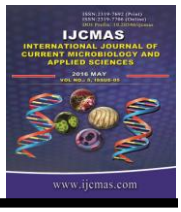
Int.J.Curr.Microbiol.App.Sci (2016) *5*(5): 322-341

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 5 (2016) pp. 322-341**
Journal homepage: http://www.ijcmas.com



Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.505.034

Effect of Organophosphate Insecticide, Dimethoate on Physiology of Common carp, *Catla catla* (Hamilton) and *Labeo rohita*

Mohammad Illiyas Hussain[1], Baidyanath Kumar[2*] and Mumtaz Ahmad[3]

[1]Research Scholar, Department of Zoology, Oriental College, Patna, India
[2]Visiting Professor, Department of Biotechnology, College of Commerce, Patna, India
[3]Head, Department of Zoology, Oriental College, Patna, India
**Corresponding author*

Keywords

LC50, Dimethoate, *Catla catla*, *Labeo rohita* Toxicity, Serum composition, Isoenzymes.

Article Info

Accepted:
15 April 2016
Available Online:
10 May 2016

A B S T R A C T

In the present investigation the effects of Dimethoate insecticide on survival chance (acute toxicity), behavioral response and blood biochemical and haematological parameters and the expression of isoenzymes in *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) after exposure to sublethal concentrations of Dimethoate was studied. It is evident that no mortality of *Catla catla* and *Labeo rohita* 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. Similarly at concentrations of 23.0, 23.5 and 24.5mg/l of Dimethoate 100% of mortality of *Labeo rohita* was recorded. *Catla catla* and *Labeo rohita* 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 glycogen content, total proteins and total lipids decreased in the blood of *Catla catla* and *Labeo rohita* under the toxicity of Dimethoate whereas the levels of total free sugars and total free amino acids increased. Dimethoate caused reduction in total proteins in the blood of both *Catla catla* and *Labeo rohita*. The acute exposure to Dimethoate resulted in a significant decrease in count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes in the experimental group. *Catla catla* and *Labeo rohita* exposed to acute effects of the Dimethoate insecticide showed significantly ($P < 0.01$) decreased concentration of alkaline phosphatase and significantly ($P < 0.01$) increased concentration of ammonia, aspartate aminotransferase, lactate dehydrogenase,creatine kinase and lactate in blood plasma. The main biochemical blood profile response of *Catla catla* and *Labeo rohita* to the acute effect of 23.5 mg/l of Dimethoate was a significantly ($P < 0.01$) decreased concentration of alkaline phosphatase and significantly ($P < 0.01$) increased concentration of NH3, LDH, AST, CK and LACT in blood plasma.

Introduction

Water pollution caused by pesticides has become a serious problem. 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.

Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish. Residual amounts of pesticides and their metabolites have been found in drinking water and foods, increasing concern for the possible threats to human health posed by exposure to these chemicals. Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels (Cerejeira *et al.,* 2003; Spalding *et al.,* 2003).

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 al.,* 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).

Lactate dehydrogenase (EC 3.1.1.27) is one of the chief enzyme of carbohydrate metabolism which catalyses the oxidation of lactate and reduction of pyruvate during anaerobic glycolysis. It is a tetrameric molecule consists of two separate loci which code for A and B subunits of this enzyme. The A and B subunits indiscriminately associate and form five tetrameric isozymes (A4, A3B1, A2B2, A1B3 and B4) (Fujio and Kaneko, 1980). Isozymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exhibit in multi molecular forms and functions (Markert and Moller, 1959). LDH electrophoretic patterns could help in investigating and to locating the pesticide stress. Stress reflects on respiratory metabolism as LDH is a key enzyme in carbohydrate metabolism and occurs virtually in all tissues. It is indicative of variation in tissue functioning as a consequence of presence, increase or decrease in the concentration of the toxicant (Jyothirmayee et al. 2005).

The present investigation depicts the effects of Dimethoate insecticide on survival chance (acute toxicity), behavioral response and blood biochemical and haematological parameters and to evaluate the expression of Isoenzymes in *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) after exposure to sub lethal concentrations of Dimethoate.

