DISEASES OF MULBERRY SILKWORM AND THEIR CONTROL

Dr.H.B.Mahesha, Yuvaraja’s College, Mysore.

Mulberry silkworm *Bombyx mori* is affected by a number of diseases caused by viruses, bacteria, fungi and protozoa. These diseases are known to occur in almost all the silkworm rearing areas of the world causing considerable damage to the silkworm cocoon crop. A number of measures have been suggested for the prevention and control of these diseases, but none of them has proved to be fool-proof with the result that one has always to be careful to eliminate the cause of primary infection as well as to prevent the cross infection. Care is also needed to be taken to see that they are not exposed to stress conditions like adverse temperature and humidity, bad ventilation and nutritional deficiency which may make them easily susceptible to various diseases.

**VIRAL DISEASES**

Viral diseases of silkworm pose a major problem to sericulture as they account for almost 70 per cent of the total loss due to diseases. Viral diseases of silkworm comprise of inclusion and non-inclusion types. The inclusion virus diseases form typical inclusion bodies. They are Nuclear polyhedrosis and Cytoplasmic polyhedrosis which can be more easily identified through ordinary microscopy. The non-inclusion type consists of Infectious flacheric and Densonuclosis which can be detected only through electron/fluorescent microscopy and serological tests.

**Nuclear Polyhedrosis**

It is one of the most serious virus diseases in tropical countries and occurs throughout the year. This disease is otherwise known as Grasseric, Jaundice, Milky disease, Fatty degeneration and Hanging disease.

**Causes of the disease**

This disease is caused by *Borrelina bombycis* virus belonging to the sub-group A of the family Baculoviridae. As the name implies, this virus multiplies and forms polyhedra (Fig. 1) in the nucleus of the tracheal epithelial cells, adipose tissue cells, dermal cells and blood cells. Occasionally the nucleus of the middle and posterior portion of silk gland cells are also affected. The viral particles are rod shaped and the size is around 330 x 80 nm. The size of the polyhedra varies from 3-6µ. The shape is usually octadecahedral or hexahedral and sometimes tetragon or trigon.
Infection mostly takes place through feeding of polyhedra contaminated mulberry leaf, rarely through wounds. Heat, cold and chemical treatments have also been known to induce this disease. Factors inducing the outbreak of this disease are high temperature and humidity, their sudden fluctuations, bad ventilation in the rearing room, ineffective disinfection of rearing room and equipments and feeding of tender leaves during late instars. Inadequate larval spacing, starvation and excessive moisture in the rearing bed have also been known to contribute towards the outbreak and spread of the disease.

**Symptoms:** During early part of the disease no symptoms are noticed except the worms being slightly sluggish. Initially the skin shows oily and shining appearance (Fig. 2). As the disease advances the skin becomes thin and fragile and the body becomes milky white with intersegmental swellings (Fig. 3). The fragile skin is prone to rupture easily, liberating the liquefied body contents containing innumerable number or polyhedra which become the source of secondary contamination. Another characteristic symptom of this disease is that the larvae become restless and crawl aimlessly along the ridges or rims of rearing trays, (fig 4) subsequently falling on the ground and dying. Death takes place after infection in about 4-5 days in the young larvae and 5-7 days in the grown-up larvae. Diseased larvae lose the clasping power of abdominal legs except the caudal legs by which it hangs with the head downwards (Fig, 5). If the infection is early the worms fail to spin the cocoons and die, whereas if the infection is late they are able to Spin the cocoons but die inside producing melted cocoons.
Prevention and control: For effective prevention of this disease, the silkworm rearing rooms, mulberry storage rooms, mounting rooms, equipments and rearing premises should be thoroughly disinfected before brushing. The eggs should be essentially surface disinfected. Silkworms should be reared under strict hygienic conditions. During rearing the diseased and dead larvae form the major source of infection with the largest quantity of fresh polyhedra available. Hence, the diseased larvae should be removed carefully without breaking the skin and disposed suitably by putting them in lime vats or by burning. Depending upon the stage of silkworm, suitable temperature and humidity should be provided. During IV and V instars fresh air circulation should be ensured by providing cross ventilation. The silkworms should be fed with nutritive rich mulberry leaf and during later stages feeding of tender leaf should be avoided. Depending upon the stage of larvae, optimum spacing and required quantum of leaf should be given. Proper bed drying is necessary before each feed to avoid accumulation of moisture in the bed.

