ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 11, pp.22730-22733, November, 2022
DOI: https://doi.org/10.24941/ijcr.44280.11.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

TOXICOLOGICAL ALTERATION OF MALATHION AGAINST FRESHWATER PREDATORY FISH

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ARTICLE INFO

Article History:
Received 24th August, 2022
Received in revised form
18th September, 2022
Accepted 25th October, 2022
Published online 30th November, 2022

Key words:
Piscicidal, *Colisa Fasciatus*, *Mystus Mystus*, LC50, Season.

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ABSTRACT

Malathion one of the earliest organophosphate insecticides is being extensively used as dust, emulsion, and vapour to control a wide variety of insect pests under different condition. Malathion, one of the most extensively studied pesticides, may induce many significant changes in fishes. The malathion has shown strong piscicidal activity in freshwater fish *Colisa fasciatus* and *Mystus mystus* for all the exposure periods (24 or 96h) in time as well as dose dependent manner. The LC_{50} values decreases from 0.096 (24h) to 0.076 (96h) in a winter season (water temp.19^0) and 0.085(24h) to 0.051 (96h) in a summer season (water temp. 20^0c) against freshwater fish *Colisa fasciatus* and LC_{50} values decreases from 0.073 (24h) to 0.047 (96h) in a winter season (water temp.19^0) and 0.095 (24h) to 0.063 (96h) in a summer season (water temp. 20^0c) against freshwater fish *Mystus mystus*. The aim of the present study the toxicological action of malathion on freshwater predatory fishes. The Pesticide exposure may also fatal to many non- target organisms like fish where it hampers its health through impairment of metabolism, occasionally leading to the death of the fish.

Citation: ***Shakuntla Bharti and Ram P.Yadav.*** **2022.** "Toxicological alteration of malathion against freshwater predatory fish*". International Journal of Current Research*, 14, (11), 22730-22733.

INTRODUCTION

If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre, and Amelioration of vector-borne diseases, then their debits have resulted in serious health implications, to man and his environment. There is now overwhelming evidence that some of these chemicals do pose potential risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993; Igbedioh, 1991; Jeyaratnam, 1985). Malathion one of the earliest organophosphate insecticides developed in 1950 (Sanjoy and Mahanta, 2016). The possibility of being contaminated by a variety of chemicals in aquatic ecosystems that run through agricultural areas is high (Ramesh and Saravanan, 2008). Water is one of the primary ways pesticides are transported from an application area to other locations in the environment. Upon entering the aquatic environment produce multiple changes in the organism by altering the growth rate, nutritional value, behavioural pattern and so on. It is exigent to find out the detrimental effects of pollutants especially pesticides on fish since they form an important link in the food chain and their contamination by pesticides imbalance the aquatic system (Al-Otaib *et al*., 2017). The pesticide, malathion S-(1,2-bis- (ethoxy-carbonyl) ethyl)-O,O-dimethyl-dithiophosphate is a commonly used which cause serious intimidation to aquatic organisms and cause severe metabolic disturbances in non- target species like fish (Sudhasaravanan and Binukumari, 2015).

The use of pesticides has increased considerably to reduce the change caused by pests to standing crops. Among these pesticides the malathion are commonly used because of their rapid biodegradability and non-persistent nature, these compounds, which frequently enter the aquatic ecosystem through agricultural runoff and spraying operations adversely, affect non-target animals such as fish (Murphy, 1996). Therefore, in the present study, an attempt has been made to study the effect of malathion on freshwater Fish *Colisa fasciatus* and *Mystus mystus* with particular reference to the concentration of the pesticide and exposure periods.

MATERIALS AND METHODS

Adult freshwater teleost fish *Colisa fasciatus* of uniform size range (length 6.3±0.86 cm) and *Mystus mystus* (7.2±11.3 cm) were collected from different water bodies of Gorakhpur district of Uttar Pradesh, India. The fish *Colisa fasciatus* and *Mystus mystus* is an important fish of captured fishery and a good experimental, material due to its convenient size, easy availability and survivability under laboratory condition. The netted fish were brought to laboratory in plastic bucket and released in to tank containing de-chlorinated tap water. The physiochemical properties of water measured in the beginning of experiment by the standard method of (APHA, 2005). Prior to experiment fish were allowed to acclimate to laboratory conditions for 7 days. Diseased, injured and dead fish (if any) were removed as soon as possible in order to prevent the decomposition of the body in the tank. Fish were used fed with commercial fish food. Acclimatised fish were used for experiments.

The temperature of the experimental water was 20±0.7°C, pH was 7.3±0.2 dissolved oxygen was 7.2±0.3 mg/L, free carbon dioxide was 5.9±0.9 mg/L and alkalinity was 107. 7.8 mg/L. Water was changed every day. Dead fish were removed as soon as possible to avoid water fouling. Fishes were fed daily on commercial fish food manufactured by Tokyu, Japan.

