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ORIGINAL RESEARCH

Cypermethrin induced stress and changes in growth of freshwater fish *Oreochromis niloticus*

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Abstract Bioassays were conducted with technical grade and commercial formulation of cypermethrin using freshwater fish *Oreochromis niloticus* as the test fish. The technical grade cypermethrin contained 92% active ingredient (a.i.) and the commercial formulation was an emulsified concentrate (EC) containing 10% a.i. (10% EC). Based on the actual concentration in water (2 h), the commercial formulation was found to be more acutely toxic to *O. niloticus* (96-h LC_{50} = 4.85 µg/L) than the technical grade cypermethrin (96-h LC_{50} = 9.74 µg/L). Exposure to sub-lethal concentrations (1.25, 2.5 µg/L) of commercial cypermethrin for 96 h produced stress on the fish, which was evident from the reduction of hepatic glycogen, reduction in the activities of alkaline phosphatase, acetylcholinesterase and catalase in liver and elevation of plasma glucose level and activities of hepatic acid phosphatase, aspartate aminotransferase and alanine aminotransferase. Exposure to these concentrations of cypermethrin for 14–28 days produced anaemia in fish. Long-term exposure (90 days) of the fish to these concentrations reduced the growth and deposition of protein and lipid in the body of fish as compared to control.It is concluded from this study that even minute concentration (1.25 µg/L) of cypermethrin (10% EC) in water can produce stress on fish. Long term exposure to such concentration of cypermethrin may also affect growth of the fish.

Keywords Pesticide · Toxicity · Tilapia · LC_{50} · Enzymes · Growth

Introduction

Cypermethrin is a 4th generation halogenated type II pyrethroid (Kaviraj and Gupta 2014) and is extensively used in tropical countries to control insect pests of cotton, cabbage, okra, brinjal, sugarcane, wheat, sunflower and many other crops. As a result, cypermethrin has been the centre of attention of many researches dealing with susceptibility of fish to agricultural run-offs. Type II pyrethroids are extensively used for pest management because of their relatively low toxicity to birds and mammals. But information on the toxicity of these pesticides to non-target aquatic organisms is scarce. Cypermethrin has already been reported as highly toxic to fish (Kumar et al. 2007; Saha and Kaviraj 2008). Sporadically, it has also been reported that the toxicity of cypermethrin to fish varies with stereochemical structure (Polat et al. 2002), mixtures of their isomers (Yilmaz et al. 2004) and formulation.

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Commercial formulations of cypermethrin have always been found to be more toxic than its active ingredient (Demetrio et al. 2014; Majumder and Kaviraj 2015) because of inert ingredients added to the commercial formulations of pesticides to improve their efficiency in field conditions (Puglis and Boone 2011).

Emulsified concentrate (EC), with 10–20% active ingredients, is the most common formulation of cypermethrin being marketed in India. Composition of inert synergists added to these formulations and their contribution to the toxicity of cypermethrin to non-target aquatic organisms are not properly known. Lethal concentrations of different formulations of cypermethrin to fish, reported so far, are based mostly on the nominal concentration of cypermethrin. In this study we determined lethal concentrations of both chemically pure (technical grade) and commercial formulation of cypermethrin to freshwater fish *Oreochromis niloticus* based on nominal as well as actual concentration of cypermethrin measured after 2 h of exposure. Comparison of the data generated was helpful to evaluate impact of inert ingredients, if any, onthe toxicity of the commercial formulation of cypemethrin to fish. Since liver is the main site of detoxification of toxicants entering into the body, researchers have made efforts to detect stress of cypermethrin on fish from the activities of hepatic enzymes related to various metabolic pathways (Tiwari et al. 2012; Adeyemi et al. 2014). Reviews by Kaviraj and Gupta (2014) have documented the effects of cypermethrin on liver glycogen, activities of alkaline and acid phosphatase, activities of enzymes related to nitrogen metabolism and lipid peroxidation of liver. Changes in haematological profile have also been used as biomarkers of cypermethrin toxicity to fish (Vani et al. 2012; Ojutiku et al. 2013). However, the effects of cypermethrin on the growth of fish are rather poorly documented (Carriquiriborde et al. 2007). Although the toxic potential of commercial cypermethrin (10% EC) gradually decreases once it is exposed to light (Saha and Kaviraj 2009a), long-term exposure (60 days) to sub-lethal concentration of this pesticide has been found to reduce the growth of the catfish *Heteropneustes fossilis* (Saha and Kaviraj 2013). A comprehensive study on the acute toxicity and the effects of sub-lethal concentrations of cypermethrin on the biochemical parameters as well as the growth of fish is lacking.

