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Effect of sub lethal concentration of imidacloprid on proximate body composition of *Labeo rohita*

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Abstract

Randomly selected 320 fingerling of *Labeo rohita* were collected from Faheem fish farm, Mattital Road, Multan. Groups of 40 fish each were exposed to subleathal concentration of imidacloprid (120 mg L^{-1}) under short (2, 4 and 8 days) and long term (16, 32 and 64 days) conditions to access the effect of this most extensively used pesticide on the body composition parameters of economically most important fresh water fish, *L. rohita*, in Pakistan. Separate control groups were used for each imidacloprid treatment. It was found that most of the studied body composition parameters remained unaffected during short and long term experimental treatments as compared to their respective control groups with the exception of the 64 days treatment where several parameters including body weight (p=0.02), dry body weight (p=0.005), % dry weight (p=0.001), total water contents (p=0.04), total fat (p=0.03), %fat wet weight (p=0.001), % fat dry weight (p=0.003), total organic contents (p=0.04), % organic content wet weight (p=0.001), total proteins (p=0.005) and %protein wet body weight (p=0.001) had significantly lower values in imidacloprid treated fish as compared to control group. Our results indicated that chronic exposure of imidacloprid can adversely affect the nutritional quality of *L. rohita*.

Keywords: Body composition, Imidacloprid, LC_{50}, Sub lethal concentration, *Labeo rohita.*

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Introduction

In developing countries, the most common form of water pollution is the wastes produced by businesses and households that are discharged directly into fresh water bodies (rivers, streams or ponds) which are also the major source of water supplies to rural and urban populations (Stanitski, 2003). Most destructing types of aquatic pollutants are generally present in such type of wastes including sewage (containing infectious pathogens), pesticides, herbicides, heavy metals, radioactive substances, oils, hydrocarbons and acid and bases like corrosive substances (Samantha *et al.*, 2005; Baki *et al*., 2011).

At present, there are more than 200 types of organic pesticides which are available in thousands of different products (Malik *et al*., 2010). These pesticides contain various heavy metals such as iron, copper, chromium, cadmium, zinc, lead, nickel, and manganese as active ingredients and have high deteriorating effect in the environment because they corrode (oxidize) in water and the resulting dissolved or suspended oxidation products become pollutants (Sharma and Agarwal, 2005; Verma *et al*., 2005). These pesticides adversely affect the nutritional quality, growth, reproduction, physiology and survival of aquatic life, the non target organisms, including major carps due to their stable and persistent existence in the environment (Hayat *et al*., 2007; Iqbal and Saima, 2008; Sudagar and Hajibeglou, 2010).

Imidacloprid acts as a neurotoxin and belongs to a chemical group called neonicotinoids. It interferes with transmission of stimuli in nicotinergic neurons of insect nervous system and is most commonly used to control the sucking insects including thrips, whiteflies, rice hoppers, soil insects, aphids, termites and beetles (Medrzycki *et al*., 2003).

Fish body contains variable amount of water, protein, lipids, ash, carbohydrates and non-protein nitrogen compounds. Energy contents of the fish body are generally determined by its chemical composition (Ali and Wootton, 2003) as these are good indicators of physiological and pathological status of fish which is effected by physiological and environmental factors and particularly nutritional status (Ali *et al*., 2005). A lot of studies has reported the body composition of various fish species under different physiological conditions (Ali *et al*., 2006; Umer *et al*., 2009; Naeem *et al*., 2011; Umer *et al*., 2011) but effect of pesticide on body composition of fish, especially *L. rohita*, is comparatively unexplored. The aim of this study was to demonstrate the effects of the most widely used pesticide, imidacloprid, on the nutritional quality of economically most important fresh water fish in Pakistan, *L. rohita*.

Materials and methods

Specimen collection

Randomly selected 320 fingerlings of freshwater Cyprinid fish, *L. rohita*, of both sexes (total body length 4.2-16.1 cm and body weight 0.83-57.79 g) were purchased from the Faheem fish farm, Mattital Road, Multan and transported to the fisheries laboratory at Bio Park in Bahauddin Zakariya University, Multan. Fish were acclimated to laboratory conditions for two weeks in fiberglass containers with recirculation aerated system (RAS). All experiments were carried out in semi-static systems with water renewal every 24h. Temperature, pH and oxygen concentration of water were maintained throughout the experimental duration following Umer *et al*. (2011).

