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Impact of Zinc Phosphide on Hematology, Behaviour and Proximate Composition of *Oreochromis niloticus*

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ABSTRACT

Zinc phosphide is a rodenticide, crystalline compound of dark grey color. It is present in many pesticides and when sprayed on plants it gains entry into food and water. **Objective:** To evaluate the effect of zinc phosphide on the hematology, behavior and proximate composition of *Oreochromis niloticus*. **Methods:** In present research, fish were given a dose of zinc phosphide in aquarium for twenty days. Fish were divided into two groups, control, and treatment, each with three replicates. Treatment group was exposed to 1mg/ml of Zinc phosphide given to treatment group twice a day for 20 days continuously. When the experiment was completed fish were anaesthetized, dissected and blood was drawn to evaluate the hematological parameters. Fish activities swimming, gill movement, mortality and morbidity were recorded. **Results:** Zinc Phosphide exhibited variable impact on proximate composition. There was a significant decrease in values of crude fat and increase in value of crude protein and total ash in comparison to control group. Fish were active during the trial period they exhibited fast movement, no rubbing against the wall, and fish didn't harm each other in the treatment group. **Conclusions:** According to these results, zinc phosphide have harmful impacts on fish health. As a result, its usage must be carefully regulated to reduce environmental contamination and safeguard aquatic ecosystems.

INTRODUCTION

The aquaculture industry is flourishing rapidly and production of aquaculture is important. It is rapidly expanding as it provides protein-based food for human consumption [1]. This expansion necessitates sustainable practices to ensure the health and productivity of cultured species, such as Nile tilapia *(Oreochromis niloticus)*. It will approximately reach to 62% of total worldwide production by 2030 [2]. About 8,563,820 km2 area of Pakistan is covered with water which includes rivers, ponds, lakes, and water lodging areas [3]. Aquaculture also contributes approximately 1% of national GDP [4]. Pesticides control pests by killing, destroying, or mitigating their ratio. About 1.8 billion people globally are involved in agriculture. Europe has the utmost contribution (31.7%) followed by Latin America, Asia, Africa, and North America, which contribute 47.6% of the worldwide pesticide trading [5]. Herbicides are the major category followed by the Fungicide (17.5%), Insecticide (29.4%) along with others (5.5%) [6, 7]. The global trading forecast was predicted to reach $59 billion in the year 2016, pursuant to World Agricultural Pesticides [8]. Particularly, 5% of the rodent species are considered serious pests. The authorized state agencies as well as the United States Environmental Protection Agency (EPA) manage rodenticides along with their usage [9]. Zinc phosphide is a crystalline compound having a dark gray color. Zinc phosphide is considered a rodenticide because

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it is used to kill small mammals such as rats, mice, squirrels, and field mice [10]. Zinc phosphide can cause acute poisoning indirectly by accidental inhalation of phosphine gas which is generated during its usage and directly by ingestion [11]. Zinc phosphide is poisonous to non-targeted mammalian organisms, wild birds as well as freshwater fish [12, 13]. Food is sprayed with pesticides, particularly fruits and vegetables. It is absorbed through seepage into the groundwater and soil, where it may end up in drinking water. They also have the potential to disperse and pollute the air [14]. Tilapia is transported all over the world and is native to Africa. Tilapia does not harm other native species [15]. Tilapia belongs to Cichlidae family which is a freshwater fish. It is wholly connected with Africa along with the Middle East [16, 17]. One of the first fish species to be cultivated in the globe is the Nile tilapia *(Oreochromis niloticus)* [18]. Tilapias grow quickly, have thick, white meat, can tolerate unfavorable water environments, consume a variety of food kinds, reproduce readily without the use of advanced hatchery equipment, and forage at the foundation of the aquatic food chain. Recent research has demonstrated that insecticides, notably zinc phosphide, may adversely affect Nile tilapia *(Oreochromis niloticus)*. In Nile tilapia, acute poisoning from zinc phosphide has been associated with death and serious health problems, such as gill tissue destruction, liver dysfunction, and oxidative stress brought on by the production of reactive oxygen species (ROS) [19, 20]. Furthermore, it was discovered that sub-lethal levels of zinc phosphide reduced the survival rates of tilapia in contaminated habitats by affecting immunological function and causing histopathological alterations [21, 22]. Fish health is seriously threatened by the bioaccumulation of zinc phosphide in tilapia tissues, and eating contaminated fish can have negative health effects on humans. The purpose of this study was to determine the possible toxicity of zinc phosphide to fish populations and the possible hazards it presents to the aquaculture sector by investigating its effects on the hematological, behavior, and proximate composition of *Oreochromis niloticus*.