Oreochromis niloticus is a freshwater fish. Although the species is exotic to India, it is widely cultured throughout the country because of its capacity to adapt to local conditions and tolerance to a wide variety of environmental conditions. The main objectives of the present study were to determine the stress produced by sub-lethal concentrations of commercial cypermethrin (10% EC) on *O. niloticus* and the subsequent impact of the pesticide on growth of the fish. Accordingly, we first determined the acute toxicity of technical grade cypermethrin containing 92% active ingredient (92% a.i.) and an emulsified concentrate containing 10% active ingredient (10% EC) on *O. niloticus* based on both nominal and actual concentrations of cypermethrin in water and then evaluated the degree of difference in toxicity between the two forms of cypermethrin. Detailed studies on the stress and growth of the fish, however, were carried out with sub-lethal concentrations of an emulsified concentrate (10% EC) of cypermethrin because this form is used in agricultural fields.

Materials and methods

Experimental design

Four experiments were performed in this study. These included acute toxicity bioassay to determine lethal concentrations of cypermethrin to *O. niloticus* and experiments with sub-lethal concentrations of cypermethrin (10% EC) on biochemical parameters (96 h), haematological parameters (28 days) and growth of *O. niloticus* (90 days).

Specimens of *O. niloticus* (mean length 4.61 ± 0.24 cm and mean weight 2.64 ± 0.16 g) were obtained from Kulia fish farm, Kalyani, West Bengal, and were acclimatized to the test conditions for 96 h before use. During acclimatization, the fish were fed a balanced diet containing 30% crude protein formulated in the laboratory (Table 1). Proximate compositions of the ingredients and the diet were determined by AOAC method (Helrich1990). Technical grade cypermethrin (92% active ingredient of (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate) was obtained from Krishi Rasayan Group of Companies, Kolkata-700020 (India), and the emulsified concentrate (10% EC) of cypermethrin was procured under the brand name Ustaad® from the United Phosphorus Ltd., Vapi-396195, Gujarat. Deep tube-well water stored in an overhead tank (temperature 31 ± 3 °C, pH 7.2 ± 0.1; free CO_2 3.37 ± 0.31 mg/L; dissolved oxygen 6.5 ± 0.2 mg/L; total alkalinity 123.28 ± 3.37 mg/L as $CaCO_3$; total hardness 141.44 ± 11.01 mg/L as $CaCO_3$) was used as the test medium in all experiments.

Table 1 Proximate composition of the ingredients and the experimental diet

Ingredients used for formulation	Proximate composition (% dry matter)					Proportion used in formulation (%)
	Crude protein	Crude lipid	Crude fibre	Moisture	Ash	
Rice bran	11.96	10.8	16.3	7.46	8.73	38.74
Mustard oil cake	31.63	12.6	8.3	7.58	9.55	38.74
Fishmeal	65.98	13.6	3.1	13.62	9.5	20.52
Vitamin premix[a]	–	–	–	–	–	1.0
Mineral premix[b]	–	–	–	–	–	1.0
Formulated diet (dry matter: 94%)	30.29	4.98	11.29	6.0	12.21	

[a] Vitamin mixture (%): (Ambiplex; Brihans Lab, Pune): Vit B_1: 7.14, Vit B_2: 2.55, Vit B_6: 1.02, VitB_{12}: 0.012, biotin: 0.025, calcium pantothenate: 2.55, niacin: 76.50, choline chloride (B4): 10.20

[b] Mineral mixture (%): (Agrimin; Glaxo India Ltd, Mumbai): copper 3.12, cobalt: 0.45, magnesium: 21.14, iron: 9.79, iodine: 1.56, zinc: 21.30, calcium: 30.00, phosphorous: 8.25

Acute toxicity bioassays

Acute toxicity bioassays were made in 15-L glass aquaria, each holding 10 L of water and five acclimatized fish. Separate bioassays were made with technical grade (92% a.i.) and formulation (10% EC) of cypermethrin. The range of concentrations of cypermethrin used in these bioassays is presented in Table 2. Three replicates were maintained for each concentration including a water control and a solvent control. The solvent control contained 0.1 ml/L acetone, because acetone was used to dissolve technical grade cypermethrin, and the maximum amount of acetone present in the highest concentration of technical grade cypermethrin tested was less than 0.1 ml/L. The experiment was carried out for 96 h. Mortality of the fish was recorded every 24 h and the dead fish were removed. No food was provided during the bioassay to avoid the interference of excretory products of the fish with the test chemical. Lethal concentrations of cypermethrin at which 50% mortality of the fish occurred (LC_{50}) and its 95% confidence limits were estimated for 96 h from the mortality data using EPA-Probit analysis version 1.5 statistical software based on the probit analysis method of Finney (1971). LC_{50} values between the active ingredient and formulation were compared following the criteria of Mayer and Ellersieck (1986), Schmuck et al. (1994) and Demetrio et al. (2014).

