

Nuclear Polyhedrosis

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Experiment No. 8: Identification of grasserie based on external symptoms - Staining and preparation of temporary slides of polyhedra of 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 Grasserie, Milky disease, etc.,

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 in the nucleus of the tracheal epithelial cells, adipose tissue cells, dermal cells and blood cells. The size of the polyhedra varies from 3-6 μ . 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.

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. As the disease advances the skin becomes thin and fragile and the body becomes milky white with inter segmental swellings. The fragile skin is prone to rupture easily and 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, 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. 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:

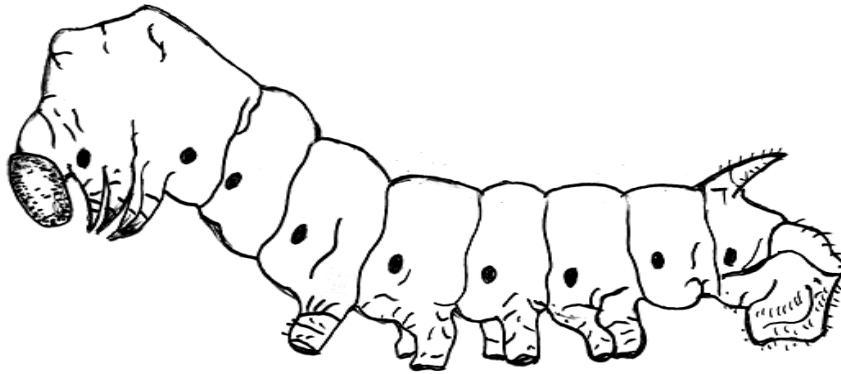
1. For effective prevention of this disease, the silkworm rearing rooms, equipments and rearing premises should be thoroughly disinfected before brushing.
2. Use of DFLs for silkworm rearing and maintenance of standard rearing conditions.

In addition to the above, use of certain bed disinfectants like Reshamkeet Oushadh *etc.*, could also prevent secondary contamination and spread of the disease.

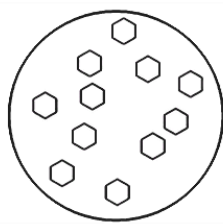
Temporary slide preparation of nuclear polyhedral bodies

1. Make a thin smear of nuclear polyhedral bodies on clean glass slide.
2. Air dry or flame dry the specimen.

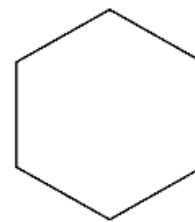
3. Fix the specimen in ethanol/methanol for 1 min and air dry.
4. Stain in Giemsa for 30-45 min (2 drops of stain in 1 ml of distilled water).
5. Wash the excess stain, air dry and observe under 10 x X 40/45 x (400-450 x) magnification.



Nuclear Polyhedrosis virus infected Silkworm



Polyhedral Bodies under Microscope



Enlarged Polyhedral Body

REFERENCES

1. Anonymous, 1990, Hand book on pest and disease control of mulberry and silkworm, United Nations, Thailand.
2. Krishnaswamy, S., Narasimhanna, M.N., Suryanarayan, S.K., and Kumararaj, S. 1976; Sericulture Manuals, Vol. 2, Silkworm Rearing, FAO, United Nations, Rome.
