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Oxidative Stress Responses in *Bufo regularis* Tadpole Exposed to Butaforce® and Termex®

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Abstract

The present study investigated the oxidative stress responses of *Bufo regularis* tadpoles exposed to Butaforce and Termex pesticides. The results indicated that Lipid Peroxidase (LPO), Glutathione peroxidase (GPx), Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Reduced Glutathione (GHS) and Glutathione Transferase (GST) in the exposed tadpoles showed significant differences from the control groups. There was a decrease in LPO, GP_x, CAT and SOD in the *B. regularis* tadpole exposed to Butaforce and Termex while an increase in GR, GSH and GST was observed among the treatment compared with the control group. It is possible that Butaforce and Termex metabolism in the *B. regularis* tadpoles may had generated reactive oxygen species (ROS) that could have interacted with enzymatic activities in the muscle of exposed tadpoles resulting in oxidative stress. The results of the present findings indicated that both pesticides were toxic to *B. regularis* tadpole resulting in alterations in the oxidatives stress parameters. Both pesticides should be used very carefully to avoid contamination of aquatic environment.

Keywords: Pesticide; Butaforce; Termex; Oxidative stress; *Bufo regularis*; Tadpole

Introduction

Butaforce is herbicide applied in agricultural land for the control of grass and other broad leaves while termex is an insecticide for the control of termites and other pests such as cockroaches, ants and mosquitoes. In Nigeria many farmers use different types of herbicides indiscriminately in the control of weeds in crop land, irrigated canals and rice fields. Both Butaforce and Termex from agricultural fields and households can be carried into aquatic habitats through runoff. Their effects are undesirable when they affect the non target organisms in the natural habitat. Literature had shown that up to 90% of the pesticides applied never reach the targets organisms [1], thus other organisms sharing the same habitat with the pest are accidently poisoned.

The oxidative stress parameters–have been used as indicators of stress in fish and other aquatic animals. Oxidative stress is a state in which the balance between the production of reactive oxygen species (ROS) and their removal by antioxidant defenses before they can cause damage is upset [2]. Antioxidants substances protect cells against the effect of free radicals. There are several enzyme systems that catalyze reactions to neutralize free radicals and reactive oxygen species. These enzymes include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione transferases (GST). These form the body's endogenous defense mechanisms to help protect against free radical-induced cell damage.

Amphibians are one of the non target biota mostly affected in contaminated habitats [3]. They are exposed to pesticides by different routes but the most common route is agricultural runoff. Tadpoles play major role in aquatic food chain and their contamination by pesticides affects the aquatic ecosystem. Tadpoles are more susceptible to pollution because of their permeable skin. Tadpoles help in controlling the populations of algae in pond since they form their major diet thus militate against algal bloom and other associated problems. They help in pest management control: as tadpoles grow they become omnivores or carnivores reducing the population of invertebrates. Bodies of still water are preferred breeding sites of several biting insects including mosquitoes and if tadpoles are present in the pond, most of these pests become diet for them [4].

If aquatic environment is altered continuously by chemical contaminants it creates danger to aquatic biota leading to decline in population. Several studies have shown decline in amphibian population due to pollution, disease outbreak and climate change [4,5]. The decline of amphibian populations has been correlated with higher agriculture activities where the use of pesticide is common [6]. The present study aimed at evaluating the effect of Butaforce and Termex on the oxidative stress parameters of *Bufo regularis* tadpoles.

