Eco. Env. & Cons. 30 (July Suppl. Issue) : 2024; pp. (S328-S332)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i04s.056

Natural occurrence of Nucleopolyhedrovirus infecting fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Noctuidae: Lepidoptera) in Jammu and Kashmir, India

Shafiya Rashid[1]*, Rakesh Kumar Gupta[1], R.S. Bandral[1], Kamlesh Bali[2], S.K. Singh[3], Susheel Sharma[4] and Kamal Kishor Sood[5]

[1]Division of Entomology, [2]Division of Sericulture, [3]Division of Plant Pathology, [4]School of Biotechnology, [5]Division of Agroforestry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, India

(Received 15 December, 2023; Accepted 23 January, 2024)

ABSTRACT

In order to manage the invasive pest the fall armyworm, *Spodoptera frugiperda* in north India, the survey was carried out for the presence of NPV infected *Spodoptera frugiperda* larvae. The NPV infected larvae were collected from different locations, the virus was confirmed by phase contrast microscopy and pathogenicity studies. Healthy larvae fed with polyhedral suspension confirmed the pathogenicity of extracted virus. The NPV isolates exhibited significantly different degree of pathogenicity against the host population, where Jammu isolate caused highest mortality (92%) with the LD_{50} value of 1.01×10^5 POBs/larva against 2nd instar larvae. while only 31.33 and 19.33% mortality was inflicted by Rajouri and Akhnoor isolates, respectively. The concentration needed to induce a lethal infection by Rajouri and Akhnoor was exceptionally high, making it challenging to pinpoint a specific concentration that would be lethal for half of the population.

Key words: *Spodoptera frugiperda, Nucleopolyhedrovirus (NPV), Phase contrast microscopy, Mortality, LD_{50}*

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is an important and widely distributed pest in tropical and sub-tropical areas of the Americas. Recent studies have demonstrated the continuous spreading of FAW (Goergen *et al.*, 2016). The fall armyworm invaded India, reported in Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Tamil Nadu in 2018 (Shylesha *et al.*, 2018; Sisodiya *et al.*, 2018) and was found in a severe form in maize fields of North East (NE) India after March 2019 (Firake *et al.*, 2019). 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.

Growing concerns about the environmental and human health impacts, coupled with the development of insecticide resistance, have spurred increased efforts to explore economically and ecologically viable alternatives for managing invasive pests like FAW. Therefore, natural biocontrol agents, particularly native strains or populations, have emerged as promising alternatives. Since baculoviruses exhibit high insecticidal activity and a narrow spectrum of hosts. Entomopathogenic viruses, primarily from the Baculoviridae family or baculovirus (BV), present a viable option for FAW control. The initial stage of infection occurs when occlusion bodies (OBs) are orally ingested by susceptible lepidopteran larvae. The gastrointestinal tract serves as the focal point for baculovirus infection.

Following ingestion, the extreme alkaline pH of the midgut triggers the degradation of the OB, leading to the release of ocular-derived virions (ODVs). These ODVs play a crucial role in the primary infection of midgut epithelial cells. They traverse the peritrophic membrane (PM) and disseminate to other body tissues, releasing nucleocapsids. The cell nucleus then becomes the epicentre for replication. In cases of infection, an afflicted larva can succumb within a relatively short period, typically ranging from 4 to 7 days (Barrera *et al*., 2011). Nucleopolyhedro viruses (NPV) are frequently isolated from infected insects gathered from fields, and their visibility under light microscopy facilitates easy detection.

During the course of the present study in maize fields, the survey was carried out for the presence of NPV infected *Spodoptera frugiperda* larvae. The NPV infected larvae were collected from different locations and the association of the NPV with *Spodoptera frugiperda* was confirmed by phase contrast microscopy and pathogenicity studies. Also, a novel virulent isolate of NPV associated with *S. frugiperda* has been characterized.

