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Effects of Oxadiazon on Nutrient Utilization and Growth of African Catfish (*Clarias gariepinus*)

Funmilola Ajani^{1,*}, Adebola Oluyinka Ajiboye²,
Omofolake Oluwatosin Oyelowo³

¹Department of Wildlife and Ecotourism Management, University of Ibadan, Ibadan, Nigeria

²Department of Animal Science and Fisheries Management, Bowen University, Iwo, Nigeria

³Department of Animal Science, University of Ibadan, Ibadan, Nigeria

Email address

funmilolajani@yahoo.com (F. Ajani), debron2005@yahoo.com (A. O. Ajiboye)

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Abstract

In this study, effects of a herbicide, Oxadiazon was investigated on the growth and nutrient utilisation of *Clarias gariepinus*. Three hundred *C. gariepinus* with mean weight 262.17 ± 72.80 g were acclimatized for 2 weeks in nine plastic tanks containing dechlorinated tap water at room temperature of $30 \pm 1.50^{\circ}\text{C}$. Experimental fish were fed twice daily at 5% body weight. Feeding was stopped 24 hours to the commencement of the bioassay. The exposure period lasted for 96 hours. Range finding and acute toxicity tests (96HLC_{50}) were conducted according to standard methods. The LC_{50} was determined following the Probit analysis method. Test fish were subjected to the sub-lethal concentration of $1/2$ and $1/5$ of the LC_{50} of Oxadiazon and the concentration was continuously renewed every two days for a period of 6 weeks. At the end of the experiment, total weight gain (TWG), percentage body weight gain (PBWG) and specific growth (SGR) rate was best in *C. gariepinus* exposed to 0.008ml/l concentration. The total feed intake (TFI) of fish exposed to 0.008ml/l and 0.02ml/l concentrations were significantly lower than the control which had no exposure. The feed conversion ratio (FCR) of 0.02ml/l concentration was significantly lower than the control and 0.008ml/l concentration. The protein efficiency ratio (PER) of control, 0.008ml/l and 0.02ml/l concentrations were not significantly different. The decrease observed in TWG, PBWG and SGR might have been related to the high concentration of Oxadiazon. To guarantee minimal negative side effects on rice field ecosystems, herbicides should have no or low toxicity, except for the target weeds.

1. Introduction

Modern sustainable paddy cultivation worldwide involves extensive use of agrochemicals such as insecticides, fungicides, especially herbicides. Herbicide demand has unique characteristics compared with other common productive inputs in rice culture systems such as land, labour, seeds, chemicals and fertilizers (Yamamoto and Nakamura, 2003). The goal of herbicide use is to kill or stunt weed infestation, allowing the rice to grow and gain a competitive advantage (Monaco et al., 2002).

Oxadiazon, a herbicide used in rice fields has been identified for other uses such as a pre-emergent or early post-emergent oxadiazole herbicide registered for commercial use on turf grown on golf courses and in apartment/condominium complexes, parks, athletic fields, playgrounds, and cemeteries. In addition, oxadiazon is used on sod farms and on

conifer nurseries and landscapes (i.e. industrial sites, ornamental, roadside plantings, woody, ornamental shrubs, vines, trees and herbaceous ornamentals). Besides weeds, herbicides can act upon other species, causing serious side effects on non-target rice field inhabiting organisms. Oxadiazon has the potential to be transferred to surface water following rainfall events, thus increasing the potential for exposure among fish and other aquatic animals (U.S.EPA 2003). Environmental fate studies indicated that potential exposure of aquatic invertebrates to this herbicide is enhanced due to its relatively high mobility and persistence in soil and water (U.S.EPA 2003).

The use of rice herbicides has been expanding enormously worldwide over the past 20 -40 years. However, herbicides are considered a “two-edged sword” (Kudsk and Streibig, 2003) since the subsequent dispersion of herbicide compounds and their degradation products in rice fields and adjacent areas with strong ecological value still threatens the integrity of ecosystems, thus resulting in serious global environmental concerns (Olofsdotter *et al.*, 1998). Herbicide residues contaminate soils and water, remain in the rice crop, enter the food chain and finally, are ingested by humans with rice foodstuffs and water (Liebman, 2001; EEC, 1991 and Benfenati *et al.*, 2007).

The effects of herbicides on estuaries, rivers and fragile coastal zones are all reflected on the reduction of fish catch (Clay, 2004). This led to concerns about environmental protection which have increased over the years from a global viewpoint (Galhano *et al.*, 2011). One of the major issues about environmental herbicide contamination in wetland rice fields is its bioaccumulation in ecosystem primary producers and its subsequent propagation through trophic chain. Therefore, there is the need to carry out the study on the effects of Oxadiazon, a herbicide used in rice fields, on the growth and nutrient utilization of the fish, *Clarias gariepinus* bearing in mind that the practice of polyculture is now being encouraged in Nigeria and this fish is the most cultured due to its hardy nature.

