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Full Length Research Paper

Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*

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Acute toxicity of butachlor, a chloroacetanilide herbicide (2-chloro-N-[2, 6-diethylphenyl] acetamide) to *Tilapia zillii*, was studied in a semi static bioassay. The 24, 48, 72 and 96 h LC_{50} values (with 95% confidence limits) estimated by probit analysis were 3.13 (2.88 to 4.61), 1.93 (0.63 to 4.41), 1.27 (0.59 to 1.92) and 1.25 (0.60 to1.85) mgl^{-1}, respectively. There were significant differences ($p<0.05$) in the LC_{50} values obtained at different exposure times and the safe levels estimated by different methods varied from 1.25×10^{-1} to 1.25×10^{-5} mgl^{-1}. There were dose and time dependent increase in mortality rate due to exposure to the herbicide. Stress signs in form of hyperactivity, erratic swimming, skin discoloration, vigorous jerks of the body followed by exhaustion and death were observed. The 96 h LC_{50} of 1.25 mgl^{-1} obtained indicate that the herbicide was toxic to *T. zillii.* Agricultural use of butachlor in the environment, especially, near water bodies, must be restricted to avoid the severe risk associated with the use of the pesticide.

Key words: *Tilapia zillii*, butachlor, toxicity, mortality, behavioral changes, safe level.

INTRODUCTION

Butachlor, (2-chloro-N-[2, 6-diethylphenyl] acetamide) is one of the most widely used chloroacetanilide herbicide for the control of annual grasses in rice fields and many broadleaf weeds. It can also be used in seed beds and seed transplant fields as well as in some crop fields such as wheat, barley, cotton, vegetables and peanuts. It reaches aquatic environment due to the proximities of the agricultural country sides to water places. The repeated and indiscriminate use of the herbicide, careless handling, accidental spillage or discharge of untreated effluents into natural water ways has harmful effects on the fish and other aquatic organisms. Butachlor contamination of 0.163 ppb has been recorded in ground water collected from tube wells adjacent rice fields in Philippines (Natarajan, 1993). The pesticide can degrade rapidly but under conditions of low temperature, low moisture, high alkalinity and lack of suitable microbial degraders, it may remain biologically active and persist in soils for a long time. Lin et al. (2000) reported that increase in sunlight enhanced photo degradation of butachlor in water and that the half life of the herbicide in non-filtered river water was shorter than filtered samples. Lin et al. (1999) also reported that surface waters with high concentrations of organic and suspended solid reduced the toxicity of pesticides possibly through photo degradation.

The contamination of aquatic ecosystems by xenobiotics has gained increased attention and several recent studies have demonstrated the toxicity of butachlor to fish and other aquatic organisms (Geng et al., 2005; 2010; Ateeq et al., 2006; Geng et al., 2005; Peebua et al.,

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Table 1. Data on fish survival at different test concentrations and sampling time intervals in *T. zillii.*

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
0.60	30	30	30	30	24	80	20
1.20	30	28	24	20	18	60	40
1.80	30	26	21	14	12	40	60
2.40	30	24	12	10	06	20	80
3.00	30	18	10	4	00	00	100

2007; Geng et al., 2010, Chang et al., 2011). Butachlor has also been reported to be carcinogenic (Ou et al., 2000) and can adversely disrupt the reproductive process and affect the thyroid and sex steroid hormones in Zebra fish (Chang et al., 2011). The fish *Tilapia zillii* is commonly found in all African countries and some other tropical countries of the world. It is an aquaculture candidate that can narrow the gap between demand and supply of animal protein in developing countries. Furthermore, *Tilapia zillii* is an attractive model species for toxicity studies because of its availability throughout the year, ease of culture, prolific reproduction, omnivorous nature and their general hardiness in culture environment.

In spite of the wide application of butachlor to farmlands by our farmers and the possible ecotoxicological impact attached to its use, there is paucity of information on its effects on many local fish species like *Tilapia zillii*. The present study thus aims at the determination of the 96 h LC_{50} of butachlor to the freshwater fish *Tilapia zillii,* their behavioral responses and the possible safe levels of the pesticide during the exposure period.

