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Immunological Changes in Two Sizes of *Tilapia guineensis* Exposed to Fenthion Formulations

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ABSTRACT
The immunological profiles of Tilapia guineensis juvenile and adult sizes were examined for the acute impacts of Fenthion formulation insecticide. The fish were treated to the chemical at five different doses over the course of 15 days: 0.00 (control), 0.05, 0.10, 0.15, and 0.20 mg/L. Six water quality factors, including temperature, pH, salinity, dissolved oxygen, nitrite, and ammonia, were assessed during the exposure. Blood samples from the fish were taken at the conclusion of the experiment, and they were examined for immunological profiles using conventional laboratory techniques. The results showed that as the chemical's concentration was increased, so did the values for ammonia and nitrite. While the dissolved oxygen values decreased. Salinity, pH, and temperature were all within the same range, though. Leucocytosis, a normal response of the fish body to attacks of foreign substances, such as fenthion, which can alter the normal physiological function in fish, increased as a result of the chemical's significant distortions in the immune profiles of T. guineensis. Significant changes (infections) that appear after exposure to this chemical include significant increases (P<0.05) in the number of lymphocytes (lymphocytosis), eosinophils (eosinophilia), and neutrophils (neutropenia), along with significant declines (monocytopenia) in each cell type.

Key words: *Immunology, Toxicants; Stress; Tilapia, Aquatic pollution.*

INTRODUCTION

Aquatic pollution is the most significant environmental issue as a result of the industrialization and green revolution's quick progress. In Pakistan, domestic trash, industrial waste, and agricultural runoff all directly into streams, ponds, and other bodies of water. Infectious pathogens, oil, hydrocarbons, radioactive materials, heavy metals, pesticides, herbicides, and various corrosive materials, such as acids and bases, are all present in these pollutants (Samantha *et al*., 2005). However, these springs are utilized to cultivate luscious fish species that are economically significant and to provide water to the local population (Stanitski *et al*., 2003). Aquatic pollution has long been a significant environmental issue on a global scale. Aquatic pollution in emerging nations has been significantly exacerbated by population growth, civilization, industrialization, and rising anthropogenic activity (Abbas et al., 2002). Aquatic pollution in developing nations appears to be mostly caused by chemical contamination as a result of untreated wastewater discharges and inadequate waste management and disposal (Atika, 2012). Less than 10% of companies in developing nations treat their effluents before releasing them into neighboring streams or rivers, according to earlier studies (Adhikari et al., 2004;Ayoola and Ajani, 2008).

Numerous biomarkers are employed in ecotoxicological investigations to assess stress reactions. Fish histopathology has recently become more significant for quick evaluation of the harmful effects of contaminants in the lab. Haematology can be used to assess the impact of a stressor at the level of the biological organization (Akinrotimi *et al*., 2013). According to Akinrotimi *et al*. (2012), evaluating blood parameters is a crucial step in determining how pollutants affect fish. As a result, it is utilized as a quick tool for determining how the pollutant affects various organs and even body tissues. The hematological parameters can be used to analyze the toxicity of chemicals on aquatic organisms like fish since they also show how an animal reacts to its surroundings (Gabriel *et al*., 2007; Ghaffar *et al*., 2014). Additionally, the environment in which fish are operating has considerable influence on their hematological traits. Hematological characteristics are therefore useful for assessing how fish react to stresses (Gabriel *et al.*, 2007). These metrics have defined reference values that can change in response to stressors like pesticides and other contaminants and are useful for identifying issues. Since fish have a close relationship to the aquatic environment, their hematological profile can reveal information about their internal health before any obvious symptoms appear (Akinrotimi *et al.*, 2007).

