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RESEARCH ARTICLE

Effect of lambda-cyhalothrin and Neemgold on some biochemical parameters in the gill, liver, and ovary of zebrafish, *Danio rerio* (Cyprinidae)

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Abstract. The aim of the present work was to study the effect of lambda-cyhalothrin and Neemgold on the total protein, total free amino acid, and nucleic acid contents in tissues (gill, liver, and ovary) of zebrafish, *Danio rerio* (Hamilton), after exposure to 96 h LC_{10}, LC_{20}, and LC_{40} of lambda-cyhalothrin and the neem-based pesticide Neemgold. It was found that the total protein content was reduced to 38, 46, and 45% in gill, liver, and ovary, respectively, after lambda-cyhalothrin exposure for 21 days at the LC_{40} dose. The total free amino acid content in the liver was enhanced to 172 and 154% of control (100%) after exposure to LC_{40} of lambda-cyhalothrin and Neemgold, respectively. However, the total free amino acid content in the gill and ovary of treated fish was significantly reduced. In addition, DNA content was reduced to 45, 41, and 41% of controls (100%) in gill, liver, and ovary, respectively, after 21 days of exposure to LC_{40} of lambda-cyhalothrin. The reduction in DNA content from the control was 51, 53, and 55% in gill, liver, and ovary, respectively, from Neemgold exposure at the same concentration (LC_{40}) and period as lambda-cyhalothrin. It was observed that all the changes were dependent on concentration as well as time. Similarly, significant reductions in RNA content were also observed in gill, liver, and ovary after lambda-cyhalothrin and Neemgold exposure. The probable causes are discussed.

Keywords: *Danio rerio*, lambda-cyhalothrin, Neemgold, toxicity, biochemical constituents

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Introduction

The control of insect pests involves the heavy use of synthetic insecticides, but the wide-spread use of these substances has led to serious problems including toxic residue on grass and toxicity to non-target organisms such as mammals, birds, and fishes. These compounds also interfere with many vital physiological functions and constituently alter the levels of various biochemical constituents in fishes (Agrahari et al. 2006, Sharma and Ansari 2011). Synthetic pyrethroids were developed as the best alternative to organophosphorus and carbamate pesticides; however, these insecticides have also proved to be detrimental to human beings, fish, and domestic animals by altering various metabolic activities (Amweg et al. 2005, Adhikari et al. 2006).

To overcome the hazards presented by synthetic chemicals, one of the best control measures is to use chemicals of plant origin because they biodegrade rapidly, are the least persistent, and are less toxic to non-target organisms. Neem (*Azadirachta indica* A. Juss) is a traditionally highly esteemed medicinal tree on the Indian subcontinent. Neem extract is considered to be of low toxicity to non-target organisms; however, water extracts from various parts of the Neem tree have been reported to cause respiratory problems and delayed growth in fishes and also to

interfere with homeostasis thus affecting performance (Omoregie and Okpanachi 1997). Deshmukh and Pariyal (1992) have also reported the toxic effects of Neemax on *Oreochromis mossambicus* (Peters) and *Gambusia* sp. These results indicate that Neem extract added to water can cause disturbances in fish populations. Consequently, it is important to identify the impact these products have on certain biochemical parameters. Water pollution biomarkers are early diagnostic tools for measuring biological effects and assessing environmental quality.

That the intake of insecticide affects the biochemical composition of fishes has been reported previously by many scientists (Kumble and Muley 2000, Prasad et al. 2002). Pesticides produce numerous physiological and biochemical changes in fish and other aquatic species by influencing the activities of several antioxidant enzymes (Regoli and Principato 1995). Pesticides also affect basic genetic materials (DNA and RNA), total protein, total free amino acids, and carbohydrates (Ansari and Kumar 1986, 1988). This provided the impetus to investigate the toxic effects of sub-lethal exposure to lambda-cyhalothrin and Neemgold on different biochemical parameters in the gill, liver, and ovary of zebrafish, *Danio rerio* (Hamilton). This fish was selected as the test species for toxicological studies on the recommendation of the International Organization for Standardization and the Organization for Economic Co-operation and Development.

