Research Paper Zoology

Study The Cytopathic Effect of BmNPV in The *BOMBYX MORI* Ovarian Cell Lines

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ABSTRACT

Insect cell culture has found many applications in the field of physiology, biochemistry, genetics, developmental biology and insect pathology. This is true with lepidopteran cells. They are commonly used to study insect viruses
In present study, four ovarian cell lines of Bombyx mori, Bm-12, Bm-1, Bm-16 Bm-17 and Bm-5 is a widely used cell line was inoculated by nucleopolyhedrovirus, BmNPV and observed their cytopathic effect. All the cell lines are susceptible to BmNPV infection and shows diverse cytopathic effect.

KEYWORDS Bombyx mori, Ovarian cell lines, BmNPV ,Cytopathic Effect.

1. INTRODUCTION

lepidopteran cell lines are commonly used to study insect viruses [1] and have been considered for production of certain pathogenic viruses and recombinant proteins by the use of the Baculovirus expression vector [4.8.13]

Bombyx mori nucleopolohedrovirus (BmNPV) is one such baculovirus, which is being used in BEV system for expression of recombinant proteins in susceptible cell lines [9.10.11]. Some lepidopterian larval cell lines are susceptible to BmNPV [2.5.6.7]

In the present study the larval ovarian cell line Bm-12, Bm-1 Bm-16, Bm-17 and a widely used, Bm-5 cell lines were also tested for susceptibility of BmNPV and Observed their cytopathic effect

2. MATERIALS AND METHODS

BmNPV was obtained from diseased larvae of the silkworm, B. mori. Turbid haemolymph of fifth instar larvae infected with BmNPV was collected through an incision on proleg. After centrifugation (3000 rpm, 10 min) the supernatant was diluted with equal volume of medium and passed through 0.45 µm membrane filter and used as an inoculum. It was stored in refrigerator at 4°C.

2.1 Inoculation of Cultures:

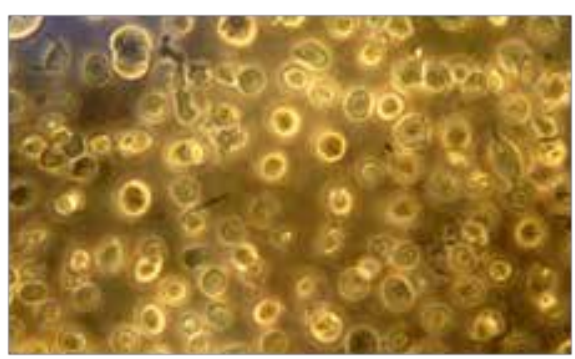
The cells of each cell line at log phase were harvested, counted and transferred to three 30 mm Falcon plastic Petri-plates at about 3×10^5 cells /ml to higher densities (1-1.8 × 10^6 cell/ml). The cultures were inoculated by adding 2-3 drops of the inoculum with a Pasteur pipette. The infected cultures were maintained at 25°C and examined every day for cytopathic effect and occurrence of occlusion bodies (OBs) in the nuclei.

3.OBSERVATION AND DISCUSSION:

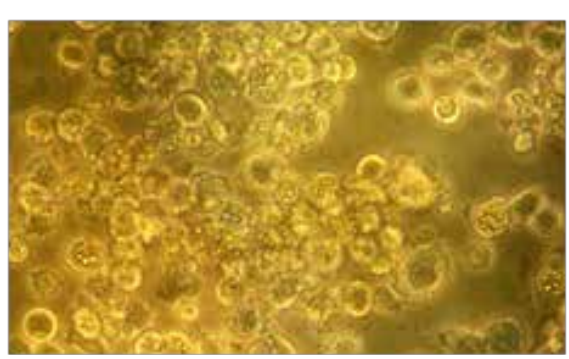
Phase Contrast Microscopy:

At early stage of infection, the cytopathic effect such as hypertrophy of nuclei, heavy clumping and adherence of the cells to the substratum of the culture flask in TNM-FH medium were prominent in all the five cell lines. Numerous large clumps of cells were observed 16-20 h post inoculation (h pi) in Bm-1, Bm-16 and Bm-12 and about 40-48 h pi small refractive OBs were appeared in the nuclei of cells. By 72 h pi OBs were prominently seen in the nuclei of aggregated and dislodged cells. The replication of BmNPV between 16 and 18 h pi and formation of OBs by 40-48 h pi in B. mori cells have also been reported earlier . Some of the cell aggregates that exhibited infected cells were removed from the infected cultures and examined under microscope. The cells were loaded with OBs in the nuclei depending on the cell size in each cell line. In contrast to Bm-1, Bm-5 and Bm-16 where the lysis of cells loaded in OBs was a common feature 96 h pi , the OBs loaded Bm-12 and Bm-17 cells remained in the infected cultures for a long time and only a few cells exhibited lysis and release of OBs by 96 h.p.i. This may be the characteristic feature of these cell lines.

