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RESEARCH

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Hyposidra talaca NPV (HytaNPV): a potential baculovirus for efficient control of the black inch worm, *Hyposidra talaca* Walker (Lepidoptera: Geometridae), a major pest of tea *Camellia sinensis* (Ericales: Theaceae (L.) O. Kuntze)

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Abstract

Background The black inch worm (BIW), *Hyposidra talaca* Walker (Lepidoptera: Geometridae), is a pest that defoliates tea leaves in India, posing a significant threat to the tea industry. Nucleopolyhedrovirus (NPV) is capable of infecting larvae of this species, which has raised the possibility of its use as a biocontrol agent.

Results Rearing larvae in a semi-synthetic artificial diet produced healthy adults, which is sufficient for mass culture of *H. talaca* to support one of the IPM components using baculovirus. In artificial diets, the NPV was evaluated for its insecticidal activity against *H. talaca*. The bioassay findings of inoculated *H. talaca* nucleopolyhedrovirus virus (HytaNPV) at various concentrations showed that it was effective in killing the BIW. Purified *polyhedral inclusion bodies* (PIBs) were estimated to a concentration of 1×10^{10} PIBs per ml by mixing with water, and various concentrations of 0.25, 0.5, 1, 2, 5, 7.5, 10 ml/l were evaluated against BIW. Both laboratory and field studies revealed that HytaNPV is an eco-friendly and ecologically safe agent for controlling BIW. Besides no residue was estimated in made tea after the seventh day of exposure, and it is nontoxic to non-target species.

Conclusion It was found that NPV is environmentally beneficial for the control of pests on tea plants and in production of pesticide-free tea. Tea ecosystems can reduce their reliance on conventional insecticides by using HytaNPV as an alternative bio-insecticide.

Keywords Black inch worm, Tea, *Hyposidra talaca*, Baculovirus

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Background

Hyposidra talaca Walker (Lepidoptera: Geometridae), also known as the black inch worm (BIW), is a major tea pest that damage tea plants, resulting in yield losses and lower quality tea (Roy et al. 2014) (Fig. 1A). In general, chemical pesticides are employed by tea farmers to combat tea pests such as this BIW (Hazarika et al. 2009). The BIW is a most devastating pest because of its polyphagous nature, migratory behavior, high reproduction rate, multiple generations, and ability to develop pesticide resistance (Antony et al. 2012).

Fig. 1 **A** Healthy larvae of black inch worm (BIW), *Hyposidra talaca*. **B** A *Hyposidra talaca* nucleopolyhedrovirus (HytaNPV) infected BIW

This insect pest is expected to cause a 10–50% loss in tea production per year (Roy et al. 2014). The invasion of BIW is increasing due to a wide range of factors, including rapid climate change over the past several decades, the abundance of forest or shade trees in and near tea plantations, and the continuous spraying of synthetic pesticides (Das and Mukhopadhyay 2014). Because of its short life cycle, rapid growth, more than seven generations per year, lack of diapause during the cold season, and rapid multiplication rate, and BIW has recently become a severe threat to the tea industry (Das et al. 2010). Nevertheless, repeated and excessive use of the chemical pesticides results in water pollution, the deterioration of beneficial microbes in the present in the soil, a recurrence, the development of insect resistance, and pesticide residues in tea (Roy et al. 2008). Adopting non-chemical control measures is critical to overcoming the present scenario. The use of biocontrol agents, particularly entomopathogens, is critical for environmentally responsible tea pest management. Planters have sought eco-friendly methods to manage various pests in tea crop because of the presence of synthetic pesticide residues in tea. These include selective pesticide use and the use of biological control agents (BCAs), which include parasitoids, microorganisms and predators. Nuclear polyhedrosis virus (NPV) has become increasingly popular as an alternative to synthetic pesticides over recent years. It has been shown that the *H. talaca* nucleopolyhedrovirus (HytaNPV) baculovirus is a highly specific and efficient biocontrol agent (Nguyen et al. 2018). These microbes are known to be harmless to animals and non-target beneficial organisms because of their host-specificity (Islam et al. 2018). In the present study, experiments were carried out to assess the HytaNPV's effectiveness against *H. talaca* in light of the aforementioned information.

Methods

Preparation of semi-synthetic artificial diet (SSAD)

The semi-synthetic artificial diet (SSAD) was prepared using dried powders of both tea leaf and tea seeds as base materials, along with other ingredients (unpublished data). Agar–agar (6.375 g) and distilled water (390 ml) were mixed with other ingredients such as tea seed powder (25 g), tea leaf powder (25 g), sorbic acid (1 g), brewer's Yeast (5 g) ascorbic acid (1.625 g), multivitamin (1.5 g), streptomycin (0.125 g), formaldehyde (40%) (2.5 ml). SSAD was placed in a Petri dish and let to harden and then placed at 4 °C for later use.

