Effect of Dimethoate on mortality and Biochemical changes of Freshwater fish *Labeo rohita* (Hamilton)

Nagaraju Bantu1, Rathnamma vakita2*

1 Department of Biochemistry, Acharya Nagarjuna University, Guntur-522510, A.P, India
2 Department of Zoology, Acharya Nagarjuna University, Guntur-522510, A.P, India

*correspondence should be addressed to Rathnamma vakita, Department of Zoology, Acharya Nagarjuna University, Guntur-522510, A.P, India; Tell: +91; Fax: +91; Email: nagaraju.bantu301@gmail.com.

**ABSTRACT**

The acute toxicity tests were conducted during certain intervals in various concentrations of Dimethoate. The physical and chemical analyses of water were carried out by following APHA methods. While treating with Dimethoate, the percentage of fish mortality was assessed during 24, 48, 72 and 96 hours. The estimated lethal concentration values along with 95% confidence limits of Dimethoate were found to be 17.532 mg/l (16.781-19.877), 17.321 mg/l (16.521-18.134), 16.721 mg/l (16.063-17.952), 16.350 mg/l (15.388-17.143), mg/l for 24, 48, 72, and 96 h, respectively. In sub lethal and lethal exposure, the glycogen and protein levels were decreased, minimum percentage of protein depletion was (22.21%) in kidney and maximum percentage was (49.80%), in liver, were observed, the depletion of glycogen content minimum in kidney (18.73%), and maximum in liver (80.14%), the fish was exposed to sub lethal and lethal concentrations for 8 days. The decrease in protein content in various tissues might also be due to inhibition of metabolizing enzymes by administration of agro chemicals. Decline protein levels in early periods due to stress in metabolic process and impairment of protein synthesis machinery in fish.

**Key words:** percentage, sub lethal, protein, liver, enzymes, and synthesis

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1. INTRODUCTION

Environmental protection has attracted the attention of the wide cross-section of people all over the world which has now become a global issue amongst scientists and researchers working in this area. Unfortunately several toxic pollutants, few are even unknown or un-identified to the biota, are being regularly introduced in large quantities into the environment, especially into the aquatic environment. Pollution of water by pesticides is an important dimension of environmental degradation. The disposal of the industrial and agricultural wastes directly into the aquatic medium burdens the ecosystem and stresses the need to analyze, the concentration of these substances in the medium as well as in the organisms. The pesticides are also found to be highly toxic not only to fish but also to other organisms which constitute food of the fish. It is estimated that approximately 3000000 people are exposed to effects of organophosphates or carbamates every year worldwide, which leads to up to 300000 deaths. Many of research workers have used the acute toxicity tests of pesticides on fish to acquire rapid estimates of the concentrations that caused direct, irreversible harm to test organism. Many of these chemicals are mutagenic (1, 2), linked to the development of cancers (3) or may lead to the developmental deficits. Worldwide pesticide usage has increased dramatically during the past two decades, coinciding with changes in farming practices and increasingly intensive agriculture. 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 (4). Pesticides were found to adversely affect a number of biological functions, thus causing harm to the non-target organisms. Organophosphate compounds are known for this persistence in the environment and accumulation in the tissues for long periods for controlling the loss of produce due to pest attack and as a consequence of the demand for producing more food, there has been an increasing use of
pesticides in India. Dimethoate is an organophosphate insecticide used to kill mites and insects systematically and on contact. In the present study, an attempt has been made to analyze the toxicity of the Dimethoate on the freshwater fish *Labeo rohita*.

### 2. MATERIALS AND METHODS

The fresh water fish *Labeo rohita* size 4-5 cm and weight 7-9 g were brought from a local fish farm located at Nandivelugu, Guntur district of Andhra Pradesh, India. The fish *Labeo rohita* were fed daily with commercial fish pellets and acclimatized to the laboratory conditions at 28 + 2°C for 15 days. During the acclimatization period daily fed with fish meal. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. The water used for acclimatization and conducting experiments was clear unchlorinated ground water and the hydrographic conditions of water are shown in the Table 1. The physical and chemical analyses of the water were carried out (5). The containers of the test media are of 10 liter capacity, where in each test five containers were used and each container consisted of ten fish.

**2.1. Procurement of commercial grade**

Dimethoate 30% EC (Rogor) manufactured by Rallis India Limited, Mumbai. It was purchased from the local market in Guntur.

