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Natural occurrence of nucleopolyhedrovirus infecting fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in Gujarat, India

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Abstract

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a new invasive pest to India. It is a notorious pestiferous insect with high dispersal ability, wide host range and high fecundity. The aim of this study is to identify the natural enemies of fall armyworm infesting maize. Nucleopolyhedrovirus (NPV) infected *S. frugiperda* larvae were found during the survey. More number of NPV infected larvae were found and collected at location 'Kanisa' (22°23'2" N, 72°41'13" E), Anand district, Gujarat. The association of NPV with *S. frugiperda* was confirmed by phase contrast microscopy and pathogenicity studies. Further, the detailed bioassay and electron microscopic studies are under progress.

Keywords: Fall armyworm, maize, nucleopolyhedrovirus, polyhedra

1. Introduction

Maize (*Zea mays* L.) is one of the important cereal crops next to wheat and rice in the world. A new invasive pest fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is recently invaded to India, reported in Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Tamil Nadu, Telangana states infesting the maize crop [8, 9]. It is native to the tropical and subtropical region of America, where it is a serious pest of corn but also known to attack more than 100 hosts. It is reported to cause major damage to economically important cultivated grasses such as rice, sorghum and sugarcane as well as horticultural crops like cabbage, beet, tomato, potato and onion besides cotton, pasture grasses, peanut, soybean, alfalfa and millets [2]. It is a notorious pestiferous insect with high dispersal ability, wide host range and high fecundity that make it one of the most severe economic pests. *S. frugiperda* can cause corn yield loss as much as 70% of whole production [1].

This pest has been the subject of many studies, but most of them focus on chemical control. To date, no plant protection program has included biological control as its cornerstone. For eco-friendly management of many insect pests, biocontrol agents are decisive components which include the natural enemies viz., predators, parasitoids and insect pathogens (fungi, bacteria, virus, protozoa and nematodes). Microbial based biopesticides offer a sustainable and cost effective management of many insect pests. Nucleo Polyhedron Viruses (NPV) are the most studied baculovirus group and commercially exploited for insect pest control [4]. NPV infect a wide range of insect pests and cause lethal epizootics in susceptible species. These are host specific pathogens and do not harm other plants and animals. Polyhedral inclusion bodies (PIBs) are produced in virus infected larvae and cause highly infectious lethal disease. The PIBs ingested by the susceptible host infect the gut cells of the larva and it spreads to other tissues of the body. An infected larva can be killed within 4 days [3]. NPV are commonly isolated from infected insects collected from fields and being visible with light microscopy can be easily detected.

Survey and surveillance programs were carried out to identify the natural enemies of fall armyworm infesting maize in Anand district of Gujarat. NPV infected larvae were found in few locations during the survey. The association of NPV with *S. frugiperda* was confirmed by phase contrast microscopy and pathogenicity studies. Further, the bioassay and electron microscopic studies are under progress.

2. Materials and Methods

Collection and extraction of polyhedral inclusion bodies (PIBs)

NPV infected larvae were found during the survey (Table 1). Collected samples were brought to the laboratory and stored at -20 °C. The infected larvae were dissected and wet smears were examined under phase contrast microscope to detect PIBs. The occlusion bodies (OBs) were isolated by following the standard methodology [4] with few modifications. The diseased larva was homogenized using a sterile pestle and mortar using 5 ml of sterile distilled water and vortexed for 2 minutes. The suspension was filtered twice through a double layered muslin cloth and then the filtrate was centrifuged at 500 rpm for 2 minutes to remove the larger particles. The supernatant was carefully transferred and centrifuged at 5000 rpm for 20 minutes to collect the pellet containing polyhedra. The pellet containing polyhedra was re-suspended in sterile distilled water (5 ml) and stored at 4 °C. The polyhedral suspension was observed under phase contrast microscope (400x) and OBs were counted using a haemocytometer.

