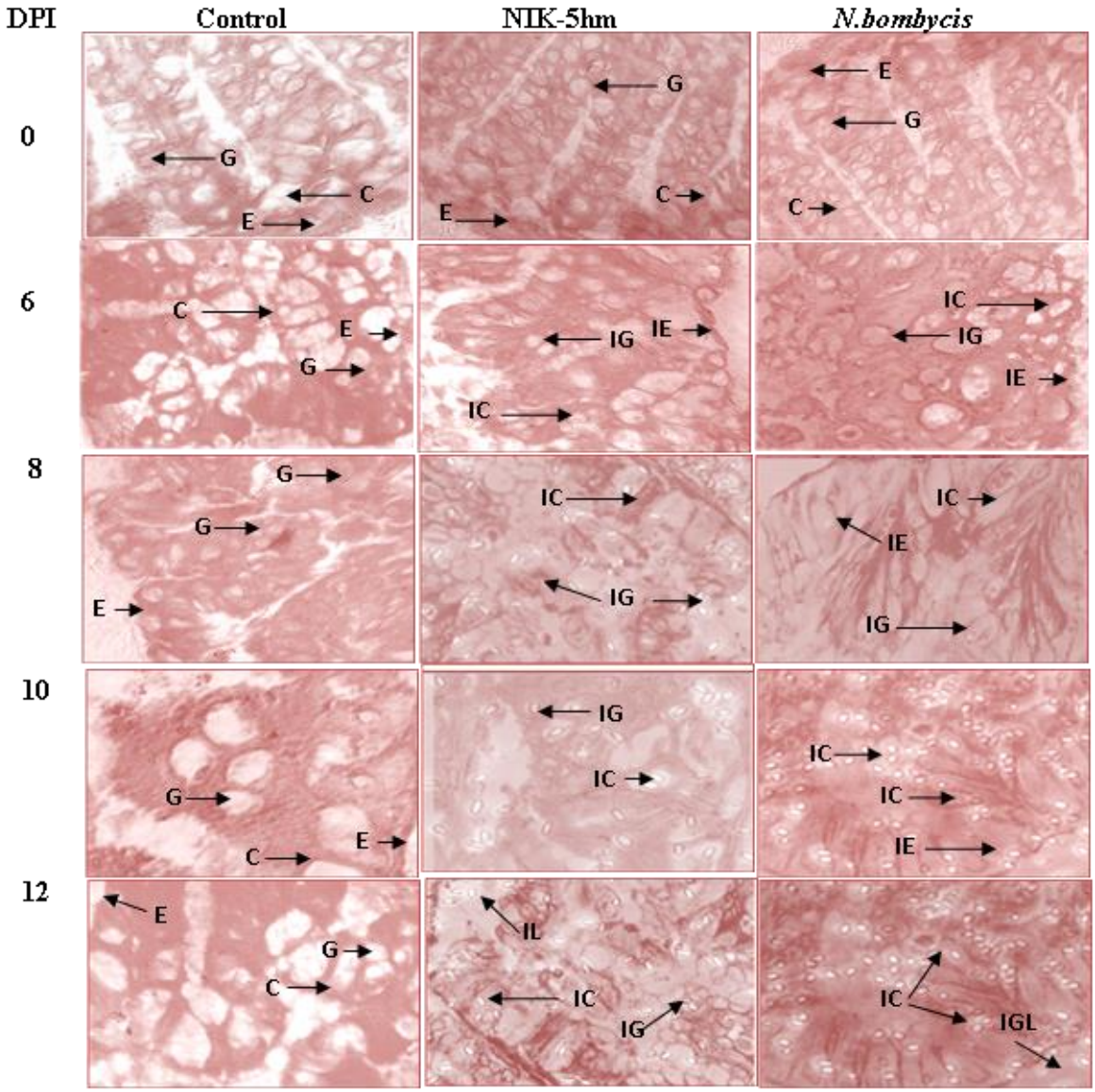
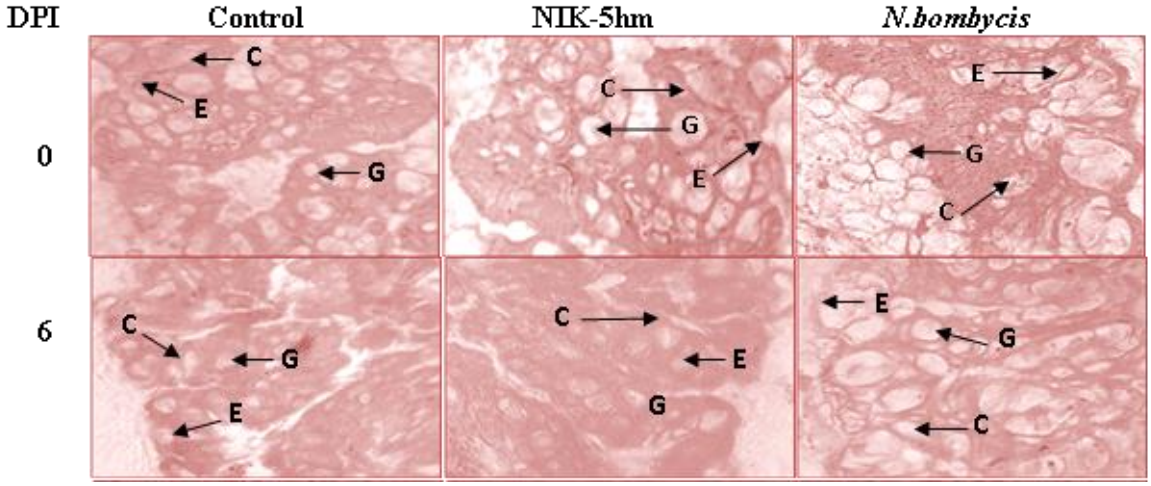


