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Research Article
Monocrotophos Toxicity to Freshwater Fish, *Clarias batrachus* (Linnaeus, 1758) at Two Different pH Levels

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Abstract

Background and Objective: Pesticides contamination in an aquatic ecosystem may affect the normal physiology and early development of aquatic animals which causes reduced fish growth, productivity and death also. Therefore, the present study has been aimed to investigate the monocrotophos 36% SL toxicity to a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) at two distinct selected levels of pH strength to know its toxicity level. **Materials and Methods:** A full scale basic routine bioassays test (96 hrs) has been carried out to understand the acute toxicity of monocrotophos 36% SL to a freshwater air-breathing fish, *Clarias batrachus* (Linnaeus, 1758) at two different selected level of pH strength (8.62±0.3 and 6.62±0.3) by a Probit Analysis software method (Finney's). Data were subjected to software, SPSS (Version 16) and Graph Pad Prism software (Version 7) for statistical analysis. **Results:** The 96 hrs LC_{50} values of monocrotophos 36% SL were reported to be 89.611 and 67.616 ppm for experimental fish, *Clarias batrachus* (Linnaeus) at pH, 8.62±0.3 and 6.62±0.3, respectively whereas safe dischargeable concentrations were documented too low as in comparison of safe or harmless concentrations. A significant decline in Dissolve Oxygen (DO) content (mg L^{-1}) and an increasing trend in free CO_2 (mg L^{-1}) were observed from 0-96 hrs during bioassay. Furthermore, Group analysis (One way ANOVA) between LC_{50} values (24, 48, 72 and 96 hrs) of monocrotophos 36% SL has also performed. Any behavioural alteration in the test fish was also documented carefully towards the course of the static bioassay test. **Conclusion:** Monocrotophos 36% SL are found to be highly toxic to test fishes *Clarias batrachus*, (Linnaeus) at lower pH, 6.62±0.3 as compared to higher pH strength, 8.62±0.3.

Key words: Acute toxicity, LC_{50} value, monocrotophos 36% SL, *Clarias batrachus* (Linnaeus), dissolve oxygen, organophosphate pesticide, public health

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fishes are affected by environmental contamination of water and therefore certain physiological and biochemical processes may be altered significantly due to the entering of pollutants in fishbody[1]. It has been previously reported that the organophosphate insecticide is more preferred in households, agriculture and public health programme for controlling a variety of insect pest due to less persistent and more effectiveness in place of organochloride, display a high degree of environmental persistent[2]. Therefore, the enhanced concentration of organophosphate has been seen in aquatic bodies which ensure the acute/chronic toxicity to the ichthyofaunal species[3-5].

Monocrotophos was reported as an organophosphate based insecticide/acaricides which are effective against a variety of pest viz: Sucking, boring and chewing insects and also acts on spider mites on some plants. Since Monocrotophos was found to be moderately toxic to the fish and it may create hazardous effects in aquatic lives. Moreover, fish are reported to be important bioindicators and integrators of toxicant due to wide distribution, free swimmers, quick response to contaminants and good food source for human[6]. Therefore, because of this, an effort has been made to investigate the monocrotophos 36% SL toxicity to a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) at two different selected levels of pH strength to compare its toxicity level in the water.

MATERIALS AND METHODS

Study area: The study was carried out at Department of Zoology Lab, J.P. University, Chapra from May-June, 2019.

Sample collection and selection: Healthy test fishes, *Clarias batrachus* (Linnaeus) of approximately equal sizes (5.0±0.6 cm) were collected and acclimatized separately in the water tank of 500 L capacity for15 days, were selected for the bioassay tests.

Stock solution preparation: Common stock solution was prepared for monocrotophos 36% SL by the standard formula:

$$N_1V_1 = N_2V_2$$

Where:

N_1 = Concentration of pesticide

V_1 = Volume of available pesticide

N_2 = Required concentration of pesticide to be prepared

V_2 = Volume of solution required for application

The series of various concentrations of monocrotophos 36% SL were prepared by adding (with the help of micropipette) the common stock solution into the measured diluents water, used in the full-scale static bioassay tests. The series of different concentrations of monocrotophos 36% SL are based on the progressive bisection of intervals on a logarithmic scale[7]. The tap water of pH, 8.62±0.3 and water of pH, 6.62±0.3 (prepared by dropping HCl solution with the help of micropipette) were utilized as experimental water during the static bioassay test.

