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Ritesh Kumar
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Roma Kumari
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

BKP Mishra
Associate Professor, Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Corresponding Author:
Ritesh Kumar
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Behavioural and morphological changes induced in the freshwater fish, *Clarias batrachus* exposed to chlorpyrifos 50% + cypermethrin 5% EC

Ritesh Kumar, Roma Kumari and BKP Mishra

Abstract
The intention of the present study is to determine the detrimental effects of a hybrid pesticide, Chlorpyrifos 50% + Cypermethrin 5% EC on the behavior and morphological aspects of an air breathing fish, *Clarias batrachus* exposed to various concentrations of the pesticide. The study revealed marked behavioral changes like altered schooling behavior, localization at the bottom, irregular and erratic swimming pattern, frequent surface visit, caudal bending, spinning of the body and delayed response to touch. The morphological changes included body de-coloration, excessive mucus secretion, thinning of the body. These changes are indicative of the toxic effects of the pesticide on the behavior and morphological aspects of the fish, *Clarias batrachus*.

Keywords: Pesticides, fish, organophosphorous, organochlorines, carbamates, synthetic pyrethroids, behavior, morphology, *Clarias batrachus*

Introduction
Since its discovery, agricultural practices have seen huge scientific progress which have led to positive as well as negative impacts. The use of pesticides is one such issue. Use of pesticides in agriculture have some benefits as well as hazards. The primary benefits is increased agricultural outputs by killing caterpillars and other crop damaging pests. However, putting aside the benefits, there are numerous harmful hazards of the use of agricultural pesticides which directly or indirectly affects the biodiversity including humans. Pesticides enhance agricultural productivity by eradicating unwanted insects and controlling disease vectors. However, over spray/use of these chemicals have resulted in surface run off of these chemicals from agricultural fields, and may contaminate nearby water bodies. These chemicals seriously affect the non-target species including fish, amphibians, vertebrates etc. According to one report, only 0.1% of these chemicals reach the specific target [1]. The significant increase of pesticide contamination has led to deleterious effects on aquatic organisms [2].

The current trend of the use of the combination of two classes of pesticides (also called hybrid pesticides) has resulted in more serious effects. A hybrid pesticide includes the combined toxicity of two or more classes of pesticides to increase spectrum of action. However, the toxic enhancement of the hybrid class of pesticides is an issue of concern for the non-target species.

In current investigation, the toxic effects of the combination of an organophosphorous and a synthetic pyrethroid (Chlorpyrifos 50% + Cypermethrin 5% EC) have been studied on the behavioral and morphological aspects of a freshwater teleost fish, *Clarias batrachus* (Family Claridae), commonly known as 'Magur' in North Bihar.. Chlorpyrifos is a synthetic organophosphorous (OP), non-systemic and broad spectrum insecticide acting as a cholinesterase inhibitor and may get entry into the body via dermal contact, ingestion and respiratory pathway. Similarly, cypermethrin is synthetic pyrethroids and a potent neuro-inhibitor and several orders of magnitude more toxic to fish than organophosphate pesticides [3]. It is potent neurotoxicant that interfere with nerve cell function by interacting with voltage dependent sodium channel and causing paralysis [4]. Fish are able to uptake and retain different xenobiotics dissolved in water via active and passive processes. They can be used to detect and document pollutants released into their environment. Sub-lethal concentration of pesticides causes structural and functional changes in the fish body [5]. Behavioral modification is one of the most sensitive indicators of environmental stress. Alteration in fish behavior can also provide important indices for ecological assessment of changes in the properties (physical and chemical) of the habitat.

2. Materials and Methods

2.1 Procurement of the test animal

Healthy and living specimens of *Clarias batrachus* (Linn.) of size roughly in the range of 15- 25 cm. and weight 150- 220 gm. were collected from the local pond in Muzaffarpur, Bihar. The fish were disinfected by bathing them in 0.1% aqueous potassium permanganate ($KMnO_4$) solution for 15 minutes. The fish were acclimatized to lab conditions for 20 days. During this period, they were fed alternatively with pieces of soyabeans and chick's intestine. The average physico-chemical conditions of the lab as well as test water were maintained during this period.

