



Comparative Assessment of Organophosphate and Pyrethroid Pesticide Toxicity on Hematological and Biochemical Parameters of Freshwater Fish *Channa punctatus* (Bloch.)

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Abstract

The food chain! Fish are a crucial source of protein for humans and other animals. When individuals or groups of fish are exposed to chemicals, it can negatively impact their growth and reproduction. In addition, when fish stay in polluted areas for long periods of time, toxins from these chemicals will accumulate in the fish's tissues. From a scientific perspective, these pollutants reduce the amount of natural food available for fish, but they also harm the habitat of the fish directly. Once fish are affected by pollutants, they can have difficulty surviving or reproducing. The effects of pesticides on aquatic ecosystems are complex, and more research is needed in order to understand how pesticides impact aquatic life. This paper will summarize literature regarding the impact of pesticides on freshwater fish specifically *Channa punctatus* (Bloch) and other aquatic animals as a result of their exposure to both organophosphate and pyrethroid classes of pesticides. The results of this study showed that both organophosphate and pyrethroid pesticides negatively impact the health of these fish. The negative effects from exposure to cypermethrin were greater than those from exposure to chlorpyrifos even when the concentrations of the two pesticides were equivalent. Overall, both types of pesticides were found to be harmful to the fish, though pyrethroids had greater toxicity than organophosphates. Thus, both hematological and biochemical parameters were determined to be good indicators of the effects of these pesticides on freshwater fish. The data presented in this paper indicate a clear need to manage and monitor for pesticide use in order to reduce potential damage to aquatic ecosystems from pesticide use.

Keywords: Organophosphate, Pyrethroid, Chlorpyrifos, Cypermethrin, Hematology, Biochemistry, *Channa punctatus*, Aquatic Toxicology

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Introduction

Pesticides have been used in modern agriculture for a long time and are one of its most significant components due to their ability to control insects, weeds and diseases that can harm crops. There is a growing demand for the food and other products of agriculture, which has led to the widespread use of many different types of pesticides globally. Within this category of products, organophosphate and pyrethroid pesticides are the most commonly used because they kill the target organisms quickly and they can kill these organisms over a large area (Aktar *et al.*, 2009). Pesticides are very important to crop production and food safety, but their use has raised significant concerns related to environmental contamination, particularly with regard to aquatic ecosystems. Pesticides are commonly found in rivers, ponds, lakes and other sources of freshwater (Schulz, 2004) due to runoff from agricultural land, accidental releases and the inappropriate disposal of pesticides. Fish are particularly susceptible to being adversely affected by pesticide contamination as water serves as a sink for many of the pollutants that are used in agriculture. Fish are excellent indicators of environmental quality because of their sensitivity to toxic chemicals and their tendency to bioaccumulate contaminants through the water around them (Van der Oost *et al.*, 2003). Fish exposed to pesticides can exhibit adverse physiological, biochemical, behavioral,

reproductive and developmental changes that may ultimately lead to mortality and population changes. Therefore, determining the effects of pesticides on fish is a critical aspect of ecotoxicology research. *Channa punctatus* (Bloch.), also known as the spotted snakehead, is one of the most common freshwater fish species found throughout South Asia, making it a valuable species for ecotoxicological research involving pesticides. The species is ecologically and economically significant, and highly sensitive to pollutants in the environment, which makes it an excellent test organism to evaluate the toxicity of aquatic environments (Nanda *et al.*, 2005). Additionally, this species has many advantages for use as an experimental model, such as its availability, adaptability to laboratory conditions, and physiological characteristics when studying the stress response of has been well-documented in studies using fish exposed to pesticide. Organophosphate pesticides are one of the most widely used types of insecticides in the world today. Chlorpyrifos, a representative organophosphate, is used extensively in agriculture to combat a variety of different insect pests. The mechanism of action for toxicity related to organophosphate chemicals is due to inhibition of acetylcholinesterase (AChE), and leads to the build-up of acetylcholine at synapses resulting in continual stimulation of nerves (Fulton & Key, 2001). Although organophosphate insecticides have less persistence in the environment compared to

organochlorines, there is still acute risk for non-target aquatic organisms associated with their use. Several studies have indicated that chlorpyrifos produces alterations in hematologic variables; metabolic disruption; and dysfunction of organs in freshwater fish (Velisek *et al.*, 2011).