BIW collection and laboratory culture

BIW was reared in the laboratory using the method reported by Das and Mukhopadhyay (2014), with changes. To maintain a stock culture under the laboratory conditions (26 ± 3 °C; 80% RH; 16L: 8D photoperiod), disease-free BIW eggs and loopers were collected from the NBRRDC experimental plot. For egg-laying, gravid females were kept individually in (46×52 cm) insect rearing boxes. They were given a 10% honey solution that had been soaked in cotton. The eggs were kept in plastic containers (10×12 cm), where the mouths of the containers were sealed with a fine cloth, the base was covered with tissue paper, and the side walls were lined with opaque black paper. After hatching, each larva was placed in a (2.5×4 cm plastic) container and fed by the SSAD, while ensuring that the culture was kept clean and hygienic. Efficacy studies were conducted on insect cultures that were sustained for more than ten generations (without being exposed to any pesticides, from F_1 to F_{10}).

Isolation of NPV from infected BIW

NPV-infected loopers (Fig. 1B) were collected from different tea gardens of Dooars, West Bengal, India, using

hand gloves and camel hair brush and kept in sterile plastic vials (separate vials were used for each looper). Collected infectious loppers were stored at 4^0C inside an airtight plastic zip bag until confirmation of presence of *polyhedral inclusion bodies* (PIBs). Using a sterile mortar and pestle and distilled water, infected larvae were macerated. The occlusion bodies were purified and isolated (homogenization, filtration, and centrifugation) by adopting a standard procedure (Antony et al. 2011). The PIBs per ml were identified with the aid of an optical microscope for confirmation and counted using hemocytometer to maintain the PIBs.

Mass production of HytaNPV formulation

The HytaNPV was prepared by using a semi-synthetic artificial diet contaminated with the respective NPV. The mass production of HytaNPV was carried out by following the methodology of Mondal et al. (2021) with modifications. The isolated POBs were diluted with distilled water @ 1 ml/1 l and spread over the SSAD using the camel painting brush. The third instar larvae of BIW raised in laboratories were allowed to feed on the contaminated diet which get infected with virus within 3–4 days and hang them self-upside down in containers, and later, the infected larvae were collected and kept in a beaker for purification. A melted larva (insect cadaver) was homogenized using mortar and pestle, mixed in 1 ml of double-distilled water in a (1.5 ml) tube. Centrifuged the re-suspended melted insect cadavers at 500 g for 2 min and transferred the supernatant to a fresh tube, which was re-centrifuged at 500 g for 2 min in order to remove insect cuticles and completed PIB isolation. After collecting the supernatant in a new tube, it was centrifuged at 2800 g (RCF) for 10 min. The white pellet was re-suspended, rinsed with 5 ml of double-distilled water, centrifuged again for 5–10 min at 2800 g (RCF), and the supernatant was collected. Viral polyhedral inclusion bodies (PIBs) settled as white pellets in the bottom of the tube were collected. The number of 25–30 larvae was used to make 10 ml PIB, which was mixed with refined sunflower oil (25 ml), polyethylene glycol 400 (2.5 g), glycerin (2.5 g), tween 20 (50 µl) and titanium dioxide (TiO_2) (15 ml) as UV absorbent for making 50 ml HytaNPV formulation containing 1×10^{10} virus particles per ml. Using the Polytron PT 2100 (Kinematica AG, Lucerne, Switzerland), the sampled larvae were homogenized and adjusted to 50 ml in sterile distilled water. A Thoma hemocytometer was used to count the POBs.

Laboratory virulence study of HytaNPV against BIW

Insects molting out of the second instar larvae, as detected by head-capsule slippage, were kept into plastic cages without food. After 16 to 20 h, the freshly molted insects were collected and given a diet for 24 h. In 1 l of water, a stock solution of prepared formulation 50 ml (25–30 larval equivalent, having PIBs of 1×10^{10}) was prepared. Seven concentrations of the HytaNPV formulations (0.25, 0.5, 1, 2, 5, 7.5, 10 ml/l) were prepared from the stock solution and used to assess the efficacy of NPV. Each larva was placed in a separate plastic cage holding a feed treated with POBs at a rate of specified dosages per gram of diet. The diet without PIBs was given to the same number of larvae as a control. Larvae were individually transferred into 30-ml glasses with fresh artificial diet after 24 h of feeding. The larvae were incubated at 25 °C under a 16-h photoperiod. The mortality rate in the PIB-treated group and one control group was monitored daily until pupation. PIBs were examined found in tissue smears prepared from dead larvae using a phase contrast microscope. The experiment was conducted five times with a completely randomized design (CRD), using 30 BIW larvae for each treatment. Until all the larvae died or pupated, the mortality data were collected every 24 h by treating the larvae to various concentration formulations. A percent mortality rate for BIW was then calculated using Abbott's method (Abbott 1925), compared to an untreated control group.

Virus yield and time of harvest

The virus was isolated and collected, and yield was recorded seven days after third larval instar of BIW were inoculated at eight different intervals. The larvae were incubated at 25 ± 2 °C and allowed to feed a diet that had been given a viral dosage of 1×10^{10} PIBs. At 0, 1, 4, 6, 8, 12, 24 and 48 h, the larvae were inoculated. As a control, a treatment that included collecting the cadavers from healthy larvae after they died was also used. With 30 larvae per replication, each treatment was replicated five times. The cadavers from dead larvae were collected and subsequently frozen. Infected and dead larval cadavers were recorded for virus production.

