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Biological Control of Cabbage Butterfly (*Pieris rapae* Linn.) Using Nuclear Polyhedrosis Virus

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Abstract

Pieris rapae Linnaeus, commonly known as cabbage white butterfly, is a serious pest of cruciferous crops around the world, particularly in high-altitude places including Benguet, Philippines, where infestation causes enormous economic losses. Traditional chemical pesticides pose environmental and health hazards, leading to the quest for long-term alternatives like Nucleopolyhedrosis Virus (NPV). This study evaluated the efficacy of NPV as a biological control agent against *P. rapae*, specifically on its performance alone and when combined with carrier materials at various time interval exposure. Results showed that all of the various treatments were effective, against cabbage butterfly larvae consistently resulting in 100 % infection, and severity of infection, with the NPV alone treatment being particularly impactful. Notably, second larval instar exhibited significantly higher susceptibility to NPV alone, causing 60% mortality at 48 hours post-infection (HPI), at 120 HPI, all treatments and larval stages achieved almost complete mortality of 95% to 100%. Optimal environmental conditions of 25 °C and 70% relative humidity accelerated infection causing mortality, especially in susceptible second larval instar. Among the carrier materials used, Oatmeal and Cornmeal had intermediate effects on NPV efficacy, whereas All-Purpose Flour produced the lowest impact. This was linked to their physical characteristics, which influence viral persistence, such as water-holding capacity. Over time, morphological changes in infected larvae progressed, including sluggishness, discoloration, paralysis, and liquefaction. Given its affordability, NPV alone proved to be the most economical option, amounting to PhP 343.50 per treatment. Carrier materials like Oatmeal shows potential for formulation stability, although, they were less practical which contributed to higher cost of the treatments. The study concludes that NPV alone and NPV with carrier materials as treatment is a promising and good biological control strategy for *P. rapae*, with optimal application targeting early instar under specific environmental conditions.

Keywords: *Pieris rapae* Linn; Biological control; Nuclear Polyhedrosis Virus (NPV); Integrated Pest Management (IPM); Carrier materials; Larval Infection; Larval Mortality.

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1. Introduction

Pieris rapae Linnaeus, or cabbage white caterpillar, is a serious pest of cruciferous crops globally, causing enormous economic damage. The insect, belonging to the order Lepidoptera and the family Pieridae, has four life stages: egg, larva (caterpillar), pupa (chrysalis), and adult (butterfly) [1,2,3] The larval stage is the most damaging since it feeds voraciously on the leaves of cruciferous plants, thereby, lowering agricultural production [4]. In the absence of management methods, entire defoliation is not unusual, and it can render 80% of the cabbage heads unmarketable [5]. In the Philippines, the authors in [6] identify *P. rapae* as a prominent crucifer pest in the Cordillera Administrative Region, including Benguet. Severe infestations, when unchecked, could result in a 30% to 50% yield reduction. The larvae feed aggressively on leaves and bore into cabbage heads, causing further economic losses. The climate and high-altitude environment in Benguet further facilitate the pest's life cycle, making it a persistent problem in agricultural production.

Traditional chemical pesticides have environmental and health hazards, leading to a search for more sustainable pest management alternatives. Among these, Nucleopolyhedrosis Virus (NPV) had emerged as a potential biological control agent. NPV, a baculovirus that infects and kills lepidopteran pests, is an environmentally acceptable alternative to chemical pesticides [7]. Recent studies showed that NPV is highly effective against *P. rapae* larvae. According to studies, NPV could cause over 80% mortality in first instar *P. rapae* larvae [8]. In a field trial, NPV also significantly reduced *P. rapae* populations, indicating their significance in integrated pest management (IPM) systems [9,10]. Spraying is usually done to deliver NPV. Timing is crucial and sprays should coincide with larval hatching, particularly targeting larvae less than 7 mm for optimum control. Effective coverage is also critical because larvae must consume virus particles to get infected [8].

NPV is delicate and readily deactivated by environmental conditions, particularly ultraviolet radiation from sunlight. Without a carrier, the virus would rapidly deteriorate and become ineffective. Also, carrier materials are critical because they operate as a protective shield, extending the virus's vitality and enabling practical application in the field. A recent study also emphasized the use of carrier materials for NPVs, such as bran and soy flour. The soy flour was found to be superior to other carriers in terms of stability, adhesion, and viral protection, which led to higher rates of mortality [11]. Benguet province is well-known for its strong vegetable agricultural business. Insect pest occurrences, such cabbage butterfly larvae, pose a threat to the farmers' livelihoods. Using NPV as a biocontrol strategy can provide a sustainable and environmentally sound pest management solution. When integrated into a carrier, NPV is better protected against sunlight and heat, extending its efficacy. Likewise, NPV with carriers adheres better to plant leaves, increasing the possibility that larvae consumed the virus while feeding. Furthermore, applying this biological control through optimal carrier formulations aids sustainable agricultural practices by creating healthier ecosystems, conserving beneficial insects like pollinators, and contributing to the long-term well-being of the agricultural area. Currently, there is no data on carrier-based NPV strategies against cabbage butterflies in the region. The data from this study can serve as a reference and baseline for future studies.Generally, the study aimed to assess the efficacy and potential of Nuclear Polyhedrosis Virus (NPV) as a biological control agent of *Pieris rapae* in crucifer crops. Ultimately, this aimed to reduce reliance on chemical pesticides and promote integrated pest management for *P. rapae*. Specifically, the study was conducted to compare the effectiveness of NPV alone and NPV + carriers on

different larval instars as affected by time interval exposure, evaluate the water holding capacity of different carriers, and compare the economic analysis of the various treatments.The study was conducted at the College of Agriculture, Department of Entomology, Benguet State University, La Trinidad, Benguet, from January to March 2025.

