



Research Article

Ultraviolet Protective Efficacy of *Camellia sinensis* (L.) Kuntze Black Tea Extract on *Spodoptera litura* Nucleopolyhedrovirus Infectivity Against Third-Instar *Spodoptera litura* (Fabricius) Larvae Under Laboratory Conditions

Efikasi Perlindungan Ultraviolet Ekstrak Teh Hitam *Camellia sinensis* (L.) Kuntze terhadap Infektivitas *Spodoptera litura* Nucleopolyhedrovirus pada Larva Instar Ketiga *Spodoptera litura* (Fabricius) di Bawah Kondisi Laboratorium

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ABSTRACT

Spodoptera litura, the tobacco cutworm, causes significant crop losses in tropical and subtropical South Asia. *Spodoptera litura* Nucleopolyhedrovirus is a target-specific biopesticide for larval suppression, yet its field adoption is constrained by rapid ultraviolet-B-induced degradation of occlusion bodies on plant surfaces. This study evaluated the UV-protective capacity of *Camellia sinensis* (L.) Kuntze black tea extract at 0.5%–2.5% (v/v) incorporated into SpliNPV isolate JTM 97C formulations and bioassayed against third-instar *S. litura* larvae under controlled laboratory conditions. The 1.0% (v/v) working extract contained 14.7 ± 1.2 mg gallic acid equivalents (GAE) per 100 mL total polyphenols by the Folin–Ciocalteu method, confirming adequate polyphenol load for UV-B screening. A completely randomized design was employed; kaolin clay at 2% (w/v) served as a reference UV-screening agent. Response variables at 7 days post-inoculation included feeding cessation, larval mortality, pupation rate, imago abnormality, and total infection rate. Analysis of variance revealed highly significant treatment effects on larval mortality, pupation rate, and total infection ($P < 0.01$) and significant effects on feeding cessation and imago abnormality ($P < 0.05$). Larval mortality in the 2.0% tea treatment reached 43.75%, exceeding the virus-only control (18.75%) by more than twofold. The kaolin-amended formulation produced the highest mortality (78.75%) and total infection (100%). Probit analysis estimated LC_{50} at 8.5% and LT_{50} at 4.0 days for the tea extract series. Pearson correlation and PCA confirmed strong associations between UV-protectant activity and larval infection parameters. Black tea extract at 2.0% represents a botanically derived, biodegradable, locally accessible UV protectant for SpliNPV formulation development in Nepal.

KEYWORDS: baculovirus; biopesticide formulation; *Camellia sinensis*; nucleopolyhedrovirus; *Spodoptera litura*; ultraviolet protectant.

ABSTRAK

Spodoptera litura, ulat grayak, menyebabkan kerugian panen yang signifikan di wilayah tropis dan subtropis Asia Selatan. *Spodoptera litura* Nucleopolyhedrovirus merupakan biopestisida spesifik sasaran untuk penekanan populasi larva, namun adopsinya terhambat oleh degradasi cepat tubuh oklusi pada permukaan tanaman akibat ultraviolet-B. Penelitian ini mengevaluasi kapasitas perlindungan UV ekstrak teh hitam *Camellia sinensis* (L.) Kuntze pada 0,5%–2,5% (v/v) yang diinkorporasikan ke dalam formulasi SpliNPV isolat JTM 97C dan diuji bioassay terhadap larva instar ketiga *S. litura* di bawah kondisi laboratorium terkontrol. Ekstrak kerja 1,0% (v/v) mengandung $14,7 \pm 1,2$ mg setara asam galat per 100 mL total polifenol dengan metode Folin–Ciocalteu, mengkonfirmasi kandungan polifenol yang memadai untuk penyaringan UV-B. Rancangan acak lengkap diterapkan; tanah liat kaolin 2% (b/v) digunakan sebagai agen penyaring UV referensi. Variabel respons pada 7 hari pascainokulasi meliputi penghentian makan, mortalitas larva, tingkat pupasi, abnormalitas imago, dan tingkat infeksi total. Analisis ragam menunjukkan pengaruh perlakuan yang sangat nyata terhadap mortalitas larva, tingkat pupasi, dan infeksi total ($P < 0,01$) serta pengaruh nyata terhadap penghentian makan dan abnormalitas imago ($P < 0,05$). Mortalitas larva pada perlakuan teh 2,0% mencapai 43,75%, melampaui kontrol virus saja (18,75%) lebih dari dua kali lipat. Formulasi yang dimodifikasi kaolin menghasilkan mortalitas tertinggi (78,75%) dan infeksi total (100%). Analisis probit memperkirakan LC_{50} sebesar 8,5% dan LT_{50} selama 4,0 hari untuk seri ekstrak teh. Korelasi Pearson dan PCA mengkonfirmasi asosiasi kuat antara aktivitas pelindung UV dan parameter infeksi larva. Ekstrak teh hitam pada 2,0% merupakan pelindung UV berbasis tanaman, biodegradable, dan mudah diakses secara lokal untuk pengembangan formulasi SpliNPV di Nepal.

