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Effect of Cypermethrin on Lactate and Succinic Dehydrogenase and Cytochrome Oxidase of Snail and Fish

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Organic pesticides though extensively used for the control of insect and snail pests are a serious environmental hazard. Synthetic pyrethroids, which came after organochlorines, carbamates and organophosphates, became popular not only because of their effecticacy but also due to their relatively faster biodegradability and low mammalian toxicity (Khan 1983). Singh and Agarwal (1986 and 1991); Singh and Agarwal (1990) reported that the synthetic pyrethroids are potent molluscicides as their toxicity to the snails Lymnaea acuminata and Indoplanorbis exustus is many times higher than carbamate and organophosphorus compounds.

We are interested in the toxic properties of pyrethroids from the point of view of using these as agents for the control of undesirable aquatic snails, provided these were not hazardous to fishes living in the same waters. Singh and Agarwal (1990) observed that the pyrethroids, cypermethrin, permethrin and fenvalerate though fatal to snails did not kill the fish Channa striatus at doses which needed to be the used against the snails Lymnaea acuminata and Indoplanorbis exustus. Although it is generally accepted that the primary target of pyrethroids is the nervous system (Wilkinson, 1976), the effect of these compound on the enzyme systems of snails and fishes have not been studied. In the present study the effect of cypermethrin ((S,R-Cyano-3-phenoxybenzyl (1R, 1S, Cis-trans)-2, 2-Dimethyl-3-(2,2-Dichlorovinyl) Cyclopropane Carboxylate) was investigated on the activity of lactic dehydrogenase (LDH), succinic dehydrogenase (SDH) and Cytochrome oxidase (Cytooxidase) in the foot and hepatopancreas of the snail, Lymnaea acuminata, Lamarck (Lymnaeidae) and the liver and muscle tissue of the Channa striatus (Bloch) (Channidae Ophicephalidae). The former is the vector of the liver flukes, Fasciola hepatica and Fasciola gigantica which cause endemic fascioliasis in cattle and livestock in the wet regions of Northern India, (Singh and Agarwal, 1981) and the latter shares the habitat of these snails.

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MATERIALS AND METHODS

Snails and fish were maintained and treated with cypermethrin, according to the method of Singh and Agarwal (1986). Adult L. acuminata (2.6 ± 0.3 cm in length) and C. striatus (10.5 ± 0.9 cm in length) were kept in glass aquaria containing 3 l dechlorinated tap water for snails and 6 l for fishes. Each aquarium contained either 30 snails or 10 fishes.

Snails and fish were treated with 40% or 80% of the LC50 of cypermethrin for 24 h. LC50 doses were determined during earlier studies carried out by Singh and Agarwal (The 24 h LC50 for cypermethrin for L. acuminata was 0.73 mg/l (Singh and Agarwal, 1986) and for C. striatus 2.3 mg/l (Singh and Agarwal, 1990). After 24 h of treatment the test animals were removed from the aquaria and washed with water. The hepatopancreas and foot of L. acuminata and liver and muscle of C. striatus were excised and used as enzyme source. Controls were kept under identical condition without any treatment.

Lactic dehydrogenase activity was measured according to Anon (1984). Fifty milligrams of foot and hepatopancreatic tissues of L. acuminata and 25 mg of liver and muscle tissues of C. striatus were homogenised in 1 ml of 0.1 M phosphate buffer, pH 7.5 for 5 min in an ice bath and centrifuged at 10,000 g for 30 min at 4°C. Supernatants were used as enzyme source. An incubation mixture containing 0.01 ml of supernatant, 0.038 m M sodiumpyruvate substrate in 0.5 ml phosphate buffer and 0.01 g NADH2 were incubated at 37°C for 45 min. After this, 0.5 ml of 2-4-dinitrophenyl hydrazine solution was added and the mixture was kept at room temperature. After 20 min, 5.0 ml of 0.4 M NaOH was mixed and left for 30 min at room temperature; optical density was measured at 540 nm and converted to the LDH units by means of a specially prepared standard curve. Enzyme activity has been expressed as nano moles of pyruvate reduced/min/mg protein.