Micro-plot field study of HytaNPV against BIW

In accordance with the methodology outlined earlier, a liquid formulation of HytaNPV comprising 1×10^{10} PIBs was prepared for the field experiment. To assess HytaNPV's performance against BIW, a micro-plot field study was conducted. In 2021, the study was conducted three times in the NBRRDC experimental field using randomized complete block designs (RCBDs). Each plot (40 m^2) had 50 tea bushes on it. The treatments comprised the HytaNPV @ 800 ml/ha (T1, containing 1×10^{10} PIBs/ml) conventional pesticide Flubendiamide 20WG@80gm/ha (T2); and control (T3). The plots with ETL above 5% threshold for each treatment were chosen and labeled. Spraying was carried out with a knapsack

sprayer, which is capable of spraying 1.5 l per plot. Bushes were thoroughly soaked to improve coverage and control. Before starting the treatment spray, the shoots were plucked, and for each treatment, the percentage of damage brought on by BIW was calculated using 10 randomly chosen bushes per replication. The loopers' population on these bushes was recorded one day before spraying and subsequently 24 h and 7 days after spraying. As soon as the harvesting was over, the first treatment spray was applied. From the randomly chosen 10 bushes each replication, the percentage control of BIW was recorded after 24 h and on the 7^{th} day after spraying. Based on the values from the pre-spray (C) and post-spray (T) assessments. The mean reduction in BIW per treatment was assessed using the method: C-T/C × 100 (Roy et al. 2015).

Large-scale field study of HytaNPV against BIW

A large-scale field trial to evaluate the effectiveness of the HytaNPV was conducted at the Tocklai Tea Research Institute (TTRI) experimental plot in Assam (27° 46′ 41.6′′ N, 95° 11′ 09.1′′ E longitude) and NBRRDC experimental plot in the Dooars (26° 53′ 0′′ N, 87° 54′ 0′′ E longitude). Three replications of each experiment were carried out using a RCBD. Each plot (100 m^2) had 150 tea bushes, and there were a total of 7 treatments (HytaNPV at 400 ml/ha (T1), @600 ml/ha (T2), 800 ml/ha (T3), and 1000 ml/ha (T4), each of which contained 1×10^{10} PIBs/ml. There were also two different recommended standard insecticides (Flubendiamide 20WG @80 g/ha (T5), Emamectin Benzoate 5%SG @ 40 g/ha (T6), and control (T7) in every replication. In accordance with the micro-plot trial description, the pre- and post-treatment evaluations were made.

Harvestable shoot yield estimation

Estimating the yield was a part of the thorough field testing as well. A regular plucking schedule was maintained for the first six rounds (7-day intervals) to record the yield (green tea leaf), and the average yield was stated in kg/plot. According to Ponmurugan and Baby (2007), the formula used to convert the harvested green leaves measured at each harvest in to made tea for each hectare was: Green leaf yield (kg) × number of bushes/ha × conversion factor (0.225).

Effect of HytaNPV on the non-target organisms

The predominant insect predator in the ecosystem of tea *Stethorus gilvifrons* Mulsant (Coleoptera: Coccinellidae) (Perumalsamy et al. 2010) was tested using laboratory (25 ± 2 °C, 75 ± 5% RH, and 16L: 8D photoperiod) bioassays, to evaluate the HytaNPV's infectivity. The larval and adult predators, collected from the tea gardens of Dooars region, were reared in the laboratory as described earlier. Larvae of the *S. gilvifrons* were used for the study. Tea leaves were cut in 4 cm diameter for making the leaf disk. From the stock culture maintained in the laboratory, ten individuals of two- to three-day-old adult red spider mites [*Oligonychus coffeae* Nietner (Acarina: Tetranychidae)] (Deka et al. 2022) were released onto each leaf disk as a source of food material for the predator (Deka et al. 2017). From the stock culture maintained in the laboratory, ten individuals of two-day-old larvae of *S. gilvifrons* were released onto each leaf disk. Spraying of different concentrations of HytaNPV at 400 (T1), 600 (T2), 800 (T3), and 1000 ml/ha (each treatment containing 1×10^{10} PIBs/ml) and an untreated control (T5) per replicate were used in the experiments. Each treatment was performed three times, and data were recorded based on their mortality; larval, pupal, adult period and emergence of adults, according to Leatemia and Isman's (2004) methodology.