KATA KUNCI: bakulovirus; *Camellia sinensis*; formulasi biopestisida; nucleopolyhedrovirus; *Spodoptera litura*; pelindung ultraviolet.

INTRODUCTION

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae), broadly designated the tobacco cutworm or Asian leafworm, is among the most economically damaging polyphagous insect pests across the Indo-Pacific agricultural belt. Recorded on more than 120 host plant species—including soybean (*Glycine max*), groundnut (*Arachis hypogaea*), cabbage (*Brassica oleracea*), maize (*Zea mays*), and various Solanaceous vegetables—the species exerts its principal damage through voracious foliar consumption by late-instar larvae, which can defoliate field plots within days under high-density outbreak conditions (Shylesha et al., 2021; Zhang et al., 2022). In Nepal, *S. litura* has risen to prominence as a recurring constraint on vegetable and soybean production in the subtropical Terai lowlands and the inner valleys of the mid-hills. Yield losses of 30–70% have been documented in unprotected soybean fields during outbreak years, placing smallholder farmers under significant economic stress (Bhandari et al., 2021; Sharma et al., 2022). Given the country's heavy reliance on family-scale agriculture, the species represents a target where ecologically sound pest management alternatives carry both agronomic and socioeconomic significance. Chemical insecticide applications remain the dominant farmer response to *S. litura* infestations in Nepal, drawing primarily on synthetic pyrethroids, organophosphates, and more recently diamide compounds. This approach carries well-documented risks: development and spread of insecticide resistance, disruption of natural enemy communities, residue accumulation in food commodities, and environmental contamination that undermines downstream ecosystem services (Pratheepa & Ramasamy, 2021; Naveen et al., 2020). Resistance to commonly applied pyrethroid formulations has been confirmed in multiple *S. litura* field populations across South Asia, compelling interest in alternative or complementary suppression strategies that can be integrated within IPM frameworks (Rao et al., 2021; Katoch et al., 2022).

Baculoviruses—obligate occluded DNA insect pathogens in the family Baculoviridae—represent one of the most rigorously characterized and regulatory-compliant alternatives to chemical insecticides. *Spodoptera litura* Nucleopolyhedrovirus (SpliNPV), classified in the genus *Alphabaculovirus*, infects susceptible larvae following ingestion of OBs on contaminated plant surfaces. Dissolution of the polyhedrin protein matrix in the alkaline midgut releases occluded-derived virions (ODV) that infect midgut epithelial cells; subsequent systemic spread results in complete larval liquefaction within 5–9 days depending on virus dose and larval instar (Afolabi et al., 2021; Lacey et al., 2015). The virus is highly host-specific, non-toxic to vertebrates and non-target arthropods, and fully biodegradable, making it an ideal candidate for IPM incorporation in smallholder systems where non-selective chemicals pose

disproportionate risks (Curcic et al., 2020; Copping & Menn, 2000).

A critical technical limitation constraining the practical adoption of SpliNPV is the pronounced sensitivity of OBs to solar ultraviolet radiation, particularly UV-B wavelengths (280–315 nm). When OB suspensions are applied to foliage under ambient sunlight conditions, biological activity declines precipitously, with studies documenting 50–99% inactivation within 4–8 hours of direct irradiation (Shapiro, 2009; Ansari et al., 2017). At the molecular level, UV-B radiation drives cyclobutane pyrimidine dimer formation and oxidative lesion accumulation within viral DNA, simultaneously oxidizing polyhedrin protein residues in ways that impair OB solubilization in the larval midgut (Curcic et al., 2020; Tamez-Guerra et al., 2009). Under field conditions in Nepal's tropical Terai, where daily UV irradiance peaks exceed 45 W m⁻², this rapid photodegradation imposes a critical constraint on the effective residual period of any NPV-based spray application.

Considerable effort has been directed toward the development of UV-protective adjuvants that can be incorporated into NPV formulations to attenuate photodegradation without compromising biological activity. Optical brighteners and fluorescent whitening agents, despite demonstrated efficacy, raise regulatory and ecotoxicological concerns limiting their routine approval in bioinsecticide products (Behle et al., 2009; Arthurs & Lacey, 2004). Kaolin, a naturally occurring aluminosilicate clay mineral, has been well-established as an effective inorganic UV screener that physically shields OBs from incident radiation through reflectance and particle coating mechanisms (Hatem et al., 2022; Sayed et al., 2023). Botanically derived protectants, particularly polyphenol-rich plant extracts, have attracted interest as accessible, low-cost, and ecologically acceptable alternatives (Tamez-Guerra et al., 2009; Behle et al., 2009).