Materials and Methods

Isolation and purification of Polyhedral Occlusion Bodies (POBs)

During May-August, 2021 and may- August 2022, intensive exploratory surveys were conducted in different regions of Jammu *viz*., Jammu, Kathua, Akhnoor and Rajouri from the maize fields, for the presence of native NPV isolates of *Spodoptera frugiperda*, from naturally infected caterpillars. Both live and dead larvae showing typical symptoms of NPV infection were collected individually in the sterile glass vials and capped with ventilation lids to allow exchange of gases and prevent moisture condensation. These infected larvae were then stored at -20°C until the isolation of Polyhedral Occlusion Bodies POBs. The infected larvae were dissected and wet smears were examined under phase contrast microscope to detect POBs. To fulfil Koch's postulates for experimental investigations, the causal organisms were reisolated after passing through susceptible healthy individuals. The percent infection was calculated by using the following formula:

$$\frac{\text{No of infected larvae}}{\text{Total no. of larvae (Healthy + infected)}} \times 100$$

Virus killed individual larvae were kept in 1ml micro-tube in 0.1% sodium dodecyl sulphate (SDS), overnight at 4°C, for obtaining the crude viral occlusion bodies. The purification of Polyhedral Occlusion Bodies (POBs) followed the method outlined by Gupta *et al*. (2007). Each larva was gently squashed with a micro-pestle and vortexed for 10 seconds in a vortex shaker. The homogenate was then filtered through three layers of muslin cloth and centrifuged at 500 rpm for 1 minute. The pellet formed by the insect debris was discarded, and the supernatant was carefully transferred to a clean tube, followed by further centrifuged at 2500 rpm for 5 minutes. After discarding the supernatant, the pellet was resuspended in 1ml distilled water by vortexing. This washing process, repeated 3-4 times, continued until a clear, white colour pellet was obtained. The virus pellet was then resuspended in 0.75 ml of distilled water and stored in aliquots at -20°C for further studies.

Dose response bioassays: evaluation of the most potent virus isolate

Considering that native isolates act as permanent regulators of insect-pest population density, there is a significant risk of developing resistance by the local population against these native isolates. Hence, evaluating the virulence of the virulent NPV strain is crucial for the development of effective viral insecticides. This evaluation is of vital importance for eco-friendly management of *Spodoptera frugiperda* in various cropping systems in Jammu. In the experiment, laboratory-reared host larvae were assessed through per os bioassay. These assessments help gauge the efficacy and potential impact of the virulent NPV strain on the target insect population.

Different virus isolates were bioassayed on 2nd instar larvae with seven different doses of NPV *viz*., 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 POBs/ larva (Plate 5) via leaf disc cut from fresh maize leaves each of 1.5 cm diameter (Plate 6). To achieve the desired doses, serial dilutions with distilled water were conducted. Using a blunt end of a glass rod, 10 µl aliquots of each viral dose from each viral isolate were spread on individual diet plugs. The diet plugs were air-dried and then placed individually in a compartmentalized acrylic insect rearing system designed by the National Bureau of Agricultural Insect Resources (NBAIR) in Bangalore, India. One larva was then placed in each compartment measuring 2×2 cm. Larvae that consumed the

entire diet plug within 24 hours were transferred to fresh uncontaminated diet and reared at 26±1°C, 16:8 (L:D) h, and 85% RH. Larvae that did not consume the entire plug were discarded. Control larvae were inoculated with distilled water. Virus induced mortality was recorded daily from first day to the fifteenth day after inoculation till pupation. Larval mortality was analyzed using Probit analysis to estimate the median lethal dose (LD_{50}) values.

Results

The findings indicated that only 2-3% of larvae were infected with the disease during the survey. In natural field conditions, most of the *Spodoptera frugiperda* larval population did not exhibit signs of viral disease. However, when transferred to the laboratory for monitoring and rearing, they succumbed to the virus, displaying characteristic symptoms. Microscopic examination using Giemsa staining of haemolymph and tissue smears from *Spodoptera frugiperda* larvae exhibiting typical symptoms revealed the presence of POBs as observed under phase contrast microscope at × 400 magnifications (Figure 1). The natural incidence of NPV in *Spodoptera frugiperda* larvae exhibited a variation ranging from 0.78% to 6.07%. The highest incidence, at 6.07%, was observed on maize crops in Jammu, while the lowest incidence of 0.78% was recorded in Akhnoor. Rajouri showed a natural infection rate of 1.98% by NPV larvae. Notably, no natural incidence of NPV was observed in Kathua (Table 1).