2. Materials and Methods

2.1. Experimental Fish and Procedure

Three hundred *Clarias gariepinus* with mean weight 262.17 ± 72.80 g were purchased from a reputable fish farm in Ibadan, Oyo State and were transported to the laboratory of the Department of Animal Science and Fisheries Management, Bowen University, Iwo, Osun State, Nigeria. The fish were acclimatized for 2 weeks in 9 plastic tanks measuring 50cm by 30cm by 30cm containing dechlorinated tap water at room temperature of $30 \pm 1.50^\circ\text{C}$. There was no mortality during the acclimation period. Water was changed every other day in order to prevent the build up of metabolic wastes. Experimental fish were fed twice daily at 5% body weight with 2mm Durante pelletized fish feed containing 45.30% crude protein. Feeding was stopped 24 hours to the

commencement of the bioassay and during the exposure period which lasted for 96 hours. This was necessary because feeding increases the rate of respiration and excretory products which may influence the toxicity of the test solutions.

2.2. Experimental Toxicant

The toxicant, Oxadiazon was purchased from a reputable Agro-chemical, Jubaili Agrotech outlet in Ibadan, Nigeria.

2.3. Experimental Procedure

The acute and chronic bioassay was conducted to investigate the toxicity of Oxadiazon on the juvenile of *Clarias gariepinus*.

2.4. Range Finding and Definitive Tests

A rapid range finding test in acute lethality studies of fish was described and evaluated. The test is based on the increase in susceptibility of fish to toxicants at low Oxygen concentrations and is commonly referred to as a residual Oxygen test. Feeding was stopped 24 h prior to and during exposure period that lasted for 96 h. Acute toxicity test (96 h LC₅₀) was conducted in the laboratory following Odiete (1999). Ten juveniles of *C. gariepinus* were randomly selected and transferred from the holding tanks into the triplicate test bowls (with 20L of water) within 30 min of preparing the toxicant mixture. The Oxadiazon concentration used were 0.5ml, 1ml, 2ml, 3ml, 4ml and 5ml L⁻¹. There was a control in which ten fish were exposed to de-chlorinated tap water only. Temperature condition was kept at room temperature and all aquaria were exposed to equal amount of natural light. The toxicant solution and test water were renewed every other day during the bioassay. Mortality was observed and recorded at 1, 2 and 4 h and subsequently every 6 h up to 96 h. Fish were considered dead when gill movement ceased and no response was observed upon gentle prodding. Dead fish were recorded and removed immediately from test solutions to avoid fouling the media. The number of dead fish was counted in every bowl at observation time and recorded.

In definitive test, a set of 10 fish specimen were exposed to nominal Oxadiazon (0.5ml, 1ml, 2ml, 3ml, 4ml and 5ml/l) concentrations in two replicates. Another set of 10 fish were simultaneously maintained in tap water without test chemical and this served as the control. Mortality was recorded up to 96hr at every 24hr interval to obtain LC₅₀ values of Oxadiazon. The LC₅₀ was determined following the Probit analysis method described by Finney (1971).

2.5. Sub-Lethal Concentration Procedure

Nine plastic containers were filled with 20 liters of water. $\frac{1}{2}$ and $\frac{1}{5}$ of the LC₅₀ of Oxadiazon was introduced into the plastic containers filled with 20 liters of water using an auto-pipette and the concentration was continuously renewed every two days. The fish samples were weighed weekly and

the new weight was used to calculate the quantity of feed to be fed to the fish.

2.6. Proximate Analysis

The proximate composition of the fish carcass and the diet were determined before and after the experiment according to the procedures described by AOAC, 1990.

2.7. Growth Parameters

$$\text{Total Weight Gain} = W_2 - W_1$$

Where W_1 = initial weight (g)

W_2 = final weight (g)

2.7.1. Percentage Body Weight Gain (PBWG)

This was calculated from the relationship between mean weight gain and the initial mean weight.

$$PBWG = \frac{\text{Weight gain}}{\text{Initial weight}} \times 100$$

2.7.2. Specific Growth Rate (SGR)

This was calculated from the difference between the final weight and the initial weight divided by the trial days.

$$SGR = \left[\frac{\ln W_2 - \ln W_1}{T_2 - T_1} \right] \times 100$$

Where $\ln W_1$ = initial weight (g)

$\ln W_2$ = final weight (g)

T_1 = initial day of the experiment

T_2 = final day of the experiment

2.7.3. Feed Conversion Ratio (FCR)

This was calculated from the relationship of feed intake and weight gain

$$FCR = \frac{\text{Feed intake}}{\text{Weight gain}}$$

2.7.4. Protein Efficiency Ratio (PER)

This was calculated from the relationship between the weight gain of the fish and the protein consumed.

$$PER = \frac{\text{Total weight gain}}{\text{Protein intake}}$$

2.7.5. Total Feed Intake (TFI)

This is sum of the total feed consumed by the fish throughout the feeding trial period.

