DISEASES OF MULBERRY SILKWORM AND THEIR CONTROL

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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

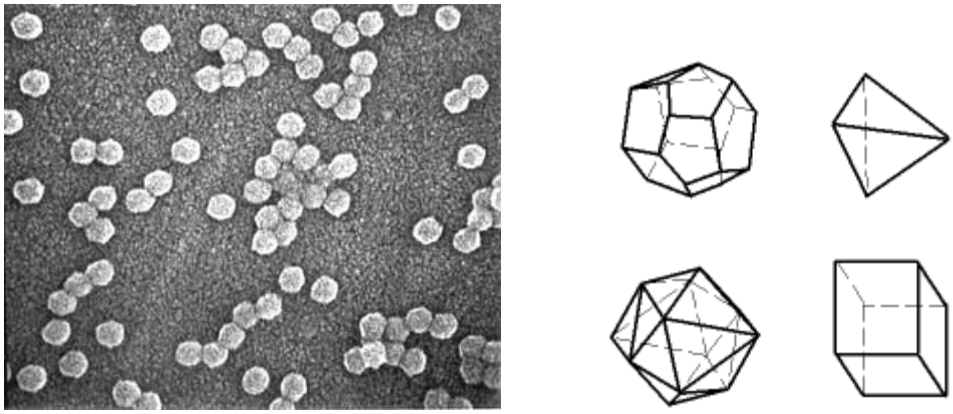
Viral diseases of silkworm pose a major problem to sericulture as they account for almost 70 per cent of the total loss due to diseases. Viral diseases of silkworm comprise of inclusion and non-inclusion types. The inclusion virus diseases form typical inclusion bodies. They are nuclear polyhedrosis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

Nuclear Polyhedrosis

It is one of the most serious viral diseases in tropical countries and occurs throughout the year. This disease is also 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.



Polyhedral Bodies under Light Microscope & their Shapes

Infection mostly takes place through feeding of polyhedra contaminated mulberry leaf, rarely through wounds. 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 of 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.

Figure. 2. Grasseri affected larvae (early stage)

Figure. 3. Grasserie affected larvae (late stage)

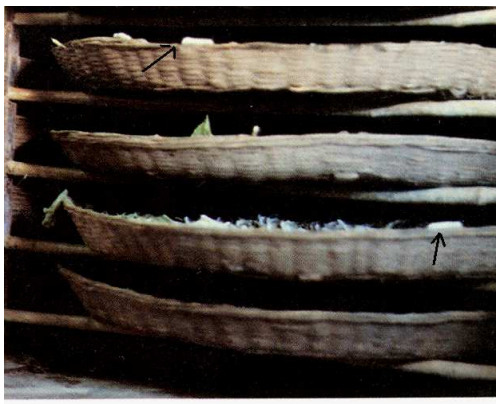


Figure. 4. Grasserie affected larvae crawling on the edges of trays

Figure. 5. Grasserie affected larva hanging with head downwards

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. Paraforrnaldehyde 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-Trichloromythyl Thio-4-Cyclohexane 1,2- Dicarboxymide), 1 % paraformaldehyde (Tri- oxymethylene) 2% Benzoic acid and 96 % 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 sq m. area during early instars and 4- 5 grams/0.1sq m. 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 kg.

Figure. 6. Dusting of "Reshamkeet Oushadh"

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 responsible for the sudden collapse of the silkworm industry of both France and Italy in 1865. 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).

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.

Symptoms*:* The symptoms of this disease can be observed in all the stages of silkworm *viz*., egg, larva, 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 (near death) 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).

Figure. 10 *Size variation in diseased larvae*

Pepper like spots on the body affected by pebrine

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.

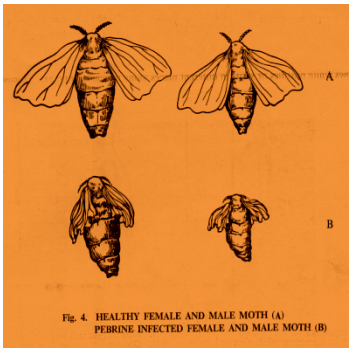
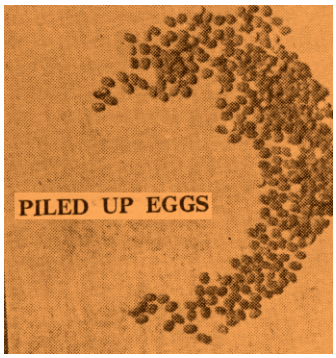


Fig. 4. HEALTHY FEMALE AND MALE MOTH (A)
PEBRINE INFECTED FEMALE AND MALE MOTH (B)



Healthy and diseased moths

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 % 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 egg shells 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.

FUNGAL DISEASES

Fungal diseases otherwise called mycosis, is caused in the silkworm by a few parasitic fungi. Two major kinds of such disease are Muscardine and Aspergillosis. Muscardine appears in various forms and depending upon the colour of spores which cover the body of the silkworm giving a characteristic colour, they have been named as white-muscardine, green-muscardine, yellow-muscardine, black-muscardine, red-muscardine etc. The more common muscardine diseases are, however, white and green-muscardine. In addition Aspergillosis is also found to

occur. Since the silkworm attacked by a fungal disease in course of time turns hard and chalky, muscardine disease is also called Calcino.

