Indian Journal of Entomology Online published Ref. No. e24873 DoI. No.: 10.55446/IJE.2024.1873

REPORT OF *MARUCA VITRATA* (F) NUCLEOPOLYHEDROVIRUS

J S Pavan[1], R D Dodiya[2], M N Rudra Gouda[1]*, B L Raghunandan[3], Nainesh B Patel[3] and C N Rajarushi[1]

[1]Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[2]Department of Entomology, B A College of Agriculture;
[3]AICRP on Biological Control of Crop Pests, Anand Agricultural University, Anand 388110, Gujarat, India
*Email: rudragoudamn@gmail.com (corresponding author): ORCID ID 0000-0002-1644-4226

ABSTRACT

This study explores the potential of nucleopolyhedroviruses (NPVs) as a sustainable solution for managing legume pod borer *Maruca vitrata* (F). Distinctive signs of viral infection, such as suspended deceased larvae on cowpea leaves, and virus spread have been observed. Microscopic examination reveals characteristic polyhedral inclusion bodies (PIBs), confirming MaviNPV as the causative agent. Symptoms, including decreased activity and mortality in younger larvae, larval-pupal intermediates and deformed pupae have been observed. Reproductive disruption is evident through the absence of an egg mass and discharge of fluid from emerged adults. Bioassay results highlight age-dependent susceptibility, with higher mortality in early instars.

Key words: *Maruca vitrata*, baculovirus, MaviNPV, pest management, biological control, larval mortality, viral pathogenicity, bioassay, polyhedral inclusion bodies (PIBs)

The crambid moth *Maruca vitrata* (F), known as the spotted pod borer or legume pod borer is a highly destructive affecting grain legumes (Agunbiade et al., 2017). Its likely origin is the Indo-Malaysian region, and its distribution encompasses South and East Asia, sub-Saharan Africa, Oceania, and Central America, including the Caribbean islands (Margam et al., 2011). This pest attacks various cultivated legumes, including *Cajanus cajan* (pigeon pea), *Vigna unguiculata* subsp. *unguiculata* (cowpea), *V. radiata* (green gram), *Phaseolus lunatus* (lima bean), and *Glycine max* (soybean) (Agunbiade et al., 2017). The larvae pose a substantial threat by feeding on tender leaf axils, flowering inflorescence, and pods, creating distinctive webbings or clusters (Sharma, 1998). This concealed feeding presents a challenge to management practices, as the webbed mass protects the larvae from natural enemies and reduces the efficacy of insecticides. In India, *M. vitrata* has been reported to cause significant yield losses of up to 84% in early pigeon pea crop (Chakravarty et al., 2017; Chatterjee et al., 2019). Currently, the primary method of controlling *M. vitrata* involves the use of chemical insecticides, as no *Maruca*-resistant varieties are available (Srinivasan et al., 2021). Frequent application of insecticides has led to increased resistance contributing to outbreaks (Mahalle and Taggar, 2018) and other adverse impacts (Lazaro et al., 1995; Ekesi, 1999. In response to these challenges, insect pathogenic viruses, such as granulovirus (GV) and cypovirus (CPV), have been explored as potential alternatives. Field observations in Kenya, Benin, and China had reported GV and CPV infections (Otieno et al., 1983). However, the weak dose-response relationship for CPV, attributed to its chronic nature, suggests limited effectiveness against boring insects (Tamo and Cherry, 1999). Nucleopolyhedroviruses (NPVs) have virulent pathogenicity making them promising candidates. While *Galleria mellonella* nucleopolyhedrovirus (GmNPV) has shown high infectivity to *M. vitrata* (Parthasarathy et al., 2004), there is a lack of reports on NPVs isolated directly. A survey spanning from 2021 to 2023 was conducted to investigate the natural infestation of microbial agents on *M. vitrata* in Gujarat and Haryana. These revealed the presence of diseased larvae on cowpea, with characteristic signs of NPV infection, including lethargy, a distinct pinkish hue, cessation of feeding, and fragile, often ruptured larval bodies. Notably, deceased larvae were observed hanging from plant tops with attached prolegs, as previously documented by Srinivasan et al. (2005).