Pyrethroid insecticides are the second most important class of synthetic insecticides due to their low toxicity (to mammals) and high efficiency as insecticides. Cypermethrin is one of the most widely used pyrethroid compounds used in agriculture and public health. Despite being viewed as safe, pyrethroids pose a significant threat to aquatic organisms, especially fish because they are absorbed through gill membranes quickly, and they have limited ability to detoxify depending on their metabolic pathway (Bradbury & Coats, 1989). The primary mode of action for pyrethroids is through disruption of voltage-gated Na⁺ channels and prolonged depolarization of nerve cell membranes, which directly leads to neurotoxic effects. Research has shown that fish exposed to cypermethrin have severe physiological stress, changes in blood parameters, and biochemical dysfunction (Saha & Kaviraj 2009).

The hematological parameters of fish are good indicators of physiological and/or pathological conditions and responses to environmental stress. Blood parameters can respond quickly to toxic substances and thus provide important information on the health of fish that have been exposed. The following hematological parameters have been shown to be altered after fish have been exposed to pesticides: red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Gabriel *et al* 2012). A decrease in RBCs and hemoglobin indicates that fish are anemic and/or have impaired oxygen transport due to damaged erythropoiesis, while increased WBCs indicate the activation of the immune system in response to the toxic stress.

Biochemical parameters are also important to measure in determining the toxicity of environmental contaminants. The enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) have important functions in metabolic processes and are routinely used as indicators of liver function. Increased enzyme activities for ALT, AST and ALP indicate that fish have experienced hepatic injury and damage to their cellular membranes due to pesticide exposure (Begum 2004). Likewise, the concentrations of total protein, albumin, and globulin provide valuable information on the nutritional status and protein metabolism of fish, as well as their level of immune competence. Changes in these biochemical parameters suggest that fish are not maintaining their physiological homeostasis due to toxicant exposure. Many studies have researched the toxic properties of organophosphate and pyrethroid insecticides; however, there is still little available information on how these two classes of pesticides compare to one another regarding the toxicity of freshwater fish. For efficient environmental risk assessment, and the creation of sustainable pest control methods, an understanding of the comparative toxicity of these two groups of pesticides is essential. Comparative studies will also provide researchers insight into the mechanism of toxicity that each pesticide has through its different modes of action and may help identify pesticide classes that present a higher risk to aquatic ecosystems. The purpose of this study

was to investigate and compare the toxic effects of chlorpyrifos (an organophosphate) and cypermethrin (a pyrethroid) on the hematological and biochemical parameters of *Channa punctatus*. This study aims to identify physiological changes in freshwater fish from the effects of pesticides by measuring changes to blood characteristics and assessing biochemical biomarkers, thereby contributing to the collective knowledge around aquatic ecotoxicology. Although freshwater ecosystems are some of the most diverse and productive ecosystems on Earth, they continue to receive increasing amounts of pollutants from anthropogenic sources. The rise in agricultural activity, industrial activity, urbanization, and population has resulted in an increase in the number of contaminants in aquatic systems. One of the most dangerous groups of chemicals that regularly pollute agricultural land is pesticides, due to their persistence, bioavailability, and toxicity to non-target organisms (Carvalho, 2017). The continuous release of pesticide residues into freshwater ecosystems has subsequently presented as a major problem within developing countries, where there is generally a culture of excessive pesticide use in agriculture; however, there is often a lack of adequate environmental monitoring to meet those levels of use.

India is one of the largest users of pesticides in the world, with insecticides comprising a large portion of the total amount used. Organophosphate and pyrethroid insecticides are widely used in crop protection programs because they are effective against many insect pests; however, less than 1% of what was applied arrives at the target, and the remaining pesticide may enter the environment through runoff, leaching, drift, or direct (off-target) application to water. Thus, aquatic organisms are continually exposed to pesticide residues, leading to detrimental ecological effects and physiological effects to those organisms.

Fish are a key member of aquatic ecosystems and serve as a primary source of animal protein for human populations. Fish readily absorb dissolved pollutants and contaminants through their gills, skin, and gastrointestinal tract due to their direct contact with the aquatic environment. Once the toxic chemicals are absorbed by the fish, they will accumulate in fish tissues and disrupt many physiological processes. Exposure to pesticides can negatively affect fish growth, reproduction, immune function, metabolism, and behavior, all of which result in decreased fitness and survival rates of fish (Authman *et al.*, 2015). Therefore, fish serve as effective biological indicators of environmental contamination and as indicators of the health of ecosystems.

