IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 13, Issue 9 Ser. II (September 2020), PP 48-53
www.iosrjournals.org

Toxic Effect of Endosulfan -Pesticide on oxidative stress parameters of Clarias Gariepinus juveniles

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Abstract
Rapidly evolving Technology has led to the introduction of several chemical substances for improved agricultural yields and protection from pests. Over the years the use of the pesticides has been limited due to lethality,accumulation in environmental organisms and persistence in the environment. In this work the effect of endosulfan a commonly used insecticide on the oxidative stress parameters of Catfish is studied. The sub lethal dose effect of endosulfan - pesticide (0.19, 0.48 and 0.95 mg/L) on the lipid peroxidation, superoxide dismutase, catalase,glutathione peroxidase, glutathione reductase and glutathione S transferase activity were investigated over a period of 21 days exposure in three replicates. The colorimetric analysis of the samples collected on day 1,7,14 and 21 for lipid peroxidation, glutathione peroxidase, glutathione reductase, glutathione S transferase,catalase and superoxide dismutase showed significant increase in a time and concentration dependent manner. The Lipid peroxidation increased from (6.32 ± 0.00^{2b} -6.64 ± 0.00^{3c}) at 0.19 mg/L, (6.26 ± 0.00^{3d} –6.74 ± 0.00^{1b}) at 0.48 mg/L and (6.32 ± 0.00^{2a} –7.12 ± 0.00^{1a}) at 0.95 mg/L of endosulfan when compared with the control (5.16 ± 0.48^{2c} –5.90 ± 0.43^{1d}); Superoxide Dismutase increased from (11.12 ± 0.00^{3c} -12.31 ± 0.00^{2a}) at 0.19 mg/L, (11.16 ± 0.10^{2a} –12.66 ± 0.00^{3b}) at 0.48 mg/L and (11.05 ± 0.00^{2b} –12.39 ± 0.00^{3d}) at 0.95 mg/L of endosulfan when compared with the control (10.18 ± 0.62^{1c} –$10.14\pm0.51^{2c)}$; Catalase activity also increased from (0.26 ± 0.00^{1a} -0.49 ± 0.00^{3a}) at 0.19 mg/L,(0.34 ± 0.00^{2b} –0.72 ± 0.00^{3b}) at 0.48 mg/L and (0.42 ± 0.00^{2c} –0.76 ± 0.00^{3c}) at 0.95 mg/L of endosulfan when compared with the control (0.23 ± 0.05^{1b} –$0.42\pm0.03^{2b)}$;Glutathione Peroxidase increased from (8.12 ± 0.00^{2c}-10.12 ± 0.00^{2b}) at 0.19 mg/L, (9.32 ± 0.00^{2a} –9.32 ± 0.00^{2a}) at 0.48 mg/L and (9.36 ± 0.00^{2a} –9.42 ± 0.00^{2a}) at 0.95 mg/L of Endosulfan when compared with the control (7.05 ± 0.46^{2b}–8.02 ± 0.56^{3a}); the Glutathione Reductase increased from (14.19 ± 0.00^{2b} -14.19 ± 0.00^{2b}) at 0.19 mg/L, (14.26 ± 0.00^{1c} –19.41 ± 0.00^{2c}) at 0.48 mg/L and (14.86 ± 0.00^{1d} –20.64 ± 0.00^{2d}) at 0.95 mg/L of endosulfan when compared with the control (13.80 ± 0.54^{1a} –$13.97\pm0.42^{1b)}$; the Glutathione S-Transferase increased from (3.29 ± 0.00^{2d} -5.73 ± 0.00^{1b}) at 0.19 mg/L, (3.56 ± 0.00^{1a} –8.28 ± 0.00^{2d}) at 0.48 mg/L and (3.36 ± 0.00^{1b} –8.92 ± 0.00^{2c}) at 0.95 mg/L of endosulfan when compared with the control (2.78 ± 0.51^{3c} –$4.71\pm0.62^{1a)}$respectively. The result suggests that endosulfan may induce oxidative stress that may overwhelm the antioxidant system of juvenile catfish especially at higher concentrations with long exposure.

Keywords*: Toxicity; endosulfan; Clarias gariepinus; Oxidative stress*

Date of Submission: 04-09-2020 Date of Acceptance: 19-09-2020

I. Introduction

The worlds' population is increasing at an alarming rate and application of agrochemicals such as fertilisers and pesticides to increase food production and ensure the continuation of the human race becomes necessary[1]. Endosulfan (ladosulfan -trade name) is a broad-spectrum organo-phosphorous insecticide which is one of the most frequently applied pesticides in agriculture for the protection against a wide variety of pests[2]. It is used widely in the rural communities and washed from nearby farmlands and accidental discharges into aquatic systems and contribute to long term eco-toxicological effects in the environment and particular to non-target aquatic organisms Due to affordability, availability and solubility of endosulfan,its utilization has increased in recent years in Africa.[3,4,5]. It then became necessary to study the effects of the insecticide on local species like catfish which will help in formulating the strategies for safeguarding aquatic organisms.

