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Exploration of *Spodoptera litura* Nuclear Polyhedrosis Virus (*Sl*-NPV) in Several Regions of East Java Province

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Abstract

Background: *Spodoptera litura is a significant pest of crops, causing substantial yield and economic losses. Overreliance on chemical insecticides has led to negative impacts on human health and the environment, as well as the emergence of pest resistance. As a sustainable alternative, Nuclear Polyhedrosis Virus (NPV) has been widely recognized as an effective biological control agent. This study aimed to explore the occurrence of S. litura NPV (Sl-NPV) across multiple regions in East Java Province, Indonesia.* **Methodology:** *Field surveys were conducted on maize and tobacco plants in 11 regions, including Mojokerto City, Mojokerto Regency, Jombang, Sidoarjo, Batu City, Malang, Lamongan, Kediri, Blitar, Pamekasan, and Bangkalan. Pathogenicity was evaluated using Koch's postulate through spray applications on second-instar S. litura larvae. This study employed isolation of NPV from naturally infected S. litura larvae showing typical NPV infection symptoms.* **Findings:** *The exploration successfully identified Sl-NPV in Pamekasan and Blitar. Pathogenicity tests revealed that the isolate from Pamekasan was capable of inducing mortality in healthy larvae, whereas the isolate from Blitar did not produce infection symptoms when applied. These results highlight variability in pathogenic potential among isolates.* **Contribution:** *The findings provide important insights for the development of local Sl-NPV isolates as environmentally friendly biopesticides to support sustainable pest management in Indonesia.*

Keywords: *East Java; Exploration; Nuclear Polyhedrosis Virus; Spodoptera litura; Sl-NPV Biological Control Agent*

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INTRODUCTION

Spodoptera litura is a versatile pest known for its extensive range of host plants. Intense larval infestations compromise crop quality and yield, leading to considerable economic setbacks. In Indonesia, findings from the Center for Forecasting of Plant Pest Organisms (National Center for Plant Pest Forecasting, 2022) indicated that *S. litura* infestations on soybean with infestation levels reaching 68.48%. The presence of holes on leaves is indicative of feeding damage caused by larvae (Rosmiati et al., 2018). *S. litura* is one of the major pests affecting various cultivated crops, therefore environmentally friendly control alternatives are required to suppress its population (Megasari et al., 2025).

The predominant approach for managing *S. litura* has been an overreliance on chemical insecticides. Nonetheless, this method has resulted in adverse effects on human health and the environment, in addition to hastening the emergence of pest resistance. Kusumah et al., (2022) indicated that Nuclear Polyhedrosis Virus (NPV) has surfaced as a noteworthy biological control agent for lepidopteran pests. Prior investigations have shown that NPV is efficient, environmentally benign, and exhibits a high degree of specificity towards target insect populations. Furthermore, it poses less risk to humans and advantageous organisms in comparison to chemical pesticides. Various forms of NPVs have been recognized, such as *S. litura* NPV (*Sl*-NPV), *Spodoptera exigua* NPV (*Se*-NPV), *Spodoptera frugiperda* NPV (*Sf*-NPV), and *Helicoverpa armigera* NPV (*Ha*-NPV).

Investigating locally adapted NPV isolates is essential, as they tend to demonstrate greater efficacy in particular environmental contexts. Field detection typically relies on observing symptoms of larval infection, which include decreased feeding activity, discoloration, body softening, increased fragility, and liquefaction (Tanuja et al., 2019). The implementation of NPV in various countries has already demonstrated its potential contribution to integrated pest management programs in Indonesia.

Although NPV has demonstrated significant potential, there is still a scarcity of information regarding the occurrence, distribution, and pathogenicity of local *S. litura* NPV (*Sl*-NPV) isolates in Indonesia. According to Sinambela et al., (2022), the majority of existing studies have concentrated on laboratory evaluations or findings from other countries, leaving a notable gap in regional exploration and characterization within East Java. This gap obstructs the advancement of efficient biopesticides derived from local isolates that are more suited to regional agroecosystems.

