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Isolation and Identification of Nuclear Polyhedrosis Virus Infecting Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in Egypt

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda*, is a newly introduced invasive pest in Egypt causing significant crop damage. In the invaded areas, fall armyworm management options are limited and primarily dependent on synthetic pesticides, raising concerns about resistance development and negative environmental and human health impacts. So, it was necessary to search for alternative solutions. Insect pathogenic viruses present a viable alternative as they are highly effective, host-specific, and have minimal harmful effects on beneficial insects and non-target organisms and promote sustainable agricultural practices. The primary objective of this research is to accurately identify the specific Nucleopolyhedrovirus NPV strain infecting fall armyworm larvae. This study identified naturally occurring Nucleopolyhedrovirus (NPV)-infected fall armyworm larvae. The NPV strain was isolated, purified, and examined microscopically. Scanning electron microscopy revealed a large quantity of polyhedra with varying shapes. Transmission electron microscopy of dissected larvae demonstrated the virus's significant impact on the cytoplasm and internal organs. Finally determining the exact NPV strain will aid in understanding its virulence and host specificity, and exploring the potential of integrating NPV with other control methods to enhance its effectiveness in sustainable agricultural practices.

INTRODUCTION

Maize (*Zea mays* L.) stands as one of the world's most economically significant cereal crops **(**Day *et al.*, 2017). *Spodoptera frugiperda*, commonly known as the fall armyworm, is recognized as an invasive and harmful pest affecting maize crops in both Africa and the Americas (Deole and Paul 2018., Shylesha *et al.*,2018 and Maruthadurai. and Ramesh 2020). It is native to several countries in America and has a significant impact on numerous crops (Dias *et al.,* 2019). FAW feeds upon the above parts of maize plants, especially the leaf whorls. Larvae can eliminate the plants within a short time (Deole and Paul 2018). Three hundred million people may be at risk of starvation due to the severe harmful impacts of the armyworm in Africa (Idrees *et al.,* 2022).

The development of multi-insecticide resistance makes *S. frugiperda* a difficult pest to control using chemical pesticides. To avoid resistance development against pesticides, other effective and safe control alternatives are highly required. Biological control agents, as nematodes, viruses, fungi, and bacteria, are among the alternatives that are currently being investigated., (Prasanna *et al.*, 2018, Bateman *et al*., 2021, Fakeer *et al.,* 2024 and Harrison *et al.*, 2019). The use. of viruses for FAW control is a viable substitute as they are virulent, have a narrow host range, with no environmental impacts. compared to chemical pesticides (Prasad and Srivastava 2016, Moscardi *et al*., 2011, Federici, 2009).

Spodoptera frugiperda Nucleopolyhedrovirus (*Sf*NPV) belongs to the family, Baculoviridae and is recognized as an alternative specific, virulent and safe to non-target insects. (Gencer *et al.*, 2022 and Barrera *et al.*, 2011). *Sf*MNPV is specific to FAW larvae, so it has great potential in pest management (Haase 2015). In nature, infection occurs by ingesting contaminated food (usually maize leaves) (CABI 2023). After ingesting infected food, the polyhedral dissolves in the midgut and releases many virions that destroy the epithelium cells and multiply in the nuclei.

The infected insect with Nucleopolyhedrovirus almost feeds upon only 7% of the food as compared with a healthy insect (Cruz*etal.*, 2018, Zakseski *et al*., 2021). The infected insect appears with yellowish skin and a liquified body. Before dying, infected. larvae climb to the highest point of the host plant, where they die in a hanged position (Cruz *et al*., 2018). After death, larvae appear with soft, darkish skin and rupture easily to release. polyhedrons, that helps in the spread of the virus (Cory 2015, Virto*et al*., 2014, Redman *et al*., 2016).

The aim of this study was to identify the Nucleopolyhedrovirus (NPV) infecting *S. frugiperda* larvae. Further, detailed molecular characterization studies are in progress.

MATERIALS AND METHODS

Extraction of Polyhedral Inclusion Bodies (PIBs) from NPV-Infected FAW larvae:

Spodoptera frugiperda Nucleopolyhedrosis virus *Sf*NPV infected larvae were collected during the survey, and brought to the laboratory.