Succinic dehydrogenase activity was measured by the method of Arrigoni and Singer (1962). 50 milligrams of foot and hepatopancreatic tissue of L. acuminata and 25 mg of liver and muscle tissue of C. striatus were homogenized in 1 ml of 0.5 M potassium phosphate buffer (pH 7.6) for 5 min in an ice bath and centrifuged at 10,000 g for 30 min at 4°C. Supernatants were preincubated with 50 micromoles succinate at 37°C for 7 min. The preincubated sample was then kept on ice and 0.05 ml aliquots from this were used for enzyme assay.

A 2.9 ml cocktail containing 100 micromoles of phosphate buffer (pH 7.6), 300 µM of KCN, 0.80 µM of Cacl2, 0.04 µM of DCIP (2,6-dichlorophenol indophenol), 50 µM of succinate, 0.05 ml of 2% PMS (phenazine methosulphate) and 0.05 ml of preincubated enzyme source was prepared. The decrease in absorbance at 600 nm was monitored for 3 min. Enzyme activity has been

expressed as micromoles dye reduced/min/mg protein.

Activity of cytochrome oxidase was measured according to Copperstein and Lazarow (1951). Hundred milligrams of foot and hepatopancreatic tissue of L. acuminata and 50 mg of muscle and liver tissue of C. striatus were homogenized in 1.0 ml of 0.33 M phosphate buffer, pH 7.4 for 5 min in an ice bath and centrifuged at 10,000 g for 30 min at 4°C. Supernatants were used as enzyme source. Enzyme activity at 25°C was measured in a 10 mm path length cuvette. 3.0 ml of reduced cytochrome C solution (1.7 x 10-5mol/l) was taken into cuvette and 0.02 ml of tissue homogenate was added. Absorption at 550 nm was monitored for 30 min. A few milligrams of pottassium ferricyanide was added (in order to completely oxidise the activity of cytochrome C) and the extinction was determined. Change in absorption was converted to arbitrary units (Anon,1984). Enzyme activity has been expressed in arbitrary units/min/mg protein.

Protein estimation was done by the method of Lowry et al.(1951).

Each experiment was replicated at least with six different animals and the values have been expressed as mean ± SE of six replicates. Student's 't' test and analysis of variance were applied to locate significant changes (Sokal and Rohlf,1973).

RESULTS AND DISCUSSION

Lactic dehydrogenase catalyzes the last step in glycolysis,as it reduces pyruvate to lactate. Lactic dehydrogenase activity in the control snails was 332 µmoles of pyruvate reduced/min/mg protein in foot and 357 µmoles of pyruvate reduced/min/mg protein in hepatopancreatic tissue (Table 1). Exposure to 40% LC50 of cypermethrin for 24 h reduced the LDH activity in foot and hepatopancreas, to 74% and 64% of controls respectively (Table1); exposure to 80% LC50 for the same period caused 54% and 60% reduction in the activity of the enzyme in foot and hepatopancreas, respectively (Fig. 1).

In case of untreated fishes LDH activity was 435 µmoles of pyruvate reduced/min/mg protein in muscle and 534 µm of pyruvate reduced/min/mg protein in liver tissue. Exposure to cypermethrin (40% and 80% LC50) for 24h caused significant reduction in LDH activity (Table 1, Fig. 1). Exposure of fishes of 40% LC50 for 24 h reduced the LDH activity in both muscle and liver to 90% of control values (Table 1). Exposure to 80% LC50 for the same period reduced the activity of this enzyme to 85% of controls in muscle and 76% in liver (Fig. 1).

Analysis of variance ($P < 0.05$) demonstrated that inhibition of LDH activity was dose dependent both in snails and fishes.