Materials and Methods

Live specimens of freshwater carp, *Catla catla* (Hamilton) and *Labeo rohita* (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, 2015. 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±20C; 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 and *Labeo*

rohita of length 6.5±1.5cm and weigh 10.5gm 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 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. LC50 values were calculated by Finney's Probit analysis (1971).

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).

Biochemical and Haematological Profile following Dimethoate Exposure

Examinations were performed after 96 h exposure period with Dimethoate at an exposure level of 96h LC50 (21.0mg/l). *Catla catla* and *Labeo rohita* in the control group were monitored concurrently. The test was performed in sixteen 300 liter tanks. Each tank contained 20 *Catla catla* and 20 *Labeo rohita* i.e. six tanks with 96hLC50 of Dimethoate, and one control tank with *Catla catla* and one control tank with *Labeo rohita*. Tanks for all treated fish and controls were replicated, Presence of the tested substance (above 80% of the nominal concentration) was ensured through a 12 h exchange of the water bath. Determination of Dimethoate concentration in water was measured using gas chromatography (Mekebri *et al.*, 2008). Forty-eight experimental (8 fish from each pesticide duplicated) *Catla catla* or *Labeo rohita* and sixteen control *Catla catla* or *Labeo rohita* were selected at random and used for haematological and biochemical examination at the end of the 96 h exposure. Blood was sampled from the vena caudalis, using an 18G x 1 1/2 in syringe. Fish were not anaesthetized prior to blood sampling, as they were calm due to low water temperature and there was no danger of tissue trauma or handling stress. Heparin was used as an anticoagulant (Heparin inj., Leciva, Czech Republic) at a concentration of 40 I.U. heparin sodium salt in 1 ml blood. The indices used to evaluate the haematological profile included erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean colour concentration (MCHC), erythrocyte haemoglobin (MCH), leukocyte count (Leuko), and the differential leukocyte count (Leukogram). The procedures were based on unified methods for haematological examination of fish (Svobodova *et al.*, 1991). Blood was sampled by v. caudalis as mentioned above. Plasma was obtained by centrifuging blood samples in a cooled centrifuge (4 °C, 837×g). Plasma samples were held at -80 0C until analysis. Biochemical indices included glucose (GLU), total proteins (TP), albumins (ALB), total globulins (GLOB),

ammonia (NH3), tricylglycerols (TAG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydro-genase (LDH), gama-glutamyl-transferase (GGT), creatine kinase (CK), lactate (LACT), alkaline phosphatase (ALP), calcium (Ca2+), magnesium (Mg), and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories Inc., Maine, USA) was used

Results and Discussion

The LC50 values of Dimethoate for 24, 48, 72 and 96 hours of exposure have been presented in Table-1 and 2. From the results it is evident that no mortality of *Catla catla* and *Labeo rohita* 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 concentrations 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. Similarly at concentrations of 23.0, 23.5 and 24.5mg/l of Dimethoate 100% of mortality of *Labeo rohita* was recorded. 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. The LC50 values of Dimethoate to *Labeo rohita* were recorded as 20.5mg/l for 96h; 21.0mg/l for 72h; 21.5, 22.0, 22.5 for 48h and 23.0mg/l, 23.5 and 24.0 for 24h.So it is difficult to compare the toxicity of Dimethoate insecticides to *Catla catla* and *Labeo rohita* 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-3 and 4) it is evident that the *Catla catla* and *Labeo rohita* 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-5 and 6) it is evident that the glycogen content, total proteins and total lipids decreased in the blood of *Catla catla* and *Labeo rohita* 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. As a consequence, the blood sugar level could increase as observed in the present experimental fish, *Catla catla* and Labeo rihita. 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* and *Labeo rohita* 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 both *Catla catla* and *Labeo rohita*. 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 (Table-5):

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 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	5	50
21.0	24	1	10
	48	2	20
	72	5	50
	96	7	70
21.5	24	2	20
	48	5	50
	72	6	60
	96	8	80
22.0	24	3	30
	48	5	50
	72	7	70
	96	8	80
22.5	24	3	30
	48	5	50
	72	7	70
	96	9	90
23.0	24	5	50
	48	6	60
	72	8	80
	96	10	100
23.5	24	5	50
	48	8	80
	72	9	90
	96	10	100
24.0	24	5	50
	48	7	70
	72	10	100
	96	10	100