LC_{50} determination

For determination of 96h imidacloprid LC_{50} values, groups of 10 juveniles *L. rohita* were exposed to one of the seven concentrations; 100, 200, 300, 400, 500, 600 and 700 mgL^{-1} of imidacloprid. An untreated control group was maintained parallel with treatments on other groups. Fish mortality was observed after 24, 48, 72 and 96 hours. LC 50 values were calculated following Iqbal *et al*. (2005).

Experimental design

After acclimation, an electronic digital balance (Chyo, Japan) was used to record the initial body weight to the nearest 0.01g and by using a Perspex measuring tray, fitted with a sheet of millimeter ruler, total length was measured to the nearest 0.01cm. Measurements were taken from the tip of mouth to the longest caudal fin ray following Abbas (2000). All fish were fed with ordinary fish diet used in fish farms (24% protein). Experiments were divided into short and long term phases. During short term experiments, fish were exposed to subleathal concentration of 120 mg L^{-1} imidacloprid for 2, 4 and 8 days (20 fish in each experiment) while in long term experiments fish was exposed to above mentioned dose for 16, 32 and 64 days (20 fish in each experiment). Separate control groups were used for each treatment (20 fish in each experiment). At the end of the experiment, fish were captured from each container and final body weight and length were recorded for each individual. All the experimental procedure and fish handling protocols were approved by Ethical Committee of Zoology Department, Institute of Pure and Applied Biology, B. Z. University Multan.

Analysis of body composition parameters

Following the sacrifice, to estimate water contents and dry body mass, fish were placed as a whole in pre-weighed aluminum foil to dry till it attained constant weight in an electric oven (Gallen Kamp, England) at 50-55 ^{o}C. 25mg of fish powder was taken in a pre weighed, heat resistant china clay crucible and ashed in a muffle furnace

(Sybron thermolyne 1300) for 5-7 hours at 500 ^{o}C and reweighed after cooling to estimate ash contents in fish body. The fat contents were estimated following dry extraction method as determined by Umer *et al*. (2011). Briefly, 50mg of fish powder was mixed in 10 ml of chloroform: methanol (1:2) mixture, left overnight, centrifuged and supernatant was transferred to an oven at 40-50 ^{o}C, for solvent evaporation, in pre weighed bottles. The lipid was weighed and total amount of fat in dry fish mass was calculated using the formula:

Total fat content of fish = (weight of fat in sample weight of sample $^{-1}$) - dry body weight

Total protein present in dry mass was calculated by subtracting ash, fat and water from the total fish mass (Umer *et al*., 2011).

Statistical analysis

All the data is expressed as mean± standard deviation (SD). Data was analyzed statistically through 2 sample student t-test to compare various parameters of each treatment with their respective controls using the Minitab computer package (version 16).

Result

General observation and behavioral response

Exposure of *L. rohita* to 120 mg L^{-1} of imidacloprid divulged influential changes in fish behaviour. An avoidence response was observed in imidacloprid treated groups and fish showed abrupt and sluggish swimming movements in various directions. Occasional jumping and hitting against the walls of tanks were also observed. *L. rohita* experienced rapid scale loss and mucous secretion. Due to pesticide, body colour changed to light brown in treated groups and it was remarkably different from control group. As the imidacloprid exposure time increased, fish tend to recover from disturbed condition and frequency of abnormal behaviour decreased but swimming speed remained slow as compared to control and it was easy to catch treated fish with hand nets as compared to untreated *L. rohita*.

LC_{50} values of imidacloprid

The 96 hr LC_{50} value for *L. rohita* treated with imidacloprid was 550 mgL^{-1}. All fishes survived at 200 mgL^{-1} while 100% mortality was observed at 900 mgL^{-1}.

