J. Biol. Control, 22(2): 479-481, 2008

Research Note

An improved method of extraction of nucleopolyhedrovirus from soil with reference to *Hyblaea puera* NPV

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ABSTRACT: **An efficient method of extraction of the nucleopolyhedrovirus of the teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae) (*Hp*NPV) from soil has been standardized. This method ensures recovery of about 34 per cent of NPV from soil.**

KEY WORDS: Extraction, *Hyblaea puera*, *Hp*NPV, Nucleopolyhedrovirus, Persistence, Soil

Soil has been referred to as the most stable medium for long-term persistence of nucleopolyhedroviruses (NPVs) (Sakkonen, 1995). For recovering NPV from field soil different methods have been used earlier. Podgwaite *et al.* (1979) used bioassays to prove the virus persistence in soil after an epizootic. Immunofluorescence technique has been reported to be successful for detecting polyhedral occlusion bodies (POBs) of *Ivela auripes* NPV in field soil (Hukuhara and Akutsu, 1987). The method of extraction as proposed by Evans *et al.* (1980) which is essentially a modified version of detergent agitation method developed by David and Gardiner (1967) has been repeatedly used in studies on NPVs with reference to temperate soils. It was used by Wood *et al.* (1994) for studies on genetically modified *Autographa californica* Nucleopolyhedrovirus (AcMNPV) wherein it could detect polyhedral concentrations down to seven occlusion bodies per gram of soil. The defoliator *Hyblaea puera* (Lepidoptera: Hyblaeidae) is a serious pest of teak, a well-known tropical timber species. As part of a study on the persistence of the nucleopolyhedrovirus of the teak defoliator, (*Hp*NPV) in soil, extraction of the virus using the method suggested by Evans *et al.* (1980) which is referred to hereafter as "standard method" was carried out. The poor recovery of *Hp*NPV prompted us to modify this extraction method, which is reported here. In this paper the efficiency of the standard method with the modified method is compared.

The virus extraction as per the method Evans *et al.* (1980) which is referred to as 'standard method' hereafter was carried out as described below. One ml of the partially purified *Hp*NPV inoculum containing 1.8×10^9 POBs was added to 25 g of soil. The soil used was loamy sand of pH 5.6-6.5 collected from a teak plantation site in Nilambur, Kerala. The virus inoculum was well mixed with soil and maintained at room temperature ($28 \pm 2^{\circ}$C) for one hour. The treated soil was then suspended in 50 ml of 1 % sodium dodecyl sulphate (SDS) which served as the elutant and mixed for 5 min in the vortex mixer. In order to complete the elution

process, the suspension was allowed to stand for one hour. The supernatant was collected thereafter and stored and the sediment was resuspended in 50 ml of SDS. After an elution of 20 min the supernatant was collected and stored and the sediment was discarded. The supernatant samples obtained in the two steps as described above were pooled and centrifuged at 1000 rpm for 5 min. The supernatant obtained was again centrifuged at 7000 rpm for 20 min, and the pellets obtained were dissolved in 5 ml of distilled water and enumerated. The *Hp*NPV retrieved at each step was quantified using an improved Neubauer haemocytometer. The experiment was replicated thrice.

The standard method was modified as follows. The duration of the process of eluting the soil with SDS was increased as detailed in the Table1. The first step of the elution process of 60 min was followed by three steps of 20 min, 15 min and 15 min respectively with collection of supernatant at each step. The supernatant collected at each step was pooled and centrifuged as per the standard procedure. The extraction procedure was repeated five times and the average yield under the two methods was estimated. The experiment was replicated thrice.

The % of *Hp*NPV retrieved in each of step of the extraction procedure under the two methods is presented in Table 1. The quantity of the virus retrieved is presented as percentage of inoculum applied to the soil. Using the standard method after two steps of elution, *Hp*NPV retrieved was 22.72 per cent. In the modified method the total yield after four steps of elution process was 38.12 per cent. The overall quantity of the virus recovered under the standard method and modified method was 16.36±7.99 percent and 34.12±8.95 respectively.