Previous studies in Indonesia have mainly focused on laboratory evaluation, molecular characterization, or efficacy testing of *Sl*-NPV isolates. Information regarding the natural occurrence, geographical distribution, and field exploration of indigenous *Sl*-NPV isolates in East Java remains limited. Consequently, the availability of local isolates adapted to regional agroecosystems for biopesticide development is still insufficient. Therefore, this study aimed to explore the occurrence of *Sl*-NPV in several regions of East Java Province and evaluate the pathogenicity of the obtained isolates against S. litura larvae. The findings are expected to provide baseline information for the development of locally adapted *Sl*-NPV-based biological control agents.

METHOD

Place and Time

The survey was conducted in maize and tobacco agroecosystems across East Java Province. Field exploration was conducted in 11 agricultural regions across East Java Province, namely Mojokerto City, Mojokerto Regency, Jombang Regency, Sidoarjo Regency, Batu City, Malang Regency, Lamongan Regency, Kediri Regency, Blitar Regency, Pamekasan Regency, and Bangkalan Regency. The purification of NPV isolates and the pathogenicity tests were conducted at the Plant Health Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" East Java. The investigation took place between September 2024 and January 2025.

Materials

The tools utilized for sampling and propagation comprised vials, isolate containers, tweezers, gloves, ice bags, ice gels, propagation containers, tissues, syringes, stationery, a 48 MP mobile phone camera, pipettes, mortar, pestle, and sprayer. The materials utilized comprised distilled water, 70% alcohol, and NPV isolate derived from infected *S. litura*.

Exploration

Isolates of NPV from infected larvae. Second instar *S. litura* larvae were used for pathogenicity testing. The investigation utilized a random sampling technique for NPV collection. Sampling activities were executed directly at designated locations via hand collection. The selection of random sampling was implemented to guarantee that every plant or location within the tobacco field possessed an equal opportunity for selection as a sample which refers to Sinambela et al., (2022). Infected *S. litura* larvae are preserved in a freezer maintained at a temperature of -20ºC.

NPV Purification

NPV purification took place in Plant Health Laboratory 2. *S. litura* larvae exhibiting NPV symptoms, identified in the observation plots, were collected. According to Bedjo (2004), the larvae underwent digestion with a mortar and pestle, resulting in a solution at a concentration of 1%, equating to 1 ml of distilled water for each larva. The gathered larvae were deceased, displaying symptoms including a change in body color to black, a soft and watery texture, and disintegration upon contact.

NPV Postulate Koch Test

The purpose of this test was to assess the pathogenic properties of the isolate (Murwantoko, 2013). The NPV isolate was administered to the feed of *S. litura* larvae. The application of the spray method involved administering five sprays, with each spray measuring 1 ml. Pathogenicity was evaluated using Koch's postulate through spray applications on second-instar *S. litura* larvae. A negative control treatment consisting of larvae without NPV exposure was included in the experiment. Observation of symptoms until the adult stage

Data Analysis

The visualization of data exploration is conducted through the use of ArcGis software. Observations of the Postulate Koch Test involved identifying symptoms in larvae through to the imago stage, and comparing the morphology of healthy and symptomatic larvae.

RESULT AND DISCUSSION

NPV Exploration

Exploration carried out on tobacco and corn in 11 provincial regions of East Java, including Mojokerto City, Mojokerto Regency, Jombang Regency, Sidoarjo Regency, Batu City, Malang Regency, Lamongan Regency, Kediri Regency, Blitar Regency, Pamekasan Regency and Bangkalan Regency (Figure 1.).

