**Review Article**

**Review on management of *Nosema bombycis* causing pebrine disease in silkworm, *Bombyx mori.***

**Abstract:** The major constraints in silk cocoon production are occurrence of the diseases in silkworm. Pebrine (protozoan disease) is a major disease of the silkworm*, B. mori* caused by microsporidian parasite, *Nosema bombycis* N. *N. bombycis* infection causing pebrine in silkworm is transmitted both horizontally and vertically, any lapse in monitoring the progeny could result in serious damage to the sericulture industry. The literature reveals that, pebrine disease is manageable but very difficult to eradicate. Early detection and elimination of pebrine disease is essential for sustenance of cocoon crop from industrial point of view. Mother moth examination is the only method that is being followed to date. Therefore there is need to develop a more effective, reliable and instant molecular diagnostic tools for early detection and management of the disease.

**Introduction:** Disease is a condition in which a state of physiological equilibrium of the host with the environment become unbalanced. The major constraints in silk cocoon production are occurrence of the diseases in silkworm. Silkworm, *Bombyx mori* L. is poikilothermic hence, it is unable to regulate its body temperature. So, even minute changes in the rearing environment makes the silkworm susceptible to many viral, bacterial, fungal and protozoan diseases. Pebrine (protozoan disease) is a major disease of the silkworm*, B. mori* caused by microsporidian parasite, *Nosema bombycis* N. The name pebrine was coined by De Quadrefagues in 1860. It is named because, appearance of pepper like spots on the diseased larvae in advanced stage of infection. This disease is also known as Kata or Matha kata in Bengali and Gantu or Gantu roga in Kannada. Microsporidian disease remained as a threat to the silk industry since time immemorial, because it is the only disease of silkworm which is transmitted both vertically and horizontally. The disease spreads quickly and takes a heavy toll of silkworm and even results in total crop failure when the infection is severe (Govindan *et al.,* 1998).

**History of the Disease:** The microsporidiosis of silkworm, commonly known as Pebrine is the earliest known menace to silk industry. Microbiological studies of silkworm were initiated after the outbreak of disease in France in 1845. Several historical evidences in various countries of the world showed that the outbreak of Pebrine disease had greatly influenced the decline of the sericulture industry in the past. The first scientific record of occurrence of the disease came from France in 1845, where the annual cocoon production of cocoons came down from 26,000 tonnes in 1853 to 4000 tonnes in 1865 due to epizootics of microsporidiosis and subsequently collapsed the French and Italian silk industry. Later in the year 1984 disease spread to Spain, Syria and Romania (Steinlaus, 1949). In India, the first report of the occurrence of the disease was from Mysore in 1866 followed by an epidemic level outbreak of the disease in Kashmir in 1878 (Baigh *et al*., 1997). It is due to the outbreak of this disease in 1878 that Kashmir lost its productive indigenous univoltine breed “Kashmir Race” some 131 years back (Kamili and Massodi, 2000).

**Causative agent:** By the end of 19th century only *Nosema bombycis* was known as the causative agent of microsporidiosis of silkworm but the advanced molecular, biological and immunological studies of this parasite revealed that there are several other microsporidia belonging to different genera, causing microsporidiosis in silkworm. These spores are different in spore shape, size as well as in pathogenicity (Table-1). Each having different morphological, pathological and antigenic characters; some infecting only midgut cells and others some specific tissues (Table-2). Many of them though infective but have demonstrated low multiplication rate in silkworm (Bhat and Nataraju, 2006). (Chitra *et al.,* 1975)have reported that one of the isolated strains of *Nosema bombycis* infects only the midgut cells which is less virulent then the normal strain which infects all the tissues of the host.

