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Toxicity of neem pesticides on a fresh water loach, *Lepidocephalichthys guntea* (Hamilton Buchanan) of Darjeeling district in West Bengal

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Abstract: *Static renewal bioassay tests were conducted to evaluate the acute toxicity of two neem based biopesticides, applied widely on tea plantation namely, Nimbecidine and Neem Gold either separately as well as, in combination to the fingerlings (mean body length- 4.46± 0.15 cm; mean body weight- 0.49± 0.15g) of a fresh water loach, Lepidocephalichthys guntea (Hamilton Buchanan) acclimatized to laboratory conditions prior to experiment. The 96 hours LC_{50} values for Nimbecidine and Neem Gold and the combination of the two were 0.0135mgl^{-1}, 0.0525mgl^{-1} and 0.0396 mgl^{-1}, respectively. The regular water quality analysis showed, that with increasing doses of biopesticides, dissolved oxygen level was lower and other parameters like pH, free carbon dioxide, total alkalinity, total hardness, chloride ions of water increased. The fish under toxicity stress suffered several abnormalities such as erratic and rapid movement, body imbalance and surface floating responding proportionately to the increase in concentrations of the toxicant biopesticides. The 96 hours LC_{50} values proved Nimbecidine more toxic than Neem Gold and the combination of the two biopesticides.*

Key words: *Toxicity, Neem pesticides, Lepidocephalichthys guntea, Tea gardens*

Introduction

Tea is an important cash crop from economic point of view of the country. Familiarly known in the country and abroad for production of quality tea, the Darjeeling district in the Northern part of West Bengal abounds in tea gardens and hill streams (perennial and seasonal). Most of these hill streams originating from the Himalayas and running through the tea gardens have a wide variety of edible and ornamental fishes. *Lepidocephalichthys guntea,* a common fresh water loach is found in this region and has both edible and ornamental value in the market.

It has been observed, that widely used chemical pesticides of the categories like, organophosphates and organochlorides cause heavy environmental pollution. Therefore, nowadays, it is propagated worldwide to use herbal pesticides in lieu of the chemical ones. Among the herbal or biopesticides, neem based pesticides namely, Nimbecidine and Neem Gold are being mostly used in the tea gardens to eradicate insect pests.

The acute toxicity values of several neem preparations and some for pure azadirachtin for laboratory animals and some non target species have been studied extensively (Gandhi *et al.*, 1988; Mahboob *et al.*, 1998; Wan *et. al*., 1996; Osuala and Okwuosa, 1993). Das *et al.* (2002), found the acute toxicity of neem, in Indian major carps, whilst, Anjaneyulu *et al.* (1999) worked on the acute toxicities of Neem Oil and Nimin (neem based product) on the aquatic hemipteran predatory insect, *Notonecta sp.* and the fingerlings of *Labeo rohita,* respectively. But there is no report of the acute toxicities of neem-based biopesticides on the fish, *L. guntea.*

Thus, the present study is aimed to determine the LC_{50} values of two neem based pesticides namely, Nimbecidine and Neem Gold separately, as well as, in combination on the fingerlings of *L. guntea* at 96 hr exposure period and some water quality parameters at the desired toxicity level.

Materials and Methods

Biopesticides : Nimbicidine (manufactured by T. Stanes and Company) and Neem Gold (manufactured by SPIC-Southern Petrochemicals Industries Limited, India) are the two neem based biopesticides used in the study. Nimbicidine is a neem oil based preparation containing a maximum of 300 mgl^{-1} (0.03%) of azadirachtin as active ingredient. Neem gold, on the other hand, is based on neem seed kernel extract. It contains a maximum of 1500 mgl^{-1} (0.15%) of azadirachtin A as active ingredient.

Test animal: Fingerlings of loach *(L. guntea)* weighing 0.49±0.09g with a mean body length of 4.46±0.15cm were collected from a local stream of Darjeeling district. Fish were brought to the laboratory and acclimatized for two weeks prior to exposure. The length and weight of the fingerlings were recorded.

Experimental procedure: The experiments were conducted in a series of plastic containers (18 litre capacity) filled with 10 litre tap water. For the test of Nimbecidine, Neem Gold and the combination of both (ratio 1:1), 0.0105, 0.012, 0.0135, 0.015 mgl^{-1}; 0.0375, 0.0525, 0.06, 0.075mgl^{-1} and 0.036, 0.0378, 0.0387, 0.0396, 0.0414, 0.0450 mgl^{-1} concentrations, respectively along with a control in triplicate were maintained. These concentrations were selected to determine the fifty percent lethal concentration

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(LC_{50}) values of the selected biopesticides for *L. guntea* exposed for 96 hours. The tests for each Nimbecidine, Neem Gold and the combination of both (1:1) were done separately.