**2.2. Preparation of stock solution**

The stock solutions were made with acetone and concentrations were taken in mg/l. Controls were maintained for each experiment and they were added with the quantity of acetone equal to the highest concentration used in the test. Precaution is taken to minimize the acetone as solvent.

**2.3. Selection of sub-lethal concentrations**

The lethal concentrations ensure death even before noticing the behavioral abnormalities. Even when the animal is exposed to low concentrations continuously, many behavioral abnormalities and physiological alterations would be observed. In the present study 1/10th of 96 hr. LC50 value (1.635 mg/l) was selected as sub lethal concentration to study the behavioral alterations and biochemical alterations (As per the recommendations of committee on toxicity studies – OECD. The concentrations of pesticides, which may normally be sub-lethal during short-term exposure, may prove to be lethal, if the exposure time is extended. Since the toxicity of the poison is a function of time, it is customary to expose the test organisms over a fixed period of time to the toxicant usually for 24, 48, 72 and 96 h. The acute toxicity (96 h LC50) of Dimethoate for the freshwater fish, *Labeo rohita* was determined in the laboratory using the semi-static method in OECD (6). The mortality rate was taken into consideration and while taking the data, dead fish was removed immediately. Pilot experiments were conducted to choose the mortality range between 10% and 90%.

Basing on the pilot experiments, the experiments were conducted to determine the toxicity in five different concentrations for 24, 48, 72, and 96 hours with organophosphate compound Dimethoate in semi-static system, in this method, the test solution and test organisms are kept in the test chambers for a specific duration of the experiment. The data of each concentration was pooled up to calculate the LC50 values by using the un-weighted regression method of probit analysis (7). The exposure was continued for 8 days at 28±1°C with photoperiod of 12D:12L. The water was renewed freshly every day to produce constant effect of Dimethoate on fish. At the end of 8 days exposure, the tissues such as liver, muscle, kidney, brain, and gill were collected by dissecting the animal and stored at – 20°C for biochemical studies.

**2.4. Estimation of Glycogen**

The total glycogen was estimated, employing the method of (8). Each tissue was homogenized in 80% Methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of 5% TCA (Trichloro acetic acid), and boiled for 15 minutes at 100°C and then cooled in running water. The solution was made up to 5 ml with 5% TCA to compensate for the evaporation and then centrifuged, from this, 2 ml of supernatant was taken into the test tube, 6 ml of conc. H2SO4 was added and the mixture was boiled for 6 minute. The mixture was cooled and the optical density was measured of 520 nm.

**2.5. Estimation of Proteins**

Each tissue was homogenized in 5% TCA and centrifuged at 3000 rpm for 10 minutes. The suspended protein residue was dissolved in 1 ml of in NaOH; 0.2 ml of the extract was taken into the test tube and 5 ml of alkaline copper solution (50 ml of 2% Na2CO3 in 0.1 N NaOH and 1 ml of 0.5% CuSO4 5H2O in 1% of sodium or potassium tartrate) was added. The contents were mixed well and allowed to stand for 10 minutes; 0.5 ml of diluted Folin phenol reagent was added. After 30 minutes the optical density was measured spectrophotometrically at 540 nm (9).
Table 1. Chemical analysis of water used for experiment

| Parameter                              | Value          |
|----------------------------------------|----------------|
| Turbidity                              | 8 silica units |
| Electrical conductivity at 28°C         | 816 micro ohms/cm |
| pH value at 28°C                       | 8.1            |
| Alkalinity:                            |                |
| i. Phenolphthalein                     | Nil            |
| ii. Methyl orange                      | 472            |
| Total Hardness (as CaCO3)              | 232            |
| Non-carbonate Hardness (as CaCO3)      | Nil            |
| Calcium Hardness (as N)                | Nil            |
| Sulphate (as SO4)                      | Trace          |
| Chloride (as Cl)                       | 40             |
| Fluoride (as F)                        | 1.8            |
| Iron (as fe)                           | Nil            |
| Dissolved oxygen                       | 8-10 ppm       |
| Temperature                            | 28 + 32°C      |

3. RESULTS AND DISCUSSION

In the present investigation the test species, *Labeo rohita* has shown differential toxicity level with the function of period. The 96 hours LC50 concentration is less than the 24 h, 48, and 72 h concentration, which shows that the more is the duration period the less is the concentration required. The observed percentage of mortality of *Labeo rohita* for Dimethoate in static tests for 24, 48, 72 and 96 hours were shown in (Table 2). The observed and estimated LC50 values of *Labeo rohita* for Dimethoate in static test for 24, 48, 72 and 96 h, parameter estimation, were shown in Table 2 and Table 3 respectively. The changes in the amount of glycogen and protein content in the fish *Labeo rohita* exposed to sub-lethal and lethal concentrations of Dimethoate for 8 days along with standard deviation was given in the Table 5, Table 6 and also graphically represented in the (Figure 2, Figure 3, Figure 4 and Figure 5) respectively. The values were expressed as mean ± S.D. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by using the computer package SPSS 20.0 v and the significance of difference was set up at (*p*< 0.05).