More than 20 000 distinct species of fish make up the oldest and most diversified group of vertebrate animals (FAO, 1997). According to Ellis et al. (2003), their immune system is very diverse and may be related to fish phylogeny. Even the most sophisticated teleost species lack lymph nodes and bone marrow, but fish do, mostly in the kidney, spleen, and thymus, where they are functionally identical. Additionally, fish have circulating white blood cells that resemble lymphocytes, granulocytes, and monocytes in mammals in terms of both function and morphology (Bowden, 2008). The innate immune system, on the other hand, is a feature shared by all multicellular creatures. Other mammalian-equivalent substances including complement, lysozyme, sodium bromide, C-reactive protein, hemolysins, and hemagglutinins have also been discovered in fish serum (Sole et al., 1996; Oakes et al., 2004; Nte et al., 2018; Nestman et al., 1980; Akinrotimi et al., 2010; Meister, 1983).

If kept in good condition, teleosts are immunologically capable creatures (Hrubec et al., 200). Fishes' nonspecific reactions include phagocytosis and inflammation. The term "innate" or "non-specific" immunity refers to defense systems that guard against infection without requiring prior exposure to a specific microbe. (Sole et al., 1996; Oakes et al., 2003) Many innate immune system components seem to have undergone little change throughout evolution. Due, at least in part, to the complexity of the immune system, differences in the integrity of disease resistance and the immune response are particularly sensitive markers of toxic insult in mammalian systems (Andreson, 1992). Therefore, immune system sensitivity to a specific toxin may be identical across species. The relationship between environmental toxicity and disease in fish populations has long been debated. The economic importance of knowing how pollutants affect fish immunity applies to both aquaculture and fisheries. In order to evaluate the immunological alterations caused by exposure to fenthion formulations in the lab, this study examined two sizes of *Tilapia guineensis*.

MATERIALS AND METHODS

Experimental Location and Fish

The study was conducted at the African Regional Aquaculture Center in Buguma, Rivers State, Nigeria, which is a branch office of the Nigerian Institute for Oceanography and Marine Research. During low tide, ponds yielded 180 *T. guineensis*, 90 of which were juvenile and 90 of which were adults. The fish were brought to the lab in six open, 50-liter plastic containers, where they acclimated for seven days.

Preparation of Test Solutions and Exposure of Fish

In the present study, commercial formulation of fenthion (containing 600 gL1 fenthion as the active ingredients), manufactured by Yufull Industry Co., Ltd China with CAS No. 55-38-9 was used to make the stock solutions. The pesticide was purchased from a commercial outlet in Port Harcourt, Nigeria. *T.guineemsis* were exposed to the chemical at the concentrations of 0.00 control, 0.05, 0.10, 0.15, and 0.20 mg/L in triplicates. Five fish were randomly distributed into each test tank. The experiment lasted for a period of 15 days. The water in the tanks was renewed daily. The fish were fed twice daily at 3% body weight with a commercial feed.

Evaluation of Immune Systems of Fish

The fishes were taken out individually using a small hand net and placed belly upward on a table. Blood samples of about 5 mL was collected from the caudal peduncle with the aid of a 2 mL plastic syringe, 2 mL of the blood was dispensed into Ethylene Diamine Tetra-acetic Acid (EDTA) anticoagulant for haematological studies .Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after appropriately diluted. Differential leukocyte counts were determined by scanning

Giemsa's stained slides in the classic manner (Hrubec *et al*., 2000). The leucocytes count was made using improved Neubauer haemocytometer after diluting the blood 1:100 with Shaw's solution (Shaw, 1930).

Evaluation of Water Quality Parameters

Water quality parameters in the experimental tanks during the study were evaluated: Water temperature was measured with mercury in glass thermometers, pH with pH meter (Model 3013, Jenway, China), and Salinity was determined with hand held refractometer (Atago products, Model H191, Japan). The values of dissolved oxygen, nitrite and ammonia were evaluated using the method described by APHA (1998).

Data Analysis

The results were analyzed using two way analysis of variance (ANOVA) followed by F-LSD post hoc test. The significance level was taken as $P < 0.05$.

RESULTS

Table 1 shows the results of the physico-chemical parameters in the experimental tanks throughout the exposure time. All fenthion formulation concentrations fell within the same range for temperature, pH, and salinity values. Nitrite and ammonia levels considerably rose, though. However, as the chemical concentration increased, the amount of dissolved oxygen decreased. Tables 2 and 3, respectively, indicate how the chemical affected the total leucocyte and differential white blood cell counts in the juvenile and adult sizes of *T. guineensis*. The result showed that the total leucocyte in the treatment groups were significantly higher ($P < 0.05$) than the control. Also the leucocyte counts in the treatment groups were significantly different ($P < 0.05$).