Channa punctatus, the spotted snakehead, is a species of freshwater teleost from the family Channidae. This species inhabits ponds, rivers, lakes, canals, and wetlands throughout the Indian subcontinent. This species is capable of acclimating to a wide array of environmental conditions and is of significant importance both from a nutritional perspective and in terms of marketability. Due to its ecological importance and sensitivity to pollutants, *C. punctatus* has been widely used as a model organism in ecotoxicological studies of heavy metals, pesticides, and industrial waste products (Mishra & Srivastava, 1983). This species thus serves as a reliable model for studying the physiological effects of pesticides.

Organophosphate pesticides have their toxic effects mainly through blocking the enzyme; acetylcholinesterase, which is responsible for breaking down acetylcholine within the nervous system. As a result of blocking this enzyme,

acetylcholine builds up in the neuromuscular junctions, causing continued excitation of nerve endings and ultimately causing neuromuscular disruption. In fish, this has been linked with abnormal swimming patterns, difficulty in breathing, decreased feeding, and changes to the blood system; Singh and Sharma 1998. With long-term exposure these kinds of pesticides will disrupt normal metabolism and organ functioning and therefore will threaten fish health. of all of the organophosphate pesticides; chlorpyrifos, which is used heavily in agriculture; has received the most interest in regards to impacts on aquatic organisms. Several prior studies have shown that fish exposed to chlorpyrifos had lower red blood cell counts, lower hemoglobin levels and hematocrit levels than fish not exposed to chlorpyrifos. Higher levels of liver enzymes have also been detected in these fish suggesting that chlorpyrifos causes liver damage and alters metabolism; Ramesh and Saravanan 2008. These previous findings suggest that chlorpyrifos has the potential to cause significant physiological effects on both blood and biochemical functions of fish even at non-lethal doses. Pyrethroid insecticides were synthesized as synthetic versions of the naturally occurring insecticides found in the chrysanthemum flower. Generally, pyrethroids are assumed to have low environmental stability because of their high toxicity to aquatic organisms due to their highly lipophilic properties and their strong affinity for Cypermethrin is a type of insecticide made from pyrethroid materials that has been linked with producing highly toxic effects in freshwater fish at very low concentrations for example Dain, *et al.*, 2005 reported severe fish toxicity from as low as 1 pm of cypermethrin. Cypermethrin produces its toxic effects primarily through disruption of sodium channel function resulting in abnormal nerve transmission as well as physiological stress. Many studies have established substantial hematological abnormalities in freshwater fish as a result of exposure to cypermethrin. Most fish respond to cypermethrin exposure with decreased RBC counts, decreased hemoglobin concentrations, and decreased PCV levels attributed to hemolysis, abnormal erythropoiesis, or increased destruction of blood cells. Many fish also have elevated leucocyte counts due to toxic stress or tissue damage Svoboda, *et al.*; 2001; indicative of an adaptive response used to maintain homeostasis during times of environmental stress. Measurements of biochemical indicators can provide valuable information regarding the functional status of tissue and organ systems in animals exposed to toxicants. The liver is uniquely positioned to participate in detoxification and metabolism but therefore, is also particularly vulnerable to damage from pesticide exposure. The most common indicators (elevated serum activities of ALT, AST, and ALP) of liver injury in fish liver damage occurs as a result of leakage of these enzyme from damaged cell membranes and perturbation of normal liver function (Yonar, *et al.*, 2011). As well as being indicators of liver injury, elevations in enzyme levels may also reflect changes in protein metabolism due to decreased protein synthesis, increased protein degradation, reduced efficiency in the use of nutrients as a result of stress. Alterations in total proteins and the related constituents of albumin and globulins represent the total amount of proteins produced by the liver and therefore are important indicators of physiological health. Several authors have reported decreased total serum protein levels in fish exposed to pesticides, likely due to decreased hepatic protein synthesis and increased metabolic demand from

detoxification processes (Das and Mukherjee, 2003). These types of changes may inhibit the immune response and reduce the overall fitness of fish populations exposed to pesticides. Despite the body of literature each assessing the toxicity of pesticides, there are few studies directly comparing the toxicity of organophosphate and pyrethroid pesticides when applied to organisms under consistent experimental conditions. Because pesticides of these two classes are chemically different and differ in mode of action, environmental behavior, and toxicological properties, comparative studies are important in determining their ecological risk. Such information is essential for governments and agencies involved with regulatory, environmental, and agricultural management to assess the impact of pesticides when required for pest control and environmental conservation.

