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Comparative Assessment of Acute Toxicity of Two Organophosphates (Monocrotophos 36% SL and Dimethoate 30% EC) to a Freshwater Fish, *Poecilia reticulata* (Peters) at Two Different Selected Level of pH

¹M.M. Prakash, ²Resham Rajput, ³Nageshwar Wast and ¹Rohit Verma

¹Department of Zoology, Govt. Holkar Science College, Indore (M.P), India ²Department of Zoology, Govt. Girls College, Khandwa, M.P., India ³PG Department of Zoology, J.P. University Chapra, Bihar, India

Abstract: An experimental routine static bioassay test has been conducted to compare the acute toxicity $(LC_{50} \text{ values})$ of two organophosphates (Monocrotophos 36% SL and Dimethoate 30% EC) to the females of a freshwater fish, Poecilia reticulata (Peters) at two different selected level of pH (8.74±0.3 and 6.74±0.3) for 96 hrs by Probit analysis statistical method. The 96 hrs LC₅₀ values of Monocrotophos 36% SL were estimated as 38.845 and 28.535 ppm however, the same values were noticed for Dimethoate 30% EC as 12.545 and 10.673 ppm at pH 8.74±0.3 and 6.74±0.3 respectively. Consequently, the 96 hrs LC₅₀ values for combination of both organophosphates (Monocrotophos 36% SL+Dimethoate 30% EC) at the pH 8.74±0.3 and 6.74±0.3 were documented as 18.259+6.633 and 14.20+4.510 ppm respectively. The Presumable safe concentration of Monocrotophos 36% SL and Dimethoate 30% EC were found to 12.584 and 3.9089 ppm and; 3.9089 and 3.3143 ppm respectively however, its Safe dischargeable concentrations were noticed as 1.0634 and 1.0844 and; 1.0920 and 1.0740 ppm respectively at pH 8.74±0.3 and 6.74±0.3. The value of Presumable safe and Safe dischargeable concentrations for the combination of both Organophosphates (Monocrotophos 36% SL+ Dimethoate 30% EC) were estimated as 5.8159+2.1025 and 1.0792+1.0774 ppm at pH 8.74±0.3 and; 4.4052+1.3992 and 1.0620+1.0570 ppm at pH 6.74±0.3 respectively. One way ANOVA (Group analysis) between Median lethal concentrations $(LC_{50}$'s) of Monocrotophos 36% SL and Dimethoate 30% EC for 24, 48, 72 and 96 hrs to females of Poecilia reticulata at pH (8.74 ± 0.3) verses and pH (6.74 ± 0.3) and pH (6.74 ± 0.3) verses pH (8.74 ± 0.3) has been also analyzed by SPSS statical software. The change in behavioral response in the test fishes was also observed carefully during the course of full scale bioassay. Results illustrates that both organophosphates are toxic to the test specimen but Dimethoate 30% EC is highly toxic as compared to Monocrotophos 36% SL since 96 hrs LC₅₀ values of later is too low however, the combined formulation of both organophosphates (Monocrotophos 36% SL+ Dimethoate 30% EC) exhibits to a higher level toxicity as compared to single ones at both selected levels of pH. Higher level of toxicity of combined formulation is may be due to synergic effect of one another at both the pH strength.

Key words: Acute Toxicity • LC₅₀ Value • Monocrotophos 36% SL • Dimethoate 30% EC and *Poecilia* reticulata

INTRODUCTION

Pesticides are extensively used in agriculture to control the insects, pests and other vectors [1] that ultimately enters into aquatic bodies like ponds, rivers and lakes. It has been noticed that pesticides can be actively toxic to fish species [2-6]. Monocrotophos is an

organophosphate based insecticide and acaricides which acts systemically and also as an contact poison. It is effective against a various kinds of pest *viz;* sucking chewing and boring insects and; spider mites on cotton, tobacco, peanuts, sugarcane and ornamentals [7, 8]. It is found to be direct acting cholinesterase inhibitor, having capacity to penetrate through the skin [9].

Corresponding Author: Nageshwar Wast, PG Department of Zoology, J.P. University Chapra, Bihar, India. Mob: +91 8109909354. It has a little environmental persistence, not accumulated in the soil and reported to be a biodegradable organophosphate. Its half life is estimated to be less than 7 days (in soil) in natural sunlight [10, 11]. Monocrotophos is found to be moderately toxic to the fish [7] and leads to reproductive damage to crustaceans if exposed to prolonged time [12].

Dimethoate (an organophosphate insecticide) is reported to be highly toxic to fish and other aquatic invertebrates. It is biodegradable and rapidly degraded in the environment and; in sewage treatment plants [13]. It is used to control a various kinds of insects viz; mites, flies, aphids and plant hoppers and also found to be inhibitor of acetylcholine cholinesterase activity which causes nerve damage, ultimately leads to death [14]. Since high persistent of organchloride creates an environmental problems and due to less persistence and being more efficient, the organophosphate insecticide have been extensively used for agricultural, household pest and in public health programme [15]. Due to this, a large scale existence of an organophosphate insecticide has been estimated in aquatic bodies which create the acute and chronic toxicity to the fish species [16, 17]. In view of this, an effort has been made to asses the comparison of acute toxicity of two organophosphates (Monocrotophos 36% SL and Dimethoate 30% EC) to a freshwater fish, Poecilia reticulata (Peters) at two selected level of pH.

