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# Insecticides induced stress response and recuperation in fish: Biomarkers in blood and tissues related to oxidative damage

Madhusudan Reddy Narra [a], Kodimyala Rajender [a], R. Rudra Reddy [a], U. Suryanarayana Murty [b], Ghousia Begum [b, *]

[a] Department of Zoology, College of Science, Osmania University, Hyderabad, 500 007, India
[b] Toxicology Unit, Biology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, 500 007, India

## HIGHLIGHTS

- Toxicity and recuperation study of insecticides in food fish.
- Biomarkers of blood and tissues related to oxidative damage.
- Results indicate high efficiency of depuration process.

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## ABSTRACT

The present research investigated the growth, blood, antioxidant response (liver), AChE (brain and muscle) and Na+/K + ATPase in gills of *Clarias batrachus* exposed to 0 (control), two insecticides, 1.65 mg $L^{-1}$ chlorpyrifos (CPF) and 2.14 mg $L^{-1}$ monocrotophos (MCP) for a fixed interval time of 3, 6, 9, 12 and 15 days and follow up depuration process in fresh water for 30 days (at an interval of 7, 15 and 30 days). The toxicants exposed fish indicated significantly ($P < 0.05$) lower weight gain and HSI. The RBC, Hb, Hct, plasma total protein, glucose, albumin, globulin and respiratory burst activity was reduced. However, WBC, plasma glucose, serum creatinine, and triglycerides were enhanced. The weight gain, HSI and all haematological parameters were reversed following depuration of CPF and MCP exposed fish. Hepatic superoxide dismutase, catalase, lipid peroxidation, reduced glutathione, and glutathione S-transferase activities were significantly activated whereas glutathione peroxidase was inhibited in both tested groups. All the antioxidant enzymes were reversed on day 15 in MCP concentration, whereas CPF on day 30 of depuration process. The inhibition of acetylcholinesterase (brain, muscle) and gill Na+/K + ATPase activities were more in CPF exposure and early recovery in MCP. The results indicated that depuration process might help in detoxification of fish and improve growth, haematological conditions, oxidative stress and AChE, Na+/K + ATPase activity. However, further studies are needed in different fish species with different toxicants to support this strategy of depuration process in order to detoxify polluted fish.

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## 1. Introduction

Since the promotion of green revolution world-wide, there is an exponential use of pesticides, this resulted in high contamination of aquatic ecosystems. Some of these compounds are toxic and/or persistent and thus potentially threaten the aquatic environment (Mugni et al., 2013), less than 0.1% of an applied pesticide reaches the target pest, rest of 99.9% as an unintended pollutant in the environment (Pimentel, 1995). The environment contaminates routes by which the pesticides can move from the site of application via drift, runoff (Campo et al., 2013), volatilization (Majewski and Capel, 1995) and leaching (Aliyeva et al., 2013).

The organophosphates (OP) attract special attention because they constitute approximately 50% of the global insecticide use (Shittu et al., 2012). Malathion, an organophosphate insecticide induces significant cellular, biochemical and histological alterations in *Labeo rohita* (Karmakar et al., 2016). OP insecticides share a common mechanism, the inhibition of acetylcholinesterase (AChE),

* Corresponding author.
*E-mail addresses:* ghousia@iict.res.in, ghousi786@yahoo.com (G. Begum).

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the enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. Toxicity of environmental pollutants results in negative effects on many organs and systems such as the detoxifying (gills and liver), central nervous and hematological systems (Kubrak et al., 2013). Chlorpyrifos (CPF) and monocrotophos (MCP) are broad spectrum OP pesticides which are widely used in agriculture and domestic purposes (WHO, 2009; USEPA, 2011). The toxic effects of CPF are increasingly threatening the health of humans and aquatic animals (Xing et al., 2012). These pesticides are AChE inhibitors and altered the structural integrity of physiological activities of the respiratory organs and induce oxidative stress that serve to detoxify reactive oxygen species of aquatic animals (Kavitha and Rao, 2007; Shweta and Gopal, 2008; Oruç, 2010). The cholinesterases and carboxylesterases are most sensitive combination of enzymes in *Planorbarius corneus* exposed to a phosphorodithioate insecticide in vitro and in vivo (Otero and Kristoff, 2016).

Oxidative stress causes imbalance of oxidants and antioxidants in favor of the oxidants, potentially leading to cell damage (Azzi et al., 2004). Animals show an ability to live in contaminated sites, principally due to defense mechanisms that allow detoxification, excretion, antioxidant protection, and the stress response (Bard, 2000). Bioaccumulation of toxic substances triggers redox reactions generating free radicals, especially free oxygen radicals, but also other reactive oxygen species (ROS) are produced, that induce biochemical alterations in fish tissues (Sayeed et al., 2003; Narra, 2016). To counteract the toxic effects of ROS, aerobic organisms use both enzymatic and non-enzymatic antioxidants to scavenge the free radicals. However, when ROS generation exceeds the capacity of the cellular antioxidants, it will cause oxidative stress and oxidative damage (Kubrak et al., 2013; Shweta and Gopal, 2008; Narra, 2016).