Table.3 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

Table.4 Behavioral response of *Labeo rohita* 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

Table.5 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)

Table.6 Biochemical composition of blood serum of *Labeo rohita* 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	450.00±11.63	70.00±5.54	18.75±1.45	140.00±6.11	390.65±15.41
20.5	470.00±14.26 NS (+4.85; r=0.75)	69.80±1.35* (-14.31; r=1.00	17.60±1.45* (-18.04; r=0.55)	160.00±10.15NS (+10.05; r=0.32)	268±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	501.00±11.25* (+21.11; r=0.81)	51.25±1.65* (-31.65; r=0.44)	10.20±1.15* (-25.21; r=0.91)	203.00±9.62* (+26.31; r=.031)	308±11.65* (-16.21; r=0.61)
22.0	512.00±12.65 (+31.15; r=0.65)	42.5±1.65* (-34.51; r=0.45)	9.25±1.16* (-30.22; r=0.91)	220.00±10.61* (+32.35; r=0.38)	281.45±17.64* (-21.51; r=0.61)
22.5	543.00±6.58* (+33.15; r=0.51)	37.80±1.17* (-38.81; r=0.15)	8.35±1.21* (-36.15; r=0.91)	231.00±11.50* (+35.15; r=0.45)	202.55±16.64* (-27.51; r=0.52)
23.0	574.25±7.45* (+36.14; r=0.53)	30.60±1.16* (-42.35; r=0.18)	6.25±1.25* (-40.16; r=0.71)	251.00±15.12* (+43.12; r=0.73)	185.16±11.65* (-32.35; r=0.61)
23.5	609±7.46* (+38.12; r=.0.64)	28.00±2.35* (-45.81; r=0.15)	6.27±1.35* (-42.15; r=0.81)	245.10±11.15* (+45.15; r=0.72)	185.14±11.50* (-41.21; r=0.51)
24.0	627.00±13.25* (48.56; r=0.85)	20.25±2.75 (-51.95; r=0.95)	4.45±1.27* (-45.16; r=0.71)	270.15±7.15* (+48.12; r=0.62)	175.27±10.90 (-46.25; r=0.61)

Table.7 Haematological profiles of *Catla catla* and *Labeo rohita* affected by acute exposure to Dimethoate

Indices	*Catla catla*		*Labeo rohita*	
	Control, n=15, $\bar{x}$± SD	Experimental group, n= 15, $\bar{x}$± SD	Control, n=15, $\bar{x}$± SD	Experimental group, n= 15, $\bar{x}$± SD
RBC (T/l)	0.80±0.15[a]	0.78±0.24[a]	0.75±0.12[a]	0.71±0.15[a]
Hb (g/l)	41.71±6.39[a]	42.69±10.30[a]	37.51±3.15[a]	32.50±0.18[a]
PCV(l/l)	0.36±0.04[a]	0.39±0.05[a]	0.30±0.05[a]	0.35±0.04[a]
MCV (fl)	460.57±8.82[a]	568.15±2.91[a]	428.25±6.88[a]	455.15±2.51[a]
MCH (pg)	53.31±5.87[a]	59.81±8.23[a]	48.25±6.17[a]	55.75±8.65[a]
MCH (g/l)	115.8.36[a]	108.7±7.68a	112.85±8.35[a]	105.55±6.55[a]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Table.8 Leukocyte differential count of *Catla catla* and *Labeo rohita* affected by acute exposure to Dimethoate

