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A simple, efficient and cost effective protocol for detection of BmNPV in the silkworm, *Bombyx mori* L.

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Abstract
The present investigation was carried out at P. G. Department of Sericulture, Poonch campus, University of Jammu for isolation and identification of various diseases. Silkworm just like all other living organisms is susceptible to infection and diseases. Various pathogens including viruses (Nuclear Polyhydrosis, Cytoplasmic Polyhydrosis), bacteria (flacherie), fungi (muscardine) and protozoa (pebrine) cause various diseases to silkworm. Among various diseases the most common and most critical one is viral diseases like Bombyx mori Nuclear Polyhydrosis (BmNPV) and Bombyx mori Cytoplasmic Polyhydrosis (BmNPV), commonly known as Grasserie and it accounts for about 13.85 to 26.03 per cent of crop loss alone. In the present investigation a simple, efficient and cost effective protocol has been designed for detection of BmNPV by using various body organs of silkworm larva like, a) using whole larva b) using haemolymph c) using larval body without alimentary canal and d) using KoH. On the basis of visual and microscopic observations it has been reported that the present larval samples were found to be infected with Nuclear Polyhydrosis (Grasserie) disease caused by BmNPV. Thus, this study aids in easy and efficient diagnosis methodology for detection of grasserie disease with minimum inputs and maximum accuracy.

Keywords: silkworm, haemolymph, grasserie, BmNPV, nuclear Polyhydrosis

Introduction
Silkworm *Bombyx mori* L. is a Lepidopteran monophagous insect which is reared only on mulberry leaf for production of high quality mulberry silk. It is commonly cultured in many sericulture practicing countries including India, which ranks second in the world's total silk production. Silkworm just like all other living organisms is susceptible to infection and diseases. Various pathogens including viruses (Nuclear Polyhydrosis, Cytoplasmic Polyhydrosis), bacteria (flacherie), fungi (muscardine) and protozoa (pebrine) cause various diseases to silkworm. Among various diseases the most common and most critical one is viral diseases like Bombyx mori Nuclear Polyhydrosis (BmNPV) and Bombyx mori Cytoplasmic Polyhydrosis (BmNPV), commonly known as Grasserie. BmNPV is most common in occurrence particularly in autumn rearing and in rainy season as the proliferation and multiplication rate of viral pathogen is quiet high during this season. Therefore, prevalence of grasserie disease in silkworm is reported to be higher during autumn and rainy season as compared to other seasons (Reddy and Rao, 2009) [5] and grasserie alone contributes about 13.85 to 26.03 per cent of crop loss (Illahi and Nataraju, 2007) [2].

Crop loss or crop failure occurs in almost all the sericulture practising countries of the world with slight variation only in the type and extent of severity. The extent of damage may vary from tropics to temperate, race to race (bivoltine and multivoltine races) and even from farmer to farmer as the rearing skills vary from person to person. Generally maximum crop loss is reported in tropical regions as compared to temperate regions and Indian sericulture Industry faces higher magnitude of crop losses due to occurrence of various diseases as compared to other countries (Rahmathulla *et al*., 2012) [4]. It is a general observation that out of 5-6 crops per year, two crops are usually lost due to diseases and other reasons and even the full potential of successful crops are partially deprived due to incidence of various diseases. Thus, the frequent outbreak of diseases is one of the main reason hindering the growth and development of Sericulture Industry in countries like India.

Material and Methods

Locale of the study: The present investigation was carried out at P. G. Department of Sericulture, Poonch campus, University of Jammu for isolation and identification of various diseases.

Collection of larval samples: Larval samples were collected from State Sericulture Development Department, Mendhar, during autumn rearing on 27th of September 2019 and processed at P. G. Department of Sericulture, Poonch campus, University of Jammu for detection of various diseases.

Methodology adopted

The present larval samples were processed for detection of disease by using various body organs like: a) Using whole larva b) Using haemolymph c) Using larval body without alimentary canal d) Using KoH

A. Using whole larva: Steps followed are depicted below

1. Take a live diseased larva and crush it in an autoclaved mortar and pestle by applying gentle force.
2. Crush the larval body to a fine homogenous paste of uniform thickness by addition of small amount double distilled water.
3. Take a fresh glass slide and make a uniform smear of crushed larva on it with the help of another fresh slide.
4. Cover the slide by gently placing a cover slip on it with the help of forcep or needle.
5. Observe the slide under light microscope.

B. Using haemolymph

1. Take a live diseased larva and give a sharp prick to its abdominal segment or appendages. Body fluid i.e., haemolymph starts oozing out.
2. Place 1 or d drops of haemolymph directly on the surface of a fresh glass slide.
3. Cover the slide by gently placing a cover slip on it with the help of forcep or needle.
4. Observe the slide under light microscope.

C. Using larval body without alimentary canal

1. Take a live diseased larva and fix it dorsally in a dissection tray. Dissect out the larva and remove the alimentary canal as it contains the maximum residual material in it.
2. Crush the rest of the larval body to a fine homogenous paste of uniform thickness by addition of small amount double distilled water.
3. Take a fresh glass slide and make a uniform smear of crushed larva on it with the help of another fresh slide.
4. Cover the slide by gently placing a cover slip on it with the help of forcep or needle.
5. Observe the slide under light microscope.