For purification of the *S. frugiperda* Nucleopolyhedrosis virus (*Sf*NPV) from larvae the method of Tompkins (1991) was used. Frozen larvae were weighed, thawed and crushed in a mortar with the addition of an equal volume of 0.01 M Tris-buffer at PH 7.3-8. The macerate was filtered through two layers of muslin with the addition of more Tris-buffer if needed, then centrifuged at 10000 g for 10 min and resuspended in the appropriate carrier fluid (Tris-buffer). The suspension was pelleted and suspended in de-ionized water. The pellet was then taken up in 1 ml of distilled water and counted using a hemocytometer.

Insect Source and Rearing Conditions:

Spodoptera frugiperda larvae were reared in the laboratory. Newly hatched larvae were introduced to small pots containing the synthetic diet (Table 1) by using a soft hairbrush. Ten larvae were reared until reaching prepupae.

Well-developed pupae were sexed. Newly emerged moths were coupled in glass jars supplied with filter paper as a site for egg-laying, and 10 % sugar solution. All experiments were carried out under laboratory conditions of temperature 26 ± 2 ^{o}C and 60 ± 5% R.H.

Virus Propagation In The Larva:

The surface of synthetic diet was sprayed by the virus suspension, and the larvae were allowed to feed, then left until complete propagation of virus. The infected larvae were identified by their pale color and liquified body according to Hunter *et al.* (1990). These larvae were taken for virus purification and counting by hemocytometer.

The larvae were reared on the synthetic diet as described by Shorey and Hale, (1965) and modified by Mabrouk *et al.* (1996).

Table 1: Component of the fall armyworm synthetic diet**.**

Agar	9.9 g.
Formaldehyde	5% (1 ml./1diet).
White bean	5.9 g.
Sorbic acid	0.5 g.
Vitamin C	3.7 g.
Yeast	12.4 g.
Nepa gin	1.9 g.
Distilled water	500 ml.

Bioassay:

The synthetic diet used was similar to that used for the routine maintenance of larvae but without formaldehyde. It was poured into plastic cups in a layer of 5mm. A standard. volume (250µl) of virus solution was sprayed onto the diet. surface then left to dry. In the control treatment, distilled water replaced the virus suspension. Five concentrations ($1.8x10^{8}$, $1.8x10^{7}$,$1.8x10^{6}$, $1.8x10^{5}$ and $1.8x10^{4}$) PIB/ml were tested for the 2nd instar larvae were used. The larvae of each instar were held in groups containing 50 larvae each. Five replicates were used for each concentration, and allowed to feed on treated synthetic diet for 48 h. Five replicates of 50 larvae as control were fed on an untreated diet for 48 h. Mortality of larvae was recorded after 48h. then calculated using the Abbott formula. **(**Abbott, 1925**)** and subjected to probit. analysis according to Finney (1971) using "LdPLine®" software.

Scanning Electron Microscopy (SEM)of semi-purified polyhedral:

For scanning. electron microscopy (SEM), semi-purified OBs were spread. onto a piece of foil paper, dried naturally during the night, sputter-coated with gold, and observed with a scanning electron microscope at EM Unit, Faculty of Agriculture, Mansoura University, Egypt

Transmission Electron Microscopy (TEM) of infected larvae:

A newly moulted 2nd instar larva was reared on a contaminated diet, then fed on an untreated one until the disease symptoms. Infected larvae were anesthetized. and fixed in a wax plate and dissected. by cutting along their backs with an entomological. scissors. The gut was removed and stored in a fixative solution until processing**.** Preparation and ultra-thin sections were observed at 80 kV using a JEOLJEM-2100 at the EM Unit, Faculty of Agriculture, Mansoura. University, Egypt.

RESULTS

Morphological characteristics of infected larva:

Infected larva was collected during the survey. Diseased larvae of *S. frugiperda* were found to harbor the virus. Figure (1), shows typical characteristics of baculovirus infection, i.e. hanging downward the plant leaf and body swollen with apus-like discharge**.**

Fig. 1: Diseased larva of *Spodoptera frugiperda* showing characteristic viral infection symptoms**.**

Efficacy of the Nuclear Polyhedrosis Virus Isolate against 2nd Instar Larvae of *S. frugiperda:*