Materials and Methods

One hundred and eight (n=180) tadpoles of freshwater *Bufo regularis* (Family: Bufonidae; order: Anura), mean length 2.01 ± 1.01 cm and mean weight 0.10 ± 0.09 g were collected using hand net from a stagnant pool at the Biological Garden of the Department of Biology, Federal College of Education, Eha-Amufu Enugu State, Nigeria. The tadpoles were transported in four twenty liters plastic aquaria containing their habitat water to the Fishery Wet Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were acclimatized for fourteen days in a large (1000 L) test plastic aquarium prior to the experiment. Water was changed daily with well aerated tap water to siphon off faecal matter and other waste materials in order to reduce ammonia content in water and the aquarium cleaned thoroughly. The tadpoles were fed with green algae three times daily. Any dead tadpoles were removed instantly

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with forceps to maintain healthy water quality. The feeding of the experimental tadpoles were stopped 24 h before the commencement of acute toxicity test as recommended by Ward and Parrish [7] and Reish and Oshida [8]. Ethical clearance (MANR/FD/2017/EC102) was obtained from the committee on Experimental Animal Care, Fishery Department, Ministry of Agriculture and Natural Resources Enugu and followed carefully. In the present study Butachlor (trade name Butaforce liquid-NAFDAC NO A5-0268, China Agro Cropcare Co. Ltd China Dacui village Huimin Nanjiang) containing 50% Butachlor as active ingredient and Termex (trade name termex 350 SC; Nariman point Mumbai Rallis India Limited) containing 350gw/v chlopyrifos and imidacloprid 30.5% SC, were purchased from an agrochemical shop in Ogbete main market, Enugu, Nigeria and were used as the stock solutions.

Determination of sub lethal concentrations

The 96 h LC_{50} values of Butaforce and Termex on *B. regularis* were 0.42 mg/L and 1.13 mg/L respectively following the prohibit analysis as described by Finney [9]. Based on the 96 h LC_{50} value obtained during the acute toxicity assay three test concentrations corresponding to I/5^{th}, 1/10^{th} and 1/20^{th} of 96 h LC_{50} of Butaforce (7 µg/L, 9 µg/L, 11 µg/L) and Termex (15 µg/L, 20 µg/L and 25 µg/L), respectively were used for *in vivo* experiment. For the Butaforce a total of 120 tadpoles were used for the experiment. The tadpoles were randomly divided into four groups of 30 tadpoles each. Each group was further randomized into three replicate experiment of 10 tadpole per replicate in 40 L glass aquaria (60 × 30 × 30 cm size). Tadpoles in the first, second and third groups were exposed to 7, 9 and 11 µg/L Butaforce while the forth group was exposed to tap water as the control. The same procedure was used for the Termex but the first, second and third groups were exposed to 15 µg/L, 20 µg/L and 25 µg/L Termex respectively while the forth group was exposed to tap water. The exposure lasted for a period of 96 h duration and renewed daily. On each sampling day three tadpoles from each of the treatment groups including the control were sacrificed. The sacrificed tadpoles were weighed and the muscle tissue harvested. EDTA containers were used to store the muscle tissues and were used to estimate biochemical and oxidative stress parameters. The tissues were quickly rinsed in cold 0.9% sodium chloride solution. The muscle tissues from each triplicate were homogenized immediately in pre-chilled potassium phosphate buffer or normal saline (1: 10 w/v, 0.1M, pH 7.0) and was centrifuged for 20 minutes at 10,500 rpm under 4°C to obtain the supernatant which was stored at 4°C for the estimation of oxidative stress activities.

Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al. [10]. Glutathione peroxidase (GP_X) was estimated according to the method of Paglia and Valentine [11]. The activity of GP_X was determined by monitoring the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase. Catalase activity was determined as described by Aebi [12]. While Superoxide dismutase (SOD) was determined using the method of Xin et al. [13]. Glutathione reductase (GR) activity was estimated by measuring the rate of conversion of NADPH using the method of Tayarani et al. [14]. The glutathione transferases (GST) Activity of the tissue fraction was determined according to the method of Habig et al. [15]. It involved the formation of a complex enzymatic conjugation of GSH with the aromatic substrate 1-chloro-2, 4-dinitrobenzene, the complex formed absorbs at 340 nm.

Statistical analysis

The data obtained were analyzed using the statistical package SPSS version 21 computer program (SPSS Inc. Chicago, IL, USA). The data were subjected to one-way analysis of variance. Duncan multiple range test was used to determine differences among treatments at 5% probability level. Results were expressed as means ± standard error.