Table 2. Mortality of Labeo rohita exposed to Dimethoate

| Conc. *(24 h)* | % mortality | Conc. *(48 h)* | % mortality | Conc. *(72 h)* | % mortality | Conc. *(96 h)* | % mortality |
|---------------|-------------|---------------|-------------|---------------|-------------|---------------|-------------|
| 15            | 20          | 14            | 20          | 13            | 20          | 12            | 20          |
Table 3. Parameter estimates for the probit analysis

| Probit a/Parameter | Estimate | Std. Error | Z  | Sig. | L. B  | U. B |
|--------------------|----------|------------|----|------|-------|------|
| concentration of pesticide | 20.571 | 6.425 | 3.202 | .001 | 7.979 | 33.164 |
| Intercept | -24.963 | 7.830 | -3.188 | .001 | -32.793 | -17.134 |

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base (10.000logarithm)). L.B=Lower bound, U.B=Upper bound at 95% confidence intervals.

Table 4. Estimated lethal concentration values and confidence limits

| Probability | 95% Confidence Limits for concentration of pesticide | 95% Confidence Limits for log(concentration of pesticide) |
|-------------|--------------------------------------------------|--------------------------------------------------|
| 15.5        | 14.5                                             | 13.5                                             | 12.5                                             | 11.5                                             | 10.5                                             |
| 111          | 110                                              | 109                                              | 108                                              | 107                                              | 106                                              |

*Estimated LC50 values in semi-static test for 24 h: 17.322 mg/L (16.781-19.877) 48 h: 17.321 mg/L (16.521-18.134), 72 h: 16.721 mg/L (16.063-17.952), and 96 h: 16.330 mg/L (15.388-17.143).
Figure 1. Probit line graph of acute toxicity of Dimethioate on fish Labeo rohita

Table 5. Effect of Dimethioate on Glycogen content in different tissues of fish Labeo rohita in sublethal and lethal concentrations– mg/g wet weight of tissue

| Tissues | Control | Sub-lethal | Lethal |
|---------|---------|------------|--------|
| Muscle  | 19.15±0.416 | 7.18±0.316(62.50%) | 5.29±0.341(72.37%) |
| Kidney  | 9.18±0.316 | 7.46±0.347(18.73%) | 6.34±0.373(30.93%) |
| Gill    | 12.50±0.314 | 8.15±0.316(34.08%) | 3.38±0.374(72.96%) |
| Liver   | 27.14±0.316 | 8.29±0.344(69.45%) | 5.39±0.377(80.14%) |
| Brain   | 10.29±0.341 | 6.46±3.47(37.22%) | 5.39±0.371(47.61%) |

Values represent mean of five individual observations, mean ± S.D and the mean difference at the *P* < 0.05 level.
Figure 2. Changes in the glycogen (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to lethal and sub-lethal concentrations of Dimethoate for 8 days. (mean±S.D, n=5 observations, p<0.05.)

Figure 3. Changes in the glycogen (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to lethal and sub-lethal concentrations of Dimethoate for 8 days in a steep slope and a flat slope direction, (mean ± S.D), n=5 observations, p < 0.05.
Table 6. Effect of Dimethoate on Protein content in different tissues of fish Labeo rohita in sub-lethal and lethal concentrations – mg/gm wet weight of tissue

| Tissues | Control | Sub-lethal | Lethal |
|---------|---------|------------|--------|
| Muscle  | 53.4±0.286 | 52.0±0.301(26.21) | 51.15±0.032(42.13) |
| Kidney  | 55.4±0.286 | 47.4±0.344(34.29) | 43.1±0.036(22.21) |
| Gill    | 94.3±0.371 | 69.4±0.347(26.40) | 56.5±0.323(40.08) |
| Liver   | 51.3±0.328 | 25.6±0.325(49.80) | 49.4±0.377(39.33) |
| Brain   | 56.2±0.340 | 58.10±0.316(33.29) | 54.4±0.281(32.02) |

Values represent mean of five individual observations, ± S.D and the mean difference at the P< 0.05 level.

