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Assessment of Toxic Effects of Cypermethrin and Nucleopolyhedrovirus in *Hyposidra talaca* Walker (Lepidoptera: Geometridae)

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Abstract: *Hyposidra talaca* Walker is a major defoliating pest of the tea plantations in the Terai region of Darjeeling foothills, West Bengal, India. A huge array of chemical pesticides, including cypermethrin (synthetic pyrethroid), are regularly used to control this geometrid pest in the tea plantations of this region. Indiscriminate use of chemical pesticides induces increasing level of tolerance to pesticides in the pests leading to control failure. Some entomopathogens of this looper pest including nucleopolyhedrovirus (NPV) have been studied to determine the potency of these agents as biopesticides. For effective pest management strategy, the information regarding the toxic effects of sub-lethal concentrations of cypermethrin and HytaNPV on *H. talaca* at their cellular, biochemical and enzymatic levels is the needs of the hour. Therefore, in the present investigation, mid-lethal concentrations of cypermethrin and HytaNPV were determined in *Hyposidra talaca* larvae reared on artificial diet by diet-incorporation and diet-coating methods, respectively, for three successive days of exposure at 24 h interval. LC_{50} of cypermethrin for 48 h exposure showed steep decrease from that of 24 h exposure, but showed little difference with 72 h exposure. It indicated immediate action of cypermethrin, a neurotoxin, on the larvae. For NPV, the estimated LC_{50} values for 24 and 48 h exposure revealed very little difference, whereas, at 72 h exposure the LC_{50} value declined steeply from the earlier values. It supports the delayed action of NPV on the larvae.

Keywords: Cypermethrin, Nucleopolyhedrovirus, *Hyposidra talaca*, Lethal concentration, Diet-incorporation, Diet-coating

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Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze, the most important agro-industrial, perennial foliage crop in the sub-Himalayan West Bengal and north-east India, is regularly attacked by huge number of tea pests (Mukhopadhyay and Roy, 2009). The black inch looper, *Hyposidra talaca* (Walker)

(Lepidoptera: Geometridae) is one of the major defoliating pests in the tea plantations in the Terai region of the northern part of West Bengal (Das *et al.,* 2010). To control this pest, conventional pest management strategy relying on the use of some synthetic chemical pesticides is mostly followed in the tea plantations of India. Among few widely used chemical pesticides, cypermethrin, a synthetic pyrethroid is one of the most preferred pesticides against the looper caterpillars (Sannigrahi and Talukdar, 2003). But injudicious use of these synthetic pesticides, especially due to 'no-threshold spray strategy' of crop growers often leads to the development of insecticide resistance as well as residual hazards (Mukhopadhyay *et al.,* 2014). In this scenario, some entomopathogens such as nucleopoly-hedrovirus (HytaNPV) isolated from *H. talaca* were investigated for characterization and virulence to assess their potentiality to be developed as commercial biopesticide in future (Mukhopadhyay *et al.,* 2010, 2011, Ghosh *et al.,* 2015). To study the effectiveness of these pesticides and entomopathogens, the lethal concentrations of these controlling agents have to be determined in different developmental stages of *H. talaca* larvae.

In this study, the response of the population of *Hyposidra talaca* against a widely used chemical pesticide, cypermethrin and a potential entomopathogen, HytaNPV, was evaluated by diet-incorporation and diet-coating bioassays to determine the LC values of cypermethrin and HytaNPV, respectively.

Materials and Methods

Insect rearing on artificial diet:

The male and female moths of *Hyposidra talaca* were collected by hand-picking method from the tea plantations in the Terai region of Darjeeling District. These were kept in 2:2 or 2:3 female to male ratio in mating containers (11 cm height × 12 cm diameter) and were allowed to mate and lay eggs in the laboratory. After hatching, ten neonates (1-12 hours old) were released in each rearing containers of 100 ml (Tarson-510010) having 1cc diet cube freely suspended by a toothpick and were reared in a BOD incubator (CHM-10 PLUS, REMI, India: Make) at 27 ± 2°C, L:D 13:11 h and RH 75 ± 5% on artificial diet following the method of Prasad and Mukhopadhyay (2015) with minor modifications. At this temperature and humidity, the growth of looper caterpillars was optimal, and the diet did not get dried very fast.

