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RESEARCH ARTICLE

Effect of the synthetic pyrethroid Deltamethrin and the neem-based pesticide Achook on the reproductive ability of zebrafish, *Danio rerio* (Cyprinidae)

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Abstract. In the present research, both male and female zebrafish, *Danio rerio* (Hamilton), were exposed to 96-h LC5 values of Deltamethrin (0.016 μg dm^{-3}) and Achook (0.025 μg dm^{-3}) for three months. The fish were then returned to normal water and allowed to breed to permit observations of fecundity and hatchability. The results show significant reductions in fecundity and hatchability in comparison to the control group. Fecundity was reduced by 54.12% in the fish treated with Deltamethrin and by 17.81% in those treated with Achook. Significant decreases in hatchability of up to 49.7% were noted in the Deltamethrin group and of 36.9% in the Achook treated fish. The number of unhatched/dead eggs increased significantly ($P < 0.05$ for each case). It was concluded that low concentrations (96-h LC5 values) of both pesticides can have a significant impact on the reproduction of zebrafish.

Keywords: Achook, Deltamethrin, sub-lethal toxicity, zebrafish

Introduction

Pesticides and herbicides are frequently used in agriculture for the eradication of pests and weeds and to increase production. They are also used to prevent disease-spreading insects like mosquitoes, flies, and termites. The indiscriminate, heavy use of chemical pesticides results in ecological degradation, deleterious effects on water taste and odor, lethal effects on non-target organisms in agroecosystems, and direct toxicity to users (Ansari and Kumar 1988, Kalavathy et al. 2001).

Synthetic pyrethriods, including Deltamethrin, are manufactured analogues of naturally occurring pyrethrins found in the flowers of *Chrysanthemum cinerariaefolium* (Naumann 1990). Deltamethrin is popular not only because of its effectiveness, but also for characteristics that allow the insecticide to work efficiently at low doses. Deltamethrin is a type-II pyrethroid compound that is highly toxic to fish, which are the most abundant aquatic organisms, while it is less toxic to birds and mammals (Venkataramudu et al. 2008). Pyrethroids are also toxic to many aquatic invertebrate species (Stephenson 1982, Paul and Simonin 2006).

Immediate fish mortality is usually not excessive in areas exposed to pesticides, but the accumulation of persistent pesticides in aquatic organisms can result in delayed mortality, reduced reproductive capabilities, altered growth rates, and reduced ability to withstand natural changes of pH, temperature, and dissolved oxygen in aquatic environments. The more widely used pesticides are persistent, and their

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toxicity is of a broad spectrum type. 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 (Cagauan 1990).

To overcome the hazardous effects of these organic pesticides, natural pesticides of plant origin have recently come into use. Plants are virtually inexhaustible sources of structurally-diverse and biologically-active substances (Istvan 2000). Some plants, like neem, contain various classes of compounds that have insecticidal, piscicidal, and molluscicidal properties. Unlike synthetic chemical pesticides, which leave harmful residues in the aquatic environment, botanical insecticides are believed to be more environmentally friendly because they biodegrade easily and leave no residues in the environment. Azadirachtin derived from neem (*Azadirachta indica*) is very effective and is used extensively in various neem-based formulations. It has been reported that neem-based pesticides are target specific and comparatively less toxic, but that long exposure to low concentrations of the crude extract of neem delayed the growth of redbelly tilapia, *Tilapia zilli* (Gervais) (Omoregie and Okpanachi 1997). Recently, it was observed that the neem-based pesticide, Achook, was toxic to zebrafish (Ansari and Sharma 2009).

It is possible to substitute organic pesticides with pesticides of plant origin. However, the literature on the toxic effects of neem-based pesticides on reproductive ability, which is very important for fish survival, is scanty. Hence, it was deemed necessary to evaluate the comparative effects of the synthetic pyrethroid Deltamethrin and the neem-based pesticide Achook on the reproductive ability of Zebrafish, *Danio rerio* (Hamilton) (Cyprinidae), after three months of pesticide stress. Zebrafish has been proven to breed readily under laboratory conditions with high yields of eggs and viable larvae. The zebrafish was selected as the test species for the current toxicological studies in accordance with recommendations of the International Organization for Standardization and the Organization for Economic Co-operation and Development.