The findings also demonstrated that *T. guineensis'* peripheral blood included three distinguishable types of white blood cells: lymphocytes, neutrophils, and monocytes. Depending on whether or not granules were present in their cytoplasm, these were categorized as either granulocytes or a granulocytes. The most prevalent leucocyte type in the blood of *T. guineensis* is lymphocytes. When compared to the treated groups, the leucocyte levels in the control group were considerably greater ($P< 0.05$). As exposure time increased, lymphocytosis developed. The second type of a granulocyte found in blood are the monocytes, which are spherical cells with oval nuclei and clumped chromatin.

The monocytes in the exposed fish decreased significantly (P 0.05) when compared to the control group. As exposure time increased, monocytopenia developed. The granulocytes in the blood of T. guineensis were the neutrophils. When compared to the control, the treated groups' neutrophil counts were considerably lower ($P< 0.05$). The granulocyte that predominated in the fish T. guineensis was the neutrophil. The fish that had been exposed to 0.10 mg/l of the chemical displayed the most severe neutropenia.

Table 1: Physico-chemical parameters of water in Experimental tanks (Meant ± SD)

Parameters	Concentrations of Carbofuran (mg/L)				
	0.00	**0.05**	**0.10**	**0.15**	**020**
Temperature (^{0}C)	29.11 ± 1.03^a	29.03 ± 1.03^a	29.01 ± 1.07^a	29.00 ± 1.02^a	29.01 ± 1.06^a
pH	6.67 ± 1.03^a	6.66 ± 1.23^a	6.67 ± 1.02^a	6.69 ± 1.67^a	6.79 ± 1.04^a
Ammonia (mg/l)	0.22 ± 0.01^a	0.36 ± 0.01^{ab}	0.48 ± 0.02^b	0.48 ± 0.02^b	0.69 ± 0.11^c
DO (mg/l)	6.69 ± 0.02^a	6.32 ± 0.21^a	5.54 ± 0.77^{ab}	4.19 ± 0.44^b	3.88 ± 0.77^b
Nitrite (mg/l)	0.03 ± 0.01^a	0.07 ± 0.01^b	0.08 ± 0.01^b	0.08 ± 0.01^c	0.14 ± 0.02^c
Salinity (ppt)	10.99 ± 1.88^a	10.90 ± 2.44^a	10.79 ± 1.90^a	10.69 ± 2.99^a	10.77 ± 3.99^a

Means within the row with different superscripts are significantly different (p<0.05).

Table 2: Immune Profiles in Juveniles of *T. guineensis* Exposed to Different Concentrations of Fenthion (Mean ± SD)

Parameters	Concentrations OF Carbofuran (mg/L)				
	0.00	**0.05**	**010**	**0.15**	**0.20**
WBC (cellsx10^9)	16.77±1.02[a]	19.03±1.84[a]	25.66±4.88[b]	29.04±3.99[b]	34.77±2.65[c]
Leucorit (%)	13.42±1.03[c]	10.66±1.87[b]	8.99±1.74[b]	7.88±1.05[b]	4.77±0.88[a]
Thrombocytes (%)	147.88±9.08[c]	125.77±3.67[c]	101.99±9.07[b]	97.41±9.44[a]	86.03±9.11[a]
Neutrophils (%)	21.38±2.77[a]	19.02±1.54[b]	16.88±1.02[b]	13.09±1.63[a]	9.22±2.77[a]
Lymphocytes (%)	61.60±1.01[c]	66.55±1.55[c]	73.80±1.43[ab]	80.18±1.52[a]	83.87±1.53[a]
Monocytes (%)	17.02±1.88[a]	14.43±1.99[a]	10.52±1.09[ab]	8.22±1.04[c]	7.09±1.44[c]

Means within the row with different superscripts are significantly different (p<0.05).