Efficacy of the *Spodoptera frugiperda* Nucleopolyhedrosis virus *Sf*NPV isolate against 2nd instars larvae of *S. frugiperda* was performed by testing five concentrations polyhedra from *Sf*NPV isolate (1.8×10^8, 1.8×10^7, 1.8×10^6, 1.8×10^5 and 1.8×10^4 PIB/ml). The data in Tables (2 and 3) and Figure (2), indicated that larval mortality increased by increasing the concentration of tested isolate. High concentrations of *Sf*NPV caused high mortalities; LC_{90} and LC_{50} for *Sf*NPV were $1.8x10^9$ and $1.8x10^5$ PIB/ml, respectively. The bioassay test revealed that *S. frugiperda* larvae exhibited disease symptoms during the first three days after exposure.

Table (2): Effectiveness of a Nucleopolyhedrovirus isolate against second instar larvae of *Spodoptera frugiperda*, assessed 10 days after treatment.

Concentrations PIB/ml*	Mortality %
$1.8x10^8$	81
$1.8x10^7$	75
$1.8x10^6$	62
$1.8x10^5$	50
$1.8x10^4$	41

*****PIB:Polyhedral inclusion body

Table 3: Vulnerability of Nucleopolyhedrovirus isolate against 2nd instar larvae of *Spodoptera frugiperda* 10 days post-treatment.

Virus isolates	LC_{90} (PIB/ml) *	LC_{50} (PIB/ml) *	Slope± SE
***Spodoptera frugiperda* Nucleo polyhedron virus (*Sf*NPV)**	$1.8x10^9$	$1.8x10^5$	0.2889 ± 0.0426

*****PIB:Polyhedral inclusion body

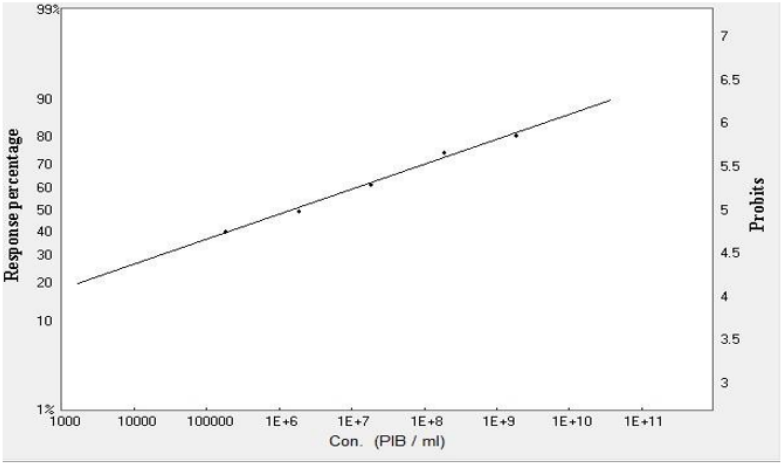

Fig. 2: Toxicity of nuclear polyhedrovirus isolate against 2nd instar larvae of *Spodoptera frugiperda* 10 days post treatment.

Scanning Electron Microscopic Studies:

The occlusion bodies (OBs) were isolated and propagated in the 2nd instar larvae fed on a contaminated synthetic diet. SEM shows a cluster of occlusion bodies (OBs) surrounded by infected cells (Fig. 3a, b, and c) OBs appear as crystalline structures of variable shapes and sizes (Fig. 3 c & d), With many enveloped virions packaged with either single or multiple nucleocapsids within an envelope (Fig. 3 a & b & c).