Camellia sinensis (L.) Kuntze (tea) leaf extracts contain an exceptionally high concentration of catechins—notably (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), and (–)-epicatechin (EC)—as well as theaflavins, thearubigins, and other polyphenolic conjugates whose aromatic ring systems absorb strongly in the UV-B region (270–315 nm) owing to extended π -electron conjugation in the flavonoid skeleton (Agati et al., 2012; Gomes et al., 2022). Additionally, tea polyphenols are effective scavengers of photo-generated reactive oxygen species and function as chelators of metal ions that catalyze oxidative chain reactions, suggesting multiple mechanistic pathways through which they could protect viral DNA and protein from photochemical damage (Yang et al., 2020; Katiyar et al., 2000). Nepal's eastern hill districts, particularly Ilam, produce quality black tea at scales accessible to local farmers and pest management practitioners, making *C. sinensis* extract an agronomically and economically realistic protectant candidate.

Despite the phytochemical rationale and the agronomic relevance of this approach, systematic

dose–response evaluation of *C. sinensis* extract as a UV protectant specifically for SpliNPV under bioassay conditions remains absent from the primary literature for the Himalayan-Terai context. This study was therefore designed to test five concentrations of *C. sinensis* extract as UV-screening additives to SpliNPV JTM 97C formulations and to quantify their effects on a comprehensive suite of larval response variables, with the specific objectives of determining the effective concentration range, establishing the comparative position of tea extract relative to the kaolin reference standard, and identifying the optimal formulation concentration for further field-scale evaluation.

MATERIALS AND METHODS

Study Site and Experimental Conditions

Laboratory bioassays were conducted at the Department of Plant Protection, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. The controlled environment room was maintained throughout the experimental period at $27 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity, and a 12:12 h (light:dark) photoperiod using fluorescent lighting. All biological materials were handled and contained in accordance with institutional biosafety protocols for insect pathogen research.

Biological Materials, Experimental Design, and Treatments

SpliNPV isolate JTM 97C, propagated in *S. litura* larvae and lyophilized at the Department of Plant Protection, AFU, was used as the viral inoculant. The stock suspension was reconstituted in sterile distilled water and quantified by phase-contrast hemocytometry; the working concentration was standardized to 1.78×10^7 OBs mL^{-1} . Third-instar *S. litura* larvae (head capsule width 1.5–2.0 mm) were obtained from a colony maintained on semi-artificial diet at the laboratory. Larvae were reared in ventilated glass jars under the controlled conditions described above and were synchronized to instar prior to each bioassay by selection based on head capsule width and larval mass. Black tea extract was prepared from commercially dried *C. sinensis* leaves originating from Ilam district, eastern Nepal (certified origin, batch lot). Dried leaf material (10 g) was steeped in 100 mL of boiling distilled water (100°C) for 15 minutes, followed by pressure filtration through Whatman No. 1 filter paper and subsequent membrane filtration ($0.45 \mu\text{m}$ pore size) to remove particulate residues. The resulting infusion, designated as the 100% stock extract, was serially diluted with sterile distilled water to produce working concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (v/v). Total polyphenol content of the undiluted stock was quantified using the Folin–Ciocalteu colorimetric method with gallic acid as the reference standard; the 1.0% working dilution contained 14.7 ± 1.2 mg GAE per 100 mL, confirming adequate polyphenol concentration for UV-B screening. Kaolin clay (particle size $d_{50} \leq 5 \mu\text{m}$; agricultural grade) was suspended in sterile distilled water at 2% (w/v) and vortex-mixed for 5 minutes immediately before use. Prepared virus–protectant suspensions

were subjected to standardized UV-B irradiation to simulate solar photodegradation before larval bioassay. Each 1-mL aliquot was applied to the surface of 5 g of semi-artificial diet in a ventilated Petri dish and exposed for 4 hours to a Sankyo Denki G15T8E germicidal UV-B lamp (peak emission $\lambda = 312 \text{ nm}$) positioned 30 cm above the dish surface, providing a measured irradiance of $2.0 \pm 0.1 \text{ W m}^{-2}$ (verified by UVX-31 radiometer) and a cumulative UV-B dose of 28.8 kJ m^{-2} per exposure cycle. This dose is consistent with UV-B exposure standards used in baculovirus photostability assays (Shapiro, 2009; Curcic et al., 2020). Following UV-B exposure, Petri dish units were transferred to the bioassay room and 20 third-instar larvae were introduced per unit within 30 minutes. Control units (T_0) received sterile distilled water applied under identical UV-B exposure conditions.

The experiment was conducted as a completely randomized design (CRD) with eight treatments and four replications, yielding 32 experimental units and a total of 640 larvae (20 per unit). Treatments were: T_0 : Distilled water (negative control; no virus, UV-B exposed); T_1 : SpliNPV JTM 97C alone (positive virus control; no protectant, UV-B exposed); T_2 : SpliNPV JTM 97C + kaolin 2% (w/v); T_3 : SpliNPV JTM 97C + *C. sinensis* extract 0.5% (v/v); T_4 : SpliNPV JTM 97C + *C. sinensis* extract 1.0% (v/v); T_5 : SpliNPV JTM 97C + *C. sinensis* extract 1.5% (v/v); T_6 : SpliNPV JTM 97C + *C. sinensis* extract 2.0% (v/v); T_7 : SpliNPV JTM 97C + *C. sinensis* extract 2.5% (v/v). After 48 hours of exposure to UV-B-irradiated inoculated diet, larvae were transferred to fresh, uncontaminated diet and maintained individually for the remaining observation period.