Materials and Methods

Zebrafish were reported from Uttar Pradesh (Ansari and Kumar 1982). They were collected from local ponds, acclimatized, and then bred in the laboratory in 25 dm^{-3} glass aquaria containing 10 dm^{-3} of dechlorinated water. The aquarium water was aerated continuously with stone diffusers connected to a mechanical air compressor. The water temperature ranged between 25 ± 2°C, and pH was maintained between 6.6 and 8.5. The fish were fed twice daily alternately with raw chopped goat liver and brine shrimp. This diet was supplemented with *Drosophila* flies once daily.

In the present study, 10 mature females and 20 mature males were procured from stocks and exposed for three months to sub-lethal concentrations of the two pesticides (LC5 values of 96-h, i.e., 0.016 $\mu g\ dm^{-3}$ Deltamethrin and 0.025 $\mu g\ dm^{-3}$ Achook). The LC50 values were determined during earlier studies by Ansari and Sharma (2009). The 96-h LC50 value of Deltamethrin was 0.121 $\mu g\ dm^{-3}$ and of Achook – 0.595 $\mu g\ dm^{-3}$.

After three months of continuous exposure with both pesticides, the adult zebra fish were returned to normal water. Three batches of three mature females and six males were placed in 25 dm^{-3} glass aquaria and bred in the laboratory using the method by Ansari and Kumar (1986) for the observation of fecundity, viability, hatchability, and the survival of eggs laid by fishes. The parents were returned to the stock culture. Unfertilized eggs were identified by their milky color and were discarded. The hatched and dead eggs/embryos were recorded from every 24 up to 72 hours. The dead embryos became white because of the coagulation or precipitation of protein. The data were compared with the control, and Student's t-test was applied to test significance using StatPlus® version 2009 computer software.

Results

During the present investigation, significant ($P < 0.05$) reduction in fecundity and hatchability

Table 1
Effect of Deltamethrin and Achook on the fecundity, fertility, hatchability, and survival of zebrafish embryos (N = 3). Data in parentheses are percentage values. All data were significant ($P < 0.05$) when Student's t-test was applied to the treated and control groups. *Changes in fecundity rate after pesticide stress as compared to the control (100%)

Groups	Concentrations ($\mu g\ dm^{-3}$)	Fecundity	Number of unfertilized eggs	Number of viable eggs	Number of eggs (hatchability %)			Number of unhatched eggs
					24-h	48-h	72-h	
Control	0.0	295.7±7.31 (100)*	37.7±1.45	258.0±7.10 (100)	65.7±0.88 (25.45)	129.7±4.98 (50.25)	43.3±0.88 (16.80)	22.0±2.30 (8.5)
Achook	0.025	243.0±3.06 (82.19)*	40.7±1.20	202.3±2.19 (100)	34.7±3.93 (17.13)	65.3±3.18 (32.29)	27.7±1.45 (13.7)	74.7±2.40 (36.9)
Deltamethrin	0.016	135.7±6.39 (45.88)*	25.67±3.28	110.0±3.51 (100)	17.0±1.52 (15.45)	26.7±2.40 (24.24)	11.7±0.33 (10.61)	54.7±1.20 (49.7)

were observed (Table 1). During the experiment, the mean number of eggs laid by the zebrafish was 295.67 ± 7.31 under normal conditions, whereas that of treated zebrafish was remarkably reduced. Fecundity was reduced by 54.12% (135.67 ± 6.39) in fish treated with Deltamethrin and by 17.81% (243 ± 3.06) in fish treated with Achook in comparison with the control group, which was considered to be 100%.