2. Materials and Methods

2.1Materials

The materials used in the study were healthy *Pieris rapae* larvae, Nucleopolyhedrosis virus (NPV), host plant (*Brassica rapa* subsp. *chinensis* (Pechay leaves), mortar and pestle, bowls, blenders, stirring rods or other mechanical mixers, weighing scale, cornmeal, oatmeal (Golden oats-instant oatmeal), all-purpose flour (APF-class I), plastic containers measuring 135 mm long x 117 mm wide x 45 mm deep with improvised cover using fine mesh net for ventilation, hygrometer, muslin cloth, pressurized hand sprayer or applicator, distilled water, tissue, forceps, disposable gloves, face mask, pen and paper.

2.2Methods

2.2.1Collection and Rearing of Pieris rapae

Adults of *Pieris rapae* were collected from healthy cabbage plants at the Crop Techno Station of Benguet Provincial Agriculture Office and were brought to the study area at Wangal La Trinidad for rearing in a large fine net tunnel under controlled conditions. The rearing temperature was maintained at 25.0 °C, and 70.0±5.0 of Relative Humidity (RH). Ten (10) 1m x 5m plots were planted with *Brassica rapa* subsp. chinensis (Pechay) for rearing *P. rapae*. From these plots, healthy and active *P. rapae* larvae were collected for bioassay test. The healthy larvae used in the bioassay test was fed with natural diet that consists of Pechay

2.2.2Procurement and Propagation of NPV

The Nuclear Polyhedrosis Virus was acquired from the Regional Crop Protection Center of the Department of Agriculture-Cordillera Administrative Region (RCPC, DA-CAR). The NPV suspension was inoculated on pechay leaves, which were fed to the fourth (4^{th}) larval instar of *P. rapae*.

2.2.3Preparation of NPV Using Different Carrier Materials

For standardized measurements, 0.2 grams of 4^{th} instar NPV-infected larvae were first weighed and measured. The infected larva was then combined with 10 mL of distilled water. The mixture was then utilized in inoculating *P. rapae* larvae. To inoculate, sixteen 4^{th} instar *P. rapae* larvae with the same size of 20 mm were dipped in the NPV solution. From the 16 infected larvae, one infected larva was combined with 10 grams of various treatments at 5 days post-infection. To guarantee uniform dispersion, the mixture was well combined in a dry, clean container [12,13]. As the carrier absorbed the NPV solution, the combination was continually blended to ensure that the virus was uniformly distributed throughout the carrier material and forms a

homogenous mixture. To preserve the virulence, the prepared carrier-based NPV was kept from direct sunlight and placed in a cool, dried area.The carrier materials used in the study were purchased from a grocery store and the public supermarket of La Trinidad, Benguet. These materials, which served as the study's treatments, included all-purpose flour, cornmeal, and oatmeal that had been finely milled. The carrier materials were sieved using a standard test sieve, No. 30, with a nominal sieve opening of 600 µm to ensure uniform particle sizes. The uniformity aided in the mixing process and distribution when applied to *P. rapae* larvae. Milled cornmeal contributed to steady matrix NPV administration, enhancing infectivity and persistence in target insects [14]. Oatmeal also improved stability and adhesion, increasing viral infectivity and mortality rates [15]. All-purpose flour, on the other hand, was shown to be an excellent solid carrier, providing a protective medium for NPV, which was critical for keeping the virus infective during administration. According to research, the flour's physical qualities promoted adherence to plant surfaces, boosting the possibility of ingestion by target larvae Reference [16].

3.Bioassay

Fresh and healthy young pechay leaves were collected from the field and were brought to the laboratory. The leaves were thoroughly washed three times in running tap water then rinsed with distilled water and air dried. The leaves were cut to uniform sizes of diameter of 5.08 centimeters or 2 inches, and four (4) cut leaves were allocated per container. The leaves were sprayed with standard NPV diluted in a liter of water. For NPV coated with all-purpose flour, cornmeal, and oatmeal as carriers, 10 grams of the prepared formulation were also diluted in a liter of water, properly mixed, and filtered before these were placed in a pressurized hand sprayer container [13].

The leaf disc method using pechay leaves was used for the study before introducing the healthy and active larvae. For spray application, the handheld pressurized hand sprayer was calibrated to ensure uniform application. Test spray on a non-experimental surface was also conducted to confirm the correct droplet size (400 microns) and coverage.

A uniform population of 640 *P. rapae* larvae at the second, third and fourth instar stages collected at the Crop Techno Station, Wangal, La Trinidad, Benguet was used for the bioassay, prior to inoculation of treated pechay leaves. The *Pieris rapae* larvae were placed in plastic containers with a diameter of 135 mm long x 117 mm wide x 45 mm deep with an improvised fine mesh net for ventilation following the treatment. After spraying, the time for the application was recorded to ensure accurate data collection.

The plastic containers with treated pechay leaves were placed at room temperature (25^{o}C) and assessed for mortality following two factors within a Complete Randomized Design (CRD). A one-time application of treatments, and observation was done for seven days. The treatments, replicated four time with each replicate having 10 subsamples were as follows:

Table A: Carrier Materials

Treatment	Description	Weight
A 1	Nuclear Polyhedrosis Virus Alone	10 grams
A 2	Nuclear Polyhedrosis Virus + All-Purpose Flour	10 grams
A 3	Nuclear Polyhedrosis Virus + Cornmeal	10 grams
A 4	Nuclear Polyhedrosis Virus + Oatmeal	10 grams

Table B: Larval Stage of *Pieris rapae*

Treatment	Larval Stage
B 1	2nd instar
B 2	3rd instar
B 3	4th instar

4.Data Gathered

Infection rate (%). This refers to the percentage of larvae showing symptoms of NPV infection using the given formula [17]. This was gathered via ocular observation just after the application of various treatments at time intervals of 30 minutes, 1, 2, 24, 48, 72, 96, 120, 144, and 168 hours after application.

$$\text{Infection Rate (\%)} = \frac{\text{Number of Infected Individuals}}{\text{Total Number of Exposed Individuals}} \text{ X } 100$$

Severity of infection. These included morphological changes in the structure and appearance of the larvae, such as body color, size, shape, and form, based on the scoring system [18] (Table 1). Continuous daily observation was conducted to track the progression and severity of infection on the larvae.