Results

Effect of Butaforce on the oxidative stress parameters of *B. regularis*

The sub-lethal concentrations of Butaforce caused some alterations on oxidative stress parameters of *B.* regularis (Table 1). Lipid peroxidase (LPO) decreased from 8.46 ± 0.07 to 5.53 ± 0.43 during 24 h while at 96 h, it decreased from 10.87 ± 0.27 to 5.16 ± 0.04 at the same concentrations. The activity of LPO significantly decreased ($p<0.05$) in the muscle tissue of treated tadpoles in comparison to the control. GP_x decreased from 10.42 ± 0.02 to 6.14 ± 0.10 during 24h while it also decreased from 10.17 ± 0.08 to 5.17 ± 0.14 at 96 h. There was concentration and time- dependent decrease of GP_X in the exposed tadpoles compared with the control group. As the concentration increases for instance from 9.00 μgL^{-1} to 11.00 μgL^{-1}, the mean value of GP_x of the exposed tadpole decreased compared to the control.

CAT (Catalase) values decreased from 0.78 ± 0.03 at 7.00 μgL^{-1} to 0.34 ± 0.01 during 24 h while at the 96 h, it decreased from 0.90 ± 0.02 to 0.24 ± 0.01. Therefore there were concentration and time-dependent decrease in the exposed tadpole. Activity of CAT significantly decreased ($p<0.05$) in muscle tissue of exposed tadpoles compared to the control group.

SOD (Superoxide dismutase) values decreased from 23.70 ± 0.68 to 16.70 ± 0.28 at 24 h and decreased from 28.32 ± 0.12 to 15.10 ± 0.37 during the 96 h. There were concentration and duration-dependent decrease in the exposed tadpoles compared to the control group.

GR (Gutathione Reductase) values increased from 18.44 ± 0.28 to 24.30 ± 0.50 at 24 h and further increased to 16.97 ± 0.31 to 27. 61± 0.25 at the 96 h. Therefore, there were concentrations and time dependent increase of GR in the exposed tadpole compared to the control group.

GSH (Reduced Glutathione) values increased from 0.50 ± 0.04 to 0.62 ± 0.01 at the 24 h whereas at 96 h it increased from 0.51 ± 0.01 to 0.78 ± 0.02. This showed concentration and time-dependent increase in the tissue of the exposed tadpoles.

Glutathione transferase (GST) increased significantly ($p<0.05$) from 1.66 ± 0.05 to 2.68 ± 0.04 at 24 h and from 3.15 ± 0.14 to 3.60 ± 0.12 at 96 h. This showed concentrations and time dependent increase in the muscle tissue of the exposed tadpoles compared to the control.

Effect of Termex on the oxidative stress parameters of *B. regularis*

Sub-lethal concentrations of Termex had effects on the oxidative parameters *B. regularis* tadpoles (Table 2). LPO values decreased from 8.46 ± 0.08 to 6.63 ± 0.40 at 24 h and further decreased further from 8.03 ± 0.03 to 4.47 ± 0.09 at 96 h exposure.

GPx values decreased significantly from 10.42 ± 0.02 to 4. 59 ± 0.20 at 24 h and further decreased from 10.07 ± 0.08 to 4.37 ± 0.13 at 96 h. Therefore, there was concentration and duration-dependent decrease of GP_X in the muscle tissue of the exposed tadpole compared to the control group.