Table 1. Natural incidence of NPV in *Spodoptera frugiperda* larvae collected from different zones of J&K

Location	No. of Larvae collected	NPV infected larvae	Percent NPV infection
Jammu	379	23	6.07
Akhnoor	257	3	0.78
Rajouri	352	7	1.98
Kathua	367	0	0

The virus isolates obtained from different locations when tested under laboratory conditions caused significantly different mortalities in the host larvae. The larvae died with symptoms of NPV disease, including flaccidity and rupturing of the cuticle, larval pupal intermediates and deformed pupae which then degraded gradually were observed

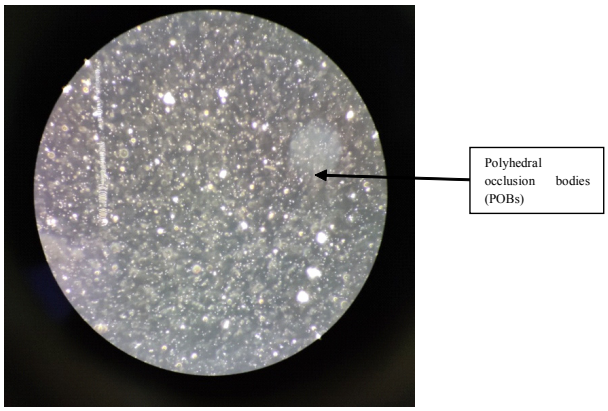

Fig. 1. Polyhedral occlusion bodies (POBs) under phase contrast microscope at 400X

(Figure 2). The infected larvae climbed to the top of the vials in which they were kept prior to death.

The variation in mortality caused by different isolates was taken as a measure for the strain selection. The mortality of *Spodoptera frugiperda* larvae caused by different isolates with seven different doses of 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 POBs/ larva via leaf disc method ranged from 92-19%, with significant differences between the doses (Jammu, F=14.17; df=7,16; P= 0.00; Akhnoor, F=133.61; df=7,16; P= 0.00; Rajouri, F=82.91; df=7,16; P= 0.00). No mortality was observed in controls and Jammu virus isolate inflicted highest mortality of 92% at a dose of 1×10^8 POBs/ larva, however, only 31.33 and 19.33% mortality was inflicted by Rajouri and Akhnoor isolates at the same dose, respectively. It was evident that the percent mortality was highest in larva inoculated with Jammu virus isolate followed by Rajouri and Akhnoor, the difference being significant between the different isolates ($\div^2$= 9.85, df= 2, p=0.007). (Table 2)

Probit analysis of mortality data of *Spodoptera frugiperda* larvae showed median lethal dose (LD_{50}) estimate of 1.01×10^5 POBs/larva against Jammu isolate (Table 3). The effect of dose on the mortality of *Spodoptera frugiperda* larvae varied upto the magnitude of 39%. The concentration required to elicit a lethal infection by Rajouri and Akhnoor isolates was so high that a reliable estimate of LD_{50} could not be obtained.

Discussion

The presence of variants exhibiting diverse biological activities could have significant implications for

Fig. 2. NPV infected larva

the advancement of biopesticides, offering the potential to selectively choose more effective naturally occurring strains. The documentation of *Spodoptera frugiperda* nucleopolyhedrovirus (SpfrNPV) is extensive, with the virus being previously identified in Gujarat, northeast India, and south India (Raghunandan *et al*., 2019; Gunda *et al*., 2020; and Sivakumar *et al*., 2021). Several workers have documented similar observations on NPV infected *S. frugiperda* infesting maize (Velasco *et al*., 2012; Vieir, *et al*., 2012). Nonetheless, this study has unequivocally established, for the first time, the presence of NPVs in natural populations of *Spodoptera frugiperda* in Jammu. Despite this, the natural incidence was found to be relatively low, ranging from 0.78% to 6.07%. The variation in natural NPV incidence suggests that diverse agro-climatic conditions may have played a crucial role in the occurrence of NPV in nature. The recorded NPV epizootics in natural conditions have been linked to stress factors such as high population density, aligning with the findings of Gupta *et al.* (2016), which highlighted the density-dependent nature of baculovirus prevalence. Fluctuations in population density are attributed to temperature variations, with the warm and humid summers in Jammu possibly contributing to the outbreak of *Spodoptera frugiperda*, consistent with the conclusions of Nivetha *et al.* (2022) and Anandhi *et al.* (2020), who suggested that weather influences population density fluctuations.

