Short Communication

Pathogenicity of a baculovirus isolated from *Arctornis submarginata* (Walker) (Lepidoptera:Lymantriidae), a potential pest of tea growing in the Darjeeling foothills of India

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**A R T I C L E   I N F O**

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**A B S T R A C T**

A granulosis virus (GV) was isolated from the diseased caterpillars of *Arctornis submarginata* (Walker) (Lymantriidae), a defoliating pest of tea from Darjeeling foothill region. The phase contrast and transmission electron microscopic studies identified the virus as granulosis virus. SDS–PAGE analysis of major protein of the occlusion bodies was found to be 31 kDa, characteristic for granulin. The total genomic DNA was isolated. The major band found was of molecular weight 16 kDa. Bioassay conducted with the occlusion bodies (OBs) of the virus showed LC50 value of 4.46 x 10^4 OBs/ml for the second instar caterpillars. Median lethal time (LT50) were 6.6 days for 1 x 10^4 OBs/ml, 5.09 days for 1 x 10^5 OBs/ml, 4.45 days for 1 x 10^6 OBs/ml, and 3.87 days for 1 x 10^7 OBs/ml concentrations. The results indicated the potential of the virus for its future application as microbial pesticide against *A. submarginata* in future.

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1. Introduction

The hairy caterpillar *Arctornis submarginata* (Walker) (Lymantriidae) is found to attack and defoliate mature tea, *Camellia sinensis* (L.) O. Kuntze in the foothills and terai of Darjeeling Himalaya, India. The geographic distribution of this insect includes North East Himalaya, Borneo and Sumatra (Schintlmeister, 1994). In recent years, high population levels of *A. submarginata* caterpillars have adversely affected the tea plantations of Darjeeling foothill region (Mukhopadhyay et al., 2007). Population could only be controlled by regular application of synthetic pesticides (pyrethroids). Since in tea, use of synthetic pesticides is being discouraged due to residue problem and safety, naturally occurring population of the pest was screened for potential microbial biopesticide, especially baculovirus so that the potential of one isolated may be further explored and exploited for biopesticidal value.

The introduction and establishment of baculovirus in an environment is intended to result in permanent suppression of target pest (Cunningham, 1995). Application of lethal doses of baculovirus, as many times as needed, have been attempted for suppression of the host (pest) population, similar to the application of chemical insecticides (Fuxa, 1991; Moscardi, 1999). GVIs are more specific than NPVs, as they have been reported only from Lepidoptera (Bilimoria, 1991; Granados, 1986; Miller, 1997; Moscardi, 1999). In Japan, two important pests of tea, the smaller tea tortrix (*Adoxophyes* sp.) and the oriental tea tortrix (*Homona magnanima*), were controlled by the granulosis virus (GV) isolated from these pests (Nishi and Nonaka, 1996). GVIs were also isolated from different crop pests, such as apple pest *Adoxophyes orana* and apple and pears pest *Cydia pomonella* (Moscardi, 1999) and also from pests like *Erinys ello*, *Phthorimaea operculella*, *Pieris rapae*, *Plodia interpunctella*, *Plutella xylostella* etc. (Moscardi, 1999).

The objective of the present study is to isolate the naturally occurring viral pathogen of *A. submarginata*, to characterize it and to determine the lethal concentration (LC50) and lethal time (LT50) of the virus through bioassay on the same pest species.

2. Materials and methods

2.1. Isolation of baculovirus

Cadavers of 5th instar *A. submarginata* (mean length 30.5 mm) were randomly collected from five different tea plantations (n = 25) of Darjeeling foothills and were individually processed after the method of Sudhaker et al. (1997) for isolation of baculovirus. Crystalline white precipitate obtained from each cadaver was stored in sterilized eppendorf tube with sterilized double distilled water and stored at −20 °C for future experiment.

2.2. Characterization of the virus

The crystalline white precipitate obtained from each cadaver were taken for phase contrast and then for transmission electron microscopy.

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2.3. Total DNA isolation and gel running

The total DNA was isolated from the virus according to the procedure of O’Reilly et al. (1992). In this procedure purified OBs were lysed in 0.1 M Na2CO3 and incubated with proteinase K (100 µg/ml) at 50 °C for overnight.

2.4. SDS–PAGE analysis of major occlusion body protein

Occlusion body proteins were analyzed in SDS–PAGE. Molecular weight marker was used to compare the molecular weight of the major protein band (Sciocco-Cap et al., 2001; Rohrmann, 1992; Tweeten et al., 1981).

2.5. Multiplication of granulosis virus

Multiplication of GV occurs in the fat body cells (Asayama, 1975). The late 2nd instar larva was infected with GV and as the third instar larvae showed typical symptoms of the granulosis after incubation at 30 °C for 2–4 days (Asayama, 1975) their fat body cells were dissected out after 4 days and smeared on glass slide and observed under phase contrast microscope for GV infection.

2.6. Bioassay

All the stages (1st–6th instars) of A. submarginata were collected from mature Tocklai vegetative clone, TV-25, during winter months (Nov–Dec, 2006) and were reared in laboratory for two generations at 27 ± 2 °C and 72 ± 2% RH with a photoperiod (L:D) of 11:13 h in aseptic conditions. Fresh tea twigs (TV-25) collected from organically maintained tea garden of North Bengal University located at Darjeeling foothill, was supplied as food. The second instar larvae were used for bioassay test.

