FACTORS AFFECTING PATHOGENICITY OF NPV PREPARATIONS TO THE CORN EARWORM, *HELIOTHIS ARMIGERA*

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Pathogenicity of *Heliothis* nuclear polyhedrosis virus (HSNPV) to the corn earworm, *Heliothis armigera*, was studied using 3 different inoculative methods. The LD50 values of 4th-instar larvae inoculated with corn-fed, diet-fed and inoculum-imbibing method were $1.85 \times 10^6$, $2.55 \times 10^5$ and $1.22 \times 10^3$ PIBs/larva, respectively. The inoculum-imbibing is more sensitive and convenient for inoculating *H. armigera* with HSNPV. The HSNPV product, Elcar®, was highly pathogenic to *H. armigera*, the LD50 values of 2nd-, 3rd- and 4th-instar larvae being 27, 83 and 1,221 PIBs/larva, respectively, as measured by the inoculum-imbibing method. The mortality of 4th-instar larvae caused by HSNPV was increased, but the incubation period was shortened with higher incubation temperatures. However, the high temperature at 35 °C caused a lower mortality, and a prolongation of the median lethal time (LT50). Stability and persistence of HSNPV preparations were better in January-February and April-May than in June-July and October-November periods when sprayed on corn silks under field conditions. The HSNPV was inactivated by weak alkaline dew (pH 8.1) collected from soybean leaves, but it remained active on those from corn, tomato and asparagus with pH 7.2-7.3. The artificial heavy rainfall of 242 mm/h for 30 min did not wash off HSNPV preparations sprayed on the corn silks.

KEY-WORDS: *Heliothis armigera*, corn earworm, nuclear polyhedrosis virus, Elcar®.

The corn earworm, *Heliothis armigera*, is a polyphagous and destructive lepidopteran pest in Taiwan. The nuclear polyhedrosis virus isolated from *Heliothis* spp. (HSNPV) is highly pathogenic to *Heliothis* larvae, but it is safe to human, animals and natural enemies (Ignoffo, 1965; Ignoffo, 1966c; Ignoffo, 1973; Ignoffo & Couch, 1981; Teakle et al., 1986). This virus has been produced, formulated as the 1st viral insecticide and registered for controlling various *Heliothis* species in North America and other countries (Ignoffo, 1973; Teakle et al., 1983; Entwistle & Evans, 1985). It is thus promising as a microbial agent for controlling *H. armigera*, *H. zea* and related species. However, efficacy of viral preparations

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is largely dependent upon stability, persistence, timing, host age, dosage, etc. when applied in the field (Roome, 1975; Boucas et al., 1980; Rogers et al., 1983). Temperature may affect larval mortality of *Heliothis* species after exposing to HSNPV (Ignoffo, 1966b; Salama et al., 1986). Sunlight UV is considered to be the most destructive factor in the inactivation of many viruses (Bullock, 1967; Ignoffo & Batzer, 1971; Ignoffo et al., 1972; Young & Yearian, 1974). The pH values of dew on foliage surfaces of cotton, soybean and tomatoes are important in inactivation of entomopathogenic viruses (Young & Yearian, 1974; McLeod et al., 1977; Young et al., 1977). Rain could cause reduction in the number of viable spores of *Bacillus thuringiensis* on plants (Frye et al., 1973); however, not much is known about its effect on viral preparations when sprayed on leaves in Taiwan. Therefore, influence of environmental factors on NPV persistence and stability needs to be examined while applying the virus to control insects under field conditions. The present study is to investigate pathogenicity of HSNPV to *H. armigera*, and factors affecting its persistence and stability.

**MATERIALS AND METHODS**

**INSECTS**

The laboratory colonies of *H. armigera* were derived from corn fields at Chia-yi county, Taiwan. Larvae were reared individually on a semi-synthetic diet filled in 1-oz plastic creamers (BioServ. Inc., N.J., USA). Rearing was carried out in a growth chamber with 12L : 12D photoperiod, at 25°C ± 1°C, 60-70% R.H., 3 generations were colonized in culture prior to HSNPV test.

**VIRUS SOURCE**

Elcar” WP., containing 0.4% *Heliothis* NPV, ca. $4 \times 10^9$ PIBs/g, was provided by the Sandoz Inc., Calif. USA, and was used for various experiments. Elcar” was suspended in deionized water at pH 6-7 in a sterile beaker as the stock suspension from which the serial dilutions were prepared.

**VIRUS INOCULATION AND PATHOGENICITY MEASUREMENT**

Newly molted 4th-instar larvae were starved for 12 h and then inoculated with virus suspensions by 3 methods. Larvae were fed on the NPV-containing diets, corn kernels soaked with virus suspensions, or allowed to imbibe the virus suspension as described by Klein (1978).

The median lethal dose (LD50) was determined on 2nd, 3rd and 4th-instar larvae using the inoculum-imbibing method at 2, 2 and 4 μl/larva, respectively. Sixty larvae in 3 replicates were inoculated in each treatment. Larval mortality was recorded until 7 days after inoculation. The LD50 values for 2nd, 3rd and 4th-instar were calculated from dosage-mortality curves by probit analysis.

**MEASUREMENT OF VARIOUS FACTORS AFFECTING HSNPV PATHOGENICITY**

For determining the optimal temperature, 20 4th-instar larvae were inoculated with $4 \times 10^4$ PIBs/larva using the inoculum-imbibing method and incubated at 15°, 20°, 25°, 30° or 35°C in each treatment. Six replications were made at each temperature.