CAT values decreased from 0.43 ± 0.02 to 0.42 ± 0.00 at 15 $\mu g\ L^{-1}$and 25 μgL^{-1} respectively at 24 h and decreased further from 0.42 ± 0.01 to 0.33 ± 0.01 at 20 μgL^{-1} and 25 $\mu g\ L^{-1}$ respectively at 96 h exposure. There

Parameter	Tissue	Conc (µgL^{-1})	Exposure Duration (h)			
			24	48	72	96
LPO nmol/protein	Muscle	Control	8.46 ± 0.07^{1c}	9.38 ± 0.09^{2b}	10.83 ± 0.06^{3c}	10.87± 0.02^{3d}
		7	8.37 ± 0.16^{3c}	4.47 ± 0.17^{1a}	7.40 ± 0.07^{2b}	6.88 ± 0.27^{2c}
		9	6.54 ± 0.07 ^{3b}	4.36 ± 0.12^{1a}	6.33 ± 0.09^{3a}	5.83 ± 0.23^{2b}
		11	5.53 ± 0.43^{3a}	3.84 ± 0.32^{1a}	6.12 ± 0.07^{1a}	5.16 ± 0.04^{2a}
$GP_{X(IU/-1)}$	Muscle	Control	10.42 ± 0.02^{1c}	10.32 ± 0.38^{1c}	10.19±0.323^{1c}	10.17± 0.08^{2c}
		7	8.21 ± 0.58^{2b}	8.47 ± 0.08^{2b}	8.28 ± 0.02^{2b}	6.87 ± 0.33^{1b}
		9	7.85 ± 0.28^{3b}	6.74 ± 0.34^{2a}	6.34 ± 0.03^{2a}	5.20 ± 0.07^{1a}
		11	6.14 ± 0.10^{2a}	6.23 ± 0.06^{2a}	6.33 ± 0.05^{2a}	5.17 ± 0.04^{1a}
CAT(unit/mg protein)	Muscle	Control	0.78 ± 0.03^{1c}	0.73 ± 0.00^{1c}	0.86 ± 0.00^{2b}	0.90 ± 0.02^{2c}
		7	0.51 ± 0.04^{2b}	0.43 ± 0.00^{2b}	0.35 ± 0.00^{1a}	0.32 ± 0.04^{1b}
		9	0.39 ± 0.01^{3b}	0.39 ± 0.00^{2b}	0.33 ± 0.00^{1a}	0.34 ± 0.01^{1b}
		11	0.34 ± 0.01^{2a}	0.29 ± 0.03^{1a}	0.30 ± 0.03^{2a}	0.24 ± 0.01^{1a}
SOD(unit/mg protein)	Muscle	Control	23.70 ± 0.68^{2a}	26.06 ± 0.23^{2c}	27.76 ± 0.33^{3c}	28.32 ± 0.12^{3b}
		7	17.86 ± 0.36^{3a}	16.64 ± 0.25^{2b}	16.33 ± 0.10^{2b}	15.44 ± 0.03^{1a}
		9	17.41 ± 0.55^{3a}	16.34 ± 0.08^{2a}	15.35 ± 0.10^{1a}	15.30 ± 0.09^{1a}
		11	16.70 ± 0.28^{2a}	15.70 ± 0.41^{1a}	15.17 ± 0.04^{1a}	15.10 ± 0.37^{1a}
		Control	18.44 ± 0.28^{2a}	16.97 ± 0.29^{1a}	16.44 ± 0.04^{1a}	16.97 ± 0.31^{1a}
GR(nmol/mg protein)	Muscle	7	21.59 ± 0.80^{2b}	19.30 ± 0.59^{1b}	20.72 ± 0.32^{1b}	21.64 ± 0.25^{2b}
		9	23.26 ± 0.64^{1c}	22.81 ± 0.29^{1c}	25.08 ± 0.30^{2c}	27.61 ± 0.25^{3c}
		11	24.30 ± 0.50 ^{1c}	24.51 ± 0.37^{1d}	26.38 ± 0.10^{2d}	27.47 ± 0.04^{3c}
		Control	0.18 ± 0.03^{1a}	0.25 ± 0.01^{2a}	0.20 ± 0.03^{2a}	0.13 ± 0.01^{1a}
GSH mg/protein	Muscle	7	0.42 ± 0.01^{1b}	0.50 ± 0.04^{1b}	0.45 ± 0.01^{2b}	0.51 ± 0.01^{2b}
		9	0.55 ± 0.02^{1c}	0.60 ± 0.01^{1c}	0.65 ± 0.01^{2c}	0.75 ± 0.01^{3c}
		11	0.62 ± 0.01^{4d}	0.66 ± 0.00^{3c}	0.67 ± 0.01^{3c}	0.78 ± 0.02^{3c}
		Control	0.74 ± 0.05^{1a}	0.75 ± 0.01^{1a}	0.72 ± 0.04^{1a}	0.75 ± 0.01^{1a}
GST mg/protein	Muscle	7	1.45 ± 0.04^{2b}	1.66 ± 0.05^{2b}	1.55 ± 0.05^{2b}	2.02 ± 0.20^{2b}
		9	1.50 ± 0.09^{2b}	2.14 ± 0.11^{3c}	2.42 ± 0.04^{3c}	3.15 ± 0.14^{3c}
		11	1.22 ± 0.21^{2b}	2.68 ± 0.04^{4d}	2.70 ± 0.00^{4d}	3.60 ± 0.12^{4d}