2.2 Bioassay test

An acute toxicity LC50 test by the static renewal bioassay method was conducted to determine the toxicity of Chlorpyrifos 50% + Cypermethrin 5% EC in the fresh water fish, *Clarias batrachus* exposed to various concentrations of the pesticide till 96 hrs. Based on the mortality observed at different concentrations during 96 hrs, LC50 value was estimated for different periods such as 24 hrs, 48 hrs, 72 hrs and 96 hrs. For exposing the test animal to sub-lethal concentration of the pesticide, $1/12^{th}$ of the 96 hrs LC50 value (i.e. 0.5 µL/Litre of water) was taken. The fish were now exposed at this concentration for 30 days. During this period, the behavioral and morphological changes were deeply observed.

3. Results

3.1 Physico-chemical characteristics of the test water

The physico-chemical characteristics of the test water were estimated by using the procedures as mentioned by APHA [6]. The water quality parameters were as follows:

Table 1: Physico-Chemical Characteristics of Water

1.1	Temperature	26° ± 2.0 °C
1.2	pH	7.12 ± 0.14
1.3	Dissolved O_2	7.42 ± 1.10 ppm
1.4	Total hardness as $CaCO_3$	164.76 ± 5.38 ppm
1.5	Total alkalinity as $CaCO_3$	148.64 ± 7.77 ppm
1.6	Chlorides	14.42 ± 1.05 ppm

3.2 Behavioural and morphological changes

The present investigation have revealed marked changes in the behaviour as well as morphological features of the fish, *Clarias batrachus* exposed to lethal and sub-lethal concentrations of the pesticides. The physical and behavioural responses were almost similar at different concentrations except that the magnitude of the response increased markedly with increasing concentration of the pesticide during LC50 estimation process. At lower concentration of the pesticide, the fish showed disrupted school behaviour, localization to the bottom of the test tank and independency in swimming. The fish moved to the corners of the test vessel which can be viewed as avoidance behaviour of the fish to the pesticide. At higher concentration, irregular, erratic and darting swimming movement as well as loss of equilibrium were observed. The fish sometimes, stood hanging vertically in water. Fish slowly became lethargic, restless and secreted mucus all over the body. It frequently visited surface to gulp fresh air. However, during sub-lethal exposure, the fish remained motionless and lethargic (showing least response towards touch and disturbances) and stayed at the bottom. Caudal bending was observed frequently in the fish exposed to comparatively higher concentration along with the spinning of the body (like a screw). During sub-lethal exposure, feeding preferences were greatly affected and consumption of food was reduced. The fish became lean and thin along with the discoloration (dullness) of their body. Also, internally, the pesticide treated fish showed alteration in the normal coloration of their liver. The size of liver has also increased. Similarly, intestine also showed pathological lesions all over its surface.

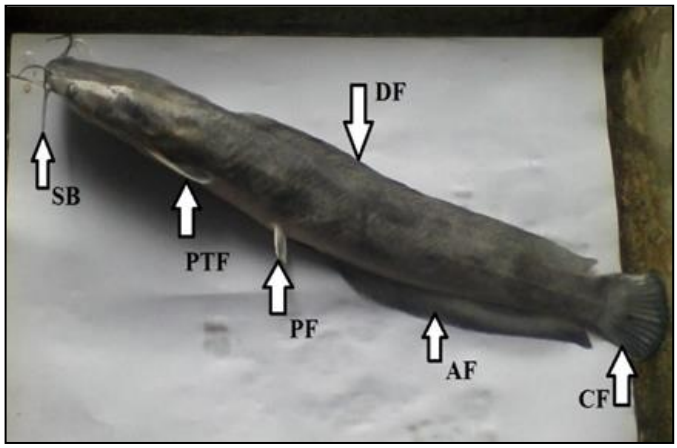

Fig 1: Fish showing body parts *viz*. caudal fin (CF), anal fin (AF), pelvic fin (PF), dorsal fin (DF), pectoral fin (PTF) and Sensory branches (SB)

