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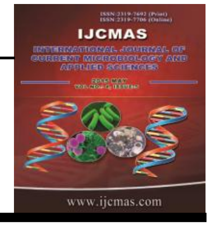

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Original Research Article

Acute Toxicity, Behavioral response and Biochemical composition of Blood of common carp, *Catla catla* (Hamilton) to an Organophosphate Insecticide, Dimethoate

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A B S T R A C T

In the present investigation the acute toxicity bioassay, behavioral response and biochemical parameters of blood of *Catla catla* to toxicity of an Organophosphate insecticide, Dimethoate were carried out in a static renewal system. It is evident that no mortality of *Catla catla* was recorded at 20.5mg/l Dimethoate up to 24 hrs. of exposure. At concentrations of 23.0, 23.5 and 24.0mg/l of Dimethoate 100% mortality of *Catla catla* was recorded at three different concentration of Dimethoate viz., 23.0mg/l, 23.5mg/l and 24.0mg/l in 96 hrs. of exposure. The LC50 values of Dimethoate at various exposure times were 21.0mg/l for about 84 hrs; 21.5mg/l for 72 hrs; 22.0mg/l for about 60hrs; 22.5mg/l for 48 hrs; 23.0mg/l for 48 hrs; and 23.5mg/l for 24 hrs. The *Catla catla* showed behavioral alterations against Dimethoate intoxication viz, uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, drowning and change in body pigmentation, muscle fasciculation, moribund lethargy, refusal of feeding and respiratory distress. These symptoms became more apparent with increase in duration of exposure at all test concentration of Dimethoate. The glycogen content, total proteins and total lipids decreased in the blood of *Catla catla* under the toxicity of Dimethoate whereas the levels of total free sugars and total free amino acids increased.

Keywords

LC50,
Dimethoate,
Catla catla,
Toxicity,
Serum
composition

Introduction

Water quality management faces greater problems today. Contaminants of varied nature exist in surface waters which include multiple chemical compounds and different products of industrial and agricultural revolution. The insecticides constitute major pollutants which contribute to the environmental problems.

Application of insecticides has contributed greatly in enhancing crop yields and also for the control of insect- borne diseases. Excessive use of broad- spectrum or non-selective insecticides damage the ecosystem, sometimes irreversibly, contaminates soil surface and ground water as well as food webs and thus compromises the health and well being of the inhabitants of the aquatic and terrestrial ecosystems.

There are many pathways by which insecticides leave their sites of application and distribute throughout the environment and enter the aquatic ecosystem. The major route of insecticides to aquatic ecosystems is through rainfall, runoff and atmospheric deposition. Another source of water contamination by insecticides is from municipal and industrial discharges. Most insecticides ultimately find their way into rivers, lakes and ponds (Tarahi Tabrizi, 2001; Honarpajouh, 2003; Bagheri, 2007; Shayeghi et at., 2007; Vryzas et al., 2009; Werimo et al., 2009; Arjmandi et al., 2010) and have been found to be highly toxic to non- target organisms that inhabit natural environments close to agricultural fields. The contamination of surface waters by insecticides causes adverse effects on growth, survival and reproduction of aquatic animals. The increase of mortality among the fish in various water bodies has drawn attention of researcher to the problems caused by insecticides runoff associated with intense agricultural practices. Different concentrations of insecticides are present in water bodies and found to be toxic to aquatic organisms especially fish (Talebi, 1998; Uner et al., 2006; Banaee et at., 2008). Fishes are highly sensitive to the environmental contamination of water. Hence insecticides, serious pollutants may significantly damage certain physiological and biochemical processes when they enter into the organs and tissues of fish (John, 2007; Banaee et al., 2011). It has been found that different kinds of insecticides can cause serious impairment to physiological and health status of fishes (Begum, 2004; Monteiro et al., 2006; Siang et al., 2007; Banaee et al., 2009). Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is important for human beings.