Fig 1: Cross section of midgut of CSR2 larva during the progressive infection of NIK-5hm and *N.bombycis*. **C**-columnar cells; **E**-epithelial cells; **G**-goblet cells, **EIC**-early infected columnar cells; **EIG**-early infected goblet cells; **GL**-infected gut lumen, **IC**-infected columnar cells; **IG**-infected goblet cells

In case of resistant PM breed similar trend was noticed, epithelium of larval midgut is the primary target of microsporidian infection, After infection by NIK-5hm and *Nosema bombycis*, epithelial cells of the midgut show various cytopathic changes it was observed the matured spores of both NIK-5hm and *Nosema bombycis* are observed in the infected tissue and also the gut lumen, but spore intensity was more in case of NIK-5hm infected tissue (Fig. 2).



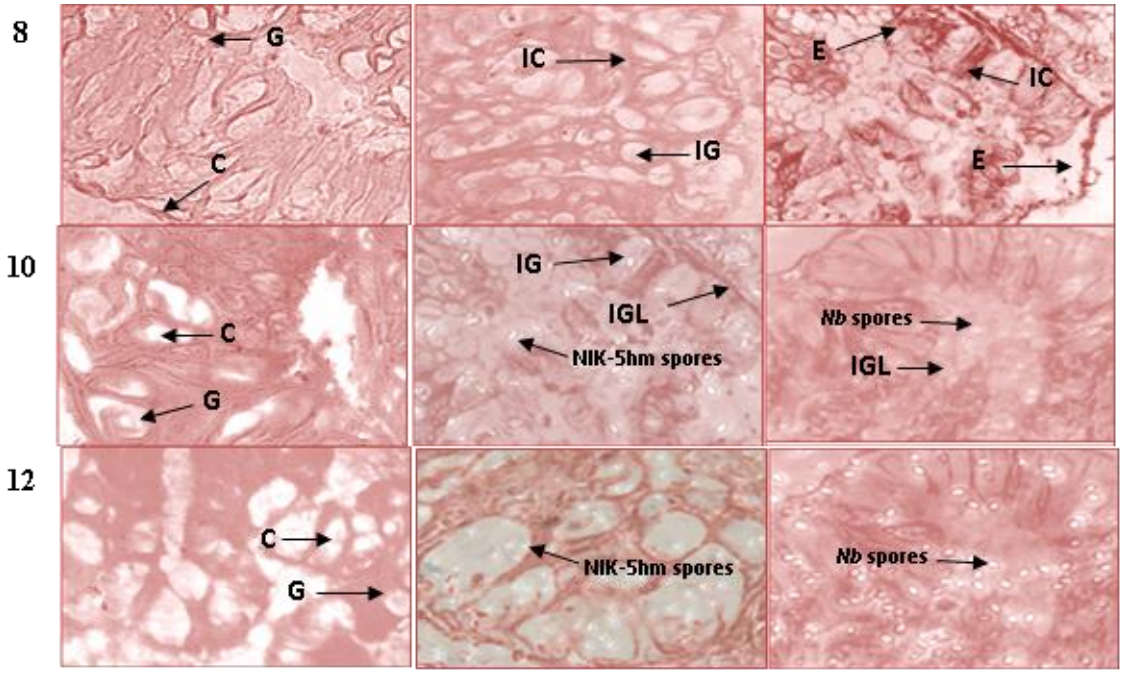


Fig 2: Cross section of midgut of PM larva during the progressive infection of NIK-5hm and *N.bombycis.* **C**-columnar cells; **E**-epithelial cells; **G**-goblet cells, **EIC**-early infected columnar cells; **EIG**-early infected goblet cells; **GL**-infected gut lumen, **IC**-infected columnar cells; **IG**-infected goblet cells

The results indicated that in both susceptible (CSR2) and resistant (PM) breeds during the progressive infection and multiplication were observed in NIK-5hm and *Nosema bombycis.* However, in CSR2 the number of infected goblet cells increased continuously while in resistant breed the infected goblet cells were less in number as the infection progressed. This may be due to the discharging of infected cells into gut lumen and the regeneration of new goblet cells from the *nidi* cells allowing physiological repair. The regeneration of the goblet cells in place of infected cells helps in prolonging the life of infected larvae. Therefore the tolerance level depends on the regenerative capacity of midgut. Ananthalaxmi (1994) studied the progressive infection due to the infection of *Nosema bombycis* and observed that in the midgut ephitheliun goblet cells are severely infected than the columnar cells [7].

The regenerative capacity of epithelial cells of midgut differs in different silkworm breeds and possibly the basis for inter breed differences in their susceptibility to NIK-5hm. The rate of multiplication in the midgut is same in both susceptible and tolerant silkworm breeds. However, in the resistant breeds the infected goblet cells are discharged into the midgut and the regenerative cells rapidly develop into new goblet cells this repair mechanism may prolonging the tolerance level of resistant breeds (PM).

4. References

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