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A Comparative Study on The Acute Toxicity Bioassay of Dimethoate and Lambda-cyhalothrin and Effects on Thyroid Hormones of Freshwater Teleost Fish *Labeo rohita* (Hamilton)

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ABSTRACT: Dimethoate (organophosphate insecticide) and Lambda-cyhalothrin (synthetic pyrithroid) are two frequently used pesticides due to their high effectiveness and rapid breakdown into environmentally safe products. The aim of this present study was to assess and compare the acute toxicity of Dimethoate (Rogor30%EC) and Lambda-cyhalothrin (5%EC) on *Labeo rohita* (Hamilton). Acute toxicity bioassay was carried out in a static renewal system to assess the toxicity. The fishes were exposed to different concentration of dimethoate and Lambda-cyhalothrin to determine the LC_{50} values for 96hrs. The mortality data obtained were analyzed by SPSS 16.0 based on Finney's Probit Analysis Statistical method. The 96h LC_{50} values for Dimethoate and Lambda-cyhalothrin were found to be 24.55 µl/L and 0.7 µl/L respectively. Effects of these two insecticides on serum T_3, T_4 and TSH were investigated under sub-lethal toxicities which cause decrease in the serum T_3, T_4 and increase in TSH level. It shows significant difference from control fish ($p < 0.05$)..

Key words: Dimethoate, Lambda-cyhalothrin, *Labeo rohita*, LC_{50} value,Thyroid hormone

INTRODUCTION

Rapid industrialization and indiscriminate use of various pesticides and chemicals for pest control from last decades have menacing effects on non-target aquatic organism's particularly commercially important fishes. Leaching of agricultural and industrial effluents into water bodies lead to contamination of aquatic environments and enter into organisms through food webs. The accumulation and persistence of pesticide residues and heavy metals in the aquatic environments have adverse effects to biological life (biomagnifications) (Amanchi and Hussain, 2010). Contamination of water bodies by pesticides in any way can lead to fish mortality, reduced fish productivity etc. The broad spectrum of activity of these pesticides often extends far beyond the pest. Fish, one of the bio-indicator species determine the acute toxicity value and to assess the quality of and meaningful procedure. Among different classes of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment (Singh *et al.*, 2009). Dimethoate [$C_5H_{12}NO_3PS_2$, IUPAC NAME: O,O-Dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate] (commercially available as Rogor 30%EC) is a acaricide and insecticide, used against a wide range of insects, including aphids, thrips, planthoppers and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables. It is also used as a residual wall spray in farm buildings for house flies and has been administered to livestock for control of botflies. The 96h LC_{50} value of Dimethoate is 21.42 mg/l of *Colisa fasciatus* (Singh, 2013), 17.9 mg/l of *Channa punctatus* (Srivastava and Singh, 2001), 2.98mg/l of *Heteropneustes fossilis* (Pandey *et al*., 2009) and 65 mg/l of *Clarias batrachus* (Begum and Vijayaraghavan, 1995). In contrast, however, Dimethoate is reported to be more toxic in carp fishes- 0.007 mg/l 96 h LC_{50} value of *Catla catla* (Kumar and Singh, 2000) and 1.60 mg/l of *Cyprinus carpio* (Singh *et al*., 2009).

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Lambda-cyhalothrin 5%EC [IUPAC NAME: 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl) methyl cyclopropanecarboxylate] $C_{23}H_{19}ClF_3NO_3$ (Type II pyrethroids) used to control a wide spectrum of insect pests, e.g.

Aphids, Colorado beetles, thrips, Lepidoptera larvae, Coleoptera larvae and adults etc. in cereals, hops, ornamentals, potatoes, vegetables, cotton, and other crops provides good control of insect borne plant viruses. Also used for control of insect pests in public health against major insect pests in a wide range of crops and having the ability to prevent a build up of mite populations. The main advantages of pyrethroids are their photo-stability, high effectiveness in low concentrations, easy disintegration and low toxicity to birds and mammals (Maud *et al*., 1998). However, fish are extremely susceptible to these substances. The main reaction involved in the metabolism of pyrethroids including Lambda-cyhalothrin in mice and rats is ester cleavage mainly due to the action of carboxyesterase (FAO /WHO, 2008).

