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ALTERATION IN METABOLIC ENZYME ACTIVITY OF MUSCLE TISSUE IN THE FISH *CYPRINUS CARPIO* (L.) ON CHRONIC EXPOSURE TO SOME PESTICIDES

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Studies were conducted to assess the effects of sub-lethal exposure of pesticides endosulfan, chlorpyrifos and malathion for 40 days on freshwater teleost, *Cyprinus carpio.* Jerky and whirling movements, surfacing, exodus trials, engulfing air, excess mucus secretion and poor response to feed were the commonly observed behavioral changes in the treated fish. A dose dependent reduction in the activity levels of selected dehydrogenase enzymes (glutamate dehyrogenase, succinate dehyrogenase, malate dehyrogenase and pyruvate dehyrogenase) was evident in the muscle tissues of the test animal. A reduction of 30.2 and 70.2 percent in activity levels of succinate dehydrogensae (SDH) was evident in 0.0001 and 0.001 ppm endosulfan treatments, respectively. The combination of pesticides appeared consequently additive in nature. The toxic effects of endosulfan in combination with chlorpyrifos and malathion inhibited the SDH activity to a further low of 71.6 percent. Lactate dehydrogenase activity in the muscle tissue was higher in pesticide-exposed fish in comparison to the control fish.

INTRODUCTION

Metabolic enzymes such as glutamate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, succinate dehydrogenase and pyruvate dehydrogenase are used as clinical biomarkers which can give an estimate of relative damage caused to the physiology of an animal on chronic exposure to pesticides (Tripathi and Verma, 2004). Pesticide absorbance and their accumulation in the tissues further result in the loss of nutritive value in the form of tissue protein dysfunction (Zinada, 2000). Pesticides are introduced in the environment because of their application in agricultural fields, stores or at homes, as well as their discharge as effluents from the pesticide-manufacturing units and by means of accidental spillage. Their ubiquitous and bioaccumulative nature results in their residual persistence in the environment. An estimated 85-90% of the pesticides do not reach even to their target organisms (Repeto and Baliga, 1996). Most of such studies on the chronic effects of pesticides report effects of individual chemicals on fishes. There have been very few studies dealing with combined effects of the pesticides endosulfan (6, 7, 8, 9, 10, 10-Hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin 3-oxide; 1, 4, 5, 6, 7, 7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite), chlorpyrifos (Phosphorothioic acid O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl) ester) and malathion ([(Dimethoxyphosphinot hioyl)thio]butanedioic acid diethyl ester; mercaptosuccinic acid diethyl ester; S-ester with O, O-dimethyl phosphorothioate) on fish physiology. Thus, present investigation aims to determine metabolic enzyme activity in commonly cultured and edible fish species, common carp (*Cyprinus carpio*) on exposure to the mentioned pesticides individually and in combination.

MATERIAL AND METHODS

Healthy specimen of *C. carpio* (about 25 g) procured from the local fish farms at Hisar (Haryana) were disinfected with 0.1% $KMnO_4$ solution and acclimated under laboratory conditions for fifteen days in 1000 liter water tanks, filled with well aerated tap water. Fish were transferred for pesticide treatments (0.0001, 0.0005 and 0.001 ppm of endosulfan, chlorpyrifos and malathion each and 0.0001 ppm of combinations: enosulfan + chlorpyrifos, malathion + chlorpyrifos, endosulfan + malathion and endosulfan + chlorpyrifos + malathion) in aquaria with 40 liter water. Three replicates for each treatment with ten fish of equal size were maintained for 40 days along with control. The fishes were fed with pelleted fish feed @ 3% of their body weight on alternate days. Important physico-chemical characteristics of the water were measured at weekly interval following standard methods (APHA, 1998) and the ranges of different parameters were: water temperature 26-30 °C, pH 7.1-7.5, alkalinity 152-155 mg/l and conductivity 270-272 µs/cm. Fish excreta and left out food were siphoned out on alternate days and water was changed maintaining the apt treatment dose level. The treated fishes were examined regularly for certain behavioral changes over the control. After 40 days, three fish from each replicate were anesthetized with MS-222 (taken randomly) and sacrificed to dissect out the muscle tissues. Activity of glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), pyruvate dehydrogenase (PDH) and malate dehydrogenase (MDH) were estimated by following the methods of Nachlas *et al.* (1960).