Preliminary or screening test: The static bioassays for 96 hrs were conducted in a test container with a 5 L capacity (filled with 4 L toxicant solution) for the evaluation of acute toxicity of monocrotophos 36% SL to the test fishes, *Clarias batrachus* (Linnaeus). Critical concentration range (highest and lowest concentrations) of selected toxicant at which most of the experimental fishes died or survived within a specified exposure period (24, 48, 72 and 96 hrs) were known by preliminary or screening tests.

Full-Scale static bioassay test: During this, the test containers of 5 L capacity (filled with 4 L toxicant solution) were placed in three rows and were labelled with the details of the experiment (Date and time of the experiment, concentration and replicate number). There are 10 acclimatized tests fishes were placed in each experimental test containers along with controls (runs simultaneously). The toxicant solutions of different concentration were renewed and replaced every 24 hrs by newly made toxicant solutions and the test was continued for 96 hrs duration. The numbers of test fishes that died (at a specified period of intervals i.e., 24, 48, 72 and 96 hrs) in each concentration of toxicant solution were observed, make in the count and removed.

Parameter calculation: The LC_{50} values, 95% confidence limits and R-values (Confidence ratio (UCL/LCL) of monocrotophos 36% SL were analyzed for different concentration and time intervals (24, 48, 72 and 96 hrs) by Probit Analysis statistical software method[8]. The presumable safe and safe dischargeable concentrations of monocrotophos 36% SL for the test fishes, *Clarias batrachus* (Linnaeus)were also calculated[9]. Behavioural changes in the exposed fish were analyzed after subjected to the various concentrations of selected toxicants. The pH of the toxicant solution was measured by a digital pH meter. Dissolve

Oxygen (DO) content (mg L^{-1}) of the toxicant solution were measured at the interval of 0, 24 and 96 hrs by using Winkler Method. The free CO_2 was analyzed at the interval of 0, 24 and 96 hrs by the titrimetric method using phenolphthalein indicator and NaOH titrant.

Statistical Analysis: The data were subjected to statical software, SPSS (Version 16) to analyse the LC_{50} values, Upper and Lower Confidence Limits and One way ANOVA whereas, Graph Pad Prism software (Version 7) were used for S.D. analysis.

RESULTS

Mortality rate (%) of *Clarias batrachus* (Linnaeus) due to exposure (upto 96 hrs) of different concentration of monocrotophos 36% SL (in ppm) at pH, 8.62±0.3 and 6.62±0.3 has been depicted by Fig. 1 and 2. Maximum mortality was observed as 10 (at 135 ppm) and minimum as 2 (at 75 ppm) at pH, 8.62±0.3 whereas, 9 and 3 test fishes died at 100 and 56 ppm, respectively during 96 hrs exposure to the toxicant. Different concentrations of monocrotophos 36% SL (based on a logarithmic scale, APHA, 2005) were utilized in full-scale bioassays (96 hrs) at both the selected pH strength, has been described by Table 1. The LC_{50} value, UCL (Upper Confidence Limits), LCL (Lower Confidence Limits) and Confidence Ratio (R = UCL/LCL) at both the selected pH strength has been elucidated in Table 2 and 3. The

Table 1: Test concentrations of monocrotophos 36% SL used in full-scale static bioassays (96 hrs) for two different selected levels of pH

pH (8.62±0.3)	pH (6.62±0.3)
Monocrotophos 36% SL (ppm)	Monocrotophos 36% SL (ppm)
135	100
115	87
100	75
87	65
75	56

*Based on a logarithmic scale (APHA, 2005)

Table 2: Median lethal concentrations (LC_{50} value) of monocrotophos 36% SL for *Clarias batrachus* (Linnaeus) at pH of 8.62±0.3

