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A New Virus Isolated from Mulberry Pyralid, *Glyphodes Pyloalis* Walker (Pyralidae: Lepidoptera)

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ABSTRACT: In present investigation larvae of mulberry pyralid, *Glyphodes pyloalis* Walker (Pyralidae: Lepidoptera) which infest mulberry plantation were collected from different sericultural farms of Kashmir valley. The larvae were macerated and the homogenate obtained was subjected to microscopic examination which revealed viral infection. The virus was purified using percoll, differs distinctly in morphology from *Bombyx mori* Nuclear polyhedrosis virus (*Bm*NPV) and was tentatively designated as (Gpv). The pathogenicity of the virus was also determined by inoculating (1×10^6/ml) of viruses to the zero day of 4th instar silkworms and the results were compared with that of *Bm* NPV. Peroral inoculation at a dosage of 1×10^6 viruses/ml caused no mortalities (larval/pupa) and all individuals survived, adults emerged successfully suggested it is avirulent to the silkworms. However, peroral inoculation of *Bm*NPV at same dosage developed disease specific symptomlogy and caused 35 larval and 32 pupal mortalities and also decreased pupation drastically.

Keywords: *Bombyx mori* Nuclear polyhedrosis, Behavioural, *Glyphodes pyloalis* and Microscopic etc.

INTRODUCTION

Insect pests of mulberry not only defoliate leaf and make it unfit for silkworm consumption but also harbors some microbial organisms and act as alternate hosts for silkworm pathogens (Kishore *et al.,* 1994; Kumbhar and Mishra 2022). These insects have been frequently observed resting in mulberry gardens and possibly deposit feaces or die on the mulberry leaves and such infested / infected mulberry leaf becomes the source of cross infection. Common cases of lepidopteron insects *viz.*, *Pieris rapae* (Pieridae), *Spodoptera deparvata*, *Spodoptera exigua*, *Spodoptera litura,* *Trchiplusiani* (Noctuidae), *Diaphania pulverulentalis* and *Tribolium freemani* have been reported to coexist with microbial infection and some of them cross-infect the silkworm (Iwano and Isihara 1991; Singh *et al.,* 2003; Bashir *et al.,* 2021). The gypsy moth, *Lymantria dispar* L, a pest on oak trees is known to host a complex of entemopathogenic microsporidia that include three genera *viz.*, *Nosema Vairimorpha* and *Endoreticulatus.* Mulberry pyralid, *Glyphodes pyloalis* Walker is a monophagous pest of mulberry cause a great loss to the mulberry wealth besides hosting some pathogens (Watanabe *et al.,* 1988; Kumar *et al.,* 2002; Zea *et al.,* 2003; Imtiyaz *et al.,* 2021). In the present study a virus was isolated from the larvae of mulberry pyralid, *G. pyloalis* and illustrated herein.

MATERIAL AND METHODS

Collection of Insect pests: Mulberry gardens of College of Temperate Sericulture Sher-i-Kashmir University of Agricultural Sciences and Technology of Kashmir, Mirgund, Silkworm Seed Station Sericulture Development Department, Jammu and Kashmir Mirgund, Baramulla and Sericulture Station Dangerpora Bandipora were surveyed during July to August 2022 for *G. pyloalis* infestation. The *G. pyloalis* larvae were collected from the infested mulberry plantation and brought to the laboratory.

Homogenization and microscopic examination: The larvae were macerated individually using mortar and pestle and the homogenate obtained was examined under microscope for presence of infection.

Prevalence of viral infection: Virus was isolated from the smear and purified following the method suggested by Nataraju *et al.* (2005) and photographed [Magnus (Model-CH20i-Bi)] at 600x magnification.

Enumeration and inoculation: The virus suspension was suspended in distilled water and was quantified using Neubauer haemocytomater as described by Cantwell (1974) to obtain inoculum dosage of (1×10^6 viruses/ml) for per oral studies. Zeroday of 4th instar were inoculated with 1×10^6 viruses/ml and observations were made on morphological/behavioral changes associated with the disease, larval/ pupal mortalities, effective rate of rearing and pupation. The data was collected compiled and presented in present article.

RESULTS AND DISCUSSION

Mulberry pyralid, *G. pyloalis* larvae were collected from tender mulberry leaves where they web the leaf together and feed on the chlorophyll content (Plate 1).

The larvae were homogonised individually using motor and pestle and the smear obtained was examined under microscope for the naturally occurring pathogens in the wild population. A virus was isolated and purified using percoll (Plate 2a) photographed [Magnus (Model-CH20i-Bi)] at 600x magnification (Plate 2b). The virus was found distinctly different from the standard strain, *Bm* NPV in morphology. The pathogenicity of Gpv in silkworm, *Bombyx mori* L. was determined by inoculating (1×10^6 of viruses/ ml) to the zeroday of 4^{th} instar silkworms. A *Bombyx mori* Nuclear polyhedrosis virus (*Bm*NPV) inoculated control was maintained for comparison. Three replications of 100 larvae in each treatment were maintained and the mortality was recorded accordingly. Peroral inoculation of Gpv at a dosage of 1×10^6 viruses / ml didn't cause any larval / pupal mortality and all the individuals attained cocooning and the adults emerged successfully suggested it is not pathogenic to silkworm, *B. mori*. However, peroral inoculation of *Bm*NPV at same dosage caused 35 and 32 larval and pupal mortalities respectively (Table 1, Fig. 1). The effective rate of rearing by number and by weight (kgs) were 9800 and 11.50 respectively in Gpv inoculated silkworm batch. Drastic decrease in effective rate of rearing by number (3322) and weight (4.30) were recorded in the *Bm*NPV inoculated silkworm batch (Table 1 and Fig. 2). The pupation of 95.83% and 82.35% in Gpv and *Bm* NPV respectively inoculated batches were recorded (Table 1 and Fig. 1).

