

Acute toxicity of two organophosphorus pesticides to fry stage of flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758)

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Abstract

The present study assessed the acute toxicity (96 h LC₅₀) of two organophosphorus pesticides (OPs), chlorpyrifos (CPF) and dimethoate (DMT) to early life stage (fry) of flathead grey mullet, *Mugil cephalus* (total length, 22 ± 2 mm, wet weight, 285 ± 4 mg). *M. cephalus* were exposed to concentrations of CPF (2, 4, 6, 8, and 10 µg/L) and DMT (300, 750, 1500, 3000 and 4500 µg/L) for 96 h. The acute toxicity indices (96 h LC₅₀) indicated that CPF (3.45 µg/L; 95% confidence limits, lower = 2.77 and upper = 4.06 µg/L) was found to be highly toxic to *M. cephalus* fry compared to DMT (854.10 µg/L; 95% confidence limits, lower = 558.23 and upper = 1176.77 µg/L). Data obtained during the present study would be helpful in monitoring OP pollution in coastal waters with *M. cephalus* as key bioindicator species.

Keywords: Chlorpyrifos, Dimethoate, LC₅₀, Mortality, Pollution, Toxicity.

Introduction

Pesticides play an important role in promoting agricultural production by protecting crops from pests and vector-borne diseases. Due to their strong insecticidal ability and wide range of applications, organophosphorus pesticides (OP) occupy the top of commonly applied pesticides/insecticides to agricultural and horticulture crops such as cotton, rice, pasture, vegetables, etc^{1,2}. The toxicity mechanism OPs in vertebrate and invertebrates is by inhibiting the activity of acetylcholinesterase—an important enzyme involved in the transmission of nerve impulses and thus leading to blocking the cleavage of acetylcholine^{3,4}. Although obvious merits such as low persistence nature and rapid biodegradation in the aquatic environment outweigh in favour of OPs, their effect on non-target organisms however, has been a major concern^{1,2}.

Pesticides may enter into the estuarine, coastal and marine ecosystems from a variety of anthropogenic sources such as industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbour activities. The primary route of pesticide pollution in aquatic ecosystems is through rainfall-runoff and spray drift⁵. Furthermore, the coastal marine environment has also been impacted by OPs pollution from different activities such as transportation of these compounds via sea surface, indiscriminate use on agricultural and horticultural crops near to seashore, direct release of agricultural wastewaters containing high concentrations⁶. According to Bhardwaj & Sharma⁷, only 0.1% of pesticide targets the pest, whilst the remainder is permeated to the environment

In view of the toxic limit for aquatic life and also the lethal concentration and confidence limits, results of acute toxicity tests are meaningful⁸. The concentration that kills 50% of the test population within a given period is called lethal concentration (LC₅₀ values). It is an essential tool to measure the standard toxicity dose. The use of fish as biological indicators for assessing the effects of pollutants and their monitoring in aquatic environments has been widely recognized^{2-5,9-12}.

The flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758) is a benthopelagic catadromous, euryhaline and eurythermal fish known to inhabit in a wide-ranging environments (marine, brackishwater and freshwater) forms a subsistence fishery in tropical, sub-tropical and temperate regions^{13,14}. Due to its unique attributes such as acceptable commercial value and adaptability to variable environmental conditions (salinity and temperature), *M. cephalus* has been considered

as a good candidate species for aquaculture. *M. cephalus* is cultured in estuaries and ponds along the east and west coasts of India and is much relished for its flesh quality and good flavour^{13,14}.

Due to its extensive distribution, occupying diverse habitats and detritus-mud feeding habit often make *M. cephalus* a good bioindicator species for assessing the pollution¹⁵⁻¹⁸. Few toxicological studies with adult stages *M. cephalus* as test species to different xenobiotic compounds have been carried out earlier^{3,15-17}. Furthermore, due to variations in the degree of sensitivity among the numerous test criteria and test species, it is important to detect adverse effects of contaminants to early life stages of organisms³. Compared to vast published literature assessing the acute toxicity of pesticides to adult fish species,^{2,3,9-12,19-21} studies on early life stages of fish which are generally regarded as the most sensitive to toxicants, are limited^{22,23}. With this background, the present study investigated the acute toxicity of two widely used OP pesticides, chlorpyrifos (CPF) and dimethoate (DMT) to fry stages of *M. cephalus*. In view of the economic importance of *M. cephalus* as well as a biomarker for the monitoring of the health of coastal habitats²⁴, the results of the present study would help in monitoring pesticide pollution, particularly by OPs.

