

Acute Toxicity and Behavioural Response in Fresh water Fish *Labeo rohita* Exposed to Floraguard and Neem-On Biopesticides

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ABSTRACT

The objective of this study was to assess the acute toxicity and behavioural changes of biopesticides Floraguard and Neem-On on the freshwater fish *Labeo rohita*. Fishes were exposed to various concentrations of these biopesticides to determine the LC₅₀ values. It was found that Floraguard is more toxic than Neem-On. Behavioural patterns were observed critically during the whole experiment. The test fish exhibited erratic swimming, increased surfacing, decreased rate of opercular movement, reduced agility and inability to maintain normal posture and balance with increasing exposure time.

Key words: Acute toxicity, Behavioural changes, *Labeo rohita*, Neem-On, Floraguard

INTRODUCTION

Several studies have assessed the toxicity of various pesticides such as organophosphate, organochlorine, carbamide and pyrethroid pesticides to the aquatic biota especially fishes^{5,12}.

The acute toxicity test is short term exposure to the test organisms under the laboratory condition. The mortality of the test animal is the most detectable response to find out the LC₅₀ concentration of experimental organisms. The wide use of fishes as test organism is probably due to their adaptability to the laboratory conditions as well as their availability and their varying degree of sensitivity to the toxic substance. Variety of

fishes are found in the Meerut region, the *Labeo rohita* was selected for the present study owing to fact that, the *Labeo rohita* inhabiting the fresh water sources is widely cultured in ponds, lakes of this region.

The present work has been carried out to study the toxicity of bio-pesticides “Floraguard” and “Neem-On” on the freshwater fish “*Labeo rohita*”. Various biopesticides belonging from different groups are available in the market. Neem-On and Floraguard were selected for present study because, not much records of toxic effects of these biopesticide on the freshwater fish were found. These biopesticide are widely used in agricultural fields in this region.

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MATERIAL AND METHODS

In the present study, the specimens of freshwater fish, *Labeo rohita* of the weight 10 ± 2 gm and length 8 ± 1 cm were selected for the experiment and were collected from Upper Ganga Canal, Jani Khurd Village, Meerut (U.P.), India. The fishes were purchased by local fishermen. Collected fishes were transported alive to the laboratory in large plastic containers filled with canal water to minimize stress and mortality. The fish were checked for injury as well as disease, if any. They were treated with 0.1% KMnO₄ solution and acclimatized to standard laboratory condition for 10 days in glass aquaria of 40 L capacity containing dechlorinated tap water (Temperature $28 \pm 2^\circ\text{C}$; total hardness 518 ± 23 mg/l; dissolved oxygen 5.6 ± 0.2 mg/l; salinity 1.2 ± 0.13 ppt and pH 7.8 ± 0.04). During the acclimatization process, the fishes were fed daily with fish food (Taiyo) purchased from the market.

NEEM-ON (Manufactured by Jai Kissan Agro Pvt. Ltd., Indore, M.P., India) and Floraguard (Manufactured by Asean Agro technologies (I) Pvt. Ltd., Nasik, Maharashtra, India) were selected for present study and were purchased from the market. Food supply was withdrawn 24 hrs preceding the experiment. The fishes then kept in the plastic through having 10 liters of water and each through contains 10 fishes. The control and experimental fishes were exposed to the biopesticides at 0, 5, 10, 20, 40, 80 and 100 ppm concentrations for 24, 48, 72 and 96 hrs respectively. Three replicates were maintained simultaneously. The data were collected and used to calculate median lethal concentrations (LC₅₀) and median lethal time (LT₅₀) values. Simultaneously behaviour changes of the fish were also observed and recorded during the exposure period.

RESULTS

In this study, *Labeo rohita* was subjected to various concentrations of Neem-On and Floraguard and both behaviour and mortality were observed during the exposure. No mortality was found in the control group of

fish. The behavioral and the swimming patterns of the fish were normal in case of control group and there was no mortality. But when the fishes were exposed to biopesticides Neem-On and Floraguard, various behavioral changes were observed. First the shoaling of fishes starts disrupting and then abnormal swimming behavior increases. The opercular movement initially increases and then decreases with rising toxicant concentration in the exposed fishes. Loss of balance increased and the color of the fish were observed to get lighter with an increased secretion of mucus. Surfacing frequency and gulping of surface water with occasional coughing increases remarkably in exposed fishes. Defecation was increased and more fecal matter was found at the bottom of the aquarium than control. Finally due to complete loss of balance, fishes sank to bottom with their ventral side facing upwards.