Different alphabetic letters show significant differences ($p< 0.05$) among butaforce concentrations within the rows while different numeric superscripts indicate significant differences among durations of exposure within the horizontal as determined by Duncan's multiple range.

Table 1: Effect of Butaforce on oxidative parameters [lipid peroxidase (LPO), Glutathione peroxidase (GP_x), Catalase (CAT), Superoxidase dismutase (SOD), Glutathione reductase (GR), Reduced glutathione (GSH) and Glutathione transferase (GST)] in *Bufo regularis* tadpole.

Parameter	Tissue	Conc (µgL^{-1})	Exposure Duration (h)			
			24	48	72	96
LPO (nmol/mg protein)	Muscle	Control	8.46 ± 0.08^{1b}	9.38 ± 0.95^{2d}	10.83 ± 0.57^{3c}	8.06 ± 0.03^{3c}
		15	6.65 ± 0.07^{1a}	5.88 ± 0.30^{3c}	6.90 ± 0.52^{2b}	6.24 ± 0.06^{2b}
		20	6.79 ± 0.32^{1a}	5.09 ± 0.11^{1b}	5.38 ± 0.04^{1b}	4.47 ± 0.09^{1a}
		25	6.63 ± 0.40^{1a}	4.41 ± 0.03^{1a}	5.45 ± 0.05^{1a}	4.47 ± 0.09^{2a}
$GP_{X(nmol/mg\ protein)}$	Muscle	Control	10.42 ± 0.02^{1d}	10.32 ± 0.38^{1c}	10.19 ± 0.32^{1c}	10.07 ± 0.08^{2b}
		15	6.26 ± 0.06^{1c}	6.14 ± 0.13^{3b}	5.36 ± 0.04^{2b}	4.78 ± 0.28^{1a}
		20	5.24 ± 0.04^{2b}	5.80 ± 0.29^{3b}	4.95 ± 0.16^{2b}	4.24 ± 0.07^{1a}
		25	4.59 ± 0.20^{3a}	4.55 ± 0.37^{1a}	4.24 ± 0.07^{1b}	4.37 ± 0.13^{1a}
CAT (unit/mg protein)	Muscle	Control	0.78 ± 0.03^{1c}	0.73 ± 0.00^{1a}	0.86 ± 0.01^{2c}	0.90 ± 0.02^{2d}
		15	0.43 ± 0.01^{1b}	0.48 ± 0.032^{1b}	0.58 ± 0.03^{2b}	0.54 ± 0.01^{3c}
		20	0.34 ± 0.01^{2a}	0.36 ± 0.01^{1a}	0.38 ± 0.02^{1a}	0.42 ± 0.01^{1b}
		25	0.42 ± 0.00^{1c}	0.35 ± 0.01^{1a}	0.35 ± 0.02^{1b}	0.33 ± 0.01^{1a}
SOD(unit/mg protein	Muscle	Control	23.70 ± 0.68^{1b}	26.07 ± 0.23^{2c}	27.76 ± 0.33^{3c}	28.32 ± 0.12^{3c}
		15	17.67 ± 0.37^{3a}	15.64 ± 0.30^{1a}	16.45 ±0.05^{2b}	15.45 ± 0.82^{1a}
		20	16.30 ± 0.07^{3a}	16.28 ± 0.08^{3b}	15.30 ± 0.08^{1a}	15.81 ± 0.09^{2b}
		25	16.80 ± 0.38^{2a}	15.45 ± 0.04^{1a}	15.27 ± 0.08^{1a}	15.54 ± 0.10^{1a}
GR(nmol/mg protein)	Muscle	Control	18.44 ± 0.03^{2a}	16.97 ± 0.29^{1a}	16.44 ± 0.04^{1a}	16.97 ± 0.31^{1a}