METHODS

Zinc phosphide (Zn_3P_2) is a crystalline powder and is grey in color, it is accessible in 2% to 10% assemblage as sugar-based or grain baits in a pellet, powder, tablet form, or paste [23]. Zinc phosphide was purchased from Sigma Scientific Lab, Lahore, Punjab, Pakistan, and it contained ≥ 19% active phosphor (P) basis, powder (CAS- No:1314-84-7) (Sigma-Aldrich). *Oreochromis niloticus* commonly known as Nile tilapia was purchased from Al-Madina Fish Hatchery Kasur, division Lahore, Punjab and transported to fish laboratory at Department of Fisheries and Aquaculture, University of Okara. Live fish was transported through sterilized plastic oxygen-filled bags and specimens with average lengths and weights of 9.31 ± 0.59cm and 21.4 ± 2.5g respectively were shifted to aquariums. Fish were acclimatized to lab conditions for two weeks in an aquarium containing tap water. Fish were fed with commercial fish feed twice a day. Waste products were siphoned daily and 30% of water was renewed. This study was conducted according to the declarations of Helsinki. After acclimation for 15 days, fish were divided into two groups and moved into six aquariums of equal size and shape. To recover against stress management, fish were kept for 24 hours with regulated oxygenation and temperature. Three aquariums were labeled as a control group and the other as treatment group for zinc phosphide. Each aquarium was stocked with 10 fish per 50L of water respectively. Zinc phosphide impact on *Oreochromis niloticus* was observed for 20 days and water-borne dose at 1 mg/ mL (0.5 mg/g) in distilled water according to the body weight. The 0.5 mg/g dose of zinc phosphide employed in this investigation was chosen in light of earlier research that investigated the harmful effects of this chemical on animals. Hinds, Henry reported the acute oral toxicity of zinc phosphide on wild house mice (Mus musculus), with LD50 values ranging from 25 to 50 g/Kg. However, for fish, the LD50 value is typically lower due to their aquatic environment and unique physiology. The World Health Organization (WHO) states that 0.1 mg/L of zinc phosphide is the highest amount that can be present in water [24, 25]. However, depending on the fish's body weight, we used a dose of 0.5 mg/g in this investigation, which is equal to 1 mg/mL in distilled water. This dosage was selected to reduce the chance of death while guaranteeing that the fish were exposed to enough zinc phosphide to cause a hazardous reaction. Water was continuously aerated artificially. Water quality parameters, total dissolved solids (144.3 ± 1.81), Dissolved oxygen (7.87 ± 0.86), Temperature (37-39°C), Electrical Conductivity (284.5 ± 0.75), and pH (8.08 ± 0.60) were monitored at regular intervals during experimental period. Proximate analysis of commercial feed was conducted at the Poultry Research Institute (PRI) Rawalpindi, Punjab. Fish were anesthetized before being dissected and sampled, and feeding was discontinued 24 hours before the experiment's conclusion. *Oreochromis niloticus* caudal vein was punctured with a 3 ml medical syringe, which was then washed with EDTA solution (as an anticoagulant) and gently shaken to minimize hemolytic anemia of blood utilized for hematological analysis. The process of collecting blood samples was completed within 30 to 40 seconds. Samples of blood were collected in EDTA tubes. Hematological parameters include white blood cells (WBC), lymphocytes (LYM), mid cells (MID), granulocytes (GRA), LYM%, MID%, GRA%, red blood cells (RBC), hemoglobin (HGB), hematocrit

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(HCT%), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red blood cell distribution width (RDW-SD), Red blood cell distribution width (RDW-CV%), Platelet (PLT), Mean platelet volume (MPV), platelet distribution width (PDW%), plateletcrit percentage (PCT%) and Platelet larger cell ratio (P-LCR%). WBCs were counted in blood smears which are stained with routine panoptic stains LYM, MID, GRA, LYM%, MID%, GRA%, RBCs were examined manually with a Neubauer hemacytometer and using a solution Natt-Herrick's as a diluent stain, Hgb was obtained by the cyanmethemoglobin process according to method of Hrubec et al., 2000 [14]. HCT was evaluated by micro centrifugation in a capillary tube. RDW-SD, PLT, RDWCV%, MPV, PCT%, PDW%, P-LCR. MCV, MCH, MCHC, were calculated by using the formula. The ash content, moisture, dry matter, crude fats, digestible, and crude protein from muscles were evaluated according to method of AOAC (2000) at the Pakistan Poultry Research Institute (PPRI) Islamabad. Crude protein and crude fat were determined by using micro Kjeldahl method along with Soxthlet appliances respectively according to method of For moisture determination, evaporating dish was cleaned, washed in addition to this it was then placed in a forced air circulating oven at temperature105ºC for 10 to 15 minutes. After 15 minutes it was taken out and cooled in desiccators then properly weighed on digital balance. Fry after a facet on filter paper and placed on a pre-weighed evaporating dish and weighed once again then these samples were placed in a forced air circulating oven for 24 hrs. at 98ºC until the sample is dried.