Significant ($P < 0.05$) decreases in hatchability of up to 49.7% in the fish treated with Deltamethrin and of 36.9% in fish treated with Achook were also observed. The number of unhatched/dead eggs increased significantly ($P < 0.05$) in both of the treated groups (Table 1), but the Deltamethrin-treated fish had significantly more dead eggs in comparison to the fish treated with Achook. The lower fecundity and hatchability noted in the group treated with Deltamethrin indicated that it is more toxic than Achook.

Thus, the results provide evidence that both pesticides cause reductions in fecundity and hatchability and alter the reproductive ability of zebrafish at very low concentrations. This indicates that "safe" neem-based products are not wholly so for zebrafish, and that this should be considered when these chemicals are used in agricultural areas near aquatic ecosystems.

Discussion

Successful reproduction is essential for the perpetuation of species, including those of aquatic organisms. Individuals of *Carassius auratus gibelo* exposed to Deltamethrin (2 $\mu g\ dm^{-3}$ for 14 days) exhibited symptoms of induced hepatic, gonadal, and renal toxicity. Decreased numbers of spermatozoa is a severe reaction that affects the reproductive potential of animals (Staicu et al. 2007). At higher concentrations of pesticides, the eggs of *Cyprinus carpio communis* L. died before hatching because the pesticide affects the activity of hatching enzymes. The mortality of hatchlings in higher numbers immediately after hatching indicates that they are more sensitive to pesticides than are embryonic stages (Kaur and Toor 1977). It was reported previously that esfenvalerate (synthetic pyrethroid) causes reduced fecundity and the failure of eggs to hatch among Australian crimson-spotted rainbow-fish, *Melanotaenia fluviatilis* (Castelnau), (Barry et al. 1995) and, reduced fry growth, delayed spawning, and reduced hatching in bluegill, *Lepomis macrochirus* Raf. (Tanner and Knuth 1996).

According to Kumar and Ansari (1984) zebrafish exposed to long-term sub-lethal concentrations of Malathion failed to spawn and exhibited skeletal deformities. Dave and Xiu (1991) found that low concentrations of copper (0.25 $\mu g\ dm^{-3}$), lead (30 $\mu g\ dm^{-3}$), mercury (0.2 $\mu g\ dm^{-3}$), and nickel (80 $\mu g\ dm^{-3}$) can interfere with the hatching and survival of zebrafish. Von Westernhagen's review (1988) cites numerous studies that indicate exposure of mature female fish to contaminants can reduce ovary weight and egg size, and increase teratological and pathological effects on larvae. Moore and Waring (2001) revealed that exposure of mature salmon parr *Salmo salar* L. to low concentrations (≤0.028 μg dm^{-3}) of cypermethrin inhibited the ability of males to detect and respond to the female priming pheromone prostaglandin F2α (PGF2α). The increase in expressive milt and the levels of plasma sex hormones were reduced in the presence of the pyrethroid as a result of impaired olfactory detection of the priming pheromone. Exposure of salmon eggs and milt to cypermethrin also reduced the level of fertilization, suggesting a further toxic impact of the insecticide on salmon reproduction (Moore and Waring 2001).

The exposure of fish to diazinon is known to affect the nervous system through the inhibition of acetyl-cholinesterase activity (Ansari et al. 1987). Sub-lethal effects of diazinon resulting in acetyl-cholinesterase inhibition can drastically affect the growth, survival, feeding, and reproductive behaviors of fish (Dutta and Meijer 2003), while sub-lethal exposure to cypermethrin (1/10 and 1/50 of 96-h LC50 at 0.139 ppm) alters the biochemical, hematological parameters and enzymes of organ tissues, and exerted stress on roho, *Labeo rohita* (Hamilton) (Indian major carp) fingerlings. Plant extracts might be useful in counteracting some of these effects (Das and Mukherjee 2003).