Material and Methods

Chemicals

The commercial-grade of OPs, chlorpyrifos (CPF) (diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl) oxy- λ^5 -phosphane) and dimethoate (DMT) (O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate) marketed under the brand names, 'PYRIBAN' (AIMCO Pesticides Ltd., Ratnagiri, India) and 'TAFGOR' (Rallis India Ltd., Akola, India), respectively were used. The commercial-grades of OPs, CPF 20% EC and DMT 30% EC were diluted with MilliQ water to prepare nominal stock solutions of 2 mg/L and 3 mg/L, respectively.

Collection and acclimatization of M. cephalus fry

The fry stages of *M. cephalus* (total length, 20 ± 3 mm; weight, 265 ± 4 mg) were collected from the brackishwater ponds connected to the River Mandovi at Old Goa, Goa (India). Immediately after being caught, the fry were transferred to 100 L tanks fitted with an aeration system and transported to the Aquaculture Laboratory, CSIR-National Institute of Oceanography, Goa (India). The fish fry were acclimatized to the laboratory conditions for one week in rectangular fibre reinforced plastic (FRP) tank (capacity, 2000 L) prior to the initiation

of experiments under a photoperiod of 16 : 8 h light : dark period. The seawater drawn from Dona Paula Bay (Lat. 15° 45' N; Long. 73° 80' E), Goa was treated by rapid sand filtration followed by cartridge filtration (20 to 1 µm) and finally disinfected by ultraviolet radiation. Due to the absence of major industries and large-scale agriculture/horticulture practices in the vicinity of seawater intake point, the natural concentrations of OPs in the seawater and sediment were undetectable.

During the acclimation, experimental animals were fed with freshly hatched nauplii of brine shrimp (*Artemia salina*) *ad libitum* daily. Optimum levels of dissolved oxygen (DO) were maintained in the acclimation tank with continuous aeration (Hi-Blow 150 air-pump, Japan). During acclimatization, the measured water parameters such as temperature (28.4 ± 0.2 °C), DO (6.5 ± 0.6 mg/L), salinity (15 ± 0.5 ppt), pH (7.8 ± 0.2), NO₂-N (< 0.05 mg/L), NO₃-N (0.85 ± 0.5 mg/L) and NH₃-NH₄ (0 mg/L) fell within the optimum range for the rearing of *M. cephalus*²⁵. The water in the rearing tank was replenished every day to prevent the built-up of metabolites (excreta, uneaten feed etc). The feeding was suspended 24 h prior to the initiation of the acute toxicity test. Experimental animals did not show any symptoms of stress or unusual behaviour during the acclimation and the mortality rate fell < 5%. The dead fry, noticed if any were removed immediately from the acclimation tank.

Acute toxicity experiments

Acute toxicity experiments were carried out in accordance with the standard procedures^{8,26}. For assessing acute toxicity (96 h LC₅₀), healthy and actively moving fry of *M. cephalus* (total length, 22 ± 2 mm; wet weight, 285 ± 4 mg) were selected from the acclimation tanks and exposed to definitive test concentrations of CPF (2, 4, 6, 8 and 10 µg/L) and DMT (300, 750, 1500, 3000 and 4500 µg/L). These concentrations were selected after range-finding acute toxicity tests. All experiments were conducted under aqueous semi-static renewal conditions and each test concentration was prepared in triplicate. A negative control (without pesticide) was included to ensure the reliability of the experimental procedure. No feeding was done during the acute toxicity test. All the experiments were performed in triplicate per pesticide.

Acute toxicity tests, separately (CPF and DMT) were performed in the glass jars (2-litre capacity) containing 1 L seawater with a stocking density of 10 fry/L. According to the time schedule of 24, 48, 72 and 96 h, mortalities in each test container were noted during the 96 h exposure (HoE). Behavioural alterations during the first 6 HoE and then at the end of 24, 48, 72

and 96 HoE were also observed. The absence of opercular movement and or no response to external stimuli were considered as indications of death. The dead organisms were removed immediately to avoid any type of bacterial contamination and the survivors were counted. In order to maintain the same stocking density, dead fry, if any were replaced with similar sized fishes reared separately in the same medium which has been exposed to the same HoE.