MATERIALS AND METHODS

Experimental Fish: Test fish, *Poecilia reticulata* (Peters) were collected from local sources, acclimatized separately in tank (300 liters capacity) for 10 days and proper food supplied regularly. Approximately equal sizes of healthy fishes $(3.00 \pm 0.3 \text{ cm})$ were selected for the experimental routine bioassay tests.

Preparation of Stock Solution: Stock solution were prepared for Monocrotophos 36% SL and Dimethoate 30% EC by using formula of $N_1V_1 = N_2V_2$. Where, $N_1 =$ Concentration of available 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 solutions of different concentrations (in ppm) were prepared by adding the stock solution into the measured diluents water with the help of micropipette. The series of different concentrations of selected organophosphates applied in the full scale static bioassay tests were based on the progressive bisection of intervals on logarithmic scales [18]. The static bioassay test (up to 96 hrs) for selected organophosphates to the test fish, *Poecilia reticulata* (Peters) were runs separately in test container with one liter water capacity containing experimental water of two different selected levels of pH, 8.74 ± 0.3 and 6.74 ± 0.3 . The tap water of pH, 8.74 ± 0.3 and water of pH strength, 6.74 ± 0.3 (prepared by adding HCl solution with the help of micropipette) were used as experimental water.

Preliminary or Screening Tests: The test range of Monocrotophos 36% SL and Dimethoate 30% EC and its combined formulation (Monocrotophos 36% SL + Dimethoate 30% EC) was taken between the highest and lowest concentrations at which most of the test fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs, since it provide a clues for the full scale bioassay test.

Full Scale Bioassay Test: The test container with one litre capacity, filled with toxicant solution were placed in three rows and each container was labeled with the details of the experiment as date and time of the experiment, pH strength, concentration, replicate number. The acclimatized test fish, *Poecilia reticulata* (Peters) of approximately equal sizes $(3.00 \pm 0.3 \text{ cm})$ were provided to these containers after about 30 minutes of the preparation of test solutions. The bioassays for test fishes were carried out for selected organophosphates both separately and in combination, for two selected level of pH (i.e. 8.74±0.3 and 6.74±0.3). There are 10 acclimatized test fishes were transferred to each experimental test containers and proper controls were run simultaneously for the same period of exposure. The test solutions were replaced with renewed and fresh toxicant solutions after each 24 hrs and the experiments were continued for 96 hrs. The number of test fishes died in each concentration of toxicants were observed carefully at the time intervals of 24, 48, 72 and 96 hrs and removed from the test solution regularly. The LC₅₀ values and the 95 per cent confidence limits for different concentration and time intervals (24, 48, 72 and 96 hrs) for selected organophosphates were analyzed by Probit Analysis methods [19]. The presumable safe or harmless and safe dischargeable concentrations of selected organophosphates experimental fishes were for calculated by using the formula of Hart et al. [20]. The change in behavioural response in the studied fishes was also noticed carefully during the course of full scale bioassay.

RESULTS AND DISCUSSION

In present investigation, the test concentrations (based on logarithmic scale [18] applied for Monocrotophos 36% SL, Dimethoate 30% EC and their combination (in ppm) used in full scale static bioassays test (96 hr) for females of *Poecilia reticulata* at two selected levels of pH (8.74 ± 0.3 and 6.74 ± 0.3) has been summarized in Table 1.

The LC₅₀ values of Monocrotophos 36% SL has been noticed in the range from 53.647 (24 hrs) to 38.845 ppm (96 hrs), the values of LCL, UCL were ranged from 32.013 to 47.371, 43.252 to 79.827 and its ratio (R) were calculated as 1.351 to 1.685 respectively at pH 8.74±0.3 (Table 2 and Fig. 1). Whereas, the 24 hrs and 96 hrs LC₅₀ values of Monocrotophos 36% SL were estimated as 42.244 and 28.535 ppm and; it's LCL, UCL and its ratio (R) were found to be in the range from 22.213 to 36.603, 32.123 to 83.008 and 1.446 to 2.267 respectively at pH 6.74±0.3 (Table 4 and Fig. 1). The LC₅₀ values varied from 18.530 (24 hrs) to 12.545 ppm (96 hrs) and; LCL, UCL and its ratio (R) were ranged from 10.237 to 15.701, 14.220 to 45.703 and 1.389 to 2.910 respectively at pH 8.74±0.3 (Table 3 and Fig. 2) however, at pH level 6.74±0.3 the LC50 values, LCL, UCL and its ratio (R) were documented in the range from 14.703 to 10.673 ppm, 9.425 to 12.905, 11.653 to 23.849 and 1.236 to 1.848 respectively for Dimethoate 30% EC (Table 5 and Fig. 2). The 96 hrs LC_{50} values of combined formulation (Monocrotophos 36% SL+ Dimethoate 30% EC) were estimated as 18.259+6.633 ppm and; its LCL, UCL and its ratio (R) were calculated as 14.940+5.465, 20.330+7.354 and 1.360+1.345 respectively at pH 8.74±0.3 (Table 6). Consequently, the 96 hrs LC_{50} values, its LCL, UCL and ratio (R) for the same were analyzed as 14.200+4.510 ppm, 12.493+3.995, 15.585+4.924 and 1.247+1.232 respectively at pH level 6.74±0.3 (Table 7).