Materials and methods

Zebrafish were collected, stocked, and bred under laboratory conditions. The aquaria were continuously aerated through stone diffusers connected to a mechanical air compressor. Water temperature was 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 shrimps. The diet was supplemented with *Drosophila* flies once daily.

For the present study, mature adult fish were obtained from the general stock culture of the laboratory. The pesticides used were lambda-cyhalothrin 2.5% EC (Royal Crop Science India, Panipat, Haryana, India) and Neemgold (0.03% Azadirachtin) purchased from Foliage Chemicals Pvt. Ltd., Puzhal, Chennai, India. The fish were exposed to LC_{10}, LC_{20}, and LC_{40} concentrations, i.e., 0.03, 0.05, and 0.09 $\mu g\ l^{-1}$, of lambda-cyhalothrin and 0.61, 1.06, and 2.18 $\mu g\ l^{-1}$ of Neemgold for 21 days continuously. The concentrations chosen for the present study were drawn from the results of our previous toxicity test experiments (Ansari and Ahmad 2010a). Fifty fishes selected randomly for each concentration of the pesticides. The water of the aquarium was renewed after every 24h, and fresh treatments of the respective concentration of the pesticides were added. Each experiment was accompanied by a control.

Five fish were taken out after the end of each exposure period (7, 14, and 21 days), the gill, liver, and ovary were excised and processed to estimate levels of nucleic acids, total protein, and total free amino acids. Nucleic acids were estimated according to the method by Schneider (1957) using diphenylamine reagent for DNA and orcinol reagent for RNA and measured at 600 nm and 660 nm, respectively. Total protein was estimated according to the method by Lowry et al. (1951) using Folin-phenol reagent, whereas total free amino acids were estimated with the method by Spies (1957) using ninhydrin reagent. The values were expressed as $\mu g\ mg^{-1}$ wet tissue. Two-way ANOVA was employed to test the significance of the data using StatPlus® version 2009 computer software purchased from Analystsoft Vancouver, Canada.

Results

During the present investigation we observed significant alterations in the total protein, total free amino acid, and nucleic acids (DNA and RNA) contents in the gill, liver, and ovary of *D. rerio* exposed to lambda-cyhalothrin and Neemgold at different concentrations and exposure periods. The total protein

Table 1
Effects of Lambda-cyhalothrin and Neemgold on total protein content (μg mg^{-1} wet tissue) in zebrafish, *Danio rerio*

			Exposure concentrations (μg l^{-1})					
			Lambda-cyhalothrin			Neemgold		
Tissue	Period (days)	Control (0.00)	LC10 (0.03)	LC20 (0.05)	LC40 (0.09)	LC10 (0.61)	LC20 (1.06)	LC40 (2.18)
Gill	7	125.35 ± 0.22 (100)	92.20 ± 0.25 (74)	85.41 ± 0.13 (68)	71.15 ± 0.39 (57)	95.25 ± 0.45 (76)	89.45 ± 0.68 (71)	75.95 ± 0.25 (61)
	14	127.45 ± 0.37 (100)	83.40 ± 0.36 (65)	80.79 ± 0.08 (63)	63.11 ± 0.47 (50)	88.40 ± 0.79 (69)	84.39 ± 0.16 (66)	66.51 ± 0.44 (52)
	21	126.95 ± 0.62 (100)	75.55 ± 0.26 (60)	68.35 ± 0.20 (54)	48.79 ± 0.85 (38)	79.55 ± 0.33 (63)	80.35 ± 0.66 (59)	54.79 ± 0.85 (43)
Liver	7	114.72 ± 0.02 (100)	95.25 ± 0.87 (78)	86.45 ± 0.18 (73)	75.95 ± 0.25 (66)	109.08 ± 0.17 (95)	101.44 ± 0.31 (88)	92.28 ± 0.17 (80)
	14	113.86 ± 0.35 (100)	88.40 ± 0.79 (78)	80.79 ± 0.08 (71)	66.51 ± 0.44 (58)	95.27 ± 0.14 (84)	86.90 ± 0.29 (76)	80.17 ± 0.23 (70)
	21	115.66 ± 0.32 (100)	79.55 ± 0.33 (67)	72.35 ± 0.26 (63)	52.79 ± 0.85 (46)	88.73 ± 0.54 (77)	82.76 ± 0.27 (72)	69.33 ± 0.14 (60)
Ovary	7	189.09 ± 0.15 (100)	160.91 ± 0.33 (85)	152.76 ± 0.32 (81)	128.37 ± 0.35 (68)	178.50 ± 0.17 (94)	163.50 ± 0.28 (86)	140.46 ± 0.16 (74)
	14	186.65 ± 0.19 (100)	151.63 ± 0.32 (81)	138.13 ± 0.24 (74)	106.86 ± 0.22 (57)	163.50 ± 0.14 (58)	150.60 ± 0.24 (81)	128.80 ± 0.29 (69)
	21	188.54 ± 0.03 (100)	134.77 ± 0.32 (71)	120.45 ± 0.11 (64)	85.39 ± 0.01 (45)	148.37 ± 0.03 (79)	137.29 ± 0.23 (73)	115.61 ± 0.32 (61)