We calculated the median lethal concentration (LC₅₀) at 72 and 96 hrs and the median lethal time (LT₅₀) for each concentration of Neem-On and Floraguard (Table 1 & 2). The mortality rate of *Labeo rohita* after treatment with various concentrations of Neem-On and Floraguard were significantly different as compared with control as shown in Figure 1 (A - D). We observed that the toxic effects of Neem-On and Floraguard increase as concentrations increase and cause the severe ($p < 0.05$) toxicity in fishes as compared to control. In between both biopesticides, Floraguard is identified as more lethal as it killed the fishes within 72 hrs. At 96 hrs, its LC₅₀ was 10.1 ppm, significantly lower than Neem-On (Table 1). The Neem-On showing higher LC₅₀ value of 24.2 ppm at 96 hrs (Table 1). The LT₅₀ values for Floraguard also showed that it was the quickest in killing fishes with the lowest LT₅₀ value of 48hrs at 40 ppm as compared to 72hrs for Neem-On at 40 ppm (Table 2).

DISCUSSIONS

Neem-On and Floraguard are biopesticides that are widely used in agriculture. They are organic research products. They act as

systemic as well as contact poison to different pest species. They are commonly applied on grapes, mango, strawberry, brinjal, chili and onion etc. The aim of present the study was to assess acute toxicity and behavioural changes of these biopesticides on fresh water fish *Labeo rohita*.

In the present study, *Labeo rohita* was subjected to different concentrations of Neem-On and Floraguard and mortality and behavioural changes were observed. Acute toxicity studies are the very first step to determining the water quality requirements of fish and the studies reveal the toxicant concentrations that cause fish mortality even at short exposure¹³. Worldwide investigations reported effect of pesticides on aquatic organism^{14,15}. The acute toxicity values of several biopesticides for different fish species have been reported earlier by many workers. Das *et al.*¹⁶, have studied the acute toxicity of neem in the fingerlings of Indian major carps i.e., *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* and the 96h LC₅₀ values were found to be 2.36, 2.04 and 2.78ppm respectively. Hassanein *et al.*¹⁷, reported the 96h LC₅₀ value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112 ppm. Bhat *et al.*¹⁸, studied the acute toxicity of Neem-On on the *Labeo rohita* and the 96h LC₅₀ value of was found to be 42.66 ppm. Bansode and Patil¹⁹ recorded 96 hrs LC₅₀ value of neem based insecticide Bioneem on the fresh water fish *Gara mullya* was to be 167.45 ppm. Ishi & Patil²⁰ recorded 96 hours LC₅₀ value of a Newtech biopesticide on fresh water cyprinid *Danio aequipinnatus* was found to be 1.5549 ppm. Ishi & Patil¹², also recorded 96 hrs LC₅₀ value of Floraguard to *D. aequipinnatus* was found to be 1.9870 ppm. In the present investigation, 96 hrs LC₅₀ values of Floraguard and Neem-On to *Labeo rohita* were found to be 10.1 ppm and 24.2

ppm respectively. The variation in the LC₅₀ values is due to its dependence upon various factors viz., sensitivity to the toxicant, its concentration and duration of exposure; type and size of the test animal and so on. From the present study, it is indicated that Floraguard is highly toxic than Neem-On to freshwater fish *Labeo rohita*.

Behavioral changes are the most sensitive indication of potential toxic effects. Impact of different pesticides on the behavior of *Labeo rohita* have been studied by various workers^{9,18,21,22}. Fishes exhibited a number of behavioral changes when they were exposed to different concentrations. The opercular movement of fishes initially increases and then gradually decreases. Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular coordination which may be due accumulation of acetylcholine in synaptic and neuromuscular junctions²³. A thick coat of mucus was observed all over the body of the fish, making the fish slimier. The fish were swimming with the belly upwards and in zig zag motion. There were also erratic and parallel movements observed in the fish, indicating loss of equilibrium while in control, the fish was swimming normally without loss of equilibrium. The fish sometimes becomes highly excited and was observed to hit the aquarium walls at a very fast speed. Due to this hyperexcitability bleeding from the snout was observed in some fishes.

So in the present investigation, it can be concluded that Floraguard and Neem-On are ecofriendly biopesticides used against different agricultural pests but they are highly toxic to non-target organisms. So these biopesticides should be used cautiously.

