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A 72-hr Median Lethal Concentration (MLC) of Neem for *Cyprinus carpio* Linn. (Cyprinidae) Juvenile

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Author's contribution

RD designed the study, performed the data analysis, wrote the protocol, and wrote the first draft of the manuscript. RD read and approved the final manuscript.

Research Note

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ABSTRACT

A study was conducted to determine the median lethal concentration of neem for common carp (*Cyprinus carpio*) as a non-target organism. Five test concentrations and a control were used in triplicates to determine the 72 hour MLC of neem gold pesticide. With the increase of dose concentration (0.05, 0.07, 0.09, 0.12 and 0.17 $ml/L^{-1)}$ and exposure duration significant increase in the mortality level was noticed. The 72 h LC_{50} values (with 95% confidence limits) of Neem Gold for common carp juvenile were found to be 0.09799 ml/L^{-1}.

Keywords: Azadirachtin; LC_{50}; neem gold; pesticide; toxic effect; Cyprinus carpio.

1. INTRODUCTION

Introduction of pesticides to aquatic ecosystems can destroy balance between species that are functional units of the ecosystem. *Azadirachta indica* is a medicinal plant widely used against agricultural pests and enters the aquatic ecosystems via agricultural runoff. The objective of aquatic toxicity tests is to estimate the "safe" or "no effect" concentration of

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toxicants that permit normal propagation of fish and other organisms in the aquatic media. In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of pest control for minimizing this damage, there have been extensive researches on pest control by substances from plants (Wan et al., 1996). One of the most promising natural compounds is azadirachtin, which is an active compound extracted from the neem tree (*Azadirachta indica*), known to possess antiviral, antibacterial and antifungal properties since time immemorial (Isman et al., 1990; Harikrishnan et al., 2003; ICAR, 1993). It is generally considered eco-friendly in comparison to other commonly used pesticides (Mordue [Luntz] and Blackwell, 1993). Therefore Neem-based insecticides are used against synthetic insecticides for the control of insect pests. However, adverse effects of azadirachtin on economically important organisms have also been reported (Schmutterer and Holst, 1987; Beckage et al., 1988; Hoelmer et al., 1991; Price and Schuster, 1991). In comparison, little attention has been focused on the effects of neem in aquatic environments. The use of pesticides in aquaculture systems to control fish diseases, parasites and other pests not only leads to high levels of residues in the animals but also may interfere with the maintenance of their homeostasis and thus affect their performance (Barton and Iwama, 1991; Wendelaar Bonga, 1997).

Azadirachtin is widely used in agro-ecosystems, forests, and recreational areas such as golf courses, but it can enter aquatic systems like streams, rivers and lakes if an accidental spill occurs. Neem has been successfully used in aquaculture systems for controlling fish predators (Dunkel and Ricilards, 1998). Aqueous extract of neem leaves and other allied products find extensive use in fish-farms for controlling fish parasites and fish fry predators chiefly the dragon-fly larvae (Martinez 2002). Although neem extract has low toxicity towards non-target aquatic life, the water extracts of the bark of this plant have caused respiratory problems in *Tilapia zilli* (Omoregie and Okpanachi, 1997) and long exposure to low concentrations of crude extract of *A. indica* has delayed the growth of this cichlid fish (Omoregie and Okpanachi, 1992).

Therefore, the purpose of the study was to figure out the effects of Neem Gold, a neem based pesticides derived from *Azadirachta indica*, on the fish, *Cyprinus carpio*, to trace out the lethal concentration for this species.