Statistical analysis

The median lethal concentrations (LC_{50} values) of CPF and DMT for different HoE (24 h, 48 h, 72 h and 96 h) and their 95% confidence limits (CL) were computed with a computer-based programme using the method of Log-Probit transformation for time and dose-mortality curves suggested by Finney's method²⁷. One-way analysis of variance (ANOVA) in conjunction with Dunnett's test was used to verify the LC_{50} values between different HoE²⁸. Significance in statistical tests was adjudged at a ' P ' = 0.05 level. All statistical analyses were performed by using computer-based GraphPad PRISM 5.0 software (Graph Pad, San Diego, CA, USA). A lowest observed effect concentration (LOEC) and no observable effect concentration (NOEC), respectively as the lowest concentration that had statistically significant mortality and the highest concentration that had no statistically significant mortality as in Pawar *et al.*¹ determined.

Results

No fish died during the acclimation period before exposure and also in control group during the experiments. Absence of mortalities in control tanks during acute toxicity test indicated that the test conditions were appropriate implying that the mortality recorded in the test solutions therefore could have been induced by pesticides. The percentages of mortality recorded in each treatment at different HoE are presented in Table 1. The mortalities in treatment tanks increased progressively with HoE and with the concomitant increase in the concentration of pesticides (Table 1) ($P < 0.05$). During the 96 h acute exposure, fish exposed to higher concentrations of (CPF > 6 $\mu\text{g/L}$ and DMT > 750 $\mu\text{g/L}$) showed pronounced behavioural and morphological changes causing abnormal swimming such as loss of buoyancy, opercular and erratic movements, mucus secretion and discoloration of the skin.

The 96 h LC_{50} values of CPF and DMT to *M. cephalus* fry were determined to be 3.45 $\mu\text{g/L}$ (95% CL, lower = 2.77 and upper = 4.06 $\mu\text{g/L}$ and; for DMT 854 $\mu\text{g/L}$ (95% CL, lower = 558.23 and upper = 1176.77 $\mu\text{g/L}$ and ($P < 0.05$), respectively (Table 2). The calculated LOEC and NOEC were 2 and 1 $\mu\text{g/L}$ for CPF and 300 and 120 $\mu\text{g/L}$ for DMT, respectively. The 96 h LC_{50}

value was 245 times higher and showed a progressively decreasing trend in DMT compared to CPF. Furthermore, during the experiment, there was no significant difference in water quality parameters between control and treatment tanks (Table 3). No significant variation ($P > 0.05$) in physico-chemical parameters of water between control and treatment tanks measured at 12 h intervals throughout the acute toxicity experiment was discernible.

Discussion

Many researchers have reported high concentrations of pesticides in agricultural wastewaters and their toxicity to aquatic organisms, especially in fish species¹⁰. In general, the pesticide contamination in the aquatic environment occurs via rainfall-runoff⁴. During the present study, the LC₅₀ levels of CPF and DMT to fry of *M. cephalus* assessed by static bioassay condition displayed varying degree of sensitivity. The 96 h LC₅₀ values of CPF and DMT to *M. cephalus* fry were determined to be 3.45 µg/L and 854.10 µg/L, respectively. Furthermore, the acute toxicity indices were known to be affected by the concentration and exposure time and the relationship between concentration and exposure time was highly significant.

The acute toxicity indices indicated that CPF was relatively more toxic to fry stages of *M. cephalus* compared to DMT. The median lethal concentrations of CPF to different fish species have been widely reported previously^{17,19,29}. Marigoudar *et al.*³, reported LC₅₀ values of 1.13 µg/L and 3.20 µg/L to fingerlings of *M. cephalus* and *Chanos chanos*, respectively. On the other hand, Schimmel¹⁷, observed that LC₅₀ values of CPF to be 5.4, 4.1 and 1.7 µg/L to *M. cephalus*, longnose killifish (*Fundulus similis*) and Atlantic silverside (*Menidia menidia*), respectively. Tilak *et al.*²⁹ has reported 96 h LC₅₀ values of technical grade CPF to three Indian major carp species (*Gibelion catla*, 350 µg/L; *Labeo rohita*, 470 µg/L; *Cirrhinus mrigala*, 650 µg/L). Rao *et al.*² reported LC₅₀ value of CPF to be 25.97 ± 0.01 µg/L for fish *Oreochromis mossambicus*. According to Pathiratne & Athauda¹¹, the LC₅₀ value of CPF to fingerlings of *Oreochromis niloticus* was observed to be 117 µg/L. Acute toxicity (96 h LC₅₀ values) of CPF to freshwater catfish, *Heteropneustes fossilis* during different reproductive phases, 0.174 mM/L (resting phase), 0.123 mM/L (preparatory phase) 0.026 mM/L (pre-spawning phase) have been reported¹⁹.