Table 3: Immune Profiles in Adults of *T. guineensis* Exposed to Different Concentrations of Fenthion (Mean ± SD)

Parameters	Concentrations OF Carbofuran (mg/L)				
	0.00	**0.05**	**0.10**	**0.15**	**0.20**
WBC (cellsx10^9)	17.09±1.05[a]	21.83±7.22[a]	27.77.76[b]	35.76±9.28[c]	44.75±9.04[c]
Leucorit (%)	16.88±7.06[c]	8.05±4.90[b]	7.05±1.04[b]	6.77±0.88[b]	5.88±0.65[a]
Thrombocytes (%)	179.44±9.03	148.45±9.03	122.02±2.32	110.88±1.71	105.88± 7.45
Neutrophils (%)	25.02±2.55[c]	21.82±1.34[b]	18.62±1.88[b]	15.70±1.06[a]	13.77±2.04[a]
Lymphocytes (%)	58.60±1.67[a]	65.36±1.33[b]	72.03±1.76[b]	75.81±1.05[c]	79.82±9.53[d]
Monocytes (%)	18.38±1.09[c]	13.42±1.04[b]	10.05±1.66[b]	9.59±1.33[b]	7.01±3.44[a]

Means within the row with different superscripts are significantly different (p<0.05).

DISCUSSION

Fish leukocytes, which are phagocytes directly associated to cell-mediated immune responses, are the primary cells implicated in immunological responses (Akinrotimi *et al.*, 2012b). This finding's increase in WBC content with rising concentrations might be brought about by the immune system and defensive mechanisms of the animal. Due to their involvement with the immune system, a number of chemical chemicals, including insecticides, produce antibodies, which may be the reason for an increase in WBC (Gabriel *et al*., 2012). According to Bersenyi *et al.* (2003), changes in the WBC counts following exposure to different toxins may indicate a decrease in the fish's non-specific immunity, while the marked increase in the WBC count may be a protective reaction in fish under stress. WBC is involved in the control of immunological function. According to Cain *et al*. (2000), leukocytosis such as lymphophenia and heterophilia, which are typical of the leucocytic response in animals experiencing stress, is generally indicated by an elevated WBC count in fish subjected to continuous dosages.

Under conditions of toxic stress, stimulation of lymphopoiesis and/or enhanced lymphocyte release from lymphomyeloid tissue may result in an increase in WBC count (Akinrotimi *et al*., 2009). According to Gabriel *et al.* (2011), the present study's significant increase in WBC count may have resulted from the fish's defensive system being activated to counter the effects of the pesticide. WBC production has increased as a preventive response to pesticide stress. These alterations are most likely the result of the immune system activating in the presence of a pesticide, which may then be a fish's adaptive response leading to a more potent immunological defense (Al-Akel & Shamsi, 1996). This discovery is consistent with findings made by prior researchers who saw comparable outcomes after subjecting some fish to detergent (Akinrotimi & Amachree, 2016).

Regarding the differential white blood cell count, the neutrophils greatly outnumbered the control whereas lymphocytes and monocytes dramatically reduced as concentrations rose. The type of immunological challenge to which the fish were exposed at a certain time and in the various sub-lethal concentrations of fenthion may be related to the decrease and increase in lymphocytes, monocytes, and neutrophils. The lower number of monocytes in *T. guineensis* that had been subjected to fenthion was consistent with Al-Attar's (2005) research on Oreochromis niloticus that had received cadmium treatment. According to Akinrotimi *et al.* (2012), cypermethrin exposure caused a decrease in lymphocytes and an increase in neutrophils in *C. gariepinus* juveniles. Akinrotimi and Amachree (2016) showed that detergent exposure to Tilapia guineensis had an effect on neutrophil number.

CONCLUSION

The data from this study show that fenthion are significant stressors. Fenthion is highly hematotoxic and damaging to *T. guineensis's* immunological condition, as evidenced by the profound changes in immunological variables that were seen. Consequently, the environmental contamination of these compounds might pose a major threat to fish populations as well as a challenge to aquaculture. The research demonstrated the need to assess the dose-response of different fish species to this chemical as toxicants in order to learn how to properly manage and track stress in aquaculture.

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