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Effects of endosulfan and quinolphos on carbohydrate metabolism of freshwater fish, *Cirrhinus mrigala* (Ham)

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Abstract
The freshwater fish, *Cirrhinus mrigala* was exposed to two pesticides i.e., endosulfan and quinolphos. The fishes were exposed to lethal and sublethal concentrations for 21 days and the changes in the carbohydrate metabolism of vital organs such as brain, gill, kidney, liver and muscle were studied.It was observed that the liver is vital organ of carbohydrate metabolism and were drastically affected by both the pesticides.

Keywords: *Cirrhinus mrigala*, endosulfan, quinolphos, carbohydrate metabolism

Introduction

The increasing human population and consequent need for more food, health services and improved living standards have boosted the development of chemical industry manufacturing pesticides, drugs, cosmetics and other chemicals for human use. These chemicals neither degraded biologically nor assimilated in the living system as they are not compatible with the living system. Over the past four decades, pesticides have become an indispensable part of world agriculture since these are applied to protect standing crops, stored grain and insect pest (Singh *et al.*, 1998) [19].

Pesticides have no doubt been a boon to the human civilization in sustaining agriculture revolution but at the same time bared its ravaging face on humanity pushing to a point of almost no return (Tilak *et al.*, 2001 [20]. Modern Agriculture practices, despite their remarkable contribution to the enhancement of crop production, have at the same time, also widely polluted the aquatic environment, (Pandey *et al.*, 2000) [15]. It has been estimated that the use of pesticides has increased the crop's yield three fold.

Indiscriminate uses of pesticides are very common in India as well as in several Asian, American and African countries. Pesticides are used by farmers for spraying directly into the crops in the fields during storage etc. These chemicals are capable of killing pests and insects but on the other hand, they are highly toxic to animals as well as human beings (Ojha and Nortan, 1992) [14].

As considerable amount of pesticides and their by products enter the fish body, through the food chain, where they are distributed and metabolised depending upon the detoxifying ability of the fish, and elicit some responses in fish which depends on the nature and concentration of pesticides as well as on the duration of retainment of these pesticides in fish body, and the ability of fish to metabolise the pesticides observed (Kaur *et.al.*, 1997 [11]; Kumar, 1999) [10].

The route of pesticide transport to different aquatic eco system has been well documented. It is well known that various pollutants, including pesticides will be carried to the freshwater by means of different processes like surface run-off, disposal through wastes, spray drift and atmospheric fall out, rain etc (Menzer *et.al.*, 1994) [12]. Natural water is the ultimate recipients of much of the chemical wastes. As far as aquatic ecosystem is concerned, it has been recognized as an important global problem, and thus, stimulated the eco-toxicologists to determine the lethal and sub-lethal impact on the fish and other aquatic organisms (Ninawae, 2000) [13].

Endosulfan ($C_9H_9Cl_6O_3S$) is a chlorinated hydrocarbon insecticide used to control pests in more than 60 countries around the world in agriculture. Endosulfan is one of the most toxic pesticides on the market today, responsible for many fatal pesticide poisoning incidents around the world(Shafiq-ur-Rehman,2006) [17].Endosulfan is an extremely toxic organochlorine pesticide to aquatic organisms which might be hampering fish health through impairment of metabolism sometimes leading to death(Kamalaveni *et al*;2001) [8]. Fishes are extremely sensitive to endosulfan and mortality has been reported a number of times as a result of endosulfan leakage into rivers.

Quinolphos is a broad spectrum insecticide which is very highly toxic to freshwater fishes. It is moderately persistent, but relatively immobile in the environment. It has the potential to bio accumulate in the tissues (Casida *et al*; 1998) [3]. It is a synthetic organophosphate, non-systemic, broad spectrum insecticide which the fish absorbs it through gills, skin or gastrointestinal tract. Then, the compound is rapidly metabolized and extracted, and may be bio-concentrated in various tissues of fish(Sambasiva Rao,1999) [16]. The accumulation of quinolphos disrupts aquatic organisms and then it finds its way into humans, birds and other living organisms through food chain.

