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Study on toxic effect of Dimethoate(Rogor) on *Ciprinus carpio (*common carp)

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Abstract
Survey conducted in agricultural areas of Rampur Basti, Kumurakata, Pubdhaniram Pathar and Garnagar region of Hojai district of Assam in October, 2021 revealed utilization of several organophosphates including Dimethoate (Rogor)by the farmers. Therefore, an investigation on acute and chronic toxicity of various organophosphates including dimethoate has been initiated in the Zoology laboratory of Rabindranath Tagore University of Hojai district in Assam. In the present study, the 24, 48, and 72 LC_{50} value of dimethoate for Cyprinus carpio(Length=20±1.5 cm; weight= 12.5± 0.5 g) is found to be 1.89, 1.78, and 1.70 mg/L respectively. Although slight behavioral change was exhibited by the fish exposed to 1.40mg/L, there was no mortality. 100% mortality of Cyprinus carpio was recorded at 2.10 mg/L of dimethoate. Study on opercular movement (OCM) revealed significant decrease (ANOVA test,F=52.15, $p < 0.01$) with rising pesticide concentration in the dimethoate exposed fishes. Behavior such as erratic swimming, imbalance, frequent surfacing was observed in fishes exposed to higher concentrations (1.60mg/L onwards). Further studies are being done to evaluate the various effect of dimethoate on this commercially important fish.

***Key words*:** *Dimethoate, Cyprinus carpio, Pesticides, Toxicity, Acute.*

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I. Introduction

Pesticides may be defined as any substance or mixture of substances intended for preventing or controlling any unwanted species of plants and animals. Pesticides in water resources adversely affect both the ecosystems and humans. Those materials have been thought as probable mutagens as they comprise constituents to trigger deviations in DNA [5]. According to the world health organization (WHO), about 10,00,000 human being are affected by acute poisoning by contact with pesticide. Each year, a death rate between 0.4 and 1.9% is recorded [2].

The use of insecticides has increased considerably throughout the world since last two decades with a record of approximately 5400 million tons in 2000. [2] They are beneficial by providing reliable, persistent and relatively complete control against harmful pests with less cost and effort, but wide use of the pesticides has led to increased contamination of aquatic ecosystem [3, 4].

The insecticides are transported to the fresh water bodies through run-off and affect the aquatic organism of that area. Their effects are less than desirable when they leave the target compartment of the agricultural ecosystem. As a result, many other organisms sharing the same environment are accidentally poisoned. Up to 90% of pesticides never reach their intended targets and fishes are one of the non-target biological groups mostly affected [5].

A number of insecticides currently in use today have mammalian toxicity and necessitates considerable precaution in their applications. Among different classes of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity less persistence and rapid biodegradability in the environment. Organophosphates accounted for 70% of the total insecticides used in US in the year 2001[6]. Dimethoate [IUPAC Name- 0, 0 dimethyl S- (N methyl carbamoylmethyl) phosphoro-dithioate], CAS No. 60-51-5, is an organophosphate available in the market by the trade name of Rogor. It is a systemic insecticide used for control of a wide variety of insect pests of fruits, vegetables and crop plants. Dimethoate is highly selective as insecticide because relative rate of degradation of toxicant by enzymes (esterases and amidases) are very low in insects as compared with those of mammals [7]. Like other organophosphates, rogor is also an acetylcholinesterase inhibitor [6], therefore, works primarily as nerve poison which is reflected in uncoordinated abnormal behavior of the fish soon after exposure.

Dimethoate is acutely toxic and is classified as a possible human carcinogen by USEPA based on occurrence of tumors in mice. In the WHO acute hazard ranking, this is rated as moderately hazardous. USEPA has registered dimethoate as a systemic organophosphate insecticide, but in 2006 it released Interim Re-registration Eligibility Decision (IRED) document for dimethoate in accordance with FQPA requirements [6]. Heavy contamination of water bodies by pesticides can lead to mass mortality of fish and other aquatic fauna. The common carp-*Cyprinus carpio* (Linn.), is a highly palatable fish and preferred for culture due to its high growth rate and prolific breeding in confined water [8]. This exotic carp was introduced in India in the year 1957, now it is well adapted in culture ponds and even migrated to the river system of Northern India.