2. MATERIALS AND METHODS

Prior to the experiment the common carp (*Cyprinus carpio*) juvenile were acclimatized for two weeks in indoor tanks filled with well water. During this period the water was constantly aerated with conventional aerators and fishes were fed daily on the commercial feed (3-5%) on the basis of their biomass. Once acclimation was achieved, 10 healthy fish were distributed in 64L aquaria and exposed to the pesticide concentrations indicated in Table 1. Test specimens were considered dead when they failed to show movements. The experiments were carried out in semi-static systems with regular batch wise renewal of test solutions after 24 hours. Feeding was stopped 24 hours before starting the tests and same procedure was continued throughout the experimental period. Neem Gold was obtained from India, Foliage Chemicals Pvt. Ltd. To determine the 72 hr. LC50 of the pesticide, 18 aquaria were divided into three replicates each with five concentrations and one control. The Neem Gold concentrations used in the experiment include 0.05, 0.07, 0.09, 0.12 and 0.17 ml/l^{-1}. Dose range was selected based on field prescribed concentrations that could affect 10% to 95% of the seedlings with logarithmic intervals. The aquaria were inspected after 6 hours in order to check organism's behavior, as well as the mortality levels. The data obtained were

analyzed by linear regression probit analysis (Finney, 1971) using probit software program (Fig. 1).

Table 1. Pesticide concentrations and fish mortalities at 72 hours, in LC50 experiments

Pesticide	Average fish length & weight	Pesticide concentration ml/l	Mean mortality	Brand
Neem Gold	6.42±0.43 cm	0	0	Foliage
(Neem oil	5.02±1.2 g	0.05	0	Chemicals
Based E.C.		0.07	1.3	Pvt. Ltd
Containing		0.09	5	
Azadirachtin		0.12	7.3	
0.03%)		0.17	10	

3. RESULTS AND DISCUSSION

The effect of different doses of Neem Gold (EC 0.03%) on the behavior of *Cyprinus carpio* was studied by acute survival tests and was proved to be sufficiently toxic on common carp species. The 72 hrs LC_{50} and LC_{99} of Neem Gold pesticide for common carps was found to be 0.098 and 0.16 ml/L^{-1} respectively (Table 2). The dose of 0.05 ml/L^{-1} concentration showed no mortality ($p>0.05$). Whereas, the dose of 0.17 ml/L^{-1} resulted in the death maximum number of fishes and heavy mortality was noted at 48 and 72 hrs. Although high mortality was also observed with 0.12 ml/L^{-1}, but no mortality was observed with this and other doses in the first 48 hrs. After the introduction of pesticide, all fishes were gathered at the bottom and corner of the aquarium. Heavy breathing and equilibrium loss was observed during the experiment. The same activity has also been reported in the catfish, *Heteropneustes fossilis,* treated with azadirachtin by Kumar et al., 2010. Table 2 shows the toxic effects of Neem Gold on common carp with 95% higher and lower confidential limits.

Schmutterer (1990) suggested that the use of plant based pesticides must be encouraged. Plant based pesticides contain active principles with low half life period and their toxic effect on the environment is not too detrimental (Das and Mukharjee, 2003). Fish exposed to higher concentrations of plant extract exhibited respiratory distresses, erratic swimming and nervous manifestations. Winkaler et al. (2007) noticed that fish exposed to neem extract concentrations exhibited damaged gill and kidney tissues. Toxicity of Nimbesidine and Neemgold on a fresh water loach *Lipidocephalichthys guntea* has been reported (Mondal et al., 2007). Toxicity of neem leaf extract have also been reported for many aquatic organisms (Mamdouh et al., 2008; Hamdy and Okail, 2008; Sharma and Alam Ansari, 2010) Compared to other synthetic pesticides used in fish farming, such as carbamates and organophosphates, neem based products are somewhat less toxic to fish species (Wan et al., 1996).