Chronic toxicity tests with some modern Pyrethroids, such as fenvalerate, flucythrinate, and permethrin, on early stages of fish revealed they were highly toxic (Curtis et al. 1985). Schmitz et al. (2001) studied the effects of the neem-based pesticide Neemazal on the life cycle of zebrafish and found a significant reduction in fecundity after exposure to 0.63 mg dm^{-3} of Neemazal. The herbicide atrazine, which appears to be a relatively harmless toxicant for fish, affected mechanisms of reproduction in the salmonids rainbow trout and brook trout at calculated 96-h LC50 values of > 18 and 6.3 mg dm^{-3}, respectively (Moore and Waring 1998). Widenfalk et al. (2004) recently showed that the microbial communities of natural sediments can be affected by low concentrations of pesticides, including by the insecticides Deltamethrin and pirimicarb.

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References

Ansari B.A., Aslam M., Kumar K. 1987 – Diazinon Toxicity: Activities of acetyl cholinesterase and Phosphatases in the Nervous Tissue of Zebra Fish, *Brachydanio rerio* (Cyprinidae) – Acta Hydroch. Hydrob. 15: 301-306.

Ansari B.A., Kumar K. 1982 – A record of Zebra danio (*Brachydanio rerio*) (Cyprinidae) from Uttar Pradesh with notes on sexual dimorphism – J. Adv. Zool. 3: 88-89.

Ansari B.A., Kumar K. 1986 – Malathion Toxicity: Embryotoxicity and Survival of Hatchlings of Zebrafish (*Brachydanio rerio*) – Acta Hydroch. Hydrob. 14: 567-570.

Ansari B.A., Kumar K. 1988 – Cypermethrin Toxicity: Effect on the carbohydrate metabolism of the Indian catfish, *Hetropneustes fossilis* – Sci. Total Environ. 72:161-166.

Ansari B.A., Sharma D.K. 2009 – Toxic effect of synthetic pyrethroid Deltamethrin and Neem Based formulation Achook on Zebrafish, *Danio rerio* – Trends Biosci. 2: 18-20.

Barry M.J., O'Halloran K., Logan D.C., Ahokas J.T., Holdway D.A. 1995 – Sub-lethal effects of esfenvalerate pulse--exposure on spawning and non-spawning Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*) – Arch. Environ. Con. Tox. 28: 459-463.

Cagauan A.G. 1990 – The impact of pesticides on rice fields vertebrates with emphasis on fish – In: Impact of pesticides on farmer health and the rice environment (Eds) P.L. Pingali, P.A. Roger, International Rice Research Inst., Kluwer Academic Publ. Philippines: 203-248.

Curtis L.R., Semi W.K., Chapman G.G. 1985 – Toxicity of Fenvalerate to developing steelhead trout following continuous or intermittent exposure – J. Toxicol. Env. Health15: 445-457.

Das B.K., Mukherjee S.C. 2003 – Toxicity of cypermethrin in *Labeo rohita* fingerlings: Biochemical enzymatic and hematological consequences – Comp. Biochem. Phys. C. 134: 109-121.

Dave G., Xiu R. 1991 – Toxicity of mercury, copper, nickel, lead and cobalt to embryos and larvae of zebrafish, *Brachydanio rerio* – Arch. Environ. Con. Tox. 21: 126-134.

Dutta H.M., Meijer H.J.M. 2003 – Sub-lethal effects of diazinon on the structure of the testis of bluegill, *Lepomis* *macrochirus*: a microscopic analysis – Environ. Pollut. 125: 355-360.

Istvan U. 2000 – Semi-natural products and related substances as alleged botanical pesticides – Pest Manag. Sci. 56: 703-705.

Kalavathy K., Sivakumar A.A., Chandran R. 2001 – Toxic effect of the pesticide Dimethoate on the fish *Sarotherodon mossambicus* – J. Ecol. Res. Bioconserv. 2: 27-32.

Kaur K., Toor H. S. 1977 – Toxicity of pesticides to embryonic stages of *Cyprinus carpio communis* Linn. – Indian J. Exp. Biol. 15: 193-196.

Kumar K., Ansari B.A. 1984 – Malathion Toxicity: Skeletal Deformities in Zebrafish (*Brachydanio rerio*, Cyprinidae) – Pestic. Sci. 15: 107-111.

Moore A., Waring C.P. 1998 – Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr – Pestic. Biochem. Phys. 62: 41-50.