It has been noticed that the LC_{50} value of Monocrotophos 36% SL and Dimethoate 30% EC for 24, 48, 72 and 96 hrs to females of *Poecilia reticulata* were found to be decrease at pH level 6.74±0.3 (Fig. 1 and 2) and; and Dimethoate 30% EC exhibits higher degree of toxicity than Monocrotophos 36% SL since the LC_{50} value of former is too low at both the selected levels of pH (Fig. 3 and 4). But, the combined formulation of Monocrotophos 36% SL+ Dimethoate 30% EC illustrates the higher level of toxicity as compared to single ones at both the selected pH strength, since the LC_{50} value of combined formulation were recorded too low and it has been also reported the lower toxicity strength of combined formulation at pH, 8.74 ± 0.3 as compared to pH, 6.74 ± 0.3 (Table 6 and 7). Larger toxicity strength of combined formulation is may be due to additive effect of one another at both the pH strength.

One way ANOVA (Group analysis) between Median lethal concentrations (LC_{so} 's) of Monocrotophos 36% SL and Dimethoate 30% EC for 24, 48, 72 and 96 hrs to females of *Poecilia reticulata* has been also calculated by SPSS statical software. Sum of Squares (SS) and Mean Square (MS) values between the Groups were estimated as 119.163 and 39.721 and; 110.888 and 36.963 for Monocrotophos 36% SL at pH 8.74±0.3 verses 6.74±0.3 and pH 6.74±0.3 verses pH 8.74±0.3 respectively (Table 9 a, b). Whereas, Sum of Squares (SS) and Mean Square (MS) values between the Groups were analyzed as 20.782 and 6.927 and; 9.874 and 3.291 at pH 8.74±0.3 respectively (Table 10 a, b).

The value of presumable safe or harmless concentration for Monocrotophos 36% SL and Dimethoate 30% EC were found to be 12.584 and 3.9089 ppm and; 9.1628 and 3.3143 ppm whereas, the safe dischargeable concentration for the same has been recorded as 1.0634 and 1.0920 ppm and; 1.0844 and 1.0740 ppm at pH 8.74 \pm 0.3 and 6.74 \pm 0.3 respectively. Eventually, presumable safe and safe dischargeable concentration for combined formulation (Monocrotophos 36% SL+Dimethoate 30% EC) were documented as 5.8159+2.1025 and 4.4052+1.3992 ppm and; 1.0792+1.0774 and 1.0620+1.0570 ppm respectively at pH 8.74 \pm 0.3 and 6.74 \pm 0.3 (Table 8).

In present investigation, the 24 hr and 96 hr LC_{50} values of Monocrotophos 36% SL has been estimated as 53.647 and 38.845 ppm (Table 2) and; 42.244 and 28.535 ppm (Table 4) at pH 8.74±0.3 and pH 6.74±0.3 respectively. Whereas, the 96 h LC50 value of Monocrotophos was analyzed as 4.9 mg/l during the study of neurotoxic effects on the nile tilapia fish (Oreochromis niloticus) by probit analysis method using a static bioassay under laboratory conditions [21]. The 48-hr LC₅₀ of Monocrotophos were recorded as 13.8 mg/l in O. niloticus [22]. However, the sub-lethal concentration of Monocrotophos were found to be higher in A. altiparanae (twice the concentration of 80 mg/l was lethal) but it was not for O. niloticus, as 2 mg/l [23]. A lethal dose of 50% of Monocrotophos was estimated as 43 μ g/ml in the larvae of the sea urchin Hemicentrotus pulcherrimus [24]. Whereas, the median

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Table 1: Test concentrations of Monocrotophos 36% SL, Dimethoate 30% EC (in ppm) and their combination used in full scale static bioassays (96 hr) for females of *Poecilia* at two selected levels of pH (8.74±0.3) and (6.74±0.3)

	pH (8.74±0.3)			pH (6.74±0.3)		
S. No.	Monocrotophos 36% SL (ppm)	Dimethoate 30% EC (ppm)	Monocrotophos 36% SL+ Dimethoate 30% EC (ppm)	Monocrotophos 36 % SL (ppm)	Dimethoate 30 % EC (ppm)	Monocrotophos 36% SL+ Dimethoate 30% EC (ppm)
1	56.0	18.0	28.0+10.0	42.0	15.0	21.0+6.5
2	49.0	15.0	24.0+8.7	37.0	13.5	18.0+5.6
3	42.0	13.5	21.0+7.5	32.0	11.5	15.0+4.9
4	37.0	11.5	18.0+6.5	28.0	10.0	13.5+4.2
5	32.0	10.0	15.0+5.6	24.0	8.7	11.5+3.7