Duration (hrs)	Monocrotophos 36% SL (ppm)	LCL	UCL	R
24	128.336	112.703	192.537	1.708
48	112.684	99.238	137.177	1.382
72	100.237	88.102	112.045	1.271
96	89.611	73.322	99.906	1.362

UCL: Upper confidence limits, LCL: Lower confidence limits and R: Confidence ratio (UCL/LCL)

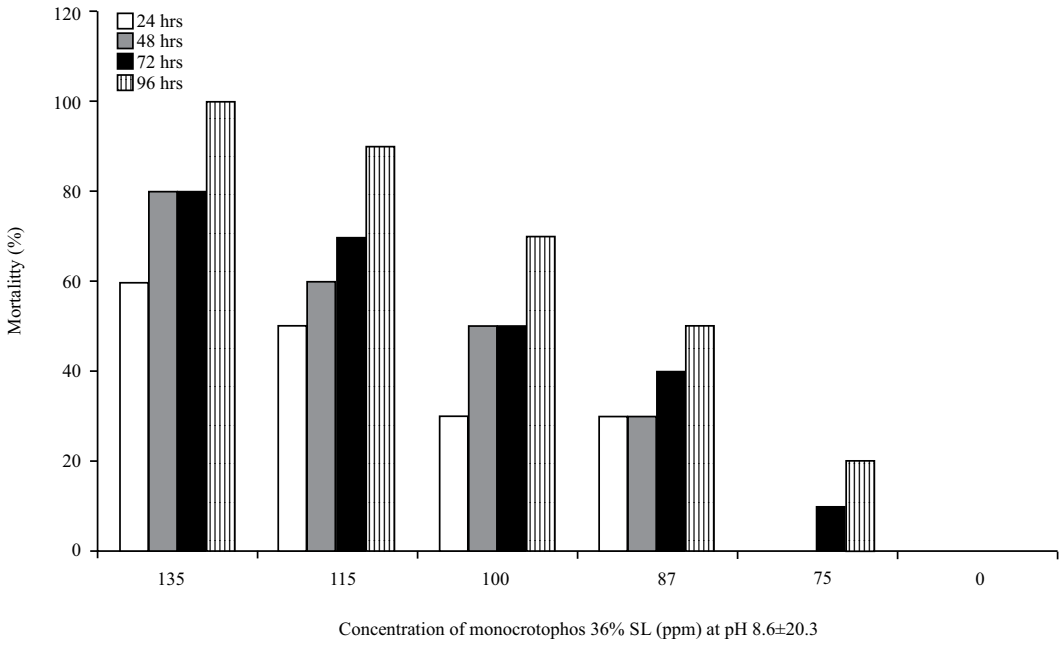

Fig. 1: Mortality rate of *Clarias batrachus* (Linnaeus) during exposure of different concentration of monocrotophos 36% SL at pH, 8.62±0.3