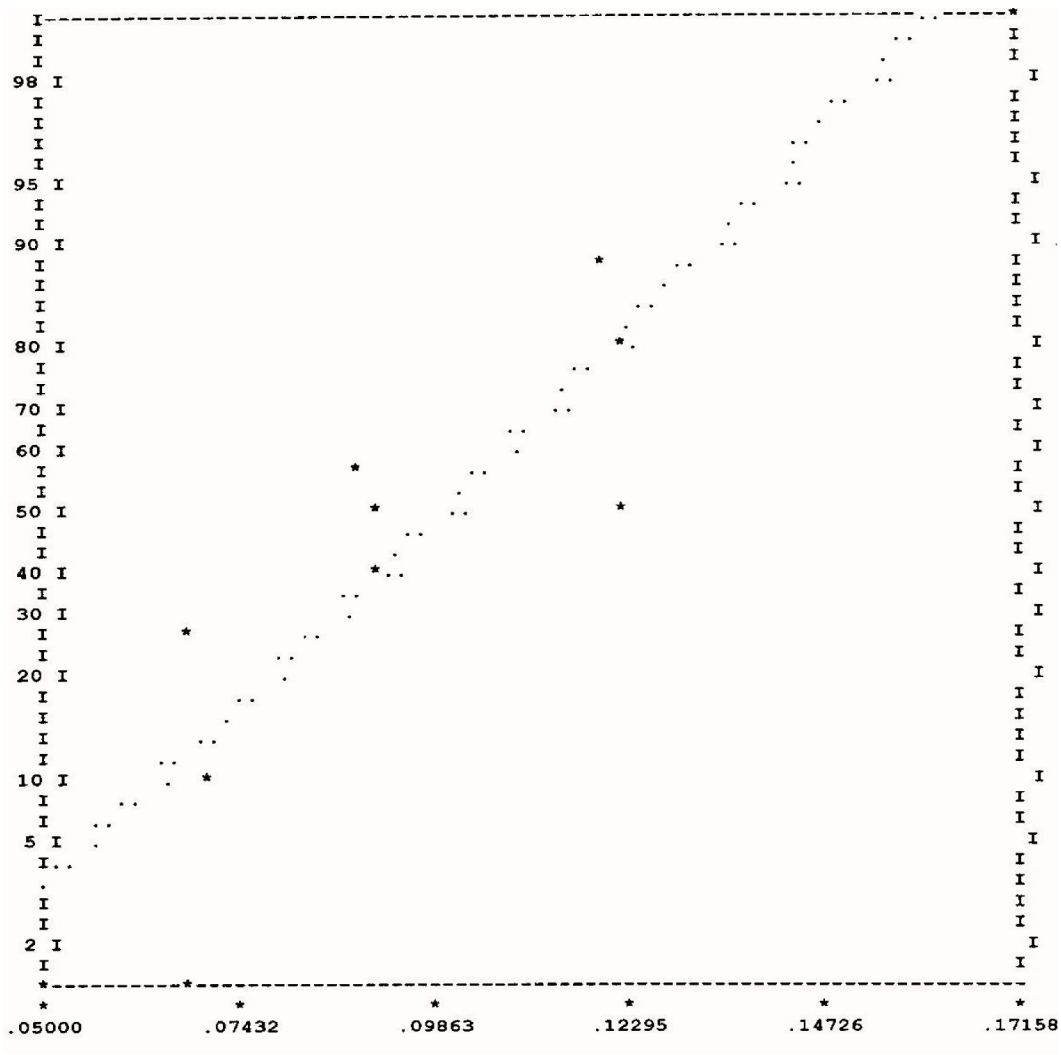

Fig. 1. Plot of probits and predicted regression line for neem gold to common carp.
X axis: Dose; Y axis: mortality

Table 2. Result of 72 hours toxicity test with 95% upper and lower confidential limits

72 hours LC	Dose	95% confidential limits			
		LCL 0.05	UCL 0.05	LCL 0.01	UCL 0.01
25	0.08064	0.07161	0.08777	0.06783	0.08994
50	0.09799	0.09092	0.10632	0.08866	0.10965
75	0.11535	0.10693	0.12817	0.10471	0.13413
99	0.15784	0.14163	0.18619	0.13783	0.20031

4. CONCLUSION

From the present study, it can be concluded that neem based pesticides are less toxic and eco-friendly, but must be handled with care while dealing with fish inhabiting areas as their excess application can affect the life of organisms living near the farming areas resulting in high mortality.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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