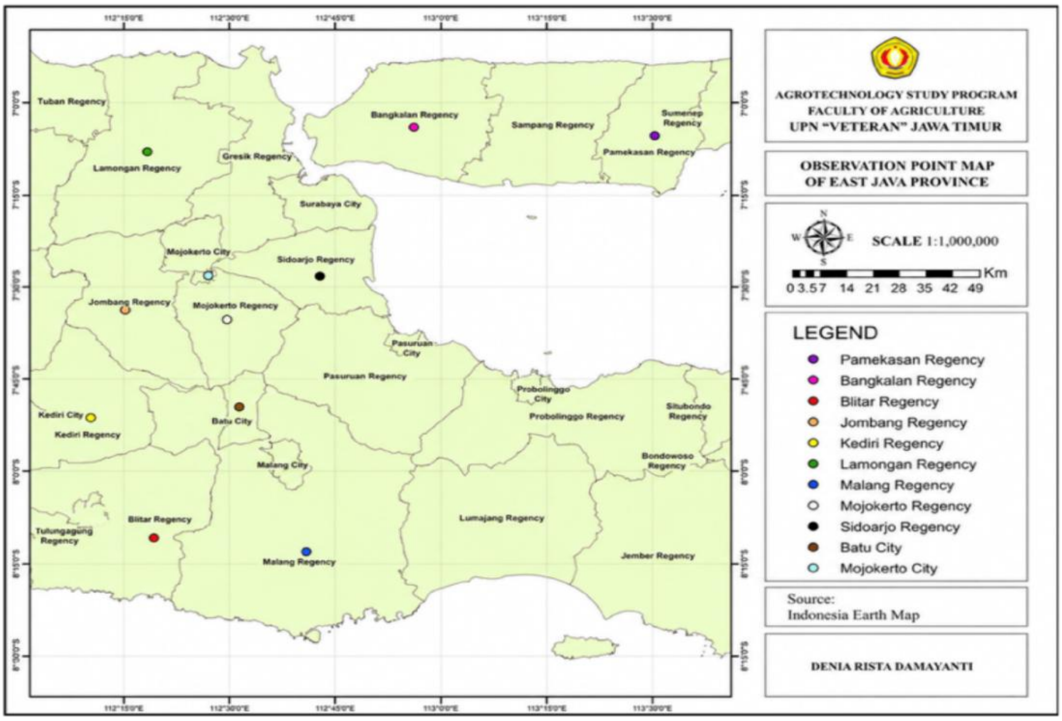

Figure 1. Location map NPV exploration.

Symptoms indicative of suspected larvae NPV were observed in the land located in the Regency of Pamekasan (Table 1.). The results of the exploration indicate that the natural distribution of NPV in East Java remains quite restricted and specific to certain locations. This condition is subject to various influences, such as the presence of the primary host (*S. litura*), the degree of chemical pesticide application, and the surrounding environmental factors. Yasin et al., (2020) indicate that the presence of NPV in a given area is closely associated with the population of the host pest. Populations that are either low or significantly suppressed as a result of chemical insecticide application decrease the chances of encountering NPV.

Hasanah & Haryadi (2022) indicate that increased sunlight exposure can hasten the breakdown of virus particles, consequently lowering infection rates in the environment.

Table 1. Results of NPV exploration in several areas in the Province East Java.

No.	Location	Dominant Crop Species (Maize/Tobacco)	Results
1.	Mojokerto City	Maize	Not found
2.	Mojokerto Regency	Maize	Not found
3.	Jombang Regency	Tobacco	Not found
4.	Sidoarjo Regency	Maize	Not found
5.	Batu City	Tobacco	Not found
6.	Malang Regency	Maize	Not found
7.	Lamongan Regency	Maize	Not found
8.	Kediri Regency	Tobacco	Not found
9.	Blitar Regency	Tobacco	Not found
10.	Pamekasan Regency	Tobacco	Found
11.	Bangkalan Regency	Maize	Not found

The exploration results indicating that NPV was exclusively detected in Pamekasan Regency are presumably associated with a combination of biotic and abiotic factors. The occurrence of NPV is highly dependent on the population density of its primary host, *S. litura*. *S. litura* NPV is capable of persisting and propagating only when larval populations are sufficiently abundant to facilitate horizontal transmission among individuals (Afandhi et al., 2022). In the present study, infection symptoms were observed solely in Pamekasan, an area recognized as a major tobacco cultivation center. In contrast, the population of *S. litura* in other regions, particularly maize-growing areas, was presumably lower.