		15	20.67 ± 0.24^{2b}	20.74 ± 1.10^{2b}	18.30 ± 0.10^{1b}	19.14 ± 0.24^{1b}
		20	21.38 ± 0.59^{1b}	24.55 ± 0.22^{3b}	23.42 ± 0.59^{3c}	24.58 ± 0.34^{2c}
		25	24.03 ± 0.21^{1c}	26.10 ± 0.37^{3b}	26.44 ± 0.06^{2d}	27.02 ± 0.18^{2c}
GSHmg/protein	Muscle	Control	0.18 ± 0.03^{1a}	0.25 ± 0.07^{1a}	0.20 ± 0.03^{1b}	0.13 ± 0.01^{1a}
		15	0.36 ± 0.02^{1b}	0.31 ± 0.46^{1a}	0.35 ± 0.01^{2a}	0.36 ± 0.03^{2a}
		20	0.43 ± 0.01^{1c}	0.55 ± 0.03^{2b}	0.47 ± 0.04^{3ab}	0.56 ± 0.05^{3b}
		25	0.44 ± 0.01^{1c}	0.48 ± 0.02^{1a}	0.56 ± 0.02^{4b}	0.59 ± 0.03^{3b}
GSTmg/protein	Muscle	Control	0.74 ± 0.06^{1a}	0.75 ± 0.01^{1b}	0.72 ± 0.04^{1b}	0.75 ± 0.01^{1a}
		15	1.35 ± 0.05^{2b}	1.38 ± 0.04^{1b}	1.50 ± 0.04^{2b}	1.68 ± 0.04^{2c}
		20	1.56 ± 0.07^{3c}	1.82 ± 0.04^{3c}	1.92 ± 0.04^{3bc}	2.15 ± 0.17^{3c}
		25	1.78 ± 0.07^{1d}	2.32 ± 0.21^{2d}	2.39 ± 0.05^{2d}	2.51 ± 0.09^{2d}

Different letters show significant differences ($p<0.05$) among Termex concentrations within the rows while different numerals indicate significant differences among durations of exposure within the horizontal as determined by Duncans multiple range test.

Table 2: Effect of Termex on oxidative parameters [lipid peroxidase (LPO), Glutathione peroxidase (GP_x), Catalase (CAT), Superoxidase dismutase (SOD), Glutathione reductase (GR), Reduced glutathione (GSH) and Glutathione transferase (GST)] in *Bufo regularis* tadpole.

was significant ($p < 0.05$) decrease of CAT in the muscle tissue of the exposed tadpoles which was concentration and time-dependent. SOD values decreased significantly from 17.67 ± 0.37 to 16.80 ± 0.38 at 15 µg L^{-1} and 25 µg L^{-1} respectively during 24 h duration whereas it reduced further from 15.54 ± 0.10 to 15.45 ± 0.82 at 96 h.

There was significant increase of GR in the muscle tissue of exposed tadpoles which was dependent on concentrations as well as exposure duration compared to the control. For example, GR values significantly increased from 18.44 ± 0.03 to 24.03 ± 0.21 at 24 h and from 16.97 ± 0.31 to 27.02 ± 0.18 at 96 h exposure.