MATERIALS AND METHODS

During the survey, polyhedral inclusion bodies (PIBs) in NPV-infected larvae were identified and subsequently collected for analysis from various locations namely Sihol (22º30’53’’ N 72º52’22’’

E), Dholi (22º49'53.8'' N 73º45'43.0'' E), Chitva (23º17'22.3'' N 73º52'14.8'' E), Rewari (28º22'73.8'' N 76º65'83'' E), Jhajjar (28º57'25.2'' N 76º65'83.3'' E), Mahendragarh (28º27'.51.4'' N 76º16'96.6'' E). The gathered specimens were stored at -20°C. The infected larvae underwent dissection, and wet smears were scrutinized under a phase-contrast microscope to detect the presence of PIBs. The isolation of occlusion bodies (OBs) followed a standardized methodology with slight modifications (Hussain et al., 2019). To assess the pathogenicity of the extracted OBs, a viral suspension of 10^8 OBs/ ml was prepared and applied to cowpea leaf discs (4 cm^2). These discs, after air-drying, were placed in small plastic containers (7x5 cm). Healthy larvae (10 individuals) collected from the field, were individually introduced into the containers, which were then covered with lids. Larvae that consumed the diet were later transferred to plastic containers containing fresh cowpea leaves and maintained at 26± 2°C and 60-70% relative humidity. For the control treatment, larvae were allowed to feed on leaves treated with sterile distilled water. The larvae were monitored for viral infection and mortality for a period of up to 8 days of post inoculation.

The effects of the extracted OBs on healthy larvae of *M. vitrata* were observed, upon hatching, larvae were transferred to clear plastic containers (22 x 15 x 7.5 cm), and they continued to be reared individually until reaching the second or early third instar on a meridic diet supplemented with equal proportions of cowpea powder at 27± 1ºC and 70± 10% relative humidity (RH). Subsequently, larvae were individually reared to pupation in clear plastic cups (4.5 cm high and 4 cm wide) with lids. Upon pupation, the larvae were sexed, and five pairs were placed in acrylic cylinders covered with netting at both ends (30 cm long and 15 cm in diameter), where they were held until adult emergence. Adult moths were sustained on a 10% (wt:vol) sugar solution provided in cotton wool within the cylinders. Eggs laid on the nylon nets attached to both ends of the acrylic cylinders were collected for further experimentation. All larvae designated for virus grow-ups and dose-response bioassays were derived from eggs subjected to surface sterilization using a 10% formalin solution. This comprehensive rearing and maintenance protocol ensured the availability of healthy and uncontaminated specimens for the subsequent stages of the research. The occlusion bodies (OBs) of MaviNPV were derived from diseased larvae identified during the survey. To propagate this isolate, laboratory-reared larvae were fed cowpea leaves treated with a suspension of purified OBs. Subsequent to this feeding, cadavers were collected, and OBs were purified using the methodology outlined by Chou et al. (1996). To quantify the purified OBs, a Neubauer hemocytometer was used under a phase-contrast microscope. To determine the median lethal concentration (LC_{50}) of MaviNPV for 3rd instar larvae (6-7 days old) leaf disc bioassay method was conducted, as outlined by Magholi et al. (2014), with a few modifications. The mortality rates of the larvae were recorded at 24 hr intervals up to 9 days post inoculation, providing a comprehensive assessment of the impact of different concentrations of the viral suspension on larval survival over time. The bioassay data were analyzed, using the probit analysis (Finney, 1971) to calculate LC_{50}, with % larval mortality calculated using Abbott formula (1925).

RESULTS AND DISCUSSION

The observed sequence of events in the progression of viral infection in *M. vitrata* larvae provides valuable insights into the dynamics of the interaction between the insect host and MaviNPV. The characteristic signs of viral infection, including the suspension of deceased larvae on cowpea leaves with abdominal prolegs, are indicative of the successful replication and spread of the virus within the insect population. The confirmation of virus presence upon collection of the deceased larvae underscores the specificity of MaviNPV for its target host (Magholi et al., 2014). Further microscopic examination of the body fluid and haemolymph revealed the presence of numerous spherical polyhedral inclusion bodies (PIBs), a hallmark feature of baculovirus infections. The average size of these PIBs (2.04± 0.22 µm) aligns with the typical dimensions observed in baculovirus infections and further supports the identification of the virus responsible for the observed mortality. The progression of symptoms, such as decreased activity and reduced feeding at 48 hr post-inoculation, followed by notable mortality in younger stage larvae on the third day, reflects the efficiency of MaviNPV in causing pathogenic effects in its host (Magholi et al., 2014). The transition of larvae to a pale pinkish color, along with the discharge of body fluid during the late instar stages, indicates the systemic impact of the viral infection on the physiology of the larvae. The observation of larval-pupal intermediates and deformed pupae, which gradually underwent degradation, highlights the profound consequences of MaviNPV infection on the normal developmental processes of *M. vitrata*. The emergence of adults from the affected pupae, coupled with the absence of an egg mass, suggests a potential

disruption of the reproductive capabilities of infected individuals. The discharge of orange-brownish liquid from the emerged adults, along with the microscopic identification of numerous spherical PIBs, further supports the persistence and propagation of the virus in the population. This phenomenon could contribute to the horizontal transmission of the virus, enhancing its spread within the insect population.