* Based on logarithmic scale (APHA, 2005)

 $\frac{\text{Table 2: Median lethal concentrations (LC_{50}'s) of Monocrotophos 36\% SL (in ppm) for 24, 48, 72 \& 96 hrs to females of$ *Poecilia* $at pH (8.74\pm0.3)}{\text{LC}_{50}'s of Monocrotophos 36\% SL (in ppm)}$

Duration (hrs)	Monocrotophos 36% SL(in ppm)	LCL	UCL	R
24	53.647	47.371	79.827	1.685
48	47.438	41.963	57.530	1.370
72	43.244	37.315	49.227	1.319
96	38.845	32.013	43.252	1.351

Table 3: Median lethal concentrations (LC₅₀'s) of Dimethoate 30% EC (in ppm) for 24, 48, 72 & 96 hrs to females of Poecilia at pH (8.74±0.3)

Duration (hrs)	Dimethoate 30% EC (in ppm)	LCL	UCL	R
24	18.530	15.701	45.703	2.910
48	15.538	13.813	19.288	1.396
72	13.581	11.694	15.538	1.328
96	12.545	10.237	14.220	1.389

Table 4: Median lethal concentrations (LC₅₀'s) of Monocrotophos 36% SL (in ppm) for 24, 48, 72 & 96 hrs to females of *Poecilia* at pH (6.74±0.3) LC₅₀'s of Monocrotophos 36% SL (in ppm)

Duration (hrs)	Monocrotophos 36% SL(in ppm)	LCL	UCL	R
24	42.244	36.603	83.008	2.267
48	35.920	31.580	44.562	1.411
72	30.843	25.308	34.991	1.382
96	28.535	22.213	32.123	1.446

 $\frac{\text{Table 5: Median lethal concentrations (LC_{50}'s) of Dimethoate 30\% EC (in ppm) for 24, 48, 72 \& 96 hrs to females of$ *Poecilia* $at pH (6.74\pm0.3)}{\text{LC}_{50}'s of Dimethoate 30\% EC (in ppm)}$

Duration (hrs)	Dimethoate 30% EC (in ppm)	LCL	UCL	R
24	14.703	12.905	23.849	1.848
48	12.745	11.105	15.665	1.410
72	11.179	9.839	12.318	1.251
96	10.673	9.425	11.653	1.236

Table 6:

Median lethal concentrations (LC₅₀'s) of Monocrotophos 36% SL+ Dimethoate 30% EC (in ppm) for 24, 48, 72 & 96 hrs to females of *Poecilia* at pH (8.74±0.3)

	LC ₅₀ 's of Monocrotophos 36% SL+ Dimethoate 30%	EC (in ppm)		
Duration (hrs)	Monocrotophos 36% SL+ Dimethoate 30% EC	LCL	UCL	R
24	26.302+9.445	23.358+8.406	36.039+12.985	1.542+1.544
48	22.581+8.136	19.527+7.064	27.042+9.725	1.384+1.376
72	19.714+7.135	16.771+6.109	21.998+7.933	1.311+1.298
96	18.259+6.633	14.940+5.465	20.330+7.354	1.360+1.345



Table 7: Median lethal concentrations (LC so's) of Monocrotophos 36% SL+ Dimethoate 30% EC (in ppm) for 24, 48, 72 & 96 hrs to females of Poecilia at pH (6.74±0.3)

	LC_{50} 's of Monocrotophos 36% SL+ Dimethoate 30%	EC (in ppm)		
Duration (hrs)	Monocrotophos 36% SL+ Dimethoate 30% EC	LCL	UCL	R
24	18.680+5.824	16.417+5.164	24.708+7.583	1.505+1.468
48	16.562+5.212	14.950+4.740	18.492+5.764	1.236+1.216
72	14.928+4.727	12.987+4.147	16.613+5.225	1.279+1.259
96	14.200+4.510	12.493+3.995	15.585+4.924	1.247+1.232
	00 00 00 00 00 00 00 00 00 00		■ pH 8.74±0.3 ■ pH 6.74±0.3	

Fig. 1: Comparison of Median lethal concentrations (LC_{50} 's) of Monocrotophos 36% SL for 24, 48, 72 and 96 hrs to females of Poecilia at pH 8.74±0.3 and 6.74±0.3

Exposure duration in hours

72 hr

96 hr

48 hr

24 hr



Fig. 2: Comparison of Median lethal concentrations (LC₅₀'s) of Dimethoate 30% EC for 24, 48, 72 and 96 hrs to females of Poecilia at pH 8.74±0.3 and 6.74±0.3



Fig. 3: Comparison of Median lethal concentrations (LC50's) of Monocrotophos 36% SL (MCP 36% SL) and Dimethoate 30% EC (DMT 30% EC) for 24, 48, 72 and 96 hrs to females of Poecilia at pH 8.74±0.3



