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Short Communication

Comparative Study of Synthetic Pyrethroid Lambda-cyhalothrin and Neem based Pesticide Neemgold on the Fingerlings of Zebrafish *Danio rerio* (Cyprinidae)

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Abstract

Toxicity tests using early life stages of fish are of great importance in assessing risk to growth, reproduction and survival in polluted environments and are important tools for good environmental monitoring. In the present study, the toxicity of Lambda-cyhalothrin and Neemgold to the fingerlings of Zebrafish was evaluated. Five different concentrations viz. 0.03, 0.06, 0.09, 0.12 and 0.15µg/l for Lambda-cyhalothrin and 0.40, 0.45, 0.50, 0.55 and 0.60µg/l were selected for Neemgold. The 96h LC_{50} value for Lambda-cyhalothrin was calculated to be 0.072, 0.066, 0.058 and 0.053µg/l and for Neemgold 0.52, 0.50, 0.49 and 0.46µg/l. The toxicity of zebrafish fingerlings was time as well as concentration dependent. The fingerlings were more sensitive to Lambda-cyhalothrin than to the Neemgold.

Keywords: Zebrafish, fingerlings, toxicity, Lambda-cyhalothrin, Neemgold.

Introduction

Synthetic pyrethroids have emerged as a new class of agricultural pesticides showing high toxicity to a wide range of insects including resistance strains, low toxicity to mammals and birds and rapid biodegradability[1]. These pesticides are used in cotton, cereals, vegetables, fruits, food storage and in animal husbandry. Most of these pesticides are extremely toxic to fish and other aquatic organisms and they are used in sufficiently high quantities to pollute waters, as is indicated by residues in fish[2].

In view of the environmental problems caused by synthetic chemicals and the growing need for alternative methods of pest control that minimize this damage, there has been extensive research on pest control by natural plant products. Azadirachtin derived from neem (*Azadirachta indica* A Juss), is very effective and extensively used in various Neem based formulations. It has been reported that neem based pesticides are target specific and comparatively less toxic. Recently, some neem based formulations were found toxic to the adult Zebrafish as well as their embryos and fingerlings. Moreover, they also affected its reproductive ability[3-7]. Also, Ansari and Ansari[8] observed that Dimethoate was toxic to the adult, embryos and fingerlings of Zebrafish and caused a significant reduction in fecundity, viability, hatchability and survival of fingerlings.

The purpose of this study is to establish a comparative account of whether Azadirachtin and Lambda-cyhalothrin to the fingerlings has any non-target effect of Zebrafish. The Zebrafish was selected as the test species according to the recommendations of the International Organization for Standardization and the Organization for Economic Co-operation and Development[9] for the early life stage toxicity.

Material and Methods

The Zebrafish, *Danio rerio,* reported from Uttar Pradesh were collected, stocked and bred in laboratory to obtain the fertilized eggs[10]. After 72h of fertilization the eggs of Zebrafish hatches. The eleutheroembryonic stage *i.e.* the time between hatching and yolk sac fry stage of Zebrafish is two days and within five days they become free swimming. For bioassay, 5-days old fingerlings were procured from the general stock. By trial and error method the maximum concentration for 100% mortality and minimum concentration for 0% mortality was calculated. Finally, five different concentrations of each pesticide were selected for the toxicity tests. For Lambda-cyhalothrin 0.03, 0.06, 0.09, 0.12 and 0.15µg/l and for Neemgold 0.40, 0.45, 0.50, 0.55 and 0.60 µg/l was selected.

Fingerlings in the batches of ten were introduced in 500 ml glass beakers containing 250 ml of dechlorinated water to observe the toxicity. Three replicates for each concentration were used. Acetone alone, in the same amount served as control. A 24-96h bioassay was performed in the laboratory to determine the $LC_{10,}$ LC_{50} and LC_{90} values at different exposure periods, lower and upper confidence limits (LCL and UCL), Slope and Chi-Square values of Lambda-cyhalothrin and Neemgold for fingerlings using StatPlus® computer programme, version 2009 purchased from analystsoft Vancouver, Canada.

Results and Discussion

LC_{50} values decreased with the increase in time. Lambda-cyhalothrin was more toxic to fingerlings than Neemgold. It is evident from result that the percent mortality of the 5-day old fingerlings of Zebrafish for Lambda-cyhalothrin is more than Neemgold. The maximum mortality was

observed after 96 h exposure *i.e*. 96% and 89% for Lambda-cyhalothrin and Neemgold respectively (table-1). Observations shows that during the exposure of Lambda-cyhalothrin, the 24 h LC_{50} of Lambda-cyhalothrin was 0.072 µg/l while after 48 and 72 h it declined to 0.066 and 0.058 µg/l respectively which decreased to 0.053 µg/l after 96 h of exposure. The 24 h LC_{50} values of Neemgold was 0.52 µg/l while for 48 and 72 h it was 0.50 µg/l and 0.49 µg/l respectively, which decreased to 0.46 µg/l after 96 h of exposure (table-2). The LC_{50} values of Neemgold for all the recorded periods is much higher than that of Lambda-cyhalothrin indicating that the synthetic pyrethroid are much toxic as compared to the natural pesticide for the early life stage of Zebrafish.