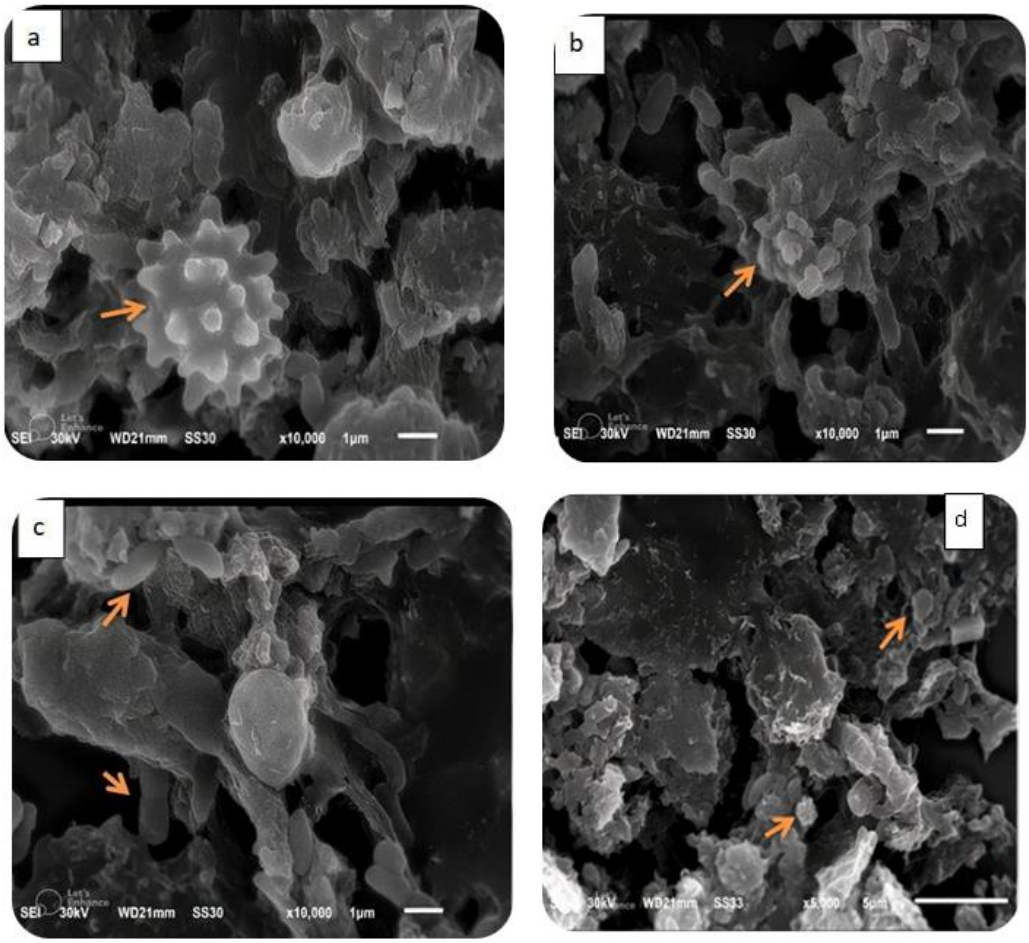

Fig. 3: Scanning photomicrographs of occlusion bodies of *Sf*NPV**.**

Transmission Electron Microscopic Studies.

-Midgut of Control Larva of *Spodoptera frugiperda:*

Control larvae showed epithelium with goblet cells (arrowhead) with well-developed microvilli (MV). Well-developed nucleus (thick arrow) at the apical region of the cell with few crumples of heterochromatin. (Fig. 4).