Phytotoxic effect, organoleptic evaluation, and tainting test

To evaluate the phytotoxic effect (stunting, yellowing, epo/hyponasty, necrosis, etc.) of HytaNPV formulation (at "X" and 2X concentrations) on tea leaves, a field experiment was carried out at the North Bengal Regional Research and Development Centre (NBRRDC) experimental plot. There were a total of three treatments: 800 ml/l (T1), 1600 ml/l of water (T2) and an untreated control (water spray) (T3), and three replications were maintained at 100 square meters per replication. Each treatment solution, T1 and T2, included 1×10^{10} PIBs per ml. A knapsack sprayer with a 400 l/ha spray volume was used to apply the spraying. Stunting, yellowing, necrosis, hyponasty, epinasty, and other parameters were recorded on day 0 (pre-treatment), 3, 7, and 14 days (post-treatment), and the damage symptoms were categorized using the Phytotoxicity Rating Scale (PRS) as: No crop response or no lesion = 0, 1–10% = 1, 11–20% = 2, 21–30% = 3, 41–50% = 5, 51–60% = 6, 61–80% = 8, 81–90% = 9, and 91–100% (Babu et al. 2008). A mini-CTC machine was used to process shoots from each plot 1, 3, 5, 7, 10 and 14 days after spraying to test whether HytaNPV treatment left a taint on the CTC black tea produced from treated leaves. The samples were sent to expert tasters who evaluated them organoleptically to determine whether they were tainted (if any).

Statistical analysis

Using SPSS17, ANOVA (analysis of variance) was used to evaluate data from laboratory and field trials. In order to analyze the laboratory bioassay of HytaNPV against BIW, the repeated-measures model was adopted. The mortality response variable was the repeated factor, and

the exposure period was the exposure interval. The days (time) and concentration were the main effects in a two-way ANOVA on the data collected for mean percent mortality. In order to standardize the variance before the analysis, the data were transformed to a log $(x+1)$ scale. To separate the means, the Tukey–Kramer honest significant difference (HSD) test was used at the 0.05 level of significance. The data from the field study of HytaNPV against BIW were angularly transformed before statistical analysis. Tukey's post hoc HSD test was used to compare the pre- and post-spray incidence of BIW between treatments, with a 95% confidence interval. For the yield of green leaves, the treatments were compared to the control and the means were separated at the 95% confidence level using HSD test. An analysis of the effects of different concentrations of HytaNPV on beneficial non-target species was conducted using the DMRT *F*-Test.

Results

The purified virus particles were obtained after processing the infected larval cadavers, which showed the typical signs of NPV infection in BIW infected larvae. Electron microscopic studies of the virus particles determined that polyhedral inclusion bodies were present in the infected dead larvae (Fig. 2).

Laboratory virulence study of HytaNPV against BIW

The in vitro bio-efficacy study indicates that HytaNPV is pathogenic to the BIW and significantly increased its mortality. The mortality of BIW larvae remained low after 3 days of treatment, ranging from 1 to 65% mortality across all treatments (Table 1). One day later, the mortality was increased further, ranging from 80 to 100% at concentrations of 7.5 and 10 ml/l and between 27 and

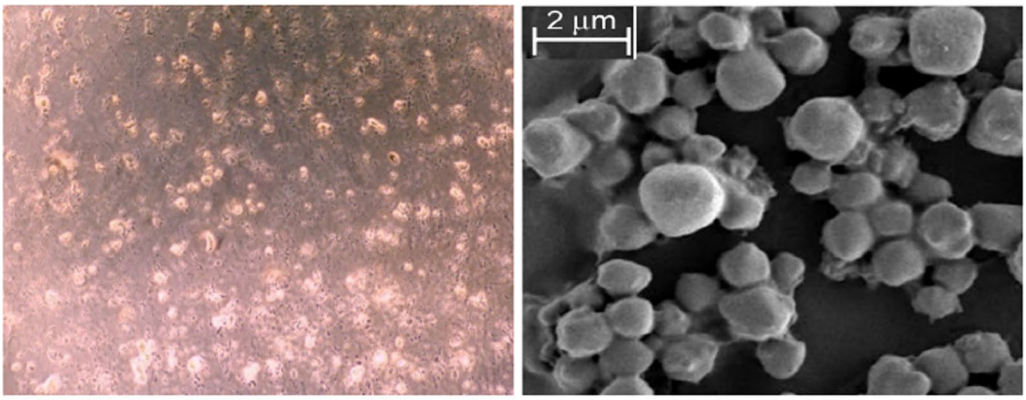

Fig. 2 **A** *Hyposidra talaca* nucleopolyhedrovirus polyhedron (observed in phase contrast microscope; 10X zoom). **B** SEM image showing the polyhedral inclusion bodies

Table 1 Mean percent mortality of the black inch worm (BIW) larvae fed on artificial diet treated with *Hyposidra talaca* nucleopolyhedrovirus (*HytaNPV*) at different concentrations for 7 days

	Mean % mortality of BIW after different treatment periods						
Exposure	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Concentrations (ml/l)							
Control	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
0.25 ml/l	0^{a}	0^{a}	0^{a}	27^{b}	37^{b}	44^{b}	62^{b}
0.5 ml/l	0^{a}	0^{a}	1^{a}	37^{c}	43^{bc}	65^{c}	81^{c}
1 ml/l	0^{a}	0^{a}	4^{ab}	43^{cd}	51^{c}	79^{cd}	100^{d}
2 ml/l	0^{a}	0^{a}	8^{b}	63^{d}	77^{d}	85^{de}	100^{ad}
5 ml/l	0^{a}	7^{b}	22^{c}	73^{de}	83^{de}	92^{e}	100^{d}
7.5 ml/l	0^{a}	17^{b}	28^{c}	80^{e}	97^{e}	100^{e}	100^{d}
10 ml/l	0^{a}	27^{b}	65^{d}	100^{f}	100^{e}	100^{e}	100^{d}
F	4.6	5.5	12.1	22.2	18.5	17.6	16.5
p	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Means that are followed by the same letter inside each column do not differ significantly (all cases DF = 7, 54, Tukey–Kramer HSD test at $p = 0.05$)