Bioassay of viral suspension was done after the procedure of Chandel et al. (2004) with some modifications. Different concentrations of OBs (1 × 10⁵, 1 × 10⁶, 1 × 10⁷, 1 × 10⁸ and 1 × 10⁹ OBs/ml) were used in the LC₅₀ (median lethal concentration) and LT₅₀ (median lethal time) bioassay, by spreading these uniformly on tea leaves (diameter 4 cm) offered as food. Leaves treated with each viral concentration were fed to second instar larvae (n = 20) and such treatments were replicated thrice. Sterile distilled water was used in control.

The mortality was observed at an interval of 24 hrs from the day of inoculation (bioassay testing). After Abbott’s correction of mortality, data were subjected to probit analysis (Finney, 1954) and the value of median lethal concentration (LC₅₀) was calculated from the regression equation. Median lethal time (LT₅₀) was also determined simultaneously following the method of Biever and Hostetter (1971)

\[ \text{LT}_{50} = a + e(c - b)/D \]

where, \(a\) = the number of hours from the initiation of the test until the reading made just before the 50% value was recorded, \(b\) = the total number of larvae dead at the reading just before 50% value was recorded, \(c = 50\%\) of the total number tested, \(d\) = the number of larvae dying in 24 h period during which the 50% mortality was reached, and \(e\) = the number of hours between mortality counts.

3. Results and discussion

The hairy caterpillar, A. submarginata was recorded as a potential defoliating pest of tea plantations of Darjeeling foothills and terai plains (Mukhopadhyay et al., 2007) of North-East India. In Indomalayan region, the species is reported to have four to five broods a year, with a high incidence from May to December (Web site: http://www.hk.geocities.com/notodontidae/lyma8.htm). In nature the larvae of A. submarginata infected with virus hang head down with flaccid body and liquefied tissue (Fig. 6). It was noted that the average viral occlusion bodies occurring in late stage larva (Vth) was 1.28 × 10⁶ OBs/ml. The typical structure of OB found in phase contrast (Fig. 1) and in transmission electron microscopy helped identification of the virus as granulosis.
virus (GV). In transmission electron microphotograph it was found that the virus was oval in shape (Fig. 4) which is the main characteristic of granulosis virus (Asayama, 1975). The major viral protein (granulin) was found to be of 31 kDa molecular weight (Fig. 2). The virus was found in the cytoplasm of fat body cell (Fig. 5) which is the main multiplication site of GV (Asayama, 1975). Typically the GV got dissolved in dilute KOH and sodium bicarbonate solution. The molecular weight of the native DNA band was found to be 16 kDa (Fig. 3). Application of GV in various doses along with food (tea leaf) showed dose dependent mortality of A. submarginata caterpillars.

Percentage mortality ranged from 45% to 88.33% in first 7 days (Table 1). The median lethal concentration (LC$_{50}$) was $4.46 \times 10^4$ OBs/ml with fiducial lower limit $4.35 \times 10^4$ and upper limit $4.57 \times 10^4$, and the median lethal time (LT$_{50}$) 6 days in case of $1 \times 10^6$ OBs/ml, 5.09 days in case of $1 \times 10^5$ OBs/ml, 4.45 days in case of $1 \times 10^4$ OBs/ml and 3.87 days in case of $1 \times 10^3$ OBs/ml concentrations. Introduction and establishment of baculoviruses in an environment is intended to result in permanent suppression of the target pest (Moscardi, 1999), which might happen through an inoculative method under augmentation strategy of non-conventional pest management.

The present finding on the killing efficacy of the granulosis virus (GV) of A. submarginata therefore, holds a great promise of its use in future as a potent microbial pesticide in tea. However, such applications warrant more experimentation on its formulation that would include search for ideal carriers, protectants (brighteners) against sunlight, spreaders, stickers etc and also testing of the baculovirus for biosafety, especially against beneficial insects.

![Fig. 3. Total genomic DNA of GV.](image)

![Fig. 4. Transmission electron microphotograph of Granulosis virus isolated from A. submarginata.](image)

![Fig. 5. Fat body cell of A. submarginata infected with GV (Granulosis virus).](image)

![Fig. 6. A Granulosis virus infected caterpillar of Arctornis submarginata hanging head-down from tea leaf.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Concentration of baculovirus (OBs/ml)</th>
<th>No. of tested larvae (2nd instar)</th>
<th>Actual mortality (%)</th>
<th>Percentage mortality (%)</th>
<th>LT$_{50}$ (days)</th>
<th>LC$_{50}$ (Bs/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^7$</td>
<td>60</td>
<td>53</td>
<td>88.33</td>
<td>3.87</td>
<td>$4.46 \times 10^4$ (With lower limit $4.35 \times 10^4$ and upper limit $4.57 \times 10^4$)</td>
</tr>
<tr>
<td>$1 \times 10^6$</td>
<td>60</td>
<td>49</td>
<td>81.66</td>
<td>4.45</td>
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</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>60</td>
<td>41</td>
<td>68.33</td>
<td>5.09</td>
<td>$4.35 \times 10^4$</td>
</tr>
<tr>
<td>$1 \times 10^4$</td>
<td>60</td>
<td>33</td>
<td>55</td>
<td>6</td>
<td>Limit $= 4.57 \times 10^4$</td>
</tr>
<tr>
<td>$1 \times 10^3$</td>
<td>60</td>
<td>27</td>
<td>45</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Regression

\[ Y = 0.341 + 19.045X \]

Heterogeneity

\[ x^2 = 94.9254 \text{ for } 1 \times 10^7 \]
\[ x^2 = 82.8169 \text{ for } 1 \times 10^6 \]
\[ x^2 = 62.2785 \text{ for } 1 \times 10^5 \]
\[ x^2 = 45.5172 \text{ for } 1 \times 10^4 \]
\[ x^2 = 34.8387 \text{ for } 1 \times 10^3 \]
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