There was significant increase of GSH in the muscle tissue of exposed tadpoles which was dependent on concentrations as well as exposure duration compared to the control. GSH values increased from 0.18 ± 0.03 to 0.44 ± 0.01 at 24 h and further increased from 0.13 ± 0.01 to 0.59 ± 0.03 during the 96 h exposure.

GST increased significantly from 0.74 ± 0.06 to 1.78 ± 0.07 at 24 h exposure and from 0.13 ± 0.01 to 2.51 ± 0.09 at 96 h exposure. This showed concentration and time dependent increase of GST in the muscle tissue of the exposed tadpole which is comparable to the control group.

Discussion

The results of oxidative stress responses in the present study showed a decrease in LPO, GP_X, CAT and SOD in *B. regularis* exposed to Butaforce and Termex. It is possible that Butaforce and Termex metabolism in the *B. regularis* tadpoles may have generated reactive oxygen species (ROS) that could have interacted with enzymatic activities in the muscle of exposed tadpoles resulting in oxidative stress and possible decrease in these parameters. The pesticides could have also affected the specific metabolic pathways of the muscle tissue. The decrease in LPO shows enzyme inactivation by Butaforce and Termex. This is contrary to the observations of Amaeze et al. [16] who observed that *Amietophryus* tadpoles exposed to refined petroleum products and unused spent engine oils indicate high level of lipid peroxidation. Radovanović et al. [17] also reported increase LPO in Asian toad *Duttaphrynus melanostictus* tadpoles exposed to deltamethrin. Other investigators also reported increase LPO in other vertebrates and pesticides, for instance zebra fish exposed to delmamethrin [18], cyprinid fish exposed to endosulfan [19], suckling rat exposed to fenthion [20] and African catfish exposed to fenthion formulations [21].

There was a significant decrease ($p<0.05$) in GP_X activity in the muscle tissue of the exposed *Bufo regularis* tadpoles compared to the control group. Contrary to this result, Radovanović et al. [17] observed an increased GP_X activity in the liver and skin of exposed adult green toad. Capkin and Altinok [22] and Nwani et al. [21] reported respectively decreased GPx activity in rainbow trout (*Oncorhynchus mykiss*) exposed to carbosulfan, and *Clarias gariepinus* exposed to fenthion respectively.

SOD is a vital antioxidant defense enzyme in almost all the cells to catalyze the dismutation of superoxide into oxygen thus protecting the cell from superoxide toxicity. SOD is involved in protection of biological systems from the actions of free radical. The present study showed that SOD value in the muscle tissue at the different Butaforce and Termex concentrations were not sufficient to scavenge ROS values thus leading to the oxidative stress. The decrease in SOD values from 24-96 h in this investigation may be attributed to different concentrations of Butaforce and Termex induced excessive ROS generation. This in turn inhibits SOD activity or inactivated antioxidant enzymes [23]. Amaeze et al. [16] reported that SOD was inhibited in *Amietophryus* tadpoles exposed to refined petroleum products and unused and spent engine oils and this observation is in line with the finding of the present study. So the decreased SOD activity may reflect oxidative stress caused by Butaforce and Termex exposure.

In contrast to this observation on reduced SOD in muscle tissue of tadpoles, Xiaohui et al. [5] observed an increased SOD in the Chinese toad tadpoles *Bufo bufo gargarizans* exposed to 3.23 mg/L spirotetramat after 4 days of exposure. However, a decreased SOD was recorded after 15 days exposure of Chinese tadpoles exposed to spirotetramat. The observation was different from some previous studies [18,21] on other vertebrates. Xiaohui et al. [5] observed greater levels of activity in antioxidant enzymes (SOD) in all experimental groups compared to acetone control group for 4 days. Only the experimental group exposed to the highest concentration of spirotetramat (3.23 mg/L) showed comparatively lower activity of SOD. Radovanović et al. [17] also recorded an increased SOD in green toad (*Bufotes viridis* L.) exposed to deltamethrin.