The exposure concentrations used were LC_{10}, LC_{20}, and LC_{40} (0.03, 0.05, and 0.09 μg l^{-1} of lambda-cyhalothrin and 0.61, 1.06, and 2.18 μg l^{-1} of Neemgold). Values are means ± SD (n=6); data were significant at $P < 0.05$ (two-way ANOVA). Values in parentheses indicate the percent change rounded to the nearest values with the control value as 100%

content was reduced to 60, 67, and 71% after lambda-cyhalothrin exposure and to 63, 77, and 79% after Neemgold exposure of the controls (100%) for 21 days in gill, liver, and ovary, respectively, at LC_{10} exposure. Whereas, after 21 days and LC_{40} exposure the total protein content was reduced to 38, 46, and 45% after lambda-cyhalothrin exposure and to 43, 60, and 61% after Neemgold exposure in comparison to controls (100%) in gill, liver, and ovary, respectively (Table 1). On the other hand, the total free amino acids content was significantly enhanced in the liver of treated fish exposed to sub-lethal concentrations of both the pesticides. Total free amino acid content was increased to 172 and 154% of controls for lambda-cyhalothrin and Neemgold in the liver of treated fishes after exposure to LC_{40} concentration for 21 days. However, the total free amino acids content in the gill and ovary of treated fish was significantly reduced (Table 2). The total free amino acids content was reduced to 49 and 46% (after lambda-cyhalothrin exposure) and 62 and 58% (after Neemgold exposure) of controls (100%) for 21 days in gill and ovary, respectively, at the LC_{40} dose (Table 2).

In addition, the DNA content was reduced to 45, 41, and 40% of controls (100%) in gill, liver, and ovary, respectively, after 21 days exposure to LC_{40} of lambda-cyhalothrin. Up to a 55% reduction in DNA content was noted in the ovary because of Neemgold at the same concentration and exposure period as that of lambda-cyhalothrin (Table 3). Similarly, RNA contents also decreased to 34, 38, and 43% after Lambda-cyhalothrin exposure and to 49, 56, and 52% after Neemgold exposure for 21 days in gill, liver, and ovary, respectively, at the LC_{40} dose (Table 4). The results showed that Lambda-cyhalothrin

Table 2
Effect of lambda-cyhalothrin and Neemgold on total free amino acid content in zebrafish, *Danio rerio*