Pesticide: Malathion, one of the earliest organophosphate insecticides is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions. Technical grade malathion 5% D.P active ingredient manufactured by Escort Crop Care Industries Bhopa Road Jat Mujheda, Muzaffarnagar (UP), India was used in the present toxicological experiments.

Toxicity Experiments: Toxicity experiments were performed by the method of Singh and Agarwal (Singh and Agarwal, 1990). Ten fishes were kept in glass aquaria containing 5L of de-chlorinated tap water. Fishes were exposed to four different concentrations of malathion which were 0.045, 0.055, 0.065 and 0.075 mg/L at summer season and 0.07, 0.80, 0.90, 0.95 in winter season against *Colisa fasciatus*; 0.065, 0.075, 0.085 and 0.095 mg/L at summer season and 0.35, 0.45, 0.55 and 0.65 in winter season against *Mystus mystus*.

Pesticides malathion were given as the final concentration (w/v) of aquatic ingredient 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 of 96h.The LC values (LC_{10} LC_{50} and $LC_{90)}$, upper and lower confidence limits (UCL, LCL) at 95% confidence limits), slope values, ‘t’ ratio and heterogeneity were calculated by POLO computer programme (Robertson, *et al*., 2007). The regression coefficient was determined between exposure time and different values of LC_{50} (Sokal and Rohlf 1973).

RESULTS

Behavioral changes due to malathion exposure: Irregular, erratic and some time jerky movements are more observable in fish. This abnormal behaviour sets after 36-40 hours manifestation of abnormal behaviour pattern is certainly a sign of in toxification, which is more under pesticides treatment. The fish exhibit a peculiar behaviour of trying to jump out from the treated water. Exposure to these pesticides, the fish resorts to erratic swimming indicating loss of equilibrium. It is that some region in the brain associated with the maintenance of equilibrium, should have been affected under experimentation periods. No such behavioural anomalies and death occurred in control group indicating that no factor other than the pesticides was responsible for altered behavioural and mortality. The mortality of freshwater teleost fish *C. fasciatus* and *Mystus mystus* due to exposure to four different concentrations of malathion for 24h, 48h, 72h and 96h in different water temperature (19ºC and 20ºC) are represented in (Table 1 and 2). The doses of different concentrations for malathion against freshwater fish *C. fasciatus* at 24, 48, 72 and 96h were 0.045, 0.055, 0.065 and 0.075 mg/L respectively at 19ºC water temperature (Table 1). However, these values were for *Mystus mystus* 0.65, 0.075, 0.85 and 0.095 mg/L for 24h, 48h, 72h and 96h at 20ºC water temperature, respectively (Table 2). In freshwater fish *Mystus mystus* due to four different concentration of pesticides malathion for exposure period 24h, 48h, 72h and 96h in different water temperature. The different concentrations of malathion for *Mystus mystus* is 0.035, 0.045, 0.055, 0.065 mg/L (Table 3) and 0.070, 0.080.0.090,0.095 mg/L for freshwater fish *Colisa fasciatus* (Table 4).

DISCUSSION

Malathion was more toxic in winter season than the comparison of summer season. The fish showed typical changes in behaviour when exposed to various concentrations of malathion. They have observed that the fishes experienced progressive lethargicity, loss of equilibrium, difficulty in respiration, exhibited convulsions, dashing against the wall of the experimental aquaria and short unpredictable bumpy body movements. Light coloured skin was observed by the on the fishes exposed to malathion with an excessive amount of mucus secretion over the body. However, Control fish behaved normally according to their observation (Salwa and Ella, 2008).

Table 1. Toxicity (LC_{10} LC_{50} LC_{90}) of pesticide (Malathion) against freshwater fish *Colisa fasciatus* (Summer season)

Exposure periods	Effective Dose	Limits		Slope value	‘g’ factor	‘t’ ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.045	0.035	0.050				
	LC_{50}=0.085	0.076	0.109	1.619±1.335	0.024	3.97	0.001
	LC_{90}=0.161	0.121	0.322				
48h	LC_{10}=0.035	0.024	0.041				
	LC_{50}=0.069	0.064	0.080	1.330±1.088	0.014	4.100	0.092
	LC_{90}=0.139	0.108	0.245				
72h	LC_{10}=0.026	0.014	0.034				
	LC_{50}=0.057	0.051	0.062	1.244±1.011	0.005	3.84	0.178
	LC_{90}=0.122	0.097	0.212				
96h	LC_{10}=0.028	0.019	0.034				
	LC_{50}=0.051	0.046	0.055	1.335±1.080	0.012	4.77	0.443
	LC_{90}=0.093	0.081	0.120				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of malathion.
- 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 pesticide (Malathion) against freshwater fish *Mystus mystus* (summer season)

