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Toxicity of neem based insecticide Bioneem (Azadirachtin) to fresh water fish *Gara mullya* (Sykes)

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Abstract
Azadirachtin derived from neem (*Azadirachta indica A. Juss*) is a very effective and extensively used target specific and comparatively less toxic pesticide. Present work is carried out to determine 96 hours LC_{50} value and safe concentration of Bioneem to fresh water fish *Gara mullya* (Sykes). The biostatic assay of neem based pesticide, Bioneem has been carried out to determine 96 hours LC_{50} values at different exposure period for fresh water fish *G. mullya*. Estimated LC_{50} values at 24, 48, 72 and 96 hours of exposure to Bioneem are 334.58 ppm, 288.66 ppm, 222.53 ppm and 167.45 ppm respectively. *G. mullya* exposed to Bioneem (Azadirachtin) shows toxic effect and neem based pesticides may cause significant death of fishes when exposed for longer duration.

Keywords: Bioneem, azadirachtin, LC_{50}, *Gara mullya*

1. Introduction
Indiscriminate use of synthetic pesticides by the human activities causes high risk to non-target organisms [1]. Excessive use of pesticide results in the inflow of toxicants, mainly in to the aquatic bodies [2, 3]. Use of natural pesticides of plant origin is the recent emphasis to minimize hazardous effects of organic or synthetic pesticides. It is possible to substitute organic pesticides with the pesticides of plant origin. Botanical insecticides are environmentally friendly and do not leave any harmful residues in the aquatic environment due to their biodegradability [4-6]. Azadirachtin is an active ingredient of neem tree (*Azadirachta indica A. Juss*). It is very effective and extensively used target specific and comparatively less toxic pesticide. However Goktepe *et al.* [7] and El-Shazly *et al.* [8] reported lethal effect of neem extracts in aquatic environments to benthic populations. Mortality due to pesticidal exposure drastically decreases the number of organisms in the food web and nutrient cycling process. The toxicity study is essential to find out toxicants limit and safe concentration, so that there will be less harm to aquatic fauna in near future. *G. mullya* is a fish of nutritive importance in tribal community of Navapur tehsil and literature about pesticidal stress on *G. mullya* is scanty. Present work is aimed to find out acute toxicity and effects of bioneem (Azadirachtin) on freshwater fish *G. mullya* by determining the LC_{50} values at various exposure periods. This type of study will help to estimate the safe level dose and strengthen the baseline data by which comparative sensitivity of neem based pesticides could be analysed.

2. Materials and Methods
G. mullya (local name - Molga) used in the present investigation ranging in length 9-10 cm and weight 5 - 7 grams were collected from Bhavare Dam of Navapur Taluka. Acute toxicity experiments were carried out from November 2011 to February 2012. Fishes were brought to the research laboratory, A.C.S College Navapur, Dis. – Nandurbar and acclimatized to the laboratory condition for ten days in well aerated and dechlorinated water glass aquarium at a room temperature of 26 ± 1 ^{0}C and pH of the water maintained was 7.2 ± 0.3. During acclimation period the fishes were fed regularly. Aeration was provided. Physicochemical parameters of water used for experimentation were studied by method given in APHA and AWWA [9]. The dead fishes were removed immediately so as to avoid depletion of dissolved oxygen (D.O.) level and infection to other fishes [10]. Technical grade Bioneem containing 2% azadirachtin was 100% water soluble. 0.2ml bioneem was dissolved in 1000ml water to prepare 10ppm solution. Concentration of bioneem was increased to prepare solutions of

different concentration (50, 100, 150, 200, 250, 300, 350, 400 and 450 ppm).