Residue analysis of cypermethrin

Residue analyses of cypermethrin were made in the samples of water collected from the aquaria used for acute toxicity bioassay. The samples of water were taken in a separating funnel for separation into water and organic phase. The organic phase was extracted with ethyl acetate and the concentration of cypermethrin in the extract,

Table 2 Range of concentrations of cypermethrin (μg/L) used in the experiments

Technical (92% a.i.)		Formulation (10% EC)	
Nominal	Actual[a]	Nominal	Actual[a]
0	0	0	0
5	4.31	1	0.92
10	8.61	3	2.77
15	12.92	5	4.61
20	17.22	7	6.45
25	21.53	9	8.30
30	25.83	14	12.91

[a] After 2 h of exposure

after centrifugation (11,200*g*) and filtration through a 0.2-μm nylon membrane filter, was determined in a gas chromatograph (Agilent 6890N) equipped with a wide bore HP column (HP-5, 30 m, 0.32 mm ID, 0.25 μm film thickness), an electron capture detector (ECD) and a 7683 B Series auto-injector. N_2 gas was used as a carrier. Concentration of cypermethrin was quantified from the calibration curve prepared from standard cypermethrin concentrations, using ChemStation software. Limit of detection (LOD) and limit of quantification (LOQ) of the instrument were 0.01 and 0.03 ppm, respectively. Nominal concentrations (*N*) used in the experiments and the respective actual concentrations (*A*) determined by the above method after 2 h of exposure are presented in Table 2.

Experiments on biochemical parameters

Experiments on biochemical parameters were also made in 15-L glass aquaria, each holding 10 L of water and five fish. Three sub-lethal concentrations of the cypermethrin formulation used for these experiments were as follows: control (0.0 μg/L), a low dose (1.25 μg/L) and a high dose (2.25 μg/L) of cypermethrin (10% EC). The low and high doses of cypermethrin were approximately 25 and 50% of the 96-h LC_{50} value of the nominal concentration of cypermethrin (10% EC) to *O. niloticus*, respectively. There were three replicates for each concentration. The fish specimens were sampled after 96 h of exposure, rinsed in deionized water and dried on blotting paper. Blood was collected directly from the heart of the sampled fish with a micro-syringe and was used for glucose estimation. Simultaneously, liver was dissected out from the sampled fish, weighed and homogenized, and the homogenate was used for the analysis of glycogen content, alkaline and acid phosphatase, aspartate and alanine aminotransferase, acetylcholinesterase and catalase activities of the liver. Plasma glucose was determined by the method of Hyvarinen and Nikkila (1962) and liver glycogen was determined by the method of Carroll et al. (1956). Protein in liver tissue was determined by the method of Lowry et al. (1951), while the activities of the enzymes acid and alkaline phosphatase, aspartate and alanine aminotransferase, acetylcholinesterase and catalase were determined, respectively, by the methods of Walter and Schutt (1974), Reitman and Frankel (1957), Ellman et al. (1961) and Luck (1974).

Experiments on haematological parameters

These experiments were also made in 15-L glass aquaria using the same treatments and experimental conditions as described above for the experiment on biochemical parameters. However, the experiment was carried out for 28 days. One half of the fish were sampled from each aquarium at the end of 14 days and the rest were sampled at the end (28 days). Blood was collected from the sampled fish by the technique described above and was subjected to the determination of different haematological parameters. Haemoglobin (Hb%) was determined by cyanomethemoglobin method following Dacie and Lewis (1968), packed cell volume (PCV) or haematocrit (Hct) was determined by Wintrobe method or Macromethod (Dacie and Lewis 1968), and total erythrocyte count (TEC) and total leucocyte count (TLC) were determined with Neubauer's improved double haemocytometer using RBC and WBC diluting fluids, respectively.

Experiments on growth

Bioassays were carried out in outdoor cement vat (diameter 90 cm and average depth 50 cm). Before stocking of fish, each vat was prepared with a 3-cm-thick layer of uncontaminated soil at the bottom and approximately 100 g cow dung was applied on it. The vats were then filled with water (400 L) and kept in this condition for 1 month prior to the start of the experiment to ensure the growth of planktons, which serve as natural food for the test fish. Fingerlings of *O. niloticus* were stocked in these experimental vats and acclimatized in this condition for 1 week before the start of the experiments. Thirty fish, irrespective of their sex, were stocked in each vat. Altogether, nine vats were arranged according to randomized block design so that the fingerlings could be reared in three replicates for each of the three test concentrations (0, 1.25 and 2.50 μg/L) of cypermethrin (10% EC). Treatments with cypermethrin were made on day 1 of the experiment (initial treatment). Then 20% of the test medium was renewed at 10-day interval with a pulse treatment of pesticides at 20% of the initial nominal concentration. Static renewal bioassay with renewal of the bioassay medium at regular interval is a standard method to compensate loss of water through evaporation, to reduce the possibility