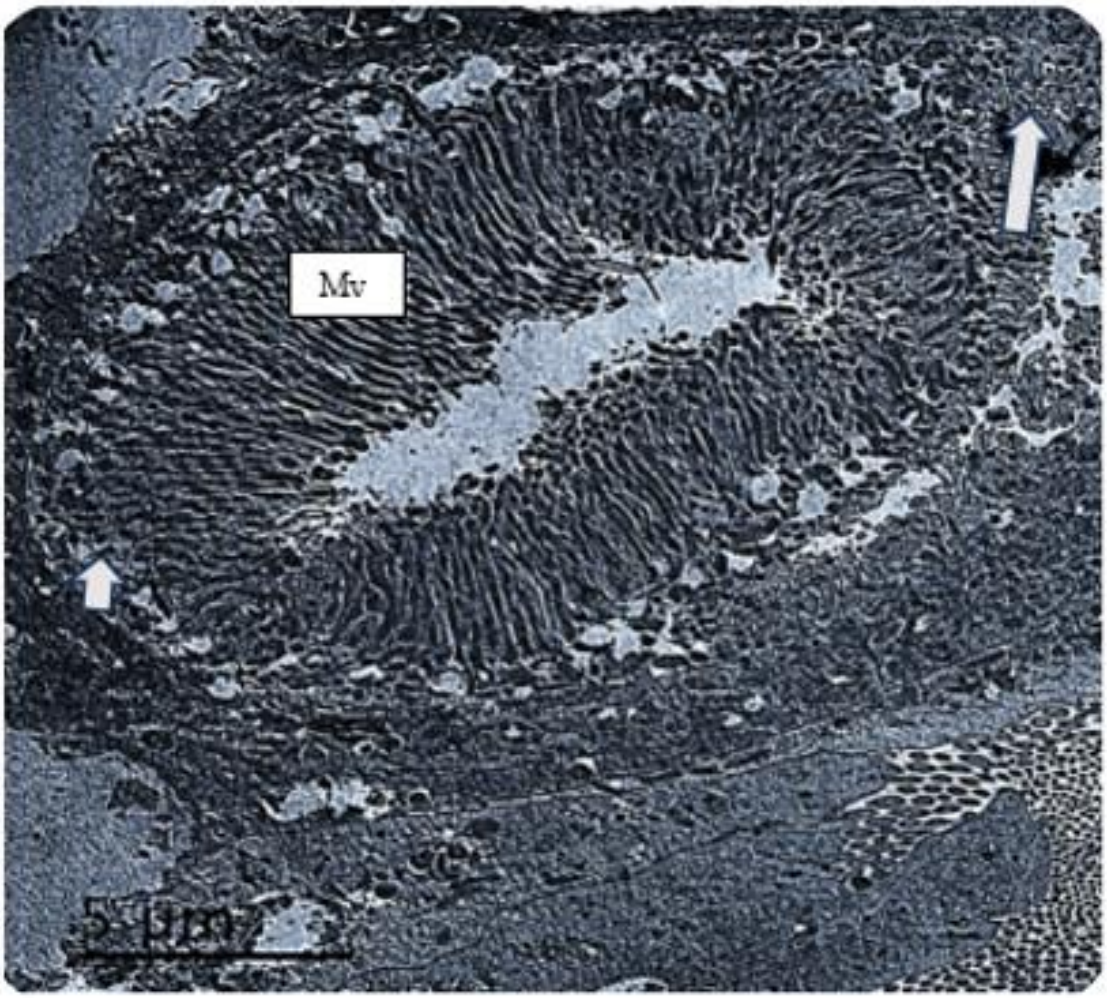

Fig. 4: Transmission electron micrograph of the midgut of *Spodoptera frugiperda* control larvae

-Midgut of the Larva of *Spodoptera frugiperda* infected with NPV Virus:

The gut of the infected larva was processed with the LC_{50} of *Sf*NPV and examined under the TEM. Early stages of infection showed degeneration of epithelial cells and detachment of microvilli (Fig. 5 a). Highly infected cells had enlarged nuclei and scattered chromatin (Fig. 5 b). Late stages of virus infection resulted in complete hydrolysis. of the epithelial gut wall and the formation of liquified gut content (Fig.5 c &d).