The aim of the present work was to study the stress response (1/10 of $LC_{50}$) of CPF and MCP toxicity in *Clarias batrachus* and recuperation by releasing fish into fresh water. The parameters analyzed were growth, survival, hepatosomatic index, respiratory burst activity, ROS-scavenging enzymes (antioxidants in liver) AChE activity (muscle and brain) and ATPase (gills), as biomarkers of stress and recuperation.

## 2. Materials and methods

### 2.1. Chemicals

All the chemicals were purchased from Sigma-Aldrich chemical Co. The test compound chlorpyrifos 20% EC [*O,O,*-Diethyl-*O*-(3,5,6-Trichloro-2-Pyridyl) phosphorothioate] and monocrotophos 36% EC [Dimethyl (E)-1-methyl-2-(methyl-carbamoyl)] were purchased from local market (NOCIL Bombay-India).

### 2.2. Experimental animals and physicochemical parameters of medium

The freshwater fish, *Clarias batrachus* (average weight 40 ± 5 g and 22 ± 2 cm length) were obtained from a local supplier and transported to laboratory in large aerated drums. They were first given prophylactic dip (2% NaCl solution for 1 min) followed by oxytetracycline treatment (15 mg $L^{-1}$) for the first three days, and then acclimatized to laboratory conditions for 4 weeks prior to the experiment, during which they were fed *ad-libitum* with commercially available pellet feed twice a day. Water was changed 100% daily at 10 a.m. to maintain the quality of water and to remove the fecal matter and excess diets. The average mean values of water quality during experimentation are; temperature 25 ± 3 °C, pH 7.4 ± 0.4, dissolved oxygen 8.24 ± 0.22 mg $L^{-1}$, total hardness 415 ± 1.2 mg $L^{-1}$ as $CaCo_3$, alkalinity 348 ± 1.6 mg $L^{-1}$ as $CaCo_3$, and chlorides 245.57 ± 1.44 mg $L^{-1}$.

### 2.3. Experimental design

The $LC_{50}$ values were determined using the semi-static method; detail methodology is described previously (Narra et al., 2011). The present experiments were performed using 1/10 of $LC_{50}$ value of CPF and MCP (1.65 and 2.14 mg $L^{-1}$). The stock solutions of insecticides were prepared by dissolving 10 mg of CPF and MCP in 10 mL of acetone. One hundred forty four healthy fishes with active movement (average weight 40 ± 5 g and 22 ± 2 cm length) were distributed in three groups (control, CPF and MCP), of 48 fish per tank. Control group was kept in fresh water with carrier solvent (acetone); CPF and MCP group were exposed to 1.65 mg $L^{-1}$ chlorpyrifos and 2.14 mg $L^{-1}$ monocrotophos for a period of 15 days (3, 6, 9, 12 and 15). Fish were fed with commercially available fish feed during the experiment but starved 24 h prior to sampling. Water was 100% renewed daily throughout the experiment (10 a.m.) and the required concentrations of insecticides were added in order to maintain the toxicants concentration constant for 15 days. After 15 days 18 fish from CPF and MCP group were released in to fresh water tanks for 30 days (7, 15 and 30) to study recuperation.

### 2.4. Sample collection

At the end of the experimental period, (3, 6, 9, 12, and 15 days of stress and 7, 15, and 30 days of recuperation), six fish per group were sampled and anaesthetized on ice for 10 min and dissected, liver, muscle and gill tissues were used for the estimation of different parameters. Blood was collected from the caudal vasculature by syringe from fish selected randomly per tank and divided into two aliquots. The blood samples were transferred to tubes (contained 1.0% (v/v) of 15% EDTA), centrifuged (9000 × *g* for 10 min at 4 °C) and plasma was collected for haematological assay. For serum parameter blood was collected without EDTA and was allowed to clot for 2 h, centrifuged (3000 × *g* for 5 min) and kept at 20 °C for analysis.

### 2.5. Growth, survival and hepatosomatic index (HSI)

Weight gain (%) = Final weight−initial weight/initial weight *X* 100

Survival (%) = Number of fish survived after 30 days/initial number of fish stocked *X* 100

Whole liver was removed and weighed to calculate hepatosomatic index (HSI) standard equation: Liver weight (g)/whole body weight (g) *X* 100 (without gall bladder).

### 2.6. Estimation of haematological parameters

The red blood cells (RBC) count was determined by using Neubauer haemocytometer after diluting the blood with Grower's solution (Voigt, 2000). The white blood cells (WBC) were measured using a Neubauer haemocytometer after dilution with Dacie's solution (Dacie and Lewis, 2001). The haematocrit (%) values were determined by using a micro haematocrit centrifuge and haemoglobin (Hb) (g $dL^{-1}$) was estimated by the cyano-methemoglobin method. Plasma proteins, serum albumin, and globulin content were analyzed by kit (Biuret method using dye reagent, Qualigens Fine Chemicals, Mumbai, India). Plasma glucose was estimated using a commercially available kit (GOD-POD based kit for estimation of blood glucose procured from Diatek, Kolkata, India). Serum creatinine and triglycerides were measured by autoanalyzer (Vitalab Selectra, Merck KGaA, Darmstadt, Germany) following

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