Dry matter was determined by using formula:

$$\% \text{ Dry Matter} = \frac{\text{Weight of sample + Weight of china dish after drying } \times 100}{\text{Weight of sample + Weight of china dish before drying}}$$

Moisture(%)=100 - Dry Matter(%)

After being thoroughly cleaned and rinsed, a crucible was put in a muffle oven set to 90°C for an hour, after which it was cooled and weighed. Following sample weighing, 5.0g of the sample was deposited in a crucible, and it was once more heated at 600°C in a muffle furnace for 24 hours. This was then put into a dryer, quickly cooled, and along with weighed to stop moisture absorption.

The formula used to determine crude ash %.

$$\text{Crude ash (\%)} = \frac{weight\ of\ ash \times 100}{weight\ of\ sample}$$

For three weeks, behavior was monitored between the hours of 9:00 am and 4:00 pm. For capturing behavior visually, a stopwatch, a multifunctional counter, an Android mobile camera, and a notebook were utilized. Fish behavior was observed again and again after short intervals. Survival, morbidity, and mortality rates were observed. Fish swimming movement was observed and recorded. Fish behavior was observed by instantaneous sampling method. Experimental data was recorded and processed using MS Excel Software. Data were subjected to 'T-test' followed by Post hoc LSD test. Statistical analysis was performed with SPSS IBM V.22. (Chicago, USA). Data significance was established at a P<0.05. The results were displayed in the form of tables and figures in addition to graphs with elaborated values as mean ± SD.

RESULTS

Zinc phosphide exhibited profound effects on hematological parameters of *Oreochromis niloticus* exposed for 20 days (Table 1). T-test showed a significant effect on MID, MCHC, GRA%, MCH, PLT, PCT, MPV,and P-LCR values of both groups. Moreover, Treatment group showed significant (p<0.05) decrease in MID, GRA, MID%, GRA %, MCH, MCHC, PLT, MPV, PCT, and P-LCR values as compared to control group. However, treatment group exhibited significant (P <0.05) increase in the LYM%, RBC, HGB, MCV, RDW- SD, HCT, RDW-CV, values as compared to control group. Furthermore, no significant effect was observed on WBCs, LYM, and PDW% values of both treatment and control groups. Data is presented as mean ± SD (n=3). Alphabets a and b indicated values that were significantly (confidence level 95 %) different among control and treatment group.

Table 1: Impact of Zinc Phosphide On Hematological Parameters of *Oreochromis niloticus* on exposure for 20 Days

Variables	Control	Treatment	T-Value	DF	p-Value
WBC (109/L)	17.9 ± 0.57ns	14.8 ± 3.12ns	1.000	2	0.42
LYM (10^9/L)	9.51 ± 0.57ns	7.97 ± 1.14ns	2.69	2	0.11
MID (109/L)	3.00 ± 0.51a	0.43 ± 0.28b	10.52	2	0.009
GRA (109/L)	5.39 ± 0.60a	0.46 ± 0.27b	15.1	2	0.004
LYM (%)	53.1 ± 0.57b	91.9 ± 1.13a	-70.0	2	0.000
MID (%)	16.7 ± 0.72a	3.20 ± 0.55b	67.5	2	0.000
GRA (%)	30.2 ± 0.59a	4.73 ± 0.78b	133.1	2	0.000
RBC (1012/L)	0.43 ± 0.05b	1.68 ± 0.21a	-4.59	2	0.04
HGB (g/dL)	4.39 ± 0.60b	6.65 ± 0.81a	-8.43	2	0.01
HCT (%)	3.00 ± 0.28b	24.6 ± 0.88a	36.05	2	0.001
MCV (fL)	77.3 ± 0.57b	136.5 ± 1.45a	-67.2	2	0.000
MCH (pg)	112.1 ± 0.63a	37.03 ± 0.88b	263.5	2	0.000
MCHC (pg)	144.6 ± 0.88a	25.4 ± 1.85b	119.2	2	0.000
RDW-SD (fL)	21.7 ± 0.57b	109.2 ± 3.84a	-26.2	2	0.001
RDW-CV (%)	20.6 ± 0.57b	21.6 ± 0.88a	-3.20	2	0.08
PLT (109/L)	634 ± 0.57a	434 ± 1.52b	200.0	2	0.000
MPV (fL)	7.3 ± 0.57a	3.69 ± 0.81b	14.3	2	0.005
PDW (%)	9.00 ± 0.57ns	10.3 ± 1.27ns	-1.74	2	0.22
PCT (%)	0.46 ± 0.005a	0.15 ± 0.02b	14.03	2	0.005
P-LCR (%)	14.2 ± 1.15a	0.89 ± 0.11b	12.7	2	0.006