Table 1: Parameters for morphological changes in NPV- infected larvae

SCORE	STAGE OF INFECTION	DESCRIPTION OF MORPHOLOGICAL CHANGES
0	Normal	The larvae exhibited no indications of infection. Normal coloration, movement, and activity.
1	Early infection	Slight discoloration (lightening or darkening), sluggish mobility, and decreased appetite.
2	Mid-infection	Color changes (e.g., whitening or darkening), soft body, lethargy and decreased mobility.
3	Late infection	Severe discoloration (e.g., dark, pale, or milky white), flaccidity, anorexia, paralysis, and the onset of body liquefaction.
4	Death	Larvae were entirely discolored, with a liquified body structure, a fragile cuticle, and releasing occluded bodies.

4.1Percent Mortality

The various treatments were compared to identify the most effective for causing rapid mortality over specific time intervals. The larvae were poked with a blunt needle, and those unable to move in a coordinated manner were considered dead. The number of dead specimens during the period of observation was recorded and computed using the formula [19].

$$\text{Mortality Rate (\%)} = \frac{\text{Number of Dead Individuals}}{\text{Total Number of Individuals}} \text{ X } 100$$

4.2Larval Feeding Consumption

This was measured by calculating the amount of leaves consumed at each instar from the second to the fourth stages. To assess overall consumption, the remaining host plant was measured after 48 hours.

4.3Water Holding Capacity

This refers to the capacity of different carriers to retain moisture after saturation. This was essential for determining how effectively carriers absorbed NPV. The saturated weight of the carrier materials was evaluated by placing equal weight samples in containers and setting them on a water-filled tray. Water was allowed to seep upward from the base until the surface of the carrier material became saturated, which took several hours or overnight. When the carrier materials were saturated, the samples were transferred to a rack to allow free drainage before weighing. Subsequently, these saturated samples, which remained in their original containers, were oven-dried for approximately 24 to 48 hours before weighing the final oven-dried weight. The following formula was used to calculate water holding capacity [20].

$$\text{WHC} = \frac{\text{FW - ODW}}{\text{ODW}} \text{ X } 100$$

Where:

WHC = water holding capacity of the carrier material (%)

FW = weight of saturated carrier material (g)

ODW = weight of oven dried carrier material (g)

4.5Temperature and Relative Humidity

Temperature and relative humidity were recorded daily. This was gathered using a hygrometer instrument placed under room conditions, with 10 data readings.

4.6Economic analysis

This was done by recording all the expenses incurred during the study. This was computed using the following formula:

$$\text{Total Cost} = \frac{\text{Total Cost of Inputs}}{\text{Overall Total Cost}} \text{ X } 100$$

5.Treatment of Data

A complete randomized design (CRD) was implemented in all experiments with a factorial design approach. The comparison for the level of significance of the treatment means was done using two-way table of means and standard deviation (StDev) with Fisher's Least Significant Difference (LSD) of 5% as post-hoc test.

6. Results and Discussion

6.1Effectiveness of NPV Alone and NPV + Carrier-Based on Different Larval

6.1.1Instars of Pieris rapae as Affected by Time Interval Exposure

Percent Infection of Different *Pieris rapae* Larval Instars Under Various Treatments at Different Time Intervals

Table 2 illustrates the results of percent infection as affected by different treatments such as Nuclear Polyhedrosis Virus alone and combined with carrier materials such as All-Purpose Flour, Cornmeal, and Oatmeal. The results showed a consistent pattern of infection over time. Initially, at 30 minutes, there were no significant differences in infection among the treatments. At 24 hours post-infection, NPV alone showed a 48.30% infection rate. In comparison, NPV + All-Purpose Flour had 39.20%, NPV + Cornmeal at 43.30%, and NPV + Oatmeal at 45.00% which were not statistically significant. At 120 to 168 hours post-infection, all treatments resulted in 100% infection demonstrating their effectiveness regardless of the carrier materials used.

Table 2: Percent infection in *Pieris rapae* larvae as affected by the treatment at different time intervals (January-March 2025, La Trinidad, Benguet)

TREATMENTS	TIME INTERVALS									
	30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
Nuclear Polyhedrosis Virus Alone	0.00	0.83	14.20	48.30	77.50	97.50	98.30	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + All-Purpose Flour	0.00	0.58	09.20	39.20	69.20	95.80	98.30	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Cornmeal	0.00	0.67	11.70	43.30	72.50	97.50	97.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Oatmeal	0.00	0.75	10.80	45.00	79.20	96.70	99.20	100.00	100.00	100.00

*No significant differences in the interaction based on the ANOVA table. Hence, pairwise comparison (post-hoc test using LSD or Tukey HSD can potentially incur type I error.

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Table 3 illustrates various larval stages of cabbage butterfly. Larval stages had 0.00% infection, which was not significantly difference at 30 minutes post-infection. However, at 24 hours post-infection, significant variations had been observed, where the 2nd larval instar had a 55.60% infection, significantly higher than the 3rd larval instar with 40.00% and 4th larval instars with 36.20%. This pattern continued after 48 hours post-infection, with the 2nd larval instar with 85.60% more severe infection than the 3rd larval instar having 71.20% but was significantly higher than the 4th larval instar with 66.90%. Results clearly shows that younger larvae were more susceptible to infection in the early stages, corroborating with the observation of this author [21]. At 72 hours post-infection, the second, third and fourth larval instars showed no significant difference in infection rate ranging from 95.00 % to 98.80 %. This suggests that, while initial susceptibility varied significantly among larval stages, nearly all larvae, regardless of developmental stage, eventually reached comparable high levels of infection, resulting to no significant differences with the later stages.