Among different classes of insecticides, organophosphates are more frequently used because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment. Dimethoate [IUPAC Name- o, o dimethyle S- (N methyl carbamoylmethyl) phosphoro- dithioate] is an organophosphate available in the market by the trade name of ROGER. It is a broad spectrum systemic insecticide active against acaridae, aphididae, aleyrodidae, coccodidea, coleopteran, collembolan, dipteral, Lepidoptera, pseudococcidae and thynoptera in cotton, cereals, fruits, vegetables, tea, coffee, tobacco and pastures (Aysal et al., 2004). Dimethoate is an inhibitor of enzyme cholinesterase and causes accumulation of acetylcholine in nerve tissue (synapses of the central and peripheral nervous system) and effectors organs with the principal site of action being the peripheral nervous system (Cope, 2004). The accumulation of acetylcholine results in a prolonged stimulation of the cholinergic receptors downstream leading to intense activation of autonomic nervous system, which depending upon the severity of acetyl cholinesterase inhibition results in tremors, convulsion, respiratory arrest and death (Breckenridge and Stevens, 2008).

The present investigation depicts the effects of Dimethoate insecticide on survival chance (acute toxicity), behavioral response and blood biochemical parameters of major carp, *Catla catla*.

Materials and Methods

Live specimens of freshwater carp, *Catla catla* (Hamilton) were collected from local pond of Patna city with the help of fisherman and carefully packaged into aerated polythene bags filled with tap water. In the laboratory fishes were disinfected by treatment of 0.05% potassium per magnate and transferred into large plastic tanks containing 500liters of dechlorinated tap water for acclimatization for 15 days. During acclimatization water of the tank was changed daily and fish were fed dried shrimp twice a day.

The experiment was conducted under natural photoperiod and temperature in the months of September – October, 2014. The physicochemical characteristics of experimental water used were as follows: pH 7.40± 0.2; dissolved oxygen 8.35±0.15mg/l; temperature 20.00±2^{0}C; free carbon dioxide 6.5±0.5mg/l; total hardness as calcium carbonate 135±5.25mg/l; and electrical conductivity 285.36±60.45 µmho/cm.

The acclimatized *Catla catla* of length 7.5±1.5cm and weight 12.0±3gm were sorted and starved for 24hr. before starting the experiment. Stock solution of Dimethoate (EC30%, Rallis India Ltd) was prepared in absolute alcohol. Five replicates, each containing ten fish were subjected to Dimethoate at eight different concentrations of 20.5, 21.0, 21.5, 22.0, 23.0, 23.5, and 24..mg/l. Control groups, each having ten fish kept in tap water containing 0.4ml/l acetone was run concurrently. All experiments were carried out in cylindrical glass aquaria containing 30 liters of test solution. All solutions (control and test) were renewed daily and dead fishes were immediately removed.

The behavioral changes and mortality of the fish were recorded at four different exposure periods viz, 24, 48, 72 and 96 hr.

The blood from the fishes were collected and subjected to biochemical analysis, such as total free amino acids (Yemm and Cocking, 1957), total proteins (Gornall et al., 1949), total free sugars (Roe, 1955), glycogen (Kemp and Kits, 1975) and total lipids (Barnes and Black Stock, 1973). The results obtained have been presented in Table-1, 2 and 3.