Fig. 4: Comparison of Median lethal concentrations (LC50's) of Monocrotophos 36% SL (MCP 36% SL) and Dimethoate 30% EC (DMT 30% EC) for 24, 48, 72 and 96 hrs to females of Poecilia at pH 6.74±0.3

 Table 8:
 Safe or harmless and Safe dischargeable concentration of Monocrotophos 36% SL, Dimethoate 30% EC and their combination (in ppm) to females of *Poecilia* at pH (8.74±0.3) and (6.74±0.3)

	pH (8.74±0.3)		pH (6.74±0.3)	
Organophosphates	Safe or harmless concentration (as ppm)	Safe dischargeable	Safe or harmless	Safe dischargeable
Monocrotophos 36% SL(in ppm)	12.584	1.0634	9.1628	1.0844
Dimethoate 30% EC	3.9089	1.0920	3.3143	1.0740
Monocrotophos 36% SL+ Dimethoate 30% EC	5.8159+2.1025	1.0792+1.0774	4.4052+1.3992	1.0620+1.0570

Table 9: One way ANOVA (Group analysis) between Median lethal concentrations (LC₅₀'s) of Monocrotophos 36% SL for 24, 48, 72 & 96 hrs to females of *Poecilia* at (a) pH (8.74±0.3) verses and pH (6.74±0.3) and (b) pH (6.74±0.3) verses pH (8.74±0.3)

Table 9(a):			
One way ANOVA	Sum of Squares (SS)	df	Mean Square (MS)
Between Groups	119.163	3	39.721
Within Groups	0.000	0	
Total	119.163	3	
Table 9(b):			
One way ANOVA	Sum of Squares	df	Mean Square
Between Groups	110.888	3	36.963
Within Groups	0. 000	0	
Total	110.888	3	
	lethal concentrations (EC for 24, 48, 72 & 96 (a) pH (8.74±0.3) vers	(LC_{50}) 's hrs to es and) of Dimethoate 30% females of <i>Poecilia</i> at
	pH (6.74±0.3) verses p	oH (8.7	4 ± 0.3) and (b)
Table 10 (a)	pH (6.74±0.3) verses p	oH (8.7	4 ± 0.3 and (b)
Table 10 (a) One way ANOVA	pH (6.74±0.3) verses p Sum of Squares	oH (8.7	$\frac{4\pm0.3)}{\text{Mean Square}}$
Table 10 (a) One way ANOVA Between Groups	pH (6.74±0.3) verses p Sum of Squares 20.782	df 3	Mean Square 6.927
Table 10 (a) One way ANOVA Between Groups Within Groups	pH (6.74±0.3) verses p Sum of Squares 20.782 0.000	df 3 0	Mean Square 6.927
Table 10 (a) One way ANOVA Between Groups Within Groups Total	pH (6.74±0.3) verses p Sum of Squares 20.782 0.000 20.782	df 3 0 3	Mean Square 6.927
Table 10 (a) One way ANOVA Between Groups Within Groups Total Table 10 (b)	pH (6.74±0.3) verses p Sum of Squares 20.782 0.000 20.782	bH (8.7)	Mean Square 6.927
Table 10 (a)One way ANOVABetween GroupsWithin GroupsTotalTable 10 (b)One way ANOVA	pH (6.74±0.3) verses p Sum of Squares 20.782 0.000 20.782 Sum of Squares	bH (8.7 df 3 0 3 df	Mean Square Mean Square Mean Square
Table 10 (a)One way ANOVABetween GroupsWithin GroupsTotalTable 10 (b)One way ANOVABetween Groups	PH (6.74±0.3) verses p Sum of Squares 20.782 0.000 20.782 Sum of Squares 9.874	bH (8.7 df 3 0 3 df 3	Mean Square 6.927 Mean Square 3.291
Table 10 (a)One way ANOVABetween GroupsWithin GroupsTotalTable 10 (b)One way ANOVABetween GroupsWithin Groups	PH (6.74±0.3) verses p Sum of Squares 20.782 0.000 20.782 Sum of Squares 9.874 0.000	bH (8.7 df 3 0 3 df 3 0	Mean Square 6.927 Mean Square 3.291
Table 10 (a) One way ANOVA Between Groups Within Groups Total Table 10 (b) One way ANOVA Between Groups Within Groups Total	PH (6.74±0.3) verses p Sum of Squares 20.782 0.000 20.782 Sum of Squares 9.874 0.000 9.874	bH (8.7 df 3 0 3 df 3 0 3	Mean Square 6.927 Mean Square

lethal concentrations of Monocrotophos has been recorded as 11.506 mg/l on euryhaline fish, *Oreochromis mossambicus* [25]. Monocrotophos has been reported as moderately toxic to Gambusia affinis, with LC_{50} value of 20.49 +/- 2.45 mg/l when fish exposed to sublethal concentration of LC_{10} (7.74 mgL/l)) with respect to the length of exposure, 24 day [26]. If *Labeo rohita* exposed to sublethal concentration of Monocrotophos (0.40 ppm) leads to a significant decrease in RBC's count leading to anemia, inhibition of erthropoiesis, haemosynthesis and also enhancement in the rate of erythrocyte