Table 3: Percent infection in *Pieris rapae* at various larval stages and time intervals (January-March 2025, La Trinidad, Benguet)

LARVAL STAGE	TIME INTERVALS									
	30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
2nd Instar	0.00^{a}	11.90^{a}	15.60^{a}	55.60^{a}	85.60^{a}	98.80^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
3rd Instar	0.00^{a}	9.40^{a}	12.50^{a}	40.00^{b}	71.20^{b}	96.90^{a}	98.80^{a}	100.00^{a}	100.00^{a}	100.00^{a}
4th Instar	0.00^{a}	0.00^{b}	6.20^{b}	36.20^{b}	66.90^{c}	95.00^{a}	96.20^{a}	100.00^{a}	100.00^{a}	100.00^{a}

Table 4 reveals the interaction among treatments and different larval stages with 0.00% infection at 30 minutes post-infection, indicating no significant differences. Infection rates increased consistently over time among carrier-based treatments and larval stages. This was evident since all groups had an infection rate of 100.00%, within 72 to 168 hours post-infection. At 24 hours post-infection, the 2nd larval instar consistently had higher infection rates ranging from 45.00% to 67.50% than the 4th larval instar ranging from 32.50% to 40.00%. However, despite 2nd larval instar being infected earlier, this did not differ significantly from the 4th larval instar indicating that carrier materials shown to be effective. This result supports earlier research of the author's Reference [22,23], who reported that carrier formulations increase the durability and infectivity of Nucleopolyhedrovirus against lepidopteran larvae.

Among the treatments, the interaction of NPV alone produced the highest infection rates in the early stages of the instars, followed by Oatmeal, Cornmeal, and All-Purpose Flour with no significant differences. For example, at 48 hours post-infection, the application of NPV alone had 97.50 %, followed by Oatmeal with 87.50 %, Cornmeal with 85.00 %, and All-Purpose Flour having 75.00 %, indicating the potential of the different carrier materials, aligns with this author's previous findings [14,16,24].

Nuclear Polyhedrosis Virus + Oatmeal has a feed consumption of 38.58 mm, and NPV alone with 35.83 mm recorded the highest percent infection among the larvae, with Oatmeal being second, followed by NPV + Cornmeal and NPV + All-Purpose Flour (35 mm and 31.63 mm, respectively) (Table 5). However, regardless of the carrier materials used, the virus itself was effective, and the larvae still feeds on the host plant making all treatments with no significant difference. The findings of this study were consistent with previous research, including prior investigations of this author [25], which showed that younger larvae were more susceptible to NPV infections. Author [26] also found greater mortality and prolonged growth durations in younger instars of *Helicoverpa armigera* following NPV infection. Reference [27] added that the first lag in apparent infection was to be expected because the pathogen needs time to infect the host and commence replicating. Likewise, the result of the study was highly consistent with recent findings [17,28], which consistently show that younger larval instars were more susceptible to baculovirus infections than older larvae, owing to factors such as thinner cuticles, higher feeding rates, and less developed immune responses. These findings support the observed higher infection rates in younger larvae within the first 24 hours post-infection.

Table 4: Interaction table on percent infection of different *Pieris rapae* larval instars as influenced by the treatments and time intervals (January-March 2025, La Trinidad, Benguet

TREATMENTS	LARVAL STAGES	TIME INTERVALS									
		30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
Nuclear Polyhedrosis Virus Alone	2nd Instar	0.00	15.00	20.00	67.50	97.00	100.00	100.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	10.00	12.50	45.00	75.00	95.00	100.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Cornmeal		0.00	10.00	15.00	55.00	85.00	100.00	100.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Oatmeal		0.00	12.50	15.00	55.00	87.50	100.00	100.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus Alone	3rd Instar	0.00	10.00	15.00	42.50	77.50	100.00	100.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	7.50	10.00	40.00	70.00	95.00	97.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Cornmeal		0.00	10.00	12.50	37.50	67.50	97.50	97.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Oatmeal		0.00	10.00	12.50	40.00	70.00	95.00	100.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus Alone	4th Instar	0.00	0.00	7.50	35.00	60.00	92.50	95.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	0.00	5.00	32.50	62.50	97.50	97.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus +		0.00	0.00	7.50	37.50	65.00	95.00	95.00	100.00	100.00	100.00

Cornmeal										
Nuclear Polyhedrosis Virus + Oatmeal	0.00	0.00	5.00	40.00	80.00	95.00	97.50	100.00	100.00	100.00

*No significant differences in the interaction based on the ANOVA table. Hence, pairwise comparison (post-hoc test using LSD or Tukey HSD can potentially incur type I error.

Table 5: Feeding consumption rate of different *Pieris rapae* larval instars

TREATMENT	TOTAL LEAF AREA CONSUMED (MM)
Nuclear Polyhedrosis Virus Alone	35.83 [ab]
Nuclear Polyhedrosis Virus + All-Purpose flour	31.63 [b]
Nuclear Polyhedrosis Virus + Cornmeal	35.00 [ab]
Nuclear Polyhedrosis Virus + Oatmeal	38.58 [a]

* Means with the same letter do not differ significantly at 5% LSD.

Severity of Infection on the Different *Pieris rapae* Larval Instars Under Various Treatments at Different Time Intervals

Findings of the study show that the severity of infection growth was gradual over time. All treatments and larval stages had a mean severity score of 0.00 (normal) at 30 minutes post-infection. All treatments for 2nd and 3rd larval instars, however, achieved a mean severity score of 4.00 at 168 hours post-infection, while 4th instar larvae nearly reached the maximum severity of infection indicating that majority of the larvae died from the infection. This demonstrates NPV infection progression time among treatments and larval stages.