**Table 1: Pathogenicity of different microsporidia infecting *Bombyx mori* (Bhat *et al.,* 2017).**

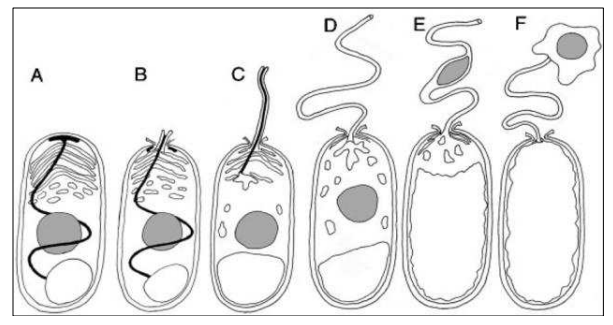
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Microsporidian isolates** | **Pathogenicity** | **Spore form** | **Spore size (µm)** | |
| Length | Width |
| ***N. bombycis*** | High | Oval | 3.8 | 2.6 |
| ***Nosema* sp. M11** | Low | Oval | 3.9 | 1.9 |
| ***Nosema* sp. M12** | Low | Ovo-cylindrical | 4.5 | 2.0 |
| ***Pleistophora* sp** | High | Oval | 2.7 | 1.6 |
| ***Thelophania* sp*.* (M 32)** | Low | Oval | 3.4 | 1.7 |
| **NIK-2r** | Low | Oval | 3.6 | 2.8 |
| **NIK-4m** | Low | Oval | 5.0 | 2.1 |
| **NIK-5hm** | High | Ovo-cylindrical | 5.0 | 3.1 |
| ***Nosema* sp*. Lbms*** | Low | Ovo-cylindrical | 4.36 | 2.14 |
| ***Microsporidian* sp*. SI*** | High | Oval | 1.73 | 1.01 |
| **NIAP-6p** | - | Oval | 5.00 | 2.40 |
| **NIAP-7g** | - | Oval | 4.60 | 2.50 |
| **NIK-5d** | - | Oval | 3.70 | 2.70 |
| **NIK-1Pr** | High | Ovo-cylindrical | 5.41 | 2.85 |
| **NIK-1Cc** | Low | Oval | 4.60 | 2.77 |
| **NIK-1Cpy** | High | Oval | 4.96 | 2.85 |
| **NIK-1So** | High | Ovo-cylindrical | 5.26 | 2.61 |
| **NIK-1Dp** | Low | Oval | 4.27 | 2.79 |
| **Msp** | - | Ovo-cylindrical | 5.38 | 2.92 |

**Table 2: Site of infection of different microsporidians in silkworm (Bhat *et al.,* 2017).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Microsporidia** | **Host tissues** | | | | | |
| **Gut epithelium** | **Malpighian tube** | **Muscle** | **Fat body** | **Silk gland** | **Gonad** |
| ***N. bombycis* NIS-001** | + | + | + | + | + | + |
| ***Nosema* sp. NIS-M11** | - | + | + | + | + | - |
| ***Vairimorpha* sp.**  **NIS-M12** | - | + | + | + | + | - |
| ***Nosema* sp. NIS-M14** | - | + | + | + | + | - |
| **Microsporidian**  **NIS-M25** | - | + | + | + | + | - |
| ***Plistophora* sp.**  **NIS-M27** | + | - | - | - | - | - |
| ***Thelohania* sp.**  **NIS-M32** | - | + | - | - | - | - |
| ***Nosema* sp. NIK-is** | + | + | + | + | + | + |
| ***Nosema* sp. NIK-2r** | + | + | + | + | + | + |
| ***Nosema* sp. NIK-3h** | - | + | + | - | + | -\* |
| ***Vairimorpha* sp. NIK-4m** | +\* | - | - | - | - | - |

(-\*): Cyst formation on the surface of gut, (+\*): Formation of spores only in heavily infected larvae, (-): No infection, (+): Infection.