Bioconcentration is possible in aquatic species for Lambda- cyhalothrin, but bioaccumulation is not likely because of rapid depuration (Wauchope *et al*., 1992). A bioconcentration factor of 858 has been reported in fish (4 species unspecified) for Lambda-cyhalothrin, but concentration was confined to non-edible tissues and rapid depuration was observed (Anomouse, 1998). The 96h LC_{50} value of Lambda-cyhalothrin to mosquito fish (*Gambusia affinis*) was estimated as 1.107 µg/l (Utku Güner, 2009). The 96h LC_{50} value of Dichlorvos to L. *rohita* was found to be 16.71ppm (Bhat *et al*., 2012). Laboratory 96h LC_{50} of Lambda-cyhalothrin was estimated to be 0.008 mg/L (C. *gariepinus*) (Ogueji *et al*., 2012). The LC_{50} values determined for endosulfan and fenvalerate to L. *rohita* at 24h were 0.6876 µg/L and 0.4749 µg/L respectively (Dr. K. Suneetha *et al*., 2012). Changes in the serum T_3 and T_4 levels were found in case of Atlantic salmon, *Salma salar* exposed to acid and lime water (Brown *et al*., 1990). Exposure of fish to lethal and sub-lethal concentrations of Dimecron also caused varying changes in serum T_3 and T_4 hormones (Thangavel *et al*., 2005).

In our present study acute toxicity bioassay was carried out in a static renewal system to determine the LC_{50} values for 96hrs. The fish were exposed to different concentration of Dimethoate and Lambda-cyhalothrin and the 96h LC_{50} values were found to be 24.55 µl/L and 0.7 µl/L respectively. Significant (p<0.05) increase in serum T_3 and T_4 levels and decrease in TSH level were found for both of the pesticides.

MATERIALS & METHODS

Experiments were conducted from the month of November to February. Healthy and active *Labeo rohita* (Hamilton) fish (20±1.4gm in weight and 12.7±0.75cm in length) were brought from local market to laboratory carefully in oxygen filled plastic bags to avoid injury and disinfected for five minutes in 0.05% $KMnO_4$ solution. Then they were transferred to glass aquarium containing about 80L dechlorinated tap water and gave sufficient oxygen supply. The fishes were acclimatized to laboratory condition for at least 14 days under 12:12 (light:dark) photoperiod. During acclimatization fishes were fed daily with commercial food pellet daily about 3% of their body weight. Fishes were maintained in static renewal conditions, where water and pesticide were completely replaced every 24h, transferring fishes to freshly prepared toxicant solutions to maintain dissolved oxygen concentration and pesticide concentration constant. Dead fishes were removed immediately to avoid fouling of water. Fishes were starved for 24 hours prior to toxicity testing and are not fed during the period of experiment. Water quality characteristics were measured by using guidelines of APHA, EPA (2005). Chlorine-free tap water having water qualities such as temperature: 26^{o}%±2^{o}C, pH: 7.2±0.4, dissolved oxygen: 7.3±0.2 ppm, total hardness: 233±1.78 ppm. Pilot experiments were run to determine the LC_{50} value for both of the pesticides through 0-100% mortality range test under laboratory condition as described by Finney (1971). Control and five concentrations were taken for each test and 10 fish were introduced in aquarium of 80L capacity. Each experiment was repeated three times to obtain constant results during 96 hours acute toxicity testing. The calculated 96h LC_{50} values were 24.55 µl/L and 0.7 µl/L for Dimethoate 30% EC and Lambda-cyhalothrin 5% EC respectively. The sub-lethal concentrations taken for experiments were 12.25 µl/L (1/2 of the 96h LC_{50} value) ,14.25 µl/L,16.25 µl/L,18.25 µl/L and 20.25 µl/L of Dimethoate and 0.2 µl/L ,0.3 µl/L, 0.4 µl/L,0.5 µl/L and 0.6 µl/L of Lambda-cyhalothrin. At the end of 96h duration fishes of sub-lethal doses were immediately anesthetized with MS222 (Ethyl 3-aminobenzene methanesulfonate salt, Sigma). Blood samples were taken from cardiac puncture and collected in anticoagulant-free centrifuge tubes. Serum was obtained by centrifuging blood at 4000 rpm for 10min. Serum samples were then stored at -80^{o}C until analysis.

The determination of serum level of T_3, T_4, and TSH was done by using ELISA kit (RFCL Limited, India) according to manufacturer's instruction. The O.D values of control and experimental samples were read within 10 min using microplate reader (BIO-RAD) with a 450 nm filter. Serum concentration of T_3, T_4, and TSH were expressed in ng/ml, µg/dl and µIU/ml respectively. The data collected from control and treated groups were represented as Mean± S.E for each group. The data were tested for statistical differences using a One-Way ANOVA followed by Duncan's Multiple Range Test (DMRT) in SPSS 16.0. Statistical differences were determined at p<0.05 levels for all analysis.