Figure 4. Change in the Total protein content (mg/gr wet weight of the tissue) in different tissues exposed to sub-lethal, lethal concentrations of Dimethoate for 8 days (mean±S.D), n=5 observations, p < 0.05
Figure 5. Change in the Total protein content (mg/gr wet weight of the tissue) in different tissues exposed to sublethal, lethal concentrations of Dimethoate for 8 days, in a steep slope and a flat slope direction. (mean±S.D.), n=5 observations, p < 0.05.

The observed percentage of mortality of *Labeo rohita* for Dimethoate in static tests for 24, 48, 72 and 96 hours were 17.532 mg/l (16.781-19.877), 17.321 mg/l (16.521-18.134), 16.72 mg/l (16.063-17.952), 16.350 mg/l (15.388-17.143), mg/l respectively. It is evident from the results that Dimethoate can be rated as toxic to fish. In the present study the control fish showed normal behavior, they were active with well-coordinated movements, jerky movements, hyper secretion of mucus, opening mouth for gasping, losing scales, hyperactivity were observed experimental group but in the toxic environment the fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium due to inhibition of AChE activity, leading to accumulation of acetylcholine in the cholinergic synapses, leading to hyper stimulation (10). In which acute chemical toxicity is determined as a 96 h LC50 value. However the environmental significance of death of individuals after short term exposure to high concentration is questionable (11). The varying degree of mortality reported in this study is consistent with the earlier reports. It has been suggested that differences in an organism’s biological adjustment and behavior response to change in water chemistry (12, 13). The toxicity of a pesticide could vary from species to species. These results have important implications for ecological risk assessments, particularly those that focus on the toxicity of individual chemicals as the basis for estimating impacts to imperiled aquatic species. Although the importance of multiple stressors is widely recognized in aquatic ecotoxicology (14), pesticide mixtures continue to pose major challenges for natural resource agencies (15). In the present study of test organism showed normal behavior in control group but jerky movements, hyper secretion of mucus, opening mouth for gasping, losing scales, hyperactivity were observed experimental group. Behavioral characteristics are obviously sensitive indicators of toxicant effect. In toxic medium of Dimethoate the fish *Labeo rohita* sank to bottom of the test chamber and independency in swimming. Subsequently fish moved to the corners of the test chambers, which can be viewed as avoidance behavior of the fish to the toxicant. In the toxic environment fish exhibited irregular, erratic, darting swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms are due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ensuing hyper stimulation. And inhibition of AChE activity is a typical characteristic of organophosphate compounds (16, 17). Gulping air and swimming at the water surface (surfacing phenomenon) were observed also with mucus secretion on the body in both the lethal and sub-lethal exposure periods (18). In sub-lethal and lethal exposure periods there is a significant decrease glycogen content in liver, kidney and followed by other tissues of freshwater fish *Labeo rohita*, relative to control group. Maximum decrease glycogen level is observed at lethal and sub-lethal exposure in all tissues but maximum in liver (80.14%), muscle (62.50%) and minimum in kidney (18.73%) as compared to other tissues were observed. Glycogen is found in all the tissues of the body but in varying amounts. Carbohydrates are stored in liver, muscle as glycogen as such that contain more energy. It has both functional and storage significance. The results indicated that the liver is vital organ of carbohydrate metabolism and were drastically affected by Dimethoate.
In almost all the tissues of fish brain, gill, kidney, liver, and muscle tested at sub-lethal and lethal concentrations of Dimethoate, a decrease in glycogen levels were noticed during the exposure periods (Table 1). Carbohydrate metabolism is mainly concerned to fulfill energy demand of animals by its aerobic and anaerobic segment. Among various organs, higher glycogen levels were observed in liver, because involvement of liver in glycogen synthesis and utilization. Glycogen is the major storage form of energy in liver and muscle tissues. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose levels. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. In almost all the vital organs brain, gill, kidney, liver and muscle at lethal and sub-lethal concentrations of Dimethoate, showed a decrease in glycogen levels were noticed during the exposure periods. There is a significant decrease proteins in liver, kidney and followed by other tissues of freshwater fish Labeo rohita after 8 days exposure relative to control. Maximum decrease protein content is observed at lethal and sub-lethal exposure in all tissues but mainly in liver (49.80%), and muscle (42.13%) as compared to kidney (22.21%) and brain (32.02%) tissue. The proteins of the body like carbohydrates and lipids also serve as a source of energy. There is no storage form of protein. The reserve protein appears to be drawn from the tissues themselves and organs such as liver, kidney and blood during the time of fasting. During the exposure periods decrease in protein content were observed in kidney, liver, muscle, gill and brain tissues. An alteration of protein metabolism was observed in fish exposed to various types of environmental stresses like metals and pesticides, we are also agreeing with these authors,