The purpose of this study is to evaluate blood and biochemical responses of *Channa punctatus* exposed to either chlorpyrifos or cypermethrin. The goals of this study are two-fold: to identify which class of pesticide results in more significant physiological disruption, and to evaluate studied biomarkers for the assessment of pesticide-related stress in freshwater environments. The results of this study have the potential to add to aquatic ecological risk assessments and provide the data needed to support the sustainable use of pesticides.

Materials and Methods

Experimental Fish-Aquarium-reared, and obtained from fish market(s) in the local area, healthy specimens of freshwater fish *Channa punctatus* (Bloch) (40-60g body weight, 12-15 cm length) were transported to the laboratory in aerated containers using dechlorinated tap water. Before pesticide experimentation began, fish were allowed to acclimatize under laboratory conditions for 2 weeks. During this time, fish were kept in glass aquaria filled with dechlorinated tap water, and were provided with commercial fish feed (twice daily). Aquatic environments were refreshed every 2 days (to ensure water quality was maintained at optimal levels) during the acclimatization period and were not fed for a period of 24 hours before the start of the pestidal (to minimize metabolic differences) (APHA 2017).

Experimental Chemicals-Two commonly used agricultural pesticides were selected for the study:

Organophosphate Pesticide

•Chlorpyrifos (20% EC)

Pyrethroid Pesticide

•Cypermethrin (25% EC)

Analytical-grade formulations were obtained from authorized agrochemical suppliers. Stock solutions were prepared using distilled water and diluted to the required experimental concentrations.

Experimental Design-Fish were randomly divided into four groups with ten fish in each group.

Group	Treatment
Control	Pesticide-free water
Group I	Chlorpyrifos exposure
Group II	Cypermethrin exposure
Group III	Chlorpyrifos + Cypermethrin exposure

The concentrations chosen were in accordance with their previously established sublethal thresholds (i.e., LC₅₀ values) for *Channa punctatus* and some similar freshwater fish (David *et al.* 2004; Velisek *et al.* 2011). The 30 day test duration occurred under semi-static conditions with the test solution being changed every 48 hours to ensure the pesticide

concentrations remained constant throughout the time of exposure.

Water Quality Parameters- Throughout the experiment, the following physicochemical parameters were monitored:

Water temperature: $26 \pm 2^\circ\text{C}$, pH: 7.2 ± 0.3 , Dissolved oxygen: 6.5 ± 0.5 mg/L, Total hardness: 120–140 mg/L as CaCO_3

These conditions were maintained within acceptable limits for freshwater fish culture.

Blood Collection-Anesthesia was induced on all fish using MS-222 (tricaine methanesulfonate) following the conclusion of the exposure event. Blood was collected from the caudal vein using a sterile disposable syringe, and a portion was transferred to an EDTA (ethylenediaminetetraacetic acid)-coated vial for the purpose of performing a hematology analysis. The remainder of the collected blood was allowed to clot prior to centrifugation at 3000 rpm for 15 minutes to produce serum that was subsequently stored at 4 degrees Celsius until analysis was performed (Blaxhall & Daisley, 1973).

Hematological Analysis

Red Blood Cell Count (RBC)-RBC count was determined using a Neubauer hemocytometer following the method described by Blaxhall and Daisley (1973).

White Blood Cell Count (WBC)-Total leukocyte count was estimated using a hemocytometer after appropriate dilution of blood samples.

Hemoglobin (Hb)-Hemoglobin concentration was measured by the cyanmethemoglobin method described by Drabkin and Austin (1932).

Packed Cell Volume (PCV)-PCV was determined using the microhematocrit technique and expressed as a percentage.

Mean Corpuscular Volume (MCV)-MCV was calculated according to Wintrobe (1934):

$$\text{MCV} = (\text{PCV} \times 10) / \text{RBC}$$

Mean Corpuscular Hemoglobin (MCH)

MCH was calculated using:

$$\text{MCH} = (\text{Hb} \times 10) / \text{RBC}$$

Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC was calculated using:

$$\text{MCHC} = (\text{Hb} \times 100) / \text{PCV}$$

Biochemical Analysis

Alanine Aminotransferase (ALT)-ALT activity was determined using the colorimetric method of Reitman and Frankel (1957).

Aspartate Aminotransferase (AST)-AST activity was estimated following the method of Reitman and Frankel (1957).

Alkaline Phosphatase (ALP)-ALP activity was measured according to the procedure described by Kind and King (1954).

Total Protein-Serum total protein concentration was determined by the Biuret method (Gornall *et al.*, 1949).

Albumin-Albumin concentration was estimated using the Bromocresol Green (BCG) method described by Doumas *et al.* (1971).