Table 6 shows that NPV alone had the highest mean severity score of 3.10 at 48 hours post infection, indicating a severe discoloration (e.g. dark, pale, or milky white), flaccidity, anorexia, paralysis, and the onset of body liquefaction. On the other hand, NPV + All-Purpose Flour had the lowest severity, with a mean of 2.33 (mid-infection), where color of larvae changes (e.g., whitening or darkening), body became soft, lethargic and decreased of mobility. At 96 HPI, NPV alone recorded a mean severity of 3.97 not significantly different with NPV + Oatmeal having 3.93, then NPV + Cornmeal having 3.83 and NPV + All Purpose Flour with 3.80, resulting into larvae to be entirely discolored, have liquified body structure, fragile cuticle, and released of occluded bodies.

Table 6: Severity of infection rating in *Pieris rapae* larvae as affected by the treatments and time intervals (January-March 2025, La Trinidad, Benguet)

TREATMENTS	TIME INTERVALS									
	30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
Nuclear Polyhedrosis Virus Alone	0.00^{a}	0.27^{a}	0.43^{ab}	1.13^{a}	3.10^{a}	3.90^{a}	3.97^{a}	3.97^{a}	4.00^{a}	4.00^{a}
Nuclear Polyhedrosis Virus + All-Purpose Flour	0.00^{a}	0.20^{a}	0.60^{a}	1.00^{a}	2.33^{c}	3.63^{b}	3.80^{a}	3.93^{a}	3.93^{a}	3.93^{a}
Nuclear Polyhedrosis Virus + Cornmeal	0.00^{a}	0.27^{a}	0.37^{b}	0.97^{a}	2.70^{b}	3.67^{b}	3.83^{a}	3.93^{a}	3.97^{a}	3.97^{a}
Nuclear Polyhedrosis Virus + Oatmeal	0.00^{a}	0.27^{a}	0.33^{b}	1.00^{a}	2.80^{a}	3.76^{a}	3.93^{a}	3.93^{a}	3.93^{a}	3.93^{a}

*No significant differences in the interaction based on the ANOVA table. Hence, pairwise comparison (post-hoc test using LSD or Tukey HSD can potentially incur type I error.

Table 7 shows that the 2nd larval instars retained the highest mean severity of 1.42 at 24 hours post-infection, with slight discoloration, sluggish mobility and decreased appetite which was significantly higher than the 3rd and 4th larval instars ranging from 0.85 to 0.80 indicating a normal coloration, movement, and activity, but did not differ significantly from one another. This suggests that younger larvae were far more vulnerable and exhibited symptoms earlier. Second and third larval stages, however, achieved a severity score of 4.00 by 120 hours post-infection, while fourth larval instars achieved a severity score of 3.98 with entirely discolored bodies, liquified body structure, fragile cuticle and in some cases, larvae released occluded bodies.

Table 7: Severity of infection at different *Pieris rapae* larval stages and time intervals (January-March 2025, La Trinidad, Benguet)

	FACTOR B									
LARVAL STAGE	MEAN									
	30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
2nd Instar	0.00^{a}	0.48^{a}	0.88^{a}	1.42^{a}	2.92^{a}	3.85^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}
3rd Instar	0.00^{a}	0.28^{a}	0.42^{b}	0.85^{b}	2.85^{a}	3.75^{a}	3.90^{a}	4.00^{a}	4.00^{a}	4.00^{a}
4th Instar	0.00^{a}	0.00^{b}	0.00^{c}	0.80^{b}	2.42^{b}	3.62^{a}	3.75^{a}	3.98^{a}	3.98^{a}	3.98^{a}

Table 8 shows the interaction among treatments and various larval stages. Findings shown that regardless of treatment, cabbage butterfly larvae in their 2nd larval instar exhibited a more rapid increase in severity followed by 3rd and 4th larval instars but no significant difference. In comparison to 3rd larval instars (0.30-0.50) and particularly 4th larval instars (0.00 for all 4th instar treatments), the 2nd larval instar consistently exhibits greater

severity scores at 2 hours post-infection (0.80 for NPV alone, 1.30 for NPV + All-Purpose Flour, and 0.60 for NPV + Cornmeal and Oatmeal). This implies that in the early hours following exposure, 2nd larval instars were more susceptible. Additionally, larvae in their 4th larval instar did not exhibit infection with a severity score of 0.00 for 24 hours after treatment. The severity of NPV alone is 1.00 at 24 hours post-infection, whereas the other treatments have a range of 0.70 to 0.80, with a stage of infection being normal to coloration, movement, and feeding. The severity of NPV alone with 2.80 at 48 hours post-infection was greater than the other three treatments ranging from 2.30 to 2.40, with color changes to darkening, decreased mobility to flaccidity and paralysis of the body. Although, results shows that the different treatments and larval stages have no significant differences with each other since severity mean were closely related, ranging from 3.60 to 4.00 mostly at 96 to 168 HPI with severe discoloration of the larvae, onset of liquefaction to liquified body structure supporting this author's observation [29].

Baculoviruses were known to exhibit the trait of NPV infection severity increasing over time, which ultimately results in larval mortality. The observed morphological changes among the larvae, which result in visible symptoms such as discoloration, flaccidity, and liquefaction before larval death, were regularly documented in the studies of this author [30,31]. These investigations highlighted how NPV was a deadly pathogen that was obligatory for its lepidopteran hosts, progressing from early stages of infection (discoloration, sluggishness) to late stages (paralysis, liquefaction) prior to death.