73% at concentrations of 0.25, 0.5, 1, 2, and 5 ml/l. The mean percent mortality of BIW larvae was concentration and time dependent. After 7 days of treatment, HytaNPV killed almost all the larvae (100%) even at a concentration of 1 ml/l, while no mortality of the larvae was observed in water spray (control).

Virus yield and time of harvest

Studies on the optimal harvest period indicated that the percentage of dead larvae in samples varied considerably by harvest period. At 0 h, the yield per 30 inoculated larvae was 1.52×10^{12} PIBs, and at 24 h, it was 1.12×10^{9} OB's. Up to 12 h after inoculation, when virus-infected larvae were used as cadavers, the PIBs yield was high, but beyond that point the virus yield started to decline (Fig. 3).

Micro-plot field study of HytaNPV against BIW

Based on the results of the micro-plot survey conducted against BIW, Table 2 summarizes the findings. After seven days of treatment, around 80% reduction of BIW was recorded against HytaNPV@400 ml/ha and 85% reduction was recorded against HytaNPV@800 ml/ha compared to a 96.5% reduction with the recommended insecticide (Flubendiamide 20WG@80gm/ha). Whereas in case of control, the normal growth of BIW was observed (Table 2).

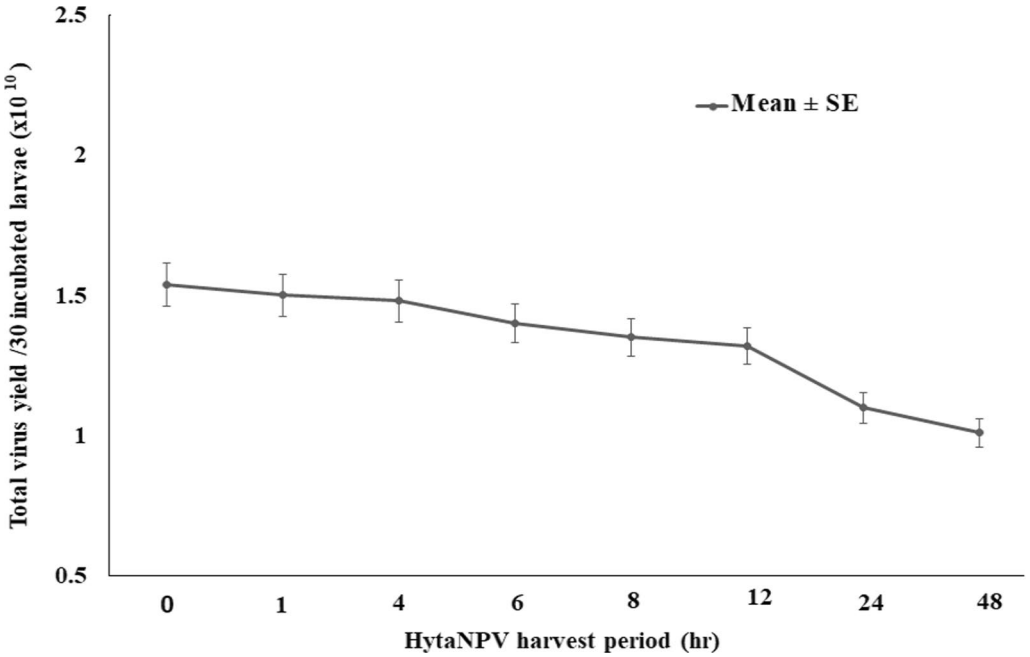

Fig. 3 Total *Hyposidra talaca* NPV yield at different harvesting periods

Table 2 Effect of *Hyposidra talaca* nucleopolyhedrovirus *HytaNPV* on the black inch worm (BIW) under micro-plot field conditions

Treatment/ Concentration	Pre-treatment observation (Average no. of (BIW) / tea bush)	Post-treatment observation Mean percent reduction of (BIW) after*	
		24 h	7 days
Control (Water spray)	18 ± 1.12	0.0[a]	0.0[a]
HytaNPV@400 ml/ha[$]	15 ± 1.22	2[a]	80[b]
HytaNPV@800 ml/ha[$]	19 ± 1.53	3[a]	85.7[b]
Flubendiamide 20WG@80gm/ha	17 ± 1.45	65[b]	96.5[b]

[*] Values represent the mean of three replications, for average no. of looper per tea bush (pre-spray incidence) and mean percent reduction of looper after 24 h and 7 days (post-spray incidence)