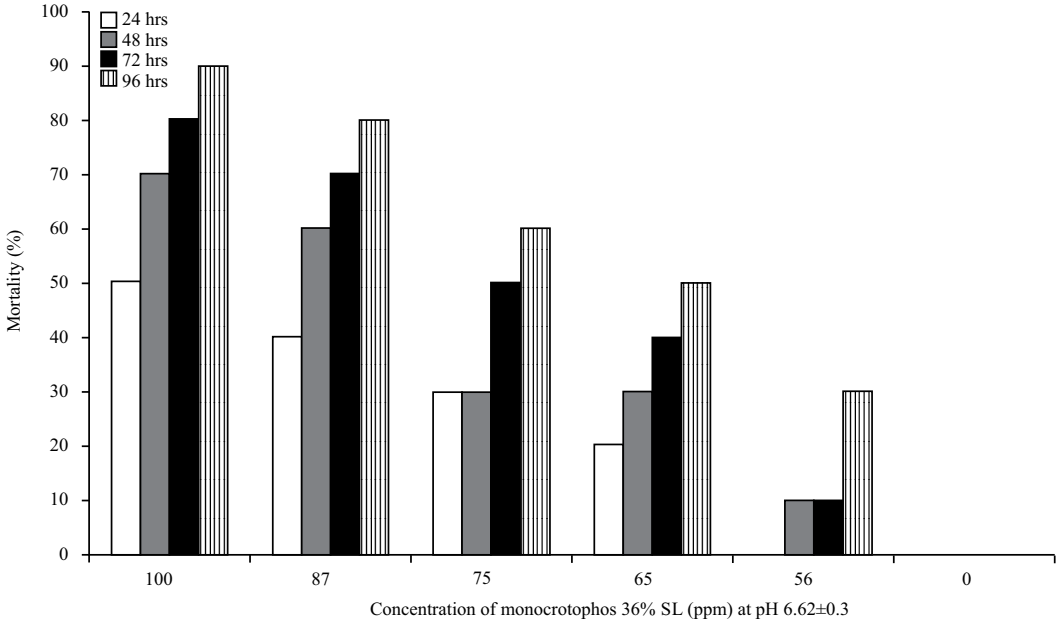

Fig. 2: Mortality rate of *Clarias batrachus* (Linnaeus) during exposure of different concentration monocrotophos 36% SL at pH, 6.62±0.3

Table 3: Median lethal concentrations (LC_{50} value) of monocrotophos 36% SL for *Clarias batrachus* (Linnaeus) at pH of 6.62±0.3

Duration (hrs)	Monocrotophos 36% SL (ppm)	LCL	UCL	R
24	95.656	84.233	142.367	1.690
48	84.235	74.299	102.168	1.375
72	76.563	66.049	87.334	1.322
96	67.616	52.638	76.983	1.462

UCL: Upper confidence limits, LCL: Lower confidence limits and R: Confidence ratio (UCL/LCL)

Table 4: Safe or harmless and safe dischargeable concentrations of monocrotophos 36% SL for *Clarias batrachus* (Linnaeus)

Concentrations	pH 8.62±0.3	pH 6.62±0.3
Safe or harmless (as ppm)	29.682	22.253
Safe dischargeable (as ppm)	1.178	1.065

Table 5: Dissolve Oxygen (DO) content (mg L^{-1}) and percentage alteration after 96 hrs during the bioassay test

	Dissolve Oxygen (DO) content (mg L^{-1})			
Concentration of monocrotophos 36% SL	0 hrs	24 hrs	96 hrs	Percentage alteration (after 96 hrs)
Control (0.00 ppm)	7.20±0.0721	7.10±0.0902	7.00±0.0551	-2.77
100 ppm (Highest concentration at pH 6.62±0.3)	7.20±0.0473	6.40±0.0666	6.00±0.0306	-16.66
135 ppm (Highest concentration at pH 8.62±0.3)	7.20±0.0346	6.60±0.1114	6.10±0.0404	-15.27

Table 6: Free CO_2 (mg L^{-1}) and percentage alteration after 96 hrs during bioassay test

	Free CO_2 (mg L^{-1})			
Concentration of monocrotophos 36% SL	0 hrs	24 hrs	96 hrs	Percentage alteration (after 96 hrs)
Control (0.00 ppm)	4.20±0.0252	4.50±0.0950	4.80±0.0451	14.28
100 ppm (Highest concentration at pH 6.62±0.3)	4.20±0.0681	7.18±0.1007	7.50±0.1114	78.57
135 ppm (Highest concentration at pH 8.62±0.3)	4.20±0.0551	7.10±0.0436	7.34±0.0854	74.76

LC_{50} value of monocrotophos 36% SL for 24, 48, 72 and 96 hrs to *Clarias batrachus* (Linnaeus) were noticed as 128.336, 112.684, 100.237 and 89.611 ppm, however, the R-values were calculated as 1.708, 1.382, 1.271 and 1.362, respectively at pH, 8.62±0.3 (Table 2). Furthermore, the 24, 48, 72 and 96 hrs LC_{50} values and R-values of