In addition to the above, use of certain bed disinfectants could also prevent secondary contamination and spread of the disease. Paraformaldehyde compounds are known to have anti-
microbial properties and various formulations involving this chemical have been prepared like Papazol in Japan and Reshamkeet Oushadh in India. The latter is a bed disinfectant formulation containing 1 per cent captan (N-Trichloromethyl Thio-4-Cyclohexane 1,2-Dicarboxymide), 1 percent paraformaldehyde (Tri-oxyethylene) 2 per cent Benzoic acid and 96 per cent slaked lime powder giving dual protection against grasserie and muscardine. It should be dusted on the larvae and bed with the help of a thin cloth at the rate of 2-3 grams/0.1 sqm. area during early instars and 4-5 grams/0.1 sqm. during IV and V instars. The dusting should be done (Fig. 6) preferably once after each moult, half an hour before resumption of feed. An additional dusting should be done on the 4th day of final instar after bed cleaning. The dusting should not be done when the larvae are under moult or preparing for moult. The quantity of Reshamkeet Oushadh required for 100 disease free layings (40,000 larvae) is between 3-3.5 kgs.

![Figure 6. Dusting of “Reshamkeet Oushadh”](image)

**PROTOZOAN DISEASES**

Protozoa which are injurious to silkworm are the parasitic ones belonging to the class Microsporidia and genera *Nosema*, *Pleistophora* and *Thelohania*. However, the major protozoan disease of the silkworm is the pebrine disease, so named due to the appearance of black peppery patches following infection.

**Pebrine**

Pebrine is a chronic and disastrous disease of the silkworm *Bombyx mori* L. It was this disease which was responsible for the sudden collapse of the silkworm industry of both France and Italy in 1965. Even though the fight against this disease in all the sericultural countries is going on since more than 100 years, the disease is not yet eliminated. However, it has been kept under check by following the techniques of strict mother moth examination for the supply of disease free silkworm eggs, in addition to disinfection and hygienic rearings. Though the
disease is under reasonable control, it appears sporadically due to infected seed and persisting secondary contamination in the rearing house.

**Causes of the disease:** Pebrine is caused by *Nosema bombycis* Nageli belonging to family Nosematidae of order Microsporidia. The pathogen infects the host through feeding of contaminated mulberry leaf (*peros*) and also by rearing infected silkworm eggs (transovarial). In addition to *Nosema bombycis*, seven different microsporidians belonging to genera *Nosema*, *Pleistophora* and *Thelohania* have been reported to infect the silkworm.

Sources of infection are rather extensive. The main source is the rearing of transovarially and surface contaminated layings. Infection also results from diseased and dead larvae, faeces of larvae, moths, diseased egg shells, larval and pupal exuviae *etc*. In the rearing bed major source of infection is the faeces of diseased larvae, contaminated tray, seat paper and dust from infected rearing and leaf storage rooms. Sometimes infection takes place through contaminated mulberry leaf from field. The excreta and dead larvae of pebrine infected wild insects may also form a source of infection.

The life cycle of *Nosema bombycis* Nageli includes three stages namely, spore, planont and meront (Fig. 7).

The mature spore is oval or ovocylindrical (Fig. 8-9). It measures approximately 3 -4x 1.5 -2.5 μ with three layered membrane: the inner, middle and outer. The sporoplasm is stretched in the form of girdle across the width of the spore and it contains a pair of nuclei. The spore has a polar capsule and polar filament. The polar filament is given out on treatment with a number of chemicals like H₂O₂ and KCl. Spores are highly refractive and appears light blue under the microscope. The spore represents the dormant stage of the pathogen and can survive in the ordinary conditions of rearing house for more than a year. It
retains its infectivity even after three years in the dried body of the female moth, in liquid medium for more than 3 weeks and in soil (or more than 2 months. But the spore is susceptible to desiccation and cannot survive for more than 6 - 7 hours in direct sunlight (39 - 40°C). It is also weak against heat, chemicals and disinfectants.