**Life cycle of *Nosema bombycis*:** The intracellular cycles of *N. bombycis* include the proliferative merogonic stage (merogony) and sporogonic stage (sponogony). The mature spore is oval or ovo-cylindrical and measures approximately 3.4-3.8 µm in length and 2.0 – 2.3 µm width, with three-layered membrane (inner, middle and outer). The spores are highly refractive, and shine bluish white under microscope exhibiting ‘Brownian movement’. The outline is smooth and the spores are heavier than water. The resistant form of the disease is spore and it remains either in an infected tissue of the body or discharged through excreta by leaving infected host tissue. *N. bombycis* infects silkworm both horizontally by ingestion of spores and transmitted to progeny by the vertical means (Han and Watanabe, 1998). The spore, when swallowed by the silkworm through contaminated food germinates under alkaline conditions inside the gut of host with the help of digestive juice and produces a long polar filament measuring 500 µm in length and 0.5 µm in width [Fig.-1], and it is more than 30 times longer than that of the lengthwise dimension of the spore, on the end of which grows a sporoplasm (Peter *et al.,* 1999). The sporoplasm has one or two nuclei and other cell organs and possesses limited membrane. The polar filaments are short our and anchor firmly to the gut wall by penetrating the epithelial cells of the alimentary canal. The sporoplasm emerging from the spore invades the cytoplasm of the host. The force that propels the everting of filament and drives the sporoplasm through the polar tube appears to be osmotic. Subsequently the polar filament gets digested in the alimentary tract. The two nuclei of the sporoplasm unite to form a uninucleate planont. The planont measures 0.5-1.5 µm and is formed in 1-2 days. The planont is sub shell, performs amoeboid movement and reproduces by binary fission. The planont which initially infects the gut later passes through the gut wall and invades the various tissues. Once the planont penetrates the host cell, it transforms in to a sedentary form and becomes localized. The stage is known as meront. Meront is an intracellular stage and has a definite cell wall which absorbs nutrients from host cell. The meront is formed in 2-3 days after infection. It reproduces by binary fission, multiple budding. When cytoplasm of the host cell is exhausted, meronts are arranged in parallel rows. The meront after massive proliferation fills up the host cells and when nutrients are depleted, sporulation takes place. The spore completes its life cycle within 4 days. Complete developmental stages of the pathogen have been studied and elucidated in detail (Takiwaza *et al.,* 1975). The mature spore is unicellular endomembranous differentiation of its sporoblast (Vavra *et al.,* 1976). These authors designated the sporoblasts as phase-I sporoblasts and Phase-II sporoblasts. The phase-I sporoblasts are characterized by the presence of a dark staining spherical body (Singh *et al.,* 2007). *Nosema bombycis* completes its relatively simple life cycle with two sporulation sequences both with diplokaryotic sporont and disporoblastic sporogony. The primary (early) sporulation sequence produces a thin-walled binucleate spore called “primary spore, internal spore or FC spore” (few coils of polar tube) with 4 coils of short polar filament and a large posterior vacuole. The primary spore can germinate quickly after formation in the infected cells and serve to disseminate infection within *Bombyx mori* (auto infection) and they are responsible for transovarial transmission because of the infection of the gonads. In cultured insect cells or in vivo, the primary spores are present at 48 h after infestation (Kawarabata and Ishihara, 1984). The second sporulation sequence produces a binucleate spore with thicker spore wall and 10 to 13coils of long polar filament in one row arrangement (Iwano and Ishihara, 1991). They are detected 72 h after infestation of the insect cell cultures (Ishihara and Sohi, 1996). These external or environmental spores are involved in the infestation of new hosts and function to infect the new host in horizontal transmission.



**Fig. 1**: Model of spore germination (Keeling and Fast, 2002). (A) Dormant spore, showing polar filament (black), nucleus (grey), polaroplastand posterior vacuole. (B) Polaroplastand posterior vacuole swells, anchoring disk ruptures, and polar filament begins to emerge and evert. (C) Polar filament continues to evert. (D) Once the polar tube is fully everted, the sporoplasm is forced into (E) through the polar tube. (F) Sporoplasm emerges from the polar tube bound by new membrane.

**Mode of infection and transmission method:**

A. Transovarial/Embryonic/Vertical Transmission: Embryonic infection occurs when the pathogen infects fourth or fifth instar larvae in which it invades the epithelial cells of the ovaries and then parasites are transferred to the oogonia, oocytes and nutritive cells. Parasitism of the oocytes may result in death of the resultant eggs after being laid. If non-infected oocytes absorbs the nutrient material from infected nutritive cells, the protozoan will pass over to the oocytes and may cause infection to the embryos (Govindan *et al.,* 1998).