The figure 1 illustrated the impact of zinc phosphide on the hematological parameters of *Oreochromis niloticus*. Data are presented as Mean ± SD (n = 3). Significant differences between the control and treatment groups are indicated by the letters a and b, representing a 95% confidence level.

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Hematological parameters measured include key indices such as Red Blood Cell count (RBC), hemoglobin (Hb), hematocrit (HCT), White Blood Cell count (WBC), and others, highlighting the physiological changes induced by zinc phosphide exposure. Figures are represented as Mean ± SD (n=3). Letters a and b indicates the values that were significantly (confidence level 95 %) different among control and treatment group.

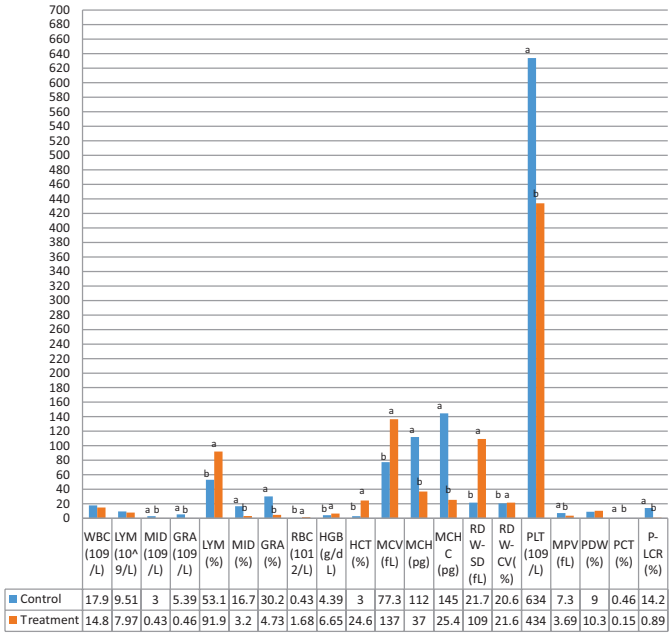

Figure 1: Impact of Zinc Phosphide on Hematology of *Oreochromis niloticus*

Zinc Phosphide exhibited variable impact on proximate composition of *Oreochromis niloticus* exposed for twenty days (Table 2). A highly significant impact was observed on crude protein (CP), crude fat (CF), and ash components in fish muscles of control and treatment group. Moreover, treatment group resulted significant ($p< 0.05$) decrease in CF, increase in CP and ash content.

Table 2: Impact of Zinc Phosphide on Proximate Composition of *Oreochromis niloticus* on Exposure for 20 Days

Proximate (%)	Control	Treatment	T-Value	DF	p-Value
CP	61.2 ± 0.71b	63.8 ± 0.80a	-26.7	2	0.001
CF	8 ± 0.80a	6.6 ± 0.75b	24.2	2	0.002
Ash	20 ± 0.63b	22 ± 0.69a	-34.6	2	0.001

A behavioral index is considered a reliable indicator to monitor response of fish. Zinc phosphide showed some impact on the behavior of *Oreochromis niloticus* on exposure for 20 days (Table 3). Fish became very aggressive after 10 days of exposure and irregular swimming was observed. Due to anemia surface agitation was observed in treatment fish.