Table 7: One way ANOVA between LC_{50} value of monocrotophos 36% SL for *Clarias batrachus* (Linnaeus)

	pH, 8.62±0.3 versus pH, 6.62±0.3 (Group-1)			pH, 6.62±0.3 versus pH, 8.62±0.3 (Group-2)		
One way ANOVA	Sum of squares (SS)	df	Mean square (MS)	Sum of squares (SS)	df	Mean square (MS)
Between groups	833.592	3	277.864	2683.399	7	383.343
Within groups	0.000	0		0.000	0	
Total	833.592	3		2683.399	7	

At both selected pH level, LC_{50} values are variables which are significantly different from each other between the group means LC_{50} values of Group-1 does not influence the same in Group-2. Moreover, there are no differences within the group and thus follows the null hypothesis

monocrotophos 36% SL to the tested fish were estimated as 95.656, 84.235, 76.563 and 67.616 ppm and1.690, 1.375, 1.322 and 1.462, respectively at pH, 8.62±0.3 (Table 3).

The R-values (less than 2) signifies the accuracy of the estimate that would be expected from the replicate. The presumable safe or harmless concentrations and a safe dischargeable concentration of monocrotophos 36% SL (in ppm) have also been calculated at both the selected pH strength (Table 4). Percentage alteration in Dissolved Oxygen (DO) content (ppm) were noticed as -2.77, -16.66 and -15.27 mg L^{-1} for control, 100 ppm (Highest Concentration at pH 6.62±0.3) and 135 ppm (Highest Concentration at pH 8.62±0.3), respectively (Table 5). Whereas, Percentage alteration in free CO_2 were reported to be 14.28, 78.57 and 74.76 mg L^{-1} for control, 100 ppm (Highest Concentration at pH 6.62±0.3) and 135 ppm (Highest Concentration at pH 8.62±0.3), respectively (Table 6). Group analysis (One way ANOVA) was also performed between LC_{50} values of monocrotophos 36% SL (in ppm) for a specified period of exposure (i.e., 24, 48, 72 and 96 hrs) to *Clarias batrachus* (Linnaeus) at pH, 8.62±0.3 versus pH, 6.62±0.3 and pH, 6.62±0.3) verses pH, 8.62±0.3 (Table 7) by SPSS Software, which exhibits that variables (LC_{50} values) are significantly different from each other.

DISCUSSION

In the current investigation, the 96 hrs LC_{50} value for monocrotophos to *Clarias batrachus* (Linnaeus) were noticed as 89.611 ppm at Ph, 8.62±0.3 (Table 2) and 67.616 ppm at pH, 6.62±0.3 (Table 3). However, monocrotophos was found to be moderately toxic to *Gambusia affinis* (LC_{50} value of 20.49±2.45 mg L^{-1}) when fish exposed to a sublethal concentration of LC_{10} (7.74 mg L^{-1}) for 24 days of exposure[10]. Further, a freshwater fish, *Clarias batrachus* were exposed to 1/10 and 1/20 of LC_{50} concentration of monocrotophos for 28 days and kept in normal water for recovery (for 21 days). A significant recovery of the enzyme was noticed at the lower concentration than that of the higher concentration[11]. The 96 hrs LC_{50} values of monocrotophos 36% SL were estimated as 38.845 and 28.535 ppm however, in combination with Dimethoate 30% EC (Monocrotophos 36% SL+Dimethoate 30% EC) these values were reported to be 18.259+6.633 and 14.20+4.510 ppm when exposed to the females of a freshwater fish, *Poecilia reticulata* (Peters) at pH, 8.74±0.3 and 6.74±0.3, respectively[12]. However, for Dimethoate 30% EC (organophosphate based insecticides) exposed *Heteropneustes fossilis* (Bloch), the 96 hrs LC_{50} values were noticed to be 25.933 and 19.109 ppm at pH, 8.32±0.3 and 6.32±0.3, respectively[13]. These observations support the findings of the current investigation in the context of the higher degree of toxicity of selected toxicants at lower pH strength (6.62±0.3) since LC_{50} values recorded too low. The sub-lethal concentration of monocrotophos was estimated higher in *A. altiparanae* (twice the concentration of 80 mg L^{-1} was lethal) than *O. niloticus*, like 2 mg L^{-1}[14]. However, in diazinon (also an organophosphate pesticide) exposed Nile tilapia (*O. niloticus*) fish, the 96 hrs LC_{50} values were found to be 2.8 ppm[15]. The 96 hrs LC_{50} values of an organophosphate pesticide, malathion were noticed as 5 μg L^{-1} to a freshwater fish, *Labeo rohita*[16]. Furthermore, there are 43 μg mL^{-1} was reported as a lethal dose of 50% of monocrotophos in the larvae of the sea urchin *Hemicentrotus pulcherrimus*[17]. Approximately, a similar pattern of observation has been estimated in the present investigation as suggested in the previous report of the above-mentioned authors.

