The Journal of Animal & Plant Sciences, 23(3): 2013, Page: 888-892
ISSN: 1018-7081

INVESTIGATION ON ACUTE TOXICITY AND BEHAVIORAL CHANGES IN *TILAPIA ZILLII* DUE TO GLYPHOSATE-BASED HERBICIDE, FORCEUP

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ABSTRACT

Glyphosate, N-(phosphoromethyl) glycine is one of the most widely used herbicide in agriculture for the control of weeds and woody shrubs. A 96 h semi-static acute toxicity bioassay was carried out to determine the LC_{50} value and behavioral responses of commercial formulation of glyphosate (forceup) on the freshwater fish *Tilapia zillii*. The 24, 48, 72 and 96 h LC_{50} values (with 95 % confidence limits) estimated by probit analysis were 477.79 (431.01-558.23), 296.43(265.45-325.52), 253.21(222.58-281.02) and 211.80 (122.27-293.55) mgl^{-1} respectively. The safe level for the herbicide varied from 2.118 to 2.118×10^{-3} mgl^{-1}. Fish exposed to higher concentration of the herbicide showed uncoordinated behavior such as erratic and jerky swimming, attempt to jump out of water, frequent surfacing and gulping of air, decrease opercula movement and secretion of mucus on the body and gills followed by exhaustion and death. Our result though indicate that commercial formulation of glyphosate (Forceup) is mildly toxic to *T. zillii*, the herbicide should be applied with caution in our environment especially near water bodies to avoid the possible risk associated with the use of the pesticide.

Key words: *Tilapia zillii*, glyphosate, toxicity, LC_{50}, behavioral changes, safe level

INTRODUCTION

Glyphosate, a broad-spectrum herbicide is one of the most frequently applied pesticides in agriculture for the control of a great variety of annual, biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs. It is also used for aquatic weed control in fish ponds, lakes, canals and slow running water (Tsui and Chu, 2008). Widely used in the rural communities, glyphosate is perhaps the most important herbicide ever developed (WHO, 1994). Glyphosate is moderately to very slightly toxic to aquatic animals and shows a high water solubility varying from 10000 to 15700 mgl^{-1} at 25^0C (USEPA, 1993). The half-life of glyphosate range from 7 to 14 days (Giesy et al., 2000) and has low vapor pressure which suggest that loss to the atmosphere from treated surfaces will be small (Battaglin et al., 2009). It reaches aquatic environment due to the proximities of the agricultural country sides to water places and has been detected in many rivers in both urban and agricultural regions (Pesce et al., 2008). Further, the indiscriminate use of the herbicide, careless handling, accidental spillage or discharge of untreated effluents into natural water ways have harmful effects on the fish population and other aquatic organisms and may contribute to long term ecotoxicological effects to resident aquatic organisms. Due to high water solubility, low persistence and extensive usage of the herbicide in the environment, exposure to non target aquatic organisms is a source of concern. Glyphosate herbicide usage has increased in recent years in Africa including Nigeria thus it became necessary to study the lethal toxicity and stress of the herbicide on local species which will help in formulating the strategies for safeguarding aquatic organisms.

The aim of the present study therefore was to determine the acute toxicity of commercial formulation of glyphosate (Forceup) and its effects on the behavior of juveniles of *Tilapia zillii*. *T. zillii* was selected for the bioassay experiments because it is indigenous to Africa and can be found in other tropical countries of the world. It is also an aquaculture candidate that can narrow the gap between demand and supply of animal protein in developing countries. The species is also an attractive model for toxicity studies because of its availability throughout the year, voracious feeding habit, prolific reproduction and their general hardiness in culture environment.

MATERIALS AND METHODS

Experimental fish specimen and chemicals: Over 300 specimens of *Tilapia zillii* (Family: Cichlidae, Order: Perciformes) juveniles were caught from nearby ponds and lakes with the help of local fishermen. The fish specimens had an average (±S.D) wet weight and length of 8.204±0.22g and 6.77±0.62cm, respectively.