RESULTS & DISCUSSION

The calculated 96h LC_{50} values were 24.55 µl/L (Fig.1) and 0.7 µl/L (Fig. 2) for Dimethoate 30% EC and Lambda-cyhalothrin 5% EC respectively. The LC_{50} values were highly significant $p<0.05$ (Table 3 and 5). In this present investigation fish were exposed to sub-lethal concentrations of the two pesticides- 12.25 µl/L (1/2 of the 96h LC_{50} value), 14.25 µl/L, 16.25 µl/L, 18.25 µl/L & 20.25 µl/L of Dimethoate and 0.2 µl/L (approximately 1/3 of the 96h LC_{50} value), 0.3 µl/L, 0.4 µl/L, 0.5 µl/L and 0.6 µl/L of Lambda-cyhalothrin. Fish mortality was increased significantly when the concentration and the time of exposure were increased (Table 1 and 2). No mortality was observed in the control group during the experiment. From the present study it was found that Lambda-cyhalothrin was more toxic to L. *rohita* than Dimethoate. Table 3, 4, 5 and 6 depicted the parameter estimates of the Probit analysis and chi square test for the acute toxicity of Dimethoate and Lambda-cyhalothrin to L.*rohita* . Serum T_3, T_4 and TSH activities of *L. rohita* were measured with respect to control fish during 96 hour. One-Way ANOVA results

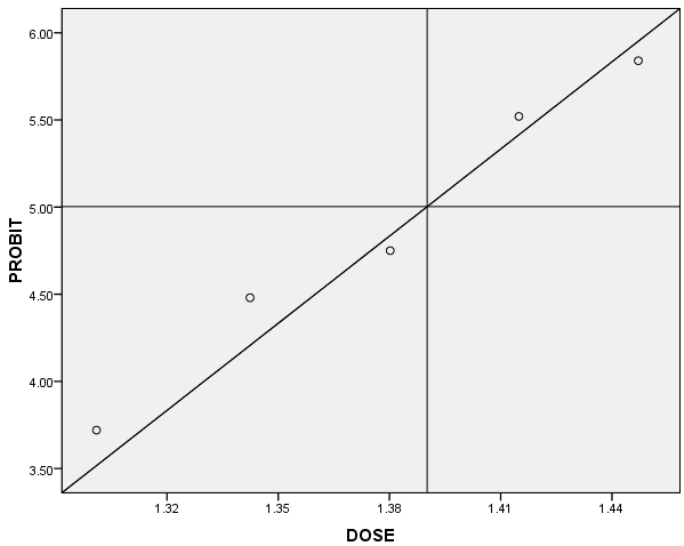

Fig. 1. Probit line graph for determination of 96hr LC_{50} of Dimethoate 30% EC to *Labeo rohita*. Dose= Log_{10} base scale.

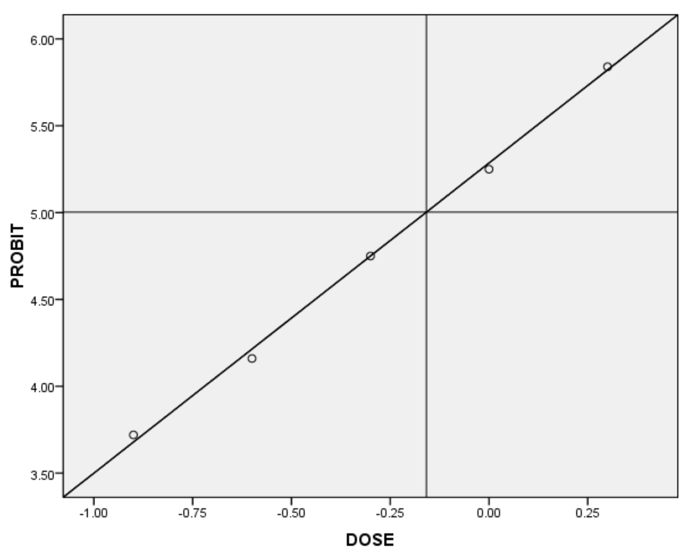

Fig. 2. Probit line graph for determination of 96hr LC_{50} of Lambda- cyhalothrin 5% EC to *Labeo rohita*. Dose= Log_{10} base scale

