Optimizing the time of harvest of nucleopolyhedrovirus infected *Spodoptera litura* (Fabricius) larvae under *in vivo* production systems

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The optimal time of harvest of nucleopolyhedrovirus (NPV) infected *Spodoptera litura* (Fabricius) under *in vivo* mass production system was investigated. Yield of virus from living and dead larvae infected previously in fifth instar larvae reared on artificial diet, at the doses 3932.4, 1966.2 and 393.24 OB/mm^2 and harvested at 5, 6, 7 and 8 days after inoculation was recorded. A maximum yield of 5.57×10^9 OB/larva was obtained at the inoculum dose of 1966.2 OB/mm^2, when exclusive harvest of cadaver was done. The study revealed that a dose of 1966.2 OB/mm^2 and exclusive harvest of cadavers were the most important parameters for the mass production of NPV.

THE tobacco caterpillar, *Spodoptera litura* (Fabricius) is a polyphagous pest attacking many crop plants[1]. Chemical control has often failed as this pest is reported to have developed resistance to many of the commercially available insecticides[2]. The development of widespread resistance to chemicals has also encouraged the development of biopesticides based on insect viruses as a means of overcoming this problem[3]. Nucleopolyhedroviruses (NPVs) are capable of originating natural epizootics in lepidopteran population[4] and have been recognized as important pest-control agents because of their insecticidal properties and their safety with respect to the environment and non-target organisms[5]. Successful control of *S. litura* with the NPV mass produced *in vivo* in *S. litura* larvae has been reported on many crops[6–10]. The virus is mass multiplied *in vivo* in *S. litura* larvae[8,11].

Information on the optimal time to harvest the infected larvae is scarce. Correct selection of harvesting time was crucial in maximizing the yield, both to achieve peak NPV production in individual larvae and to avoid losses[3]. Also, differences in biological activity of the virus harvested from living or dead larvae have been reported[12–14]. Hence, optimization of the period of harvest plays a crucial role in economic production of NPV. This communication describes a study conducted at Biocontrol Laboratory, Tamil Nadu Agricultural University, Coimbatore to determine the optimal period of harvest of nucleopolyhedrovirus (SpltNPV)-infected larvae of *S. litura* for obtaining maximum yield and without reduction in biological activity.

Pre-weighed fifth instar larvae were allowed to feed on diet treated with viral doses of 3932.4, 1966.2 and 983.1 OB/mm^2 and incubated at 25 ± 1°C. The infected larvae were harvested at five, six, seven and eight days after inoculation, which contained both live and dead larvae depending upon the time interval between inoculation and harvest. For comparison, a treatment of harvesting the cadavers as and when the larvae died was also included. Each treatment was replicated thrice with 15 larvae per replication. An untreated control was also maintained. The larvae/cadavers were collected starting from the fifth day onwards, weighed and frozen immediately. Observations on cadaver weight and OB yield were recorded. The strength of the OB was assessed using a double-ruled Neubauer haemocytometer (Weber, England). The samples were diluted 100 to 1000 fold and counted[15] by phase contrast microscopy (five counts per haemocytometer and three sub-samples per suspension were measured to reduce counting and dilution errors).

Analysis of variance was carried by completely randomized design[16] and means were separated by Duncan's new multiple range test[17].