A too low safe dischargeable concentration of monocrotophos 36% SL were obtained in the present investigation as compared to safe or harmless concentrations at both the selected pH strength (Table 4) which are in agreements with the findings of Prakash[12], who analyzed the safe dischargeable concentration of monocrotophos 36% SL as 1.0634 and 1.0844 ppm and safe or harmless concentrations as 12.584 and 3.9089 ppm at pH 8.74±0.3 and 6.74±0.3, respectively. Whereas, the presumable safe and safe dischargeable concentrations of Chlorpyriphos 50%+Cypermethrin 5% EC (mixture of organophosphates and pyrethroid based in secticide) were analyzed in the

ranges between 4.381-32.216 and 1.044-1.069 ppb, respectively for juveniles, males, females and mixed population of *Poecilia reticulata*[18]. But the safe/harmless and safe dischargeable concentration of Chlorpyriphos 20% EC (other organophosphates) to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters 1859) were reported to be in the ranges from 1.987-41.821 and 1.042-1.103 ppb, respectively[19]. Further, the Safe dischargeable and Safe/harmless concentration of organophosphates, Dimethoate 30% EC to a freshwater fish, *Heteropneustes fossilis* (Bloch) were noticed as 1.0664 and 1.0716 ppm and 8.9164 and 6.3751 at pH, 8.32±0.3 and 6.32±0.3, respectively[13]. It clearly illustrates a very low safe dischargeable concentration as compared to a Safe/harmless concentration for both the pH levels, which support the findings of the current investigation.

A decreasing trend in Dissolve Oxygen (DO) content (mg L^{-1}) has been reported significantly in the current investigation from 0-96 hrs (Table 6) suggested the more consumption of dissolved oxygen by test fish under stress condition which are also in agreement with the observation of Pandey[5] and Singh[20]. However, free CO_2 (mg L^{-1}) were found to be increased significantly from 0-96 hrs (Table 7) which may be due to very high metabolic activities under the pressure of selected toxicants. The previous report illustrates that in pesticide exposed fish, the hypoxic condition is observed mainly due to damage of gills which reduced the oxygen uptake[3]. A similar decreasing trend in DO content (6.96±0.85-5.83±0.21 mg L^{-1}) and increasing tendency in free CO_2 (4.00±0.00-9.33±2.31 mg L^{-1}) were noticed for an organophosphate, sumithion exposed striped catfish, *Pangasianodon hypophthalmus*[21].

An abnormal behavioural response has been reported in monocrotophos 36% SL exposed test fishes such as restlessness, increased opercular movement, erratic and jerky movements, abundant mucus-like slimy materials around the body, more excitement with jumping tendency, reddish colour of the gills. A more or less similar inspection regarding behavioural alteration has been noticed in the current investigation as suggested by the previous authors[12,13,22-24]. Since pesticides have been intensively used to control disease and various kind of pest and fish production are negatively affected due to pesticides toxicity in the food chain[25]. Therefore, it has been suggested to the practical users of organophosphate (specifically monocrotophos 36% SL) to utilize its concentration in a controlled way to minimize the health hazards of aquatic lives, particularly fishes.

CONCLUSION

Monocrotophos 36% SL was found to be acutely toxic to the test fish, *Clarias batrachus* (Linnaeus) at both the selected level of pH (higher degree at low pH) since it affects DO content, free CO_2 in water and also creates behavioural alteration about the test fishes. Eventually, it may cause deterioration of fish health and other aquatic species which ultimately affect the health of human beings.

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