[$] Containing PIBs of 1×10^{10}. According to Tukey's post hoc honestly significant difference (HSD) test, values in the same column with different superscripts are statistically different at $p < 0.05$

Large-scale field study of HytaNPV against BIW

Data from field evaluations of HytaNPV against BIW in Assam (TTRI Experimental Plot) and Dooars (NBRRDC Experimental Plot) are presented in Table 3. The average number of BIW seen before treatment in the Assam region ranged from 13.9 to 15 before any treatment was applied. The ANOVA revealed that there was a statistically significant difference [$F=6.2$, df = 1 (between the groups), df = 6 (within the groups), $p<0.05$] between all of the treatments once it related to decreasing the number of BIWs. High concentrations of HytaNPV (800 and 1000 ml/ha, each containing 1×10^{10} PIBs/ml) were considerably more effective ($p<0.05$) in decreasing the mean population of BIW than lower concentrations (400 and 600 ml/ha). High concentrations of 800 and 1000 ml/ha were as effective in reducing the mean population of BIW as were the standard insecticides recommended for tea, Flubendiamide 20WG and Emamectin benzoate 5%SG ($p>0.05$). There was no BIW mortality in the control group plot (Table 3).

The average number of BIW in the Dooars region ranged 15.9–16.9 before the spray. The ANOVA revealed that plots sprayed with HytaNPV at 800 and 1000 ml/ha concentrations reduced BIW by a greater percentage than those sprayed with lower concentrations of HytaNPV (400 and 600 ml/ha) [$F=9.8$, df = 1 (between the groups), df = 6 (within the groups), $p<0.05$]. HytaNPV at high concentrations comparably controlled BIW to Flubendiamide 20WG and Emamectin benzoate at 5%SG ($p>0.05$) (Table 3).

Harvestable shoot yield estimation

As shown in Table 4, the average yields from the first six plucking rounds (at an interval of 7 days) throughout the experimental periods. The yield in the untreated control group was considerably low, demonstrating the improved effectiveness of the various formulations evaluated ($F=7.8$, df = 6, $p<0.05$). The plots sprayed with Flubendiamide 20WG at 80 g/ha, Emamectin benzoate 5%SG at 40 g/ha, and HytaNPV at 800 and 1000 ml/ha had considerably greater tea leaf yield than the plots sprayed with 400 and 600 ml/ha of HytaNPV ($p<0.05$) (Table 4).

Effect of HytaNPV on the non-target organisms

In vitro tests on *S. gilvifrons,* one of the prevalent insect predators in tea ecosystem showed that the formulation HytaNPV did not result in any larval mortality (Table 5). The larval and pupal duration, emergence of adults, following HytaNPV treatment and untreated control, showed non-significant difference ($p<0.05$), proving that HytaNPV was safe for *S. gilvifrons*.

Phytotoxic effect, organoleptic evaluation, and tainting test

At 800 ml/l (T1) and 1600 ml/l (T2) of water, the phytotoxic study was carried out independently (1×10^{10} PIBs/ml). The tea leaves and harvestable shoots did not exhibit any phytotoxic effects at any of the concentrations, according to observations made on the signs of phytotoxicity. There were no obvious signs of damage on the tip and surface of leaves, wilting, necrosis, hyponasty or epinasty. Similarly, following the application of HytaNPV tea shoots were plucked on day 1, 3, 5, 7, 10 and day 14 and

Table 3 Bio-efficacy of *Hyposidra talaca* nucleopolyhedrovirus (*HytaNPV* in controlling the black inch worm (BIW) in large-scale field study after 7 days

Treatments	Concentration /ha	Assam		Dooars	
		Population of BIW/bush (Pre-treatment)[#]	% Reduction of BIW population[#]	Population of BIW/bush (Pre-treatment)[#]	% Reduction of BIW population[#]
HytaNPV	400 ml[$]	14.6 ± 0.2^{a}	45.3^{b}	16.4 ± 0.9^{a}	42.3^{b}
	600 ml[$]	14.2 ± 0.3^{a}	52.3^{b}	16.3 ± 0.7^{a}	50.3^{b}
	800 ml[$]	14.0 ± 0.5^{a}	85.3^{a}	16.7 ± 1.0^{a}	81.2^{a}
	1000 ml[$]	15.0 ± 0.4^{a}	90.4^{a}	16.9 ± 0.5^{a}	87.5^{a}
Flubendiamide 20WG	80 g	13.9 ± 0.9^{a}	97.5^{a}	15.9 ± 0.9^{a}	90.1^{a}
Emamectin benzoate 5%SG	40 g	14.2 ± 0.3^{a}	93.4^{a}	16.7 ± 1.0^{a}	88.6^{a}
Control	Water	14.6 ± 0.2^{a}	0^{c}	16.4 ± 0.9^{a}	0^{c}
F value	-	8.4	9.5	10.2	12.3
p value		< 0.0001	< 0.0001	< 0.0001	< 0.0001

[#] Values for each location's pre-spray looper population (average) and looper population reduction (%) are the average of two consecutive seasons