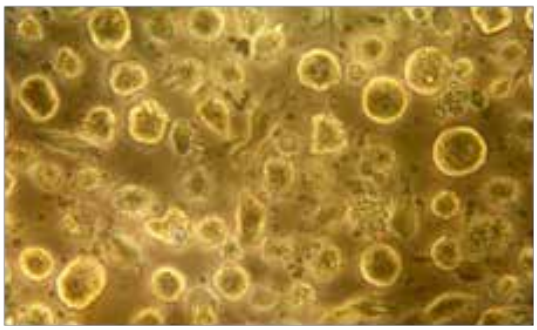
All the cell lines were susceptible to BmNPV infection and shows diverse cytopathic effect.



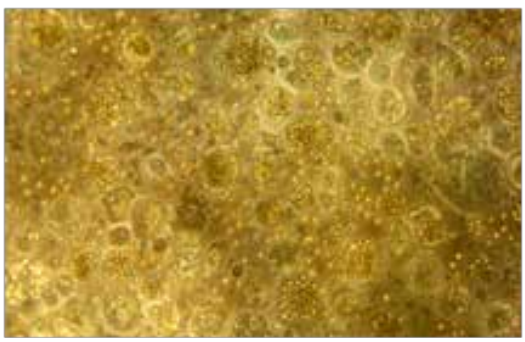
Bm-12 Healthy



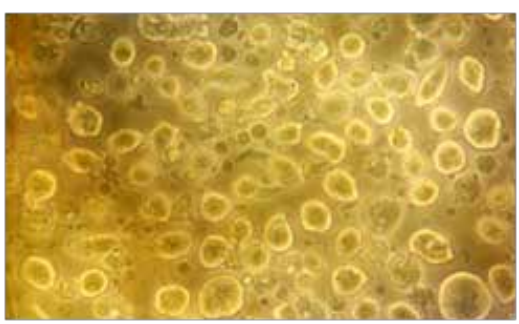
Bm-12 Infected



Bm-1 Healthy



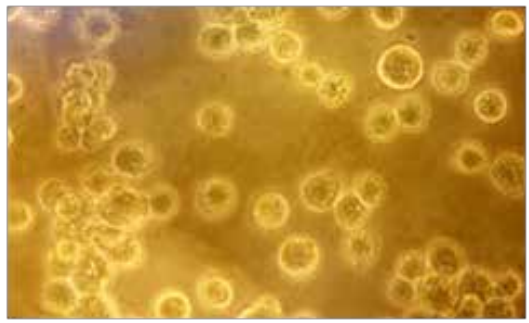
Bm-1 Infected



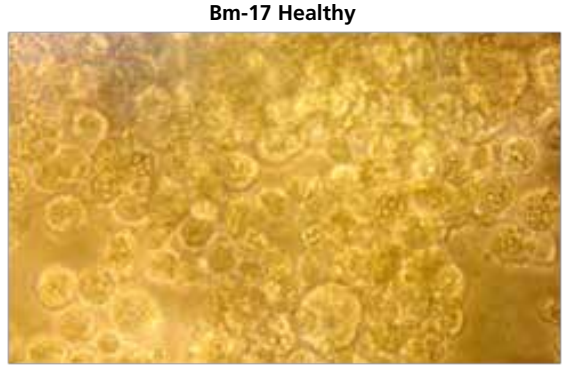
Bm-16 Healthy



Bm-16 Infected



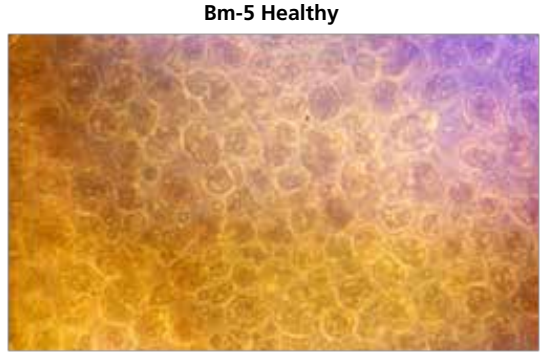
Bm-17 Healthy



Bm-17 Infected



Bm-5 Healthy



Bm-5 Infected

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