Diet preparation:

The artificial diet was prepared following the method of Prasad and Mukhopadhyay (2015) by mixing different ingredients (11.675 g casein, 10 g cellulose powder, 0.35 g choline chloride, 1 g cholesterol, 11.675 g dextrose, 2.075 g potassium sorbate, 1.675 g sodium alginate, 0.9 g sucrose, 3.35 g Wesson's salt mixture, 16.675 g wheat germ) in 300 ml 3% autoclaved agar medium and mixing more ingredients [1.28254 g ascorbic acid, 0.114 g inositol, 0.0025 g folic acid, 1 g linoleic acid , 1.675 g sodium alginate, 0.9 ml wheat germ oil and vitamin mixture (30 mg thiamine mono nitrate, 30 mg vitamin B_2, 9 g vitamin B_6, 300 mg nicotinamide, 150 mg calcium pantothenate, 4500 mcg folic acid, 45 mcg vitamin B_{12}, 450 mg vitamin C, 300 mcg biotin, 400 mg tocopheryl acetate)] after cooling down the mixture to 60-70°C in the laminar hood. The diet was provided ad libitum before getting dried or consumed. The larvae were segregated into smaller groups to new rearing containers as they grew bigger for their free movement.

Chemical Pesticides:

The bioassay was done with a chemical pesticide, Cypermethrin 10% EC (Brand name: Ustad, manufactured and marketed by UPL Ltd.). It is one of the most widely used synthetic pyrethroids to control looper caterpillars in the tea plantations of terai region (Sannigrahi and Talukdar, 2003). It is a sodium channel modulator leading to paralysis of the organisms (Abubakar *et al.,* 2000).

Entomopathogenic virus:

The bioassay was also done with an entomopathogenic virus, *Hyposidra talaca* nucleopolyhedrovirus (HytaNPV), a potent biopesticide alternative to the regular chemical pesticides (Mukhopadhyay *et al.,* 2010, 2011, Ghosh *et al.,* 2015). Occlusion bodies (OBs) of HytaNPV, collected from the cadavers of NPV infected *H. talaca* larvae (Ghosh *et al.,* 2015), were procured from the stocks maintained in the Genetics and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Darjeeling, India. This HytaNPV had been reported to be pathogenic to *H. talaca* larvae reared on natural diet (tea) in the laboratory condition (Mukhopadhyay *et al.,* 2011).

Bioassay:

Diet-incorporation method:

For bioassay using cypermethrin, laboratory-based diet-incorporation method was performed following the standard method (Perez *et al.,* 1997) with slight modification. In this method, the artificial diet was modified by supplementing with graded concentrations of the aqueous solution of cypermethrin, at the ratio of 9:1 (9 parts diet and 1 part aqueous solution of pesticide) during the preparation of diet when the temperature of the diet slurry dropped to 40°C, as high temperature can disintegrate the insecticides (Durmusoglu *et al.,* 2015). Final concentrations of cypermethrin in artificial diet were 2 mg/l, 5 mg/l, 10 mg/l and 20 mg/l. Double distilled water was added to the artificial diet instead of the pesticide for control experiment. Three replicates, each with ten healthy 1–day old 5th instar, *H. talaca* larvae procured from second generation, were used for control and experiment with contaminated food having graded concentrations of pesticide/ entomopathogen. The larvae were kept starved for 4 h before releasing them into the rearing containers of 100 ml (Tarson-510010) having 1cc diet cube.

Diet-coating method:

For bioassay using HytaNPV, diet-coating method was performed following the standard method (Zamora-Avilés *et al.,* 2013) with minor modifications. In this method, 1 ml NPV suspension of different concentrations (1 x 10^4, 1 x 10^5, 1 x 10^6 and 1 x 10^7 OBs/ml) was added onto the surface of the unmodified artificial diet cube (1 cc), thoroughly swirled and air-dried. For control experiment, the untreated (virion free) diet was provided. Three replicates, each of the ten healthy 1–day old 5th instar larvae, procured from second generation, were used. The larvae were kept starved for 4 h before providing them diet during experiment.