Table 2. Mortality of *Spodoptera frugiperda* larva with different virus isolates of Spfr NPV in dose response bioassay, inoculated via leaf disc method

Isolate	Dose (POBs/larva)	No. of larva per replicate	Larval mortality due to NPV(Mean ±SE)	Percent larval mortality
Jammu	control	50	0.00^{a}	0.00^{a}
	1×10^{2}	50	4.00 ± 0.58^{b}	8.00 ± 1.15^{b}
	1×10^{3}	50	9.33 ± 0.33^{c}	18.67 ± 0.67^{c}
	1×10^{4}	50	16.67 ± 0.33^{d}	33.33 ± 0.67^{d}
	1×10^{5}	50	24.33 ± 0.67^{e}	48.67 ± 1.33^{e}
	1×10^{6}	50	33.67 ± 0.33^{f}	67.33 ± 0.67^{f}
	1×10^{7}	50	40.33 ± 0.67^{g}	80.67 ± 1.33^{h}
	1×10^{8}	50	46.33 ± 0.33^{h}	92.67 ± 0.67^{h}
Rajouri	control	50	0.00^{a}	0.00^{a}
	1×10^{2}	50	0.00^{a}	0.00^{a}
	1×10^{3}	50	1.00 ± 0.58^{ab}	2.00 ± 1.15^{ab}
	1×10^{4}	50	2.33 ± 0.33^{ab}	4.67 ± 0.67^{ab}
	1×10^{5}	50	3.33 ± 0.67^{b}	6.67 ± 1.33^{b}
	1×10^{6}	50	10.33 ± 0.67^{c}	13.33 ± 0.67^{c}
	1×10^{7}	50	15.67 ± 0.67^{d}	20.67 ± 1.33^{d}
	1×10^{8}	50	6.67 ± 0.67^{e}	31.33 ± 1.33^{e}
Akhnoor	control	50	0.00^{a}	0.00^{a}
	1×10^{2}	50	0.00^{a}	0.00^{a}
	1×10^{3}	50	0.33 ± 0.33^{a}	0.67 ± 0.67^{a}
	1×10^{4}	50	1.00 ± 0.58^{ab}	2.00 ± 1.15^{ab}
	1×10^{5}	50	$2.33\pm0.0.33^{bc}$	4.67 ± 0.67^{bc}
	1×10^{6}	50	3.00 ± 0.58^{c}	6.00 ± 1.15^{c}
	1×10^{7}	50	5.67 ± 0.33^{d}	11.33 ± 0.67^{d}
	1×10^{8}	50	$9.67\pm0.0.33^{e}$	19.33 ± 0.67^{e}

Mean ±SE followed by different letters with the same column within the same larval instar are significant at $p<0.05$; SE= Standard error of mean; Control= Distilled water

Table 3. Median lethal dose (LD_{50}) with 95 per cent confident limits, regression equation and correlation determination of different virus isolates of SpfrNPV in dose response bioassay, inoculated via leaf disc method

Isolate	LD_{50} (POBs/larvae)	95% Confidence interval	χ^2/df	Regression equation y= bx +a	R^2
Jammu	1.01×10^5	65233.07-157441.99	2.85/22	3.09x+1.54	0.39

Regression equation; Y= mortality percentage and X= dose
R^2 is a correlation determination between the dose and mortality value

Conclusion

The exploratory survey conducted for the isolation of local strain of *Spodoptera frugiperda* led to the discovery of only three virus strains from the four different locations of Jammu. Three baculovirus isolates from wild *Spodoptera frugiperda* larvae exhibiting virus symptoms, which showed typical refractile, spherical occlusion bodies of variable sizes under phase contrast microscope were recovered in the present study. Healthy larvae fed with polyhedral suspension confirmed the pathogenicity of extracted virus. The NPV isolates exhibited significantly different degree of pathogenicity against the host population, where Jammu isolate caused highest mortality (92%) while only 31.33 and 19.33% mortality was inflicted by Rajouri and Akhnoor isolates, respectively. Thus NPV can serve as a 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.