[$] Pre-spray looper population is the mean of three replications ± SE before treatments, and reduction of looper population (%) is the mean of three replications ± SE obtained after seven days of treatment for each season. The sample contains 1×10^{10} PIBs/ml. According to Tukey's post hoc honestly significant difference (HSD) test, values in the same column with distinct superscripts are substantially different from one another at $p<0.05$

Table 4 Effect of *Hyposidra talaca* nucleopolyhedrovirus (*HytaNPV*) on the yield of harvestable shoots of tea

Treatments	Concentration /ha	Assam		Dooars	
		*Green leaf yield (Kg/Plot)	Processed tea (kg/ha/year)	*Green leaf yield (Kg/Plot)	Processed tea (kg/ha/year)
HytaNPV	400 ml[$]	$2.21^{b} \pm 0.19$	385 ± 4.25	$2.01^{cd} \pm 0.19$	375.5 ± 4.25
	600 ml[$]	$2.25^{ab} \pm 0.18$	389 ± 4.36	$2.05^{bc} \pm 0.18$	386 ± 4.36
	800 ml[$]	$2.31^{a} \pm 0.16$	400 ± 4.45	$2.11^{ab} \pm 0.16$	400 ± 4.45
	1000 ml[$]	$2.33^{a} \pm 0.23$	403.5 ± 4.21	$2.13^{a} \pm 0.23$	402.5 ± 4.21
Flubendiamide 20WG	80 g	$2.35^{a} \pm 0.29$	406.1 ± 4.19	$2.12^{a} \pm 0.29$	404 ± 4.19
Emamectin benzoate 5%SG	40 g	$2.34^{a} \pm 0.22$	404 ± 4.37	$2.14^{a} \pm 0.22$	406 ± 4.37
Control	Water	$1.81^{bc} \pm 0.14$	248 ± 4.25	$1.75^{d} \pm 0.14$	238 ± 4.25
F value	–	7.3	8.4	9.1	11.2
p value		< 0.0001	< 0.0001	< 0.0001	< 0.0001

[*] For green leaf yield, values indicate the mean of three replications ± SE. (six rounds of leaf plucking following weekly intervals for two seasons)

[$] Containing 1×10^{10} PIBs/ml. Tukey's post hoc honestly significant difference (HSD) test indicates that values in the same column that are both followed by the same letter are not statistically different from one another at the 95% confidence interval ($p < 0.05$)

Table 5 Effect of *Hyposidra talaca* nucleopolyhedrovirus (*HytaNPV)* on a few biological aspects of non-target beneficial organisms (*Stethorus gilvifrons*) in the tea environment

Treatment	Concentrations conidia/ml/ha	Larval mortality (%)	Larval period (days)	Pupal period (days)	Adult emergence (%)
HytaNPV	400 ml[$]	0.00^{a}	8.21 ± 2.02^{a}	4.52 ± 0.11^{a}	98.00^{a}
	600 ml[$]	0.00^{a}	8.18 ± 2.02^{a}	4.51 ± 0.11^{a}	97.00^{a}
	800 ml[$]	0.00^{a}	8.19 ± 2.02^{a}	4.45 ± 0.12^{a}	98.00^{a}
	1000 ml[$]	0.00^{a}	8.23 ± 2.13^{a}	4.50 ± 0.12^{a}	98.00^{a}
Untreated control	–	0.00^{a}	8.25 ± 2.23^{a}	4.48 ± 0.12^{a}	100.00^{a}
CD (5%)	–		1.51	1.26	14.02
CV (%)	–		5.64	13.61	7.02

Means in a column with similar letters are not statistically different at 5% (DMRT *F*-Test, $p > 0.05$). [$]Containing 1×10^{10} PIBs/ml

processed in the mini-CTC machine. According to tea tasters report, the made tea samples exhibited acceptable organoleptic qualities and no taint.

Discussion

BIW populations were significantly reduced at 800 and 1000 ml/ha concentrations of HytaNPV formulations in field studies conducted at both locations. Furthermore, the formulation did not have a statistically significant effect on the natural enemies present in the tea ecosystem. NPV was thought to be effective and was reported against different species of tea loopers, viz. *Biston suppressaria* Guenee (Lepidoptera: Geometridae), *H. talaca* and *Hyposidra infixaria* Walker (Lepidoptera: Geometridae) (Antony et al. 2011), and other tea pests such as *Ectropis obliqua* nuclear polyhedrosisvirus (EcobNPV), *E. obliqua* single nucleocapsid nucleopolyhedrovirus (EcobSNPV) against *E. obliqua* (Deka and Babu 2021). Insect viruses that are associated with tea pests have been the subject of investigation by scientists since the late 1970s. The use of insect-specific viruses (ISV) has shown to be an effective natural method for managing tea caterpillar infestations. ISVs are some options that are intriguing and might perhaps act as a biocontrol agent. More than ninety-five percent of the 82 virus species that have been identified as being related with tea insects have been reported from China (Ye et al. 2014). All the following have been used effectively as large-scale biocontrol agents in tea farming in China: *E. obliqua* NPV (EcobNPV), *B. suppressaria* NPV (BusuNPV), *Andraca bipunctata* GV (AnbiGV), *Euproctis pesudoconspersa* NPV (EupeNPV), and *Adoxophyes orana* GV (AdorGV). More than 90% of the first and second generations of *B. suppressaria* killed after ten days of being sprayed with polyhedral suspensions containing *BusuNPV* at a rate of 3×10^{12} PIB/ha (Peng et al. 1998). In Japan, tea pests