In each concentration of the pesticide/entomopathogen contaminated diet as well as for control, the larval mortality was recorded for three successive days at 24 h interval. Moribund insects were considered as dead (Gurusubramanian and Bora, 2007).

Statistical analysis:

A concentration-probit mortality curves for each and every concentration of the pesticides were obtained using LdP Line software. Using Abbott's formula, the mortality data was converted to corrected per cent mortality by Abbott (1925) and subjected to probit analysis (Finney, 1971) to obtain different LC values.

Results and Discussion

The 1-day old 5th instar H. talaca larvae (n=30) showed a concentration dependent response with the pesticide/entomopathogen. The mortality recorded were 2, 3, 5 and 12 after 24 h exposure; 3, 5, 7 and 15 after 48 h exposure and 4, 6, 10 and 15 after 72 h exposure of graded concentrations of 2 mg/l, 5 mg/l, 10 mg/l and 20 mg/l of the cypermethrin, respectively (Table 1). The mortality of 1-day old 5th instar H. talaca larvae (n=30) was found as 1, 1, 6 and 10 after 24 h exposure; 1, 3, 6 and 11 after 48 h exposure and 2, 4, 9 and 12 after 72 h exposure of graded concentration of 1 x 104, 1 x 105, 1 x 106 and 1 x 107 OB/ml of the NPV, respectively (Table 2). No mortality was found in control experiment. From the concentration dependent mortality data, the LC_{50} of cypermethrin for 24, 48 and 72 h, were determined as 38.62 mg/l, 25.49 mg/l and 22.19 mg/l, respectively while, the LC_{50} of NPV at 24, 48

Table 1: Dose-mortality response of cypermethrin on 5th instar *Hyposidra talaca* larvae (number of larvae =30) for three days exposure

Exposure time (h)	Concentration of cypermethrin (mg/l)	No. of tested larvae (5th instar larvae)	Actual mortality	Linear Probit	Mortality (%)
24	2	30	2	3.2976	6.667
	5	30	3	3.8245	10.000
	10	30	5	4.2230	16.667
	20	30	12	4.6216	40.000
48	2	30	3	7.6880	10.000
	5	30	5	18.0570	16.667
	10	30	7	29,9927	23.333
	20	30	15	44.5948	50.000
72	2	30	4	3.7991	13.333
	5	30	6	4.2564	20.000
	10	30	10	4.6022	33.333
	20	30	15	4.9481	50.000

Table 2: Dose-mortality response of HytaNPV on 5th instar *Hyposidra talaca* larvae (number of larvae =30) for three days exposure

Exposure time (h)	Concentration of HytaNPV (OBs/ml)	No. of tested larvae (5th instar larvae)	Actual mortality	Linear Probit	Mortality (%)
24	1×10^4	30	1	2.9702	3.333
	1×10^5	30	1	3.5096	3.333
	1×10^6	30	6	4.0490	20.000
	1×10^7	30	10	4.5885	33.333
48	1×10^4	30	1	3.1982	3.333
	1×10^5	30	3	3.6852	10.000
	1×10^6	30	6	4.1722	20.000
	1×10^7	30	11	4.6591	36.667
72	1×10^4	30	2	3.5183	6.667
	1×10^5	30	4	3.9450	13.333
	1×10^6	30	9	4.3717	30
	1×10^7	30	12	4.7984	40

Table 3: Results of two bioassay methods for cypermethrin and HytaNPV on the population of 5th instar larvae of *Hyposidra talaca* (number of larvae =30)

Method of bioassay	Insecticide/ entomopathogen	Exposure time (h)	LC_{50}*	Slope ± SE
Diet-incorporation method	Cypermethrin	24	38.62 (19.56-495.41)	1.3240 ± 0.4225
		48	25.49 (14.59-136.00)	1.2912 ± 0.3803
		72	22.19 (12.56-133.23)	1.1490 ± 0.3568
Diet-coating method	HytaNPV	24	5.79×10^7 (9.89×10^6- 1.43×10^{10})	0.5394 ± 0.1562
		48	5.01×10^7 (8.04×10^6- 1.55×10^{10})	0.4870 ± 0.1419
		72	2.97×10^7 (4.7×10^6- 8.92×10^9)	0.4267 ± 0.1264

* expressed in mg/l for cypermethrin and in OB/ml for HytaNPV

and 72 h, were determined as 5.8 x 107, 5 x 107 and 3 x 107 OB/ml, respectively, using LdP line software (Table 3).