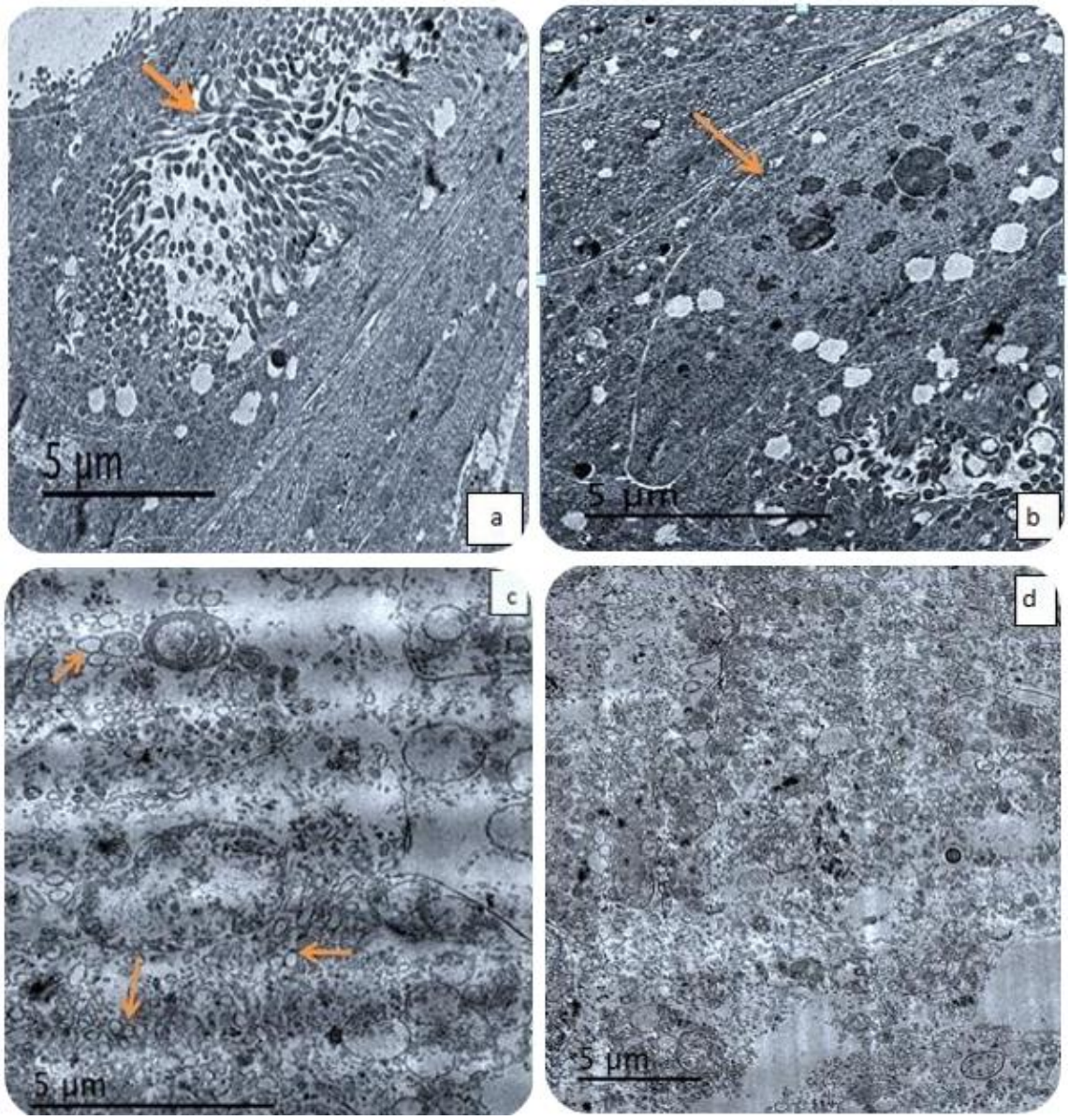

Fig. (5): Transmission electron micrograph of the midgut of *Spodoptera frugiperda larvae* infected with the nuclear polyhedrosis virus

DISCUSSION

Fall armyworm (FAW) is a very dangerous insect pest of maize in Asia and Africa, and it can be controlled using insecticides. Extensive usage of pesticides developed insect resistance. (Carvalho *et al.,* 2013 and Leonardo *et al.,* 2017). Virus infections were noticed on insect-infested areas in nature (Il'inykh and Ul'yanova 2005). These infections keep the pest populations below the economic range. (Coryand Myers2003).

NPV is characterized by its high epidemic levels and safety to natural enemies, so they could be effective biopesticides and suitable alternatives in *S. frugiperda* management. Significant researches have been done on the infectivity. of NPVs in lepidopteran pests**.** Raghunandan *et al.* (2019) reported. the natural infection of *Sf*NPV in Gujarat, while Firake and Behere (2020) reported it from Meghalaya. In this study, isolating and characterizing a NPV associated with FAW on maize has been succeeded.

Scanning microscopic studies showed the tetrahedral structure of *Sf*NPV occlusion bodies. Similar results of the shapes in OBs have been described in several NPVs (Rios Velasco *et al*., 2012; Kumar *et al.*,2015 and Hussain *et al*., 2019).

Natural occurrences of Nucleopolyhedrovirus (NPV) infected *S. frugiperda* larvae were found during the survey. This result was observed also by Il'inykh. and Ul'yanova (2005). These infections keep the populations under the economic range (Cory and Myers (2003).

We examined the isolate under scanning and electron microscope. Results of scanning microscopic examinations, showed occlusion bodies (OB) of NPV with irregular shape and size. Federici (1995), Harrison and Hoover (2012), reported that. the OB structures of NPVs are irregular in their morphological structures. In another, study the polyhedral of the Lymantria dispar MNPV-NM isolate were observed in irregular shape with the average diameter of 1.62 ± 0.33 lm.