Result and Discussion

The LC50 values of Dimethoate for 24, 48, 72 and 96 hours of exposure have been presented in Table-1. From the results it is evident that no mortality of *Catla catla* was recorded at 20.5mg/l Dimethoate up to 24 hrs. of exposure. At concentrations of 23.0, 23.5 and 24.0mg/l of Dimethoate 100% mortality of *Catla catla* was recorded at three different concentration of Dimethoate viz., 23.0mg/l, 23.5mg/l and 24.0mg/l in 96 hrs. of exposure. The LC50 values of Dimethoate at various exposure times were 21.0mg/l for about 84 hrs; 21.5mg/l for 72 hrs; 22.0mg/l for about 60hrs; 22.5mg/l for 48 hrs; 23.0mg/l for 48 hrs; and 23.5mg/l for 24 hrs. The percentage mortality of *Catla catla* increased with increased concentration of Dimethoate and with decreased exposure time. The present findings gain support from the work of Anoop *et al*., (2010) who also recorded LC50 values of Dimethoate in *Heteropeunistis fossilis*. Shukla (1995) reported the LC50 value of Dimethoate for *Colisa fasciatus* as 13.0mg/l for 24 hrs, 11.4mg/l for 48 hrs, 10.0mg/l for 72hrs and 9.3mg/l for 96hrs. Vittozzi and Angelis (1991) reported 0.78mg/l and 0.79mg/l as 96 hrs LC50 values of Dimethoate for blue gill and trouts respectively. The 96hrs LC50 value for Dimethoate for *Lebister reticulates* has been reported as 19mg/l (Gupta et al., 1984). The 96hrs LC50 value for Dimethoate to the fish Cyprinus carpio has been reported as 26.11mg/l (De Mel and Pathiratne, 2005). The median lethal concentration (LC50) of Dimethoate to fresh water food fish, *Clarius batrachus* has been recorded as 65ppm by Begum (1993). The acute toxicity values of Dimethoate (96hrsLC50) for fish species found in Canada ranged from 6mg/l for blue gill

(Lepomes macrochirus) to 22.4mg/l for carp (*Cyprinus carpio*, 7 days LC50) (C.C.M.E, 1999).Sweilum (2006) reported 40mg/l concentration of Dimethoate as LC50 for 96hrs exposure to fish Nile tilapia (*Oreochromis niloticus*). In the present investigation the LC50 values of Dimethoate to *Catla catla* were recorded as 21.5mg/l for 72hrs; 22.0mg/l for 60hrs; 22.5mg/l and 22.0mg/l for 48hrs and 23.5mg/l and 24.0mg/l for 24hrs of exposure. So it is difficult to compare the toxicity of Dimethoate insecticides to *Catla catla* because the toxicity is also influenced by several factors like temperature, hardness, pH and dissolved oxygen content of the test water. The result is also in accordance of Schimmel et al., (1976).

The interrelationship between ambient temperature and susceptibility of fish to toxicants appear to be a common feature. A wide range of insecticides have been found to increase the toxicity at higher temperature (Macek and Cope, 1969; Muirhead-Thomson, 1971).

The mechanism involved in the increase of susceptibility of fish to toxicants with rise in temperature is not well understood (Singh and Narain, 1971), though effect on general metabolism and respiration rate could largely be involved (Mackek et al., 1969; Wedemeyer et al., 1976; Gordon and McLeay, 1977). Rise in water temperature reduces the solubility of oxygen in water which could affect fish physiology. It could increase the metabolic rate (oxygen demand) of fish (Davis, 1975), limiting the affectivity of blood oxygen and hemoglobin affinity for oxygen (Bohr effect), thus resulting in low dissolved oxygen levels and greater accumulation of waste products and lowering the resistance of fish to stress. Reduced solubility of oxygen in water at higher temperatures could also increase the ventilation at gills and the respiration rate (Jones et al., 1970), causing a larger quantity of water to move across the gill epithelium, thus increasing the possibility of greater uptake of contaminants from the medium and intensifying the stress.

From the result (Table-2) it is evident that the *Catla catla* showed behavioral alterations against Dimethoate intoxication. Uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, drowning and change in body pigmentation, muscle fasciculation. Moribund lethargy, refusal of feeding, respiratory distress became more apparent with increase in duration of exposure at all test concentration of Dimethoate.

The results of water quality of the tap water used in the present investigation were in the normal range which suggests that the parameters of the test water were not the cause of fish mortality. However, temperature, hardness, pH, alkalinity and biological factors such as sex, age, health, weight and physiological status are reported to have profound effects on the acute toxicity of organophosphate pesticide, Dimethoate. The present findings gain support from the work of Singh 2013 and Singh (2009) who reported a more or less similar results on acute toxicity and behavioral response of Dimethoate to an air breathing fish, *Colisa fasciatus* and common carp, *Cyprinus carpio* respectively.

From the result (Table-3) it is evident that the glycogen content, total proteins and total lipids decreased in the blood of *Catla catla* under the toxicity of Dimethoate whereas the levels of total free sugars and total free amino acids increased. In animals, any stress could inflict excessive energy demand, which is immediately fulfilled by blood glucose.