CAT activity was generally reduced in the muscle tissue of tadpoles exposed to Butaforce and Termex respectively. The decrease in CAT activity could be as a result of the damaging effects of H_2O_2 that results from degradation of anion superoxide by SOD. A time and concentration- dependent decrease was also noticed in CAT activity. This observation suggested that CAT is responsible for the catabolism of hydrogen perioxide generated in the muscle tissue of tadpoles exposed to Butaforce and Termex. The decrease in the activities of CAT noticed from 24 h-96 h exposure to Butaforce and Termex, were sufficient to prevent the rise of LPO in the muscle. It might be due to binding of Butaforce and Termex to CAT or by inhabiting CAT synthesis. The decrease observed in the CAT activity in muscle of tadpole exposed to Butaforce and Termex with respect to the control could be due to oxyradical production. The decreased observed in the present study is similar to the findings of Sharma and Ansari [18] on the effect of deltamethrin in the muscle of zebra fish. In contrast, Radovanović et al. [17] observed an increased of CAT activities in the liver and skin of exposed adult green toad. The concentration and time dependent decrease in the CAT activity observed in this study is in agreement with the report of other researchers [24,25]. Similar findings were reported by other researcher in *Channa punctata* Bloch [26]. The decrease CAT activity may be enhanced by the flux of superoxide radicals (O^{2-}) induced by toxicants [27]. Mekail and Sharafaddin [28] noted that the activity of CAT was decreased in brain, liver and kidney of weanling rat treated with diazinon, carbaryl and cyhalothrin. A concentration and time-dependent decrease in the CAT was also observed by Rosety et al. [29] in sea bream gills exposed to Malathion.

The present study indicated concentration and time dependent increase values in the GR, GSH and GST. The increase in GR values obtained may be as a result of the ability of the enzyme to sustain the recycling GSSH to GSH. GSH is a non enzymatic antioxidant that protects the cells from undesirable changes induced by xenobiotic [21].

Similar to the findings of this study is that of Xiaohu et al. [5] who reported that GSH of tadpole exposed to spirotetramat showed significant increase ($p<0.5$) after exposure. The non enzymatic (GSH) antioxidants provide adequate defense and help to scavenge ROS. The increase in GSH levels could be a protective response of cells against Butaforce and Termex – induced oxidative stress in the tadpole. The reports by previous researchers were in line with the observed increased GSH on exposure of *Rana esculenta* to methoxychlor [30]; Rana daunching tadpoles exposed to atrazine [31]; green toad exposed to deltamethrin [17] and fish exposed to other pesticides [19,21].

There was a significant increase ($p<0.5$) in the GST values in the muscle tissue of *B. regularis* exposed at to the pesticides at different concentrations from 24-96 h. GST Enzymes play a vital role in cellular detoxification of various xenobiotics. These enzymes make the xenobiotic soluble in water and neutralize the toxic agents which protect cells against toxicity. The increase in GST activities in the present study may be responsible for the decrease in other enzyme activities such as LPO, GP_x, SOD, and CAT. The elevated level of GST activities observed in this study is similar to the reports of Ezemonye and Tongo [32] on adult frog response for serum, brain and liver gastrointestinal tract and lung after diazinon exposure. Other studies observed increased GST in the tissue of the exposed amphibian, for instance in *Rana esculenta* exposed to methoxychlor [30]; *Rana daunching* tadpoles exposed to atrazine [31] and green toad exposed to deltamethrin [17].

Conclusion

The present study indicated a decrease in LPO, GP_x, CAT and SOD in the *B. regularis* tadpole exposed to Butaforce and Termex while an increase in GR, GSH and GST among the treatment groups compared with the control. While the present study focused on the modulation of these parameters on a short time basis (24 to 96 h), further studies on the effects of the two pesticides on the studied parameters on long term basis is strongly recommended. In all, pesticides should be applied with serious caution to avoid eco-toxicological impacts on the aquatic ecosystem and by extension on non-target organisms like tadpoles from the ecological food chain.

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