The LC_{50} for 3rd larval instars of *M. vitrata* exposed to different concentrations (1 x 10^4 to 1 x 10^9 POB's/ml) of MaviNPV was determined to be 2.3 x 10^6. Experiment revealed a pattern of high larval mortality in early instars, which gradually decreased in full-grown tested larvae. The observed trend of increasing LC_{50} values with the age of larvae suggests a potential dilution effect of the virus inoculum, likely associated with the corresponding increase in larval weight. This phenomenon aligns with findings of Ayyub et al. (2019), who investigated the pathogenicity of V-SpltNPV against 2nd, 3rd, and 4th larval instars of *S. litura* at varying concentrations (1 x 10^4 to 1 x 10^8 POB's/ml) revealed a high mortality rate ranging from 37.65% to 96.82% in early instar larvae, along with an increase in LC_{50} values with advancing larval age. These results also resonate with the findings of Kaur et al. (2021), who conducted bioassays against, *S. litura*, noting second instar larvae were more susceptible and easier to kill than the fourth instar larvae. The observed results align with the research conducted by Kindozandji et al. (2022), wherein they documented variable levels of MaviMNPV-induced larval mortality, ranging from 15± 0.63% to a complete mortality of 100± 00%. The extent of larval mortality was noted to be dependent on the specific developmental stages of the larvae under investigation. This parallel finding further supports the efficacy of MaviMNPV as a potent agent for inducing mortality in the target insect population, emphasizing its potential applicability across different larval stages. Collectively, these observations emphasize the age-dependent susceptibility of larvae to viral infection, with implications for the effective use of MaviNPV as a biological control agent.

In summary, the observed progression of MaviNPV infection in *M. vitrata* larvae reveals distinctive signs of viral replication, including suspended deceased larvae on cowpea leaves with abdominal prolegs. Confirmation of the virus, identification of polyhedral inclusion bodies (PIBs), and symptoms such as decreased activity and mortality in younger larvae underscore the efficacy of MaviNPV. Larval-pupal intermediates and deformed pupae indicate profound developmental consequences, while the absence of an egg mass suggests reproductive disruption. The discharge of orange-brownish liquid from emerged adults, along with numerous PIBs, supports virus persistence. Bioassay results demonstrate age-dependent susceptibility, with higher mortality in early instars. These findings enhance our understanding of baculovirus–insect dynamics, crucial for effective biological control strategies.

ACKNOWLEDGEMENTS

We personally thanks to those helped us in performing survey during our research period.

AUTHOR CONTRIBUTION STATEMENT

JSP: Survey, Lab experiments, data collection analysis, draft preparation and revision of the draft; RDD: collected specimen and literature; MNR: Survey and reviewed manuscript, and RBL, NBP & CNR: Conceptualization, reviewing and editing of the manuscript

CONFLICT OF INTREST

No conflict of interest.

REFERENCES

Agunbiade T A, Coates B S, Datinon B, Djouaka R, Sun W, Tamo M, Pittendrigh B R. 2014. Genetic differentiation among *Maruca vitrata* F. (Lepidoptera: Crambidae) populations on cultivated cowpea and wild host plants: implications for insect resistance management and biological control strategies. PLoS One 9(3): e92072.

Agunbiade T A, Coates B S, Sun W, Tsai M R, Valero M C, Tamo M, Pittendrigh B R. 2017. Comparison of the mitochondrial genomes of the Old and New World strains of the legume pod borer, *Maruca vitrata* (Lepidoptera: Crambidae). International Journal of Tropical Insect Science 37: 125-136.

Ayyub M B, Nawaz A, Arif M J, Amrao L. 2019. Individual and combined impact of nuclear polyhedrosis virus and spinosad to control the tropical armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), in cotton in Pakistan. Egyptian Journal of Biological Pest Control 29(1): 1-6.