Table 3: Impact of Zinc Phosphide on Behavior of *Oreochromis niloticus* on Exposure for 20 Days

Variables	Control	Treatment
Erratic Swimming	No	Yes
Coloration	No Change	Shiny
Opercular Movement	Remained Same	Fast
Gill Movement	Remained Same	Fast
Feeding	Active	Active
Rubbing Against Wall	No	Yes
Aggression	No	Yes
Inappetence	No	No
Flared Opercula	No	Yes
Loss of Equilibrium	No	Yes
Hanging Head Up In Water	Yes	No

DISCUSSION

Pesticide exposure to organisms over an extended period poses a constant risk to public health. This study mimics environmental fish toxicity by administering a waterborne dose of zinc phosphide (1 mg/mL or 0.5 mg/g), which represents real-world contamination conditions in aquatic ecosystems. Pesticides and rodenticides found in agricultural runoff frequently end up accumulating in freshwater systems.The aquactic animals come into contact with this contaminated water and eating these contaminated aquatic animals puts a large portion of the human population at risk [27]. In most cases, pesticides quickly changed the hematological fish characteristics [28]. Hematological parameters are good indicators of the severe impacts of many toxic compounds mainly pesticides and industrial effluents containing heavy metals so these parameters are signs of internal homeostasis and the physiological condition of exposed organisms. Significant drops in hemoglobin, hematocrit, and red blood cells were seen mostly after contact with fipronil, which highlights the anemic state of fish. Possible causes of the reduced hemoglobin include its oxidation to methemoglobin, a reduction in gas exchange, and damage brought on by free radicals. Reduced blood parameter levels also indicate ineffective hematopoietic tissue function, improper osmoregulatory mechanisms, and increased RBC damage in blood-forming organs. However, after 20 days of exposure to zinc phosphide, *Oreochromis niloticus* showed a significant increase in values of red blood cells, hemoglobin, hematocrit, lymphocytes, mean corpuscular volume, red blood cell distribution width-SD, and red blood cell distribution width -CV when compared to the control group. Red blood cells aid in cellular respiration and transport oxygen from the gills to tissues. Pesticide stress is responsible for a large increase in these metrics. According to Far, Roodsari there is momentous escalation

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in the value of MCV and reduction in MCH on dosing Oncorhynchus mykiss with diazinon [29]. David, Sangeetha showed that there is a significant increase in values of MCV and MCH on dosing Cirrhinus mrigala with Deltamethrin [30]. Conversely, there is significant increase in Red Blood Cell calculation has been explained in Prochilodus lineatus showing a herbicide clomazone. Ghaffar, Hussain exposed Labeo rohita to 0.03-0.15mg/L of fipronil for almost nine days [31]. Directories of lymphocytes, erythrocytes in addition monocytes are reduced while total leukocyte counts, as well as neutrophils augmented prominently. Erythrocytes displayed variety of nuclear abnormalities. Furthermore, Ghaffar, Hussain discovered the toxic effects of fipronil on Cyprinus carpio treated with different concentrations (0 - 0.10mg/L) for just about 12 days [32]. Fish in groups that were given inappropriate doses showed significant abnormalities in both biochemical and clinical-hematological markers. Hematocrit, hemoglobin, and Red blood cell counts were reduced generally total leukocyte count, mean corpuscular volume, neutrophils, lymphocytes, and monocytes were mainly amplified. Organophosphate-induced anemia caused by a significant drop in Hb level has been reported in Barbonymus gonionotus subjected to quinolphos in the modern era [32]. Present research showed that treatment of *Oreochromis niloticus* with zinc phosphide resulted significant decrease in MID, GRA, MID%, GRA%, MCH, MCHC, PLT, MPV, PCT, P-LCR. There is a substantial reduction in MCH, MCHC and substantial increase in MCV on treating Channa punctatus with Deltamethrin [33]. According to studies, contaminated water systems of zinc phosphide and other pesticides, can have a major effect on aquatic life [34]. Comparable hematological abnormalities have been shown in similar experimental setups, such as exposing fish to organophosphate pesticides as deltamethrin, diazinon, and fipronil [29, 31, 32]. Future studies on zinc phosphide should concentrate on long-term exposure studies, ecotoxicological assessments across species, and an understanding of its molecular mechanisms of toxicity. Investigating its environmental destiny, bioaccumulation impacts on fish behavior and physiology, as well as the impact of co-contaminants, will aid in determining its overall ecological risks. Furthermore, a study on remediation tactics and their possible effects on non-target species is critical for enhancing environmental management and regulatory procedures.

CONCLUSIONS

In conclusion, this study focused on how zinc phosphide contamination affects hematological parameters, flesh, and behavior of *Oreochromis niloticus*. This study also highlighted possible ecological runoff related concerns posed by zinc phosphide and other toxicants, adding to the understanding of their effects on aquatic ecosystems and human health through fish eating.

Authors Contribution

Conceptualization: IS, SY, FK
Methodology: IS, FK, MF, MIH
Formal analysis: IS, SY, FK, MF, MIH
Writing, review and editing: IS, SY, NI, MSS, MH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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