The median lethal concentrations of cypermethrin for the fifth instar *H. talaca* larvae showed a sharp decrease with the duration of the exposure from 24 h to 48 h. It indicated that cypermethrin is a fast-acting pesticide as this synthetic pyrethroid is neurotoxic that acts on the nervous system of the caterpillar within few hours of the application of the pesticide (Ghatak *et al.,* 2008). Das *et al.* (2010) reported an LC_{50} value for cypermethrin on the 3rd instar larvae of *H. talaca* using leaf-dip bioassay which is about eight and half times more than our results for 5th instar *H. talaca* larvae. It might be due to difference in the method of bioassay as well as for different developmental stages. Ghosh (2021) reported higher LC values for emamectin benzoate, a second generation avermectin, using leaf-dip method (17.03, 2.80 and 0.93 at 24 h, 48 h and 72 h, respectively), in comparison to the diet-incorporation method (2.86, 1.34 and 0.89 at 24 h, 48 h and 72 h, respectively). The leaf-dip method is a residue bioassay in which the pesticide is spread evenly on the leaf surface only. But in diet-incorporation method the pesticide is homogeneously mixed with the artificial diet and thus in spite of same concentration of the pesticide used for the two bioassays, the pesticide molecules will be more in case of incorporation method (Ghosh, 2021). In a comparative study, Das *et al.* (2010) have shown higher LC value for cypermethrin in the 3rd instar larvae of *H. talaca* than *H. infixaria* at same developmental stages that indicated higher tolerance of *H. talaca*. Even the field dose of 500 ppm of cypermethrin for tea loopers (Gurusubramanian *et al.,* 2008) is much less than the LC_{95} value for both congeners of *Hyposidra* (Das *et al.,* 2010). It also indicates higher tolerance of these looper caterpillars against the synthetic pyrethroid (cypermethrin). Comparatively, lower LC values for emamectin benzoate (2.86, 1.34 and 0.89 at 24 h, 48 h and 72 h, respectively), for 5th instar *H. talaca* larvae than cypermethrin (38.62, 25.49 and 22.19 at 24 h, 48 h and 72 h, respectively) were estimated using diet incorporation method (Ghosh, 2021). This also indicated higher tolerance of *H. talaca* for cypermethrin than emamectin benzoate.

Slow decrease of LC_{50} value of NPV in 5th instar *H. talaca* larvae from 24 h post-infection to 48 h post-infection with a steep decline of LC_{50} value after 72 h exposure indicated the delayed activity of NPV. After ingestion of occlusion bodies (OBs) they have to be dissolved and the released virions have to invade the columnar epithelial cell of the mid-gut to establish and spreading of infection (Harrison and Hoover, 2012). The leaf-dip bioassay of four HytaNPV isolates on 2nd instar *H. talaca* larvae resulted lower LC_{50} values than our results (Mukhopadhyay *et al*., 2011, Dasgupta *et al.,* 2016). Unlike cypermethrin, emamectin benzoate and *Bt*-based biopesticide (Ghosh, 2021), a low LC value for NPV in leaf-dip method cannot be explained on the basis of the bioassay method. The higher LC value for NPV in this study might be due to advanced developmental stages of the larvae which indicate that the tolerance to the entomopathogen increases along with the advancement of the developmental stage.

Conclusion

In the present study, the LC values for a regularly used chemical pesticide (cypermethrin) and a potential entomopathogenic agent (NPV) which can be developed as a potent biopesticide against the major defoliating tea pest, *Hyposidra talaca* reared on artificial diet, have been estimated. This information will open up new research opportunities regarding the effect of cypermethrin (a chemical pesticide) and NPV (a future biopesticide) on the physiological parameters of this major defoliator pest of tea at the sub-lethal concentration that can help planters to opt an efficient pest management strategy.

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