The slope values shown in the table are steep which indicate that the test animals are very sensitive to the minor changes in concentrations of the toxicant. The LC_{50} values of the pesticides showed significant ($p<0.05$) negative correlation with exposure time.

Table-1

Effect of Lambda-cyhalothrin and Neemgold on the % mortality of 5-day old fingerlings of Zebrafish

Pesticide Used	Concentrations (µg/l)	Fingerling toxicity			
		% Mortality of fingerlings			
		24-h	48-h	72-h	96-h
Lambda-cyhalothrin	0.00	NIL	NIL	NIL	NIL
	0.03	6	11	16	21
	0.06	15	23	39	47
	0.09	24	33	63	74
	0.12	41	64	70	80
	0.15	53	69	84	97
Neemgold	0.40	4	9	13	18
	0.45	10	21	35	40
	0.50	19	28	55	68
	0.55	36	57	63	76
	0.60	49	60	78	89

Table-2
Summary of Probit Analysis of table 1

Pesticide Used	Exposure Duration (h)	Effective concentration (µg/l)			Confidence limits of LC_{50} (µg/l)		Slope	Chi-square Values
		LC_{10}	LC_{50}	LC_{90}	LCL	UCL		
Lambda-cyhalothrin	24	0.044	0.072	0.086	0.059	0.082	1.33	0.21
	48	0.040	0.066	0.079	0.054	0.075	1.31	0.28
	72	0.035	0.058	0.073	0.046	0.064	1.30	0.39
	96	0.033	0.053	0.064	0.041	0.059	1.28	0.48
Neemgold	24	0.40	0.52	0.66	0.50	0.54	1.24	0.22
	48	0.39	0.50	0.65	0.48	0.53	1.23	0.74
	72	0.37	0.49	0.64	0.47	0.51	1.21	0.76
	96	0.38	0.46	0.57	0.41	0.49	1.19	0.78

Toxicity test with embryos and fingerlings are valuable for assessing potential impacts on growth, reproduction and survival of fish in polluted environment and are important tools for good environmental monitoring[11]. The fingerlings exhibited normal swimming behaviour in the control while erratic swimming was observed among fingerlings in the treated media. In some cases the fingerlings exhibited inconsistent jumping, loss of balance and decrease in opercula beat. The erratic swimming may be most likely due to the inhibition of acetylcholinesterase (AChE). This view is also reported by Rendan-van-Osten *et al.*[12] and in fishes, exposure to sub-lethal doses of Diazinon is known to affect the nervous system by inhibition of AChE activity. Inhibition of AChE activities in fish altered behavioral patterns in the laboratory. AChE inhibition could drastically affect growth, survival, feeding and reproductive behaviours of fishes[13]. Recently, Ansari and Ahmad[5] studied the comparative toxicity of pyrethroid Lambda-cyhalothrin and Neemgold to the embryo of Zebrafish and reported that embryos were more sensitive to Lambda-cyhalothrin than to Neemgold.

Mondal *et al*.[14] reported the toxicity of two Neem based pesticides Nimbecidine and Neemgold on a freshwater loach, *Lepidocephalichthys guntea*. The effects of Azadirachtin, the primary active ingredient of Neem on insects include feeding and oviposition deterrence, growth inhibition, fecundity and fitness reduction[15]. It has also growth regulatory effects on larval insects like disruption of moulting, growth inhibition and malformation which may contribute to mortality. There are also effects on allatropin and juvenile hormones[16]. Adverse effects on ovarian development, fecundity and fertility have also been reported[6].

Grabriel and Okey[17] reported that aqueous leaf extracts of *Lepidagathis alopecuroides* is highly toxic to catfish hybrid fingerlings. Several studies have shown that plant toxins at low concentrations are highly toxic to all groups of aquatic fauna[3]. Similar changes were observed in fresh water fish exposed to sub-lethal concentrations of monocrotophos and fenvalerate in *Labeo rohita* by Tilak *et al*.[18]. Sheil *et al.*[19] studied the effect of diazinon on early life stage of Zebrafish. It is probable that the fish exposed to pesticides caused circulatory failure and death of embryos prior to hatching.

During the development sensitivity may change with some compounds showing higher sensitivity in embryos whereas, others are more toxic to larvae[20]. Marty *et al.*[21] also reported that early life stages of *Oryzias latipes* were the most sensitive to toxic chemicals. In earlier study we also reported that the chorion of Zebrafish provide no protection to the developing embryo exposed to Neem pesticides Neemgold and Azacel[4, 7].

Conclusion

During the present study we concluded that early life stages of Zebrafish are very sensitive to low level of Lambda-cyhalothrin than Neemgold in aquatic environment and significantly affect its population. Therefore, these pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic environment and human beings. Moreover, extensive investigations should be done for their safe use in aquaculture.

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