of depletion of dissolved oxygen in the test medium and to reduce the possibility of loss of toxicant through volatilization and/or adsorption to test vessels (USEPA 2002). The experiment was continued for 90 days. During the experiments, the fish were hand fed the formulated diet (Table 1) twice daily at an interval of 10 h up to apparent satiation of the fish. Daily survey was made on the behaviour and mortality of the experimental fish. Water samples were collected from each vat every 15 days and dissolved oxygen, free carbon dioxide, temperature, total hardness and total alkalinity of the sampled water were determined by standard method (APHA 1995). All fish from each outdoor vat were sampled at the end of 90 days and the lengths (cm) and weights (g) of the sampled fish were recorded. Three sampled fish from each vat were subjected to biochemical analyses to determine crude protein, crude lipid and ash contents of the fish following the AOAC method (Helrich 1990). Growth was determined by percent increase in weight, specific growth rate (SGR), feed conversion ratio (FCR) and apparent net protein utilization (ANPU) using standard formulae (Bagenal 1978; Castell and Tiews 1980; Adams and Mclean 1985; Steffens 1989).

Statistical methods

The data obtained from the experiments on haematological, biochemical and growth parameters were subjected to single-factor ANOVA followed by least significant difference (LSD) test to verify the significance of difference between treatments at 5% level of probability (Gomez and Gomez 1984).

Results

Acute toxicity

96-h LC_{50} values of technical grade (92% a.i.) and formulation (10% EC) of cypermethrin to *O. niloticus* are presented in Table 3. The formulation was found to be more toxic to *O. niloticus* than the technical grade cypermethrin. In both groups, fish exposed to acute doses exhibited abnormal behaviour. Initially, fish showed frequent surfacing, increased opercular movement and faster swimming activity though within 5–9 h of treatment gradual lethargy was noted in their movement. Deposition of thick mucus layer was found in the buccal cavity and gills of exposed fish probably to minimize the irritating effects of toxicant coming in close contact with body. Sign of heavy internal haemorrhage around pharynx was noticed in the dead fish.

Effects of sub-lethal concentrations of cypermethrin

Liver glycogen and plasma glucose

Changes in liver glycogen and plasma glucose levels of control and cypermethrin (10% EC)-treated *O. niloticus* are presented in Fig. 1. There was a gradual decrease in hepatic glycogen level from 24 to 96 h of exposure, while plasma glucose level increased during this period. The effects were dose dependent, with the treatment of 2.5 µg/L cypermethrin showing more acute effects than that of 1.25 µg/L of cypermethrin.

Hepatic enzyme activities

Effects of cypermethrin on hepatic enzyme activities of *O. niloticus* are summarized in Table 4. Activities of the enzymes alkaline phosphatase, acetylcholinesterase and catalase decreased significantly ($P < 0.05$) in

Table 3 96-h LC_{50} values (µg/L) of cypermethrin and 95% confidence limit (CL) to freshwater teleost *Oreochromis niloticus*

Cypermethrin	Nominal		Actual (2 h)	
	LC_{50}	95% CL	LC_{50}	95% CL
Technical (92% a.i.)	10.71	8.79–12.75	9.74	8.10–11.35
Formulation (10% EC)	5.25	4.35–6.16	4.85	4.02–5.68

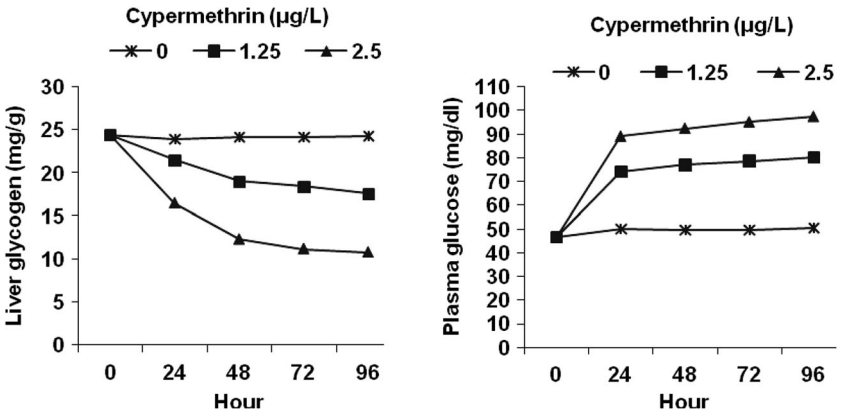

Fig. 1 Effects of cypermethrin (10% EC) on liver glycogen and plasma glucose of *Oreochromis niloticus*

