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Effect of Fish Size and Treatment Conditions on the Piscicidal Activity of *Nerium indicum* Latex Powder

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ABSTRACT- Comparative toxicity of *Nerium indicum* latex powder (NILP) was studied against two common predatory and weed fishes of different body size in laboratory condition and cemented and muddy pond. LC₅₀ of NILP for predatory fish *Channa punctatus* was 2.13 mg/L, 7.80 mg/L and 19.26 mg/L to small size, middle size and large size fish respectively in laboratory condition after 24 h exposure periods. Similar trends were also observed in case of cemented and muddy ponds but doses were 1-4 times higher than laboratory conditions. Similar trend of result was also observed against weed fish *Mystus mystus*. No further mortality was observed beyond 24 h in all set of experiments suggested fast degradation of the toxicant in water.

Key-words- Toxicity, Fish, Botanicals, Nerium indicum, Piscicidal Activity

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INTRODUCTION

One of the most important factors that adversely affect the aquaculture production is the presence of pests, predators and competitors of cultivated organisms. The predatory and weed fishes share and better utilized the cultured carp habitats as well as food also [1,2]. So, the removal of these unwanted fish population is necessary before the seed of cultured carps was added. Application of piscicides is the best method for eradication of unwanted fish population [3]. There are many kinds of piscicides of different effectiveness are used [4,5]. The chemically derived piscicides (toxaphene) are long lasting and its toxicity remains persist up to years and detoxify slowly, as its concentration reaches to level suitable for re-stocking [6]. On the other hand, the plant derived piscicides has gained unprecedented impetus because they detoxify rapidly i.e., easily biodegradable with less hazard of environmental contamination and have high piscicidal properties and safe for operator [7-9].

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It has been reported that the toxicity of plant products is affected by several factors such as size of fish, temperature and treatment conditions (in Laboratory condition and in Ponds). The aim of this study was to assess the piscicidal action of *Nerium indicum* latex powder (NILP) against different size of fresh water predatory fish *Channa punctatus* and weed fish *Mystus mystus* in different treatment conditions i.e., in Laboratory condition and in cemented and in muddy Pond.

MATERIALS AND METHODS

Test Organisms

Hundred fish *Channa punctatus* of different size (small size 2.5 ± 0.83 cm, middle size 7.5 ± 0.83 cm and large size 15.7 ± 1.35 cm in total length) and hundred *Mystus mystus* (small size 1.8 ± 0.52 cm, middle size 5.0 ± 0.35 cm and large size 10.2 ± 1.83 cm in total length) were collected from Mahesara lake of Gorakhpur district. The collected fishes were stored in plastic tank containing 100 L of de-chlorinated tap water for acclimatization to laboratory condition for seven days. Injured and dead fish (if any) were removed immediately after they were found. Water was changed every day. Fishes were fed with commercial food.

Latex of *Nerium indicum* plant was collected from botanical garden of DDU Gorakhpur University, Gorakhpur and lyophilized at -40° C. The wet weight of 1 ml latex of *Nerium indicum* was 780 mg and dry weight

(lyophilized at -40° C) was 200 mg. The freeze dried *Nerium indicum* latex powder (NILP) was used for the toxicity tests.



Fig. 1: Nerium indicum

Toxicity Experiments

The method of Singh & Agarwal [10] was used for the toxicity evaluation of latex powder. The latex powder was first dissolved in water to achieve the desired concentration and then poured into glass aquaria. Ten fishes of nearly similar size were put in glass aquaria containing 10L de-chlorinated tap water. Six aquaria were set up for each dose. Sixty fishes of nearly similar size were put in pond of the size 29.28 m² in area and 9.19 m³ in water volume. Pond experiment was also replicated three times. Fishes were exposed for 24 h, 48 h, 72 h or 96 h to four different concentration of NILP. The NILP were added in the beginning of experiment. The fishes were not fed during the experiment and no aeration was given to the test media. Water analysis for various physico-chemical parameters, viz. temperature, pH, dissolved O₂, total ammonia, free

CO₂ and total alkalinity were carried out every week. Water temperature ranged from 27.4-28.6 ^oC. The control group was kept in similar condition without any treatment. The mortality was recorded every 24 h up to the observation period of 96 h. Dead fishes were removed immediately after they were found. Percent mortality has been expressed as mean±SE of six replicates. Toxicity data were computed through POLO plus computer program of Robertson [11].

