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Toxicological Alteration of Dimethoate (rogor) Insecticide Against Freshwater Fish *Colisa fasciatus* and *Mystus mystus*

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Abstract

Like other organophosphates, dimethoate is an acetylcholinesterase inhibitor which disables cholinesterase, an enzyme essential for central nervous system function. Dimethoate insecticide can be used for both indoor and outdoor purposes to eliminate a number of insect pests under different condition. Dimethoate, one of the most extensively studied pesticides, may induce many significant changes in fishes. The aim of the present study is to determine the toxicological action of dimethoate on freshwater teleost fishes. The Dimethoate has shown strong piscicidal activity in freshwater fish *Colisafasciatus* and *Mystusmystus* for all the exposure periods (24 or 96h) in time as well as dose dependent manner. The LC_{50} values decreases from 0.084 ppm (24h) to 0.059 ppm (96h) in winter season (water temp.19^0) *Colisafasciatus* and LC_{50} 0.028 ppm (24h) to 0.010 ppm (96h) in summer season (water temp. 23^0). The LC_{50} decreases 0.039 ppm (24h) to 0.022 ppm (96h) in winter season against *Mystusmystuus* and LC_{50} 0.040 ppm (24h) to 0.015 ppm (96h) in summer season. The pesticide exposure may also fatal to many non- targeted organisms like fish where it hampers its health through impairment of metabolism, occasionally leading to the death of the fish.

1. Introduction

Pesticides are employed routinely in the integrated farming practice to protect crops and animals from insects, weeds and diseases. Liberal use of pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce is posing great danger to aquatic environment. These pesticides are carried into aquatic ecosystem by surface runoff from sites of application, where they enter the organisms through food webs and also through contact in water. Therefore, the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides. These pesticides are found to be highly toxic not only to fish but also to other organisms which constitute food of the fishes. (**Saeed *et al*., 2005**; **Singh *et al*., 2009**)
According to WHO estimate nearly three million cases of pesticide poisoning occur annually (**WHO,1992**). 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 (**Randy *et al*., 2004**). Like other organophosphates, rogor is also an acetylcholinesterase inhibitor, therefore, works primarily as nerve poison which is reflected in uncoordinated abnormal behaviour of the fish soon after exposure to pesticide. Contamination of aquatic ecosystem by pesticide can cause acute and chronic poisoning of fish and other organism (**Heger, 1995**).

2. Material and methods

2.1 Fish

Adult freshwater teleost fish *Colisa fasciatus* of uniform size range (length 6.3±0.86 cm; width 3.6±0.49 cm; weight 2.4±0.24 g) and *Mystus mystus*(length 7.2±11.3 cm;3.6-10 g; width 7.5±8.5 cm)were collected from different water bodies of Gorakhpur district of Uttar Pradesh, India and kept in glass aquaria containing 50L of de-chlorinated tap water for 7 days to acclimatize them to laboratory conditions. Water quality was measured according to the method of (**APHA, 2005**). The temperature of the experimental water ranged from 23.4 to 28.6^{0}C. The other parameters were within the following range: total alkalinity was 43-62 ppm, pH 6.8-7.7, dissolved oxygen 7.8-10.3 mg/L. Dead fish were removed as soon as possible to avoid water fouling. Fishes were fed daily on commercial fish food manufactured by Tokyo, Japan.

2.2 Pesticide

Dimethoate (Rogor) is one of the earliest insecticides widely used against vegetables and fruit sucking aphids, mites, saw flies and boring insects on cereals, cotton, chilli, tobacco and oil seeds. Pesticides are also well known for causing more toxic effects in teleost fish (**Scott *et al*.,1967; Jackson,1968**).

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2.3 Toxicity Experiments

Ten fishes were kept in glass aquaria containing 5L of de-chlorinated tap water. Fishes were exposed to four different concentrations of dimethoate (rogor) against fishes *Colisa fasciatus* and *Mystus mystus*. Organophosphates pesticides dimethoate was given as the final concentration in the test aquaria. Control fishes were kept in de-chlorinated tap water only. Each set of experiment was replicated six times. Mortality was recorded every 24h during the observation period.

Treatments	Concentration used (ppm)		
		Colisa fasciatus	*Mystus mystus*
Rogor (Dimethoate)	Summer	0.010, 0.015, 0.020, 0.25	0.015, 0.025, 0.035,0.045
	Winter	0.055,0.065,0.075, 0.085	0.020, 0.030, 0.040, 0.050

The LC values (LC_{10}, LC_{50},LC_{90}) upper and lower confidence limits (UCL, LCL at 95% confidence limits), slope values, 't' ratio and heterogeneity were calculated by using POLO computer programme (**Robertson *et al*, 2007**). The regression coefficient was determined between exposure time and different values of LC_{50} (**Sokal, 1973**).

3. Results

Like other organophosphates dimethoate (rogar) is also an acetylcholinesterase inhibitor therefore, works primarily as nerve poison which is reflected in uncoordinated abnormal behaviour of the fish soon after exposure to pesticide.