RESULTS AND DISCUSSION

Exposure to various sub-lethal concentrations of the pesticides resulted in abnormal behavioral changes in *C. carpio*. The treated fishes exhibited jerky and whirling swimming movements and were seen surfacing and engulfing air. It was evident that endosulfan treated fish were slow to reach feed unlike the active control group fishes and experienced a profuse mucus secretion. The observed behavioral anomalies were in agreement with many of the earlier reports on toxicity to various teleosts exposed to different toxicants (Joshi and Rege, 1980; Naqvi and Hawkins, 1988; Haya, 1989; Agarwal, 1991; Jensson and Toledo, 1993; Saligo *et al.*, 1996; Banaee *et al.*, 2008). David (2004) exposed *C. carpio* to various sub-lethal concentrations of endosulfan and observed that control group fishes were alert to slightest disturbances, whereas fish exposed to endosulfan behaved abnormal. Irregular, erratic and darting movement, imbalanced swimming activity, surfacing and gulping of air were also reported. Jenkins *et al.* (2003) also recorded behavioral changes like hyper-excitability, fast swimming activity, increased frequency of coughing and yawning and profuse secretion of mucus in *C. carpio* exposed to acute (10 ppb) and sub-acute (5 ppb) concentrations of endosulfan. The erratic whirling movements may be due to obstructed function of neurotransmitter (David *et al.*, 2002) while surfacing to engulf air might be due to the hypoxic condition (Devi, 1991). The process of excessive mucus secretion is a defense mechanism for protecting fish from lethal toxic contacts (Mukhopadhyay 1983a; Mukhopadhyay and Konar, 1984). Mucus acts as an efficient barrier to prevent entry of toxicant into the fish body (Bucher and Hofer, 1993).

Activity levels of GDH, SDH, PDH and MDH demonstrated dose dependent inhibition in *C. carpio* muscles except LDH activity that appeared enhanced (Table 1). Results illustrate 49.71, 70.2, 58.33 and 69.2 percent inhibited enzyme activity on 0.001-ppm endosulfan exposure for GDH, SDH, PDH and MDH, respectively. LDH activity exhibited an increase of 12.8, 30.81 and 51.1 percent in 0.0001, 0.005 and 0.001 ppm endosulfan treatments. The toxic effects of different combinations of pesticides were severe than the individual pesticide treatments (Table 2). Endosulfan in combination with chlorpyrifos and malathion exhibited a reduction of 63.2, 71.6, 60.2 and 55.6% in GDH, SDH, PDH and MDH activities, respectively and 76.4% stimulation in LDH activity in *C. carpio* muscles. Malathion appeared to be the most toxic pesticide. Results of present study are in accordance with studies conducted by Rao *et al.* (1984) and Rao (2006), which explained that due to decreased respiratory rate, the activity levels of SDH decreases in muscle tissues of the fish. This infers depressed tissue oxidative metabolism under pesticide exposure. Verma *et al.* (1982) also observed inhibition of SDH and PDH activity and stimulation of LDH activity in the other body tissues like brain, liver, kidney and gills of *Notopterus notopterus* when exposed to (Na-PCP) for thirty days.

Elevation of LDH activity was also reported in the skeletal muscles of *C. carpio* on exposure to copper sulphate, paraquat and methidation (Asztalos and Nemcsok, 1985) and in liver of juvenile pink snapper on exposure to Na-PCP (Tugiyono, 2002). Increased LDH activity in muscle tissues indicate metabolic changes in chemically stressed fish, considered as a good indicator of anaerobic activity in a tissue (Dickson *et al.*, 1993). Fox and Rao (1977) reported that MDH and SDH enzymes involved in phosphorylation were inhibited significantly in hepatopancreas of blue crab (*Callinectes sapidus*) when exposed to sodium pentachlorphenate and 2,4-dintrophenol. The increase in LDH activity may reflect an increased dependence on anaerobic carbohydrate metabolism by the muscles of the fish on exposure to pesticides. Thus, sub-lethal levels of endosulfan, chlorpyrifos and malathion affect the efficiency of tissue metabolites and disturb their physiology.