Table 8: Interaction table on severity infection of different *Pieris rapae* larval instar as influenced by the various treatments and time intervals (January-March 2025, La Trinidad, Benguet)

TREATMENTS	LARVAL STAGES	TIME INTERVALS									
		30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
Nuclear Polyhedrosis Virus Alone	2nd Instar	0.00	0.50	0.80	1.60	3.30	4.00	4.00	4.00	4.00	4.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	0.40	1.30	1.50	2.30	3.75	4.00	4.00	4.00	4.00
Nuclear Polyhedrosis Virus + Cornmeal		0.00	0.50	0.60	1.40	3.00	3.80	4.00	4.00	4.00	4.00
Nuclear Polyhedrosis Virus + Oatmeal		0.00	0.50	0.60	1.40	3.10	3.90	4.00	4.00	4.00	4.00
Nuclear Polyhedrosis Virus Alone	3rd Instar	0.00	0.30	0.50	0.80	3.20	3.95	4.00	4.00	4.00	4.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	0.20	0.30	1.00	2.40	3.70	3.80	4.00	4.00	4.00
Nuclear Polyhedrosis Virus + Cornmeal		0.00	0.30	0.50	0.80	2.90	3.60	3.80	4.00	4.00	4.00
Nuclear Polyhedrosis Virus + Oatmeal		0.00	0.30	0.40	0.80	2.90	3.80	4.00	4.00	4.00	4.00
Nuclear Polyhedrosis	4th Instar	0.00	0.00	0.00	1.00	2.80	3.80	3.90	3.90	4.00	4.00

Virus Alone										
Nuclear Polyhedrosis Virus + All-Purpose Flour	0.00	0.00	0.00	0.70	2.30	3.50	3.60	3.80	3.80	3.80
Nuclear Polyhedrosis Virus + Cornmeal	0.00	0.00	0.00	0.70	2.20	3.60	3.70	3.80	3.90	3.90
Nuclear Polyhedrosis Virus + Oatmeal	0.00	0.00	0.00	0.80	2.40	3.60	3.80	3.80	3.80	3.80

*No significant differences in the interaction based on the ANOVA table. Hence, pairwise comparison (post-hoc test using LSD or Tukey HSD can potentially incur type I error.

6.2Percent Mortality of Different Pieris rapae Larval Instars Under Various Treatments at Different Time Intervals

As shown in Table 9, there was 0.00 % mortality rate for all treatments during the 30 minutes, 1 hour, and 2 hours post-infection. After 24 hours post-infection, there were no significant difference among treatments with NPV alone at 15.00 %, NPV + Cornmeal having 11.70 %, and NPV + Oatmeal with 13.30 %. However, the mortality rate was much lower in NPV + All-Purpose Flour with 7.50 % than in NPV alone, suggesting a delayed first onset of mortality. There was a significant difference after 48 hours post-infection, with NPV alone showing the highest mortality rate of 60.00 %. In contrast, NPV + Oatmeal has a mortality of 52.50 %, followed by NPV + Cornmeal with 44.20 %, and NPV + All-Purpose Flour with 37.50 %. This suggests that after 48 hours post-infection, NPV alone was significantly effective than the other treatments and the mortality rate was lower when carriers mixed with NPV, typically All-Purpose Flour.

While the carriers may hinder the rate of infection or ingestion efficiency, the increased mortality with NPV alone might be the result of a more direct and undiluted exposure to the virus. All treatments had overall mortality after 120 to 168 hours post-infection, ranging from 98.30 % to 100.00 %, with no significant difference among the treatments. This indicates that the carrier materials still show a potential option apart from NPV alone.

Table 9: Percent mortality of different *Pieris rapae* larval instars as affected by the carrier-based NPV treatment and time intervals (January-March 2025, La Trinidad, Benguet)

TREATMENTS	TIME INTERVALS									
	30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
Nuclear Polyhedrosis Virus Alone	0.00[a]	0.00[a]	0.00[a]	15.00[a]	60.00[a]	85.80[a]	94.20[a]	99.20[a]	100.00[a]	100.00[a]
Nuclear Polyhedrosis Virus + All-Purpose Flour	0.00[a]	0.00[a]	0.00[a]	7.50[b]	37.50[d]	72.50[d]	86.70[b]	98.30[a]	98.30[a]	98.30[a]
Nuclear Polyhedrosis Virus + Cornmeal	0.00[a]	0.00[a]	0.00[a]	11.70[a]	44.20[c]	77.50[c]	90.80[a]	98.30[a]	99.20[a]	99.20[a]
Nuclear Polyhedrosis Virus + Oatmeal	0.00[a]	0.00[a]	0.00[a]	13.30[a]	52.50[b]	81.70[b]	91.70[a]	98.0[a]	98.30[a]	98.30[a]

*No significant differences in the interaction based on the ANOVA table. Hence, pairwise comparison (post-hoc test using LSD or Tukey HSD can potentially incur type I error.

Findings of the study also show that at 24 hours post-infection, 2nd larval instar had the highest mortality at 18.10 %, which significantly higher from 3rd and 4th larval instars with respective means of 11.20 % and 6.20 % (Table 10). This indicates that 2nd larval instar was more susceptible to early infection causing mortality than 3rd and 4th larval instars. At 72 hours post-infection, 2nd larval instars maintained the highest mortality with 85.60 %, significantly higher from 3rd and 4th larval instars respectively, with 74.40 % and 78.10 %. The 2nd, 3rd, and 4th larval instars at 120 hours post-infection have complete mortalities ranging from 95.60 % to 100.00 % with no significant differences. This indicates that while younger larvae succumb faster, the NPV ultimately achieved comparable high mortality rates among larval stages. It was also observed that the 4th larval instar exhibited deformed bodies and wings upon pupation (Plate 2 and 3).