B. Horizontal Transmission: i) Contamination in rearing bed:Secondary contamination takes place in the rearing bed through the faecal matter of the infected larvae, contaminated tray, dust from the contaminated room and dead larvae infected with disease. ii)Through mulberry leaf: Improper disposal of silkworm litter, infected larvae and use of incompletely decomposed silkworm bed refuse in mulberry fields as manure may form a source of contamination to the mulberry leaves. iii)Through layings:Incubation of layings in contaminated room without proper disinfection, results in surface contamination of eggs and larvae get infected (Govindan *et al.,* 1998).

**SYMPTOMS OF THE DISEASE:** Govindan *et al.,* 1998 stated that symptoms of this disease are observed in all the developmental stages of silkworm as given below

1. **Egg Stage:** The egg count of the infected moth are very less and eggs fail to attach to the egg sheets firmly. The eggs laid by infected moth are mostly unfertile and may be dead. Heavily infected eggs are generally seen in lumps.
2. **Larval stage**

* The infected larvae shows poor appetite, Irregular moulting retarded growth and development results in disparity of size
* Black pepper like spots are also noticed on the skin of heavily infected larvae
* Pebrinised larvae generally die

1. **Pupal stage**

* Infected pupae have swollen and blackish abdomen
* Heavily infected pupae fail to metamorphose into an adult
* Cocoons spun by infected silkworm may become flimsy and malformed

1. **Moth stage**

* Infected moths have deformed wings, distorted antennae and discoloured body
* Infected moths do not exhibit typical mating behaviour and have poor fecundity
* In pebrinised moths, accessory glands are infected, which results in laying loose egg

**Management of the pebrine disease:** For successful prevention and timely curtailing of pebrine disease all the possible detective methods should be employed effectively.

**1. Predictive test:** The suspected silkworm in early instars and faeces will be subjected for detection of spores. First sampling must be done during first and second instar, another sampling must be made from fourth instars upto the mounting period to identify second stage of contamination.

**2. Pupal test:** When the seed cocoon lots arrive to the market, random sampling of cocoons suspected to be pebrinised will be made and the presence of the pathogen in pupae is confirmed before the disposal of seed cocoons to the grainages.

**3. Forced eclosion test:** The confirmity for the presence of pathogen is made even before the moth emergence so that if the lot contains more pebrine, atleast those cocoons can be used for reeling silk.

* In this test, a small number of seed cocoons from each batch is preserved at temperature of 32 which facilitates early emergence by 1-2 days
* The early ecloded moths are examined and if found to be pebrinised, the whole batch of cocoons is sent for reeling after proper stifling

**4. Mother moth examination method:**

For the examination of mother moths, the standard method of Fujiwara (1984) is followed

**Step 1.** Collection of mother moths and labelling

**Step 2**. Mother moths crushed in a domestic mixer for 2 minutes by adding 4ml of 0.6% of potassium carbonate solution in the ratio of 1:4

**Step 3.** The homogenate is allowed to settle for 3-5 minutes in a beaker

**Step 4.** The un-macerated tissue and debris is separated and discarded by decantation. The bottom liquid is filtered by using filter paper

**Step 5.** The filtered sample is centrifuged at 3000 rpm for 3 minutes

**Step 6.** Later the supernatant liquid is decanted and pellet is dispersed in a few drops of 2% KOH solution over a cyclomixer

**Step 7.** Two smears from each sample is examined under microscope (600X). The intensity of infection is estimated on the basis of the number of spores/field



**Fig. 2: Steps followed during mother moth examination. Source: TNAU Agritechportal**

**5. Delayed mother moth examination**

* For delayed mother moth examination method (Sing *et al.,* 2004), the moths after oviposition were collected in groups in perforated cardboard boxes/covers and preserved in an isolated, well-ventilated room at 25-30ºC for a period of 3-4 days and examined under microscope (600X) at 4 days interval.
* Moths were examined as per the procedure of mother moth examination.

**6. Immunological techniques**

* Immunological techniques have been applied for detection of pebrine and identification of species of *N. bombycis* and closely related species with the use of
* Double immunodiffusion technique
* Slide agglutination test:
* Antibody sensitized latex
* Fluorescent antibody technique

These tests could be mainly used for early detection thereby enabling adoption of adequate preventive and control measures (Govindan *et al.,* 1998).