The mortality of freshwater teleost fish *Colisa fasciatus* and *Mystus mystus* due to exposure to four different concentrations of dimethoate (Rogar) for 24h, 48h, 72h and 96h in different water temperature (19^0C and 23^0C) are represented in (table 1-4). The LC_{50} values of dimethoate for freshwater fishes *Colisa fasciatus* at 24h, 48h, 72h and 96h were 0.084 (24h), 0.073 (48h), 0.065 (72h) and 0.059 (96h) in winter season and 0.028 (24h), 0.023 (48h), 0.016 (72h) and 0.010 (96h) in summer season respectively (Table 1 and 2). However, the LC_{50} values were decreased 0.040 (24h), 0.028 (48h), 0.019 (72h) and 0.015 (96h) (Table 3) in summer season and LC_{50} values decreased from 0.039 (24h), 0.032 (48h), 0.026 (72h) and 0.022 (96h) (Table 4) in winter season against freshwater fish *Mystus mystus* respectively. Dimethoate was more toxic in winter season than the comparison of summer season.

The slope values were steep and the results were found to be within the 95% confidence limits of LC values. The't' ratio was greater than 1.96 and the heterogeneity factor was less than 1.0. The 'g' value was less than 1.00 at all probability levels (tables 1-4).

Table 1 Toxicity (LC_{10} LC_{50} LC_{90}) of dimethoate (rogor) against freshwater fish *Colisafasciatus* at different time interval (winter season).

Exposure period	Effective Dose	Limits		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.050	0.040	0.056				
	LC_{50}=0.084	0.079	0.096	1.871±1.626	0.030	4.407	0.094
	LC_{90}=0.143	0.117	0.218				
48h	LC_{10}=0.044	0.035	0.050				
	LC_{50}=0.073	0.069	0.078	1.707±1.475	0.026	4.836	0.146
	LC_{90}=0.121	0.104	0.161				
72h	LC_{10}=0.039	0.029	0.046				
	LC_{50}=0.065	0.061	0.069	1.693±1.456	0.021	4.833	0.152
	LC_{90}=0.109	0.096	0.139				
96h	LC_{10}=0.041	0.035	0.046				
	LC_{50}=0.059	0.056	0.062	2.100±1.786	0.029	6.121	0.393
	LC_{90}=0.085	0.080	0.095				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of rogor.
- Concentrations given are the final concentration (w/v) in aquarium water.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL = Lower confidence limit; UCL = Upper confidence limit.

Table 2 Toxicity (LC_{10} LC_{50} LC_{90}) of dimethoate (rogor) against freshwater fish *Colisafasciatus* at different time interval (summer season).

Exposure period	Effective Dose	Limits		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.010	0.008	0.012				
	LC_{50}=0.028	0.022	0.046	1.054±0.570	0.005	3.89	0.037
	LC_{90}=0075	0.145	0.259				
48hs	LC_{10}=0.006	0.002	0.008				
	LC_{50}=0.023	0.018	0.043	0.836±0.447	0.001	3.12	0.116
	LC_{90}=0.094	0.048	0.816				
72h	LC_{10}=0.004	0.001	0.006				
	LC_{50}=0.016	0.014	0.021	0.786±0.418	0.000	3.12	0.215
	LC_{90}=0.068	0.038	0.425				
96h	LC_{10}=0.003	0.001	0.005				
	LC_{50}=0.010	0.008	0.012	0.856±0.452	0.000	3.74	0.447
	LC_{90}=0.033	0.024	0.071				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of rogor.
- Concentrations given are the final concentration (w/v) in aquarium water.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL = Lower confidence limit; UCL = Upper confidence limit.

Table 3 Toxicity (LC_{10} LC_{50} LC_{90}) of pesticides dimethoate(rogor) against freshwater fish *Mystusmystus* at different time interval (summer season).

Exposure period	Effective Dose	Limits		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.007	0.002	0.011				
	LC_{50}=0.040	0.033	0.060	0.351±0.227	0.001	3.48	0.131
	LC_{90}=0.236	0.118	1.590				
48h	LC_{10}=0.004	0.001	0.008				
	LC_{50}=0.028	0.022	0.035	0.335±0.215	0.000	3.41	0.129
	LC_{90}=0.179	0.096	1.033				
72h	LC_{10}=0.003	0.000	0.006				
	LC_{50}=0.019	0.013	0.023	0.340±0.217	0.000	3.47	0.141
	LC_{90}=0.118	0.072	0.452				
96h	LC_{10}=0.005	0.002	0.007				
	LC_{50}=0.015	0.011	0.018	0.429±0.268	0.001	5.091	0.537
	LC_{90}=0.046	0.038	0.063				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of rogor.
- Concentrations given are the final concentration (w/v) in aquarium water.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL = Lower confidence limit; UCL = Upper confidence limit.

Table 4 Toxicity (LC_{10} LC_{50} LC_{90}) of dimethoate (rogor) against freshwater fish *Mystusmystus* at different time interval (winter season).