Table 4 Changes in activities of hepatic enzymes of *O. niloticus* after 96-h exposure to cypermethrin (10% EC)

Parameter	0.0 µg/L	1.25 µg/L	2.5 µg/L
Alkaline phosphatase[1]	13.28 ± 0.07[a]	8.95 ± 0.08[b]	7.29 ± 0.07[c]
Acid phosphatase[1]	6.39 ± 0.06[a]	7.67 ± 0.12[b]	8.86 ± 0.07[c]
Aspartate aminotransferase[2]	2.28 ± 0.02[a]	3.04 ± 0.04[b]	3.58 ± 0.03[c]
Alanine aminotransferase[2]	5.71 ± 0.02[a]	6.98 ± 0.04[b]	9.55 ± 0.04[c]
Acetylcholinesterase[3]	0.73 ± 0.02[a]	0.48 ± 0.01[b]	0.38 ± 0.02[c]
Catalase[4]	13.37 ± 0.04[a]	11.06 ± 0.09[b]	9.36 ± 0.08[c]

Data are mean ± SD ($n = 3$); means with dissimilar superscripts in the same row indicate the least significant difference between the means at $P < 0.05$

[1] µg PNP/mg tissue protein/30 min

[2] µM pyruvate/mg tissue protein/h

[3] µM acetylthiocholine iodide/mg tissue protein/min

[4] µM H_2O_2 decomposed/mg tissue protein/min

cypermethrin treatments as compared to control. The higher the concentration of cypermethrin, the lower the activities of these enzymes. On the contrary, the activities of the enzymes acid phosphatase, aspartate aminotransferase and alanine aminotransferase increased significantly ($P < 0.05$) in both treatments of cypermethrin as compared to control. There were also significant differences in activities between the two sub-lethal concentrations (1.25 and 2.5 µg/L) of cypermethrin.

Haematological parameters

The changes in haematological parameters of *O. niloticus* exposed to control and cypermethrin are summarized in Table 5. The results indicated that TEC, haemoglobin concentration (Hb), PCV, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) decreased significantly ($P < 0.05$) in both sub-lethal concentrations of cypermethrin (1.25 and 2.5 µg/L) as compared to control. On the other hand, total leucocyte count showed significant elevation in cypermethrin-treated *O. niloticus* than the control. There was no significant difference in these parameters between the exposure periods of 14 and 28 days.

Effects on growth

Long-term exposure (90 days) to sub-lethal concentrations of cypermethrin increased mortality and reduced growth of *O. niloticus* as compared to control. The growth parameters are summarized in Table 6. Fish exposed to 2.5 µg/L cypermethrin showed more reduction in weight gain (%), SGR, protein efficiency ratio

Table 5 Changes in haematological parameters of *O. niloticus* exposed to cypermethrin

Parameters	Days of exposure	0.0 μg/L	1.25 μg/L	2.5 μg/L
Total erythrocyte count (10^6/mm^3)	14	2.81 ± 0.03^a	2.74 ± 0.04^b	2.62 ± 0.03^c
	28	2.86 ± 0.05^a	2.68 ± 0.06^b	2.51 ± 0.02^c
Haemoglobin concentration (g/dl)	14	8.72 ± 0.05^a	7.77 ± 0.04^b	7.08 ± 0.09^c
	28	8.83 ± 0.05^a	7.34 ± 0.05^b	6.74 ± 0.06^c
Packed cell volume (%)	14	25.58 ± 0.16^a	23.68 ± 0.07^b	21.99 ± 0.69^c
	28	25.67 ± 0.14^a	22.53 ± 0.11^b	20.96 ± 0.21^c
Mean cell volume (fl/cell)	14	91.05 ± 1.12^a	86.55 ± 1.13^b	83.92 ± 0.64^c
	28	89.66 ± 1.26^a	84.18 ± 1.76^b	83.51 ± 0.99^c
Mean cell haemoglobin (pg/cell)	14	31.04 ± 0.17^a	28.41 ± 0.45^b	27.04 ± 0.25^b
	28	30.86 ± 0.58^a	27.43 ± 0.51^b	26.84 ± 0.01^b
Mean corpuscular haemoglobin concentration (g/dl)	14	34.09 ± 0.17^a	32.82 ± 0.24^b	32.22 ± 0.31^b
	28	34.41 ± 0.36^a	32.58 ± 0.33^b	32.14 ± 0.40^b
Total leucocyte count (10^3/mm^3)	14	23.55 ± 0.13^a	24.74 ± 0.07^b	25.96 ± 0.08^c
	28	23.94 ± 0.09^a	25.59 ± 0.15^b	26.86 ± 0.07^c