Afandhi et al., (2022) reported that in maize agroecosystems in East Java, the population of *S. litura* has gradually been displaced by *Spodoptera frugiperda* (fall armyworm), resulting in a decline in the infestation intensity of *S. litura* compared to previous conditions. Consequently, the probability of detecting *Sl*-NPV in maize ecosystems has also decreased.

These findings demonstrate a potential novelty, as information regarding the natural distribution of local NPV isolates in East Java remains limited. Similar observations have been reported in previous studies. Sinambela et al., (2022) successfully isolated and characterized indigenous Sl-NPV from naturally infected S. litura larvae in North Sumatra, indicating that natural infections can occur in specific locations where environmental conditions favor virus persistence and transmission. Likewise, several studies have reported that the prevalence of baculovirus infections varies among regions due to differences in host population density, climatic conditions, cropping systems, and agricultural management practices.

The detection of *Sl*-NPV in tobacco fields in Pamekasan Regency indicates that entomopathogenic viruses are still capable of naturally persisting within specific agroecosystems that support both host and pathogen development. Exploration of biological control agents across diverse environmental conditions is essential for identifying local isolates with potential as biological control agents against *S. litura* (Chang et al., 2021).

The absence of *Sl*-NPV in the majority of observation sites is presumably associated with the low abundance of host larvae in the field. Reduced populations of *S. litura* may limit the opportunity for horizontal virus transmission among larvae, thereby decreasing the likelihood of naturally detecting the virus within the environment (Hussain et al., 2021).

Table 2. Comparison results observation symptom NPV infection and literature.

Parameter	Observation result	Reference
Body color	 Larvae appeared blackish and shiny.	 Infected larvae generally exhibit a dark and glossy appearance (Sinambela et al., 2022).
Body texture	Larval bodies became soft and fragile.	NPV infection causes tissue softening and integument fragility (Ali et al., 2017).
Body posture	Larvae exhibited an inverted "V"-shaped position before death.	NPV infected larvae commonly hang with the posterior prolegs attached to plant surfaces, forming an inverted "V" posture (Kusumah et al., 2023).
Body integrity	Larvae underwent lysis after death.	Advanced infection leads to body liquefaction and tissue breakdown (Ali et al., 2017).

Identification NPV Symptom

An exploration of NPV was conducted on land tobacco in the Pamekasan Regency, specifically in Desa Sana Tengah, Kecamatan Pasean. According to Rahmianna (2016), symptoms of NPV infection can be observed in the pupal and adult stages of *S. litura*. Symptoms observed include color changes in larvae. According to Ali et al., (2017), symptoms of infection are characterized by alterations in body color, swelling, and the mortality of larvae accompanied by the distinctive release of viral fluid. The observation results indicate the presence of one *S. litura* larva exhibiting symptoms of NPV infection (Table 2). Since only a limited number of naturally infected larvae were recovered during exploration, the present findings should be considered preliminary observations requiring further quantitative validation.

The findings from field observations and literature presented in Table 2 align with the larval symptoms, which are characterized by a blackish coloration and exhibit lysis upon being pressed. Study Kusumah et al., (2023), noted a primary indicator of NPV infected larvae is their hanging body posture, characterized by hind legs positioned in a "V" formation. The larvae that are infected experience notable physical transformations, as their integument becomes highly delicate and prone to breakage upon contact. Upon the rupture of this integument, a viscous, brownish fluid is expelled, which harbors viral particles. This infectious fluid arises from cell lysis caused by NPV infection within the organism.

The infection symptoms observed in the larvae, including darkening of body coloration, softening of body tissues, and eventual lysis, are consistent with the typical characteristics of nucleopolyhedrovirus infection in Lepidopteran larvae. Infected larvae generally undergo severe tissue damage as a consequence of viral replication within the host cells (Kaur et al., 2021). Infected larvae exhibited fragile integuments that ruptured easily upon contact. This condition resulted from the deterioration of tissue structures during the formation and accumulation of viral particles within the host cells (Li et al., 2021).