Table 6 (19, 20). Stress proteins are considered to be general indicators of sub-lethal cellular protein damage. The quality of protein is dependent on the rate of protein synthesis, or on rate of its degradation. The quality of the protein may also be affected due to impaired incorporation of amino acids into polypeptide chain (21-23) suggested that the fish exposed to pesticides may compensate any possible protein loss by increasing its protein synthesis (24), concluded that compensatory production of enzymes lost as result of tissue necrosis or to meet increased demand to detoxify the pesticides might have necessitated enhanced synthesis of enzyme proteins. Increase in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis. The changes in total protein content of various tissues of Channa punctata when exposed to carbaryl and 1-Naphthol were reported by (25, 26) reported that there is marked decrease in the protein content of various tissues like kidney, liver, muscle, exposed to sub-lethal and lethal concentrations of Cypermethrin. The survival ability of animals exposed to stress mainly depends on their protein synthetic potential. The degradation of protein suggests the increase in proteolytic activity and possible utilization of their products for metabolic purposes and cause damage to tissues (27), similar decreasing trend was also observed by (26, 28), reported that the depletion of glycogen content when exposed to sub-lethal concentration of Rogor to freshwater fish, Heteropneustis fossil (29) reported that chlorodecone induced changes in carbohydrate metabolism of the freshwater cat fish Heteropneustis fossil were studied, both hepatic and muscle glycogen concentrations decreased significantly (30, 31) concluded that Malathion is highly toxic to fish and due to its toxicity protein content was reduced in the kidney, reported that exposure of fish to sub-lethal concentration of Malathion decreased the protein content in the gill (-22 to –39%) over the control group during the experimental period of 30 days (31, 32) reported that fish Channa punctata exposed to insecticide Dimethoate exhibited a significant dose-dependent decrease in total protein, glycogen, pyruvate level and cytochrome oxidase activity in liver, muscle and gonad tissues (33) reported that the decline in protein level indicates an acceleration of protein catabolism during Cypermethrin and fenvalerate intoxication (34) reported that exposed to sub-lethal concentration of fenvalerate both technical grade and 20% EC, the total protein content was decreased in gill, liver, kidney, brain and muscle of Channa punctatus (35) reported that the muscle protein of Lepidocephalecthyes thermals at different sub-lethal concentrations decreased in all selected sub-lethal concentrations (36) reported that effects of Cartap Hydrochloride and urea in freshwater fish Oreochromis mossambicus, glucose level appears also to be related at least in part to the detoxification mechanisms particularly in the hepatic tissue, which is the major site for metabolism in animals (37) reported that the glycogen depletion was observed when chloropyryphos an organophosphate exposed to freshwater fish Catla catla, Labeo rohita and Cirrhinus mrigala (38) reported that carbohydrate metabolism is disturbed when L. rohita is exposed to fenvalerate; alterations in the blood glucose level indicate the variations in the carbohydrate metabolism of the fish under toxic stress (39) reported that decrease in the level of glycogen content was observed in all tissues with increase in exposure periods (38) reported that the glycogen depletion was observed, when chloropyryphos, an organophosphate exposed to freshwater fish Catla catla, Labeo rohita, Cirrhinus mrigala (27) reported that the effects of sub-lethal doses of Dimethoate and Malathion on growth parameters in Oreochromis niloticus, glycogen, protein and lipid in fish muscle gradually decreased with increased pesticidal concentrations, the effects of sub-
lethal doses of Dimethoate and Malathion in Oreochromis niloticus bio accumulated in the liver was higher than in gill or muscle.

4. CONCLUSION
In the present study, a comparison of lethal concentration values and behavior change indicated that Dimethoate was less toxic than other pesticides to fishes. The results of these studies may provide information to selection of acute toxicity to be considered in field Ecotoxicology and biomonitoring efforts designed to detect the bioavailability of Dimethoate.

Funding/ Support
Not mentioned any Funding/ Support by authors.

ACKNOWLEDGMENT
Not mentioned any ACKNOWLEDGMENT by authors.

AUTHOR CONTRIBUTION
This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST
The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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