The time of harvesting plays a crucial role in determining productivity and hence optimizing the period of harvest remains central towards the economic production of NPV. In the present study on optimization of the harvest period, it was revealed that the number of cadavers harvested increased over a period of time. Highest mortality was recorded at the inoculum dose of 3932.4 OB/mm^2, however, it was on par with 1966.2 OB/mm^2. A negative relationship between the inoculum dose and yield was noticed, which may be due to the higher inoculation rate, killing the larvae before they reach their maximum weight and consequently resulting in lower yields[3,18–20]. With large doses of NPV, there are reports that virions from the inoculum are able to pass across the mid-gut epithelia directly into the haemocoel to initiate secondary infection in other body tissues without the need for a primary replication cycle, thus speeding up the development of infection[21]. The weight of the infected larvae collected both as dead or live decreased over a period of time, being highest at the inoculum dose of 1966.2 OB/mm^2. When exclusive collection of cadavers was done, a maximum weight of 43.5 ± 2.4 mg (Table 1) was recorded at the lowest dose of inoculum, i.e. 983 OB/mm^2. The observed increase in size of larvae infected with low and intermediate doses may be attributed to the presence of the *egt* gene in the baculovirus genome[22]. Similarly, the yield per larva and yield per gram of body weight also increased progressively in relation to time of harvest. A maximum yield of 5.57×10^9 OB/larva was obtained when exclusive collection of cadavers was done at the inoculum dose of 1966.2 OB/mm^2 (Table 1). The yield per gram of body weight was highest in the treatment consisting of exclusive cadaver harvest. Estimating the virus yield per unit weight of larva gives some indication as to how much and

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Table 1. Effect of inoculum dose and period of harvest on production of SpltNPV

Inoculum dose (OB/mm^2)	Days to harvest	Larval mortality (%)	Larval weight at harvest (mg)	Yield/larva ($\times 10^9$ OB)	Yield/g of body weight ($\times 10^8$ OB)
3932.4	5	6.67c	54.3 ± 2.8a	0.144e	2.664c
	6	8.89c	48.7 ± 5.1b	0.638d	13.425bc
	7	8.89c	45.9 ± 1.2c	0.821c	17.974bc
	8	78.57b	35.8 ± 3.6d	1.123b	31.947b
	As cadavers	100.00a	34.5 ± 4.2e	1.884a	55.693a
1966.2	5	2.22c	61.0 ± 2.6a	0.129e	2.060c
	6	6.99c	60.7 ± 2.2b	0.141d	2.401c
	7	13.81c	60.6 ± 1.8c	1.528c	26.168b
	8	63.81b	59.1 ± 1.0d	2.069b	31.931b
	As cadavers	97.78a	38.3 ± 4.3e	5.572a	149.408a
983.1	5	0.00c	57.8 ± 2.2a	0.126e	2.186c
	6	0.00c	57.1 ± 2.0b	0.205d	3.579c
	7	6.67c	52.9 ± 5.3c	1.282c	21.116c
	8	48.89b	44.1 ± 3.8d	2.193b	52.258b
	As cadavers	87.18a	43.5 ± 2.4e	3.486a	82.154a

*Means followed by similar letters are not significantly different ($P = 0.05$) by DMRT.

how rapidly the insect is being converted into virus polyhedra[23]. The maximum OB yield of 1.5×10^{10} OB per gram of body weight (Table 1) obtained at the inoculum dose of 1966.2 OB/mm^2, indicates effective conversion of insect into virus polyhedra. Smits and Vlak[13] suggested a premature harvest of *Spodoptera exigua* (Hübner) larvae between 4 and 7 days post-inoculation. They reported that production of the polyhedra did not increase after the seventh day. However, this was not true in the present study because yield of the virus was high only when the infected larvae were collected as cadavers. The yield per larva and per gram of body weight was higher at lower doses of inoculum, which is in agreement with earlier reports for *Helicoverpa armigera* (Hübner)[19]. One of the problems involved in harvesting dead larvae, was that they were often heavily contaminated with bacteria[24]. Changes in the production system to include harvesting only live infected larvae led to a major reduction in bacterial contamination, but reduced the maximum yield of virus[25] up to 20%. Therefore, reduction in a key parameter such as yield of virus would result in a reduction in the fitness of a virus as it will increase the cost of production[23]. In order to be competitive with traditional pest control methods, baculoviruses must be cost-effective. At present, the only viable option for large-scale production of baculoviruses is through *in vivo* replication of the virus, most often in the homologous host reared on artificial diet. Production *in vivo* is straightforward, but yields can be highly variable, as can the costs. This variability is critical to the economic success or failure of baculovirus production. Even for non-commercial ventures, optimization of virus yield is crucial for minimizing costs[3].

