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Field evaluation of *Lymantria obfuscata* multiple nucleopolyhedrovirus for the management of Indian gypsy moth in Jammu & Kashmir, India

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ABSTRACT

The Indian gypsy moth (*Lymantria obfuscata*) is a pest of national significance, which attacks a wide range of forest and fruit crops in India. The outbreaks of this pest over the past few years spurred interest towards sustainable biointensive approaches for quality fruit production of apple and walnut for agricultural export in Jammu & Kashmir (J & K). The exploratory survey's were conducted for the isolation of native strains of *L. obfuscata* multiple nucleopolyhedrovirus (LyobMNPV) and field evaluation was conducted to determine the most promising strain as a potential biological control agent of this pest. The virus was recovered from different locations of J&K with its natural incidence varying from 0.77 to 7.43 percent. This is the first report from the Indian sub-continent and worldwide of the occurrence of NPVs in natural larval populations of *L. obfuscata*. The *in vivo* mass production was optimised by inoculating *per os* late 3rd instar *L. obfuscata* larvae with an inoculum dose of 1.44×10^5 OBs/larva and followed by a 10 day incubation period. To prevent the disintegration of larval cuticle and microbial contamination, moribund larvae were harvested with an average yield of 1.42×10^8 OBs/larva. The field application of LyobMNPV against the host populations on apple and willow with the pre-standardised dosage of 2.5×10^{12} OBs/ha reduced the larval population density by 25–63%. In the year following application, the larval population density in the treated blocks was 19–28% lower as compared to controls on both apple and willow. This suggests that the virus has considerable potential as a biocontrol agent and the timely application with LyobMNPV on non-fruit trees such as willow which are the preferred hosts for egg laying may help to initiate viral epizootics to keep population outbreaks under check.

1. Introduction

The "gypsy moth", *Lymantria* species are widespread and important defoliators of various types of cultivated and wild plants in the United States, Canada, Europe, Asia and Africa (Pogue and Schaefer, 2007; Dobesberger, 2002). Unlike winged but flightless European gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), wingless Indian gypsy moth, *Lymantria obfuscata* Walker (Lepidoptera: Lymantriidae) which occurs in the Indian subcontinent is a polyphagous pest infesting a wide variety of fruit trees such as apple, pear, apricot, plum and walnut; wild and ornamental trees and shrubs, including oak and rose species, oleaster, seabuckthorn, barberry, elm trees, willow, poplar, aspen and birch. *L. obfuscata* is univoltine and overwinters in the form of egg masses. The caterpillars first eat the buds and then leaves of trees. During the recent past, populations of *L. obfuscata* attained outbreak proportions in Jammu and Kashmir (J & K) and Himachal Pradesh (H. P.) leaving the trees completely leafless that leads to serious damage and significant financial losses, thus posing a serious threat to the horticulture industry and to the ecological integrity of forested ecosystems (Singh et al., 2007; Munshi et al., 2008; Thakur et al., 2015). Interestingly, during population outbreaks rural communities living adjoining to infested areas did not allow the cattle to feed on the infested leaves apprehending some animal related health problems, although adverse effects on animal health have not been proved scientifically yet.

Since, the Himalayan region is a known hot spot of crop diversity with an eco-fragile climate, treatment with chemical pesticides

Abbreviations: IPM, Integrated Pest Management; NPV, nucleopolyhedrovirus; LyobMNPV, *L. obfuscata* multiple nucleopolyhedrovirus; OBs, occlusion bodies; SDS, sodium dodecyl sulphate.

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may be undesirable. Even though insecticides are still advocated as a reliable and practical option to control this pest, it is very difficult to make applications in huge forest areas at farmers personal level or without large scale community interventions sponsored by government agencies. Fortunately, many natural enemies of this pest have been reported coupled with the use of biorationals, namely sex pheromones for trapping moths (Beroza et al., 1973; Punjabi et al., 1974; Masoodi et al., 1990). However, in practice field use of pheromone traps is limited and still not fully explored for managing this pest.