Six replicates of each group of ten fishes with body weight 5-7 gm and 8-10 cm in length were selected for experimentation and exposed to different concentrations of Bioneem in plastic troughs containing 15 litres of water. Water with test concentration of pesticide was replaced after every 24 hrs up to 96 hrs and resulting mortality was recorded in the range of 10% to 100% for each concentration for the duration of 24, 48, 72 and 96 hours. Collected data was then analysed statistically by probit analysis method [11]. LC_{10} and LC_{50} values, the variance, fiducial limits, chi square test, lethal dose were calculated for 24, 48, 72 and 96 hours. Safe concentration of Bioneem was calculated by method described by Hart *et al.* [12] using the formula,

$$\text{Safe Concentration} = \frac{48 \text{ hrs TLM} \times 0.3}{S^2}$$

$$\text{Where } S = \frac{24 \text{ hrs TLM}}{48 \text{ hrs TLM}}$$

Where TLM is median tolerance limit or LC_{50}

3. Results

Results obtained after toxicity evaluation of *G. mullya* exposed to various concentrations of Bioneem is cited in Table.1. The LC_{10} values for 24, 48, 72 and 96 hours exposure to Bioneem are 206.01 ppm, 163.49 ppm, 97.90 ppm and 60.11 ppm respectively. The LC_{50} values for 24, 48, 72 and 96 hours exposure to Bioneem are 334.58 ppm, 288.66 ppm, 222.53 ppm and 167.45 ppm respectively. The calculated accuracy for the log LC_{50} values are summarised in the Table.1 under the column Variance 'V'. The standard error (accuracy or variance) values of LC_{50} for Bioneem for 24, 48, 72 and 96 hours exposure are 3.06ppm, 3.25 ppm, 3.55 ppm and 3.58 ppm respectively. The minimum and maximum fiducial limits for 95% confidence at 24, 48, 72 and 96 hours in ppm of Bioneem are – 0.90 to 5.95; -1.07 to 5.99; -1.34 to 6.04; -1.48 to 5.93 respectively.

The χ^2 values are summarized in Table.1 under the column χ^2.These values were used to test the homogeneity of the data. The safe concentration of the pesticide Bioneem was calculated and expressed in Table.1 under the column of safe concentration 'C'. The safe Concentrations for Bioneem is 40.45 ppm. Lethal dose for bioneem at various exposure periods were entered in Table.1 under the column 'Lethal Dose'.

Table 1: Lethal dose and safe concentration of Bioneem to fresh water fish *Gara mullya* (Sykes)

Time of Exposure	LC_{10} value in PPM	LC_{50} value in PPM	Variance	$\chi 2$ value	Fiducial limits		Lethal Dose in PPM	Safe Conc. 'c' in PPM
					m_1 PPM	m_2 PPM		
24 Hours	206.01	334.58	3.06	0.23	- 0.90	5.95	8029.92	40.45
48 Hours	163.49	288.66	3.25	0.49	- 1.07	5.99	13856.10	
72 Hours	97.90	222.53	3.55	0.41	- 1.34	6.04	16022.57	
96 Hours	60.11	167.45	3.58	0.48	- 1.48	5.93	16075.74	

4. Discussion

Nowadays no. of biopesticides are developed that replaces organic pesticides. Active ingredients of plant based pesticides are with low half-life period and causes less detrimental effect on the environment [13]. Aquatic organisms including fishes plays an important role of bio indicator for environmental pollution. They are highly sensitive to various xenobiotics. Pesticides are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant [14, 15]. Pesticides based on azadirachtin may have direct adverse effects on aquatic organisms and their toxicity depends on various factors [16]. The mortality of *G. mullya* due to Bioneem (Azadirachtin) increases with increase in time of exposure period. The evaluation of LC_{50} concentration of pollutants is an important step before carrying out further studies on physiological changes in animals. Several investigators worked on acute toxicity of neem biopesticide. Hassanein *et al* [17] reported the 96h LC_{50} value of a neem biopesticide on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm. Suresh Babu *et al*.,[18] investigated toxic effect of neem plant on survival of fresh water cat fish *Pangasius hypophthalmus* and determined LC_{50} values at different exposure periods which was 165.72 mg L-1 for 24h; 95.17mg L-1 for 48h; 62.48 mg L-1 for 72h and 55.76 mg L-1 for 96h. The 96h LC_{50} of Dichlorvos and Neem-On was found to be 16.71ppm, 42.66ppm respectively on the fish *Labeo rohita* [19].

In the present investigation, 96 hours LC_{50} value of Bioneem to *G. mullya* was found to be 167.45 ppm and safe concentration reported for *G. mullya* was 40.45 ppm. From the study it can be concluded that although pesticides derived from Neem tree are considered to be less toxic and environment friendly but their excess application in fish inhabiting areas affect life of non-target organisms including fishes.

5. References

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