**PREVENTION AND CONTROL**

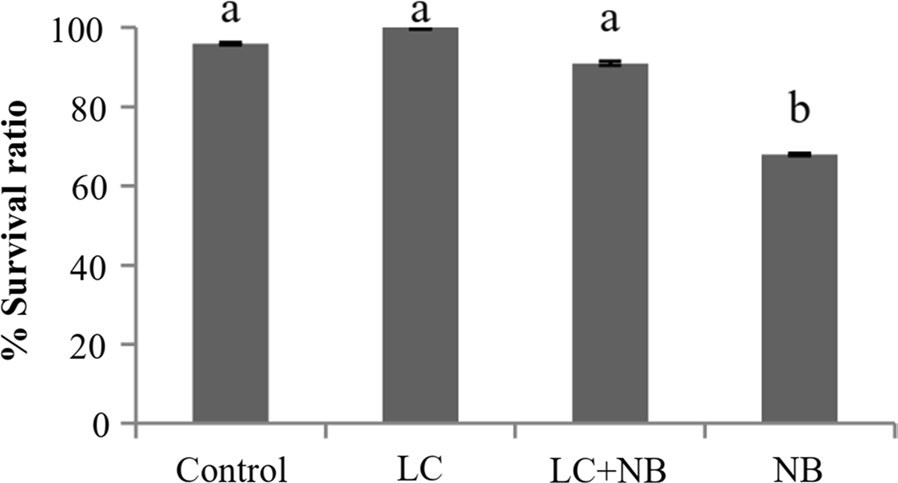
* The disease is transmitted both vertically and horizontally, this unique characteristic of the disease has made it difficult to completely eliminate it from the silkworm crops
* The disease has become more complex now because of the occurrence of different strains of microsporidians infecting the silkworm
* So, it is essential that one should have a thorough knowledge of the available methods of detection of pebrine pathogen can be detected timely and effectively which further helps to plan for successful prevention of the disease

1. In grainages only disease- free layings will be allowed for rearing.
2. Two rounds of effective disinfection of rearing house and appliances may have to be ensured once immediately after cocoon harvest with 3% formalin and the other just prior to the next brushing with 5% bleaching powder solution
3. Surface disinfection of incubated eggs two days before hatching with 2% formalin for 5 min to overcome the contamination during incubation
4. Rearing of silkworm breeds most tolarent or resistant to silkworm pathogen is the best option against the loss due to disease
5. Collection and disposal of diseased larvae
6. Prompt destruction of pebrine infected rearings by burning.
7. Avoid contamination of mulberry leaf with silkworm pathogens
8. Maintain hygienic conditions in egg production room and rearing sites.
9. Maintain personal hygiene by proper disinfection of hand and foot during rearing
10. Bed cleaning and disinfection at regular intervals
11. Regulate humidity in rearing bed by dusting slaked lime at the time of every moult
12. Provide good quality and sufficient quantity of mulberry, requisite spacing and ventilation especially during fourth and fifth instars
13. The earliest method suggested by Pasteur based on selection of pathogen free eggs through careful systematic examination of mother moths for pathogens, after egg laying has been one of the most effective methods even today to avoid the disease in the silkworm crops (Govindan *et al.,* 1998).

**Table 3. Efficacy of different bed disinfectants against the spread of pebrine in silkworm rearing (Irfan *et al.,* 2003).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Treatment** | **No. of larvae/bed** | **Pebrine disease** **(%)** | **Healthy larvae (%)** | **Disease reduction over control (%)** |
| **1**  **2**  **3**  **4** | Vijetha  Labex  Resham Jyothi  Inoculated control | 100  I00  100  100 | 09.55  16.00  14.66  22.11 | 90.4  84.00  83.34  77.89 | 52.28  27.63  33.69 |
|  | S.E ± |  | 1.32 |  |  |
|  | C.D at 5% |  | 4.58 |  |  |

The data on the efficacy of different bed disinfectant formulations against the spread of pebrine in the rearing bed were presented in Table 3 which showed that all the bed disinfectant formulations had limited efficacy in preventing the spread of pebrine in rearing bed. However, among the treatments, Vijetha was found to be comparatively more effective in reducing the spread of pebrine with 52.28% reduction in pebrine infection followed by Resham Jyothi (33.69%) and Labex (27.63%) (Irfan *et al.,* 2003).



