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INSECTICIDAL ACTIVITY OF NATIVE ISOLATES OF *SPODOPTERA FRUGIPERDA* MULTIPLE NUCLEOPOLYHEDROVIRUS FROM SOIL SAMPLES IN MEXICO

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The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is the main insect pest of corn in Latin America. Control of FAW larvae requires 2-4 applications of chemical insecticides (Hruska & Gould 1997). FAW larvae are susceptible to infection by a baculovirus, specifically the *Spodoptera frugiperda* nucleopolyhedrovirus (SfMNPV) (Shapiro et al. 1991). Use of this virus may serve as an alternative to chemical control; it would reduce the risk of resistance development and environmental pollution; due to its specificity, biosecurity, persistence and virulence level (Fuxa 2004). Essentially all isolates used against FAW have been isolated from infected insects. There are no prior reports on obtaining and using SfMNPV isolates from soil. In this study the biopesticidal activities of different isolates of native NPV on FAW larvae were measured.

FAW larvae were obtained from a colony established under controlled conditions (25 ± 2°C, 12:12 L:D h and 50-60% RH). Larvae were reared on an artificial diet (Southland Products Incorporated). Corn plots were sampled in Coahuila, Nuevo Leon and Nayarit, Mexico. Soil samples were taken by scraping away the upper 5 cm of soil, and then collecting soil at depths of 5-10 cm. Each sample consisted of 600 to 800 g soil. Samples were processed according to the methodology described by Richards & Christian (1999). Thus each 25 g sample of sieved soil was incorporated twice in 100 ml of artificial diet, and first and second FAW instars were placed on the amended diet. Larvae that died of polyhedrosis disease were considered to be infected by a NPV isolate. These NPVs isolates were amplified *in vivo* in FAW third instars and purified by filtration and centrifugation as described by Muñoz et al. (2001). The concentrations of viral occlusion bodies (OBs) were quantified with a hemacytometer and stored in aliquots of 500 µl of distilled water at 0°C until required.

The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) of each viral isolate recovered was determined by the diet surface contamination technique. The diet surface in each container was inoculated with 1 of 7 NPV concentrations ranging from 2.0×10^1 to 4.0×10^6 OBs/mm^2; and 20 FAW larvae per concentration were infected in each of 3 replicates. Larvae used as the control in the bioassay were placed in cups with artificial diet treated only with sterile distilled water, i.e., no virus. Mortality was measured daily for 25 d. Based on the highest mortality and the shortest time to death among toe 10 isolates, the most infective isolate, i.e., SfMNPV-AN_2, was selected (Table 1). Bioassays were conducted on all 5 FAW instars (Table 2). The LC_{50} and LT_{50} were determined using 7 concentrations ranging from 1.0×10^1 to 1.0×10^7 OBs/mm^2. Larval mortality was recorded every 12 h for 25 d. These bioassays were replicated 3 times.

Mortality was corrected by Abbott's formula (Abbott 1925), and the means of treatments were separated using the Tukey's test ($P < 0.05$). The LC_{50} values were calculated by the probit method using the statistical program SAS (SAS 2002). LT_{50} values were estimated with the Generalized Linear Modeling Program (GLM).

Of the 120 soil samples collected, 10 samples were positive for SfMNPV. These 10 isolates of NPV were shown to be pathogenic against FAW third instars with mortalities ranging from 82 to 100%. Also, the virulence of these isolates varied as reflected in the LC_{50} and LT_{50} values (Table 1). The isolate SfMNPV-AN_2 from Coahuila proved to be the most infective and caused 100% mortality. The LT_{50} values ranged between 6 to 11 d (Table 1). Martínez et al. (2003), reported mortalities of 63-100% and a LC_{50} of 3.4×10^4 OBs/larva, and a LT_{50} of 3.9 d in third instar FAW treated with SfMNPV. We found the first 2 instars to be the most susceptible (Table 2). The LT_{50} values ranged from 4 d in the first instar to 8 d in the fifth instar. Data on pathogenic isolates of NPV in FAW larvae have been reviewed by Escribano et al. (1999). The first instar was the most susceptible to SfMNPV; this result substantiated by Cisneros et al. (2002). The time and the concentration required for the virus to cause larval death both increase with succeeding instars (Martínez et al. 2003). Based on these results we conclude that in soils in Mexico there are native isolates of nucleopolyhedrovirus with potential for use in biological control of the FAW.

TABLE 1. MEDIAN LETHAL CONCENTRATION LC_{50} OF NATIVE ISOLATES OF SFMNPV ON THIRD INSTAR OF *SPODOPTERA FRUGIPERDA*.