Conflict of Interest: None

References

Anandhi, S., Saminathan, V. R., Yasotha, P., Sharavanan, P. T. and Rajanbabu, V. 2020. Seasonal dynamics and spatial distribution of fall armyworm *Spodoptera frugiperda* (JE Smith) on Maize (*Zea mays* L.) in Cauvery Delta Zone. *Journal of Pharmacognosy and Phytochemistry*. 9(4): 978-982.

Barrera, G., Simón, O., Villamizar, L., Williams, T. and Caballero, P. 2011. *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: Genetic and phenotypic comparison of field isolates from Colombia. *Biological Control.* 58(2): 113-120.

Firake, D., Behere, G., Babu, S. and Prakash, N. 2019. Fall Armyworm: Diagnosis and Management. *An Extension Pocket Book. Umiam-*793: 103.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. and Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PloS One.* 11(10): 165632.

Gunda, G.K.T., Champatiray, P., Chauhan, M. and Chauhan, P. 2020. Occurrence of Nuclear Polyhedrosis Virus of invasive fall armyworm, *Spodoptera frugiperda* (JE Smith) in Meghalaya, North East India. *Current Science*. 118(12): 1876.

Gupta, R.K., Gani, M., Kaul, V., Bhagat, R.M., Bali, K. and Samnotra, R.K. 2016. Field evaluation of *Lymantria obfuscata* multiple nucleopolyhedrovirus for the management of Indian gypsy moth in Jammu & Kashmir, India. *Crop Protection*. 80: 149-158.

Gupta, R.K., Raina, J.C. and Monobrullah, M.D. 2007. Optimization of *in vivo* production of nucleopolyhedro virus in homologus host larvae of *Helicoverpa armigera*. *Journal of Entomology*. 4: 279-288.

Nivetha, T. K., Srinivasan, G., Shanthi, M., Gurusamy, A. and Vellaikumar, S. 2022. Seasonal incidence and influence of weather factors on the incidence of *Spodoptera frugiperda* (JE Smith) on maize. *The Pharma Innovation Journal*. 11(6): 407-410.

Raghunandan, B.L., Patel, N.M., Dave, H.J. and Mehta, D.M. 2019. Natural occurrence of nucleopolyhedro virus infecting fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) in Gujarat, India. *Journal of Entomology and Zoology Studies*. 7(2): 1040-1043.

Shylesha, A. N., Jalali, S. K., Gupta, A. N. K. I. T. A., Varshney, R. I. C. H. A., Venkatesan, T., Shetty, P. R. A. D. E. E. K. S. H. A. and Raghavendra, A. 2018. Studies on new invasive pest Spodoptera frugiperda (JE Smith)(Lepidoptera: Noctuidae) and its natural enemies. *Journal of Biological Control*. 32(3): 1-7.

Sisodiya, D.B., Raghunandan, B.L., Bhatt, N.A., Verma, H.S., Shewale, C.P., Timbadiya, B.G. and Borad, P. K. 2018. The fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae); first report of new invasive pest in maize fields of Gujarat, India. *Journal of Entomology and Zoology Studies*. 6(5): 2089-2091.

Sivakumar, G., Kannan, M., Babu, S. R., Mohan, M., Kumar, M. S., Raveendran, P. and Kumar, P. R. 2020. Isolation and characterization of indigenous nucleopolyhedrovirus infecting fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in India. *Current Science*. 119(5): 860-864.

Vieira, C.M., Tuelher, E.S., Valicente, F.H. and Wolff, J.L.C. 2012. Characterization of a *Spodoptera frugiperda* multiple nucleopolyhedrovirus isolate that does not liquefy the integument of infected larvae. *Journal of Invertebrate Pathology*. 111(2): 189-192.