Table 1. **Mortality table for Dimethoate 30% EC**

CONC.(µl/L)	Log10 CONC.	TOTAL NO.	NO.DEAD	%MORTALITY	PROBIT
CONTROL	-	10	0	0	-
18	1.2552	10	0	0	-
20	1.3010	10	1	10	3.72
22	1.3424	10	3	30	4.48
24	1.3802	10	4	40	4.75
26	1.4149	10	7	70	5.52
28	1.4471	10	8	80	5.84
30	1.4771	10	10	100	-

Table 2. **Mortality table for Lambda-cyhalothrin 5% EC**

CONC.(µl/L)	Log10 CONC.	TOTAL NO.	NO.DEAD	%MORTALITY	PROBIT
CONTROL	-	10	0	0	-
0.0625	-1.2041	10	0	0	-
0.125	-0.9030	10	1	10	3.72
0.25	-0.6020	10	2	20	4.16
0.5	-0.3010	10	4	40	4.75
1	0	10	6	60	5.25
2	0.3010	10	8	80	5.84
4	0.6020	10	10	100	-

Table 3. Parameter Estimates of 96h LC_{50} Probit Analysis for Dimethoate 30% EC

	Parameter					95% Confidence Interval	
		Estimate	Std. Error	Z	Sig.	Lower Bound	Upper Bound
PROBIT[a]	a.conc	17.217	5.901	2.917	.004	5.650	28.783
	Intercept	-23.828	8.456	-2.818	.005	-32.284	-15.372

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.).

Table 4. Chi-Square Tests of 96h LC_{50} Probit Analysis for Dimethoate 30% EC

		Chi-Square	df[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	1.545	4	.819[b]

a. Statistics based on individual cases differ from statistics based on aggregated cases.
b. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

of acute exposure to Dimethoate and Lambda-cyhalothrin indicated significant decrease in serum T_3 (Fig. 3 and 6) and T_4 (Fig. 4 and 7) ($p<0.05$) with increasing dose exposure when compared to control**.** Similar results were reported in teleost fish Channa punctatus exposed to mercuric chloride (Bhattacharya *et al*. 1998). Unlike, T_3 and T_4 significant increase in TSH (Fig. 5 and 8) with increasing dose exposure was found ($p<0.05$). The magnitude of reduction and incretion was comparatively more in Lambda-cyhalothrin. Serum T_3 reduction was more pronounced than T_4. Reduction in body weight of fish was also found with increasing concentration of both of the pesticides.

Now-a-days, most of the research works focussed on effects of EDC (Environmental Disrupting Chemicals) on reproduction, sex steroids (Tyler *et al.*, 1998) and thyroid hormones (Eales and Brown *et al.*, 2005). Suppressed level of thyroid hormones found on salmonoid fishes depicts physiological stress responses (Pickering, 1993; McDonald & Milligan, 1997; Pankhurst & Van Der Kraak, 1997; Shreck et al. 1997). Fishes are known to respond to environmental pollutants by altering the serum hormone levels especially of T_3. Significant reduction in serum T_3 level was found in case of *S.mossambicus* after Dimecron exposure (Thangavel *et al.*, 2005). Various studies have established that environmental contaminants affect plasma thyroid hormone levels and cause thyroid dysfunction in fish (Warning *et al.*, 1997). Both T_3 and T_4 regulate growth, development and metabolism of fish and also involved in many of the biological functions (Griffin 2000). T_4 is the predominantly circulating hormone, though T_3 is metabolically more active and changed according to metabolism. Presence of T_3 in lesser amount may indicate a rapid turnover from T_4 to T_3 before usage (Sterling *et al.*, 1973). A study of Mexican pesticide applicators exposed to ethylenebis (dithiocarbamate) revealed an increase in TSH (Steenland *et al.*, 1997).

Table 5. Parameter Estimates of 96h LC_{50} Probit Analysis for Lambda-cyhalothrin 5% EC

						95% Confidence Interval	
	Parameter	**Estimate**	**Std. Error**	**Z**	**Sig.**	**Lower Bound**	**Upper Bound**
PROBIT[a]	CONC	1.916	.459	4.174	.000	1.016	2.816
	Intercept	.288	.234	1.229	.219	.054	.522

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.).