Succinic dehydrogenase is the active regulatory enzyme of the tricarboxylic acid cycle. In case of untreated control snails,

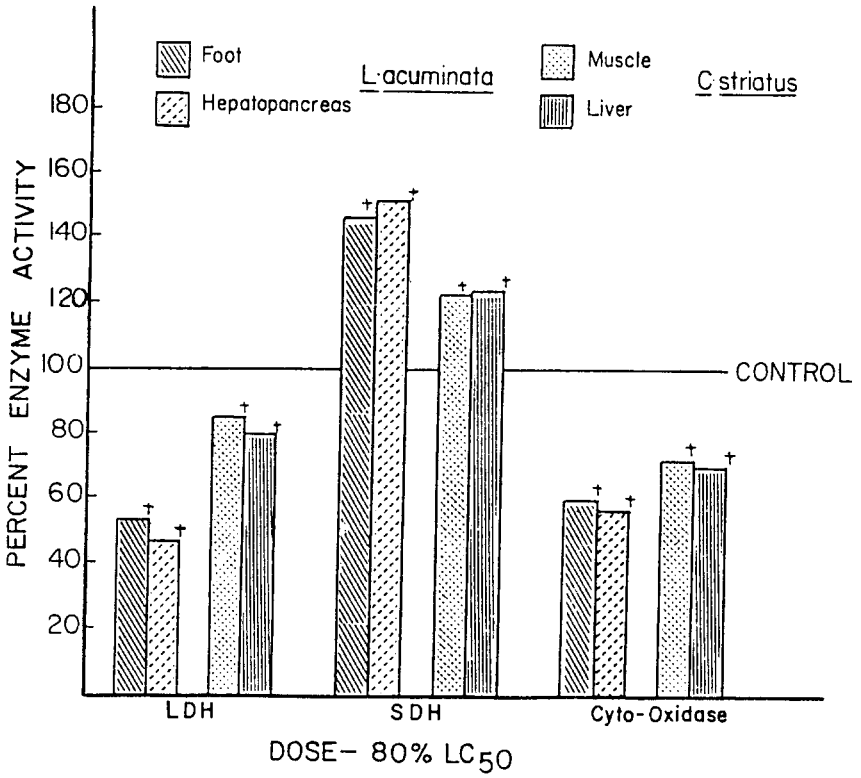

Figure 1. Figure showing percent change in the lactic dehydrogenase, succinic dehydrogenase and cytochrome oxidase activity in foot and hepatopancreatic tissue of Lymnaea acuminata and muscle and liver tissues of Channa Striatus exposed to 80% LC50 of cypermethrin for 24h.

Data are means for six replicates with control taken as 100%.

†, Significantly ($P < 0.05$) different between controls and treated groups, when studnet's 't' test was applied.

succinic dehydrogenase activity was 38 μ moles of dye reduced/ min/mg protein in foot and 41 μ moles of dye reduced/min/mg protein in hepatopancreatic tissue. Exposure to 40% LC50 of cypermethrin for 24 h enhanced the SDH activity in foot and hepatopancreas, to 127% and 129% of control respectively (Table1). Exposure to 80% LC50 for the same period enhanced it to 152% and 154% of controls, respectively (Fig. 1).

Succinic dehydrogenase activity in untreated C. striatus was 50 μ moles of dye reduced/min/mg protein in muscle and 55 μ moles of dye reduced/min/mg protein in liver tissue. Exposure to

Table 1. *In vivo* changes in the activity of lactic dehydrogenase, succinic dehydrogenase and cytochrome oxidase in the tissues of *Lymnaea acuminata* and *Channa striatus* following exposure to sublethal doses of cypermethrin for 24 h.

Tissue	LDH		SDH		Cyto-oxidase	
	Control	40% LC50	Control	40% LC50	Control	40% LC50
L. acuminata						
Foot	331.6±0.91 (100)	248.0±1.22* (74)	38.20±0.16 (100)	48.64±0.26* (127)	12.70±0.25 (100)	7.30±0.16* (57)
Hepato-Pancreas	357.1±0.67 (100)	231.3±0.41* (64)	40.75±0.24 (100)	62.96±0.13* (129)	14.38±0.22 (100)	6.75±0.11* (46)
C. striatus						
Muscle	434.6±1.57 (100)	395.3±1.56* (90)	50.31±0.17 (100)	57.61±0.16* (114)	25.16±0.13 (100)	21.23±0.05* (84)
Liver	533.7±1.70 (100)	480.8±2.98* (90)	55.27±0.19 (100)	65.46±0.27* (118)	28.04±0.10 (100)	22.56±0.19* (80)

