

## Viral Disease in Silkworm – Nuclear Polyhedrosis Virus (*Bombyx mori* L.)

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ARTICLE ID: 58

### Abstract

*Bombyx mori* L. (Lepidoptera: Bombycidae) is very important economically as a silk producer in sericulture, and the insect is used as a model in biotechnology (Goldsmith *et al.*, 2005). The mulberry silkworm life cycle is affected by a number of diseases caused by viruses, bacteria, fungi and protozoa. The genetic resistance of the silkworm to viral diseases is mainly controlled by polygenic mechanism. Resistance of silkworm to nucleopolyhedrosis virus (*BmNPV*), cytoplasmic polyhedrosis virus (*BmCPV*) and infectious flacherie virus (*BmIFV*) is controlled by polygenes. *BmNPV* is considered to cause the dreaded viral disease in sericulture industry. In silkworm, gut proteases, lipase and red fluorescent protein plays a major role in the defense mechanism against viral pathogens. Earlier, techniques have been developed to detect this viral disease such as the enzyme-link immunosorbent assay (ELISA), DNA hybridization, colloidal textile dye-based dipstick immunoassay and western blot analysis. PCR is an extremely sensitive technique which amplifies target DNA sequences and PCR amplification of conserved fragment enabled the detection of low level of viral DNA. PCR technique and polyhedrin gene (*polh*) used to detect early infection of Grasserie virus (*BmNPV*) in silkworm.

**Key words:** silkworm, viral disease, resistance

### Introduction:

The silkworm *Bombyx mori* is exploited both as a powerful biological model system and also as a tool to convert leaf protein into silk. Silkworm larvae often suffer from viral infections causing heavy losses to the economy of the silk industry. Silkworm has a greater biotic potential and the resistance is continuously offered by the environment which includes biotic and abiotic factors thus influencing the cocoon production qualitatively and quantitatively. The abiotic environmental resistance component largely includes the weather

factors such as temperature, relative humidity, photoperiod etc., in respect of silkworm and unfavourable weather conditions that lead to poor harvest of mulberry. The abiotic factors usually affect the growth and development of silkworm and predispose the silkworm to the biotic causes i.e., infectious diseases. The biotic factors responsible for low cocoon crop production are the silkworm diseases caused by protozoa, fungi, bacteria and viruses. Extent of crop loss due to fungal disease caused by white muscardine and green muscardine was 5-20 %, virus disease caused by grasserie (NPV) was 15-20%, bacterial disease reduces the crop loss upto 10-15%, and pebrine gives 5-10% damage respectively. The history of sericulture reveals devastating impact of microsporidiosis in several sericultural countries resulting in severe damage to sericulture industry. Though, the disease is known from very remote times, it attracted the attention of sericulturists only during the 17th century. The first scientific record of the occurrence of the disease came from European countries in 1809 and the disease wiped off sericulture there.

### **Viral Diseases of Silkworm**

There are four silkworm diseases are caused by viruses viz., Nuclear polyhedrosis, Cytoplasmic polyhedrosis, Infectious flacherie and Densonucleosis virus. These diseases are a major problem to sericulture, as they account for 70-80 percent of the total loss of silkworms from diseases. There are 2 types of viral diseases they are

- Inclusion / occluded type (Polyhedral)
- Non-inclusion / non-occluded type (non-polyhedral)

The inclusion virus forms typical inclusion bodies they are Nuclear polyhedral virus (NPV) and Cytoplasmic polyhedral virus (CPV) which can be more easily identified through ordinary microscope. Whereas, Non-inclusion type consists of Infectious flacherie virus (IFV) and Densonucleosis virus (DNV) which can be detected only through electron microscope or fluorescent microscope and serological tests.

### **Nuclear Polyhedrosis Virus:**

Grasserie disease of *Bombyx mori* is caused by a nuclear polyhedral virus. In India it is called by various local names such as “*Halu-hula*” in Karnataka and “*Rasa*” in West Bengal, “*Palu purugu*” in Andhra Pradesh and “*Pal pocchi*” in Tamil nadu, indicating the milky fluid condition of the haemolymph of diseased worms. It is also popularly known as “*Milky disease*”, “*Hanging disease*” and “*Fatty degeneration disease*”. Sometimes nuclear polyhedrosis and

cytoplasmic polyhedrosis occur simultaneously in silkworms and disease is called as “Jaundice”.

Among the viral diseases in silkworm, Nuclear polyhedrosis is most common and severe than other viral diseases and in all sericultural countries and it prevails to an extent of 35-58%. It usually affects the fourth and fifth instar silkworms under natural conditions.