Data are mean ± SD ($n = 3$); means with dissimilar superscripts in the same row indicate the least significant difference between the means at $P < 0.05$

Table 6 Changes in growth parameters and mortality of *O. niloticus* exposed to cypermethrin (10% EC) for 90 days

Parameters	0.0 μg/L	1.25 μg/L	2.5 μg/L
Initial weight (g)	2.64 ± 0.16	2.64 ± 0.16	2.64 ± 0.16
Weight gain (%)[1]	128.39 ± 8.36^a	74.72 ± 10.89^b	51.79 ± 9.45^c
FCR[2]	3.08 ± 0.21^a	4.19 ± 0.53^b	4.95 ± 0.98^c
SGR (%/day)[3]	0.92 ± 0.04^a	0.62 ± 0.07^b	0.46 ± 0.07^c
PER[4]	1.09 ± 0.07^a	0.81 ± 0.12^b	0.70 ± 0.12^c
HSI[5]	1.10 ± 0.04^a	1.57 ± 0.09^b	1.83 ± 0.12^c
ANPU (%)[6]	20.85 ± 1.12^a	13.61 ± 1.60^b	10.74 ± 1.54^c
Mortality (%)	4.04 ± 1.22^a	17.39 ± 1.26^b	21.75 ± 1.30^c

Values are the mean of three replicates ± SD; means with dissimilar superscripts in the same row indicate the least significant difference between the means at $P < 0.05$

[1] Weight gain (%) = [{final wt (g) − initial wt (g)}/initial wt (g)] × 100

[2] Feed conversion ratio = food given/weight gain

[3] Specific growth rate (%/day) = $\{(\log_e W_2 - \log_e W_1)/T\} \times 100$, where W_1 = initial body weight (g), W_2 = final body weight (g) and T = days of exposure

[4] Protein efficiency ratio = increase in weight of fish (wet weight)/weight of protein in feed (dry weight)

[5] Hepatosomatic index = [{wet weight of liver (g) without gall bladder}/wet body weight] × 100

[6] Apparent net protein utilization (%) = (net increase in carcass protein/amount of protein consumed) × 100

(PER) and ANPU than those treated by 1.25 μg/L cypermethrin. FCR and hepatosomatic index (HSI) increased significantly ($P < 0.05$) in fish treated with 2.5 μg/L cypermethrin than in those treated with 1.25 μg/L cypermethrin treatment and control. Proximate compositions of carcass of the sampled fish are presented in Table 7. The results clearly indicated that crude protein, crude lipid and ash contents increased from their initial values. However, the rate of increase was significantly lower in cypermethrin-treated fish in comparison to control. Among the cypermethrin-treated fish, the values reduced with the increase of cypermethrin concentration.

Table 7 Proximate composition (% wet weight basis) of carcass of *O. niloticus* exposed to cypermethrin (10% EC) for 90 days

	Initial	Final		
		0.0 μg/L	1.25 μg/L	2.5 μg/L
Crude protein	11.13 ± 0.27	15.40 ± 0.24[a]	13.56 ± 0.24[b]	12.43 ± 0.24[c]
Crude lipid	2.73 ± 0.14	4.78 ± 0.12[a]	3.63 ± 0.14[b]	3.22 ± 1.12[c]
Ash	1.89 ± 0.08	3.01 ± 0.07[a]	2.49 ± 0.06[b]	2.29 ± 0.07[c]

Values are the mean of three replicates ± SD; means with dissimilar superscripts in the same row indicate the least significant difference between the means at $P < 0.05$

Discussion

Acute toxicity

96-h LC_{50} values of technical grade (92% a.i.) and commercial (10% EC) cypermethrin to *O. niloticus* as observed in the present investigation are, respectively, close to 96-h LC_{50} values of technical grade (98%) alpha-cypermethrin to *Poecilia reticulata* (9.43 μg/L; Yilmaz et al. 2004) and commercial cypermethrin (10% EC) to freshwater fish *Colisa fasciatus* (6.0 μg/L; Singh et al. 2010). The present results indicate that the emulsified concentrate (EC) of cypermethrin is more toxic than the technical grade (active ingredient) cypermethrin. This has also been confirmed by the toxicity studies of cypermethrin on various species of invertebrates (Demetrio et al. 2014; Majumder and Kaviraj 2015). Several studies have demonstrated that the inert ingredients added to the formulated products increase its toxicity (Puglis and Boone 2011). Quotient (LC_{50} of X/LC_{50} of Y) is used to interpret and compare the acute toxicity data between technical grade (X) and formulation (Y) of pesticides. Mayer and Ellersieck (1986) assumed the formulation as more toxic when the quotient was more than 1, while Schmuck et al. (1994) observed that there was a natural variability of quotient between 0.5 and 2.0 and considered the formulation as more toxic when the quotient was more than 2. Evaluating the results of the present study, it was revealed that the formulation was more toxic than the technical grade cypermethrin according to the criteria of Mayer and Ellersieck (1986) and Schmuck et al. (1994). Since only one single point (LC_{50}) for the concentration–effect function was considered under both criteria, Demetrio et al. (2014) proposed to accept the criteria as valid only when the concentration effect lines were parallel. Log-probit model regression lines for log concentration versus probit values of mortality for *O. niloticus* following 96-h exposure to technical grade (T) and formulation (F) of cypermethrin were parallel to each other (Fig. 2).