2.8. Statistical Analysis

The results obtained were subjected to descriptive statistical analysis. The data on different concentration of Oxadiazon on *Clarias gariepinus* were compared statistically using one way analysis of variance (ANOVA) and Duncan's

multiple range tests were used in the comparison of treatment means and significant level was set at $p < 0.05$.

3. Results

The proximate analysis of the experimental diet was carried out and is presented in Table 1. The parameters determined were crude protein (45.30%), crude fibre (5.20%), crude fat (13.96%), ash (4.50%), moisture content (10.20%) and carbohydrate (31.04%). The proximate composition of the fish carcass exposed to Oxadiazon at different concentrations is shown in Table 2. The crude protein values of the fish carcass ranged between 25.40% and 28.60%. The crude protein values of fish exposed to 0.008ml/l concentration and control were significantly higher $p < 0.05$ than fish exposed to 0.02ml/l concentration. Similarly, the carbohydrate values of 0.008ml/l and control were significantly higher $p < 0.05$ than 0.02ml/l exposure to Oxadiazon. The values recorded for crude fibre showed that fish fed control and 0.02ml/l concentration were significantly higher $p < 0.05$ than 0.008ml/l concentration. The values recorded for crude fat of fish exposed to 0.02ml/l concentration was significantly higher $p < 0.05$ than control and 0.008ml/l concentration. The moisture content of fish exposed to 0.02ml/l concentration was significantly higher $p < 0.05$ than 0.008ml/l concentration and control. The ash values revealed that control, 0.008ml/l and 0.02ml/l concentrations were not significantly different.

Table 3 shows the growth performance and nutrient utilization of *Clarias gariepinus* exposed to different concentrations of Oxadiazon. The TWG of control was significantly higher $p < 0.05$ than 0.008ml/l and 0.02ml/l concentrations. Similarly, PBWG of control was significantly higher $p < 0.05$ than 0.008ml/l and 0.02ml/l concentrations. The values recorded for SGR revealed that control was significantly higher $p < 0.05$ than 0.008ml/l and 0.02ml/l concentrations. The TFI of fish exposed to 0.008ml/l and 0.02ml/l concentrations were significantly lower than the control which had no exposure. The FCR of 0.02ml/l concentration was significantly lower than the control and 0.08ml/l concentration. The PER of control, 0.008ml/l and 0.02ml/l concentrations were not significantly different.

Table 1. Proximate composition of the feed fed to *Clarias gariepinus* exposed to Oxadiazon at different concentrations.

Parameters determined	Value
Crude protein (%)	45.30
Crude fibre (%)	5.20
Crude fat (%)	13.96
Ash (%)	4.50
Moisture content (%)	10.20
Carbohydrate (%)	31.04

Table 2. Proximate composition of the experimental fish exposed to Oxadiazon at different concentrations.

Parameters	Initial	Control	0.008ml/l	0.02ml/l
Determined				
Crude protein (%)	26.90±0.43	28.60 ^a ±1.34	26.96 ^a ±0.26	25.40 ^b ±1.82
Crude fibre (%)	1.20±2.41	1.22 ^a ±0.36	1.17 ^b ±0.72	1.25 ^a ±0.53
Crude fat (%)	5.80±0.21	5.92 ^b ±0.82	4.68 ^b ±0.23	6.70 ^a ±0.27
Ash (%)	0.23±0.47	0.22 ^a ±0.61	0.20 ^a ±0.81	0.25 ^a ±0.16
Moisture	27.60±2.51	28.96 ^b ±0.64	26.90 ^b ±0.26	32.80 ^a ±0.32
Content (%)				
Carbohydrate (%)	68.70±0.62	64.04 ^a ±0.92	66.99 ^a ±0.75	60.40 ^b ±0.47

Table 3. Growth performance and nutrient utilization indices of *Clarias gariepinus* at different concentrations.

