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Development and evaluation of Immunochemical Tools for Diagnosis and Quality Control of *Helicoverpa armigera Nucleopolyhedrovirus* (*Ha*NPV)

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Helicoverpa armigera nucleopolyhedrovirus (*Ha*NPV; Family: *Baculoviridae*; Genus: *Nucleopolyhedrovirus*) is a natural pathogen of *Helicoverpa armigera* (Insecta: Arthropoda) larvae and has proven to be a good candidate for the biocontrol of this pest on legume crops. In this study various immunochemical tools were developed using the polyclonal antibodies raised against the polyocclusion body (POB) protein (polyhedrin) and evaluated for the detection and quantification of *Ha*NPV in insect larvae and viral insecticide prepartions. Indirect immunofluorescence assay and Western immunoblot assay were developed for detection of POBs in homogeneates of *Ha*NPV-infected larvae. Direct antigen coating (DAC)-enzyme-linked immunosorbent assay (ELISA) and Indirect Competitive (IC)-ELISA were developed for detection and quantification of polyhedrin protein in insect extracts. The sensitivity of DAC-ELISA is 30 ng/ml of *Ha*NPV polyhedrin in 5 µg/ml of insect total protein extracts. But in DAC-ELISA there was competition between insect and viral proteins for binding to the ELISA plate surface reducing the sensitivity of the assay. To eliminate this, IC-ELISA was developed, which has sensitivity of 0.156µg/ml of *Ha*NPV polyhedrin in 20 µg/ml of total insect protein extracts. The concentration of polyhedrin for 50% competitive inhibition (IC_{50}) was calculated to be 1.14 µg/ml. This test is equally effective in detecting polyhedrins of heterologous NPVs such as, *Spodoptera litura Nucleopolyhedrovirus* (0.31 µg/ml) and *Amsacta albistriga Nucleopolyhedrovirus* (0.32 µg/ml). A simple purification protocol was standardized for extraction of total polyhedrin from NPV preparations of 6×10^9 to 4.68×10^7 POBs /ml. The purity of the extracted polyhedrin was assayed in SDS-PAGE and evaluated in both DAC as well as IC-ELISA with sensitivity of 9.375×10^7 POBs /ml. The ELISA results were comparable to light microscope counting of POBs. Application of ELISA and Western immunoblot assay in bioassay experiments suggested that the 4th instar larvae is better for virus innoculation, and virus harvesting 9 days after inoculation for maxumum virus yield, and less bacterial contamination. These diagnostic tools are convenient, rapid and inexpensive for routine detection and quantification of *Ha*NPV.