Indices	*Catla catla*		*Labeo rohita*	
	Control, n=15, $\bar{x}$± SD	Experimental group, n= 15, $\bar{x}$± SD	Control, n=15, $\bar{x}$± SD	Experimental group, n= 15, $\bar{x}$± SD
Leukocyte (G/l)	13.15±3.56[a]	10.22±5.03[a]	11.25±2.51[a]	8.18±2.11[a]
Lymphocyte (G/l)	12.54±3.11	9.94±4.08[a]	10.55±2.11[a]	7.25±2.21[a]
Monocyte(G/l)	0.01±0.03[a]	0.01±0.05[a]	0.01±0.05[a]	0.01±0.04[a]
Neutrophile granilocute segments (G/l)	0.51±0.19[a]	0.25±0.21[b]	0.42±0.17[a]	0.27±0.20[b]
Neutrophile granilocute bands (G/l)	0.05±0.07[a]	0.02±0.04[a]	0.03±0.05[a]	0.01±0.03[a]
Developmental phases – myeloid sequence (G/l)	0.03±0.05[a]	0.01±0.02[b]	0.03±0.04[b]	0.01±0.03[b]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Table.9 Bioghemical profiles of blood plasma of *Catla catla* and *Labeo rohita* affected by acute exposure to Dimethoate

Indices	*Catla catla*		*Labeo rohita*	
	Control, n=15, $\bar{X}\pm$ SD	Experimental group, n= 15, $\bar{X}\pm$ SD	Control, n=15, $\bar{X}\pm$ SD	Experimental group, n= 15, $\bar{X}\pm$ SD
GLU (mmol/l)	3.64±0.75a	4.07±1.84a	3.55±0.15[a]	4.70±1.12[a]
TP(G/l)	36.60±5.14a	39.33±4.30[a]	35.45±4.10[a]	40.15±2.10[a]
ALB(G/l)	6.80±2.71a	8.60±0.99[a]	5.75±1.72a	7.55±0.12[a]
GLOB (G/l)	29.80±2.81[a]	30.87±2.47[a]	27.75±2.18[a]	30.15±2.15[a]
TRIG(mmol/l)	0.97±0.12[a]	0.86±0.19[a]	0.95±0.15[a]	0.82±0.14[a]
ALT (µkat/l)	0.08±0.02[a]	0.08±0.02[a]	0.07±0.04[a]	0.07±0.03[a]
Ca^{2+}(mmol/l)	2.53±0.18a	2.81±0.38a	2.13±0.15a	2.70±0.31a
.ChE (µkat/l)	2.03±1.30a	2.52±0.99a	2.00±0.95a	2.48±0.71a
PHOS (mmol/l)	1.46±0.22a	1.39±0.16a	1.40±0.25a	1.36±0.11a
NH_3 (mmol/l)	555	720**	515	630**

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)
Significant ** $P < 0.01$

Table.10 Isoenzyme profiles of blood plasma of *Catla catla* and *Labeo rohita* affected by acute exposure to Dimethoate

Indices	*Catla catla*		*Labeo rohita*	
	Control, n=15, $\bar{X}\pm$ SD	Experimental group, n= 15, $\bar{X}\pm$ SD	Control, n=15, $\bar{X}\pm$ SD	Experimental group, n= 15, $\bar{X}\pm$ SD
AST (µkat/l)	4.3±0.11	6.0**±0.15	4.0±0.17	6.5**±0.07
LDH (µkat/l)	18.0±0.15	25.0**±1.12	15.5±0.6	26.0**±0.14
CK (µkat/l)	11.0±0.12	15.0**±1.60	10.0±0.41	13.0**±0.13
ALP (µkat/l)	0.8±0.15	0.65**±1.23	0.7±0.16	0.63**±0.17
ALCT(mmol/l)	3.5±0.18	9.5**±1.05	3.0±0.17	8.5**±0.15

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**	**45.00**	**38.80**	**30.70**	**27.00**	**21.25**

Similarly the reduction in total proteins in blood of *Labeo rohita* at different concentrations of Dimethoate on 48 hrs of exposure was recorded in the following sequence (Table-6):

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	**70.0**	**61.0**	**51.25**	**42.50**	**37.80**	**30.60**	**28.00**	**20.25**

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). A more or less similar result was observed in case of Labei rohita. Similarly increase in total amino acids and total free sugars were 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. A similar pattern of increase in total amino acids and total free sugars was recorded in *Labeo rohita* after Dimethoate exposure at concentration of 24.0mg/l for 24hrs. 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* and *Labeo rohita* under study indicates the utilization of lipids to meet the energy demand during the stress caused by Dimethoate (Table-5 and 6). 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.