Compared to extensive literature in assessing the toxicity of CPF, relatively few studies on the acute ecotoxicity of DMT have been carried out in a number of fish species (Table 4). A LC₅₀

value of 14840 µg/L to fingerlings of *O. niloticus* has been reported¹¹. Singh *et al.*³⁰ reported the 96 h LC₅₀ of DMT to fingerlings of *Cyprinus carpio* to be 1600 µg/L. According to Lingaraja & Venugopalan³¹, LC₅₀ value of DMT to estuarine teleost (*Terapon jarbua*) was 700 µg/L. The median lethal concentrations (96 h LC₅₀) of DMT to Stinging catfish (*Heteropneustes fossilis*)²⁰ and Striped/Banded gourami (*Trichogaster fasciatus*)²¹ have been reported to be 2.98 mg/L and 21.65 mg/L, respectively. From these limited studies, it appears that DMT is relatively less toxic compared to CPF.

Our results are in good consonance with the previous reports indicating high toxicity of CPF and DMT to a variety of fish species^{2,11,17,29,30}. A wide range in responses by aquatic organisms following exposure to OPs depending on the compound, exposure time, water quality and species have been reported². A significant variation in acute toxicity (LC₅₀ values) of CPF and DMT to different fish species might be related to species-specific response, size of fish used for conducting bioassay, type of rearing water (freshwater or seawater/brackishwater) and interaction of pesticides with biotic/abiotic factors^{11,29,32}. Relatively higher sensitivity of early life stages of fish and invertebrates to toxicants compared to adult organisms have been previously reported^{1,23}.

During the present study, LOEC and NOEC values were calculated to be 2 and 1 µg/L for CPF and 300 and 120 µg/L for DMT. Marigoudar *et al.*³, reported the impact of sublethal concentration of CPF on average NOEC and LOEC values to be 0.09 and 0.17 µg/L for *M. cephalus* and 0.16 and 0.32 µg/L for *Chanos chanos*, respectively. During the present study, 96 h LC₅₀ values showed that CPF is highly toxic to fry stages of *M. cephalus* as compared to DMT. Relatively higher permeation by CPF into the lipid-rich tissues of aquatic animal attributable to its lipophilic nature leading to elevated levels of bioaccumulation, and responsible for severe damage or death of the organism has been reported^{9,30,33}. However, further research to understand the processes by which different OPs affect physiology and histology of different fish species is warranted.

Considering vulnerability of estuarine habitats and the associated fish assemblages potentially being impacted by a variety of anthropogenic influences have a direct influence on the food resources, distribution, diversity, breeding, abundance, growth, survival and behaviour of both resident and migrant fish species³. The effect of pesticide toxicity to other aquatic organisms,

including ichthyofauna which constitute the food chain in addition to agriculture crops has been documented^{12, 34} *M. cephalus* is a key indicator of coastal environment pollution, which feeds primarily on soft mud and other detritus. Owing to its detritivorous feeding habit, the likelihood of uptake of xenobiotic compounds (organics, pesticides, and heavy metals) by *M. cephalus* is quite high if the feeding areas are contaminated by these pollutants that settle from the water column or become bound to sediment³⁵. The greater vulnerability of *M. cephalus* to pesticide pollution in comparison to other filter-feeding fish species such as Tilapia attributing to its detritivorous feeding behaviour has been reported³⁶. Consumption of such pesticide contaminated fishes may adversely affect the health of human beings. Therefore, the results of the present study are useful for monitoring OP pollution, especially CPF and DMT with *M. cephalus* as key bioindicator species in the estuarine, coastal and marine environments.

In conclusion, our results demonstrated that CPF showed higher toxicity compared to DMT to fry stages of *M. cephalus* under similar experimental conditions. In both tested OPs, the rate of mortality was directly proportional to the concentration and exposure time. Lastly, the results of this study indicated that fry stages of *M. cephalus* are highly sensitive to OPs and thus could meet the requirement of bioindicator species.

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Authors Contribution:

Conceptualization: ASD, APP and SVS

Formal analysis: ASD and APP

Software: ASD and APP

Supervision: RAS and APP

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Table 1. The recorded mortality (%) in fry stages of *Mugil cephalus* for each tested concentration ($\mu\text{g/L}$) of chlorpyrifos (CPF) and dimethoate (DMT) at the end of different hours of exposure. Mortality data of three replicates (mean \pm SD).