Survey conducted in Hojai agricultural and market areas revealed use of several organophosphates including Dimethoate (Rogor). Previously, impact of common agricultural pesticide Imidaclopridon the mortality and growth of *Polypedates teraiensis* tadpoles in acute and chronic exposure was studied in Zoology laboratory of Rabindranath Tagore University by Tamuly Dulumoni, Basumata Caroline, Nath Ratul and Dey Mithra. In that study, mean percent mortality increased significantly ($p < 0.05$) with increased pesticide concentration and exposure time. Percent mortality of tadpoles was significantly affected by concentrations ($F = 52.15$, $p < 0.01$). In exposure to sublethal concentration of 2.091, 1.045 and 0.697 mg/L, the percent mortality of tadpoles was significantly different ($F = 8.87$, $p = 0.002$) to the control except at 0.697 mg/L ($p = 0.09$). The weight of the tadpoles decreased in all the concentrations with maximum reduction on 21 day [9]. Present study is being conducted to evaluate the direct toxic effect of rogor on*C. Carpio* by estimating LC_{50} value and then sublethal exposure of different concentrations of toxicant on mortality.Alterations in fish behavior, particularly in non-migratory species, can also provide important indices for ecosystem assessment. As any change in the behavior of fish indicates the deterioration of water quality, the effect of this organophosphate on behavior of the exposed fish was also studied.

II. Materials And Method

1. Survey:

The study was mainly carried out in the agricultural fields of Rampur Basti, Kumurakata, Pubdhaniram Pathar and Garnagar region of Hojai distric in October, 2021. The important crops grown in this region are Rice, Vegetables, Sugarcane, Banana and Jute. A total of 17 farmers from four different regions of Hojai were interviewed. All of them were actively engaged in the application of pesticides and therefore were directly exposed to them. During the second phase of the survey, 9 shops were visited all over Hojai and information about different pesticides was collected.

2. *Cyprinus carpio communis* Linn.

Common carp (Length=20±1.5 cm; weight= 12.5± 0.5 g) were collected from local ponds in the month of November. They were brought to the Zoology Laboratory of Rabindranath Tagore University carefully in plastic bags containing water, avoiding any injury and after giving a bath of 0.5% $KMnO_4$ solution for 2 minutes; they were transferred to a glass tank of 500L capacity for acclimatization in laboratory condition for two weeks.

During acclimatization fish were fed daily with rice bran mixed with mustard oil cake in the ratio of 2:1. Leftover food in the tank was removed daily when water of the tank was changed. Dead fishes, whenever located were removed immediately to avoid fouling of the tank water.

3. Dimethoate:

Stock solution of 1 mg/ml dimethoate was prepared in absolute alcohol. Variable quantities of stock solution were added to same volume of water in different plastic troughs. The pesticide was mixed thoroughly by stirring with a glass rod before adding fishes in to the plastic troughs.

4. Exposure of the fish to the pesticide and recording of data:

For range-finding test, the fishes were sorted out and separated in to six groups of four fish in each trough. They were exposed to 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L concentrations of Dimitheoate. Each group was placed in a glass trough containing 10L of water. After determining the range, they were exposed to final concentration of 1.40, 1.50,1.60, 1.70, 1.80 and 2.10 mg/L of Dimethoate to determine LC_{50} values for 24, 48 and 72 hrs. Four replicates were taken for each concentration. A control was run simultaneously containing 2 ml. of absolute alcohol in 10 liter water. During assay no food was administered to fishes. Mortality in each group was recorded, and the dead fishes were removed immediately. Fish mortality data obtained with respect to time was analyzed by SPSS based on Finney's Probit Analysis method for determination of LC_{50} values and 95% lower and upper confidence limits. Behavioral responses of fishes were noted during first 6 h and at 24, 48, 72 hours of exposure.