Specimens were subjected to a prophylactic treatment by bathing twice in 0.05 % potassium permanganate ($KMnO_4$) for two min to avoid any dermal infections. The specimens were then acclimatized for two weeks under laboratory conditions. They were fed commercial trout pellets daily at 2% body weight (BW) during acclimatization. For the present study, commercial formulation of glyphosate (400g/l glyphosate-isopropylamine salt) with trade name 'Forceup' manufactured by Zhejiang Linghua Group Ltd Linghu Huzhou was purchased from the local market and used.

Acute toxicity bioassay: Acute toxicity bioassay to determine the 96 h LC_{50} values of commercial formulation of glyphosate (Forceup) was conducted with definitive test in a semi-static system in the laboratory as per the standard methods (APHA, AWWA, WPCE, 2005). The range finding test was carried out prior to determine the concentrations of the test solution for definitive test. The experiment was conducted in glass aquaria (60cm×30cm×30cm size) containing 40L of de-chlorinated and aerated water. The test solution was changed on every alternate day to counter-balance the decreasing pesticide concentrations. During the treatment, fish behavior was observed daily. In definitive test, a set of 10 fish specimen were randomly exposed to glyphosate (Forceup) herbicide (108, 216, 324, 432 and 540 mgl^{-1}) concentrations. Another set of 10 fish were simultaneously maintained in tap water, without test chemical, and considered as control. The experiment was set in triplicate and mortality of the fish due to glyphosate (Forceup) exposure was recorded up to 96 h at every 24 h interval to obtain LC_{50} values of the test pesticides. The LC_{50} of glyphosate (Forceup) was determined following the probit analysis method described by Finney (1971). The safe level of the test pesticides was estimated by multiplying the 96 h LC_{50} with different application factors (AF) and was based on Hart et al. (1948), Sprague et al. (1971), Committee on water Quality Criteria (CWQC, 1972), National academy of Sciences/ National Academy of Engineering (NAS/NAE, 1973), Canadian Council of Resources and Environmental Ministry (CCREM, 1991) and the international Joint Commission (IJC, 1977). The physicochemical properties of test water, namely temperature, total alkalinity, pH, dissolved oxygen, conductivity and total hardness were analyzed using standard methods (APHA, AWWA, WPCE, 2005).

Data analysis: The data obtained were statistically analyzed by statistical package SPSS (Version 16). The data were subjected to one way analysis of variance (ANOVA) and Duncan's multiple range test to determine the significance difference at 5 % probability level.

RESULTS

Physico-chemical parameters of the test water: The physico-chemical characteristics of the test water are listed in Table 1. The water temperature ranged from 24.70 to 26.10 ^{0}C during the experimentation. The pH of the water ranged from 7.00 to 7.29, which was slightly higher than neutral. Dissolved oxygen varied from 6.00 to 6.08 mgl^{-1}. The conductivity value ranged from 250 to 290 μMcm^{-1} whereas total hardness and alkalinity varied from 172 to 186 mgl^{-1} and 138.60 to 186.40 mgl^{-1} as $CaCO_3$ respectively during the experimental period.

Behavioral response of fishes to test concentrations: The behavioral response of the exposed fish was observed in the exposed fish as well as in the control. Normal swimming behavior and natural colour were observed in the control throughout the exposure period and in the lowest (108 mgl^{-1}) concentration of the herbicide at 24 h exposure period. In tanks with higher concentration of test chemical, the fish swam erratically with jerky movements and hyperactivity while body pigmentation was greatly reduced. Faster opercula movement, surfacing and gulping of air were observed. With increase in duration of the exposure, swimming and body movements were retarded and copious mucus was secreted and deposited at the buccal cavity and gills. Later, fish lost balance, became exhausted, lost consciousness owing to respiratory incumbency and finally settled down passively at the bottom of the tank with the operculum wide open and ultimately died.