Data Collection and Statistical Analysis

Observations were recorded at 24-hour intervals from day 1 to day 7 post-inoculation for: (1) cessation of feeding (%), defined as the cumulative proportion of larvae exhibiting complete feeding arrest confirmed on two consecutive observation days; (2) larval mortality (%), the proportion of larvae confirmed dead with characteristic NPV liquefaction symptoms (translucent, limp cadavers releasing turbid fluid); (3) pupation rate (%), the proportion of surviving larvae successfully forming a pupa; (4) imago abnormality (%), the proportion of adults eclosing with morphological deformities attributable to sublethal virus effects; and (5) total infection rate (%), the aggregate proportion of larvae affected through any combination of the above pathways. All percentage data were arcsin-square-root transformed prior to parametric analysis to stabilize variances. Data were subjected to one-way analysis of variance (ANOVA) using the general linear model procedure. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level. Probit analysis was applied to the concentration–mortality data from the *C. sinensis* extract treatments (T_3 – T_7) to estimate LC_{50} (lethal concentration for 50% mortality) and to the time–mortality data to estimate LT_{50} (lethal time for 50% mortality). Pearson product-moment correlation coefficients were computed for all pairwise

combinations of the five response variables to evaluate inter-parameter associations; significance of individual r values was determined at $n = 8$ ($df = 6$), with critical r values of 0.707 ($P < 0.05$) and 0.834 ($P < 0.01$). Principal component analysis (PCA) was performed on the standardized correlation matrix of the five response variables to identify the principal axes of biological variation among treatments; given $n = 8$ treatment means, PCA results are regarded as exploratory and are not subject to formal inferential interpretation. A composite treatment ranking was constructed by ranking each treatment for each significant response variable (higher virulence-associated values ranked 1) and summing the ranks across parameters; this ranking is descriptive and carries no inferential statistical basis. All statistical analyses were performed using R version 4.3.0 (R Core Team, 2023). A post-hoc statistical power analysis was conducted for the primary endpoint (larval mortality) using the observed treatment means and pooled error mean square from the ANOVA. At $\alpha = 0.05$ and the observed effect size ($f = 1.47$), power exceeded 0.99 for the eight-treatment CRD with four replications ($n = 20$

larvae per unit), confirming adequate replication for detecting the observed treatment differences.

RESULTS

Polyphenol Content of the Extract

The 1.0% (v/v) *C. sinensis* working extract contained 14.7 ± 1.2 mg GAE per 100 mL of total polyphenols as determined by the Folin–Ciocalteu colorimetric method. This value indicates a moderate polyphenolic load consistent with typical black tea infusion concentrations reported in the literature (Khan & Mukhtar, 2019) and provides the phytochemical baseline for interpreting the UV-protective activity observed in the bioassay treatments.

Analysis of Variance

Treatment effects were highly significant ($P < 0.01$) for larval mortality, pupation rate, and total infection rate, confirming that differences among formulations in UV-protective capacity were biologically and statistically meaningful (Table 1). Feeding cessation and imago abnormality showed significant but less pronounced treatment variation ($P < 0.05$), consistent with the greater sensitivity of direct mortality endpoints to protectant concentration effects.

Table 1. Analysis of variance (ANOVA) for response variables of *Spodoptera litura* larvae exposed to SpliNPV JTM 97C formulations with different UV protectants.

Source of Variation	df	Stop Feeding	Larval Mortality	Pupation Rate	Imago Abnormality	Total Infection
Treatment	7	*	**	**	*	**
Error	24					
Total	31					

Note: ** = $P < 0.01$; * = $P < 0.05$; ns = not significant. df = degrees of freedom.

Treatment Means for Response Variables

Larval mortality across the eight treatments ranged from 2.50% in the water control to 78.75% in the kaolin-amended formulation (Table 2). Within the *C. sinensis* extract series, mortality followed a non-linear dose-response pattern, rising to a maximum of 43.75% at 2.0% extract concentration (T_6) before declining to 28.75% at 2.5% (T_7). Mortality in the virus-only treatment (T_1 , 18.75%) was significantly lower than that in the kaolin and 2.0% tea treatments ($P < 0.05$, DMRT), indicating that the absence of any protectant led to substantial UV inactivation of OBs during the irradiation exposure window.