MATERIALS AND METHODS

Experimental fish specimen and chemicals

The juveniles of freshwater fish *Tilapia zillii* (Family: Cichlidae, Order: Perciformes) were caught from nearby rivers, ponds and lakes with the help of local fishermen. The fish specimens had an average (±S.D) wet weight and length of 8.304 ± 0.13g and 6.17 ± 0.15cm, respectively. To avoid bias, fish of similar length and weight range were selected and used. Specimens were subjected to a prophylactic treatment by bathing twice in 0.05% potassium permanganate ($KMnO_4$) for 2 min to avoid any dermal infections. The specimens were then acclimatized for 2 weeks under laboratory conditions in semi-static systems. They were fed with commercial trout pellets daily at 2% body weight (BW) during acclimatization. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. The fish were treated in accordance with the rules conforming to principles of Laboratory Animal Care as set by set by the Institutional Animal Ethics Committee (IAEC) of the University. For the present study, commercial formulation of butachlor (50% EC) with trade name “butaforce” manufactured by Anhui Futian Agrochemical Ltd Shanghai, China, with CAS NO A5-0268 was purchased from the local market and used.

Acute toxicity bioassay

Acute toxicity assay to determine the 96 h LC_{50} values of butachlor 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 (60 × 30 × 30 cm size) containing 40 L 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 nominal butachlor (0.60, 1.20, 1.80, 2.40, 3.00 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 butachlor exposure was recorded up to 96 h at every 24 h interval (Table 1) to obtain LC_{50} values of the test pesticides. The LC_{50} of butachlor 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, dissolved oxygen; pH, total hardness, conductivity and total alkalinity 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 presented in Table 2. The water temperature varied from 23.50 to 25.50°C, pH ranged from 7.15 to 7.80 while dissolved oxygen varied from 6.30 to 6.80 mgl^{-1}. The conductivity value ranged from 252 to 300 µM cm^{-1} whereas total hardness varied from 174 to 180 mgl^{-1}as $CaCO_3$ during the experimental period.

Table 2. Physico-chemical properties of the test water.

Characteristic	Mean	Range
Air temperature (°C)	25.5	25.40-26.42
Water temperature (°C)	24.66	23.50-25.50
PH	7.20	7.15-7.80
Dissolved oxygen (mgl^{-1})	6.55	6.30-6.80
Conductivity (μMcm^{-1})	254	252-300
Total hardness (mgl^{-1})	176	174-180

Table 3. Lethal concentration of butachlor (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
LC_{10}	2.14^a (1.61-2.40)	1.17^a (0.01-1.65)	0.53^b (0.04-0.90)	0.52^b (0.04-0.87)
LC_{20}	2.47^a (2.13-2.77)	1.40^a (0.01-1.89)	0.72^b (0.10-1.10)	0.69^b (0.11-1.07)
LC_{30}	2.75^a (2.47-3.25)	1.57^a (0.03-2.17)	0.89^b (0.20-1.31)	0.87^b (0.21-1.27)
LC_{40}	3.00^a (2.70-3.86)	1.75^{ab} (0.18-2.67)	1.07^b (0.36-1.55)	1.05^b (0.37-1.51)
LC_{50}	3.26^a (2.88-4.61)	1.93^{ab} (0.67-4.41)	1.27^b (0.59-1.92)	1.25^b (0.60-1.85)
LC_{60}	3.54^a (3.07-5.53)	2.12^a (1.30-14.23)	1.50^a (0.89-2.59)	1.48^a (0.89-2.46)
LC_{70}	3.87^a (3.27-6.74)	2.36^{ab} (1.69-18.06)	1.81^{ab} (1.21-4.05)	1.79^b (1.20-3.75)
LC_{80}	4.30^a (3.51-8.53)	2.67^{ab} (1.96-65.67)	2.24^{ab} (1.54-7.69)	2.22^b (1.54-6.87)
LC_{90}	4.96^a (3.86-11.84)	3.16^a (2.26-95.11)	3.02^a (1.98-20.48)	3.00^a (1.98-17.40)

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

Behavioral response of fishes to test concentrations

Fish exposed to different concentrations of the pesticide displayed behavioral abnormalities in response to the test chemical. At the initial exposure, fish that were alert stopped swimming and remained static in position in response to the sudden changes in the surrounding environment. After some time, fish in the experimental groups tried to avoid the test water by swimming very fast, jumping and displaying other random movements. In tanks with higher concentrations of the test pesticide swimming of the fish was erratic with vigorous jerky movements along with hyper excitation. Faster opercula movement, surfacing and gulping of air were observed. Body pigmentation was decreased while copious mucus which covered the buccal cavity, body and gills were secreted. Later, the fish lost their balance, consciousness, engaged in rolling movement and became exhausted and lethargic owing to respiratory incumbency. Soon, they settled down passively at the bottom of the tank with the operculum wide open and ultimately died.