including *Adoxophyes honmai* Yasuda (Lepidoptera: Tortricidae) and *Homona magnanima* Diakonoff (Lepidoptera: Tortricidae) have all been effectively controlled with Entomo Pox Viruses (EPVs), Granulo Viruses (GVs) and the NPV (Nakai et al. 2003). The signs of NPV infection in *B. suppressaria* were described earlier, which determined that NPV was virulent against *B. suppressaria* in *in vitro* experiments (Mukhopadhyay et al. 2011). The identification of NPVs, EPVs and GVs in the lepidopteran pests of various tea-growing regions, together with their deadly effectiveness with a focus on cross infectivity, opens new avenues for converting this viral disease into a biopesticide.

In the field, tea loopers (*H. talaca*) are highly susceptible to NPV, a member of the baculovirus family Baculoviridae (Deka et al. 2021). HytaNPV is a member of the group II alphabaculovirus family, and its DNA genome is 139,089 base pairs long with a GC content of 39.6% (Nguyen et al. 2018). There is a highly conserved region of 527 bp in the polyhedron gene of HytaNPV, and it has 98% sequence similarity with the NPVs of *H. infixaria* and *B. suppressaria (*Dasgupta et al. 2016*)*.

NPV formulations found to be more effective against *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) damaging maize crops than synthetic insecticides (Armenta et al. 2003), *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) damaging cotton (Yasin et al. 2020), cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) (Abid et al. 2020), and tea pests, viz. *B. suppressaria*, *H. talaca* and *H. infixaria* (Antony et al. 2011), *E. obliqua* (Ma et al. 2006). The present study also reported similar results, and the formulation HytaNPV was effective as well ($p < 0.05$) than the Flubendiamide 20WG and Emamectin benzoate 5%SG against BIW.

Nawaz et al. (2019) reported that NPV-based biopesticides are selective to target pest species and less dangerous to natural enemies. Similarly in this study HytaNPV had no adverse effect on the non-target insects. The relationship between NPV and insect predators has often been found to be neutral for the predator and favorable for the virus, since the predator may transmit viral OBs after ingesting on a virus-infected lepidopteran larva (Armenta et al. 2003).

The plots sprayed with HytaNPV in the present investigation yielded substantially more than the control plots. The harvested tea shoots from the plots treated with our specially formulated HytaNPV was comparable to that from the plots treated with the recommended synthetic pesticides Flubendiamide 20WG and Emamectin benzoate 5%SG. As well as being non-phytotoxic to tea, HytaNPV was found not to taint tea, as observed by expert tasters assessing the phytotoxicity of tea leaves. In contrast, a number of tea plant harvestable shoots demonstrated phytotoxic effects and insecticide residue levels that exceeded EU recommendations, which indicated that natural biopesticides could be used to decrease the load of synthetic pesticides in the tea ecosystem. HytaNPV can thus be commercialized as a natural eco-friendly insecticide for the management of BIW in tea plantations and is a potential key component of integrated pest management strategies in the future due to the percentage reduction of pest populations (BIW), phytotoxicity, and non-pathogenic to non-target organisms.

Conclusions

The present study concluded that the formulation of HytaNPV effectively controlled BIW at both locations for multi-seasons, while also significantly reducing the pest population. HytaNPV's effectiveness was comparable to that of the synthetic pesticide. Furthermore, the developed HytaNPV exhibited acceptable organoleptic qualities on harvestable shoots without any phytotoxic effects.

Abbreviations

HytaNPV	*Hyposidra talaca* NPV
BIW	Black inch worm
NPV	Nucleopolyhedrovirus
IPM	Integrated pest management
BCAs	Biological control agents
SSAD	Semi-synthetic artificial diet
NBRRDC	North Bengal Regional Research and Development Centre
l	Liter
ml	Milliliter
PIBs	Polyhedral inclusion bodies
RCBDs	Randomized complete block designs
TTRI	Tocklai Tea Research Institute
WG	Water dispersible granule
CTC	Crush tear curl

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Author contributions

BD did conceptualization, laboratory and field evaluation, statistical analysis and prepared the manuscript; AB: designed and guided the laboratory as well as the field trials, reviewed and edited the writing, SS isolated the PIBS and assisted during the laboratory and field study; BK and GT gave technical support during preparation and confirmation of PIBs. All authors read and approved the final manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated and analyzed for the current study are presented in this manuscript, and the corresponding author has no objection to the availability of data and materials.

Declarations

Ethics approval and consent to participate
Not applicable. The study was conducted using *Hyposidra talaca,* a major tea pest that are abundant in the tea ecosystem hence does not need ethical approval.

Consent for publication
Not applicable.

Competing interests
The authors declare that they do not have competing interests.

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