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Original Research

Effects of Mixtures of Paraquat and NPK (15:15:15) Fertilizer on the Serum Biochemistry of *Clarias Gariepinus* (Burchell, 1822) Juveniles

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ABSTRACT: The use of different agrochemicals in agriculture to boost productivity is a common practice in Nigeria. These agrochemicals find their way into aquatic environments and cause deleterious effects on aquatic organisms and subsequently on humans who is at the top of the food chain. These effects were observed in the changes in serum biochemical parameters of African Catfish *Clarias gariepinus* following exposure to sub-lethal concentrations of mixtures of agrochemicals (paraquat and NPK 15:15:15 fertilizers) in this study. Sublethal amounts of paraquat and NPK 15:15:15 fertilizer were administered to test animals in three treatment groups. Treatment groups 1, 2, and 3 received concentrations of 0.25, 0.75 and 1.25 mg/L corresponding to 10, 30 and 50% of the LC_{50} value which was recorded at 2.5mg/L. Results of Alanine amino transferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Creatinine (CRT),and Urea (U) showed an increase and deviation from the normal control values at $p<0.05$. It can be stated that the combination of agrochemicals used in this study induced stress in the fish and consequently resulted in the impairment of both liver and kidney functions. The higher levels of serum biochemical values are a clear indication of fish stress.

Keywords: Serum biochemistry, *clarias gariepinus*, agrochemicals, toxicity

INTRODUCTION

To meet the exponential increase in human population, there has been a need to intensify farming as well as carry out diverse agricultural practices. An increase in the application of pesticides and fertilizers is a direct implication of this need. Agricultural use of pesticides has been recorded to have a major impact on water quality and life in water, and this has led to serious environmental consequences (Virginia Cooperative Extension 2009).Pesticides include all chemicals that are used to control pests, they may comprise herbicides, fungicides, rodenticides, and insecticides (insects) amongst others. Unregulated use of these pesticides in Nigeria and several other third-world countries has resulted in the inability of farmers and end users to follow the manufacturer's application instructions. The resultant effect is the indiscriminate use and, in some cases, outright misapplications, and an increased plausibility of mixtures of these agrochemicals ending up as contaminants in water bodies like wetlands, streams, rivers, and lakes. Although agricultural activities permit the direct and intentional release of pesticides into the environment to eliminate agricultural pests, it has been recorded that over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species both on land and in water (Miller 2004). Sources of agrochemical contamination of water include field run-offs, aerial spray drifts, and the escape from production sites and storage tanks.

Fish are very important components of all aquatic environments, and billions of people rely on fish for either food or income. The African Catfish (*Clarias gariepinus)* is a fish species indigenous to most water bodies in Africa, this fish species is known for its resistance to disease, fast growth, high feed conversion efficiency, aerial respiration, and generally, great resilience to stressors (Solomon and Boro 2010). Reports on the effects of some agrochemicals-related stressors on fish have previously focused on solitary stressors, such as exposure to Organophosphates (Nwani *et al.* 2015) and NPK 15-15-15 fertilizer (Asuquo and Essienibok, 2014). In 2012, Tiwari *et al.* recorded the impact cypermethrin had on some biochemical parameters in*L. Rohita* fingerlings. Results showed significant alteration of the protein and carbohydrate metabolism of the fishin both liverand muscle tissues. Changes in serum biochemical parameters in response to combined-agrochemicals exposure were studied in *Clarias gariepinus* and results expressed an elevation in biochemistry values of treated fish as against the control (Onwude *et al.,* 2022).There is, however, still limited data on the effects of multiple agrochemical mixtures and/or components on fish. Thus, an investigation of the effects of mixtures of multiple agrochemicals on this fish species may provide knowledge on agrochemical-induced impacts. Consequent to the above, this study assessed the Serum Biochemistry profile of *Clarias gariepinus* following exposure to mixtures of paraquat (herbicide) and NPK 15:15:15 (fertilizer).

MATERIALS AND METHODS

Fish sampling

384 healthy juveniles of African catfish *Clarias gariepinus* of the same cohort with similar weights of 40-60g were obtained from the hatchery unit of Pipar fisheries, Karshi Abuja. Acclimatization commenced immediately for 14 days in aerated plastic receptacles containing non-chlorinated water and placed under natural photoperiod (OECD, 2019). Fecal and unconsumed feed were removed daily during the process of water change.

Test compounds

Paraquat (herbicide) and Liquid NPK 15:15:15 (fertilizer) were used for this study.