Table.1 Percentage lethality of *Catla catla* after exposure to eight different concentrations of Dimethoate

Concentration of Dimethoate in mg/l	Time of exposure in hrs.	Number of fish died out of ten	% death
20.5	24	0	0
	48	1	10
	72	3	30
	96	4	40
21.0	24	1	10
	48	2	20
	72	4	40
	96	6	60
21.5	24	2	20
	48	2	20
	72	5	50
	96	7	70
22.0	24	2	20
	48	3	30
	72	6	60
	96	8	80
22.5	24	3	30
	48	5	50
	72	6	60
	96	9	90
23.0	24	3	30
	48	5	50
	72	7	70
	96	10	100
23.5	24	5	50
	48	7	70
	72	9	90
	96	10	100
24.0	24	5	50
	48	7	70
	72	9	90
	96	10	100

Table.2 Behavioral response of *Catla catla* after exposure to eight different concentrations of Dimethoate

Symptoms observed	Concentration of Dimethoate in mg/l															
	20.5				21.0				21.5				22.0			
	Time of exposure															
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Lethargy	+	+	++	M	+	++	+++	M	+	++	M	M	++	+++	M	L
Increased mucus	+	++	++	+++	+	++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
Skin discoloration	-	+	+	++	+	++	++	+++	-	+	++	++	++	+++	+++	+++
Muscle fasciculation	+	+	++	+++	+	++	+++	+++	+	++	+++	+++	+++	+++	+++	+++
Respiratory distress	+	+	++	++	+	++	++	+++	++	+++	+++	+++	+++	+++	+++	+++
Feeding behavior	N	N	N	N	N	N	LA	LA	N	LA	LA	LA	LA	LA	LA	RF

Symptoms observed	Concentration of Dimethoate in mg/l															
	22.5				23.0				23.5				24.0			
	Time of exposure in hrs.															
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Lethargy	+++	M	L	L	M	L	L	L	L	L	L	L	L	L	L	L
Increased mucus	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Skin discoloration	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Muscle fasciculation	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Respiratory distress	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Feeding behavior	LA	LA	RF	RF	LA	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF

Degree of intensity of the symptoms: '+' above normal; '++' moderate; '+++' severe
'M' moribund; 'L' lethal; 'N' normal feeding; 'LA' loss of appetite; 'RF' refusal of feed

As a consequence, the blood sugar level could increase as observed in the present experimental fish, *Catla catla*. As a consequence, the glycogen reserve in the blood could be subjected to glycogenolysis with the resultant depletion. This is in conformity with the findings of Mukhopadhyay and Dehadrai (1980), Parithabhanu and Subramanian (2006) and Natarajan (1989) in pesticide- treated fishes. The reduction in the glycogen content in the blood of present experimental fish, *Catla catla* could also be due to the inhibition of glycogenesis, as also observed by Kabeer et al., (1984) in bivalves treated with pesticide.

In the present investigation, Dimethoate caused reduction in total proteins in the blood of *Catla catla*. The control fish showed 75.00mg/dL of total proteins in their blood. The reduction in total proteins in blood of *Catla catla* at different concentrations of Dimethoate on 48 hrs of exposure was recorded in the following sequence:

Concentration of Dimethoate in mg/l	20.5	21.0	21.5	22.0	22.5	23.0	23.5	24.0
Total proteins in mg/dL	72.80	61.65	52.25	45.0	38.80	30.70	27.0	21.25

Similarly reduction in total glycogen content as compared to control was recorded and showed significant decline in its content (4.58mg/dL) at 24.0mg/l of Dimethoate on 48 hrs of exposure. Reduction in total lipids in blood of *Catla catla* was recorded to be 179.27mg/dL at 24.0 mg/lof Dimethoate on 48 hrs of exposure as compared to control

(396.65mg/dL). Similarly increase in total amino acids and total free sugars was recorded to be 630.00mg/dL and 272.15mg/dL respectively at 24.0mg/l Dimethoate on 24 hrs of exposure as compared to control.