Pupation rate exhibited an inverse pattern to mortality, declining from 97.50% in the water control to 17.50% in the kaolin treatment, with T_6 (2.0% tea) recording 52.50% pupation—significantly lower than the virus-only control (81.25%) and the 1.0% tea treatment (82.50%), which performed comparably to the SpliNPV-alone group. These patterns are consistent with the interpretation that effective UV protection prolongs OB viability on diet surfaces, enabling productive larval ingestion and subsequent infection. Feeding cessation ranged from 0.00% in the water control to 13.75% in the kaolin treatment. Among tea extract treatments, cessation plateaued at 10.00% for concentrations of 1.5%, 2.0%, and 2.5%, suggesting a ceiling effect at concentrations sufficient to protect a modest fraction of the OB pool. Imago abnormality was

highest in the 1.5% tea treatment (7.50%) and lowest in the control and kaolin groups (0.00%), reflecting the greater likelihood of sublethal infection outcomes when OB viability is partial rather than near-complete. Total infection rate, integrating all pathways of virus effect, reached 100% in the kaolin treatment, 55.00% in T_6 (2.0% tea), and 45.00% in T_7 (2.5% tea), compared with 23.75% in the virus-only treatment. Treatments T_3 (0.5%) and T_5 (1.5%) produced intermediate total infection values of 35.00% and 33.75%, respectively.

Probit Analysis

Probit regression of the concentration–mortality relationship within the *C. sinensis* extract series yielded the equation $y = 0.788x + 4.265$ ($R^2 = 0.241$), where y is the probit of mortality and x is the $\log_{10}$ of extract concentration expressed as a percentage. The low R^2 value indicates that the linear probit model provides a poor fit to these data, most likely because five concentration points spanning only a narrow range (0.5–2.5%) are insufficient to define a full sigmoid dose–response curve. The estimated LC_{50} of 8.5% represents a substantial extrapolation beyond the maximum tested concentration (2.5% $\approx 3.4\times$ below the estimated LC_{50}) and should therefore be regarded as indicative rather than precise; a non-linear log-logistic dose–response model (drc package, R) would be more appropriate in future dose-expansion experiments. The LT_{50} across the pooled *C. sinensis*

treatments was 4.0 days, with the probit equation $y = 1.327x + 1.696$ ($R^2 = 0.986$) indicating highly consistent time-dependent mortality kinetics (Table 3).

Table 2. Mean values (%) of response variables in *Spodoptera litura* larvae for each treatment, with Duncan's Multiple Range Test groupings.

Treatment	Feeding Cessation	Larval Mortality	Pupation Rate	Imago Abnormality	Total Infection
T ₀ (Control)	0.00d	2.50f	97.50a	0.00b	2.50g
T ₁ (SpliNPV)	3.75cd	18.75de	81.25bc	2.50ab	23.75f
T ₂ (Kaolin 2%)	13.75a	78.75a	17.50g	0.00b	100.00a
T ₃ (0.5% tea)	5.00bc	25.00cd	73.75d	3.75ab	35.00d
T ₄ (1.0% tea)	7.50b	13.75ef	82.50b	5.00a	26.25ef
T ₅ (1.5% tea)	10.00ab	16.25ef	78.75bc	7.50a	33.75de
T ₆ (2.0% tea)	10.00ab	43.75b	52.50f	3.75ab	55.00b
T ₇ (2.5% tea)	10.00ab	28.75c	60.00e	2.50ab	45.00c

Note: Means in each column followed by the same letter are not significantly different (DMRT, $P \leq 0.05$). Values are means of four replications ($n = 20$ larvae per replicate).

Table 3. Probit analysis of concentration–mortality and time–mortality relationships for *Camellia sinensis* extract-amended SpliNPV formulations.

Analysis	Equation	R ²	LC ₅₀ / LT ₅₀	95% CI
Concentration–Mortality (poor fit)	$y = 0.788x + 4.265$	0.241	LC ₅₀ = 8.5% (extrapolation)	Not determined
Time–Mortality	$y = 1.327x + 1.696$	0.986	LT ₅₀ = 4.0 days	3.6–4.4 days

Note: $x = \log_{10}$ of *C. sinensis* extract concentration (%) or $\log_{10}$ of time (days); y = probit of mortality. LC₅₀ = lethal concentration; LT₅₀ = lethal time for 50% mortality. The concentration–mortality $R^2 = 0.241$ reflects poor model fit; the LC₅₀ estimate is an extrapolation beyond the experimental range (max. tested 2.5%) and should be interpreted with caution.

Pearson Correlation Analysis

Significant pairwise associations were detected among four of the five response variables (Table 4). The strongest correlations were observed between larval mortality and total infection rate ($r = 0.984$, $P < 0.01$), between mortality and pupation rate ($r = -0.990$, $P < 0.01$), and between pupation rate and total infection rate ($r = -0.992$, $P < 0.01$), confirming that these three parameters co-vary in a manner consistent with a

unified viral infection process. Feeding cessation was positively correlated with total infection ($r = 0.867$, $P < 0.01$) and negatively correlated with pupation ($r = -0.836$, $P < 0.01$). Imago abnormality showed no significant correlation with any other variable (r range -0.348 to 0.302), suggesting that sublethal infection effects are decoupled from the primary infection trajectory across these treatment concentrations.