Toxicity test

Acute toxicity test: determination of LC_{50}

Six (6) concentrations (0.00, 2.00, 2.50, 3.00, 3.50, and 4.00mg/l) of mixture of equal amounts (50:50 ratio) of paraquat and NPK fertilizer were added in six equal-sized plastic receptacles and stirred with a glass rod to obtain a homogenous mixture. Eight (8) fishes were transferred to individual receptacles containing the agrochemicals. Feeding was discontinued 24 hours before the experiment and during the 96-hour test period (OECD, 2019).The experiment was continued for 96 hrs of each concentration. Water was changed daily to keep the agrochemical concentration constant during the 96 hrs period. Two replicates per test concentration were used to avoid test repetition due to system failure and to provide a stronger statistical baseline. Each test chamber contained an equal volume of test solution (10 liters). Dead fish were removed daily and recorded. At day 4, the mortality percentages were calculated following the probit analysis method (Finney 1979). The LC_{50} value for mixtures of paraquat and NPK fertilizer was recorded as 2.5 mg/l.

Chronic toxicity test: sub-lethal test for 56 days

Two hundred and forty (240) fish were transferred to twelve individual receptacles (20 fish each). These receptacles were divided into the Control group and treatment groups 1, 2, and 3 (totaling four groups and two replicates). Following the determination of the 96 h LC_{50} value of African catfish *C. gariepinus* for Paraquat and NPK 15:15:15 by probit analysis, recorded as 2.5 mg/L (96 hr LC50), three test concentrations of 0.25, 0.75 and 1.25 mg/L placed in treatment group 1, 2, and 3, corresponding to 10, 30 and 50% of LC_{50}, respectively, were used for the experiment.

Sample extraction and analysis

Four (4) fish were randomly picked for serum biochemical studies at an interval of 0, 7, 14, 28, and 56 days. Biochemical analysis was carried out on the blood of the fish which was obtained by bleeding the fish through the caudal vein. The blood was collected in plain bottles and allowed to clot. The use of a bench centrifuge was applied, and the blood was spun at 3000 RPM for 5 minutes. The Serum was separated from the plasma using an automated pipette and dispensed into sample tubes which were put into the biochemistry analyzer (Merck microlab 300) loading tray. Parameters such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Creatinine (CRT), and Urea (U), were investigated.

Statistical analysis

The results were expressed as mean±SD error. The data were subjected to a two-way analysis of variance (ANOVA) and differences between groups were considered statistically different for p values ≤ 0.05. The results were further analyzed by Duncan's multiple range tests, in statistic package for social science 17.0 (SPSS Inc. Chicago, Illinois, USA).

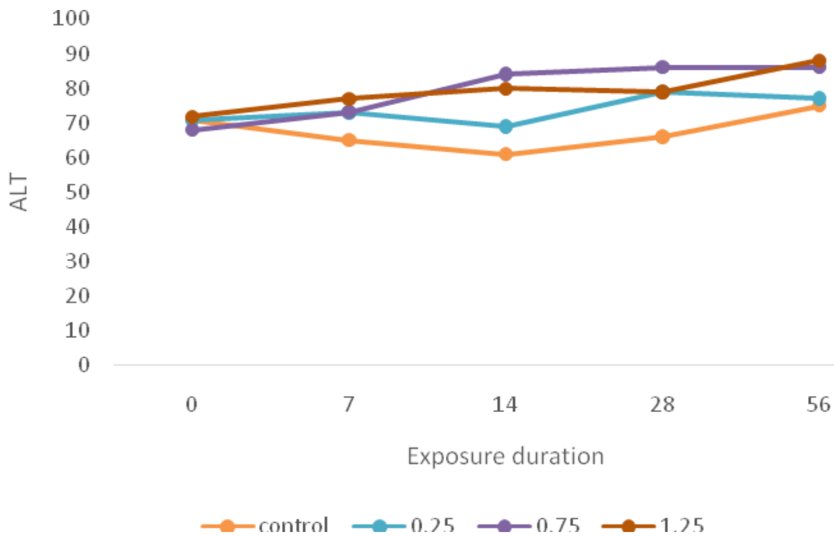

Figure 1: The values of ALT of *C. gariepinus* exposed for 56 days to different concentrations of a mixture of Paraquat and NPK 15:15:15 fertilizer.