The results of haematological profile of the control and experimental fish, *Catla catla* and *Labeo rohita* under the study period are given in Table 7. Compared to the control specimens, those after the acute exposure to Dimethoate at the concentration of 23 mg/l had no effect on the haematological indices studied (RBC, Hb, PCV, MCV, MCHC, MCH and Leuko). It was evident that the acute exposure to Dimethoate resulted in a significant decrease in count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes in the experimental group. The results of examinations of the leukocyte profile of control and experimental fish, *Catla catla* and *Labeo rohita*, are given in Table 8.

The results of biochemical blood plasma profile of the control and experimental fish *Catla catla* and *Labeo rohita* under the study period are given in Table- 9. The experimental fish, *Catla catla* and *Labeo rohita* exposed to acute effects of the Dimethoate insecticide showed significantly ($P < 0.01$) decreased concentration of alkaline phosphatase and significantly ($P < 0.01$) increased concentration of ammonia, aspartate aminotransferase, lactate dehydrogenase,creatine kinase and lactate in blood plasma. The rest of the indices (GLU, TP, ALB, GLOB, TRIG, ALT, ChE, Ca2+, PHOS) were comparable in the two groups during the study (Table- 10). The present findings gain support from the work of Velisek *et al.*, (2006), Bradbury and Coats (1989b), Davis *et al.*, (1993), Polat *et al.*, (2002), Masopust (2000) etc who also observed a more or less similar result in rainbow trout and other fish.

In the present investigation with major carp, *Catla catla* and *Labeo rohita*, a significant decrease in count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes in the experimental group was observed. No significant differences were observed in the levels of RBC, Hb, PCV, MCV, MCHC, MCH and Leuko. However, Atamanalp et al. (2002a) and Atamanalp and Yanik (2003) found a significant increase ($P < 0.05$) in the levels of RBC and a significant decrease ($P < 0.05$) in the Hb, MCH, MCHC, thrombocyte count and erythrocyte in rainbow trout after exposure of cypermethrin insecticide. The main biochemical blood profile response of *Catla catla* and *Labeo rohita* to the acute effect of 23.5 mg/l of Dimethoate was a significantly ($P < 0.01$) decreased concentration of alkaline phosphatase and significantly ($P < 0.01$) increased concentration of NH3, LDH, AST, CK and LACT in blood plasma. Dimethoate caused an increase in plasma ammonia level perhaps due to an increase in amino acids catabolism and a failure of ammonia excretion mechanisms (Svoboda, 2001). The activities of enzymes in blood plasma can be also used as a relevant stress indicator. The enzymes used for the purpose are above all LDH, CK and transaminases (ALT and AST). A significant increase in the activity of the above mentioned plasma enzymes indicate stress-based tissue impairment (Svoboda, 2001). After acute exposure to Dimethoate, a significant increase ($P < 0.01$) in AST level was found in experimental carps in comparison to control specimens. Increased activities of both transaminases indicated amplified transamination processes. An increase in transamination occurs due to amino acid input into the TCA cycle in order to cope with the energy crisis during pyrethroid-based stress (Philip *et al.*, 1995). The increase in LDH level indicated metabolic changes, i.e. the glycogen catabolism and glucose shift towards the formation of lactate in stressed fish, primarily in the muscle tissue (Simon *et al.*, 1983). On the other hand, Atamanalp et al. (2002b) found changes in the concentration of calcium and phosphor in rainbow trout following cypermethrin exposure. Jee et al. (2005) found an increase in levels of serum glutamic-acid-oxylacetic-acid-transaminase, glutamic-acid-pyruvic-acid-transaminase, glucose and alkaline phosphatase and a decrease in the concentration of plasma total protein, albumin, cholesterol and lysozyme in Korean rockfish (Sebastes schlegeli) exposed to cypermethrin.

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.

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How to cite this article:

Mohammad Illiyas Hussain, Baidyanath Kumar and Mumtaz Ahmad. 2016. Effect of Organophosphate Insecticide, Dimethoate on Physiology of Common carp, *Catla catla* (Hamilton) and *Labeo rohita.Int.J.Curr.Microbiol.App.Sci.* 5(5): 322-341.
doi: http://dx.doi.org/10.20546/ijcmas.2016.505.034