**Fig 3.** The effects of *L.casei* (LC) and *N. bombycis* (NB) on survival ratio of *B.mori* larvae

Note: Diferent letters on the top of each bar represent diference identifed by the Analysis of Variance coupled with Duncan’s Multiple Range Test at 95% signifcant level. Bars represent 1 unit of standard deviation, Breed - polyvoltine hybrid DokBua

Siripuk *et al.* (2021) investigated on the supplementation of *Lactobacillus casei* reduces the mortality of *Bombyx mori* larvae challenged by *Nosema bombycis* and the study revealed that higher survival percentage for silkworm larvae supplemented with *L. casei* (100.00 ± 0.00%) followed by control larvae (96.00 ± 0.33) and larvae receiving both *L. casei* and *N. bombycis* (91.00 ± 0.33%) and less survival percentage was observed in pebrine-infected larvae not receiving *L. casei* (68.00 ± 0.33% (Fig. 3).

**Table 4: Effects of *L. casei* and *N. bombycis* challenge on Thai polyvoltine strain,**  **DokBua of the silkworm, *B. mori* (Siripuk *et al.* 2021).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatments** | **Larval weight of fifth instar (g)** | **Cocooning ratio (%)** | **Pupation ratio (%)** | **Cocoon weight (g)** | **Cocoon shell weight (g)** |
| ***Lactobacillus casei* (108 cell/mL) followed by *N. bombycis* (106 spores/mL)** | 2.70 ± 0.96a | 95.00 ± 0.00ab | 95.00 ± 0.33a | 1.33 ± 0.33a | 0.23 ± 0.33a |
| **No treatment** | 2.55 ± 0.48a | 96.00 ± 0.33b | 100.00 ± 0.33b | 1.23 ± 0.67a | 0.20 ± 0.57a |
| ***Lactobacillus casei* (108 cells/mL)** | 2.84 ± 0.48b | 96.00 ± 0.57a | 100.00 ± 0.57c | 1.40 ± 0.58**a** | 0.23 ± 0.67**a** |
| ***N. bombycis* (106 spores/mL)** | 2.12 ± 0.96 | 93.00 ± 0.33 | 87.00 ± 0.66 | 1.27 ± 0.33 | 0.19 ± 0.33 |

**Statistically signifcant diferences as measured by Duncan multiple range test are denoted with diferent letters**

Siripuk *et al.* (2021) reported that *L. casei* supplementation had positive impact on the growth characters. As compared to larvae receiving only *N. bombycis*, the 5th larval instar weight was signifcantly higher in all groups. *N. bombycis* infection signifcantly lowered the cocooning ratio, but *L. casei* treatment made the ratio remain on an intermediate level. The pupation ratio was 100% in the groups not infected by *N. bombycis*. The *L. casei* treatment resulted in a signifcantly higher pupation ratio in *N. bombycis* infected larvae (95% with *L. casei* vs. 87% without *L. casei*). The highest cocoon weight and cocoon shell weight were obtained in those larvae receiving *L. casei*, although this diference was not statistically significant (Table 4).

**Table 5: Effect of carbendazim treatment on *N. bombycis* infected silkworms** (**Jyothi *et al..,* 2004)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Average weight of ten larvae at fifth instar of sixth day (g)** | **Larval mortality (%) due to** | | **ERR (%)** | **Single cocoon weight (g)** | **Cocoon shell ratio (%)** | **Pebrinized cocoons** |
| **pebrine** | **toxicity** |
| **TRI-1** | 36.12 | 4.40 | 0.0 | 90.80 | 1.69 | 18.98 | 100 |
| **TRI-2** | 36.97 | 0.0 | 23.20 | 71.60 | 1.70 | 19.08 | 13.92 |
| **TRI-3** | 34.64 | 0.0 | 55.80 | 41.00 | 1.71 | 18.14 | 0.0 |
| **TRI-4** | 38.69 | 0.0 | 0.0 | 95.40 | 1.72 | 20.23 | 0.0 |
| **Infected and untreated** | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Uninfected and untreated** | 39.36 | 0.0 | 0.0 | 95.20 | 1.69 | 20.13 | 0.0 |
| **F-test** | HS | HS | HS | HS | HS | HS | HS |
| **CD at 5%** | 0.64 | 1.04 | 4.98 | 5.48 | 0.076 | 0.78 | 1.70 |
| **CD at 1%** | 0.84 | 1.37 | 1.37 | 7.23 | 0.10 | 1.03 | 2.25 |