At 48 hours post-infection, the interaction of the treatments became more visible among the larval stages (Table 11). For 2nd larval instars, NPV alone with 70.00% and NPV + Oatmeal having 62.50%, next to NPV + Cornmeal with 60.00%, and NPV + All-Purpose Flour with 50.00%. Similar trends were observed on the 3rd and 4th larval instars, where NPV + All-Purpose Flour generally showed lower mortality (e.g., 35% for 3rd larval instars, 27.5% for 4th larval instars) with no significant difference with other treatments. Interaction on the mortality rates for all treatment and larval stages increased over time and converged to 95 to 100% by 120 hours post-infection with no significant differences, indicating the treatments used were effective and comparable in causing mortality to cabbage butterfly larvae.

It was also observed that the interaction among treatments and larval stages rise in mortality, especially for the 2nd larval instars mostly at 48 hours post-infection, having recorded temperature of 25.2 °C with 70% relative humidity (Plate 4). The temperature and relative humidity could have been an ideal condition for viral replication and disease progression among the 2nd larval instars, resulting to increase in mortality rate. This result is corroborated by the research of author [32], who observed that NPV was more efficient at moderate temperature (22 °C-25 °C) and high relative humidity (>70%), while author [33] added, the role of relative humidity in sustaining viral occlusion bodies.

Table 10: Percent mortality in *Pieris rapae* as affected by different larval stages and time intervals (January-March 2025, La Trinidad, Benguet)

LARVAL STAGE	TIME INTERVALS									
	30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
2nd Instar	0[a]	0[a]	0[a]	18.1[a]	60.6[a]	85.6[a]	94.4[a]	100[a]	100[a]	100[a]
3rd Instar	0[a]	0[a]	0[a]	11.2[b]	46.9[b]	78.1[b]	88.1[b]	100[a]	100[a]	100[a]
4th Instar	0[a]	0[a]	0[a]	6.2[c]	38.1[c]	74.4[b]	90[ab]	95.6[a]	96.9[a]	96.9[a]

Figure 2: Infected *Pieris rapae* larvae that developed into pupae

Figure 3: Early pupation of infected larvae which developed deformed wings upon adult emergence

Table 11: Interaction table on percent mortality of different Pieris rapae larval instars as affected by various treatments and time intervals (January-March 2025, La Trinidad, Benguet)

TREATMENTS	LARVAL STAGES	TIME INTERVALS									
		30 MIN.	1 HR.	2 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.	120 HRS.	144 HRS.	168 HRS.
Nuclear Polyhedrosis Virus Alone	2nd Instar	0.00	0.00	0.00	22.50	70.00	92.50	97.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	0.00	0.00	15.00	50.00	77.50	90.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Cornmeal		0.00	0.00	0.00	15.00	60.00	85.00	95.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Oatmeal		0.00	0.00	0.00	20.00	62.50	87.50	95.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus Alone	3rd Instar	0.00	0.00	0.00	15.00	62.50	85.00	92.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + All-Purpose Flour		0.00	0.00	0.00	5.00	35.00	72.50	82.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Cornmeal		0.00	0.00	0.00	12.50	37.50	75.00	87.50	100.00	100.00	100.00
Nuclear Polyhedrosis Virus + Oatmeal		0.00	0.00	0.00	12.50	52.50	80.00	90.00	100.00	100.00	100.00
Nuclear Polyhedrosis Virus Alone	4th Instar	0.00	0.00	0.00	7.50	47.50	80.00	92.50	97.50	100.00	100.00

Nuclear Polyhedrosis Virus + All-Purpose Flour	0.00	0.00	0.00	2.50	27.50	67.50	87.50	95.00	95.00	95.00
Nuclear Polyhedrosis Virus + Cornmeal	0.00	0.00	0.00	7.50	35.00	72.50	90.00	95.00	97.50	97.50
Nuclear Polyhedrosis Virus + Oatmeal	0.00	0.00	0.00	7.50	42.50	72.50	90.00	95.00	95.00	95.00

*No significant differences in the interaction based on the ANOVA table. Hence, pairwise comparison (post-hoc test using LSD or Tukey HSD can potentially incur type I error.

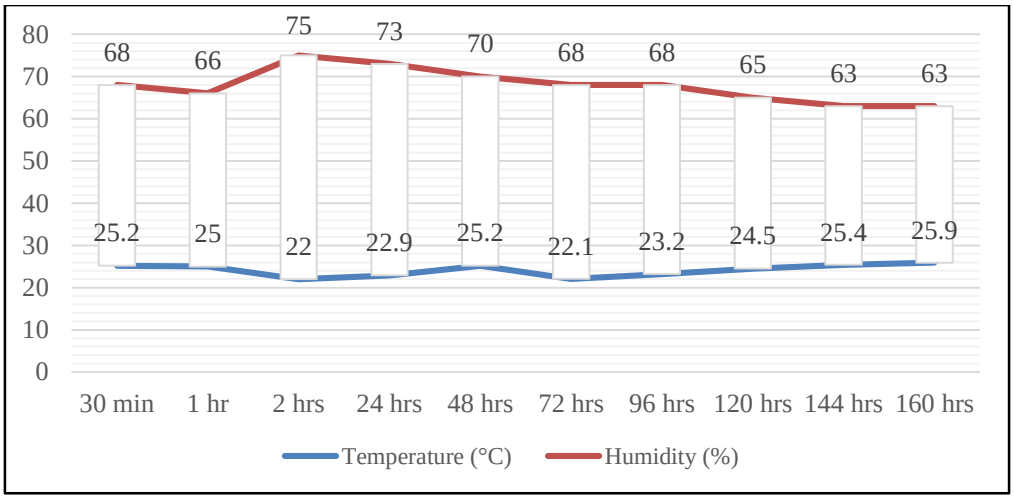

Figure 4: Temperature and relative humidity during the conduct of the study

6.3Morphological Changes of Pieris rapae as Affected by Treatments

The early infection stage in *P. rapae* larvae was indicated by decreased mobility and a slight darkening of body color. This aligns with the author's findings [34,35], who reported behavioral abnormalities in infected larvae, such as sluggish locomotion and decreased appetite, before succumbing to the infection. As the infection progresses, severity increases, often leading to flaccidity and paralysis, specifically among the 2nd and 3rd instar larvae. In some cases, larvae exhibit liquefaction or the characteristic "tree top disease" or "wipfelkrankheit," where they hang upside down (Plate 6), a phenomenon also observed by author [36]. Furthermore, the study found that not all infected larvae resulted in immediate mortality. Many experienced severe disease progressions, including anorexia (cessation of feeding), paralysis, and a fragile cuticle. Some infected larvae have gone liquefaction, releasing occluded bodies (Plate 7). This was most frequently observed during the study

among 4th larval instar, corroborating with the author's findings [29].