RESULTS

The toxicity of NILP against fresh water weed fish Mystus mystus and predatory fish Channa punctatus is presented in Table 1 and 2. Table 1 shows, 24 h exposure of small size fish Mystus mystus treated with 0.6 mg/L NILP caused 20% mortality and 1.0 mg/L caused 80% mortality, middle size fish on exposure to 1.5 mg/L caused 25% and 4.5 mg/L caused 90% mortality and exposure with 7.0 mg/L caused 30% and 19.0 mg/L caused 90% mortality in large size fish under laboratory condition. Similar trends were also observed in case of experimental and muddy ponds but the doses were higher than laboratory condition. The LC₅₀ of NILP against small size, middle size and large size fish Mystus mystus was 1.04 mg/L, 2.70 mg/L and 9.80 mg/L respectively after 24 h in laboratory condition. Similar trend of result was also observed against predatory fish Channa punctatus. The LC₅₀ of NILP against small size, middle size and large size fish Channa punctatus was2.13 mg/L, 7.80 mg/L and 19.26 mg/L respectively after 24 h in laboratory condition. No mortality was observed in the control group. Prolongation of exposure period up to 96 h did not cause any further mortality in fishes after 24 h, suggested fast degradation of toxicant in water.

In both fish species, the LC_{50} values increased with increasing fish size and also vary to different treatment conditions i.e., lowest in laboratory condition, followed by experimental (cemented) and muddy ponds.

Table 1: Toxicity (Mean±SE) of freeze dried latex powder of *Nerium indicum* plant on different size of weed fish *Mystus mystus* after 24h exposure period in different experimental conditions

Small Size	Μ	Middle Size Large		ge Size	
Doses (mg/L)	Percent	Doses (mg/L)	Percent	Doses (mg/L)	Percent Mortality
	Mortality		Mortality		
Control	-	Control	-	Control	-
0.6	20.0 ± 2.83	1.5	25.0±2.83	7.0	30.0±1.83
0.8	40.0±0.0	2.5	60.0 ± 2.45	11.0	51.0±2.31
1.4	60.0 ± 2.45	3.5	$80.0{\pm}1.85$	15.0	80.0 ± 2.45
1.8	80.0 ± 4.01	4.5	90.0±4.01	19.0	90.0±2.31
LC_{50}	1.04	LC_{50}	2.70	LC_{50}	9.80
	(0.92-1.16)		(2.47-2.93)		(8.86-10.80)
Slope value	2.87 ± 0.46	Slope value	4.04 ± 0.52	Slope value	4.21±0.57

In laboratory condition

In Experimental Pond

Control	-	Control	-	Control	-
1.0	10.0±2.31	3.0	30.0±4.91	15.0	21.0±4.91
1.5	40.0±2.31	5.0	60.0±0.0	20.0	47.0±4.90
2.0	70.0±1.83	7.0	80.0±1.83	25.0	58.0±4.01
3.0	90.0±2.45	9.0	90.0±2,45	30.0	75.0±2.81
LC_{50}	1.62	LC_{50}	4.14	LC_{50}	21.71
	(1.53-1.81)		(3.21-4.89)		(20.19-23.32)
Slope value	4.15±0.55	Slope value	4.32±0.55	Slope value	4.64 ± 0.78

In Muddy Pond

Control	-	Control	-	Control	-
3.5	10.0±2.83	8.0	30.0±2.83	32.0	21.0±2.31
4.0	40.0±1.83	10.0	50.0±4.90	36.0	30.0±1.83
4.5	60.0±2.45	12.0	80.0±1.83	40.0	55.0±3.37
5.0	80.0±2.31	14.0	90.0±2.45	44.0	73.0±2.45
LC ₅₀	4.28 (4.17-4.39)	LC ₅₀	9.53 (9.02-9.98)	LC ₅₀	38.95 (37.77-40.30)
Slope value	13.11±1.69	Slope value	7.65±1.06	Slope value	10.59±1.72

- No further mortality was observed after 24h exposure periods
- -, no mortality in control group
- Concentrations given are final concentrations (w/v) in laboratory and pond
- Regression coefficient shows that there was significant (P<0.05) negative regression between exposure time and different LC value
- Values in parentheses are LCL and UCL of LC values
- LCL = Lower confidence limit; UCL = Upper confidence limit