For each experiment, the required concentrations were prepared from fresh stock solution of the pesticides by adopting dilution techniques (APHA, 1998). Five acclimatized fingerlings of uniform size were exposed in each concentration. Behaviour of the test fish fingerlings was observed and the dead fish were removed and recorded from time to time during 96 hr exposure period. The water in the containers were changed every 24 hr and a constant concentration of Nimbecidine and Neem Gold was maintained during the period of exposure. Some water quality parameters such as, dissolved oxygen (DO), free carbon dioxide, total alkalinity, total hardness and chloride ion of the test media were recorded daily. The physico-chemical characteristics of test water (tap water of the laboratory) were, pH:6.43; DO:4.2 mgl^{-1}; free carbon dioxide:6.0 mgl^{-1}; total alkalinity:15.4 mgl^{-1}; total hardness: 27.73 mgl^{-1} and chloride ion:13.84 mgl^{-1}.

Results and Discussion

The cumulative mortality percentage of *L. guntea* fingerlings exposed to different concentrations of Nimbecidine, Neem Gold and the combination of these two biopesticides are presented in Table 1. Hundred percent mortalities (LC_{100})occurred in fish at the doses of 0.015 mgl^{-1}, 0.075 mgl^{-1} and 0.045 mgl^{-1} and there was no mortality (LC_0) found at the doses of 0.0105 mgl^{-1}, 0.0375 mgl^{-1} and 0.036 mgl^{-1} in case of Nimbecidine, Neem Gold and the combination of the two, respectively. These data clearly suggest that Nimbecidine (LC_{50} – 0.0135 mgl^{-1}) has a greater toxicity than either Neem Gold (LC_{50} – 0.0525 mg/l) or the combination of the two biopesticides (LC_{50} – 0.0396 mgl^{-1}**).** This may be due to the oily layer over the water, produced by the application of Nimbecidine, which reduces the oxygen uptake of fish fingerlings and causes mortality at a faster rate.

In all the cases, after application of these biopesticides rapid movement and then surface floating of test fish fingerlings indicated stressed condition. The mucous secretion over the gill epithelium and blood oozing further supported stressed condition.

Table - 1: Cumulative mortality percentage of fingerlings of loach, *Lepidocephalichthys guntea* exposed to different concentrations of Nimbecidine, Neem Gold and the combination of the two, for 96 hr

Concentrations (mgl^{-1})	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Nimbecidine				
Control	00	00	00	00
0.0105	00	00	00	00
0.012	00	10	10	10
0.0135	10	20	30	50
0.015	40	60	80	100
Neem Gold				
Control	00	00	00	00
0.0375	00	00	00	00
0.0525	00	00	30	50
0.06	00	50	60	90
0.075	10	60	80	100
Nimbecidine + Neem Gold				
Control	00	00	00	00
0.036	00	00	00	00
0.0378	00	00	00	10
0.0387	00	00	10	20
0.0396	00	10	30	50
0.0414	00	20	30	70
0.0450	30	60	80	100

The summary of the selected water quality parameters of the acute toxicity test in case of Nimbecidine, Neem Gold and the combinations of the two are shown in Table 2, 3 and 4, respectively. The mean pH values varied from 6.28 – 6.83 with a gradual increase in the concentrations of biopesticides. The pH of the test water always remained in slightly acidic condition.

In the present study, the DO content recorded, showed a gradual decrease with increasing dose of biopesticides. In case of Nimbecidine at a concentration of 0.015mgl^{-1} the mean DO was as low as 1.25 mgl^{-1} whereas, Neem Gold with a concentration of 0.075 mgl^{-1}, the mean DO was 2.69 mgl^{-1}. According to Swingle (1967), at DO between 1-5 mgl^{-1}, the fish

Table - 2: Influence of various concentrations of Nimbecidine on some water quality parameters during 96 hr LC_{50} test

Parameters	Concentrations (mgl^{-1})			
	0	0.012	0.0135	0.015
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Temperature (^{0}C)	29.0 ± 1.65	28.5 ± 2.08	29.5 ± 1.73	29.0 ± 1.41
pH	6.45 ± 0.14	6.48 ± 0.10	6.55 ± 0.13	6.63 ± 0.21
Dissolved oxygen (mgl^{-1})	3.80 ± 0.23	2.55 ± 0.19	2.40 ± 0.27	1.25 ± 0.19
Free carbon dioxide (mgl^{-1})	6.42 ± 0.22	6.63 ± 0.15	6.64 ± 0.11	10.75 ± 2.22
Total alkalinity (mgl^{-1})	14.92 ± 0.81	14.93 ± 1.09	15.50 ± 0.41	16.00 ± 2.16
Total hardness (mgl^{-1})	21.11 ± 0.76	21.63 ± 0.63	22.40 ± 0.49	22.46 ± 1.0
Chloride ions (mgl^{-1})	15.13 ± 1.41	15.40 ± 0.34	15.52 ± 1.5	16.75 ± 1.60