**TRI-1: 2 mg/worm/day (from II instar to spinning), TRI-2: 6 mg/worm/day (from II instar to spinning), TRI-3: 8 mg/worm/day (from II instar to spinning), TRI-4: 2 mg/worm/day (from II to III instar and 8 g/worm/day from IV instar to spinning). TRI- Treatment infected, ERR- Effective rate of rearing, HS- Highly significant Breed-NB4D2**

Jyothi *et al.* (2004) reported that among all the treatments, TRI-4 shows better effect in all the parameters such as average weight of average weight of ten larvae at fifth instar of sixth day (38.69 g**)**, larval mortality due to pebrine (0.0 %) and toxicity (0.0%), ERR (95.40%), single cocoon weight (1.72 g), cocoon shell ratio (20.23) and pebrinized cocoons (0.0). Because, observation of the ultrastructural changes, revealed that carbendazim has a profound effect on the cellular organization of the parasite and also interferes very effectively in the progression of development of the spores causing abnormalities in both the merogonic and sporogonic stages of *N. bombycis* in the silkworm. However, the host-cell organization remained normal (Table 5).

**Table 6: Curative effect of carbendazim treatment on *N. bombycis* infected silkworm *B. mori* after different post-infection (pi) periods (Jyothi *et al.,* 2004)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Average weight of ten larvae at fifth instar of sixth day (g)** | **Larval mortality due to pebrine (%)** | **ERR (%)** | **Single cocoon weight (g)** | **Cocoon shell ratio (%)** | **Pebrinized cocoons** |
| **TRI-24** | 40.03 | 0.0 | 93.66 | 1.59 | 20.14 | 0.0 |
| **TRI-48** | 39.01 | 0.0 | 94.33 | 1.58 | 20.04 | 0.0 |
| **TRI-72** | 38.30 | 0.0 | 93.33 | 1.68 | 17.94 | 38.78 |
| **TRI-96** | 37.60 | 4.33 | 93.0 | 1.49 | 16.48 | 68.23 |
| **TRI-120** | 35.40 | 4.98 | 93.16 | 1.28 | 16.14 | 100 |
| **Infected** | 0.0 | 97.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Uninfected** | 40.88 | 0.0 | 96 | 1.65 | 20.16 | 0.0 |
| **F-test** | HS | HS | HS | HS | HS | HS |
| **CD at 5%** | 1.20 | 1.68 | 1.75 | 0.09 | 0.53 | 5.79 |
| **CD at 1%** | 1.74 | 2.45 | 2.55 | 0.12 | 0.77 | 8.43 |

**TRI-24: After 24 h pi till spinning, TRI-48: After 48 h pi till spinning, TRI-72: After 72 h pi till spinning, TRI-96: After 96 h pi till spinning, TRI- 120: After 120 h pi till spinning. TRI- Treatment infected, ERR- Effective rate of rearing, HS- Highly significant Breed-NB4D2**

Jyothi *et al.* (2004) revealed that the larval weight was significantly high in all treatments from TRI-24 to TRI-120 when compared with infected control. During the larval stage, mortality caused by pebrine was not observed up to 72 h pi but was observed in the 96 and 120-h pi batches. The moths that emerged from the 24- and 48-h pi batches showed no pebrine infection. Infection rate was significantly high in moths that emerged from batches where treatment of the chemical started after 72 and 120 h pi. The percentage of infected moths was 38.78% in TRI-72, 68.23% in TRI-96 and 100% in TRI-120 batches. Hence, the administration of the drug should start within 48 h pi before the parasite establishes itself in the host tissues (Table 6).