Human beings are also sensitive to pesticide toxicity as aquatic organisms, so biomagnifications may play an important role to create toxicity in other organisms. Hence the present investigation is aimed to evaluate the comparative toxicity risk of two pesticides,endosulfan and quinolphos to the test species and to determine the sub-lethal effect of quinalphos on the carbohydrate metabolism of the fresh water fish, *Cirrhinus mrigala*.

Material and methods

Experimental set up

The freshwater fish *Cirrhinus mrigala* (Hamilton) is an edible and commercially valuable fish widely cultured in India. Live fish of size 6-7 ±1cm and 6-8 g weight were brought from a local fish farm and acclimatized at 28 ± 2 ^{0}C in the laboratory for one week. The stock solutions for Endosulfan 35% Emulsifiable Concentrate (EC) and quinolphos 20% Emulsifiable Concentrate (EC) were prepared in 95% acetone to yield a concentration of 100mg/100ml which were further diluted with distilled water to get a working solution. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a capacity of ten liters. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from ten percent to ninety percent for 24 hrs in static tests. The concentration that produced fifty percent mortality in test species noted. LC_{50} values were calculated by Finney's Probit analysis (1971).

Analysis of water quality parameters

The physico-chemical parameters of water such as temperature, turbidity, pH, total hardness, total suspended solids, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), sulphates and Dissolved Oxygen were estimated during the course of the study according to the standard protocols APHA.

Estimation of Carbohydrates

The total carbohydrates was estimated by the method of Carool *et al.* (1956). O.5% homogenate of tissues were prepared in 10% trichloro acetic acid (TCA). The homogenates were centrifuged at 1000 rpm for 15 minutes. To 0.2 ml of the supernatant, 5 ml of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and the colour was read at 620 mm in a spectrophotometer, using blank, which consists TCA and anthrone reagent in the same proportion. The values were expressed as mg/100 mg wet tissues.

Result and Discussion

Before the start of experiment, the water used for maintenance of fish was analyzed for its quality. Thus, Table 1 gives the values of different water quality parameters which are in normal range indicating good water quality for experimentation.

The change in total carbohydrates in the muscle, liver and intestine of *C. mrigala* was studied with response to the chronic toxicity of quinolphos. The total carbohydrate showed drastic reduction when the fish was reared in different sub lethal concentrations of the quinolphos. For instance the carbohydrate content of the liver of *C.mrigala* reared in endosulphan was 37.84 at lowest concentration 0.0155 and 42.66 in high concentration of 0.0620. (Table 1).Meanwhile the carbohydrate content of the liver of *C.mrigala* reared in quinolphos was 33.84 ppm at lowest concentration 0.0155 ppm and 41.01 ppm in high concentration of 0.0620(Table-2). Likewise, the muscle carbohydrates content of *C. mrigala* treated with endosulphan was 12.33 at 0.0155 ppm and 13.55 at 0.0620 ppm endosulphan when compared to those fishes reared in control medium. Similarly, the muscle carbohydrate of *C.mrigala* treated with quinolphos was 8.21 at 0.0155 ppm and 12.01 at 0.0620 ppm. The carbohydrate content in the gills was 7.61 at 0.0155 ppm and 9.86 at 0.0620 ppm in endosulphan reared fishes. Similarly, the carbohydrate content in the gills was 7.61 at 0.0155 ppm and 8.63 at 0.0620 pp, quinolphos reared fishes.

Sub-lethal concentration of quniolphos caused a significant decrease in the carbohydrates content in all the tissues like liver, muscle and intestine of *C.mrigala*. Similar response was reported by Govindan *et al.* (1994) [7] in *Gambusia affinis* and Amali (1995) [1] in *L. rohita*.

Generally, during stress conditions fishes require extra energy to restore the normal physiological and biochemical changes for better survival. This excess energy needed may be obtained by oxidising the body reserves such as the carbohydrate, protein and lipids (Ganesan *et al.*, 1989) [4]. Further, among these organic constituents, the carbohydrates is the first to come to resume fish from the persisting stress caused by an xenobiotic by providing energy (Kasturi and Chandran, 1997) [9].