D. Using KoH

1. Take a live diseased larva and crush it in an autoclaved mortar and pestle by applying gentle force.
2. Crush the larval body to a fine homogenous paste of uniform thickness by addition of 2-3 ml of liquid KoH.
3. Take a fresh glass slide and make a uniform smear of crushed larva on it with the help of another fresh slide.
4. Cover the slide by gently placing a cover slip on it with the help of forcep or needle.
5. Observe the slide under light microscope.

Results

On the basis of visual and microscopic observations it has been reported that the present larval samples were found to be infected with Nuclear Polyhydrosis (Grasserie) disease caused by BmNPV. Evidences of diagnosis in support of current results are given below:

a. On the basis of visual examination the larval samples were reported to be infected with grasserie disease (Fig.1).
b. Presence of polyhedral structures indicated the presence of BmNPV (Fig.2)
c. The microscopic observation of a drop of haemolymph from diseased larvae revealed the presence of large number of hexagonal structures i.e. the polyhedral (Fig.3)
d. NPV infected silkworm larvae depicted the overlapping intersegmental region
e. Presence of spot with somewhat reddish ting in the hypodermal and epicutilce of the thoracic region indicated the infection of BmNPV (Fig.4)
f. Presence of fruiting bodies in the haemolymph of infected larvae confirmed the presence of BmNPV infection (Fig.5).

Fig 1: A. Grasserie larva B. Oozing of milky fluid C. Polyhedron of BmNPV and D. Hanging of diseased larva

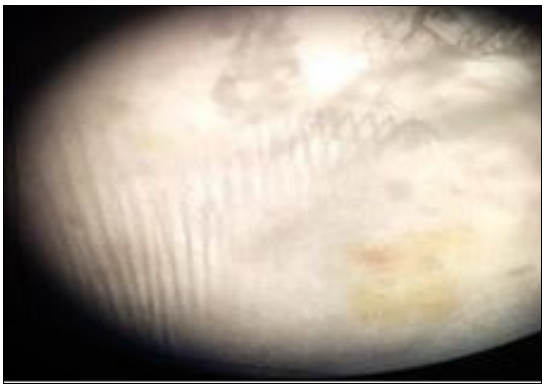

Fig 2: (1.1) Polyhedral structure

Fig 3: Polyhedral structure observed in the haemolymph of diseased larva

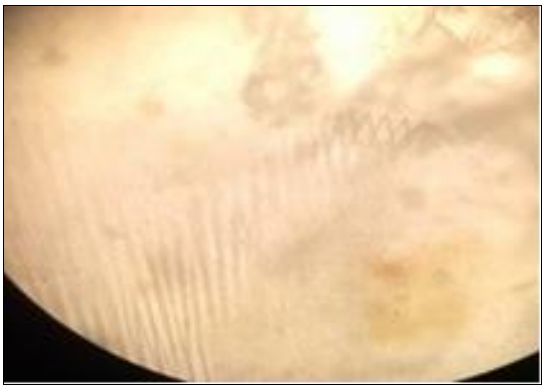

Fig 2.2: Close-up view of Polyhedral

Fig 4: Arrow indicates the region with reddish spot in the thoracic region indicating the presence of polyhedral bodies

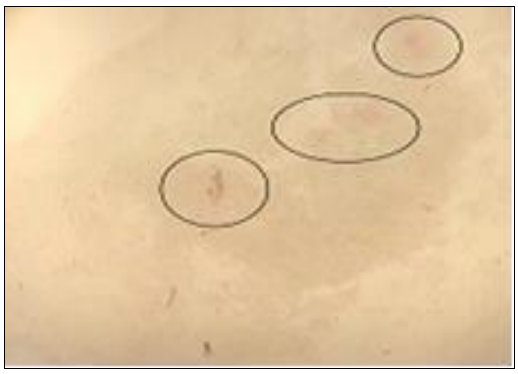

Fig 5: Fruiting bodies indicating the BmNPV infection.

Discussion

Silkworm *Bombyx mori* L. being susceptible to viral diseases particularly in autumn and rainy season demands for extra care and maintenance of hygienic conditions Sharma *et al*., 2019 [6]. Among the silkworm diseases, Nuclear Polyhydrosis (Grasserie) poses a major threat to ultimate crop production i.e., the cocoon production. Presence of spot with somewhat reddish ting in the hypodermal and epicutilce of the thoracic region indicated the infection of BmNPV. This observation lies in close conformity with Smith and Xeros, 1953, in which they reported that after 48 hours of infection with BmNPV, presence of tiny red colored granules was observed. The microscopic observation of diseased larva revealed the presence of large number of hexagonal structures i.e. the polyhedron which confirms the diagnosis conducted by Heng *et al*., 1985 [1] and Nataraju *et al*., 2005 [3].

Conclusion

Based on above observation it has been concluded that the present sample of silkworm larvae are reported to be infected with Nuclear Polyhydrosis (Grasserie) disease caused by BmNPV. Presence of polyhedral structures and fruiting bodies etc. clearly indicated the incident of grasserie disease in the studied sample.

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