To examine the pathogenicity of extracted OBs, the suspension was fed to healthy larvae of *S. frugiperda*. Viral

suspension of 10^8 OBs/ml was prepared and 25 microliters of viral suspension was spread on maize leaf discs (4 cm²), air dried and placed inside a small plastic container (7x5 cm). Healthy larvae (10 No.) of 2nd-3rd instar (5 No.) & 3rd-4th instar (5 No.) collected from the field were released individually into the containers which were then covered with a lid. The larvae consumed the diet were transferred to plastic container containing fresh maize leaves and maintained at 26 ± 2 °C and 60-70% relative humidity. In control treatment larvae were allowed to feed on leaves treated with sterile distilled water. Larvae were observed for viral infection and mortality up to 8 days post inoculation.

3. Results and Discussion

The majority of dead larvae of *S. frugiperda* suspected of viral infection hang on maize leaves with abdominal prolegs (Fig. 1) and collected diseased larvae were found to harbour the virus. Observations of discharged body fluid and haemolymph under a phase contrast microscope revealed the large number of spherical PIBs (2.04 ± 0.22 µm) formed by virus (Fig. 2 & Fig. 5).

Table 1: Details of location and crop where NPV infected larvae of *Spodoptera frugiperda* were collected.

Sl. No.	Place	GPS co-ordinates	Crop and its stage	Date of collection	Number of infected larvae collected
1	Kanisa (Khambhat tehsil)	22°23'2'' N 72°41'13'' E	Fodder maize (Local variety), vegetative stage (35 DAS), 0.2 ha	19/03/2019	122
				27/03/2019	186
				02/04/2019	26
2	Sandesar (Anand tehsil)	22°31'7'' N 72°52'47'' E	Fodder maize (Local variety), vegetative stage (25 DAS), 0.15 ha	02/04/2019	2
3	Sihol (Petlad tehsil)	22°30'53'' N 72°52'22'' E	Fodder maize (Local variety), vegetative stage (20 DAS), 0.2 ha	02/04/2019	6

Note: DAS – Days after sowing,

At 48 h post inoculation larvae were found less active with reduced feeding. Mortality was recorded in younger stage larvae (3 No.) on the third day after treatment. Larvae turned pale pinkish and body fluid was discharged during late instars (Fig.3). Larval-pupal intermediates (3 No.) and deformed pupa (1 No.) which then degraded gradually were observed (Fig. 4a & 4b). Adults (2 female, 1 male) were emerged from

the pupae and no egg mass was recorded. Orange-brownish liquid discharge was noticed from the emerged adults and microscopic observation of the same revealed the numerous spherical PIBs (Fig. 6a & 6b). Several workers have documented similar observations on NPV infected *S. frugiperda* infesting maize [5, 6, 7, 10].



Fig 1: Nucleopolyhedrovirus (NPV) infected larvae of fall armyworm, *Spodoptera frugiperda* infesting maize

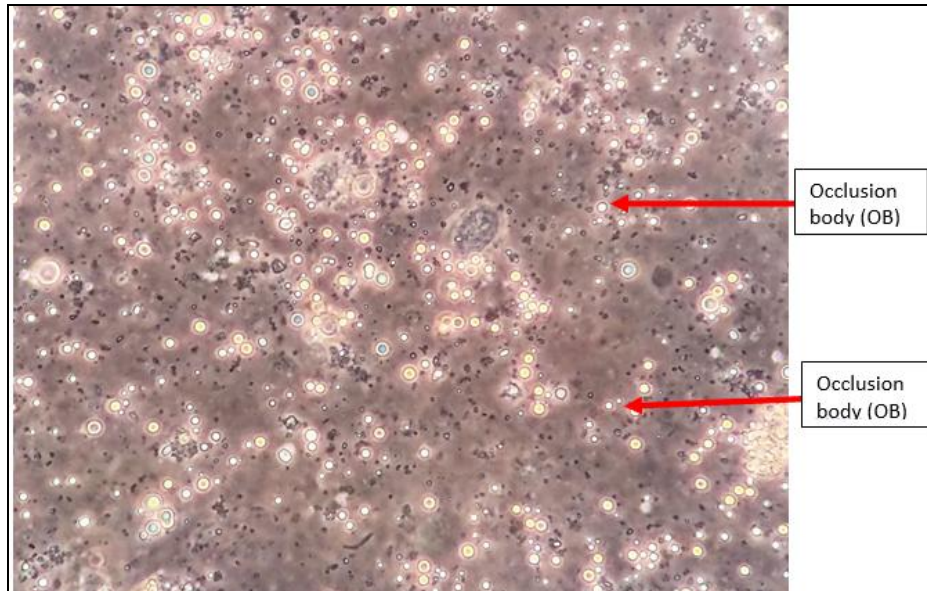


Fig 2: Phase contrast micrograph (400x) of discharge body fluid obtained from infected larvae showing occlusion bodies