Table 6. Chi-Square Tests of 96h LC_{50} Probit Analysis for Lambda- cyhalothrin 5% EC

		Chi-Square	**df[a]**	**Sig.**
PROBIT	Pearson Goodness-of-Fit Test	.336	4	.987[b]

a. Statistics based on individual cases differ from statistics based on aggregated cases.

b. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

Table 7. 96h LC_{50} Value for freshwater teleost *L. rohita* after exposure to Dimethoate and Lambda- cyhalothrin

Pesticide	**96h LC_{50} Value(µl/L)**	**Regression Equation**
Dimethoate 30%EC	24.53	Y= -18.1667+16.6667*X
Lambda-cyhalothrin 5%EC	0.7	Y= 5.2857+1.7857*X

After exposure of both of the pesticides, *Labeo rohita* showed altered behaviour. These were found more and prominent in case of Lambda-cyhalothrin than Dimethoate. In low concentration of both pesticides, the responses were of a lesser degree. Control fish behaved normally, fish aggregated in the bottom of aquarium. Irregular, erratic and darting swimming movements, loss of balance, drowning and change in body pigmentation became more apparent with increase in duration of exposure at all test concentrations. Rapid jumping was found in Lambda-cyhalothrin treated fish immediately after pesticide addition. They slowly became lethargic, hyperactive and secreted copious amount of mucus all over their body. With increasing exposure their opercular movements became least and died with their mouth opened. Similar alterations in behaviour of dimethoate exposed fish have been reported earlier in Heteropneustes *fossilis* (Pandey *et al.*, 2009) and (Singh *et al.*, 2009). Saxena *et al.*, (1997) noticed similar abnormal behavioural pattern in *Channa orientalis* during the exposure of Nuvan and Dimecron.

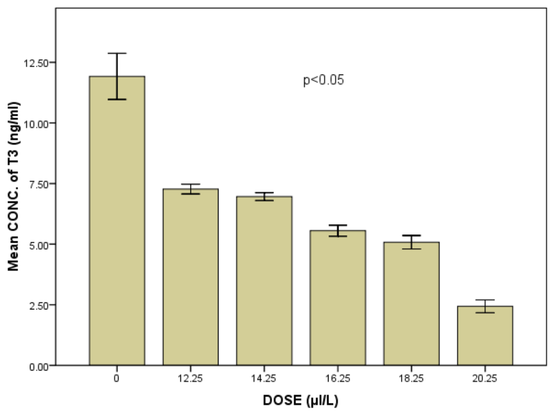

Fig. 3. Estimation of serum T_3 in *L. rohita* exposed to Dimethoate 30% EC for 96 hrs. '0' indicates 'control' fish. Data are represented as Mean± S.E (n=6). Values are significantly different (*p* < 0.05) from each other according to Duncan's multiple range tests. 'F'= 188.69 (*p*=0.000).

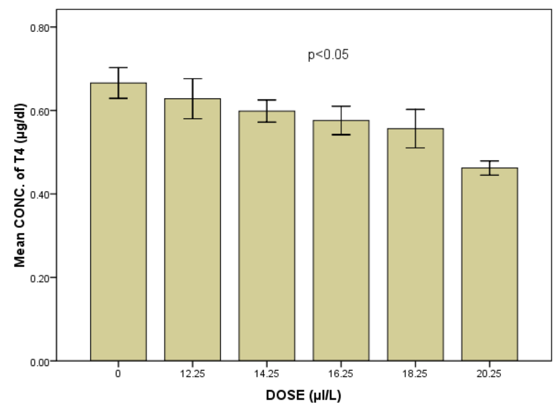

Fig. 4. Estimation of serum T_4 in *L. rohita* exposed to Dimethoate 30% EC for 96 hrs. '0' indicates 'control' fish. Data are represented as Mean± S.E (n=6). Values are significantly different (*p* < 0.05) from each other according to Duncan's multiple range tests. 'F'= 14.458 (*p*=0.000).

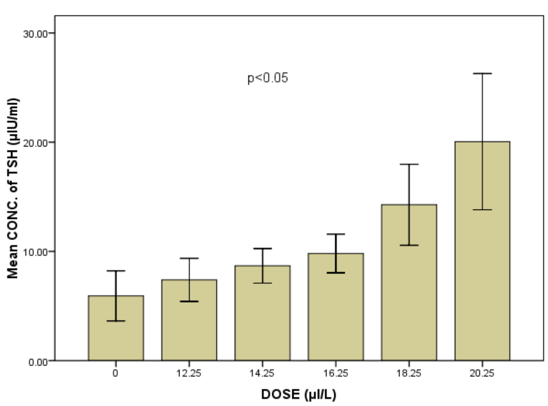

Fig. 5. Estimation of serum TSH in *L. rohita* exposed to Dimethoate 30% EC for 96 hrs. '0' indicates 'control' fish. Data are represented as Mean± S.E (n=6). Values are significantly different (*p* < 0.05) from each other according to Duncan's multiple range tests. 'F'= 9.828 (*p*=0.000).