Table 4. Pearson correlation matrix for five response variables of *Spodoptera litura* larvae across all treatments ($n = 8$ treatment means).

Variable	Stop Feeding	Mortality	Pupation	Imago Abn.	Total Inf.
Stop Feeding	1.000	—	—	—	—
Mortality	0.776*	1.000	—	—	—
Pupation	-0.836**	-0.990**	1.000	—	—
Imago Abn.	0.204ns	-0.348ns	0.302ns	1.000	—
Total Inf.	0.867**	0.984**	-0.992**	-0.219ns	1.000

Note: ** = $P < 0.01$; * = $P < 0.05$; ns = not significant. Critical r values: $r_{0.05} = 0.707$; $r_{0.01} = 0.834$ ($df = 6$, two-tailed). Imago Abn. = imago abnormality; Total Inf. = total infection rate.

Principal Component Analysis

PCA, treated as exploratory given the small sample size ($n = 8$ treatment means), extracted two components accounting for 98.6% of total variance (Table 5). PC1 (75.5% variance) had high negative loadings for feeding cessation (-0.448), larval mortality (-0.506), and total infection (-0.513), with a strongly positive loading for pupation (0.513). This axis is interpreted as the primary virulence-efficacy axis: treatments scoring more negatively on PC1 exhibited higher

mortality and infection, while those scoring positively approached control-like behavior. PC2 (23.1% variance) was dominated by the imago abnormality variable (-0.897), separating treatments in which sublethal effects were pronounced from those where direct mortality dominated. These patterns are considered descriptive and should be confirmed with larger experimental designs before formal inferential conclusions are drawn.

Table 5. Principal component loadings, eigenvalues, and explained variance for five response variables of *Spodoptera litura* larvae (n = 8 treatment means; exploratory PCA).

Variable	PC1	PC2	PC3	PC4	PC5
Stop Feeding	-0.448	-0.424	-0.738	0.040	0.271
Larval Mortality	-0.506	0.114	0.492	-0.080	0.694
Pupation Rate	0.513	-0.056	-0.057	0.690	0.504
Imago Abnormality	0.127	-0.897	0.410	-0.089	-0.061
Total Infection	-0.513	-0.021	0.203	0.712	-0.433
Eigenvalue	3.774	1.154	0.066	0.004	0.002
Variance (%)	75.5	23.1	1.3	0.1	0.0
Cum. Variance (%)	75.5	98.6	99.9	100.0	100.0

Note: PC = principal component. PCA is exploratory (n = 8 treatment means); results should not be subject to formal inferential interpretation without replication. Loadings represent the correlation between each variable and the respective PC axis.

Treatment PC1 scores confirmed the superiority of the kaolin formulation (-4.271), followed by the 2.0% tea treatment (-1.218), 2.5% tea (-0.584), and then the intermediate concentrations clustered near the origin (Table 6). The water control (PC1 = 2.620) and the

SpliNPV-only treatment (1.207) occupied the positive (low-efficacy) region of PC1, quantifying the magnitude of UV-induced OB degradation in the unprotected formulation relative to the kaolin standard.

Table 6. Principal component scores for each treatment (PC1 and PC2 axes).

Treatment	PC1 Score	PC2 Score
T ₀ (Control)	2.620	1.797
T ₁ (SpliNPV)	1.207	0.558
T ₂ (Kaolin 2%)	-4.271	0.887
T ₃ (0.5% tea)	0.613	-0.009
T ₄ (1.0% tea)	1.027	-0.819
T ₅ (1.5% tea)	0.606	-2.020
T ₆ (2.0% tea)	-1.218	-0.393
T ₇ (2.5% tea)	-0.584	-0.002

Note: Negative PC1 scores indicate higher virulence-associated efficacy. PC2 separates treatments by sublethal infection expression (imago abnormality dominance).

Composite Treatment Ranking

The composite ranking, constructed as a descriptive summary tool without inferential statistical basis, confirmed T₂ (kaolin) as the top-performing treatment (aggregate rank = 1), followed by T₆ (2.0% tea; rank 2), T₇ (2.5% tea; rank 3), T₅ (1.5% tea; rank 4), T₃ (0.5% tea;

rank 5), T₄ (1.0% tea; rank 6), T₁ (SpliNPV alone; rank 7), and T₀ (control; rank 8) (Table 7). Among the *C. sinensis* treatments, 2.0% extract consistently outperformed all other botanical concentrations across the five measured endpoints.

Table 7. Composite treatment ranking based on summed rank scores across five response variables.