S.D = Standard Deviation; n = 12

Table - 3: Influence of various concentrations of Neem Gold on some water quality parameters during 96 hr LC_{50} test

Parameters	Concentrations (mgl^{-1})			
	0	0.0525	0.060	0.075
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Temperature (^0C)	27.8 ± 1.34	26.5 ± 1.0	28.0 ± 0.82	29.0 ± 0.82
pH	6.62 ± 0.17	6.63 ± 0.13	6.68 ± 0.15	6.83 ± 1.17
Dissolved oxygen (mgl^{-1})	4.75 ± 0.29	3.94 ± 0.31	3.67 ± 0.29	2.69 ± 1.28
Free carbon dioxide (mgl^{-1})	5.01 ± 0.57	5.85 ± 0.44	5.90 ± 0.42	8.3 ± 2.91
Total alkalinity (mgl^{-1})	15.83 ± 0.44	15.85 ± 0.87	16.08 ± 0.70	16.1 ± 0.52
Total hardness (mgl^{-1})	25.46 ± 0.77	25.50 ± 1.29	25.83 ± 0.85	25.85 ± 1.46
Chloride Ion (mgl^{-1})	12.03 ± 1.85	12.5 ± 2.12	12.78 ± 0.48	14.18 ± 1.26

S.D = Standard Deviation; n = 12

Table - 4: Influence of various concentrations of Nimbecidine and Neem Gold in combination on some water quality parameters during 96 hr LC_{50} test

Parameters	Concentrations (mgl^{-1})				
	0	0.0378	0.0387	0.0396	0.0414
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Temperature (^0C)	26.19 ± 1.91	24.75 ± 2.75	25.75 ± 0.96	27.25 ± 0.96	27.0 ± 1.83
pH	6.28 ± 0.12	6.34 ± 0.18	6.45 ± 0.06	6.46 ± 0.21	6.79 ± 0.37
Dissolved oxygen (mgl^{-1})	5.14 ± 0.21	4.13 ± 0.25	3.56 ± 0.27	3.34 ± 0.33	3.24 ± 0.42
Free carbon dioxide (mgl^{-1})	4.76 ± 1.05	5.93 ± 1.23	6.90 ± 1.21	6.43 ± 0.85	8.35 ± 2.12
Total alkalinity (mgl^{-1})	15.15 ± 1.07	15.8 ± 0.72	15.9 ± 0.67	16.62 ± 0.58	17.8 ± 0.86
Total hardness (mgl^{-1})	23.54 ± 1.32	23.55 ± 1.37	24.2 ± 1.30	26.0 ± 0.16	26.66 ± 0.91
Chloride ion (mgl^{-1})	11.72 ± 1.10	11.85 ± 1.51	13.05 ± 1.53	14.05 ± 1.44	15.27 ± 1.23

S.D = Standard Deviation; n = 12

survive but the reproduction is poor and growth is slow if exposure is continued and with DO less than 5.0 mgl^{-1} is not considered conducive for fish growth (Mondal and Barat, 2004).

The free carbon dioxide in a waterbody is mostly derived from the decomposition of organic matter and the respiration of organisms. The present findings suggest, higher mean of free carbon dioxide in Nimbecidine at concentration 0.015 mgl^{-1} (10.75 mgl^{-1}) Neem Gold at concentration 0.075 mgl^{-1} (8.3 mgl^{-1}) and at the concentration of 0.0414 mgl^{-1} in the combination of both the biopesticides (8.35 mgl^{-1}) (Table 2, 3 and 4). Higher free carbon dioxide levels in these cases may be due to higher doses of biopesticides.

The total alkalinity here refers to bicarbonate alkalinity only, as carbonate alkalinity was absent throughout the study period. Lower alkalinity was recorded in all the experiments and a relatively higher mean of total alkalinity was found in case of the combination of two biopesticides (Nimbecidine and Neem Gold) at a concentration of 0.0414 mgl^{-1} (17.8 mgl^{-1}).

Kannan (1991) suggested, that hardness of water ranging between 0-60 mgl^{-1} can be regarded as soft water. In the present study, the mean hardness values of water in all the cases varied between 21.11 – 26.66 mgl^{-1} which can be considered soft in nature.

Higher concentration of chloride in water is an index of pollution of animal origin and there is a direct relation between chloride concentration and pollution level (Munawar, 1970). In our case, lower chloride concentrations were found in all the cases but a little higher concentration was found in case of higher doses of biopesticides.

Thus, it can be concluded, that Nimbecidine and Neem gold separately, as well as in combination, are highly toxic to the fingerlings of loach, *L. guntea.* If the rampant use of these biopesticides continue, it may cause high mortality of the fingerlings of loach, *L. guntea* and thus, reduce the gene pool of the fish.

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