**Table 7: Efficacy of benzimidazole derivatives in larval mortality (Bhat *et al*., 2012)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Mortality and disease reduction due to treatment (%)** | | | |
| 0.25% | 0.50% | 1.00% | Control |
| **Metronidazole** | 1.33 ± 0.57 (85.74) | 1.00 ± 0.00 (89.28) | 0.66 ± 0.57 (92.92) | 9.33 ± 0.57 |
| **Albendazole** | 1.33 ± 0.57 (85.74) | 0.33 ± 0.57 (96.46) | 0.00 ± 0.57 (100.0) |
| **Tinidizdazole** | 1.66 ± 0.57 (82.20) | 1.66 ± 1.15 (82.20) | 1.00 ± 1.00 (89.28) |
| **Ornidizole** | 2.00 ± 1.00 (78.56) | 1.33 ± 0.57 (85.74) | 0.33 ± 0.57 (96.46) |
| **Mebendazole** | 0.00 ± 1.00 (100.00) | 0.00 ± 0.57 (100.0) | 0.00 ± 0.57 (100.0) |
| **Satranidizole** | 1.66 ± 0.57 (82.20) | 2.00 ± 1.00 (78.56) | 0.66 ± 0.57 (92.92**)** |

**Each value is mean ±SD of three replications; values within parenthesis indicate % reduction in mortality. Breed-Lamerin breed**

Bhat *et al*. (2012) reportedthat among six drugs tested, mebendazole were found effective against microsporidian spores at all the concentrations. Rest of the drugs *viz.,* metronidazole, albendazole, ornidazole and satinidizole were found effective only at 1%. However, tinidizole was not found effective against the microsporidian spores at any of the concentrations tested. The results showed that among tested benzimidazole derivatives, mebendazole (0.25 to 1.00%), albendazole (1.00%) were found effective in reduction of larval mortality to an extent of 100% (Table 7).

**Table 8. Efficacy of benzimidazole derivatives on suppression of infection at moth stage Bhat *et al*. (2012).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Disease suppression at moth stage due to treatment (% )** | | | |
| 0.25% | 0.50% | 1.00% | Control  59.38 ± 4.78 |
| **Metronidazole** | 25.18 ± 2.88 (59.59) | 24.56 ± 0.66 (58.63) | 23.40 ±1.54 (60.59) |
| **Albendazole** | 27.36 ± 1.06 (53.92) | 22.76 ± 1.19 (61.67) | 22.94 ± 0.51 (61.36) |
| **Tinidizdazole** | 25.56 ± 5.24 (56.95) | 28.41 ± 1.98 (52.15) | 27.44 ± 1.45 (53.78) |
| **Ornidizole** | 23.60 ± 3.55 (60.25) | 30.04 ± 1.44 ( 49.41) | 24.14 ± 1.81 (59.34) |
| **Mebendazole** | 22.44 ± 1.02 ( 62.20) | 22.51 ± 2.63 ( 62.09 | 23.79 ± 0.92 (59.93) |
| **Satranidizole** | 24.58 ± 2.52 ( 58.60) | 24.05 ± 2.86 ( 59.49) | 27.05 ± 1.61 (54.44) |

Each value is mean ±SD of three replications; values within parenthesis indicate % reduction in mortality. Breed-Lamerin breed

Bhat *et al*. (2012) reported that the percent of infected moths was significantly low in all treatments as compared to the inoculated control. It is recorded 59.38% in inoculated control was reduced to 22.44 to 30.04% in treated batches (Table 8).

**CONCLUSION**

*N. bombycis* infection causing pebrine in silkworm is transmitted both horizontally and vertically, any lapse in monitoring the progeny could result in serious damage to the sericulture industry. The literature reveals that, pebrine disease is manageable but very difficult to eradicate. The disease has become more complex now because of the perpetual incidence of different strains of microsporidian infection in silkworm. Early detection and elimination of pebrine disease is essential for sustenance of cocoon crop from industrial point of view. Mother moth examination is the only method that is being followed to date. Therefore there is need to develop a more effective, reliable and instant molecular diagnostic tools for early detection and management of the disease.

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