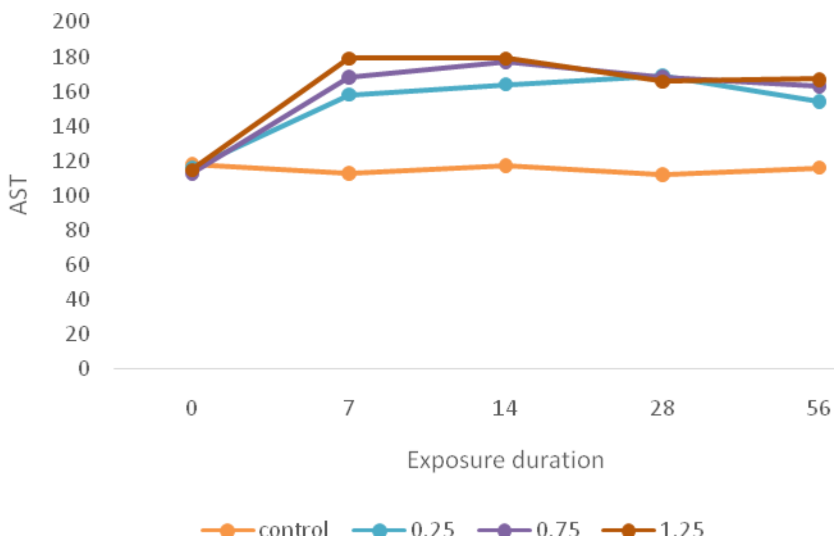

Figure 2: The values of AST of *C. gariepinus* exposed for 56 days to different concentrations of a mixture of Paraquat and NPK 15:15:15 fertilizer.

RESULTS

The variations in ALT, AST, ALP, UN, and CRT are indicated in Fig. 1-5. Values for ALT of treated fishes with 0.00, 0.25, 0.75 and 1.25 mg/L of the mixture of Paraquat and NPK 15:15:15 fertilizer for 56 days ranged from 61.86 to 75.75 u/L in the control group, and 69.03 to 77.96; 68.75 to 86.65 and 72.27 to 88.05 u/L in the treatment group 1, 2 and 3 respectively. AST values of treated fishes similarly increased from a range of 112.63 to 118.27 u/L in the control group, to a range of 116.74 to 169.86; 113.05 to 177.35 and 115.24 to 179.67 u/L in the treatment group 1, 2 and 3 respectively (Figures 1-5). The ALP values of fish followed the same trend of

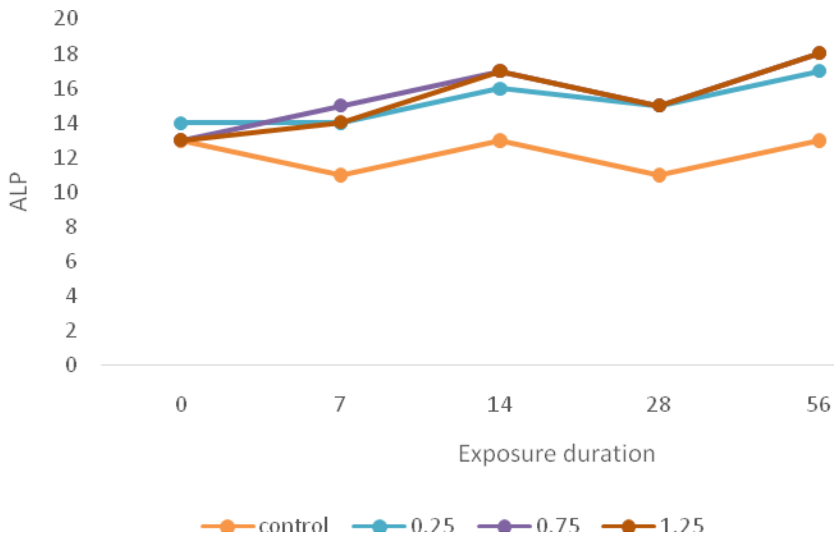

Figure 3: The values of ALP of *C. gariepinus* exposed for 56 days to different concentrations of a mixture of Paraquat and NPK 15:15:15 fertilizer.

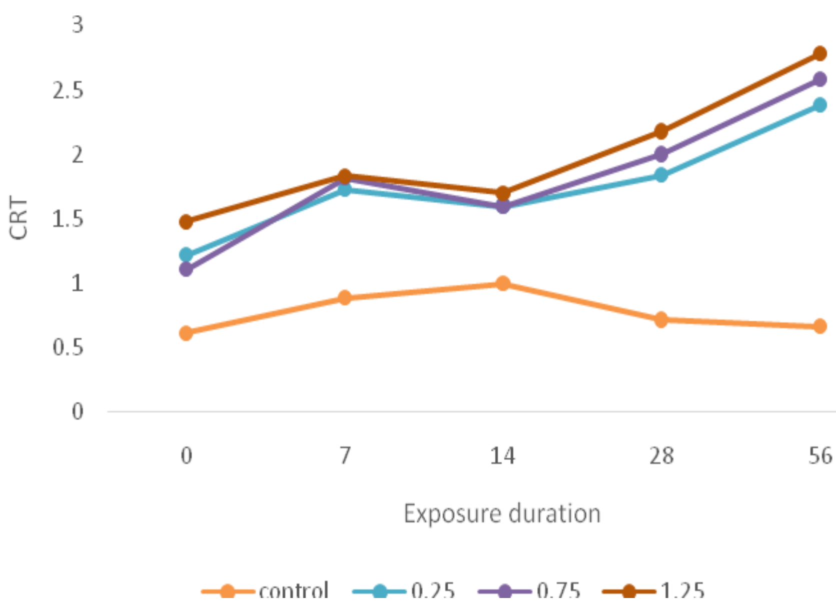

Figure 4: The values of CRT of *C. gariepinus* exposed for 56 days to different concentrations of a mixture of Paraquat and NPK 15:15:15 fertilizer.