Table: 1. Median lethal concentration (LC₅₀) values of heavy metals against Floraguard and Neem-On against *Labeo rohita*. (Numbers in parenthesis represent 95% confidence limits)

Time (h)	At 72h	At 96 h
Biopesticides	LC ₅₀	LC ₅₀
Floraguard	16.7 (5.9 – 31.8)	10.1 (2.8 – 18.2)
Neem-On	66.7 (32.2 – 688.7)	24.2 (12.8 – 43.8)

Table: 2. Median lethal time (LT₅₀) values of Floraguard and Neem-On against *Labeo rohita*

LT ₅₀ (in hrs)		
Dose (ppm)	Floraguard	Neem-On
5	72	84
10	72	72
20	60	72
40	48	72
80	48	72
100	48	72
χ^2 (log-rank test)	34.29	27.55
<i>p</i>	< 0.0001	< 0.0001

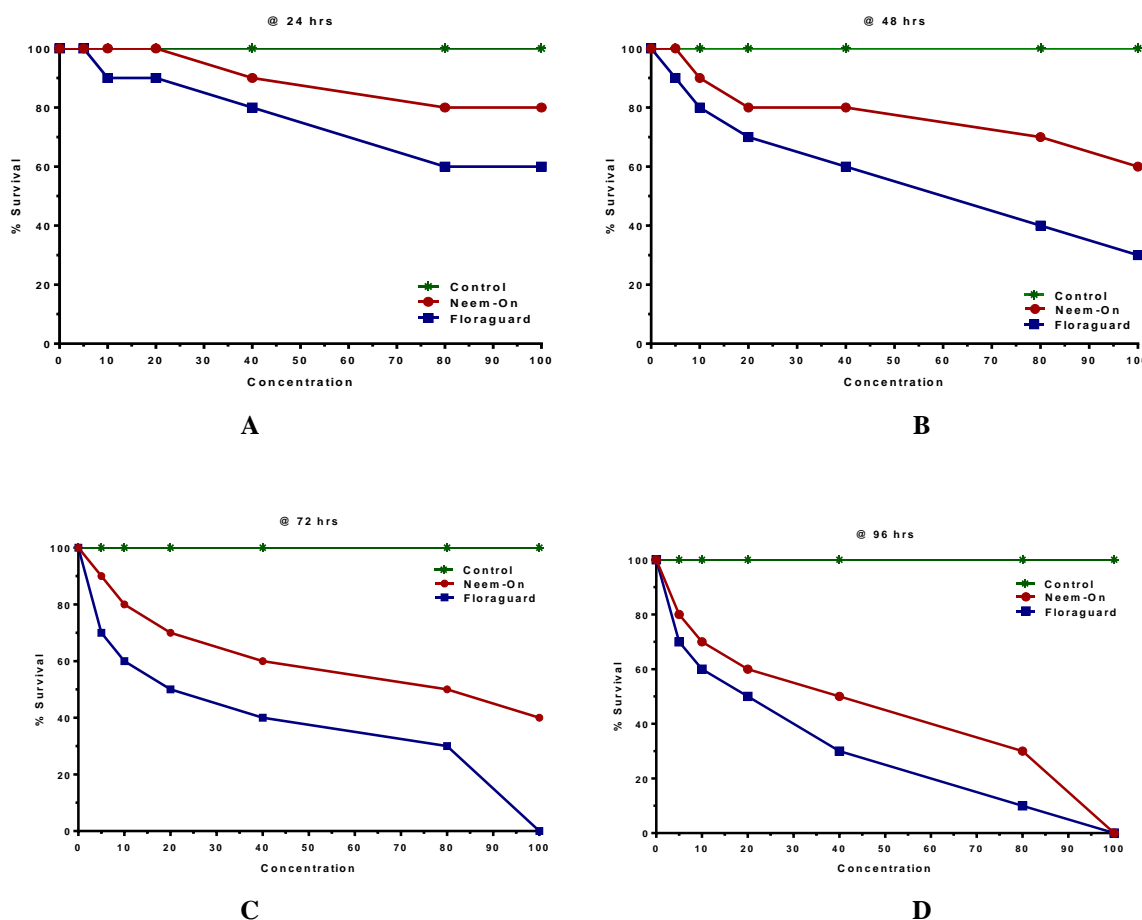


Figure: 1. Toxicity response curves of *Labeo rohita* at (A) 24 h; (B) at 48 h; (C) at 72 h and at 96 h to different concentrations of Floraguard and Neem-On Biopesticides.

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