Median Lethal concentration and application factor: The number of fish dead for commercial formulation of glyphosate (Forceup) of doses 108, 216, 324, 432 and 540 mgl^{-1} were examined depending on the duration of exposure (24, 48, 72 and 96 h) in *Tilapia zillii* (Table **2**). The herbicide concentration of 540 mgl^{-1} showed the highest fish mortality of 100 % at both 72 and 96 h exposure periods while no mortality was recorded in the control throughout the experiment. ~~al.~~ The LC_{50} values (with 95 % confidence limits) of different concentrations of glyphosate (Forceup) in *T. zillii* were found to be 477.79 (431.01-588.23), 296.43 (265.45-325.52), 253.21 (222.58-281.02) and 211.80 (122.27-293.55) mgl^{-1} respectively for 24, 48, 72 and 96 h exposure time (Table **3**). A time and dose-dependent increase in mortality rate was observed; thus, as the exposure time increased from 24 to 96 h, the median lethal concentration required to kill the fish was reduced. There were significant differences ($p < 0.05$) in LC_{10-90} values obtained for different times of exposure. During the experimental period no mortality was recorded in the control experiment. The safe level of glyphosate (Forceup) estimated by different methods at 96 h exposure, are presented in Table **4** The values of safe level of Forceup in *Tilapia zillii* varied from 21.18×10^{-1} to 2.118×10^{-3}.

Table 1 Physico-chemical properties of the test water

Characteristics	unit	Mean	range
Temperature	^0C	25.80	24.70-26.10
Alkalinity	mgl^{-1}	140.50	138.60-186.40
PH	-	7.16	7.00-7.27
Dissolved oxygen	mgl^{-1}	6.02	6.00-6.08
Conductivity	μMcm^{-1}	254	250-290
Total hardness	mgl^{-1}	178	172-186

Table 2 Data on fish survival at different test concentrations and sampling time intervals in *T. zillii* exposed to Glyphosate-based herbicide (Forceup)

Exposed concentration (mgl^{-1})	Number exposed	Number of fish alive at different time intervals (hours)					% mortality
		24	48	72	96	% survival	
0.00	30	30	30	30	30	100	00
108	30	30	30	30	24	80	20
216	30	30	24	18	18	60	40
324	30	24	12	09	09	30	70
432	30	18	06	04	03	10	90
540	30	12	03	00	00	00	100

Table 3 Lethal concentration of Glyphosate-based herbicide (Forceup) (mgl^{-1}) (95% confidence intervals) depending on exposure time for *T. zillii* (n=10 in three replicates)

Lethal concentration	Exposure time (h)			
	24	48	72	96
LC10	292.74^a(223.08-335.03)	191.29^b(149.78-221.31))	157.28^c(119.43-185.55)	98.62^d(20.48-153.57)
LC20	346.35^a(289.64-385.47)	222.33^b(183.72-250.70)	185.21^c(148.94-212.46)	128.21^d(39.06-185.74)
LC30	391.00^a(343.92-433.59)	247.79^b(212.11-275.26)	208.38^c(174.07-235.02)	154.92^d(61.40-215.87)
LC40	433.69^a(390.21-489.36)	271.84^b(238.83-299.37)	230.46^c(198.14-257.14)	182.10^d(88.83-249.68)
LC50	477.79^a(431.01-558.23)	296.43^b(265.45-325.52)	253.21^c(222.58-281.02)	211.80^d(122.27-293.55)
LC60	526.38^a(470.14-644.83)	323.24^b(293.02-356.38)	278.19^c(248.45-309.09)	246.35^d(161.61-359.36)
LC70	583.85^a(511.98-758.25)	354.62^b(322.93-396.01)	307.67^c(277.09-345.14)	289.58^d(205.68-472.56)
LC80	659.12^a(595.47-1040.09)	395.23^b(358.31-452.44)	346.16^c(311.45-396.99)	349.89^c(255.26-695.63)
LC90	779.83^a(649.51-1258.69)	459.35^b(409.14-550.53)	407.63^c(361.15-488.86)	404.87^c(322.04-471.82)

Values with different alphabetic superscripts differ significantly ($p<0.05$) between exposure time within lethal concentration.