5. Physicochemical characteristics of water were determined which was as follows:
Temperature 26 ± 2°C, pH 7.3 ± 0.5, and Dissolved oxygen was 7.4 ± 0.5 mg/L.

6. Behavioral responses of fishes were noted during first 6 h and at 24, 48, 72 hours of exposure. Opercular movement (OCM) per minute was also recorded at same interval of exposure and the values were plotted in Figure 1. The observed values of OCM were analyzed by two way ANOVA.

III. Result

1. Mortality: Although slight behavioral change was exhibited by the fish exposed to 1.40mg/L, there was no mortality. 100% mortality of *Cyprinus carpio* was recorded at 2.10 mg/L of dimethoate.

2. LC_{50} value: LC_{50} values of dimethoate for 24, 48, 72 h and their respective 95% confidence limits as calculated by Probit analysis by SPSS, 1.5 version statistical software are given in table 1.

Table 1: LC50 Values and 95% Lower & Upper Confidence Limits for Dimethoate at different intervals for the fish *Cyprinus carpio*

Duration (Hour)	LC50 Values	95% lower confidence limit	95% upper confidence limit
24	1.89	1.78	1.99
48	1.78	1.72	1.84
72	1.70	1.63	1.74

3. Behavior: Within a few minutes of exposure to higher concentrations (1.60 mg/L onwards), the carps exhibited a number of abnormalities in their behavior as follows:

i. The fishes appeared excited, the swimming became erratic.

ii. After about an hour, the fishes suddenly started surfacing more frequently.

iii. The surface water in test troughs appeared foamy.

iv. In concentrations more than 1.80mg/L, the fishes could hardly swim upwards and remained in the bottom of the troughs most of the time.

4. The Opercular movement: The OCM was decreased with rising pesticide concentration in the exposed fishes. The results are statistically significant (ANOVA test,$F=52.15$, $p < 0.01$).

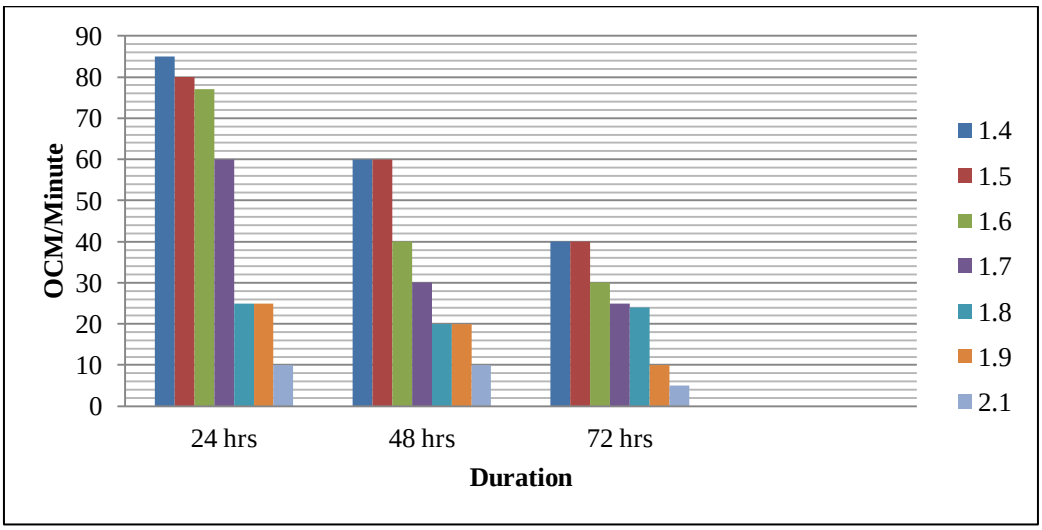

Figure 1: Two-way ANOVA of Opercular movement in pesticide exposed fish. Pesticide Concentration effect: Significant $F=50.15$, $p<0.01$.