Exposure periods	Effective Dose	Limits		Slope value	‘g’ factor	‘t’ ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.053	0.037	0.061				
	LC_{50}=0.095	0.088	0.112	2.171±1.982	0.032	3.61	0.000
	LC_{90}=0.170	0.134	0.319				
48h	LC_{10}=0.042	0.023	0.052				
	LC_{50}=0.079	0.073	0.085	2.004±1.820	0.018	3.45	0.117
	LC_{90}=0.149	0.121	0.268				
72h	LC_{10}=0.038	0.020	0.048				
	LC_{50}=0.070	0.061	0.075	2.058±1.863	0.015	3.51	0.126
	LC_{90}=0.129	0.109	0.201				
96h	LC_{10}=0.036	0.020	0.046				
	LC_{50}=0.063	0.052	0.068	2.278±2.054	0.007	3.752	0.157
	LC_{90}=0.108	0.097	0.142				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of malathion.
- 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 (Malathion) against freshwater fish *Colisa fasciatus* at different time interval (winter season)

Exposure periods	Effective Dose	Limits		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.071	0.065	0.075				
	LC_{50}=0.096	0.092	0.102	3.929±3.679	0.058	5.191	0.012
	LC_{90}=0.128	0.116	0.156				
48h	LC_{10}=0.060	0.050	0.066				
	LC_{50}=0.087	0.083	0.091	3.055±2.837	0.041	4.80	0.157
	LC_{90}=0.125	0.112	0.154				
72h	LC_{10}=0.054	0.043	0.061				
	LC_{50}=0.081	0.077	0.084	2.194±2.726	0.031	4.53	0.283
	LC_{90}=0.120	0.108	0.148				
96h	LC_{10}=0.054	0.045	0.060				
	LC_{50}=0.076	0.072	0.079	3.115±2.872	0.036	5.226	0.407
	LC_{90}=0.106	0.099	0.120				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of malathion.
- 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 pesticides (Malathion) against freshwater fish *Mystus mystus* at different time interval (winter season)

Exposure period	Effective Dose	Limits		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.037	0.030	0.042				
	LC_{50}=0.073	0.065	0.091	1.286±0.999	0.023	4.43	0.101
	LC_{90}=0.142	0.107	0.259				
48hs	LC_{10}=0.032	0.025	0.036				
	LC_{50}=0.062	0.057	0.071	1.063±0.819	0.018	4.85	0.245
	LC_{90}=0.122	0.096	0.193				
72h	LC_{10}=0.026	0.018	0.031				
	LC_{50}=0.054	0.050	0.060	0.953±0.729	0.011	4.63	0.230
	LC_{90}=0.113	0.090	0.181				
96h	LC_{10}=0.024	0.016	0.028				
	LC_{50}=0.047	0.043	0.051	0.953±0.725	0.011	5.011	0.756
	LC_{90}=0.094	0.079	0.131				

- There was no mortality in control groups.
- Batches of ten fishes were exposed to four different concentrations of malathion.
- 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.

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 1.0 at all probability levels (tables 1-4). Malathion and carbaryl are esterase inhibitor neurotoxicants, with acute cholinergic effect preceded by inhibition of acetylcholinesterase (Mount and Brungs, 1967). Being neurotoxicants, they interfere with many vital physiological functions (Barber *et al*., 1999) and consequently alter the levels of various body constituents (Rao and Rao, 1983; Arasta *et al*., 1996) in fishes. Inhibition of AChE resulted in accumulation of acetylcholinesterase (AChE), which causes twitching of muscle leading to tetanus and eventual paralysis of the muscle. Paralysis of respiratory muscle may lead to death.

Due to the agents being neurotoxicants the physiology of several systems may be affected, which results in disturbance of metabolic systems of the fishes (Begum and Vijayaraghvan, 1999). Salwa and Ella, 2008 conducted an empirical study on toxicity of malathion and its effect on the activity of acetylcholinesterase in various tissues of the grass carp, *Ctenopharyngodon idella*.

The Acute toxicity (LC_{50} values) reported by them for 24h, 48h, 72h, 96h, were 3.728 mg/l, 2.838 mg/l, 2.444 mg/l, and 2.138 mg/l respectively for grass carp. The safe concentration of malathion was 0.0513 mg/l. Mount and Brungs, 1967 had recommended that the safe level of malathion for fishes can beat some point between 1/15 and 1/45 of the 96h LC_{50} concentration (Rand and Petrocelli, 1988).

It is clear from the present study that all the group of pesticides used in this study has potent piscicidal activity in both the season i.e. winter and summer. Pollution of the aquatic environment by malathion adversely affect the metabolism of economical important fish *C. fasicatus* and *M. mustus*. So, the use of malathion in water bodies or fields adjoining the water bodies should be undertaken only after careful consideration.

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