When live spores enter into the silkworm through mulberry leaf, they germinate in the gut due to high alkalinity and potassium ions. As a result the polar filament is extruded and the sporoplasm along with two nuclei creeps through it and injects into the midgut tissues. 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 µ and is formed in 1-2 days. The planont is sub globular with a strong refractive nucleus without 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 susceptible tissues.

Once the planont penetrates the host cell, it transforms in to a sedentary form and becomes localized. This 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 spherical or pear shaped and is formed in 2 - 3 days after infection. It reproduces by binary fission, multiple fission or by 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. From the germination of the spores to sporulation is the entire developmental cycle of the pebrine protozoan.

**Symptoms:** The symptoms of this disease can be observed in all the stages of silkworm viz., egg, larvae, pupa and adult. These symptoms form an important criterion for identifying the disease.
In the egg stage, poor egg number, lack of adequate adherence to the substratum, lack of egg uniformity, more of unfertilized and dead eggs, poor and irregular hatching are some of the symptoms. Sometimes infected eggs cannot hatch out and hatched larvae may also die.

Larvae show poor appetite, retarded growth and development leading to un-uniformity in size (Fig. 10). Larvae moult irregularly and show sluggishness. Transovarially infected larvae die before third moult but those which are heavily infected die during first instar itself. The larval body shows wrinkled skin with rustic brown colour and in the moribund stage they do not rot but remain rubbery. The affected gut becomes opaque and the silkgland shows white pustules in different places along its length. Sometimes black irregular pepper like spots are noticed on larval skin (Fig. 11).

The infected pupae are flabby and swollen with lusterless and softened abdomen. Sometimes irregular black spots are noticed near the rudiments of the wing and abdominal area. Highly infected pupae fail to metamorphose into adults. The moth emergence is delayed and improper. They have clubbed wings with distorted antennae and do not mate properly. The scales from wings and abdominal area easily come off. In infected moths if the accessory glands are infected the moth may lay eggs with less gluey substance resulting in their detachment from the egg cards.
**Prevention and control:** The fundamental measure for the prevention and control of this disease is to produce healthy eggs, so as to avoid embryonic infection. This can be achieved by conducting systematic mother moth examination. The other methods are to conduct effective disinfection of rearing rooms, equipments and surroundings and maintenance of strict hygienic conditions during rearing. It is essential to surface disinfect the layings in 2 percent formalin for 10 minutes before incubation. Such surface disinfection though practiced in grainages should be repeated again after release from cold storage as also by farmers. If the eggs are in advanced stage of embryonic development surface disinfection is done with 1 per cent formalin for 5 minutes. The room and equipments must be washed and disinfected before incubation.

Young silkworms should be reared under hygienic conditions. As a precaution test examination of unhatched blue eggs, dead eggs, hatched larvae and eggshells can be done and if pebrine is detected, such eggs should not be brushed and if brushed the larvae should be destroyed. Similarly predictive examination could be conducted by utilizing unequal larvae, late moulters, faecal matter and exuviae for the detection of pebrine spores. These tests may not only minimize the chances of rearing transovarially infected layings, but also check cross contamination and spread of the disease. Infected silkworms, faeces and mulberry field pests are important sources of infection and should be properly disposed of to prevent cross infection and spread of the disease.

During seed production in addition to mother moth examination, care should be taken to prevent contamination from other sources. The equipments used for one lot should not be used for the other till they have been thoroughly cleaned and disinfected. Eggs after surface disinfection should be dried and stored in a separate room away from egg production and examination room.

Besides, the above preventive/corrective measures, it has been reported that immersing of the silkworm eggs in hot water, high temperature treatment of the pupae, dipping of the eggs in hot hydrochloric acid minimize the incidence of pebrine. Chemotherapy of *Nosema* infection has been reported through a number of antimicrosporidian drugs like fumagillin, benomyl, bengard, bavistin, ethyl and methyl thiophanate and some of their analogues with positive results, but preventive methods have always been found to be better than the curative measures.