Differences in biological activity of the virus harvested from live or dead larvae were also reported. Ignoffo and Shapiro[12] found that *Helicoverpa zea* (Boddie) NPV processed from live larvae was less potent than that from dead hosts by about 7–9 times. The yield per larva was more in the former case. This situation also applied to *S. exigua* NPV, where virus from dead larvae was about 50% more active[13]. Shapiro and Bell[14] monitored the yield and biological activity of *Lymantria dispar* (Linnaeus) NPV at different times after infection. OB harvested from dead larvae was up to 7 times more active than OB collected from living infected larvae. Virus yield increased up to 2×10^9 OB per larva during the first 11 days and then remained constant. The difference in biological activity was linked to poor stability of non-enveloped NPVs and also that the number of virions in unfinished occlusion bodies may be lower[5]. Considering the above observations, exclusive harvest of cadavers for *S. litura* should be adopted in the virus production systems. By harvesting the infected larvae at the right time, the number of larval equivalents required for spraying will be reduced and subsequently the cost of production will be reduced. Microbial contamination in the end-product can be further reduced by better sanitary conditions during rearing and virus production and by use of a highly purified inoculum, preferably produced in cell culture[13]. Hence, a dose of 1966.2 OB/mm^2 and exclusive harvest of infected larvae as cadavers is recommended for economic production of SpltNPV.

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Record of rhodoliths from Aramda Reef Member (Late Pleistocene to Holocene) of Chaya Formation, Dwarka–Okha area, Gujarat and their paleoenvironmental significance

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Rhodoliths are defined as 'certain more or less nodular forms of nongeniculate coralline algae with a nucleus and a number of concentric thallus layers around them'. In the present study we have recorded rhodoliths from Units II, III and IV of Aramda Reef Member (Late Pleistocene to Holocene) of Chaya Formation, Dwarka–Okha area, Gujarat. Unit II rhodoliths are multispecific, discoidal, columnar concentric type and multispecific, ellipsoidal, branching class III and class IV type. Unit III rhodoliths are multispecific, ellipsoidal, laminar, concentric type and multispecific, spheroidal, laminar boxwork type. Unit IV rhodoliths are multispecific, spheroidal, laminar, concentric type and multispecific ellipsoidal, laminar, concentric type and multispecific, spheroidal, branching class IV type. The thin-section study of rhodoliths reveals the presence of nongeniculate algal genera such as *Lithoporella, Lithothamnion, Lithophyllum*, *Porolithon* and *Sporolithon* and geniculate coralline alga *Amphiroa.* The rhodoliths and other algal assemblages point to high energy conditions with turbulence during the deposition of Units II and IV and low to moderate energy conditions during the deposition of Unit III of Aramda Reef Member.

CORALLINE algae have been known as the most important agents in the building of carbonate sequences since the early Palaeozoic[1]. Many studies have shown that coralline algae are reliable palaeoenvironmental indicators[2–6]. The calcified skeletal tissue enables coralline algae to construct algal reefs[4,7,8] and rhodoliths[5,9]. Rhodoliths are defined as 'certain more or less nodular forms of unattached nongeniculate coralline algae which have developed around a nucleus and which usually consist of a number of concentric thallus layers'[10]. Rhodoliths containing monospecific and multispecific nongeniculate coralline algae have been described from several parts of world like Norway[11], France[12], Bermuda[9,13], Ireland[14], Malta[15–17], Florida[18], Italy[19] and Australia[20]. From India, Rao *et al.*[21] have documented carbonate nodules containing foraminifera and coralline algae from Late Quaternary deposits along the shelf break between Goa and Cape Comorin and discussed their significance in environment and sea-level changes. In the present

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