Tissue	Period (days)	Control (0.00)	Exposure concentrations (μg l^{-1})					
			Lambda-cyhalothrin			Neemgold		
			LC10 (0.03)	LC20 (0.05)	LC40 (0.09)	LC10 (0.61)	LC20 (1.06)	LC40 (2.18)
Gill	7	48.25±0.38 (100)	35.20±0.21 (73)	33.68 ± 0.34 (70)	30.53 ± 0.20 (63)	45.42 ± 0.22 (94)	41.61 ± 0.14 (86)	39.86 ± 0.45 (83)
	14	46.66 ± 0.34 (100)	31.95 ± 0.18 (68)	30.15 ± 0.27 (65)	28.23 ± 0.26 (61)	43.21 ± 0.45 (90)	40.28 ± 0.27 (86)	36.29 ± 0.26 (78)
	21	45.95 ± 0.48 (100)	28.60 ± 0.39 (62)	26.95 ± 0.19 (59)	22.65 ± 0.67 (49)	37.28 ± 0.27 (81)	34.12 ± 0.24 (74)	28.40 ± 0.23 (62)
Liver	7	40.02 ± 0.37 (100)	45.82 ± 0.26 (114)	54.33 ± 0.29 (136)	61.24 ± 0.28 (153)	42.36 ± 0.27 (106)	48.77 ± 0.29 (122)	53.89 ± 0.14 (135)
	14	41.48 ± 0.28 (100)	49.51 ± 0.29 (119)	58.48 ± 0.20 (141)	65.36 ± 0.29 (158)	47.23 ± 0.17 (114)	50.46 ± 0.33 (122)	57.99 ± 0.19 (140)
	21	40.45 ± 0.24 (100)	52.53 ± 0.14 (130)	63.58 ± 0.35 (157)	69.60 ± 0.33 (172)	48.29 ± 0.25 (119)	54.09 ± 0.14 (134)	62.14 ± 0.15 (154)
Ovary	7	56.50 ± 0.08 (100)	48.20 ± 0.21 (85)	43.47 ± 0.36 (77)	38.33 ± 0.20 (68)	54.42 ± 0.22 (96)	47.81 ± 0.14 (85)	41.86 ± 0.25 (74)
	14	56.26 ± 0.19 (100)	43.95 ± 0.18 (78)	37.85 ± 0.27 (67)	33.83 ± 0.26 (60)	51.26 ± 0.45 (91)	41.28 ± 0.27 (73)	36.89 ± 0.26 (66)
	21	55.85 ± 0.20 (100)	41.60 ± 0.39 (74)	34.95 ± 0.19 (63)	25.45 ± 0.67 (46)	46.48 ± 0.27 (83)	39.12 ± 0.24 (70)	32.40 ± 0.23 (58)

The exposure concentrations used were LC_{10}, LC_{20}, and LC_{40} (0.03, 0.05 and 0.09 μg l^{-1} of lambda-cyhalothrin and 0.61, 1.06, and 2.18 μg l^{-1} of Neemgold). Values are mean ± SD (n=6); data were significant at $P < 0.05$ (two-way ANOVA)
Other details as in Table 1

is more toxic because the reduction in all the macromolecules was more pronounced than it was with Neemgold.

Discussion

The effects of Neem-based pesticides on non-target organisms have been studied in terrestrial ecosystems; however, little attention has been focused on aquatic environments. Hence, in the present investigations the toxic effects of Lambda-cyhalothrin and Neemgold on certain biochemical parameters of zebrafish were studied. Azadirachtin, a botanical pesticide derived from the neem, is generally considered less harmful to the environment than other more commonly used pesticides (Mordue and Blackwell 1993). Neem is a natural insect growth regulator as well as an antifeedant. However, several studies have shown that plant toxins at low concentrations are very toxic to all groups of aquatic fauna (Tiwari and Singh 2003, Goktepe et al. 2004). Recently, some pesticides were found to be toxic to adult, embryo, and fingerling zebrafish (Ansari and Sharma 2009, Ansari and Ahmad 2010b, Ahmad and Ansari 2011, Ahmad et al. 2011, Ansari and Ansari 2011), and cause reduced reproductive ability (Sharma and Ansari 2010).

During chronic periods of stress, proteins are a source of energy. The depletion of the protein fraction in gill, liver, and ovary might be due to their degradation and the possible utilization of degraded products for metabolic purposes. Singh et al. (1996) also reported declines in the protein constituent in different fish tissues exposed to sub-lethal concentrations of pesticides. The protein content might also be affected by impaired incorporation of amino acids into polypeptide chains.

Table 3
Effects of lambda-cyhalothrin and Neemgold on DNA content (μg mg^{-1} wet tissue) in zebrafish, *Danio rerio*.