It was found that in short term experimental groups (2 and 4 days), fish exposed to imidacloprid had higher values of few body composition parameters (Tables 1, 2) but most of the parameters remained unaffected, statistically non significant, when compared between control and imidacloprid treated groups. Total length of the fish was significantly reduced (p=0.04) in fish exposed to imidacloprid for 8 days as compared to their untreated control group. Chronic exposure of imidacloprid severely affected the growth and nutritional quality of *L. rohita*. Percent fat in dry

body weight was significantly higher (p=0.012) in control fish as compared to fish exposed to imidacloprid for 16 days (Tables 3, 4). Effect of imidacloprid was most severely observed on nutritional quality in 64 days treatment where several body composition parameters including body weight (p=0.02), dry body weight (0.005), % dry weight (p=0.001), total water contents (p=0.04), total fat (p=0.03), % fat wet weight (0.001), % fat dry weight (p=0.003), total organic contents (p=0.04), % organic content wet weight (p=0.001), total proteins (p=0.005) and % protein wet body weight had significantly lower values for imidacloprid treated fish as compared to control group (Table 4). Our results indicated that chronic exposure of imidacloprid can adversely affect the nutritional quality of *L.rohita*.

Table 1: Effect of imidacloprid exposure on weight, length and water components of *Labeo rohita* under short term experimental conditions. All the data is expressed as mean±standard deviation (SD). *P*-values are the 2 sample t-test results for the comparison of specific parameter between 2, 4 and 8 days treatment with their respective controls.

Parameter	Control	Imidacloprid exposure for 2 days	*p*-Value	Control	Imidacloprid exposure for 4 days	*p*-Value	Control	Imidacloprid exposure for 8 days	*p*-Value
Total Length (cm)	8.9 ±1.8	10.1 ±1.1	0.08(NS)	9.9 ±0.9	10.6 ±0.9	0.12(NS)	10.9 ±2.2	9.3 ±0.5	0.048*
Total Weight (g)	7.8 ±4.0	11.119 ±4.9	0.11(NS)	8.8 ±2.0	11.2 ±2.8	0.044*	12.3 ±10.2	6.4 ±1.5	0.11(NS)
Dry Weight (g)	1.3 ±0.7	2.06 ±1.0	0.05*	1.7 ±0.4	2.1 ±0.6	0.10(NS)	1.8 ±1.9	0.8 ±0.2	0.12(NS)
% Dry Weight	16.7±2.3	18.5±2.9	0.14(NS)	19.17±1.10	18.77±1.34	0.47(NS)	13.01±3.45	11.97±0.86	0.37(NS)
Total Water (g)	6.5±3.4	9.1±4.0	0.13(NS)	7.13±1.59	9.08±2.17	0.036*	10.49±8.37	5.66±1.31	0.10(NS)
% Water	83.3±2.3	81.5±2.9	0.14(NS)	80.83±1.10	81.23±1.34	0.47(NS)	86.99±3.45	88.03±0.86	0.37(NS)

Table 2: Effect of imidacloprid exposure on ash, fat, organic and protein contents of *Labeo rohita* under short term experimental conditions. All the data is expressed as mean±standard deviation (SD). *P*-values are the 2 sample t-test results for the comparison of specific parameter between 2, 4 and 8 days treatment with their respective controls.