The infectivity of virus extracted from soil was compared with fresh virus through leaf disc bioassays (Evans and Shapiro, 1997). Early fifth instar larvae of *H. puera* of uniform age and weight range (80-110 mg) were used for the bioassay. Leaf discs of 0.5 cm x 0.5 cm size were prepared from freshly collected tender teak leaves. The leaf disc was placed in the sterilized plastic rearing tube and 10 μl of the virus suspension was placed on to each leaf disc. When the inoculum was dried up, the leaf discs were presented to the test larvae and allowed to feed for set period 3- 5 h. After complete feeding, larvae were transferred to individual vials containing semi-synthetic diet free of formaldehyde. The doses used were $1x10^2$, $1x10^4$ and $1x10^5$ OBs per larva. Each treatment was replicated thrice with 20 larvae per replication. Bioassay using fresh virus sample served as the control. Observations on the mortality of larvae infected with the virus were made at specific time points. The data were subjected to probit analysis to calculate the median lethal dose (LD_{50}). Mean percentage mortality were compared using ANOVA and the LSD to test the statistical significance (P d"0.05). All analyses were done using the statistical software, SPSS Version 10. The results indicated that the infectivity of the virus extracted from soil was similar to that of the control (Table 2). The higher recovery of the virus under the modified method is attributable to the additional steps of elution process adopted in the new method, which might have enhanced the viral recovery. The bioassay indicated that additional steps of elution with SDS has not affected the infectivity of the virus. However, as SDS is of alkaline nature, too much exposure of the virus to it would be harmful affecting the virulence. Hence, the duration of elution of the virus with SDS fixed in this method may be treated as optimum.

The efficiency of the extraction method is critical in estimating the physical and biological persistence of NPV in soil. The standard method suggested by Evans (1980) was essentially a modified version of detergent agitation method developed by David and Gardiner (1967), which was repeatedly used in temperate soils. However, this method was not found very efficient with reference to the tropical soil. The present study suggests that in NPV persistence studies, extraction procedure needs standardization with respect to the particular NPV and the soil type. The modified method thus appears to be more effective with respect to HpNPV as it enhanced viral recovery from soil. It is expected that the method will equally be suitable for other NPVs of the tropical region.

Table 1. *Hp*NPV retrieved at each step during extraction from soil using the standard and the modified method[a]

Extraction Steps	Time (minutes)	Retrieved *Hp*NPV (%)[b](Mean±SD)	
		Standardmethod	Modifiedmethod
Elution with SDS	60	13.04±3.74	11.64±5.25
Elution with SDS	20	9.68±4.81	9.66±3.26
Elution with SDS	15	NA	9.08±1.55
Eluting with SDS	15	NA	7.74±2.90
Interim total yield	22.72	38.12	
Centrifugation (1000rpm)	5	19.42±8.87	36.50±8.61
Centrifugation (7000rpm)	20	16.36±7.99	34.12±8.95
Final yield	16.36±7.99	34.12±8.95	

[a] Initial polyhedral concentration, 1.8×10^9 POBs per 25g soil; NA- Elution not performed

[b] Mean of three replicates

Table 2. Biological activity of *Hp*NPV retrieved from soil

Nature of virus	Effective initial no. of insects	LD_{50} (x 10^4 OBs)	95% fiducial limits (x 10^4 OBs)	Slope (± SE)
Untreated	67	0.77	0.098 - 14.11	0.325 (0.126)
Extracted	145	0.93	0.173 - 5.61	0.333 (0.094)

ACKNOWLEDGEMENTS

The work was carried out as part of a research project funded by the Department of Biotechnology, Govt. of India. The work also forms part of the Ph.D thesis of the senior author.

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(Received: 13.08.2007; Revised: 13.11.2007; Accepted: 10.12.2007)