This is suggestive of degradation of proteins and glycogen with the resultant increase of total free amino acids and sugars. The present findings gain support from the work of Kabeer et al., (1984) in pesticide- treated mollusks and Joyce Shoba Rani and Janaiah (1991) in *Clarius batrachus* under pesticide toxicity. Nagabhushanam et al., (1983) has reported that the free amino acids serve as supplementary energy source under the condition of emergency during chronic stress.

The decline in the total lipids in the blood of *Catla catla* under study indicates the utilization of lipids to meet the energy demand during the stress caused by Dimethoate. According to Srinivas et al., (1991), the endogenous fat is the only source of energy requirements in animals during prolonged stress. This is in agreement with the findings of Jeba Kumar (1993) and Govindan (1994) who have reported the decrement of lipids in the tissues of fishes during stress caused by insecticides. Thus Dimethoate has disrupted the normal functioning of cells with resultant alterations in the fundamental biochemical mechanisms in fishes. This in turn would result in the mortality of fishes on prolonged exposure to the Dimethoate, an organophosphate.

It is concluded that Dimethoate is highly toxic to fish which is greatly reflected in behavioral and biochemical alterations resulting in death. Further studies on toxicity of Dimethoate in laboratory and field on various fish species may help in deciding the judicious use of this insecticide.

Table.3 Biochemical composition of blood serum of Catla catla in control and 48 hrs. of exposure to eight different concentrations of Dimethoate

Concentration of Dimethoate in mg/l	Total free amino acids in mg/l	Total proteins in mg/l	Glycogen in mg/l	Total free sugars in mg/l	Total lipids in mg/l
Control	455.00±11.63	75.00±5.54	20.75±1.45	150.00±6.11	396.65±15.41
20.5	475.00±14.26 NS (+4.85; r=0.75)	72.80±1.35* (-14.31; r=1.00	17.60±1.45* (-18.04; r=0.55)	161.00±10.15NS (+10.05; r=0.32)	258±22.11NS (-9.59;r=0.75)
21.0	485.00±12.21NS (+11.75; r=0.51)	61.65±1.35* (-12.12; r=0.41)	15.45±1.35* (-20.05; r=0.70)	175.00±9.75* (+20.55; r=0.07)	345±21.24* (-13.38; r=0.61)
21.5	505.00±11.25* (+21.11; r=0.81)	52.25±1.65* (-31.65; r=0.44)	11.20±1.15* (-25.21; r=0.91)	205.00±9.62* (+26.31; r=.031)	312±11.65* (-16.21; r=0.61)
22.0	515.00±12.65 (+31.15; r=0.65)	45.00±1.65* (-34.51; r=0.45)	9.35±1.16* (-30.22; r=0.91)	225.00±10.61* (+32.35; r=0.38)	286.45±17.64* (-21.51; r=0.61)
22.5	545.00±6.58* (+33.15; r=0.51)	38.80±1.17* (-38.81; r=0.15)	7.35±1.21* (-36.15; r=0.91)	237.00±11.50* (+35.15; r=0.45)	205.55±16.64* (-27.51; r=0.52)
23.0	576.25±7.45* (+36.14; r=0.53)	30.70±1.16* (-42.35; r=0.18)	6.35±1.25* (-40.16; r=0.71)	247.00±15.12* (+43.12; r=0.73)	195.16±11.65* (-32.35; r=0.61)
23.5	612±7.46* (+38.12; r=.0.64)	27.00±2.35* (-45.81; r=0.15)	5.27±1.35* (-42.15; r=0.81)	255.10±11.15* (+45.15; r=0.72)	187.14±11.50* (-41.21; r=0.51)
24.0	630.00±13.25* (48.56; r=0.85)	21.25±2.75 (-51.95; r=0.95)	4.58±1.27* (-45.16; r=0.71)	272.15±7.15* (+48.12; r=0.62)	179.27±10.90 (-46.25; r=0.61)

*= Significant at p>0.05; NS= not significant; + indicates increase over control; - indicates decrease over control

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