Table 4 Estimate of safe levels of Glyphosate-based herbicide (Forceup) at 96 h exposure time

Chemical	96 h LC_{50} (mgl^{-1})	Method	AF	Safe level(mgl^{-1})
Glyphosate (Forceup)	211.80	Hart et al. (1948)*	-	5.52
		Sprague (1971)	0.1	21.18
		CWQC (1972)	0.01	2.118
		NAS/NAE (1973)	0.01-0.00001	$21.18\text{-}2.118\times10^{-3}$
		CCREM (1991)	0.05	10.59
		IJC (1977)	5% LC_{50}	10.59

*$C=48h\ LC_{50}\times0.03/S^2$, where C is the presumable harmless concentration and $S=24h\ LC_{50}/48h\ LC_{50}$

DISCUSSION

Acute toxicity studies are the very first step in determining the water quality requirements of fish and the studies reveal the toxicant concentrations that cause fish mortality even at short exposure (Pandey et al., 2005). Behavioral changes are the most sensitive indicators of potential toxic effects in fishes (Banaee et al., 2011). The observed behavioral alterations in the studied formulation of glyphosate (Forceup) are consistent with previous reports on glyphosate-based herbicides (Ayoola, 2008; Langiano and Martinez, 2008; Lushchak et al., 2009; Nwani et al., 2010; Shiogiri et al., 2010, 2012) and other pesticides like malathion (Kumar et., 2009), chlorpyriphos (Ali et al., 2009); profenofos (Pandey et al., 2011), atrazine (Nwani et al., 2011) and butachlor (Chang et al., 2011). Pragatheeswaram et al. (1987) reported that such abnormal and altered movements are considered to be the result of excessive elimination of skeletal minerals while Pandey et al. (1990) attributed the secretion of mucus over the body and dispigmentation to dysfunction of the endocrine gland under toxic stress causing changes in the number and area of mucus glands and chromatophores.

The LC_{50} value reported in the present study for commercial formulation of glyphosate (Forceup) is higher than 1.05 mgl^{-1} and13.6 mgl-1 reported by Ayoola (2008) and Langiano and Martinez (2008) when glyphosate and glyphosate-based herbicide (Roundup) were exposed to *Oreochromis niloticus* and *Prochilodus lineatus* respectively. The LC_{50} obtained in our present study for commercial formulation of glyphosate (Forceup) is also higher than the 108 mgl^{-1} and 3.74 mgl^{-1} obtained by Clements et al. (1997) and Shiogiri et al. (2012) when bullfrog tadpoles and neotropical fish (*Piaractus mesopotamicus*) were exposed to glyphosate-based herbicide roundup for 48 h. Our LC_{50} value however is lower than the 620 mgl^{-1} and 975 mgl^{-1} 96 h LC_{50} reported by Neŝkovic et al. (1993) and Shiogiri et al. (2010) when *Cyprinus carpio* and *Palloceros caudimaculatus* were each exposed to glyphosate and glyphosate commercial formulation ((Rodeo®) herbicides respectively. The result indicates that our test herbicide is less toxic than some of the previously reported formations of glyphosate. Further, the previous literature indicates that the toxicity of glyphosate-based herbicides varies from one species to another species and even in strains of the same species. Toxicity of chemicals to aquatic organisms has been reported to be affected by temperature, pH, dissolved oxygen, size and age, type of species, water quality, concentration and formulation of test chemicals (Gupta et al., 1981; Young, 2000; Kumar et al., 2012). The safe level obtained for glyphosate (Forceup) in the present study varied from 2.118 to 2.118×10^{-3}. However, due to large variation in safe levels as determined by different methods, the estimates of safe levels cannot be guaranteed (Buikema et al., 1982). Extrapolation of laboratory data to field is not always meaningful value and hence it is difficult to decide on acceptable concentration based on laboratory experiments that may be considered 'safe' in the field (Elmegaard et al., 2000; Pandey et al., 2005).

Conclusion: It is concluded from the present study that the commercial formulation of glyphosate (Forceup) could be less toxic to *T. zillii* than other herbicides widely used in Nigeria. Most of the behavioral and physiological abnormalities were recorded mainly at the higher concentrations. However, the use of the pesticide in the environment especially near water bodies must be regulated to avoid the possible risk that may be associated with the herbicide contamination. More studies related to toxicity in *T. zillii* exposed to glyphosate and its formulations are necessary to understand the mechanisms and toxicokinetics of the herbicide.

Acknowledgements: We acknowledge TETFUND (Tertiary Education Trust Fund), Nigeria and the DERIC, Ebonyi State University, Abakaliki for providing the fund and conducive environment, respectively, to carry out the research.

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