Median lethal concentration and application factor

Median lethal concentration (LC_{50}) is the concentration of a test chemical, which kill 50% of the test organism in a particular length of exposure, usually 96 h. The LC_{50} values (with 95% confidence limits) of different concentrations of butachlor in *T. zillii* were found to be 3.13 (2.88 to 4.61), 1.93 (0.63 to 4.41), 1.27 (0.59 to 1.92) and 1.25 (0.60 to 1.85) 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 $LC_{10\text{-}90}$ values obtained for different times of exposure. During the experimental period no mortality was recorded in the control experiment. The estimated safe level of butachlor as calculated by multiplying the 96 h LC_{50} with different application factors are given in Table 4. The values of safe level of butachlor in the *Tilapia zillii* varied from 1.25×10^{-1} to 1.25×10^{-5} mgl^{-1}.

DISCUSSION

Acute and chronic toxicity tests are widely used to evaluate the toxicity of chemicals on non-target organisms (Santos et al., 2010). The 96 h LC_{50} of 1.25 mgl^{-1} obtained indicate that the herbicide is toxic to *T. zillii.* The toxicity is both concentration and time dependent thus, accounting for the differences in the values obtained at different concentrations and exposure times. When *T. zillii* juveniles were exposed to 0.60 mgl^{-1} butachlor con-

Table 4. Estimate of safe levels of butachlor at 96 h exposure time.

Chemical	96 h LC_{50} (mgl^{-1})	Method	AF	Safe level (mgl^{-1})
Butachlor	1.25	Hart et al. (1948)*	-	2.30×10^{-2}
		Sprague (1971)	0.1	1.25×10^{-1}
		CWQC (1972)	0.01	1.25×10^{-2}
		NAS/NAE (1973)	0.01-0.00001	1.25×10^{-1}-1.25×10^{-5}
		CCREM (1991)	0.05	6.25×10^{-2}
		IJC (1977)	5% LC_{50}	6.25×10^{-2}

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

centrations, only 20 % died after 96 h, whereas all the fish (100 %) died after 96 h expose at 3.00 mgl^{-1} butachlor concentration. Previously reported LC_{50} values of butachlor in *Oreochromis niloticus* (Wang et al., 1992), *Heteropneustus fossilis* (Ateeq et al., 2005) and *Channa punctatus* (Tilak et al., 2007) were 0.880 mgL^{-1}, 2.34 ppm and 247.46 ppb, respectively. Geng et al. (2005) reported 96 h LC_{50} of 1.40 mgl^{-1} when *Rana japonica* was exposed to butachlor while Gobic and Gunasekaran (2010) obtained 96 h LC_{50} of 0.515 mg/kg for *Eisenia fietida*. From the observed results, 96 h LC_{50} value for butachlor in *T. zilli* was higher than the 10.20 and 381.9 μgl^{-1} obtained when similar fish, Nile tilapia (*Oreochromis niloticus*) was exposed to endosulfan (Werimo and Willen, 2010) and alachlor (Peebua et al., 2007), respectively thus indicating that endosulfan and alachlor are more toxic to tilapia than butachlor. The previous literature clearly indicates that the toxicity of butachlor varies from one species to another 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; Nwani et al., 2010). The safe level obtained for butachlor in the present study varied from 1.25×10^{-1} to 1.25×10^{-5} mgl^{-1}. However, the large variation in safe levels determined by various methods has resulted in controversy over its acceptability (Buikema et al., 1982; Pandey et al., 2005). Kennega (1979) emphasized that the major weakness in calculation of application factor (AF) is its dependence on LC_{50} values. Behavioral changes are the most sensitive indicators of potential toxic effects (Banaee et al., 2011). The behavioral and swimming patterns of the fish in the control group were normal but for the experimental groups abnormal swimming behavior increased with increasing concentration and exposure time. Bekeh et al. (2011) reported similar behavioral responses in Tilapia (*Oreochromis niloticus*) exposed to butachlor. The behavioral symptoms observed during butachlor exposure to *T. zillii* also agree with the observations reported by other authors reporting toxicity of pesticides in fish (Kumar et al., 2010, Pandey et al., 2011; Banaee et al., 2011, Chang et al., 2011; Altinok et al., 2012).

Our results from the present study on butachlor toxicity support the conclusion that *T. zillii* is sensitive to the pesticide and their mortality rate is dose and time dependent. This study also shows the significance of behavioral parameters in assessing the hazards of the pesticide to fish. Agricultural use of butachlor in the environment especially near water bodies must be restricted to avoid the sever risk associated with the use of the pesticide.

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