Globulin-Globulin concentration was calculated as:

$$\text{Globulin} = \text{Total Protein} - \text{Albumin}$$

Statistical Analysis-Mean \pm Standard Error of the Mean (SEM) were the values shown for the data. A one-way ANOVA test and Tukey post hoc test were used to determine the difference between each group. A statistically significant difference was defined as $p < 0.05$. All statistical analysis was completed using SPSS version 25.0.

Results

Effect of Chlorpyrifos and Cypermethrin on Hematological Parameters of *Channa punctatus*

Table 1. Hematological parameters in control and pesticide-exposed fish

Parameter	Control	Chlorpyrifos	Cypermethrin	Combined Exposure
RBC ($\times 10^6/\text{mm}^3$)	3.52 ± 0.08^a	2.98 ± 0.07^b	2.61 ± 0.06^c	2.24 ± 0.05^d
WBC ($\times 10^3/\text{mm}^3$)	24.3 ± 0.7^a	29.8 ± 0.8^b	33.7 ± 0.9^c	38.5 ± 1.1^d
Hemoglobin (g/dL)	10.82 ± 0.24^a	9.21 ± 0.20^b	8.42 ± 0.18^c	7.58 ± 0.16^d
PCV (%)	34.6 ± 0.8^a	30.4 ± 0.7^b	27.8 ± 0.6^c	24.9 ± 0.5^d
MCV (fL)	98.3 ± 2.1^a	102.0 ± 2.3^b	106.5 ± 2.4^c	111.2 ± 2.6^d
MCH (pg)	30.7 ± 0.7^a	30.9 ± 0.6^a	32.2 ± 0.7^b	33.8 ± 0.8^c
MCHC (%)	31.3 ± 0.6^a	30.3 ± 0.5^b	29.2 ± 0.5^c	28.1 ± 0.4^d

Different superscripts (a–d) indicate significant differences at $p < 0.05$.

Compared to the control group, the exposure (toxicity) to chlorpyrifos or cypermethrin reduced RBC count, then lowered hemoglobin concentration (output) and then lowered packed cell volume (PCV). The biggest impact came from those in combined exposure. All treated groups had significant increases in WBC indicating an activated immune response; cypermethrin shows more evident hematological effects than chlorpyrifos alone.

Effect of Pesticides on Biochemical Parameters

Table 2. Biochemical parameters in control and treated fish

Parameter	Control	Chlorpyrifos	Cypermethrin	Combined Exposure
ALT (U/L)	24.8 ± 0.7^a	36.4 ± 1.0^b	42.8 ± 1.1^c	51.6 ± 1.3^d
AST (U/L)	68.5 ± 1.6^a	87.2 ± 2.1^b	96.7 ± 2.3^c	112.8 ± 2.7^d
ALP (U/L)	74.2 ± 1.8^a	95.3 ± 2.2^b	108.6 ± 2.5^c	124.7 ± 2.9^d
Total Protein (g/dL)	5.82 ± 0.14^a	4.91 ± 0.12^b	4.38 ± 0.10^c	3.86 ± 0.09^d
Albumin (g/dL)	2.94 ± 0.08^a	2.48 ± 0.06^b	2.16 ± 0.05^c	1.84 ± 0.04^d
Globulin (g/dL)	2.88 ± 0.07^a	2.43 ± 0.06^b	2.22 ± 0.05^c	2.02 ± 0.04^d

Different superscripts (a–d) indicate significant differences at $p < 0.05$.

There was a significant rise in ALT, AST and ALP enzyme activity in fish exposed to pesticides showing liver dysfunction and metabolic stress. The highest enzyme level activity was found in the combined pesticide exposed fish group. All treated groups had a significant decrease in total protein, albumin and globulin concentrations indicating impaired protein synthesis as well as physiological disturbances. Exposure to cypermethrin led to more alterations in biochemical measurements than chlorpyrifos.