- This disease was recorded as early as in 18th century in insects and it belongs to Baculoviridae. These viruses are easily recognizable from other viruses because of the presence of unique bodies - the polyhedra.
- In 1959, Ito *et al.*, observed these bodies in diseased silkworm larvae and named them as “*Polyhedral granules*” and the disease as “*Polyhedral disease*” or ‘*Polyhedrosis*’ (Prell, 1926).
- Lehninger (2001) named the virus as *Borrelina bombycis*.
- Mazzone (1985) identified that the rod shaped particles known as ‘*virions*’ in the polyhedra were the infectious ones causing the disease.
- Sarma *et al.*, (1994) identified that occluded virions entered the silkworm through *per oral* and attacked the nucleus of the fat, muscle and nerve cells and multiplied many folds.

#### **Etiology:**

Grasserie disease is caused by occluded *Bombyx mori* NPV (*BmNPV*) or Baculovirus which belongs to the subgroup A of the family Baculoviridae. The viral particles are rod shaped measures about 330×80nm in size, consisting of membranes, a capsid and an envelope. It contains double stranded DNA (Approx. 8%), protein (77%), lipids and carbohydrates. Only the nucleic acid part of virion is infective, the protein part is non-pathogenic. The group of viral particles are surrounded by proteinacious material (i.e., Polyhedrin) and the cluster of 2 virus forms polyhedra which appears as hexagonal but rarely tetragonal and the size of polyhedra varies from 2-6µ. Polyhedra are easily visible under light microscope while individual virions are submicroscopic (i.e., seen under electron microscope).

#### **Life cycle of Nuclear Polyhedrosis virus:**

- The most common route of infection by *BmNPV* in silkworm is *per os* but rarely through wounds. Silkworms get infected when they feed on mulberry leaves contaminated with *BmNPV* polyhedra or free virions.

- In the digestive tract, the polyhedra on ingestion begin to dissolve within 0.5 min and are dissolved completely by alkaline digestive fluid within 3 min liberating the polyhedral derived virions (PDV).
- The prolonged exposure of released virions to the gut juice in the midgut, for several minutes results in their inactivation. And also some of the virions are inactivated by red fluorescent protein and excreted through faeces, but those PDV's / free virions pass through peritrophic membrane and invade midgut columnar epithelial cells. The initial replication takes place in these cells.
- Within 15 min to 4h from *per os* infection, nucleocapsid moves to nucleus and attaches to nuclear pore where uncoating (discharge of nucleic acid) takes place predominantly in the cytoplasm.
- Within 6 h of viral infection DNA replicates and in the next 10 – 20 h, budded virus (BV) / extra cellular viral (ECV) buds are formed on the surface to infect other cells and tissues like midgut connective tissues, haemocytes, trachea, fatbody (adipose tissues), hypodermis etc.
- After, 18 – 24 h, the polyhedrin protein (10 kD; 30kD) assembles in the nucleus of infected cell and the virus particles become embedded in the proteinaceous occlusions.
- The virions and hexa or tetra gonol polyhedra progressively increase in their number and size within in the nucleus causing the swelling of nucleus which gradually leads to bursting. The free virus, polyhedra and cellular debris in the haemolymph gives milky-white colour.
- The polyhedra are responsible for horizontal transmission among the susceptible worms while the budded viruses (BV) / extra cellular virus (ECV) are responsible for secondary and cell to cell infection in the host. Midgut epithelial cells are first infected by virus and are followed by midgut connective tissues, haemocytes, trachea, fatbody, hypodermis and other tissues.
- Virus doesn't affect the malphigian tubules and glandular tissues. Infection of dermal cells results in their dissolution leaving only the chitinous skin which then bursts easily. The dead corpses become black immediately due to decaying. If infection is at the later part of V instar, the larvae spin irregular cocoons but the pupae die inside the cocoons causing staining / blackening / melting of the cocoons.

**Symptoms:**

- Swelling of intersegmental areas.
- Shining and yellowish body.
- Hyper activeness and aimless crawling around the edge of a rearing tray.
- Sometimes there may be overlapping of inter segmental membrane.
- The haemolymph become turbid, milky white and on injury the milky haemolymph oozes, followed by shrinkage and death.
- Corpses of the infected worms blacken and rotting due to secondary infection by bacteria.
- Nuclei of various cells / tissues contain polyhedral bodies.
- Epithelial cells and all other infected tissues become abnormal.

**Conclusion:**

Knowledge of evolutionary ancient mechanisms of innate immunity of invertebrates play a major role not only as the first barrier of defense against disease but efficiently providing adaptive immunity with specific information about infectious and neoplastic danger. The mulberry silkworm, domesticated and mass reared for several centuries, presumably has weak-ened immune system which has made the insect highly vulnerable to bacterial and viral infections. Information on the organization and function of the immune system in the silkworm in general and with reference to viral infections in particular is scanty. There are a few recent reports on the presence of antiviral proteins, against some DNA and RNA viruses, which strongly suggests the presence of a functional antiviral immune system in the silkworm.

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