Values are mean ± SE of six replicates.
Doses (w/v) have been expressed as final concentration in aquarium water.
Values in parenthesis indicate per cent enzyme activity with control taken as 100%.
*, Significant ($P < 0.05$) when student's 't' test was applied between treated and control groups.
LDH - μ moles of pyruvate reduced/min/mg/protein.
SDH - μ moles of dye reduced/min/mg protein.
Cyto-oxidase - arbitrry unit/min/mg protein.

cypermethrin (40% and 80% LC50) for 24 h caused significant enhancement in SDH activity (Table 1, Fig. 1). Exposure of fishes to 40% LC50 for 24 h enhanced the SDH activity in muscle and liver to 114% and 118% of controls respectively (Table 1) while exposure to 80% LC50 for the same period enhanced the activity of this enzyme to 126% and 130% of controls respectively (Fig. 1).

Change in SDH activity both in fish and snail depended on the dose of Cypermethrin used.

In case of untreated snails cytochrome oxidase activity was 13 units/min/mg protein in foot and 14 units/min/mg protein in hepatopancreatic tissue. Exposure to 40% LC50 of cypermethrin for 24 h reduced the cyto-oxidase activity in foot and hepatopancreas, to 57% and 46% while exposure to 80% LC50 brought the activity down to 42% and 33% controls respectively (Table 1, Fig. 1).

Cytochrome oxidase activity in untreated C. striatus was 25 Units/min/mg protein in muscle and 24 units/min/mg protein in the liver tissue. Treatment with 40% and 80% LC50 of cypermethrin for 24 h reduced the cyto-oxidase activity in muscle and liver to 84% and 80% at the lesser dose, and 72% and 68% of controls respectively at the higher dose (Table 1, Fig. 1).

Analysis of variance ($P < 0.05$) showed that inhibition of cyto-oxidase activity both in case of snail and fish was dose dependent.

It is evident from the date given above that the synthetic pyrethroid, cypermethrin, reduces the activities of lactic dehydrogenase and cytochrome oxidase and enhance the activity succinic dehydrogenase in the foot and hepatopancreatic tissue of the snail L. acuminata and muscle and liver of the fish C. striatus. It thus affects both the anaerobic and aerobic metabolism of the treated animals. The alternations in the activity of LDH, SDH and cyto-oxidase were greater in the hepatopancreatic and liver tissues as compared to the foot and muscle tissues of the snails and fish respectively; it seems that the pesticide is taken up by the hepatopancreas/liver tissue for detoxification.

Narahashi(1983) has stated that there are two types of pyrethroids: Type I are those pyrethroids which do not have an alpha cyano group. These cause repetitive discharges, increase in depolarization after potential and slow sodium current during the polarization in the nerve cells. Type II pyrethroids, like cypermethrin, deltamethrin and fenvalerate which possess an alpha cyano group, cause membrance depolarization without repetitive discharges, no increase in depolarization after potential and a long tail current. This difference between type I and Type II are thought to be only quantitative by Narahashi (1983) with no difference at the qualitative level. Had this been the case, and if pyrethroids had been changing the nerve memberane

permeability alone then pyrethroids possessing a cyano group would have been less toxic than the type I pyrethroids. However, our studies show the toxicity of cyano group containing pyrethroids, viz. cypermethrin and deltmethrin is higher than type I pyrethroids (Singh and Agarwal, 1990; Sahay et al., 1991).

Pyrethroids reportedly change the nerve membrane permeability of Na+. While such changes are said to be totally independent of metabolic energy. The Na+, K+ pump cannot function without using the ATP bound energy. It is likely pyrethroids which possess a cyano group affect the electron transport chain and thereby slow down the cation exchange pump. This might also be contributing to the changes in the shape of the action potentials by type II pyrethroids.

We do not fully understand the reason for the increase in the activity of SDH after exposure to cypermethrin. Gupta and Kapoor (1975) also reported, an increase in the activity of SDH in malathion exposed rats. It appears that the stress produced by an adverse effect on aerobic and anaerobic metabolism causes the SDH activity to rise. The inhibition of cytochrome oxidase by cypermethrin may be due to the presence of the cyano group, which is also an inhibitor of cyto-oxidase, (Wilkinson, 1976; Lehninger, 1984).

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