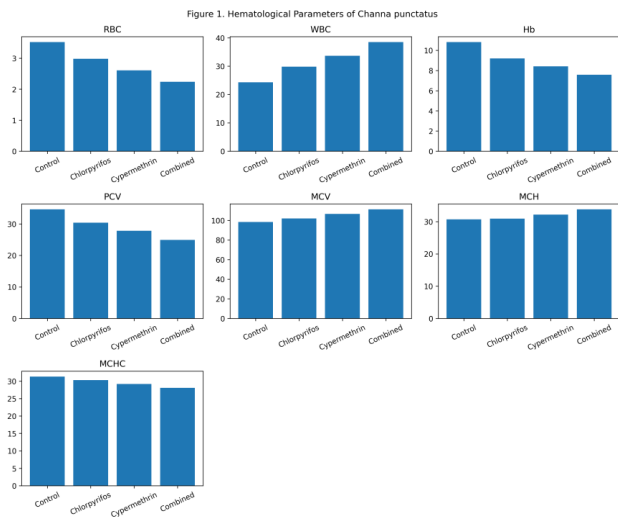


Figure 1. Comparative effects of chlorpyrifos, cypermethrin and combined exposure on hematological parameters (RBC, WBC, Hemoglobin, PCV, MCV, MCH and MCHC) of *Channa punctatus*. Values are expressed as Mean \pm SE (n = 10). Different superscript letters indicate statistically significant differences among experimental groups (p < 0.05).

Chlorpyrifos and cypermethrin caused a decrease in red blood cell count (RBC), hemoglobin level and packed cell volume (PCV); however, white blood cell count was significantly higher when compared to controls. The combined exposure group had the most dramatic changes in hematology. Fish exposed to cypermethrin had much larger deviations from normal values than did fish exposed to chlorpyrifos; thus indicating higher toxicity.

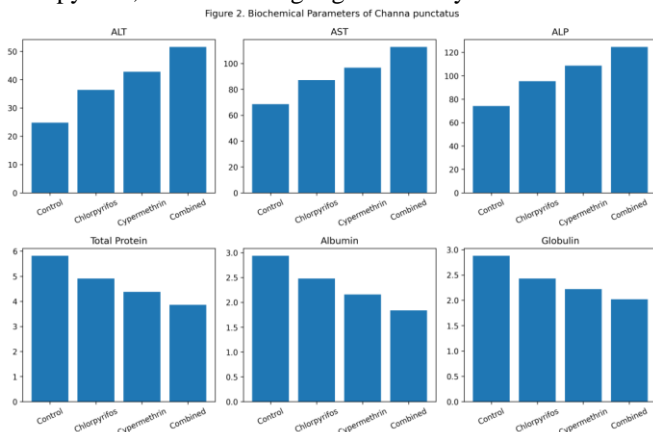


Figure 2. Comparative effects of chlorpyrifos, cypermethrin and combined exposure on biochemical parameters (ALT, AST, ALP, Total Protein, Albumin and Globulin) of *Channa punctatus*. Values are presented as Mean \pm SE (n = 10). Different superscript letters indicate significant differences among treatment groups (p < 0.05).

In fish exposed to pesticides, parameters relating to their health have markedly changed. ALT, AST and ALP activity were all elevated in the fish exposed to both insecticides. The concentration of total protein, albumin and globulin were significantly decreased. The fish that were exposed to both cypermethrin and chlorpyrifos (combined exposure) experienced the highest level of ALT, AST and ALP activity, while simultaneously having the lowest concentration of total protein, albumin and globulin (lowest concentration). Cypermethrin caused more pronounced disruption to biochemistry than chlorpyrifos suggesting a greater potential for hepatotoxicity.

Comparative analysis revealed that both insecticides induced significant physiological stress in the fish (*Channa punctatus*); however, cypermethrin caused greater hematological and biochemical effect than chlorpyrifos. Exposure to both insecticides resulted in increased/toxicity which may indicate that the two insecticides have either additive or synergistic interactions.

Overall Toxicity Ranking

Combined Exposure > Cypermethrin > Chlorpyrifos > Control

The results indicate that pyrethroid pesticides may pose a greater risk to freshwater fish health than organophosphate pesticides under similar exposure conditions.

Discussion

This study was designed to evaluate the effects of chlorpyrifos (an organophosphate) and cypermethrin (a pyrethroid) on key physiological indicators (i.e., hematological and biochemical parameters) of the freshwater fish *Channa punctatus*. Based on the results, exposure to either pesticide led to measurable physiological disruptions, with cypermethrin exhibiting a greater degree of toxicity than chlorpyrifos. Moreover, the most dramatic physiological changes occurred in fish exposed to both pesticides combined; thus suggesting that the combined toxicity associated with these two pesticides is at least additive and possibly synergistic. Changes in hematological parameters are highly sensitive indicators regarding environmental stressors and exposure to toxicants in fish. In the present study, fish exposed to either of the two pesticides had a significantly decreased RBC count, reduced hemoglobin concentration, and lower packed cell volume (PCV). Collectively, these results are indicative of the development of anemia and inadequate oxygen-carrying capacity of blood. Similar findings have been reported in other studies, where fish exposed to organophosphate and pyrethroid pesticides exhibited similar reductions in RBC and hemoglobin concentrations (Svoboda *et al.*, 2001; David *et al.*, 2004). The reduction in RBC counts could be due to increased destruction of RBCs, decreased erythropoiesis; or damage to erythropoietic tissue. Reduced hemoglobin concentrations could also result in inadequate oxygen transport, resulting in tissue hypoxia and metabolic disturbances.