Ishikawa *et al.* (1966) found. a mixture of nuclear inclusion bodies with different forms in one of. the infected larvae in Japan. Sridhar Kumar *et al.* (2011) reported OBs of three major Lepidopteran pests with multiple nucleocapsids in each envelope with bacilliform shapes. Thus, several studies on the insect pests of this group of viruses support the present. investigations.

The study reinforced the potential of *Spodoptera frugiperda* Nucleopolyhedrovirus (*Sf*NPV) as a control method for *S. frugiperda,* revealing that its efficacy increases by increasing the concentration particularly when applied against young larvae. These results align with previous studies indicating higher mortality rates in young larvae upon NPV exposure Duan and Otvos (2001), Pavan *et al.* (2024). Similar findings were recorded by El-Sabagh (2017). Gencer *et al*. (2022) studied the effect of *Helicoverpa armigera* NPV (Hear NPV) isolate on second-instar *Heliothis* larvae in Türkiye. Results showed that the virulence of the virus increases against young instars with LC50 of 1.9×10^4. In another study, the LC_{50} of NPV isolates tested against 2^{nd} instar *Helicoverpa armigera* (*H. armigera*) larvae were 2.3×10^4 OBs/ml (Kumar *et al.,* 2011). The LC_{50} of Here SNPV was 0.7×10^5 OBs/ml 3^{rd} instar *H. armigera* larvae. The decrease in mortality in late instars has been recorded in on *H. armigera* larvae (Eroglu *et al*., 2018). The utilization of NPVs presents a promising alternative for *S. frugiperda* management, particularly when focused on young larvae and employed at adequate concentrations.

The midgut acts as the main virus way of entry into the insect host (Blissard,1996). It is surrounded with a cuticle, which acts as a barrier to infection. So, the midgut is the primary way for virus entry (Sajjadian & Hosseininaveh 2015, Saxena *et al.*,2018). However, before interacting with the epithelial cells, virus must overcome two barriers: the digestive juices of midgut and the peritrophic membrane (Tatiana *et al*.1999). After infection of larva, the polyhedra are solubilized in the alkaline medium of the gut lumen (pH ranging from 10 to 12), and release occlusion derived virus (ODV) (Tatiana *et al.*,1999). The ODV penetrates the peritrophic membrane in order to get into the columnar epithelial cells (Ros 2020, Harrison *et al.*,2018 Volkman, 1990; Wang and Granados, 1998). Previous studies explained that proteins found in the occlusion body may be responsible for the disruption of the peritrophic membrane (Matos *et al.,* 1999).

Uncontrolled. cell division and the development of spherical cells with. a big nucleus that occupies most of the cell are two of the main. effects of epithelial cells. The lysosomes separated from the cells, displacing them with small, white. Vacuoles. Nucleus shrinkage, nuclear envelope disintegration, chromatin clumping, and ribosome accumulation were noted. These results align with those of Salama and Sharaby (1993) and Pandy *et al*. (2009). According to reports, the ability to digest food is decreased by

hypertrophy. and the displacement of epithelial cells from the basal lamina (Barbeta *et al.,* 2008). Lysed microvilli are totally detached from the cell. The same results were noted by Knaak and Fiuza (2005), who noticed that *Bacillus thuringiensis* and the nuclear polyhedrosis virus-treated *Anticarsia gemmatalis* cells had their microvilli disrupted. and their cells lysed.

Conclusion

Nucleopolyhedrovirus that infects fall armyworm, *S. frugiperda* was identified and examined under scanning microscope. The results showed. occlusion bodies of variable sizes and shapes. Un infected larvae fed with polyhedral inclusion bodies confirmed the pathogenicity of isolated virus. NPV is a safe biopesticide and can lead to significant reductions in pest populations, particularly when used in conjunction with other control methods

Future research could investigate the efficacy of NPVs against other pest species and explore potential synergistic effects when combined with other control methods. Additionally, evaluating the long-term effects of NPV applications on the ecosystem and non-target organisms is crucial for ensuring sound pest management system.

Declarations

Ethical Approval: Not applicable

Competing Interests: The authors declare no conflicts of interest.

Authors' Contributions: SB and ME were contribution in methodology, validation, investigation, resources and data curation, SB writing original draft preparation, ME and SB writing, review and editing.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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