Baculoviruses are naturally involved in the regulation of insect populations and individual strains often have a very narrow host range and are, therefore, harmless to non-target organisms (Groner, 1986). Therefore, their use is being encouraged by international concerns for a reduction in pesticides in the environment, as well as by the development of resistance to chemical insecticides by target pests (Ahmad et al., 2003; Martin et al., 2005). The widespread use of nucleopolyhedrovirus (NPV) is well documented against temperate insect pests such as codling moth, gypsy moth, apple tortrix moth and tent caterpillars of fruit crops in different parts of world. However, in developing countries like India their biocontrol potential has not been explored so far. Since their production requires less capital expenditure they can be mass produced locally and are particularly suitable for use in India. Also, baculoviruses as biological control agents may be merited in forest areas where sensitive habitat may be impacted by defoliation and levels of infection in insect populations could be scaled up to viral epizootics. However, to the best of our knowledge, NPV of this pest has not been reported and used hitherto. Since native strains are always preferred for sustainability, adaptability and efficacy in given agroecosystem, the present study was planned to select an NPV isolate for use against the host species in a control program designed for fruit growers in J & K. The successful use of baculoviruses as microbial insecticides depends on the development of large-scale virus production methods. Optimization with respect to the host insect, insect diet, insect age, virus dosage, incubation, environment and preservation of virus infectivity are some of the major factors (Carter, 1984; Monobrullah and Nagata, 2000; Gupta et al., 2007), besides selection of harvesting time is crucial in maximizing the yield, both to achieve peak NPV production in individual larvae and to avoid losses (Grazywacz et al., 1998). Hence, its economic production was optimized prior to field scale applications. Here we report the field efficacy of newly isolated *L. obfuscata* multiple nucleopolyhedrovirus (LyobMNPV) in India.

2. Materials and methods

2.1. The virus

The virus used in this study was originally isolated from *L. obfuscata* larvae showing typical disease symptoms of NPV infection collected from different zones of J & K during intensive exploratory surveys (Table 1; Fig. 1). The extraction, purification and standardization of OBs were done as per the methods given by Gupta and Tara (2013). Preliminary baculovirus identification was done by light microscopy by spreading smears from infected larvae thinly across a microscope slide followed by Giemsa staining and then examined through a phase-contrast microscope at 1000× magnification under oil immersion (Gupta et al., 2013). The virus was also identified through electron microscopy and viral *polh* gene amplified as described before (Jehle et al., 2006).

Table 1
Locations of collecting diseased insects with their altitude, latitude and longitude.

S. No.	Collection area	Altitude (ft)	Latitude/Longitude
1	Kupwara	5371	75°31′ N/74°15′ E
2	Harwan	5426	34°57′N/74°30′ E
3	Pulwama	5273	33°54′ N/74°53′ E
4	Banihal	5869	33°25′ N/75°12′ E
5	Bhaderwah	5295	32°58′ N/75°43′ E
6	Bani	4200	34°32′ N/73°55′ E

2.2. The host insect

The laboratory culture of *L. obfuscata* was established from field collected eggs from different locations of J & K. The eggs after surface decontamination were placed on filter paper to air-dry and kept at 28 ± 2 °C, 60% RH, and 16L: 8D photoperiod as per the method described earlier (Grisdale, 1969). Following egg hatching, the larvae were reared on the wheat germ based artificial diet as suggested by Stockhoff (1993) in sterile glass jars (30 × 20 × 10 cm) capped with ventilated lids. The diet was supplemented regularly to ensure sufficient food and proper care was taken for sanitation. The emerging adults were allowed to mate and oviposit. The egg masses obtained from laboratory culture were layered between cotton gauze and stored at 4 °C in a cardboard box. In the subsequent year, these eggs masses were removed from cold storage in early March, surface sterilized and allowed to hatch. Egg masses began to hatch from mid-March and were reared together until the second instar stage and were later used in the experiments.

2.3. Estimation of natural incidence of the virus

To estimate the natural incidence of the virus, ten trees were randomly selected from an infested area at each location and larval sampling was carried out by dividing the tree canopy into top and bottom levels selecting four shoots representing four directions. Diseased and healthy larvae present on each shoot were collected and reared in the laboratory. The larvae showing typical symptoms of NPV infection were counted and the NPV infection was confirmed by microscopic examination of the larval smear through Giemsa staining. The percentage infection was calculated by the following formulae:

$$\frac{\text{No. of larvae infected}}{\text{Total no. of larvae(healthy + infected)}} \times 100$$

2.4. Dose response and time response bioassays: selection of a potent isolate

Selection of a more virulent NPV isolate is key to the development of effective viral insecticides, as such mortality caused by different geographical isolates of LyobMNPV to the host larvae were evaluated through *per os* bioassay. The virus isolates were bioassayed on laboratory reared freshly moulted second-instar host larvae, that had been starved for 12 h and then inoculated with six doses of NPV viz., 1.8×10^2, 3.6×10^3, 7.2×10^4, 1.44×10^5, 2.88×10^6 and 5.76×10^7 OBs/larva using artificial diet as described earlier. For determining the time-response, the larvae were inoculated with a dose of 7.2×10^4 OBs/larva to produce similar mortality rates in order to facilitate comparisons among the isolates. Serial dilutions with sterile distilled water were performed to achieve the desired doses. Aliquots of 10 μl of each viral dose from each viral isolate were spread on each diet plug (square diet plugs measuring 7 mm × 7 mm × 5 mm) using the blunt end of a glass rod. It was previously ascertained that the larvae were able to consume the entire diet plug within 24 h and 10 μl was sufficient to