The reduction of the carbohydrates content in the tested tissues of *C.mrigala* is attributed to the utilization of reserve carbohydrate, possibly through anaerobic glycolysis to meet the extra energy requirement at the hypoxia caused by the pesticides in the media. A similar observation was also made in *C. carpio* (Gluth and Hanke, 1985) [5]. Exposure to pesticides might interfere with any one of the carbohydrate metabolic pathways and reduce the total carbohydrate content in the tissues of fishes.

The liver and muscle are the two active sites where storage and metabolism of glycogen take place. The changes in the carbohydrate content in the liver and the muscle of *C.mrigala* observed in the present study elucidate the inverse relationship between the concentration of pesticides and the fall in the content of carbohydrate. Further, the liver plays an important role in the metabolism of carbohydrates in that it is also the first organ to deal with any foreign molecule. The observed carbohydrate loss in the liver and muscle may be attributed to the toxicogenic effects of quinolphos on the tissue energy reserve.

Gross effects on metabolism of changes in nutritional state or in the endocrine balance of an animal may be studied by observing changes in the concentration of blood metabolites. The changes that occur in metabolic balance of the intact animal are due to shifts in the pattern of metabolism in individual tissues which are usually associated with changes in availability of metabolites or change in the activity of key enzymes. Basis and Tazeem (1995) [2] reported that the fish experiencing insecticide toxicity enhanced the glycogenolytic activities several times leading to a fall in carbohydrate level in tissues. Depletion in the levels of total carbohydrates in the fish *Cirrhinus mrigala*, exposed to quinolphos was because of their utilization to overcome the pesticide stress (Amali *et al.*, 1995) [1]. Gopalarao (2006) [6] had reported that reduction in glycogen level in the muscle and other tissues of *C. punctatus* exposed to cypermethrin was due to the differential prolonged nuclear activity. In the present study in *C. mrigala*, the carbohydrate metabolism was found affected due to pesticide stress and this has resulted in the reduction of total carbohydrate level in the test tissues. Thus the results indicated that the liver is vital organ of carbohydrate metabolism and were drastically affected by both the pesticides.

Table 1: Estimation of physico-chemical parameters of water used for experimentation

Water Parameter	Value
Temperature	28 ± 2^{0}C
Turbidity	7.7 silica units
pH value at 280c	7.20
Total Hardness as(CaCo3)	165 (mg/L)
Total suspended solids (TSS)	3.4 (mg/L)
Conductivity	182(mg/L)
BOD	7-12 ppm
Sulphates	Trace amounts
Phosphates	Trace amount
Dissolved Oxygen(DO)	6-7 mg/L

Table 2: Effect of different sublethal concentrations of endosulphan on Carbohydrate content in different tissues of *C. mrigala.*

Duration of exposure	Tissues	Control	Concentrations of endosulfan (ppm)		
			0.0155	0.0310	0.0620
21 days	Muscle	25.05 ± 0.01	12.33 ± 0.02	12.76 ± 0.080	13.55 ± 0.02
	Liver	75.82 ± 0.282	37.84 ± 0.045	39.16 ± 0.060	42.66 ± 0.01
	Gill	12.93 ± 0.045	7.61 ± 0.045	8.91 ± 0.032	9.86 ± 0.01

Table 3: Effect of different sublethal concentrations of quinolphos on Carbohydrate content in different tissues of *C. mrigala.*

Duration of exposure	Tissues	Control	Concentrations of quinolphos (ppm)		
			0.0155	**0.0310**	**0.0620**
21 days	Muscle	25.05 ± 0.01	8.21 ± 0.045	9.76 ± 0.080	12.01 ± 0.02
	Liver	75.82 ± 0.282	33.84 ± 0.045	35.16 ± 0.060	41.01 ± 0.01
	Gill	12.93 ± 0.045	7.61 ± 0.045	7.91 ± 0.032	8.63 ± 0.03

Conclusion

The present work indicates that both endosulfan and quinolphos caused a drastic change in the carbohydrate metabolism of fish *Cirrhinus mrigala.* On the basis of above mentioned findings, it may be suggested that indiscriminate use of this pesticides in water bodies should not be encouraged in the agricultural practices.

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