The significant increase in WBC counts seen in the pesticide-exposed treatment groups could be an indication of an adaptive immune response to stress due to pesticide exposure. Fish exposed to toxic materials typically show signs of leukocytosis, which is commonly associated with activation of fish's defense mechanisms (Gabriel *et al.*, 2012) and an inflammatory response to tissue damage as a result of exposure to toxic materials. Elevated WBC levels are indicative of the fish's attempt to prevent further damage and to restore normal physiological function from the effects of toxic materials. The highest WBC count from the three groups that were given 3 different types of pesticides is the reason that those groups had an increased level of immune activation and stress from multiple exposures to pesticides. The absolute alteration of MCV, MCH and MCHC in the blood of fish that were treated with pesticides confirms that the use of pesticides did alter the hematological parameters. An increase in MCV and MCH suggests that there were possible compensatory mechanisms for anemia, while the

decrease in MCHC indicates the potential for morphological changes and altered hemoglobin production. The findings in this study are similar to those previously reported in literature evaluating the effects of pesticides on freshwater fish (Mishra & Srivastava, 1983; Velisek *et al.*, 2011). Collectively, the hematological alterations seen in this study are indicative of the adverse effects on the blood physiology of *Channa punctatus* by both chlorpyrifos and cypermethrin. Biochemical biomarkers also provide valuable information regarding the function and metabolic health of organs. The present study showed that there were significantly elevated levels of ALT, AST, and ALP in the blood of pesticide-exposed fish. These enzymes are commonly used as indicators of the structural or cellular integrity of the liver and of cellular damage to the liver. Increased serum activity of these enzymes generally indicates a leak of enzymes from damaged hepatocyte membranes and/or a disruption of the normal function of the liver (Begum, 2004). The liver, as the primary organ of detoxifying and metabolizing xenobiotic compounds, is very susceptible to damage from pesticides. The elevations in ALT and AST in fish exposed to pesticides indicate that there is a stress response in the liver and that the metabolism of amino acids is altered. Fish that were exposed to chlorpyrifos, cypermethrin, malathion and other pesticides have shown similar increases in their enzyme activities (Ramesh & Saravanan, 2008; Yonar *et al.*, 2011). The cypermethrin-treated fish showed a greater level of enzymatic activity than those treated with organophosphates, indicating that pyrethroid pesticides can cause more damage to the liver than organophosphates under similar conditions of exposure. Furthermore, the very large increase in enzymes in the group exposed to both pesticides provides evidence of cumulative toxicity. Alkaline phosphatase (ALP) is an important enzyme in regard to membrane transport and phosphate metabolism. The significant increase in ALP activity in the fish exposed to pesticides indicates some form of tissue breakdown, alteration to the membrane's permeability and increased metabolic activity within the organism itself. Evidence of similar findings exists in a number of different species of fish that were exposed to agricultural contaminants (Das & Mukherjee, 2003). Because of the increased ALP activity measured in the fish, it has been proposed that it may be a useful biomarker for pesticide-induced physiological stress. A significant decrease in amount of total protein, albumin and globulin has also been recorded after exposure to pesticides. Proteins are essential for growth, metabolism, immune function and to maintain cellular integrity. Decreased levels of protein may arise from decreased synthesis of protein, increased degradation of protein, or increased use of amino acids as energy sources under stress conditions (Begum, 2004). Since the liver is the primary site of protein synthesis, it could directly lead to the decreased levels of serum protein due to pesticide-induced liver damage. Albumin assists in the maintenance of osmotic balance, as well as being a vehicle for different metabolites, whereas globulins are dependent on immune function. The current research indicates that there has been a dramatic decrease in the levels of serum albumin and globulin, which implies that normal functioning, such as physiological processes utilize protein for building tissue and protecting against disease, has been interrupted by the impact of these two pesticides. Fish exposed to the insecticides Chlorpyrifos and Cypermethrin have previously been observed to have decreased serum protein levels (Das &