elevated values following treatment. The control group recorded values ranging from 11.84 to 13.85 u/L; while treatment groups 1, 2 and 3 recorded values ranging from 14.03 and 17.90; 13.66 and 18.26; and 13.94 to 18.04 u/L respectively. CRT values of fish after exposure recorded high values in the treatment group 1, 2 and 3 which ranged from 1.22 to 2.38; 1.60 to 2.58 and 1.48 to 2.78 mg/dL respectively as against the control group of between0.62 to 1.00 mg/dL. Urea values of fish were similarly high in comparison to the control value which

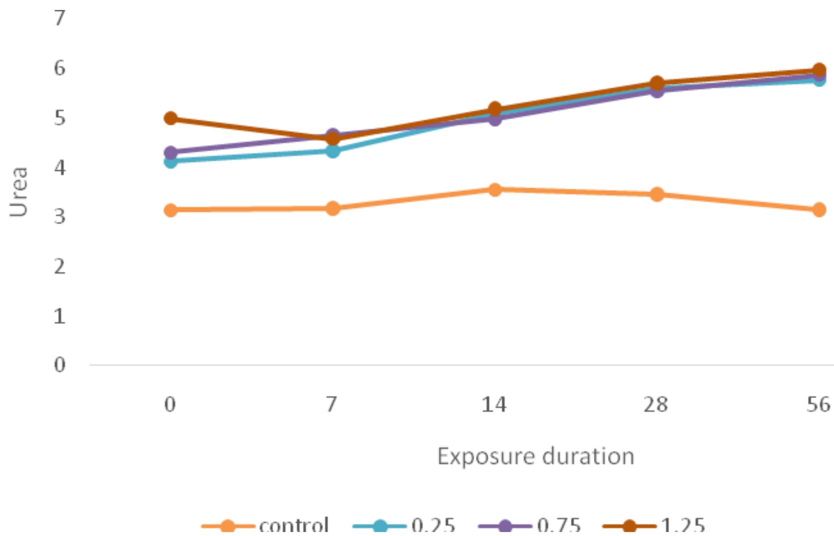

Figure 5: The values of Urea of *C. gariepinus* exposed for 56 days to different concentrations of a mixture of Paraquat and NPK 15:15:15 fertilizer.

ranged from 3.15 to 3.55 mg/dL; and 4.13 to 5.77; 4.31 to 5.87 and 4.58 to 5.97 mg/dL in the treatment group 1, 2 and 3 respectively.

DISCUSSION

Generally, activities of serum ALT, AST and ALP have been commonly used in the diagnosis of fish diseases; it has also been used in the detection of tissue damage caused by aquatic or environmental pollution of all kinds. Even minor cellular damage is reflected in an increase of these enzyme activities in the extracellular fluid or serum (Palanivelu *et al*. 2005) and indicates stress-based tissue impairment. From this study, it was deduced that *Clarias gariepinus* juveniles were stressed progressively with time before death. Values of AST, ALT, ALP, CRT and Urea were evaluated, and results showed a significant increase at $p<0.05$ in the treatment group. The increase in the activity of serum enzymes was explained as the result of the destruction of liver cells and increased cell permeability leading to a leakage of the enzymes from the damaged liver cells into serum (El-Ezabi, 2001). The increased activities of serum ALT and AST also show that there is a high probability of enhanced transamination in response to the demand for higher energy by the fish (Saravanan *et al.,* 2011). This buttresses the fact that stress is an energy-demanding process and animals mobilize energy substrates to cope with stress metabolically. This present study agrees with the findings of Das *et al.,* 2014 who studied the effect of nitrite to Indian carps; John 2007, in his study of the effects of metasystox and sevin to *Mystus vittatus;* and Jee *et al*. (2005) who recorded an increase in activities of serum ALT, AST, and LDH in Korean rockfish (*Sebastes schlegeli*) exposed to cypermethrin. Indicators of renal failure are seen in an increase in CRT and Urea values. It can be stated that the different agrochemicals used in this study induced stress in the fish and consequently resulted in the impairment of both liver and kidney functions. The high levels of serum biochemical values are a clear indication of fish stress. There is a need for more research on how the application of a variety of agrochemicals can affect the biochemical parameters of fish species. Such knowledge will come in handy for the prediction of potential impacts and the consequent designing of appropriate management strategies for the protection of fish and other aquatic animals and at the same time, not disrupting agricultural activities or productivities (Kumar and Gautam. 2014).

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