Exposure period	Effective Dose	Limits		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.017	0.012	0.020				
	LC_{50}=0.039	0.036	0.044	0.546±0.374	0.009	5.75	0.116
	LC_{90}=0.091	0.072	0.136				
48h	LC_{10}=0.014	0.009	0.017				
	LC_{50}=0.032	0.029	0.035	0.512±0.346	0.007	5.87	0.136
	LC_{90}=0.074	0.061	0.104				
72h	LC_{10}=0.012	0.008	0.015				
	LC_{50}=0.026	0.023	0.029	0.532±0.355	0.005	6.02	0.162
	LC_{90}=0.060	0.051	0.077				
96h	LC_{10}=0.010	0.006	0.013				
	LC_{50}=0.022	0.019	0.025	0.585±0.387	0.004	5.89	0.446
	LC_{90}=0.050	0.043	0.061				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of rogor.
- Concentrations given are the final concentration (w/v) in aquarium water.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL = Lower confidence limit; UCL = Upper confidence limit.

4. Discussion

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. Contamination of aquatic ecosystem by pesticide can cause acute and chronic poisoning of fish and other organism (**Omitoyin, 2006**). The pesticides are found to damage vital organs of fish (**Velmurugan *et al*, 2007; Singh *et al*., 1996**), skeletal system and cause biochemical alterations in the exposed fishes (**Singh *et al*., 2002; Srivastav *et al*., 1997; Mishra *et al*., 2004; Begum *et al*., 1995**).

The LC_{50} values of Dimethoate for certain air-breathing teleosts are reported to be very high as in *Clarias batrachus* (**Srivastava VK *et al*., 2001**). Toxicity of dimethoate to *Colisa fasciatus* is relatively lower when compared to other air breathing fishes. The 96h LC_{50} value of dimethoate (21.42mg/l) found that is higher than 17.9 mg/l of *Channa punctatus* (**Pandey *et al*., 2009**) and 2.98 mg/l of *Heteropneustes fossils* (**Begum *et al*., 1995**). However, dimethoate is reported to be far less toxic to *Clarias batrachus* (**Begum *et al*., 1995**) with 65 mg/l as 96 hr LC_{50} value. In contrast, very low LC_{50} values have been reported for dimethoate in carp fishes. 0.007ppm is reported as 96hr LC_{50} value for dimethoate in *Catla catla* and 1.60 mg/l in *Cyprinus carpio* (**Kumar *et al*., 2000**).

Wide differences in LC_{50} values of air breathing and carp fishes reflect greater resistance of air breathing fishes to dimethoate which probably occurs due to their ability to adaptively shift towards aerial breathing in contaminated water. Different LC_{50} value of dimethoate in different fish species occur probably due to differences in susceptibility and tolerance related to differences in rates of accumulation, biotransformation and excretion of toxicant. Loss of balance and drowning reflect the progression towards death as fish succumbs to the continued high exposure of dimethoate. Similar alterations in behaviour of dimethoate exposed fish have been reported earlier in *Heteropneustes fossilis* (**Pandey, 2009**).

In spite of having short life and low chronic toxicity to mammals, their extensive applications may affect fish and other wildlife population. Recently a great deal of attention has been paid to evaluate the hazardous effects of organophosphorus pesticides on physiology of many non-target organisms, particularly fishes (**Dalela *et al*., 1978; Dalela *et al*., 1981; Ghosh,1985; Ghosh, 1989; Ghosh, 1986; Singh,1984; Verma, 1978**). (**Mandloi,1995**) in his toxicity experiments, of five organophosphorus pesticides on fingerlings of *Cyprinus carpio*, reported that 100% mortality was observed at 5 and 2.5 ppm of emulsified phenthoate and quinalphos, respectively. Apart from their well-known cholinesterase inhibitory effect, the organophosphorus pesticides have been found to affect the physiology of fishes (**Dalela *et al*., 1978; Deodhat *et al*., 1984**). Organophosphate compounds have been reported to produce biochemical changes in tissue of fish. Cythion has been reported to reduce protein level in brain, liver and ovary in fish *Channa punctatus* (**Ram *et al*., 1985**).

The slope line is an index of the susceptibility of the target animal to the pesticides used. A steep slope is indicative of rapid absorption and onset of effects. Even though the slope alone is not a reliable indicator of toxicological mechanism, it is a useful parameter for such a study. Since the LC_{50} of the pesticides of different concentrations lay with in the 95% confidence limits, replicate tests of random samples would indicate the concentration response lines in the same range (**Singh *et al*., 2009**).

5. Conclusion

The considering the hazards of pesticides, it becomes necessary to think about the danger of pesticides and make an effective strategy to keep them far from non-target organisms as far as possible. It is believed that the rogor (dimethoate) is toxic to the freshwater predatory fishes in aquatic medium.

Conflict of Interest

The authors report no conflicts of interest. The authors are responsible for the content of the paper.

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