Postulate NPV Test

Symptoms of NPV infection in the field have been observed returning after the application of NPV isolates found in Sana Tengah Village, District Pamekasan. Infection symptoms were observed in the larval, pupal, and imago stages. Larvae exhibit symptomatic lysis, characterized by a blackish coloration, a soft texture, and an inverted "V" shape. *S. litura* did not successfully form pupae during the pre-pupa phase. Visible infected imago exhibiting disabilities, characterized by absent antennae and compromised wings (Figure 2).

Observation of symptom results aligns with the findings of Bedjo (2012), which indicated that larvae infected with the virus exhibit behavioral and physical alterations, including reduced movement, changes in skin color to gray, a glossy appearance, and an increase in body size. As the NPV infection advances, the larvae will weaken and ultimately perish. The viral infection induces distinct morphological alterations, particularly on the surface of the larva's body. The cuticle exhibits brittleness and a decline in its structural integrity, rendering it particularly vulnerable to mechanical damage.

The pupae of Spodoptera litura were unable to develop normally due to viral infection. According to Rahmadhini et al., (2025), toxins or harmful substances produced by organisms such as viruses, bacteria, and fungi can inhibit the development of pupae into adults. The observation results demonstrated that infected larvae exhibited reduced feeding activity prior to death. This finding is consistent with Zhang et al., (2021), who reported that the feeding activity of *S. litura* strongly influences its physiological development and susceptibility to pathogenic agents.

Figure 2. *S. litura* infected with NPV. (a) Larva undergoes lysis; (b) Larva turns black and forms a "V" shape; (c) Pupa fails to form; (c) Comparison of healthy and infected adults.

The *Sl*-NPV isolate obtained from Pamekasan was capable of inducing infection symptoms in healthy larvae, indicating the pathogenic nature of the local isolate. The biological activity of local NPV isolates may vary among regions depending on the genetic characteristics of the virus and the environmental conditions from which the isolate originated (Elmenofy et al., 2021).

The successful infection of *Sl*-NPV observed in this study demonstrates the potential application of the virus as an environmentally friendly bioinsecticide for controlling *S. litura*. According to Syahroni & Haryadi (2020), the use of NPV is considered safer for non-target organisms compared with synthetic insecticides. According to Fitriana et al., (2021); and Grewal et al., (2021), In addition to baculoviruses, other biological control agents such as *Beauveria bassiana*, *Aspergillus oryzae*, and *Metarhizium rileyi* have also been reported to possess pathogenic activity against *S. litura* and may be integrated into biological control programs.

Wang et al., (2021) explained that entomopathogenic fungal infections in *S. litura* larvae can cause physiological disturbances and suppression of the insect immune system, thereby increasing larval mortality caused by pathogenic infections. The utilization of biological control agents in pest management has become increasingly important in reducing the negative impacts associated with excessive chemical pesticide application. Khan et al., (2021) and Sarwar et al., (2021) reported that combining biopesticides with other biological control agents can enhance the effectiveness of controlling *Spodoptera* sp.

CONCLUSION

This study represents a preliminary exploration of indigenous *Sl*-NPV isolates in East Java Province. Among eleven surveyed locations, naturally infected larvae were detected only in Pamekasan Regency, indicating a limited distribution of *Sl*-NPV under the surveyed agroecosystem conditions. The obtained isolate successfully reproduced characteristic infection symptoms in healthy *S. litura* larvae, confirming its pathogenic potential. However, this study was limited to symptom based identification and pathogenicity observation without quantitative mortality analysis, LT50 estimation, microscopic examination, or molecular confirmation of the virus. Therefore, further studies are required to validate the isolate and determine its efficacy under laboratory and field conditions. The novelty of this study lies in providing baseline information on the natural occurrence of local *Sl*-NPV isolates in East Java. These findings contribute to future development of environmentally friendly *Sl*-NPV based biocontrol agents for sustainable management of *S. litura* populations.

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