Tissue	Period (days)	Control (0.00)	Exposure concentrations (μg l^{-1})					
			Lambda-cyhalothrin			Neemgold		
			LC10 (0.03)	LC20 (0.05)	LC40 (0.09)	LC10 (0.61)	LC20 (1.06)	LC40 (2.18)
Gill	7	42.35 ± 0.32 (100)	32.20 ± 0.223 (76)	28.80 ± 0.30 (68)	24.15 ± 0.39 (57)	38.25 ± 0.0.45 (90)	35.45 ± 0.68 (84)	29.95 ± 0.25 (71)
	14	40.45 ± 0.37 (100)	30.40 ± 0.36 (75)	27.69 ± 0.78 (68)	22.11 ± 0.67 (55)	35.40 ± 0.79 (88)	30.39 ± 0.16 (75)	26.51 ± 0.43 (66)
	21	43.95 ± 0.92 (100)	28.55 ± 0.26 (66)	24.35 ± 0.20 (55)	19.72 ± 0.35 (45)	31.55 ± 0.33 (72)	29.35 ± 0.66 (67)	22.45 ± 0.85 (51)
Liver	7	35.28 ± 0.15 (100)	28.55 ± 0.10 (81)	26.14 ± 0.26 (74)	23.15 ± 0.57 (66)	32.52 ± 0.31 (92)	28.12 ± 0.31 (80)	26.35 ± 0.23 (75)
	14	34.40 ± 0.19 (100)	25.00 ± 0.18 (73)	21. 80 ± 0.29 (63)	19.16 ± 0.22 (53)	28.45 ± 0.21 (78)	24.47 ± 0.29 (71)	23.16 ± 0.19 (62)
	21	37.15 ± 0.21 (100)	21.89 ± 0.17 (59)	18.93 ± 0.27 (51)	15.18 ± 0.23 (41)	26.43 ± 0.16 (71)	22.32 ± 0.23 (60)	19.81 ± 0.11 (53)
Ovary	7	39.27 ± 0.20 (100)	32.59 ± 0.30 (80)	27.71 ± 0.19 (70)	23.77 ± 0.26 (57)	35.52 ± 0.34 (90)	33.65 ± 0.29 (81)	27.43 ± 0.15 (67)
	14	41.32 ± 0.29 (100)	29.60 ± 0.32 (72)	23.71 ± 0.29 (57)	19.16 ± 0.19 (46)	31.23 ± 0.25 (76)	30.10 ± 0.25 (73)	26.20 ± 0.25 (63)
	21	40.85 ± 0.19 (100)	25.94 ± 0.29 (64)	19.71 ± 0.20 (48)	16.16 ± 0.19 (40)	29.19 ± 0.27 (71)	26.86 ± 0.32 (61)	22.60 ± 0.09 (55)

The exposure concentrations used were LC_{10}, LC_{20}, and LC_{40} (0.03, 0.05, and 0.09 μg l^{-1} of lambda-cyhalothrin and 0.61, 1.06, and 2.18 μg l^{-1} of Neemgold). Values are means ± SD (n=6); data were significant at $P < 0.05$ (two-way ANOVA)
Other details as in Table 1

Furthermore, any obstruction in RNA synthesis can also affect protein levels since it plays an important role in protein synthesis. In the present study, a significant decline in RNA level was also observed in the treated fish. Hussein et al. (1996) noted a decrease in total protein in *Oreochromis niloticus* (L.) treated with atrazine herbicide. A decrease in liver protein content would suggest intensive proteolysis, which, in turn, contributes to increased free amino acids being fed into the TCA cycle as keto acids, thus supporting the view of Kabeer et al. (1978). High concentrations of total free amino acid content could stem from the protein degradation and tissue breakdown as well as the inhibition of protein synthesis. Recently, Sharma and Ansari (2011) reported a decrease in biochemical constituents in different tissues of *D. rerio* exposed to sub-lethal concentrations of Deltamethrin and Achook, which supports our current observations.

The decreased DNA content noted during the present study might have been caused by the direct action of Lambda-cyhalothrin and Neemgold on DNA synthesis. It is known that DNA functions as a primer in DNA and RNA polymerase reactions (Haqqi and Adhami 1979), and the inhibition in DNA content can result in the inhibition of both DNA and RNA synthesis. We presume either that Neem-based pesticides interfere with or block some metabolic pathways necessary for RNA synthesis, or even that pesticides have a direct impact on it. Swietla and Zuk (1978) reported that herbicides act as inhibitors of nucleic acid and protein synthesis, while Katherine et al. (2007) recorded significant reductions in the proteins of herbicide-exposed fish.