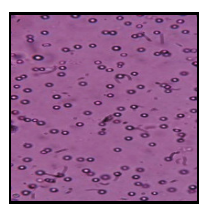
Insects and non-insect pests are known to inflict damage to mulberry in sericulture practicing countries of the world (Haripriya *et al*., 2023), however infestation varies from region to region and even from different seasons of the same region (Zeya *et al.,* 2003; Imtiyaz *et al.,* 2021). Hassan and Mir (2018) reported *G. pyloalis* walker a manophagous pest of mulberry takes a heavy toll especially during July to October. Zeya *et al.* (2003) reported 20-25 percent damage to mulberry foliage due to *G. pyloalis* during July to October. *G. pyloalis* infested leaf is unfit for silkworm consumption and if such leaf is utilized for rearing, silkworm develop constipation and are unable to defecate besides having negative effects on economic characters (Aruga, 1994; Hassan and Mir 2018a). Non availability of quality mulberry leaf during late summer is perhaps one of the important reason for not picking up the second commercial silkworm rearing at large scale. A galore of researchers have reported that the insect pests not only damage the mulberry wealth but also act as carrier for a number of silkworm pathogens (Watanabe *et al.,* 1988; Sharma *et al.,* 1989; Samson *et al*., 1999; Bashir *et al.,* 2011). Sporadic disease outbreak during rearing are due to umpteen reasons however frequent visits of alternate hosts to mulberry farms and deposit excreta or die on the mulberry plants becomes source of secondary infection for cross infection (Kishore *et al.,* 1994). Recently mulberry pyralid, *G. pyloalis* besides being a major pest of mulberry also co-exists with microbial infection (Imtiyaz *et al.,* 2021). In present study virus was from isolated from the wild population of *G. pyloalis* found different from *Bombyx mori* polyhedrosis virus in morphology. Pathogenicity of the virus was determined by inoculating 1×10^6 viruses /ml to 1^{st} day of 4^{th} instar silkworms through *per os* which neither establish disease nor cause any mortality suggests its avirulence. Although site of infection and histopathology have not yet been conducted however the preliminary experiment suggested that the virus is different from *Bombyx mori* Nuclear polyhedrosis virus however further studies need to be carried out for the logical conclusion.

a. Skeletonization of mulberry

b. *Glyphodes pyloalis* larvae

Plate 1: a) Skeletonization of mulberry leaves by *G. pyloalis* infestation and b) Collection of *G. pyloalis* larvae.

a. Purified *G. pyloalis* virus



b. Microphotograph of isolated virus

Plate 2: a) Purified virus isolate and b) its microphotograph.

Table 1: Determination of pathogenicity of virus (Gpv) against silkworm, *B. mori.*

Treatments	Mortality due to viral inoculation		Effective rearing rate by		Pupation (%)
	Larval	Pupal	No.	Wt (Kgs)	
Gpv	0.00	0.00	9800	11.50	95.83
Bm NPV	35.00	32.00	3300	4.300	82.35

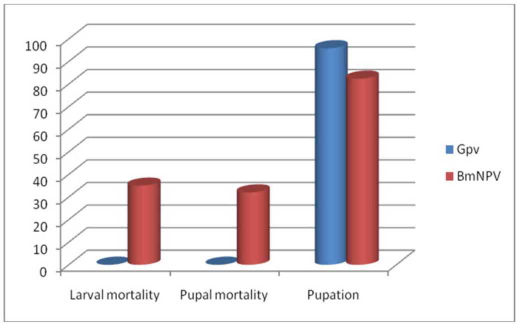


Fig. 1. Mortality due to Gpv and *Bm* NPV in silkworm, *B. mori*.

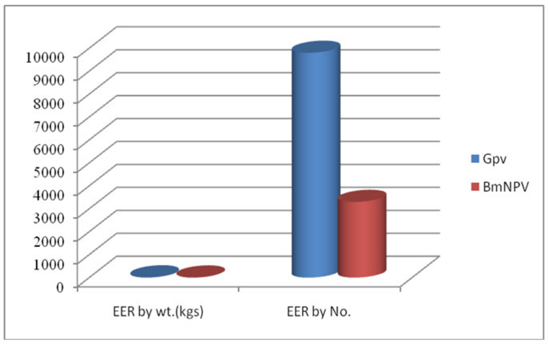


Fig. 2. Effect of Gpv and *Bm* NPV on effective rate of rearing by weight and number.

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Conflict of Interest. None.

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