CPF					DMT				
Conc. ($\mu\text{g/L}$)	24 h	48 h	72 h	96 h	Conc. ($\mu\text{g/L}$)	24 h	48 h	72 h	96 h
0.00	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.00	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
2.0	13.33 \pm 1.5	20.0 \pm 0.0	23.33 \pm 3.2	30.0 \pm 0.0	300	10.0 \pm 0.0	16.66 \pm 2.4	20.0 \pm 0.0	30.0 \pm 0.0
4.0	30.0 \pm 0.0	43.33 \pm 2.2	53.33 \pm 2.0	56.66 \pm 4.2	750	20.0 \pm 0.0	36.66 \pm 4.6	40.0 \pm 0.0	50.0 \pm 0.0
6.0	36.66 \pm 4.6	46.66 \pm 4.4	60.00 \pm 0.0	66.66 \pm 2.2	1500	33.33 \pm 2.2	40.0 \pm 0.0	46.66 \pm 4.0	60.0 \pm 0.0
8.0	43.33 \pm 2.2	56.66 \pm 4.2	66.66 \pm 4.40	80.0 \pm 0.0	3000	46.66 \pm 4.0	50.0 \pm 0.0	60.0 \pm 0.0	70.0 \pm 0.0
10.0	53.33 \pm 2.0	70.0 \pm 0.0	76.66 \pm 4.0	90.0 \pm 0.0	4500	56.66 \pm 4.2	60.0 \pm 0.0	70.0 \pm 0.0	80.0 \pm 0.0

Table 2. The 96 h LC₅₀ values (µg/L) with 95% confidence limits (in parentheses) for fry-stage of *Mugil cephalus* to chlorpyrifos (CPF) and dimethoate (DMT) at different periods of exposure. Different superscript letters in the same column significantly differ ($P < 0.05$).

Exposure period	CPF (µg/L)	DMT (µg/L)
24 h	9.39 (7.35–14.78) ^a	3397.74 (2466.54–5565.10) ^a
48 h	5.79 (4.74–7.22) ^b	2564.09 (1756.66–4604.50) ^b
72 h	4.32 (3.46–5.18) ^c	1600.61 (1156.25–2286.59) ^c
96 h	3.45 (2.77–4.06) ^d	854.10 (558.23–1176.77) ^d

Table 3. Water quality parameters (mean \pm SD) recorded in treatment and control aquaria with fry stages of *Mugil cephalus* during acute exposure experiment chlorpyrifos (CPF) and dimethoate (DMT). Superscripts sharing similar letter in the same row are not significantly different ($P > 0.05$).

Parameter	Control	CPF	DMT
Temperature ($^{\circ}$ C)	28.4 \pm 0.20 ^a	28.0 \pm 0.22 ^a	28.6 \pm 0.24 ^a
Salinity (ppt)	15.0 \pm 0.5 ^b	15.0 \pm 0.4 ^b	15.0 \pm 0.4 ^b
Dissolved Oxygen (mg/L)	6.5 \pm 0.6 ^c	6.2 \pm 0.4 ^c	6.1 \pm 0.4 ^c
pH	7.8 \pm 0.2 ^d	7.4 \pm 0.4 ^d	7.3 \pm 0.2 ^d

Table 4. Comparison of LC₅₀ values for chlorpyrifos (CPF) and dimethoate (DMT) obtained in the present study with those reported for other teleosts.

CPF			
Common name	Scientific name	96 h LC₅₀ values (µg/L)	Reference
Mozambique tilapia	<i>Oreochromis niloticus</i>	25.97	[2]
Milk fish	<i>Chanos chanos</i>	3.20	[3]
Grey mullet	<i>Mugil cephalus</i>	1.13; 5.4	[3]; [17]
Longnose killifish	<i>Fundulus similis</i>	4.1	[17]
Atlantic silverside	<i>Menidia menidia</i>	1.7	[17]
Catla	<i>Gibelion catla</i>	350 ; 1660	[29]; [40]
Rohu	<i>Labeo rohita</i>	470 ; 2350	[29]; [40]
Mrigal	<i>Cirrhinus mrigala</i>	650 ; 2350	[29]; [40]
Chola barb	<i>Puntius chola</i>	219	[41]
Common carp	<i>Cyprinus carpio</i>	8; 149	[42; 43]
DMT			
Jarboa terapon	<i>Terapon jarbua</i>	700	[31]
Stinging catfish	<i>Heteropneustes fossilis</i>	2980	[20]
Common carp	<i>Cyprinus carpio</i>	1600	[30]
Striped Gourami	<i>Trichogaster fasciata</i>	21650; 9300	[30]; [37]
Guppy	<i>Poecilla reticulata</i>	19000	[38]
Blue gill	<i>Lepomis macrochirus</i>	6000; 22400	[39]