Table 1. Alterations in activity levels of selected dehydrogenase (µg of formazan formed/mg protein/h) in muscles of *C. carpio* exposed to different pesticides (concentration in ppm)

Enzyme	Control	Endosulfan			Chlorpyrifos			Malathion			CD at 5%
		0.0001	0.0005	0.001	0.0001	0.0005	0.001	0.0001	0.0005	0.001	
GDH	0.162 ±0.014	0.133 ±0.009 (18.1)	0.109 ±0.012 (32.6)	0.081 ±0.010 (49.71)	0.120 ±0.009 (25.8)	0.101 ±0.011 (37.43)	0.076 ±0.012 (53.03)	0.113 ±0.014 (30.10)	0.090 ±0.018 (44.2)	0.063 ±0.015 (61.2)	0.007
LDH	0.442 ±0.011	0.499 ±0.016 +(12.8)	0.578 ±0.010 +(30.81)	0.674 ±0.014 +(51.1)	0.517 ±0.006 +(16.9)	0.606 ±0.011 +(37.2)	0.783 ±0.014 +(56.2)	0.546 ±0.012 +(23.5)	0.638 ±0.014 +(44.41)	0.733 ±0.018 +(66.13)	0.007
SDH	0.686 ±0.011	0.479 ±0.006 (30.2)	0.352 ±0.010 (48.72)	0.204 ±0.018 (70.2)	0.561 ±0.006 (18.2)	0.434 ±0.015 (36.43)	0.382 ±0.009 (54.19)	0.506 ±0.011 (27.11)	0.415 ±0.011 (39.5)	0.266 ±0.008 (61.16)	0.005
PDH	0.313 ±0.012	0.225 ±0.014 (28.1)	0.192 ±0.015 (38.81)	0.130 ±0.008 (58.33)	0.277 ±0.013 (11.66)	0.218 ±0.018 (30.3)	0.175 ±0.007 (44.16)	0.244 ±0.011 (22.15)	0.202 ±0.007 (35.61)	0.166 ±0.018 (47.08)	0.007
MDH	0.382 ±0.016	0.282 ±0.016 (26.23)	0.233 ±0.012 (39.01)	0.118 ±0.009 (69.2)	0.327 ±0.011 (14.4)	0.290 ±0.017 (24.2)	0.201 ±0.013 (47.4)	0.312 ±0.011 (18.2)	0.271 ±0.021 (29.15)	0.179 ±0.007 (53.18)	0.005

Values are expressed as mean± S.E. of 6 observations; Values given in parenthesis are percent reduction over control + : indicates percent increase over control

Table 2. Alterations in activity levels of selected dehydrogenases (µg of formazan formed/mg of protein/h) in muscles of *C. carpio* exposed to different combinations of pesticides (each 0.0001 ppm) for 40 days

Treatment	GDH	LDH	SDH	PDH	MDH
Control	0.186±0.018	0.396±0.014	0.633±0.015	0.292±0.011	0.414±0.010
Endosulfan + Chlorpyrifos	0.144±0.010 (22.4)	0.509±0.013 +(28.7)	0.442±0.014 (30.2)	0.185±0.016 (36.5)	0.270±0.017 (34.7)
Chlorpyrifos + Malation	0.076±0.013 (36.7)	0.634±0.009 +(60.08)	0.515±0.011 (18.6)	0.224±0.013 (23.16)	0.336±0.010 (18.8)
Malathion + Endosulfan	0.095±0.011 (49.1)	0.570±0.013 +(44.0)	0.314±0.007 (50.3)	0.150±0.011 (48.6)	0.222±0.012 (46.35)
Endosulfan + Chlorpyrifos + Malathion	0.068±0.008 (63.2)	0.699±0.011 +(76.4)	0.180±0.013 (71.6)	0.116±0.008 (60.2)	0.184±0.014 (55.6)
CD at 5%	0.027	0.025	0.027	0.026	0.026

Values are expressed as mean±S.E. of 6 observation; alues given in parenthesis are per cent reduction over control + indicates percent increase over control

Unlike present studies Bhaskar and Govindappa (1986) witnessed elevation in SDH and MDH activities along with depletion in LDH activity in *Tilapia mossambica* during acclimation to acid and alkaline medium and suggested that from decreased LDH activity, inhibited rate of mobilization of lactic acid from glycotytic pathway may occur.

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