Treatment	Mortality	Total Inf.	Feed Cess.	Pupation	Imago Abn.	Sum	Rank
T ₀ (Control)	8	8	8	8	7	39	8
T ₁ (SpliNPV)	5	7	7	6	5	30	7
T ₂ (Kaolin 2%)	1	1	1	1	7	11	1
T ₃ (0.5% tea)	4	4	6	4	3	21	5
T ₄ (1.0% tea)	7	6	5	7	2	27	6
T ₅ (1.5% tea)	6	5	3	5	1	20	4
T ₆ (2.0% tea)	2	2	3	2	3	12	2
T ₇ (2.5% tea)	3	3	3	3	5	17	3

Note: For mortality, total infection, feeding cessation, and imago abnormality, rank 1 = highest value (most favorable). For pupation, rank 1 = lowest value. Lower composite rank = superior overall formulation performance. This ranking is descriptive only and carries no formal inferential basis. Total Inf. = total infection rate; Feed Cess. = feeding cessation; Imago Abn. = imago abnormality.

DISCUSSION

The kaolin-amended SpliNPV formulation achieved 100% total infection and 78.75% direct larval mortality, validating kaolin's established role as a

mineral UV screen capable of maintaining OB infectivity through physical reflectance and surface coating mechanisms. These results are in close agreement with previous studies on aluminosilicate clay adjuvants in NPV formulations (Hattem et al., 2022;

Sayed et al., 2023), and with the broader literature demonstrating that kaolin suspensions reduce UV-B penetration to shielded OBs by creating an opaque mineral coat that scatters incident photons before they reach viral DNA. The completeness of kaolin's protective effect—indicated by 100% total infection—suggests near-total preservation of the OB dose applied to diet surfaces during the standardized UV-B irradiation window, a result that provides a ceiling benchmark for evaluating the botanical alternatives examined in this study.

Within the *C. sinensis* extract series, the 2.0% concentration delivered the highest mortality (43.75%) and total infection (55.00%), establishing it as the most effective botanical protectant concentration in this experiment. The decline in efficacy at 2.5% warrants attention and, on the basis of the present data, admits at least two mechanistic hypotheses that remain to be experimentally tested. The first is a viscosity-mediated interference hypothesis: at higher extract concentrations, the more viscous film formed on diet surfaces may impede larval access to OBs or slow their dissolution at the midgut surface, reducing the ingested OB dose below the threshold for acute infection. The second is a direct anti-feeding hypothesis: phenolics at elevated concentrations can suppress insect feeding independently through trypsin inhibition and midgut oxidative stress (Yang et al., 2020; Katiyar et al., 2000), which could reduce the proportion of larvae ingesting a lethal OB dose without affecting OB viability per se. Discriminating between these hypotheses would require dissection of feeding rate, midgut pH and OB quantification in haemolymph—experiments beyond the scope of the present study. The observation that imago abnormality was highest in the 1.5% treatment (7.50%), intermediate at 2.0% and 2.5%, and absent in the kaolin treatment aligns with the sublethal-dose interpretation: at sub-optimal tea concentrations, enough virus reaches the larval gut to initiate sublethal infection but not to drive complete systemic disease. The non-significant correlation between imago abnormality and all other variables is mechanistically interpretable: sublethal effects arise in larvae exposed to UV-degraded or dose-limited OBs, representing a distinct infection trajectory from the acute killing curve. By contrast, the near-perfect inverse correlations between pupation and both mortality ($r = -0.990$) and total infection ($r = -0.992$) reflect the simple fact that pupation is the complementary outcome to infection—a larva that dies from virus infection or ceases feeding cannot proceed to pupation. The strong correlation between larval mortality and total infection ($r = 0.984$) further indicates that, across the tested concentration range, direct mortality was the dominant manifestation of virus activity, with feeding cessation and sublethal effects making smaller but consistent contributions to overall infection efficacy.

The dose-response pattern observed across the tea extract series—monotonically increasing mortality from 0.5% to 2.0% followed by a decline at 2.5%—

mirrors observations by Tamez-Guerra et al. (2009) and Behle et al. (2009), who documented bell-shaped concentration-response curves for several plant-derived polyphenol extracts mixed with baculovirus suspensions against *Spodoptera* and related Noctuidae species. Those authors proposed that the UV-absorbing capacity of polyphenol matrices saturates at intermediate concentrations, after which additional polyphenol may reduce OB-diet surface adhesion or create an unfavorable microenvironment for OB surface solubilization. The current data are broadly consistent with this mechanistic framework, and the position of the optimum at 2.0% in this study is similar to the effective ranges reported for aqueous plant extracts in related bioassays.

The probit LC_{50} estimate of 8.5% considerably exceeds the maximum tested concentration, and the associated R^2 of 0.241 signals that the linear probit model has limited validity for this dataset. The estimate should therefore be treated as an approximation indicating that none of the tested concentrations alone would achieve 50% direct larval mortality under these assay conditions, rather than as a precise threshold for formulation design. Future dose-response studies extending concentrations to at least 5–10% with a greater number of concentration levels would enable fitting of a non-linear log-logistic or Weibull model and would yield more reliable LC_{50} estimates with confidence intervals. The LT_{50} of 4.0 days and the high R^2 of 0.986 for the time-mortality probit confirm consistent virus action kinetics across the tea extract series, indicating that the protectant modulates the magnitude of infection initiation rather than the intrinsic time course of viral disease progression once larvae have ingested a sufficient OB dose.