Table 4
Effects of lambda-cyhalothrin and Neemgold on RNA content (µg mg^{-1} wet tissue) in zebrafish, *Danio rerio*

Tissue	Period (days)	Control (0.00)	Exposure concentrations (µg l^{-1})					
			Lambda-cyhalothrin			Neemgold		
			LC10 (0.03)	LC20 (0.05)	LC40 (0.09)	LC10 (0.61)	LC20 (1.06)	LC40 (2.18)
Gill	7	27.65 ± 0.36 (100)	21.10 ± 0.28 (76)	19.20 ± 0.29 (69)	16.71 ± 0.23 (60)	24.19 ± 0.29 (87)	21.49 ± 0.20 (78)	18.22 ± 0.18 (66)
	14	26.33 ± 0.64 (100)	17.21 ± 0.05 (65)	15.21 ± 0.24 (58)	13.29 ± 0.06 (50)	22.25 ± 0.13 (85)	19.58 ± 0.12)74	16.40 ± 0.14 (62)
	21	29.32 ± 0.29 (100)	14.55 ± 0.21 (50)	12.36 ± 0.27 (42)	10.02 ± 0.09 (34)	20.01 ± 0.17 (68)	17.04 ± 0.23 (58)	14.44 ± 0.15 (49)
Liver	7	24.49 ± 0.17 (100)	18.54 ± 0.28 (76)	15.99 ± 0.19 (65)	12.63 ± 0.19 (52)	23.63 ± 0.37 (96)	18.18 ± 0.17 (74)	17.03 ± 0.23 (69)
	14	23.15 ± 0.26 (100)	16.48 ± 0.17 (71)	13.89 ± 0.25 (60)	10.67 ± 0.21 (46)	19.55 ± 0.29 (84)	17.20 ± 0.50 (74)	15.11 ± 0.31 (65)
	21	25.09 ± 0.19 (100)	14.61 ± 0.29 (58)	12.67 ± 0.24 (50)	9.48 ± 0.25 (38)	19.47 ± 0.12 (78)	15.45 ± 0.35 (62)	14.08 ± 0.28 (56)
Ovary	7	24.26 ± 0.26 (100)	22.10 ± 0.28 (91)	19.20 ± 0.29 (79)	16.71 ± 0.23 (69)	22.19 ± 0.29 (91)	20.49 ± 0.20 (84)	18.22 ± 0.18 (75)
	14	27.53 ± 0.16 (100)	20.21 ± 0.05 (73)	16.21 ± 0.24 (59)	13.29 ± 0.06 (48)	20.25 ± 0.13 (74)	18.58 ± 0.12 (67)	15.40 ± 0.14 (56)
	21	25.72 ± 0.21 (100)	18.55 ± 0.21 (72)	14.36 ± 0.27 (56)	11.02 ± 0.09 (43)	19.01 ± 0.17 (74)	17.04 ± 0.23 (66)	13.44 ± 0.15 (52)

The exposure concentrations used were LC_{10}, LC_{20}, and LC_{40} (0.03, 0.05 and 0.09 µg l^{-1} of lambda-cyhalothrin and 0.61, 1.06 and 2.18 µg l^{-1} of Neemgold). Values are mean ± SD (n=6); data were significant at $P < 0.05$ (two-way ANOVA)
Other details as in Table 1

Conclusions

During the present study, it was found that so-called safer, ecofriendly neem pesticides are toxic to zebrafish, and they alter the basic cell constituents of total protein, total free amino acids, and nucleic acids (DNA and RNA) in vital organs like the gill, liver, and ovary. This study will help scientific planning to assist the structural and functional aspects of aquatic systems and unplanned development, which has toxic effects on water bodies, and particularly on fish. Therefore, the present study is important for fishery management and will provide information and methods for monitoring toxicity and safe limits of test chemicals that can be used in toxicological, industrial, and pollution laboratories.

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Author contributions. B.A.A. and D.K.S. designed the experiments, computed the results, and prepared the manuscript. M.K.A. and S.A. maintained the culture of zebrafish and performed the biochemical estimations.

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