destruction in haemopoietic organs has been observed [27]. Study reveals that the components of proteins, carbohydrates and ninhydrine positive substances were found to be decreased significantly at different time intervals (24, 48, 72 and 96 h) due to methyl parathion (an organophosphate pesticide) exposure to different tissues of fresh water cat fish, Heteropneustis fossilis as compared to control [28].In vivo toxicity of Monocrotophos has been assessed on key metabolites and enzymes of the protein metabolism in some tissues of the freshwater fish clarias batrachus, exposed to 1/10 and 1/20 of LC₅₀^s concentration for 28 days [29]. According to these authors, the enzymes activities were recovered more significant at the lower concentration as compared to the higher concentration. Results of the present assessment also exhibit more or less conformities to the finding of these authors.

The present investigation exhibits the 24 hr and 96 hr LC₅₀ values as 18.530 to 12.545 ppm (Table 3) and; 14.703 to 10.673 ppm (Table 5) at pH 8.74±0.3 and pH 6.74±0.3 respectively for Dimethoate. However, the 24, 48, 72 and 96 hr LC₅₀ values for dimethoate on freshwater airbreathing catfish, Heteropneustes fossilis (Bloch) were estimated to be 3.38, 3.23, 3.08 and 2.98 mg/l respectively [30]. Whereas, the 96-h LC₅₀ value for 3-day old larvae of an South American characid fish Curimbata (Prochilodus lineatus) has been analyzed for Dimethoate 40% as 10.44 mg/l (8.03 mg/l - 13.57 mg/l) and were found to be equal to its recently-hatched larvae (p = 0.76) but reduced larval mobility was seen [31]. It has been reported that the 96-hour LC₅₀ value for Dimethoate in rainbow trout was found to be 6.2 ug/l and 48-hour LC₅₀ value in Daphnia magna, a small freshwater crustacean was noticed as 2.5 ug/l [13]. However, the LC₅₀ value were analyzed in the range from 40 to 60 mg/l for Dimethoate in mosquito fish [8]. Further, Shadegana & Banaee [32] were exposed 16 and 32 µg/l dimethoate to a common carp, Cyprinus carpio for 14 days and evaluated its sub-lethal toxicity. According to these authors, malondialdehyde (MDA) and catalase (CAT) in the liver and kidney and; alkaline phosphatase (ALP) and catalase (CAT) were found to be increased in gill, whereas, lactate dehydrogenase (LDH), glucose 6-phsphate dehydrogenase (G6PDH) and glycogen were reported to be decreased in liver. The 96-hour LC₅₀ value for juvenile of Cyprinus carpio var. communis was estimated as 1.1 ppm in static bioassay system by Probit analysis method for dimethoate [33]. It has been observed that Dimethoate induces oxidative stress and also alters the biochemical parameters in a freshwater snail, Galba truncatula, were exposed to 0, 100, 200 and 400?µg/l in laboratory conditions for 14 days [34]. Previously, the 96 hr LC₅₀ value were also evaluated for other organophosphates, Chlorpyriphos 20% EC to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters 1859) as 7.009, 14.575, 130.777 and 51.924 ppb however, safe or harmless and; safe dischargeable concentration were estimated to be 1.987, 4.962, 41.821 and 17.08 ppb and; 1.103, 1.042, 1.063 and 1.062 ppb respectively [35]. The experimental routine static bioassay test have been conducted by using combined formulation of organophosphates and pyrethroid based insecticide (Chlorpyriphos 50% + Cypermethrin 5% EC) to the the juveniles, males, females and mixed population of Guppy, Poecilia reticulata (Peters 1859) and its 96 hr LC₅₀ value were reported to be 13.396, 18.845, 261.866 and 106.255 ppb respectively. Further, the presumable safe or harmless concentration and safe dischargeable concentrations of Chlorpyriphos 50% + Cypermethrin 5% EC were found to be ranges from 4.381 to 32.216 and 1.044 to 1.069 ppb respectively [36]. Whereas, the LC₅₀ values of dimethoate for fingerlings of common carp, Cyprinus carpio were statically analysed as 1.84, 1.78, 1.68 and 1.61 mg/L for 24, 48, 72 and 96 h respectively [37]. According to these authors [37], dimethoate is highly toxic to fingerlings of Cyprinus carpio and severely affects its physiology and behaviour. Further, the 96 h LC50 values of Dimethoate for fingerlings and adults of Zebrafish, Danio rerio was found to be 21.64 µg/l and 60.00 µg/l respectively and; concentration and time dependent toxicity of Dimethoate has also been observed [38].

Authors of the current investigation noticed that the results are in accordance with the findings of relevant authors (discussed above) in context of LC_{s0} value, safe or harmless and safe dischargeable concentrations for both the selected organophosphates in bioassay.