Fig 3: Pinkish discoloration and discharge of body fluid* from NPV infected larvae (* Indicated in arrow)



Fig 4a: Larval-Pupal intermediates due to NPV infection



Fig 4b: Deformed pupa due to NPV infection

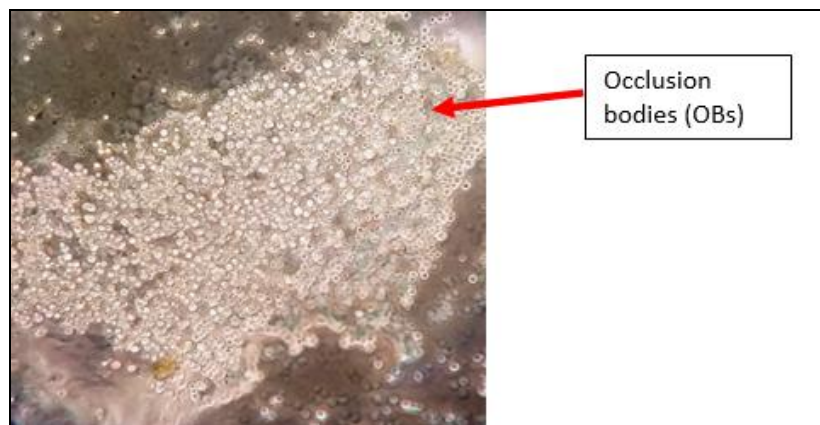


Fig 5: Phase contrast micrograph (400x) of occlusion bodies (OBs) extracted from diseased larva



Fig 6a: Orange-brownish liquid discharge from emerged adults infected with NPV

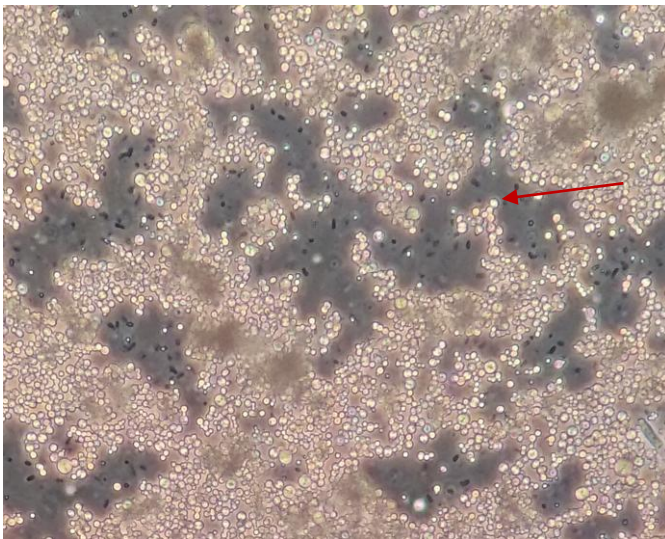


Fig 6b: Phase contrast micrograph (400x) of orange-brownish liquid discharge showing OBs (indicated in arrow)

4. Conclusion

Nucleopolyhedro virus infecting fall armyworm, *S. frugiperda* was identified, which showed typical refractile, spherical occlusion bodies of variable sizes under phase contrast microscope. Healthy larvae fed with polyhedral suspension confirmed the pathogenicity of extracted virus. Further, the bioassay and characterization of OBs by electron microscopy are under progress. NPV is safe biopesticide and it could be an ideal component for integrated management of fall armyworm which is a new invasive pest and major threat to food security.

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6. Conflicts of Interest

Authors declare that there is no conflict of interest

7. References

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