Chakravarty S, Agnihotri M, Jagdish J. 2017. Seasonal abundance of predatory bugs, *Eocanthecona furcellata* (Wolff.) and *Rhynocoris fuscipes* (F.) and its olfactory responses towards plant and pest mediated semiochemical cues in Pigeonpea ecosystem. Legume Research-An International Journal 40(2): 351-357.

Chatterjee M, Yadav J, Vennila S, Shashank P R, Jaiswal N, Sreevathsa R, Rao U. 2019. Diversity analysis reveals genetic homogeneity among Indian populations of legume pod borer, *Maruca vitrata* (F.). 3 Biotech 9**:** 1-8.

Chou C M, Huang C J, Lo C F, Kou G H, Wang C H. 1996. Characterization of *Perina nuda* Nucleopolyhedrovirus (PenuNPV) Polyhedrin Gene. Journal of Invertebrate Pathology 67(3): 259-266.

Ekesi S. 1999. Insecticide resistance in field populations of the legume

pod-borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae), on cowpea, *Vigna unguiculata* (L.), Walp in Nigeria. International Journal of Pest Management 45(1): 57-59.

Finney D J. 1971. Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of information in medicine 10(04): 237-245.

Hussain B, Sivakumar G, Kannan M, War, A R, Ballal, C R. 2019. First record of a nucleopolyhedrovirus infecting brown-tail moth larvae, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae) in India. Egyptian Journal of Biological Pest Control 29: 1-5.

Kaur M, Josh, N, Sharma S, Kalia A. 2021. Pathogenicity of Nucleopolyhedrovirus (NPV) against *Spodoptera litura* (Fabricius). Journal of Biological Control 35(4).

Kindozandji A, Dannon A E, Nondichao A N, Amevoin K, Glitho I A, Srinivasan R, Tamo M. 2022. Assessing lethal doses of the baculovirus MaviMNPV and the effect of its combination with neem oil on the survival of *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) larval stages in laboratory. International Journal of Tropical Insect Science 42(3): 2143-2149.

Lazaro A A, Heong K L, Canapi B, Gapud V, Norton, G W. 1995. Farmers' pest management knowledge, attitudes and practices in San Jose, Philippines: a baseline survey. Integrated Pest Management Collaborative Research Support Program (IPM CRSP) Working Paper 95-2.

Magholi Z, Abbasipour H, Marzban R. 2014. Effects of *Helicoverpa armigera* nucleopolyhedrosis virus (HaNPV) on the larvae of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Plant Protection Sciences 4(50).

Mahalle R M, Taggar G K. 2018. Insecticides against *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) on pigeonpea. Pesticide Research Journal 30(2): 235-240.

Margam V M, Coates B S, Ba M N, Sun W, Binso-Dabire C L, Baoua, Murdock L L. 2011. Geographic distribution of phylogenetically-distinct legume pod borer, *Maruca vitrata* (Lepidoptera: Pyraloidea: Crambidae). Molecular Biology Reports 38: 893-903.

Otieno W A, Odindo M O, Okeyo-Owuor J B, Sabwa D M. 1983. Studies on the legume pod-borer, *Maruca testulalis* Geyer-VII. Field surveys on pathogenic microorganisms. International Journal of Tropical Insect Science 4(1-2): 211-215.

Sharma H C. 1998. Bionomics, host plant resistance, and management of the legume pod borer, *Maruca vitrata*-a review. Crop protection 17(5): 373-386.

Srinivasan R, Lee S T, Lo Y J, Talekar N S, 2005. *Maruca vitrata* Nuclear Polyhedrosis Virus (MvNPV), a new candidate in the management of *Maruca vitrata* (F.) (Lepidoptera: Pyralidae). Proc. of the Fifth Asia Pacific Congress of Entomology, October 1821, 2005, Jeju, Republic of Korea. p. 57.

Srinivasan R, Tamo M, Malini P. 2021. Emergence of *Maruca vitrata* as a major pest of food legumes and evolution of management practices in Asia and Africa. Annual Review of Entomology 66: 141-161.

Tamo M, Cherry A. 1999. Evaluation of a viral pathogen for *Maruca vitrata*. Project Annual Report. International Institute of Tropical Agriculture, Ibadan. p. 10.

(Manuscript Received: December, 2023; Revised: January, 2024;
Accepted: January, 2024; Online Published: February, 2024)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e24873