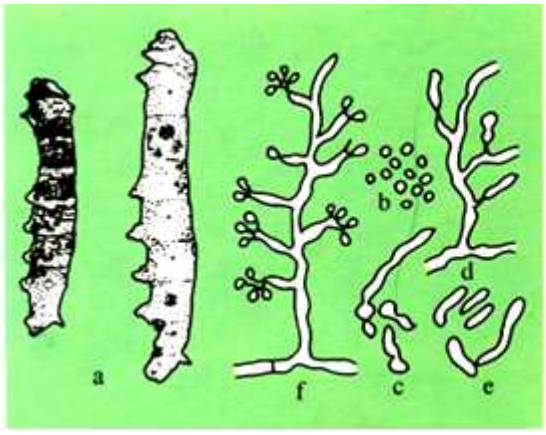
White Muscardine: It is the most common and widely prevalent fungal disease found in all sericultural countries. This disease occurs usually during rainy and winter seasons under moderate to low temperature and high humidity conditions.

Causes of the disease: This disease is caused by different species of *Beauveria* of which the most virulent is *Beauveria bassiana*. This fungus belongs to the family Moniliaceae, order Moniliales of class Fungi imperfecti. Infection is mainly by body contact, rarely through wounds and not by ingestion. Main sources of infection are the mummified larvae, infected seat paper, tray and dead wild lepidopteron larvae from the mulberry field. The disease is highly contagious as the conidia are air borne.

The developmental cycle of *Beauveria bassiana* consists of three distinct stages namely conidium, vegetative mycelium and aerial mycelium.

The conidium is colorless, globular or rarely oval in shape and porcelain white when gathered in a mass. Under favourable conditions of temperature and humidity the conidium germinates within 8-10 hours of coming in contact with the body of silkworm. On germination the conidium not only sends out its germ tube but also secretes chitinase which facilitates the germ tube to penetrate the body wall for further multiplication. The germinating tube of the conidium after invading the blood of the larvae develops into vegetative hyphae. At the tip of the hyphae round or oval shaped short hyphae develops. These often detach themselves and elongate to form vegetative hyphae.

The vegetative hypha comes out of the skin to form aerial hyphae bearing innumerable conidiophores. These conidiophores give rise to small branches which bear one or two conidia.



Development cycle of Beauveria bassiana
(a) Affected larva
(b) Conidia
(c) Germnation of conidia
(d) Formation of cylindrical spores
(e) Cylindrical spores
(f) A. Conidiophore with conidia

Symptoms: At the early stage of infection symptoms are not distinct, but as the disease advances, moist specks appear on the skin. At this stage, larvae lose appetite and become inactive. The body of the larvae becomes limp, loses its skin elasticity, stops movement and finally they die. Before death, symptoms of diarrhea and vomiting appear (Fig. A). After death, the body is initially soft, but within 6-8 hours it becomes stiff and hard (Fig. B). At this stage the body is pink in colour. This is due to the multiplication of *Serratia marcescens,* a secondary bacterium. One to two days later, wooly aerial hyphae grow out between inter-segmental membranes. Subsequently the whole body is covered with white powdery conidia except the chitinous parts of the head. The larvae, unlike other diseases do not rot or decay but remains hard (Fig. C) as the fungus secretes double oxalate - crystals of ammonium and magnesium.


Fig A

Fig B

Fig C

In case of pupal infection the pupae slowdown their reaction to outside stimuli. The thorax shrinks and abdomen is wrinkled. The aerial hyphae and conidia grow up to one third of its ordinary weight inside the cocoons. Such cocoons sound like dried cocoons when shaken. During moth stage the body is hardened and the wings fall of easily.

Prevention and control: Before the commencement of silkworm rearing, rooms, appliances and rearing surroundings must be thoroughly disinfected with 2 percent formalin or 5 percent bleaching powder solution. This disease can be kept under check by avoiding low temperature and high humidity during rearing as they are more ideal for fungal growth. The rearing bed should as much as possible be kept thin and dry in order to avoid the germination of conidia and spread of the fungus. If the disease is found during rearing, the trays, seat papers, cleaning nets, foam pads etc., must be disinfected and replaced. Diseased worms should be removed

carefully before they get mummified and should be placed in lime jars or destroyed by burning or deep burying, with a disinfectant spray. Similarly the bed refuse along with the faeces should be disposed of properly. Mulberry pests in the garden should be controlled as they get easily infected with this pathogen, later becoming an important source for cross contamination to the silkworm.