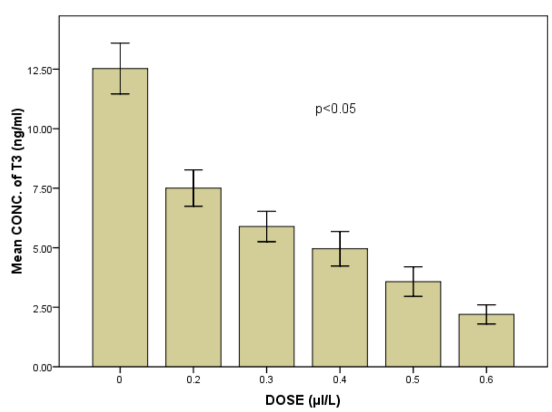

Fig. 6. Estimation of serum T_3 in *L. rohita* exposed to Lambda- cyhalothrin 5% EC for 96 hrs. '0' indicates 'control' fish. Data are represented as Mean± S.E (n=6). Values are significantly different (*p* < 0.05) from each other according to Duncan's multiple range tests. 'F'= 99.259 (*p*=0.000).

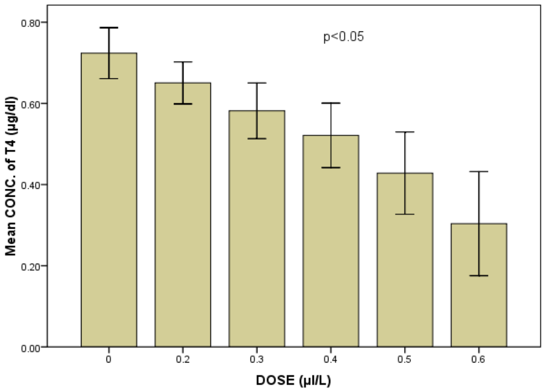

Fig. 7. Estimation of serum T_4 in *L. rohita* exposed to Lambda- cyhalothrin 5% EC for 96 hrs. '0' indicates 'control' fish. Data are represented as Mean± S.E (n=6). Values are significantly different (P < 0.05) from each other according to Duncan's multiple range tests. 'F'= 12.537 (*p*=0.000).

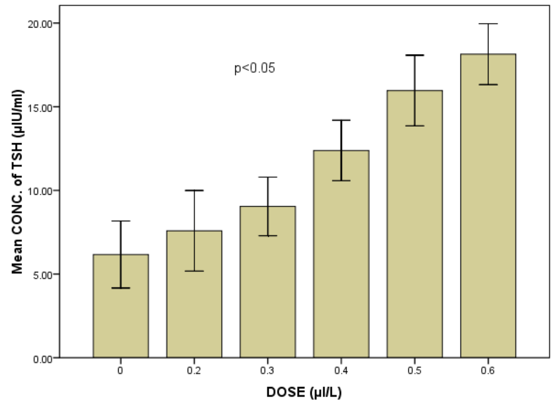

Fig. 8. Estimation of serum TSH in *L. rohita* exposed to Lambda- cyhalothrin 5% EC for 96 hrs. '0' indicates 'control' fish. Data are represented as Mean± S.E (n=6). Values are significantly different (P < 0.05) from each other according to Duncan's multiple range tests. 'F'= 22.998 (*p*=0.000).

Reduction in body weight and sluggish behaviour during exposure with respect to control also indicate this fact. So it may be stated that the weight reduction and sluggish behaviour of fish was to cope up with pesticide exposure and thereby reducing toxic impact. On the other hand causes behind the mild changes in T_4 level is still unclear. The decreased secretion of thyroid hormones could bring physiological alterations in their potential roles in regulating osmoregulation, growth and metabolism (Waring and Brown 1997) which was also supported by the reduction in body weight and sluggishness in this present study. Elevations in serum TSH levels with increasing dose exposure may be due to negative feedback of low amount of circulating T_3 and T_4 at the peripheral level to hypothalamo-pituitary-thyroid (HPT) axis.

CONCLUSIONS

The analysis of data from the present investigation demonstrated that Lambda-cyhalothrin is highly toxic compared to Dimethoate. Both of the pesticides had profound impact on behaviour and thyroid hormone levels of *L. rohita* in sub-lethal concentrations and also had adverse effects on fish physiology. An altered behaviour and thyroid hormone level of treated fish was probably due to impaired metabolism and pesticide induced stress.

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