Biochemical parameters

Reduction of hepatic glycogen content in *O. niloticus* due to cypermethrin treatment as observed in the present study is a common symptom of stress exhibited by fish when exposed to type II pyrethroids (Kaviraj and Gupta 2014). Similar effects of cypermethrin have been reported for *Clarias batrachus* (Begum 2005), *H. fossilis* (Saha and Kaviraj 2009b) and *Ophiocephalus punctatus* (Shruti et al. 2011) and *Labeo rohita* (Tiwari et al. 2012). Carbohydrate serves as the instant source of energy during stress. Accordingly, glycogen of liver is broken down (glycogenolysis) to meet the energy demand raised during cypermethrin stress, resulting in the reduction of hepatic glycogen content. On the other hand, an increase in plasma glucose level due to cypermethrin treatment, as observed in the present study, has also been observed on *H. fossilis* (Saha and Kaviraj 2009b) and *O. niloticus* (Firat et al. 2011) probably due to an increase in hepatic glucose-6-phosphatase activity which enhances glycogenolysis and glucose synthesis from extrahepatic tissue proteins and amino acids (Firat et al. 2011). Gluconeogenesis is another pathway to increase plasma glucose level during the stress of pesticide to fish (Saravanan et al. 2011). This is initiated by aminotransferase enzymes, which provide strategic links between protein and carbohydrate metabolism to meet the demand of energy under stressed condition (Neelima et al. 2013). Increased activities of hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as observed in the present study indicate active catabolism of amino acid to meet the immediate energy demand under cypermethrin stress (Kumar et al. 2011). In the first

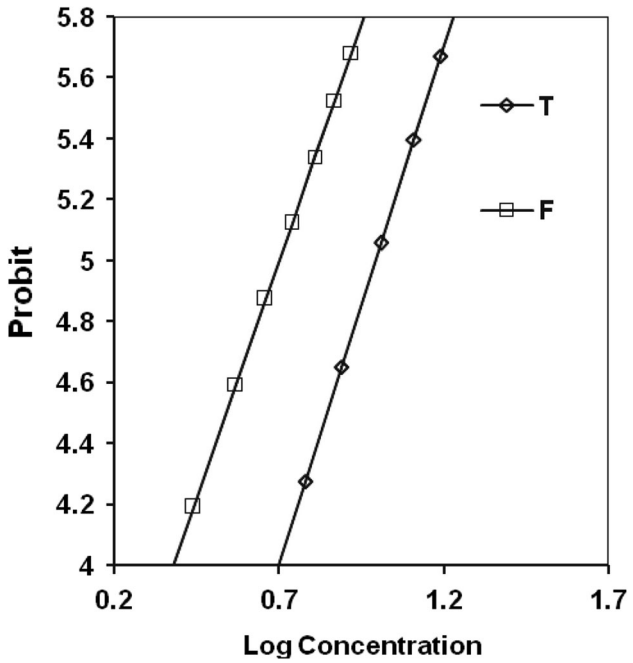

Fig. 2 Log-probit model regression lines for log concentration (actual) versus probit values for *Oreochromis niloticus* following 96-h exposure to technical grade (*T*) and formulation (*F*) of cypermethrin

phase of catabolism, α keto acids are formed through transamination. Gluconeogenesis is promoted by the incorporation of keto acids into tricarboxylic acid (TCA) cycle via active transdeamination (Neelima et al. 2013). Studies on cypermethrin-induced increase in the activities of hepatic AST and ALT in *C. batrachus* (Begum 2005), *Cirrhinus mrigala* (Prasanth and Neelagund 2008), *Channa punctatus* and *C. batrachus* (Kumar et al. 2011), *L. rohita* (Tiwari et al. 2012), *Catla catla* (Vani et al. 2012) and *Prochilodus lineatus* (Loteste et al. 2013) indicate that fish utilize amino acids to overcome the stress of cypermethrin.