Mukherjee, 2003; Velisek *et al.*, 2011). As indicated by the biochemistry of the present study, the long-term exposure to pesticides will adversely affect the general health of fish and the ability of fish populations to resist disease. The extent of changes observed in both haematological and biochemical variables among the fish exposed to Cypermethrin was considerably greater than those of Chlorpyrifos. Aged literature verifies that pyrethroids are more toxic to aquatic species than other pesticides (Bradbury & Coats, 1989; Saha & Kaviraj, 2009). Fish are very susceptible to the effects of pyrethroids, since pyrethroids can easily pass through the gill membranes of fish, and fish have lower detoxification rates than they do to other types of insecticides. Therefore, even small amounts of cypermethrin can cause significant physiological stress to the fish. In the present study, the fish that were exposed to the combined treatment of chlorpyrifos and cypermethrin exhibited the highest level of toxicity to them. This indicates that there was at least an additive, if not a synergistic, effect of the exposure to both chemicals. In the environment, aquatic organisms are commonly exposed to a mixture of contaminants rather than isolated contaminants. Therefore, the combined toxicity of those contaminants must be assessed for accurate environmental risk assessments to aquatic organisms. The physiological changes observed in the fish of the present study as a result of exposure to either pesticide strongly suggest that multiple classes of pesticides will have significant impacts on freshwater fish populations. The current results provide evidence that the haematological and biochemical indices can be used as reliable indicators of pesticide toxicity in fish, and that they can be measured as an early sign of physiological stress prior to the occurrence of any observable effects (e.g. behaviour transitions or lesions). Thus, using these indices as part of an overall monitoring program supports monitoring and assessment of the health of aquatic ecosystems. In conclusion, the results of this study provide evidence that the physiological condition of *Channa punctatus* was negatively impacted by chlorpyrifos and cypermethrin, and that cypermethrin is the more toxic of the two pesticides. This study provides support for the need for more responsible use of pesticides and more proactive management to reduce the potential for contamination of freshwater ecosystems and protect aquatic species biodiversity.

Conclusion

Data from the present study compared toxic effects of chlorpyrifos (an organophosphate) and cypermethrin (a pyrethroid) on hematological and biochemical parameters in freshwater fish, *Channa punctatus*. The results demonstrated that exposure to both pesticides resulted in significant physiological disturbances in fish which indicates that both pesticides pose a risk to aquatic organisms. Significant differences in blood parameters and serum biochemistry showed that exposure to pesticides negatively impacted the overall health and metabolic function of experimental fish. Pesticide treated fish had significantly lower red blood cell (RBC) counts, hemoglobin concentrations and packed cell volumes (PCV), which indicates that fish developed anemia and were unable to transport oxygen appropriately. Elevated white blood cell (WBC) counts in fish from pesticide treated groups indicate activation of the immune system and have undergone physiological stress. Additionally, alterations in erythrocyte indices confirm that hematological abnormality exists due to exposure to pesticides. The elevated activities of

ALT, AST and ALP in the serum indicate that pesticide exposure results in liver dysfunction and tissue damage. The decrease in total protein, albumin and globulin concentrations indicate impaired protein metabolism and disruption to normal physiological processes. These data validate that both chlorpyrifos and cypermethrin negatively affect liver function and metabolic homeostasis in *C. punctatus*. Comparative evaluations indicated that cypermethrin produced greater hematological and biochemical changes than chlorpyrifos, suggesting that pyrethroid pesticides may pose a greater risk to freshwater fish exposed in the same manner. Exposure to both pesticides demonstrated the greatest toxicity, suggesting either additive or synergistic interactions among the two classes of pesticides. This has important ramifications, because aquatic organisms in natural ecosystems are often exposed to chemical mixtures rather than individual chemical contaminants. The current study demonstrates that hematological and biochemical biomarkers serve as effective early indicators of pesticide-induced stress in aquatic ecosystems and thus could be used as early warning indicators of physiological impairment. Biomarkers may also be included in environmental risk assessment programs as part of an overall assessment of the ecological risk of indiscriminate pesticide use and thus also indicate the need for adequate management practices that minimize the contamination of freshwater resources. The current research supports the conclusions that both chlorpyrifos and cypermethrin represent significant population level threats to the health of *C. punctatus*, with cypermethrin being comparatively more toxic. Furthermore, the present study provides information regarding fish exposure to pesticide-induced physiological disturbances, as well as relevant data for monitoring the environment, aquatic toxicology, and developing sustainable agricultural practices aimed at preserving freshwater biodiversity.

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