Moore A., Waring C.P. 2001 – The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.) – Aquat. Toxicol. 52: 1-12.

Naumann K. 1990 – Synthetic pyrethroid insecticides: structure and properties – Berlin: Springer-Verlag.

Omoregie E., Okpanachi M.A. 1997 – Acute toxicity of water extracts of bark of the Neem plant, *Azadirachta indica* to the cichlid *Tilapia zillii* – Acta Hydrobiol. 39: 47-51.

Paul E.A., Simonin H.A. 2006 – Toxicology of Three Mosquito Insecticides to Crayfish – B. Evironm. Contam. Tox. 76: 614-621.

Schmitz A., Schafers C., Trob R., Ruch B. Kleeberg H. 2001 – Effects of Neemazal on fish: Full life cycle test with the Zebrafish, *Danio rerio* – Proceeding of the 2. Workshop "Neem and Pheromones'' University of Uberaba, Brazil, May 15-16, 36-49.

Staicu A.C., Munteanu M.C., Costin D., Costache M., Dinischiotu A. 2007 – Histological changes in Deltamethrin-induced intoxication in *Carassius auratus gibelio* (*Pisces-cyprinidae*) – Biotechnol. Anim. Husb. 23: 619-626.

Stephenson R.R. 1982 – Aquatic toxicology of cypermethrin. I. Aacute toxicity to some freshwater fish and invertebrates in laboratory tests – Aquat. Toxicol. 2: 175-185.

Tanner D.K., Knuth M.L. 1996 – Effects of esfenvalerate on the reproductive success of the bluegill sunfish, *Lepomis macrochirus* in littoral enclosures – Arch. Environ. Con. Tox. 31: 244-251.

Venkataramudu M., Reddy M.N., Chennaiah K., Naik M.J., Indira P. 2008 – Sub-lethal toxicity of deltamethrin in relation to sex: Enzymatic studies in the freshwater fish *Channa punctatus* – J. Indian Soc. Toxicol. 4(1).

Von Westernhagen H. 1988 – Sub-lethal effect of pollutants on the fish egg and larvae – In: The Physiology of Developing Fish - Eggs and Larvae. Fish physiology vol. XIA (Eds) W.S. Hoar and D.J. Randall, Academic press, San Diego, 546: 253-346

Widenfalk A., Svensson J.M., Goedkoop W. 2004 – Effects of the pesticides capton, deltamethrin, isoproturon and pirimicrab on the microbial community of a freshwater sediment – Environ. Toxicol. Chem. 23: 1920-1927.

Streszczenie

Wpływ syntetycznego pyretroidu Deltamethrin oraz naturalnego pestycydu Achook na zdolności rozrodu danio pręgowanego, *Danio rerio* (Cyprynidae)

W pracy porównano wpływ syntetycznego pyretroidu Deltamethrin oraz pestycydu o nazwie Achook bazujący na drzewie neem (Miodła indyjska, *Azadirachta indica*) na płodność i przeżywalność ikry danio pręgowanego, *Danio rerio* (Hamilton). Doświadczenie polegało na trzymiesięcznej ekspozycji samców i samic danio pręgowatego na preparaty Deltamethrin (0,016 μg dm^{-3}) lub Achook (0,025 μg dm^{-3}). Następnie ryby przeniesiono do zbiorników z czystą wodą, określono płodność i przeżywalność ikry. W porównaniu do grupy kontrolnej (ryby przetrzymywane w czystej wodzie) stwierdzono istotne obniżenie płodności i przeżywalności ikry (tab. 1). Deltamethrin wpłynął na redukcję ilości składanej ikry o 54,1%, a Achook o 17,8%. W porównaniu do grupy kontrolnej, przeżywalność ikry pochodzącej od ryb poddanych działaniu preparatu Deltamethrin była niższa o 49,7%, a Achook o 36,9%. Wyniki dotychchczasowych badań wskazują, że pestycyd o nazwie Achook jest mniej szkodliwy dla danio pręgowanego) niż syntetyczny pyretroid Deltamethrin.