In current investigation, Monocrotophos 36% SL exposed fishes exhibits uncoordinated behavioural response such as whirling movements, sometimes erratic and jerky movements, jumping tendency, increased opercular movement, plentiful mucous secretion at higher concentration and also reddish colour of the gills has been noticed during and after death. Whereas, Dimethoate 30% EC exposed test fishes exhibits more excitement and try to jump out of container, movement in uncontrolled manner, convulsions, increase in opercular movement, abundant mucous secretion and jerky movements. Fishes settled down on bottom with belly upside and also reddish colour of the gills (might be due to haemorrhage) has been observed during and after death. Therefore, it has been suggested to those, while using organophosphate particularly, Monocrotophos 36% SL and Dimethoate 30% EC, the attention should be focused on controlled concentration to prevents deterioration of fish health and other aquatic organisms in water bodies.

CONCLUSIONS

Monocrotophos 36% SL has been recommended to be moderately toxic as compared to Dimethoate. Eventually, this study has a great importance from agricultural point of view since it helps to manage the health of aquatic fauna, particularly fishes to a greater extent. Therefore, the mentioned organophosphates should used in controlled way in India in order to reduce its potential risk to threat the health of the human beings, ultimately.

REFERENCES

- Yonar, E.M., S.M. Yonar, M. Sener, U.S. Silici and M. Dusukcan, 2012. Protective role of propolis in chlorpyrifos-induced changes in the haemotological parameters and oxidative antioxidative status of Cyprinus carpio. Food and Chemical Toxicology, 50: 2703-2708.
- Moore, A. and C.P. Waring, 2001. The effect of a synthetic pyrethroid pesticide on some aspect of reproduction in Atlantic salmon (*Salmo salar L*). Aquatic Toxicology, 52(1): 1-12.
- Shrivasatava, S., S. Singh and K. Shrivastava, 2002. Effect of carbaryl on glucose content in the brain of Heteropneustes fossilis. Journal of Ecotoxicology & Environmental Monitoring, 12(3): 205-208.
- Sheikh, N. and S.G. Yeragi, 2004. Effect of Rogor 30E (Organophosphate) on muscle protein in the fresh water fish Lepidocephale cthyesthermalis. Journal of Ecotoxicology & Environmental Monitoring, 14(3): 233-235.

- Eder, K.J., C.M. Leutenegger, B.W. Wilson and I. Werner, 2004. Molecular and cellular biomarker responses to pesticide exposure in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Marine Environmental Research, 58(2-5): 809-813.
- Visvanathan, P., C. Maruthanayagam and M. Govindaraju, 2009. Effect of malathion and endosulfan on biochemical changes in Channa punctatus. Journal of Ecotoxicology & Environmental Monitoring, 19(3): 251-257.
- The Agrochemicals Handbook, 1994. Third Edition. Royal Society of Chemistry Information Systems, Unwin Brothers Ltd., Surrey, England.
- 8. Meister, R.T., 1992. Farm Chemicals Handbook '92. Meister Publishing Company. Willoughby, OH.
- American Conference of Governmental Industrial Hygienists (ACGIH), 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices: Sixth Edition, Volume I, ACGIH. Cincinnati, OH.
- U.S. Environmental Protection Agency, 1985. Pesticide Fact Sheet No. 72: Monocrotophos. U.S. EPA. Washington, DC.
- Smith, G.J., 1993. Toxicology & Pesticide Use in Relation to Wildlife: Organophosphorus & Carbamate Compounds. C.K. Smoley. Boca Raton, FL.
- 12. Briggs, Shirley, 1992. Basic Guide to Pesticides. Hemisphere Publishing. Washington, DC.
- Cheminova Agro A/S., 1991.Material Safety Data Sheet : Dimethoate. Cheminova, Lemvig, Denmark. (June 11).
- Van Scoy, A., A. Pennell and X. Zhang, 2016. Environmental fate and toxicology of Dimethoate. Reviews of Environmental Contamination and Toxicology, 237: 53-70.
- Oruc, O.E., N. Uner, Y. Sevgiler, D. Usta and H. Durmaz, 2006. Sublethal effects of organophosphate diazinon on the brain of Cyprinus carpio. Drug and Chemical Toxicology, 29(1): 57-67.
- Velmurugan, B., M. Selvanayagam, E.I. Cengiz and E. Unlu, 2007. The effects of monocrotophos to different tissues of freshwater fish Cirrhinus mrigala. Bulletin of Environmental Contamination and Toxicology, 78(6): 450-454.
- Aker, W.G., X. Hu, P. Wang and H.M. Hwang, 2008. Comparing the relative toxicity of malathion and malaoxon in blue catfish Ictalurus furcatus. Environmental Toxicology, 23(4): 548-554.