Isolates [a]	N [b]	% Mortality[c]	Lower limit	LC_{50} (95%)[d]	Upper limit	Slope (±SE)	Intercept (±SE)	χ^2
$SfMNPV\text{-}NAV_1$	420	52.63	6.7×10^4	1.9×10^5	9.5×10^5	0.52 ± (0.07)	-2.73 ± (0.34)	0.98
$SfMNPV\text{-}NAV_2$	420	70.00	3.7×10^5	1.5×10^6	2.8×10^7	0.53 ± (0.10)	-3.27 ± (0.59)	0.96
$SfMNPV\text{-}NAV_4$	420	89.47	5.5×10^5	7.4×10^5	9.9×10^5	2.93 ± (0.39)	-17.20 ± (2.32)	0.77
$SfMNPV\text{-}NAV_6$	420	85.00	1.0×10^4	1.2×10^4	1.5×10^5	1.22 ± (0.10)	-5.02 ± (0.41)	0.99
$SfMNPV\text{-}NAV_8$	420	90.00	9.9×10^3	2.3×10^4	4.8×10^4	0.72 ± (0.08)	-3.12 ± (0.37)	0.96
$SfMNPV\text{-}NAV_9$	420	78.95	6.8×10^4	1.0×10^5	1.7×10^5	0.55 ± (0.06)	-2.75 ± (0.28)	0.96
SfMNPV-CAD	420	80.00	9.7×10^4	1.2×10^5	1.4×10^5	1.25 ± (0.13)	-6.34 ± (0.67)	0.83
SfMNPV-NAY	420	94.74	5.6×10^3	7.5×10^3	9.8×10^3	2.06 ± (0.23)	-7.98 ± (0.92)	0.99
$SfMNPV\text{-}AN_1$	420	72.22	5.4×10^4	6.5×10^4	7.7×10^4	1.53 ± (0.18)	-7.35 ± (0.90)	0.87
$SfMNPV\text{-}AN_2$	420	100.00	4.3×10^2	5.7×10^2	7.2×10^2	2.86 ± (0.34)	-7.92 ± (0.97)	0.99

NPV Isolates, $SfMNPV\text{-}NAV_{1, 2, 4, 6, 8 and 9}$: isolated from corn plots at Navidad, Nuevo León; SfMNPV-CAD isolated from Cadereyta, Nuevo León; SfMNPV-NAY isolated from Nayarit; $SfMNPV\text{-}AN_{1, 2}$ isolated from Coahuila, México.

[b]Number of insects treated;

[c]Percent mortality with the highest concentration ($4.0x10^6$ OBs/mm^2).

[d]LC_{50} values were expressed as OBs/mm^2 on the diet surface. Twenty 20 larvae per NPV concentration, 7 concentrations per isolate and 3 replicates; 20 untreated larvae (control) per replicate were used. SE = Standard error; χ^2 = Goodness of fit test.

TABLE 2. MEDIAN LETHAL CONCENTRATION AND MEDIAN LETHAL TIME TO DEATH OF THE $SFMNPV\text{-}AN_2$ ISOLATE DETERMINED IN EACH OF THE FIVE *SPODOPTERA FRUGIPERDA* INSTARS.

Instar	N[a]	Lower limit	LC_{50} (95%)[b]	Upper limit	Slope (±SE)	Intercept (± SE)	χ^2	LT_{50} (95%) (days)
First	420	6.1×10^1	1.15×10^2	1.7×10^2	0.58 ± (0.04)	-1.61 ± (0.13)	0.72	3.96
Second	420	2.6×10^2	3.6×10^2	4.8×10^2	0.87 ± (0.07)	-2.23 ± (0.22)	0.99	5.88
Third	420	2.6×10^2	5.7×10^2	1.4×10^3	2.86 ± (0.34)	-7.92 ± (0.97)	0.83	6.55
Fourth	420	1.3×10^5	4.3×10^5	4.5×10^6	1.07 ± (0.31)	-6.02 ± (1.72)	0.74	7.01
Fifth	420	1.1×10^7	2.4×10^7	4.4×10^7	1.42 ± (0.21)	-10.54 ± (1.64)	0.81	7.82

[a]Number of insects treated

[b]LC_{50} values were expressed as OBs/mm^2 of diet surface; 20 larvae per NPV concentration, 7 concentrations per isolate and 3 replicates were used; also 20 untreated larvae (control) per replicate were used.

SE = Standard error; ^{2} = Goodness of fit test.

Summary

The FAW is the main insect pest of corn in Latin America. The larvae are susceptible to *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV). Ten isolates collected from soil of corn plots infested with FAW larvae in Coahuila, Nuevo Leon and Nayarit, Mexico were evaluated to control this pest. Bioassays were performed to determine the biological response of the third instar to SfMNPV infection in order to select the most infective isolate. The diet surface contamination technique was used. The isolate SfMNPV-AN_2 from Coahuila was the most highly infectious. Additional bioassays of the same isolate were performed in the 5 FAW instars to determinate mortality. LC_{50} increased as the size of the insect increased from first to fifth instar. A similar pattern occurred with LT_{50}. This study achieved the isolation from the soil of highly virulent SfMNPV isolates.

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