The higher PC1 loading of mortality (−0.506) relative to feeding cessation (−0.448) along the virulence axis reflects the greater discriminatory power of the mortality endpoint for separating UV-protected from UV-degraded formulations. The second principal component, dominated by imago abnormality, accounts for variation that is essentially orthogonal to treatment efficacy as commonly defined, and likely represents biological noise associated with instar variation and diet-related developmental variability among larval cohorts. For formulation evaluation purposes, direct mortality and total infection rate together constitute the most informative endpoint pair, a conclusion supported by their near-unity correlation. A particular agronomic advantage of *C. sinensis* extract as a UV protectant is the availability of locally produced black tea in Nepal's eastern hill districts at costs accessible to smallholder farmers. Commercial-grade black tea from Ilam district contains 12–18% total polyphenols on a dry-weight basis (Khan & Mukhtar, 2019), and the polyphenol content of 14.7 ± 1.2 mg GAE per 100 mL measured in the 1.0% working extract falls within the range demonstrated to provide meaningful UV-B absorption in in vitro assays (Gomes et al., 2022). The preparation procedure requires only boiling water and basic filtration equipment, making it

practicable under field or extension station conditions without sophisticated laboratory infrastructure.

Several limitations of the present study deserve acknowledgment. First, the experimental design did not include a dark-stored virus control (OBs stored without UV-B exposure); consequently, the absolute efficacy loss attributable to the UV-B treatment cannot be formally partitioned from background biological variability among larval cohorts. Future studies should include paired UV-exposed and dark-stored replicates for each formulation to quantify the photodegradation effect independently. Second, laboratory UV-B irradiation at a fixed dose (28.8 kJ m⁻²) represents a single-point approximation of the temporal and spectral complexity of outdoor solar UV exposure. Field UV flux in Nepal's Terai varies with altitude, season, cloud cover, and canopy architecture, and can substantially exceed or fall below the laboratory dose applied here. The UV-protective gains observed at 2.0% tea extract under controlled conditions should therefore be validated in field trials that capture the full range of agroclimatic UV environments encountered in smallholder soybean and vegetable production systems. Third, the rain-wash-off and re-deposition dynamics that modify biopesticide residual activity on crop surfaces under field conditions were not represented in this study and constitute an additional variable requiring evaluation before field-scale recommendations can be made.

From the perspective of IPM program design in Nepal, these results support a developmental pathway toward botanically enhanced biopesticide formulations that retain the ecological safety profile of SpliNPV while extending its effective residual period on crop surfaces. A formulation combining SpliNPV with 2.0% *C. sinensis* extract could be positioned as a tank-mix additive applied during the evening hours—when UV intensity is lowest—to maximize OB persistence through the following dawn and morning, the periods of highest larval feeding activity on *S. litura*'s typically nocturnal feeding cycle. Complementary field trials under the agro-ecological conditions of Nepal's Terai and mid-hills are warranted to validate the UV-protective efficacy observed here under the higher UV flux, variable humidity, and rainfall-wash-off dynamics characteristic of outdoor cropping environments.

CONCLUSIONS

Camellia sinensis black tea extract incorporated at 2.0% (v/v) into SpliNPV JTM 97C formulations significantly enhanced larval mortality (43.75%) and total infection (55.00%) in *S. litura* third-instar larvae compared to the unprotected virus control (18.75% mortality; 23.75% total infection), establishing it as the most effective botanical UV protectant concentration among those tested. The kaolin reference standard (78.75% mortality; 100% total infection) outperformed all tea extract treatments, defining the ceiling for OB protection under standardized UV-B irradiation conditions. Pearson correlation analysis confirmed strong mechanistic linkages between the primary

infection parameters, and principal component analysis (exploratory, n = 8) objectively quantified the virulence hierarchy across all eight formulations. Composite treatment ranking consistently placed the 2.0% tea extract as the superior botanical option, followed by 2.5% and 1.5% concentrations. These findings establish a phytochemically rational and agronomically feasible basis for incorporating Nepali-produced *C. sinensis* extract as a UV protectant in SpliNPV biopesticide formulations. Two directions merit priority in future research: (1) field-based evaluation of SpliNPV + 2.0% *C. sinensis* extract under ambient UV conditions across Nepal's Terai soybean and vegetable production systems, including paired dark-stored controls and rainfall-wash-off assessments, to quantify the realized persistence and efficacy gain relative to unprotected virus; and (2) formulation optimization studies combining *C. sinensis* extract with low-dose kaolin or other UV-screening minerals to achieve synergistic protection while minimizing mineral residue deposition on marketable produce.

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