Parameter	Control	Imidacloprid exposure for 2 days	*p*-Value	Control	Imidacloprid exposure for 4 days	*p*-Value	Control	Imidacloprid exposure for 8 days	*p*-Value
Total Ash (g)	0.0023±0.0	0.0032±0.0	0.19(NS)	0.0036±0.00	0.0041±0.00	0.34(NS)	0.005±0.00	0.003±0.00	0.17(NS)
% Ash (Wet body weight)	0.0270±0.009	0.0292±0.005	0.76(NS)	0.040±0.01	0.036±0.00	0.13(NS)	0.036±0.00	0.041±0.00	0.015**
% Ash (Dry body weight)	5.5196±1.7	4.4607±1.6	0.16(NS)	6.41±1.56	4.94±1.39	0.04*	10.25±6.86	12.31±1.26	0.38(NS)
Total Fat (g)	0.002±0.00	0.003±0.00	0.01**	0.0021±0.00	0.0034±0.00	0.0087**	0.005±0.00	0.003±0.00	0.25(NS)
% Fat (Wet body weight)	0.02±0.01	0.03±0.01	0.28(NS)	0.024±0.01	0.030±0.00	0.05*	0.042±0.02	0.045±0.02	0.80(NS)
% Fat (Dry body weight)	30.08±3.63	32.35±6.21	0.34(NS)	0.13±0.04	0.16±0.01	0.033*	0.333±0.12	0.373±0.20	0.59(NS)
Total organic contents (g)	1.29±0.69	2.06±0.96	0.05*	1.69±0.42	2.12±0.64	0.091(NS)	1.77±1.86	0.77±0.17	0.12(NS)
% Organic contents (Wet body weight)	16.72±2.34	18.52±2.87	0.14(NS)	19.13±1.10	18.73±1.34	0.47(NS)	12.98±3.45	11.93±0.86	0.37(NS)
% Organic contents (Dry body weight)	94.48±1.65	95.54±1.61	0.16(NS)	93.59±1.56	95.06±1.39	0.040*	89.75±6.86	87.69±1.26	0.38(NS)
Total Protein (g)	1.29±0.69	2.05±0.96	0.05*	1.69±0.42	2.112±0.63	0.10(NS)	1.77±1.85	0.76±0.17	0.12(NS)
% Protein (Wet body weight)	16.70±2.33	18.49±2.86	0.14(NS)	19.11±1.09	18.70±1.33	0.47(NS)	12.93±3.44	11.88±0.85	0.37(NS)
% Protein (Dry body weight)	99.71±0.08	99.70±0.03	0.63(NS)	99.67±0.05	99.64±0.01	0.16(NS)	99.37±0.15	99.28±0.18	0.25(NS)

Table 3: Effect of imidacloprid exposure on weight, length and water components of *Labeo rohita* under long term experimental conditions. All the data is expressed as mean±standard deviation (SD). *P*-values are the 2 sample t-test results for the comparison of specific parameter between 16, 32 and 64 days treatment with their respective controls.

Parameter	Control	Imidacloprid exposure for 16 days	*p*-Value	Control	Imidacloprid exposure for 32 days	*p*-Value	Control	Imidacloprid exposure for 64 days	*p*-Value
Total Length (cm)	12.6 ±1.6	12.9 ±2.4	0.74(NS)	10.5±2.7	11.9±2.8	0.26(NS)	10.0±0.8	9.4±0.4	0.11(NS)
Total Weight (g)	17.3 ±8.2	20.4 ±11.6	0.50(NS)	14.2±15.2	18.7±14.3	0.50(NS)	8.5±2.0	6.7±0.7	0.025*
Dry Weight (g)	3.2 ±1.6	3.9 ±2.3	0.49(NS)	3.4±1.9	3.3±1.9	0.89(NS)	1.5±0.4	1.0±0.1	0.0051**
% Dry Weight	19.62±8.51	18.79±1.51	0.77(NS)	36.57±25.65	27.71±20.83	0.41(NS)	17.27±1.82	14.55±1.15	0.0012***
Total Water (g)	14.02±6.86	16.52±9.35	0.51(NS)	10.80±15.67	15.49±15.10	0.50(NS)	7.02±1.64	5.74±0.61	0.041*
% Water	80.38±8.51	81.21±1.51	0.77(NS)	63.43±25.65	72.29±20.83	0.41(NS)	82.73±1.82	85.45±1.15	0.0012***

Table 4: Effect of imidacloprid exposure on ash, fat, organic and protein contents of *Labeo rohita* under long term experimental conditions. All the data is expressed as mean±standard deviation (SD). *P*-values are the 2 sample t-test results for the comparison of specific parameter between 16, 32 and 64 days treatment with their respective controls.