Table 2: Toxicity (Mean±SE) of freeze dried latex powder of *Nerium indicum* plant on different size of predatory fish *Channa punctatus* after 24h exposure period in different experimental conditions

In Laboratory condition

Small Size		Middle Size		Large Size	
Doses (mg/L)	Percent	Doses (mg/L)	Percent Mortality	Doses (mg/L)	Percent Mortality
_	Mortality			-	
Control	-	Control	-	Control	-
1.5	20.0±2.83	5.0	20.0±2.45	12.0	30.0±2.45
2.0	40.0±0.0	7.0	50.0±2.31	22.0	55.0±4.91
2.5	60.0 ± 2.80	9.0	60.0±1.83	30.0	67.0±1.83
3.0	90.0±1.81	11.0	80.0±2.34	36.0	80.0±2.45
LC_{50}	2.13	LC_{50}	7.80	LC_{50}	19.26
	(2.02 - 2.02)		(7.16-8.51)		(16.54-21.72)
Slope value	6.65 ± 0.85	Slope value	3.87±0.68	Slope value	2.69±0.48

In Experimental Pond

Control	-	Control	-	Control	-
3.0	40.0±2.31	8.0	20.0±1.83	32.0	25.0±2.31
3.5	60.0±1.83	10.0	50.0±2.31	36.0	55.0±1.83
4.0	80.0±2.45	12.0	80.0±2.45	40.0	73.0±2.45
4.5	90.0±2.31	14.0	90.0±2.31	44.0	83.0±2.31
LC ₅₀	3.22	LC_{50}	9.61	LC_{50}	35.82
	(3.04-3.36)		(9.08-10.06)		(34.65-36.83)
Slope value	8.81±1.42	Slope value	7.48±1.03	Slope value	11.94±1.77

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Control	-	Control	-	Control	-
5.0	10.0±2.83	18.0	20.0±1.83	51.0	20.0±4.91
7.0	30.0±2.83	20.0	50.0±1.83	55.0	50.0±4.90
9.0	60.0±4.01	22.0	70.0±2.31	60.0	70.0±2.45
11.0	80.0±2.83	24.0	90.0±2.45	65.0	90.0±2.31
LC ₅₀	8.01	LC ₅₀	19.86	LC ₅₀	55.84
	(7.56-8.50)		(18.85-20.68)		(54.76-56.84)
Slope value	5.97±0.77	Slope value	17.92±2.20	Slope value	19.25±2.46

In Muddy Pond

- No further mortality was observed after 24h exposure periods
- Other details as given in Table 1

DISCUSSION

It is clear from our result, that the *Nerium indicum* latex powder has potent piscicidal activity against predatory fish *Channa punctatus* and weed fish *Mystus mystus*. Mortality caused by the NILP showed a significant positive correlation between dose and mortality (Table 1, 2). The positive correlation between dose and mortality was noted because increase concentration of pesticides in aquarium water resulted in more intake or entry of pesticides in the body of animals. This trend is also depend upon several factors such as rate of penetration, nature of slope, variability and maximal effects of active moieties [12].

The body size or different stages of fish influence the acute toxicity of NILP. It is assumed that size of target organisms will increase proportional to body weight, thus larger organisms require higher doses to achieve a detectable effect [13,14].

The different treatment conditions also affect the toxicity of latex powder. It is clear from the result that the latex powder is very effective in laboratory condition followed by cemented and earthen pond. Chiayvareesajja [15] reported that under natural conditions many factors such as temperature, photo-stability, adsorption by soil particles etc. have a possible influence on toxicity and toxicant degradation.

Dawson [16] found that rotenone disappeared two to three times faster in earthen ponds than in concrete ponds. Detoxification of phostoxin occurred in four days in laboratory tanks at 23° C and in one day in earthen ponds at 30° C [17].

A comparison of the toxicity of the synthetic pesticides i.e organophosphate: Dimethoate (24 h LC_{50} –17.92 mg/L) and carbonate: Carberyl (24 h LC_{50} –18.51 mg/L) compounds [18] with NILP (24 h LC_{50} –19.26 mg/L) against fresh water fish *Channa punctatus*, is nearly similar to pure synthetic pesticides in laboratory condition.

It is believed that further purification and extraction of active compounds from latex powder will almost give better results than the synthetic piscicides for eradication of unwanted fish population from fish culture ponds and ultimately save the water bodies from pesticidal pollution.

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