Changes in glucose metabolism were also linked with the activities of hepatic alkaline phosphatase, which were decreased in cypermethrin-treated *O. niloticus* as compared to control. Similar effects of cypermethrin on alkaline phosphatase activities were found in the liver of *Heterobranchus bidorsalis* (Gabriel et al. 2011), *H. fossilis* (Saha and Kaviraj 2009b) and *L. rohita* (Tiwari et al. 2012). Liver alkaline phosphatase promotes glycogen synthesis by inactivating the enzyme phosphorylase. Reduced activities of hepatic alkaline phosphatase may lead to the breakdown of glycogen in order to get necessary energy to overcome the stress. Some researchers reported increased activities of alkaline phosphatase in the serum of fish exposed to cypermethrin, which might be due to the efflux of enzymes from the liver into bloodstream (Firat et al. 2011; Meenambal et al. 2012). On the other hand, the activity of acid phosphatase was elevated in cypermethrin-exposed *O. niloticus* as compared to control. These results are in agreement with the findings of Kumar et al. (2014) on *C. batrachus*. Acid phosphatase is a lysosomal enzyme that hydrolyses the ester linkage of phosphate esters and cause autolysis of cell after its death. Increased acid phosphatase activity may be due to hepatocellular damages in *O. niloticus* exposed to cypermethrin.

Acetylcholinesterase (AChE) is distributed in synaptic region and induces nerve impulse propagation by converting acetylcholine into acetic acid and choline. Inhibition of this enzyme can cause hyperexcitability. This reaction was evident in the acute toxicity experiment with cypermethrin in the present study. Cypermethrin probably interacts with the hydrophobic aromatic surface region of acetylcholinesterase and inhibits the activities of this enzyme as observed in *L. rohita* (Tiwari et al. 2012), *C. batrachus* (Kumar et al. 2014), *Channa striatus* (Ahmed et al. 2015) and *Cyprinus carpio* (Neelima et al. 2015).

Cypermethrin also produces oxidative stress in fish. This was evident from the decreased activity of catalase in the liver of *O. niloticus* exposed to sub-lethal concentrations of cypermethrin, as observed in the present study. Catalase is an enzymatic antioxidant. Reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide and hydroxyl radicals are formed in fish upon exposure to pollutants. Catalase scavenges

reactive oxygen species, converts them into less reactive species and prevents lipid peroxidation. Accordingly, the activity of malondialdehyde (MDA) is increased and that of catalase is decreased as oxidative defense in fish (Marigoudar et al. 2013; Kaviraj and Gupta 2014). Catalase activity thus serves as a potential oxidative stress biomarker in fish toxicity studies. Tripathi and Bandooni (2011) observed decreased activity of catalase in the liver of *C. batrachus* treated with alphamethrin, a racemic mixture of two isomers of cypermethrin. Cypermethrin-induced reduction in the catalase activity of spermatozoa has also been observed in *Oncorhynchus mykiss* (Kutluyer et al. 2016).

Haematological parameters

Decline in total erythrocyte count, Hb content, PCV and MCHC values as observed in the present study is an indication of anaemia. Anaemia is probably caused by the inhibition of erythropoiesis and chemosynthesis as well as the destruction of erythrocytes (Adhikari et al. 2004; Jee et al. 2005; Akinrotimi et al. 2012). In addition, WBC is liberated from the spleen into the blood to counter the cypermethrin-induced stress (Akinrotimi et al. 2012), resulting in an increase in leucocyte count (Adhikari et al. 2004; Ojutiku et al. 2013) as also observed in the present study.

Effects on growth and biochemical composition

Results of the present study indicated that the growth of *O. niloticus* is reduced when exposed to sub-lethal concentrations of cypermethrin for 90 days. This was due to poor efficiency in feed conversion, net protein utilization and subsequent poor deposition of protein and lipid in the muscle. There are evidence that cypermethrin can reduce the protein level of serum (Vani et al. 2012; Kannan et al. 2014), gills and liver (Begum 2005), muscle and kidneys (Begum 2007) in several species of fish. Gijare et al. (2011) also found that the lipid content was decreased in *O. punctatus* due to cypermethrin treatment. The toxic impact of cypermethrin is quickly removed from water (Saha and Kaviraj 2009a). But the results of the present study indicate that the stress produced by 1.25–2.5 µg/L cypermethrin (10% EC) on biochemical and haematological parameters is carried and reflected on feed conversion and deposition of protein.

Conclusion

It is concluded from the present study that cypermethrin (10% EC) is highly toxic to *O. niloticus.* Concentration of cypermethrin as low as 1.25 µg/L is capable of inducing changes in biochemical and haematological parameters of the fish after short-term exposure.These changes are indication of stress on the fish. The stress also affects growth of the fish, if itis exposed to cypermethrin for long period.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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