Parameter	Control	Imidacloprid exposure for 16 days	*p*-Value	Control	Imidacloprid exposure for 32 days	*p*-Value	Control	Imidacloprid exposure for 64 days	*p*-Value
Total Ash (g)	0.01±0.00	0.02±0.05	0.33(NS)	0.01±0.00	0.01±0.00	1.0(NS)	0.003±0.00	0.010±0.02	0.39(NS)
% Ash (Wet body weight)	0.04±0.01	0.08±0.13	0.35(NS)	0.08±0.06	0.06±0.05	0.52(NS)	0.04±0.01	0.15±0.36	0.33(NS)
% Ash (Dry body weight)	3.93±1.53	5.07±3.60	0.38(NS)	3.94±1.99	4.37±2.71	0.69(NS)	7.35±2.21	6.91±2.59	0.68(NS)
Total Fat (g)	0.004±0.00	0.004±0.00	0.85(NS)	0.01±0.00	0.01±0.00	0.75(NS)	0.003±0.00	0.002±0.00	0.0030**
% Fat (Wet body weight)	0.027±0.01	0.02±0.01	0.16(NS)	0.07±0.04	0.06±0.04	0.63(NS)	0.037±0.01	0.028±0.00	0.0010***
% Fat (Dry body weight)	0.13±0.03	0.10±0.02	0.012**	0.20±0.05	0.22±0.05	0.43(NS)	0.22±0.03	0.19±0.03	0.032*
Total organic contents (g)	3.23±1.64	3.85±2.27	0.50(NS)	3.37±1.89	3.25±1.89	0.89(NS)	1.47±0.43	0.97±0.15	0.0047**
% Organic contents (Wet body weight)	19.58±8.50	18.71±1.45	0.76(NS)	36.50±25.59	27.65±20.78	0.41(NS)	17.23±1.82	14.4±1.24	0.0010***
% Organic contents (Dry body weight)	96.07±1.53	94.93±3.60	0.38(NS)	96.06±1.99	95.63±2.71	0.69(NS)	92.65±2.21	0.97±0.15	0.68(NS)
Total Protein (g)	3.22±1.64	3.84±2.26	0.49(NS)	3.36±1.89	3.24±1.89	0.89(NS)	1.47±0.43	0.97±0.15	0.0051**
% Protein (Wet body weight)	19.55±8.49	18.69±1.45	0.76(NS)	36.43±25.56	27.59±20.74	0.41(NS)	17.20±1.81	14.37±1.24	0.001***
% Protein (Dry body weight)	99.65±0.03	99.48±0.64	0.42(NS)	99.60±0.04	99.57±0.03	0.076(NS)	99.55±0.03	98.73±2.52	0.33(NS)

Discussion

A number of factors including food, space, chemicals, season, temperature, salinity and physical activity are known to affect fish growth. Proximate analysis of parameters indicating nutritional quality and energy content are good indicators of physiological condition of fish (Umer *et al*., 2011).

During the last two decades or so, production and use of pesticides has substantially increased all over the world. Use of pesticides has increased crop production but it is also a fact that only a small portion of the applied pesticides reach the final biological target in the field and major part of these pesticides are ultimately released into the environment, where they are responsible for variety of ecological and environmental problems such as toxicity to non-target organisms and accumulation in environment. Environment and human health are directly or indirectly at risk due to polluted soil, surface and ground waters exposures (Tisler *et al*., 2009).

Bayer AG, Nihon TN and Seizo KK commercially introduced imidacloprid to the market in 1991 and its demand is increasing since then (Tomlin, 1997; Tomizawa and Casida, 2005). imidacloprid can pass into water bodies by spray drift or by run-off after application although it is not intended to be used in water. Only few toxicity studies have been reported on the effects of imidacloprid on aquatic organisms despite its increasing use. Bayo and Goka (2005) have reported that imidacloprid has teratogenic effects such as spinal cord deformities in embryos at very low, sub lethal concentrations in Japanese madaka fish (*Oryzias latipes*). No data is available, to date, regarding the effect of imidacloprid, on nutritional quality of *L. rohita*, the most economically important fresh water fish in Pakistan. Our results indicated that chronic exposure of imidacloprid can adversely affect the nutritional quality of *L. rohita*. As this was a pioneer study on the topic, further studies are recommended on the same topic to demonstrate the effect of various doses of imidacloprid on food quality of *L. rohita* as well as on other commercial fish.

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