Fig 2: Image showing increased mucus secretion over body due to pesticide exposure

Fig 3: Image showing de-coloration (dullness) of the body in the fish exposed to pesticide

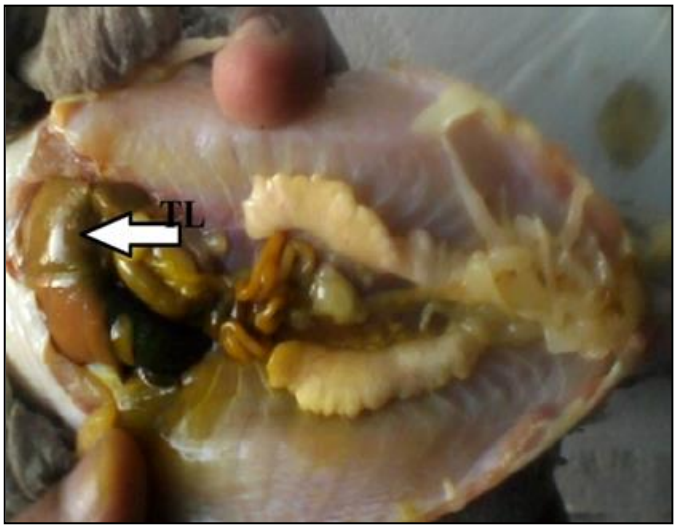

Fig 4: Image showing change in liver colour (TL exposed to pesticide).

4. Discussion

Behavioural changes such as disrupted school behaviour, localization to the test tank bottom, irregular, erratic and darting swimming movement, loss of equilibrium etc. may be due to the inhibition of acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine (ACh) in cholinergic synapses ensuing hyper-stimulation [7, 8]. These findings are in agreement with observations made by Hulya *et al*. in the sentinel freshwater fish, *Oreochromis niloticus* exposed to sub-lethal concentration of diazonin [9]. ACh is the primary neurotransmitter in the sensory and neuromuscular system in most species. Activity of ACh system is vital to normal behaviour and muscular function and represents a prime target on which some toxicants exert detrimental effects. Once bound, OP compounds are considered irreversible inhibitors. An excess secretion of mucus may be a strategy to avoid dermal contact with toxicant. Mucus forms a barrier between the body and the toxicant. Ural and Simsek also observed disrupted school behaviour, gulping of air as well as surface visit [10]. Frequent surface visit may be due to elevated oxygen demand during exposure period [11]. Caudal bending may be a sort of paralysis due to inhibition of muscular AChE activity resulting in blockage of neural transmission. This produces rapid twitching of the voluntary muscles followed by paralysis [12]. Changes in the body colour may be caused by impairment of pituitary functions reflected by reduction in number and size of chromatophores and their pigment content [13]. Body colour change has also been observed in *Cyprinus carpio* exposed to $HgCl_2$ [14]. Depression in feeding behaviour is a common response of fish under stressful conditions. Reduced feeding behaviour over a long period of time may have a clear impact on growth and reproductive physiology [15]. Dembele *et al*. indicated that the abnormalities in fish behaviour exposed to OP pesticide (chlorfenvinphos, chlorpyrifos and diazonin) could be related to failure of energy production or the release of stored metabolic energy which many cause severe stress, leading to the death of the fish [16]. Chawanrat *et al*. reported that inhibition of brain AChE activity is an early process of sub-lethal exposure of chlorpyrifos in hybrid catfish [17].

5. Conclusion

It is very much evident from our present investigation that the findings are in much similarities with the findings of several previous workers. Thus, the present study has revealed the deleterious effects of the hybrid pesticide, Chlorpyrifos 50% + Cypermethrin 5% EC on the behavioural and morphological changes in the freshwater teleost fish, *Clarias batrachus.* The fish showed marked changes in its behaviour and morphology which was in a way the outcome of the altered neurological/nervous coordination due to the pesticide.

6. References

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