Figure 6: Infected *Pieris rapae* larvae in upside down position

Figure 7: Liquified body structure of the 4th instar *P. rapae* larvae

6.4Water Holding Capacity of Various NPV-Based Carrier Materials

The Nuclear Polyhedrosis Virus + Oatmeal treatment exhibited the highest water holding capacity of 95.25% garnering the highest feeding consumption rate of 38.58 mm which was not significantly different with NPV + Cornmeal with WHC of 82.60% and feeding consumption rate of 35.00 mm. On the other hand, All-Purpose Flour, had the lowest WHC of 78.46% (Table 12) which corresponded with the lowest feeding consumption rate of 31.63. This aligns closely with the results of author [37] who reported a WHC of 95.08% for Oatmeal, reinforcing the notion that Oatmeal was superior in retaining moisture and provides dietary stimulant that

promotes feeding among the larvae compared to All-Purpose Flour, which had a lower capacity due to its composition and less appealing to the cabbage butterfly larvae. Reference [38] further highlighted that the high WHC of Oatmeal was ascribed to its high β-glucan content, a soluble fiber that absorbs water and forms a viscous gel.

With the higher protein content of Oatmeal such β-glucan, this might aid physically (e.g., viscosity when hydrated) on the potency of viral particles, aligning with the author's observation [39]. Additionally, in line with these results, reference [40] highlighted that All-Purpose Flour tends to absorb less water because it had finer particles and less porosity and surface area. It has also less fiber due to milling. The densely packed structure of all-purpose flour possibly decreased absorption capacity by requiring a slower rate of water penetration through the tiny particles.

Moreover, it was observed that the pechay as host plant using leaf disc method was not completely consumed by the larvae and was dried three days after the larvae were introduced. However, even when the pechay leaves were dried, mortality occurred in all of the treatments and larval stages. This indicates that the NPV, which was absorbed by the carrier materials, was the main cause of mortality. The peak of mortality at about 120 hours, observed in the study was in line with the normal incubation period of NPVs in lepidopteran larvae, during which the virus multiplies and ultimately kills the host, as observed by author [41].

Table 12: Water holding capacity of the various carrier materials

Carrier Materials	Total Mean
All-Purpose Flour (APF)	78.46^{c}
Cornmeal	82.60^{b}
Oatmeal	95.25^{a}

* Means with the same letter do not differ significantly at 5% LSD.

6.5Economic Analysis

The study's expenditures, amounted to PhP 5,921.00, distributed among protective structures (PhP 1,938.00), monitoring equipment (PhP 150.00), and labor (PhP 2,000.00). The materials accounted to about PhP 1,833.00 which comprised about 30% of the total expenses.

According to the findings of this study, the application of NPV alone was the most effective in progressing from infection to mortality, having the low cost amounting of PhP 343.50 per treatment. This shows that it is the most economically viable and efficient option. However, among the carrier materials, the NPV + Oatmeal treatment demonstrated potential as a carrier, although, with higher cost amounting to PhP 542.50 per treatment which makes it less affordable. Treatments with Cornmeal amounting to PhP 523.50 and All-Purpose Flour amounting to PhP 423.50 also shown to be effective but requires higher cost (Table 13).

The NPV alone treatment simplifies preparation and applications. While other treatments that incorporate carrier

materials such as All-Purpose Flour, Cornmeal, and Oatmeal may have potential benefits, their significantly higher input costs, could be a barrier for many farmers to apply the technology.

Table 13: Cost of expenses per treatment

TREATMENT	TOTAL ESTIMATED COST PER TREATMENT (PhP)
Nuclear Polyhedrosis Virus Alone	343.50
Nuclear Polyhedrosis Virus + All-Purpose Flour	423.50
Nuclear Polyhedrosis Virus + Cornmeal	523.50
Nuclear Polyhedrosis Virus + Oatmeal	542.50
Total:	1,833.00
Overall Total Cost:	5,921.00

7. Conclusion

This study examined the biological control against *Pieris rapae* using Nuclear Polyhedrosis Virus to reduce reliance on chemical pesticides and promote integrated pest management strategies. The study showed that NPV alone was the most effective treatment causing mortality for *P. rapae* and economically viable treatment followed by NPV + Oatmeal, NPV + Cornmeal, and NPV + All-Purpose Flour. In terms of percent infection, severity of infection, and percent mortality, 2nd larval instars were the most susceptible, followed by 3rd and 4th larval instars. These results were directly influenced by feeding consumption and environmental factors like temperature of 25 °C and relative humidity greater than 70% recorded during the study. Morphological changes like sluggishness, discoloration, paralysis, and liquefaction appeared gradually over time. Additionally, an increase in time exposure of the different larval instars to the various treatments corresponds to a higher percent infection, severity of infection, and percent mortality.

Carrier materials can alter NPV efficacy, such as water retention which directly influenced viral transmission. Oatmeal and Cornmeal as carriers exhibited intermediate efficacy, while All-Purpose Flour had the lowest result. Further, NPV alone is the most economical treatment at PhP 343.50 offering a significant advantage due to its lower cost and ease of use.

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"*Namwaw ay maid labas ken dakayo amin. Adik masubadan amin ay naimpasek ay badang you. Iya-iyaman*".

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