Parameters	Control	0.008ml/l	0.02ml/l
Initial weight (g)	260.17 ^a ±72.80	262.53 ^a ±43.77	263.67 ^a ±34.36
Final weight (g)	391.33 ^a ±104.72	330.73 ^b ±73.44	318.07 ^b ±49.89
Weight gain (g)	131.16 ^a ±57.06	68.20 ^b ±32.11	54.40 ^b ±15.56
Percentage weight gain (%)	48.64 ^b ±14.86	25.31 ^a ±8.06	27.60 ^a ±2.70
Specific growth rate	1.36 ^a ±0.39	0.84 ^a ±0.24	0.09 ^a ±0.08
Total feed intake (g)	194.57 ^a ±26.02	82.47 ^b ±38.42	27.27 ^c ±6.01
Feed conversion ratio	1.39 ^a ±0.32	1.49 ^a ±0.98	0.42 ^a ±0.03
Protein efficiency ratio	0.02 ^a ±0.01	0.02 ^a ±0.02	0.05 ^a ±0.01

Means with the same superscript along the row are not significantly different $p > 0.05$

4. Discussion

At the end of the experiment, total weight gain, percentage body weight gain and specific growth rate was best in *C. gariepinus* juvenile exposed to 0.008ml/l concentration. Fish exposed to 0.02ml/l had the least weight gain, percentage body weight gain and specific growth rate. The decrease observed in weight gain, percentage body weight gain and specific growth rate might have been related to the high concentration of Oxadiazon. This result supports the findings of Shirasu (1987) when he carried out 24 months oral chronic toxicity study in rats. Oxadiazon was fed in the diet for 104 weeks at 0, 3, 10 and 100ppm to rat. At 100ppm, there was a reduction in body weight of the rats between 4% and 11%.

Rhonepoulenc (1974) observed the influence of Oxadiazon on the reproduction of rats. Oxadiazon in corn oil was administered to pregnant albino rats by oral gavage at nominal levels of 0, 10, 20, 40, 60, 100 and 500mg/kg on days 6-15 of gestation. Maternal body weight gain decreased. Teratology study in rabbit which was reported by life science research, UK (1987) showed maternal toxicity, evidenced as decreased weight gain after the start of treatment which was observed at the highest concentration of Oxadiazon of 60 and 180mg/kg. Also, at 180mg/kg, the group of Zealand white rabbits also exhibited reduced food consumption.

According to European Food safety Authority, short-term toxicity studies with Oxadiazon were conducted between 1963 and 2002. Dose level of 20mg/kg bw/day in dogs showed decreased body weight in both the 90 days and one-year studies at dose level of 60mg/kg bw/day. Rhone-poulenc (1990b) evaluated bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*) for potential toxicity to Oxadiazon. Herbicide levels of 0.5 and 10ppm produced no

abnormal behaviour on bluegill sunfish. Higher levels of Oxadiazon 12 to 55 ppm however, did result in loss of equilibrium and death. Kudo *et al.*, (1981) reported decrease in body weight gain in rats at 50.9mg/kg/day through their feed for 24 months.

Macrae *et al.*, (1982) also reported decrease in total weight and body weight gain of mouse fed with 254mg/kg/day of Oxadiazon through their feed for 105 weeks. Tesh *et al.*, (1987) teratology study in rat showed decrease in total weight and body weight gain at 3.0mg/kg/day of oxadiazon in their feed for 10 days. He also conducted an experiment on New Zealand white rabbit on effect of Oxadiazon at 20mg/kg/day for 13 days and a decrease in body weight gain was recorded. Chapman (1989) administered Oxadiazon orally (capsule) to beagle dog for 52 weeks at dose level 0, 5, 20, 60 and 200mg/kg/day and a decrease in body weight gain at the highest concentration was observed. A research was conducted by Nippon Institute of Environmental Toxicology, Tokyo (1981) on Fischer rats. Oxadiazon was fed *ad libitum* at dose levels 0, 10, 100, 1000 and 3000ppm for 2 years and the rats showed decrease in body weight and food consumption.

Weatherholtz (1970) conducted 13 weeks experiment on dietary administration of Oxadiazon to rats at 1000mg/kg/day and observed decrease in body weight in the rats. Macanulty (1988) fed rats with Oxadiazon at 1.84mg/kg/day for 14 weeks and the result showed decreased in body weight of the rats. Rhodia (1975), carried out chronic toxicology and carcinogenic study with Oxadiazon in Sprague Dawley rats. Diet mixed with Oxadiazon was made available to the rats *ad libitum* for 24 months at dose levels of 0, 50, 100, and 500ppm and result showed decrease in body weight at the highest concentration of Oxadiazon in the diet of the rats.

5. Conclusion

The present environmental concerns about rice field herbicide residues in water, soil and rice feedstuffs will probably not vanish in the next years. Not enough has been done to reduce the risks caused by herbicide pollution in rice agriculture. Even though the switch to low-dose agents has significantly reduced herbicide consumption, most surface water and ground water samples still contain herbicides, sometimes at levels harmful for human health and for environment.

To guarantee minimal negative side effects on rice field ecosystems, herbicides should have no or low toxicity, except for the target weeds. Improved formulations will be needed to reduce off-target deposition, improve retention on target, and enhance uptake and translocation.

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