IV. Discussion

This study on toxicity of Dimethoate on *Cyprinus carpio* revealed that mortality due to exposure to a toxicant depends upon the sensitivity of the species used for the experiment [6]. The LC_{50} values also vary in the same species as has been found in this study. There are reports on sensitivity of some air-breathing teleost species such as *Clarius batrachus* [11] and *Channa punctatus* (Srivastava, V.K. and A. Singh, 2001) [12] to dimethoate which are very high. The LC_{50} value of Dimethoate in *C. batrachus* is 65 mg/L for 96 h, and 17.9 mg/L for 96 h in *C. punctatus*. However, in *Heteropneustes fossilis* [11] very low LC_{50} value for24, 48, 72 and 96 h dimethoate exposure is recorded as 3.38, 3.23, 3.08 and 2.98 mg/L.

In carps, very low LC_{50} values have been recorded by some researchers. In *Catla catla*, [13] the LC_{50} value for 96 h is reported as 0.007 ppm and in the present study the 24, 48, and 72 LC_{50} value of dimethoate for

Cyprinus carpio is found to be 1.89, 1.78, and 1.70 mg/L respectively. Again, De Mel, *et al.* [8] has reported very high LC_{50} value (26.11mg/L) of dimethoate for 96 h in *Cyprinus carpio* fry (size 20 – 34 mm). In air breathing fishes, LC_{50} values are much higher than in carps for the same pesticide, probably because the fishes with accessory respiratory organs can adaptively shift towards aerial breathing when the water is contaminated. Even in carps, the variation in LC_{50} values may be attributed to the different tolerance limit of different species. This is in agreement with Sprague[14]who observed variation in LC values for the same species and toxicant depending on size, age and condition of test species along with experimental factors.

Behavioral changes observed in the exposed carp fingerlings, appear to be the manifestation of dimethoate toxicity resulting into hypoxic condition as studied in *Anabas testudineus* after exposure to monocrotophos [15].

Decreased opercular movement with increasing concentration of the pesticide was observed in this study. Reduction in oxygen consumption in *Cyprinus carpio* has also been reported after sublethal exposure of copper [16] and antimony chloride by some workers [17]. There is however a report on increased opercular rate and coughing in the common carp exposed to industrial effluents [6] Carlson *et al.* have shown that there is direct relationship between coughing response with concentration of pollutant in water [18].

As shown by Rao J.V. *et.al.*, 2005, erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and neuromuscular junctions. Tremors, gradual loss of equilibrium and drowning are caused by adverse effects of organophosphate on central nervous system. Observations of the behavioral responses of tadpoles exposed toImidaclopridpesticides were conducted during 96 h acute toxicity test by Tamuly D. *et al.* The control group showed normal behavior during the test period. Tadpoles exposed to different concentrations of pesticides showed the similar response. The treated tadpoles showed abnormal behavior, like hyperactivity followed by loss of balance, erratic movements, becoming motionless, lying down on the bottom and finally leading to dead [9].

V. Conclusion

It may be concluded that carps are very sensitive to dimethoate than some teleosts. The erratic behavior and decrease in opercular movement of the exposed fishes is attributed to the direct toxic effect on the nervous system of the carps. Further studies on toxicity of dimethoate and its combinations with other pesticides in field conditions are required. There is an urgent need of investigationon the effect of dimethoate and other pesticides on fish and mammalian species at cellular levelsto specifically pinpoint the exact nature of damage to the animals. USEPA has considered Dimethoate as acutely toxic and classified it as a possible human carcinogen. Therefore, there is huge scope for studies in this direction in future.

Survey conducted by Zoology department from 1st October 2021 to 4th October 2021 revealed utilization of several insecticides/Herbicides/Fungicides in Hojai area including Dimethoate. Interview of the farmers working in agricultural land fields of Rampur Basti and Raikata, Hojai also revealed that the farmers are using these toxicants without any proper knowledge on dosage per land area of crop fields. Therefore, there is an urgent need to organize awareness campaigns in the said areas to prevent unscrupulous use of these poisonous substances for their own benefits and to minimize adverse effect of the toxicants on ecosystem of the area.

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