In addition to the above, anti-muscardine powders can be fruitfully used to control the outbreak and spread of this disease. A few methods of application of the same are given below:

Application of formalin chaff:

In this method formalin solution of required concentration depending on the silkworm instar is mixed with burnt paddy husk and sprinkled on the larval body and bed (Fig.). The concentration of formalin required is 0.4 per cent during I and II instars, 0.5 percent in III ins tar, 0.6 percent in IV instar and 0.8 per cent during V ins tar. The paddy husk is charred or burnt either by burning or roasting in a pan without making ash. Depending on the instar of larvae, the required strength of formalin is mixed with the burnt paddy husk in the ratio of 1:10 by volume and mixed thoroughly. Then it is sprinkled evenly on the larvae and covered with a paraffin or double fold newspaper. After 1/2 an hour the paper cover is removed and feed is given. Formalin chaff application should not be done when larvae are preparing for moult or under moult. Application of formalin chaff can be done before brushing on the newly hatched larvae and after each moult 1/2 an hour before the resumption of feeding. The frequency of application of formalin chaff should be increased depending on the incidence of disease.

Application of Formalin Chaff

Application of Dithane M 45 *(Zinc ion Manganese ethylene oxide bisdithio carbomate) or captan (N-Trichloromethyl Thi0-4-Cyclohexane 1,2-Dicarboximide).*

These are the two commonly available fungicides used for the control of

muscardine. These fungicides are used at a concentration of 1 per cent during, I, II and III instars and 2 per cent during IV and V instars in combination with levigated China clay or Kaolin. The ingredients are thoroughly mixed and tied in a thin cloth and dusted on newly born larvae and after each moult 1/2 an hour before the resumption of feed. An additional dusting should be done on the 4th day of final instar after bed cleaning (Fig.). The quantity required is 2-3 grams per 0.1 sq m. area during I, II and III instars and 4-5 grams during IV and V instars. The dustings should not be done when the larvae are preparing for moult or are under moult. The dusting frequency should be increased if the intensity of infection is high.

Application of "Reshamkeet Oushadh"

It is a bed disinfectant formulation used to prevent both muscardine and grasserie. Its constitution, method of application and quantity required has already been indicated in the part covering the control of grasserie disease.

Dusting of Bed disinfectant

BATERIAL DISEASES

Bacterial diseases affecting silkworm are collectively known as flacherie due to the flaccid nature of the diseased larvae. The incidence of flacherie is high during hot and humid seasons. In general, massive out-break of these diseases are uncommon but depending upon poor disinfection, accumulation of faeces in the rearing trays, feeding of mulberry leaves with contamination, improper handling and unsafe use of bacterial pesticides, large scale loss in crops sometimes occur. Bacterial diseases of silkworms are divided into three major types namely bacterial septicemia, bacterial diseases of the digestive tract and bacterial toxicosis.

Septicemia:

This is a condition where bacteria multiply enormously in the blood (haemolymph) of the larvae, pupae and moths. Septicemia during the larval stage leads to larval mortality whereas the infection in pupal and moth stages leads to a

large number of melted cocoons affecting the egg production in the grainages.

Causes of the disease: This disease is caused by the multiplication of a large number of bacteria, *bacilli*, *streptococci* and *staphylococci* in the haemolymph. The route of infection is through injury or wounds and rarely perorally. Two major types of bacterial septicemia are generally observed, one is the black thorax septicemia caused by *Bacillus* sp. belonging to the family Bacillaceae of the order Eubacteriales size 1-1.5 x 3 microns, spores subterminal, gram-positive and the other is the red septicemia or serratia septicemia caused by the *bacillus Serratia marcescens* Bizio size 0.6-1.0 x0.5 microns non-sporulating and gram negative. The former is more resistant to disinfectants than the latter except for lime emulsion.

Symptoms: They have some common symptoms like sluggish movement, decreased appetite, straightened body, swollen thorax, shrinkage of abdominal segments, vomiting and bead like faeces and loss of clasping power of legs. Further, the body becomes soft and discolored and the body wall ruptures easily emitting foul smelling fluid.

Difference in the symptoms of two diseases are that, in case of the black thorax septicemia, the blackening starts from the thorax and extends to the dorsal vessel till the whole body blackens and rots (Figs.) whereas in the latter case the whole body softens taking a slightly reddish tinge (Fig.). Septicemias are generally acute diseases, spreading quickly, the time clasping from the time of infection to death at 28°C being about 10 hours. At higher temperature and under epidemic conditions they may die within 5-6 hours.

Black Thorax Septicemia

Red Septicemia

Prevention and control: High temperature and humidity conditions are most favourable to the propagation of the bacteria responsible for these diseases and so

these diseases occur chiefly in the seasons having high temperature and humidity. They normally follow wound infection. The bacteria enter generally through the wound and multiply in the haemolymph, disrupting the normal physiological functions, causing septicemia. The 5th instar larvae are more prone to injury and these diseases thus occur mostly in the later part of this stage. *An* effective means of control of these diseases can be the maintenance of hygienic condition so that these bacteria do not occur on the mulberry leaves, in the rearing room and rearing equipment. Care should be taken to avoid injury to the worms, overcrowding of trays and accumulation of faeces in the rearing bed.

References:

1. Hand book on pest and disease control of mulberry and silkworm, CSB, India.
2. Internet.