- American Public Health Association (APHA), 2005. American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Sandard methods for the examination of water and wastewater, 21st Edn, American Public Health Association, Washington D.C.
- Finney, D.J., 1971. Probit Analysis, University Press, Cambridge, pp: 333.
- Hart, W.B., P. Doudoroff and J. Greenbank, 1945. The evaluation of the toxicity of industrial wastes, chemical and other substances to freshwater fishes. Atlantic Refining Co. (Phill), pp: 317.
- Thangnipon, W., P. Luangpaiboon and S. Chinabut, 1995. Effects of the organophosphate insecticide, monocrotophos, on acetylcholinesterase activity in the nile tilapia fish (*Oreochromis niloticus*) brain. Neurochemical Research, 20(5): 587-91.
- Tejada, A.W. and C.M. Bajet, 1990. Fate of pesticide in rice- fish ecosystem. Philip. Agricultural Laguna, 73: 153-163.
- Cruz, A.L., 2002. Sub-lethal concentrations of monocrotophos affect aggressive behavior of the fishes Astyanax altiparanae Garutti & Britski (Teleostei, Characidae) and Oreochromis niloticus (Linnaeus) (Teleostei, Cichlidae). Revista Brasileira de Zoologia, 19(4): 1131-1138.
- 24. Yao, D., S. Ru and H. Katow, 2010. The neurotoxic effects of monocrotophos on the formation of the serotonergic nervous system and swimming activity in the larvae of the sea urchin Hemicentrotus pulcherrimus. Environmental Toxicology and Pharmacology, 30(2): 181-187.
- 25. Rao, J.V., 2004. Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, Oreochromis mossambicus. Ecotoxicology and Environmental Safety, 59(2): 217-222.
- Rao, J.V. G. Begum, V. Sridhar and N.C. Reddy, 2005. Sublethal effects of monocrotophos on locomotor behavior and gill architecture of the mosquito fish, Gambusia affinis. Journal of Environmental Science and Health B, 40(6): 813-25.
- Tamizhazhagan, V., 2015. The toxicity effect of Monocrotophos 36% EC on the haematology, Labeo rohita (Hamilton, 1882). International Journal of Current Pharmaceutical Research, 7(4): 92-95.

- Rao, T.B., K. Thirupathi and Y. Venkaiah, 2018. Effect of methyl parathion (an organophosphate) on biochemical contents of fresh water cat fish Heteropneustes fossilis (bloch). International Journal of Pharmaceutical Sciences and Research, 9(7): 2869-2874.
- Narra, M.R., G. Begum, K. Rajender and J.V. Rao, 2011. In vivo impact of monocrotophos on biochemical parameters of a freshwater fish during subacute toxicity and following cessation of exposure to the insecticide. Z Naturforsch, 66 C 507-514.
- Pandey, R.K., R.N. Singh, S. Singh, N.N. Singh and V.K. Das, 2009. Acute toxicity bioassay of dimethoate on freshwater airbreathing catfish, Heteropneustes fossilis (Bloch). Journal of Environmental Biology, 30(3): 437-440.
- Campagna, A.F., M.N. Eler, E.L. Espindola, J.A. Senhorini, R.F. do Rego and L.O. Silva, 2006. Dimethoate 40% organosphosphorous pesticide toxicity in Prochilodus lineatus (Prochilodontidae, Characiformes) eggs and larvae. Brazilian Journal of Biology, 66(2B): 633-640.
- 32. Shadegan, M.R. and M. Banaee, 2018. Effects of dimethoate alone and in combination with Bacilar fertilizer on oxidative stress in common carp, Cyprinus carpio. Chemosphere, 208: 101-107.
- 33. Qayoom, I., F.A. Shah, M. Mukhtar, M.H. Balkhi, F.A. Bhat and B.A. Bhat, 2016. Dimethoate Induced Behavioural Changes in Juveniles of Cyprinus carpio var. communis under Temperate Conditions of Kashmir, India. Scientific World Journal, 16: 1-6.

- Banaee, M., A. Sureda, S. Taheri and F. Hedayatzadeh, 2019. Sub-lethal effects of dimethoate alone and in combination with cadmium on biochemical parameters in freshwater snail, Galba truncatula. Comparative Biochemistry and Physiology C Toxicology & Pharmacology, 220: 62-70.
- Wast, N., K. Tiwari, A.K. Gupta, M.M. Prakash and S. Gaherwal, 2015. Assessment of Acute Toxicity of Chlorpyriphos 20% EC to the Guppy, Poecilia reticulata (Peters, 1859). Global Veterinaria, 14(2): 239-243.
- Wast, N., A.K. Gupta, M.M. Prakash and S. Gaherwal, 2014. Acute Toxicity of Chlorpyriphos 50% + Cypermethrin 5% EC to the Guppy, Poecilia reticulata (Peters, 1859). Global Veterinaria, 12(3): 393-398.
- Singh, R.N., R.K. Pandey, N.N. Singh and V.K. Das, 2010. Acute toxicity and behavioral responses of common carp *Cyprinus carpio* (Linn.) to an organophosphate (dimethoate). World Journal of Zoology, 5(3): 183-188.
- Ansari, S. and B.A. Ansari, 2011. Embryo and Fingerling Toxicity of Dimethoate and Effect on Fecundity, Viability, Hatchability and Survival of Zebrafish, *Danio rerio* (Cyprinidae).World Journal of Fish and Marine Sciences, 3(2): 167-173.