Catfish (Claridae family, order siluriformes)has average length of 1-1.5 m, weigh up to 60 kg with flat body head, broad terminal mouth, four pairs of barbels and large shrub-like arborescent breathing organs made up of modified gill arches. They are found in freshwater, lakes, rivers and swamps and human made habitats

such as oxidative ponds and urban sewage system in Africa, the middle east, Brazil and Indonesia and are highly esteemed group of fishes, with high growth rate, commanding high market value as they form part of food chain, hardy in nature that enable them tolerate difficult aquatic conditions[6].

In Oxidative stress, cells are destroyed due to imbalance between the level of free radicals and the enzyme systems that neutralizes their effects[7]. Production of reactive oxygen species due to metabolism of pesticides attack biomolecules like lipids by oxidation, disrupts cellular redox status and may cause certain aging disease conditions[8]. When the environment is polluted,fish a biological indicator is used to determine the associated dangers in nearby water environments due to direct leaching effect on the agrochemicals or through the food chain of ecosystem indirectly[9]. The present study aims at determining the oxidative stress factors in juveniles of *Clarias gariepinus* exposed to endosulfan within 21 days.

II. Materials and Methods

Experimental fish and acclimatization: Four hundred and fifty juveniles of Clarias gariepinus were obtained from Sacentourist game village, Idemili LGA. Anambra state, Nigeria in 200 litre capacity plastic containers and transported to Applied Biology Special Laboratory ESUT,Agbani, Enugu State, Nigeria. The fish were acclimated to laboratory conditions for 14 days and fed with commercial feed (6 mm Coppens fish feed for agriculture). The container was cleaned and the water changed every morning during the acclimatisation. The fish was not fed for 48 hours before and during the exposure time. A triplicate set of 10 fish specimen was randomly exposed to different concentrations (0.19, 0.48 and 0.95 mg/L) of endosulfan in 10 litres of dechlorinated and aerated tap water to determine the 96hour lethal concentration (96h LC50) value. Based on the LC50 of endosulfan at 96hours, the effect of the sub-lethal concentrations of 0.19, 0.48 and 0.95 mg/L on the oxidative stress parameters for 1,7,14 and 21 days were determined with sets of 10 fish. Fish in tap water served as the control with (0.00mg/L) of endosulfan.

Procedure Methodology

The evaluation of oxidative stress parameters involves the determination of Lipid peroxidation (LPO) by measuring the malondialdehyde (MDA) formation as described by [10].Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 at 30℃ as described by [11]. Catalase (CAT) activity was assayed from the liver homogenate as described by [12] while the activity of glutathione peroxidase (GPX) was determined by monitoring the rate of NADPH oxidation at 340nm by the coupled reaction with the specific activity determined using the extinction 6.22mMcm^{-1} [13]. Glutathione reductase (GR) was estimated by measuring the rate of conversion of NADPH using the method of [14]. Glutathione S-transferase activity (GST) was measured by the extent of conjugation of reduced glutathione (GSH) with -1-chloro-2,4-dinitrobenzene (CDNB) and the proportionate change in the absorption was determined at 340 nm[15].

Statistical analysis: The statistical data were shown as the mean ± sem. The significant differences of the data were analysed using analysis of variance (ANOVA) from SPSS statistical package (version 17).

III. Results

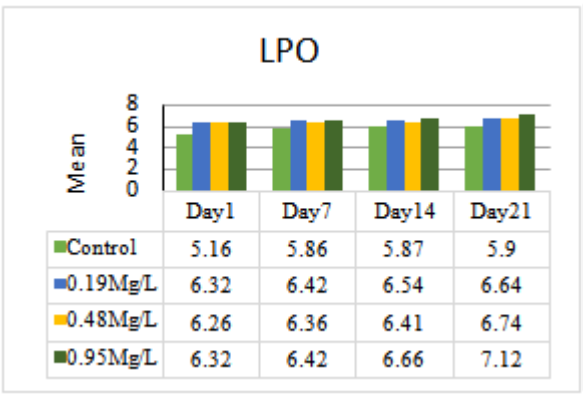
In this study, the data showed that the oxidative stress parameters (LPO,SOD, CAT, GPX, GR, GST) significantly increased with time and concentration.

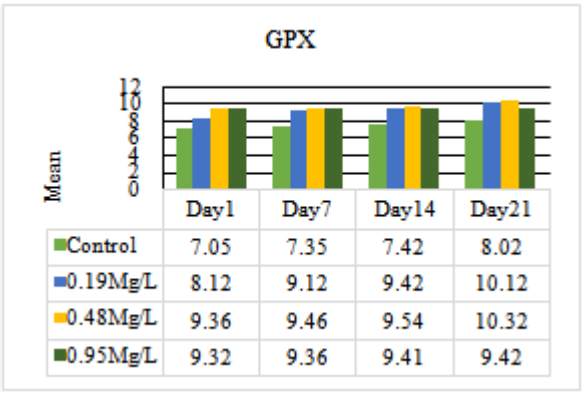
Table 1 show the effects of sublethal concentrations of endosulfan on Oxidative stress parameters indices of *C.gariepinus at* day 1, 7,14 and 21.The lipid peroxidation increased from (6.32 ± 0.00^{2b} -6.64 ± 0.00^{3c}) at 0.19 mg/L , (6.26 ± 0.00^{3d} –6.74 ± 0.00^{1b}) at 0.48 mg/L and (6.32 ± 0.00^{2a} –7.12 ± 0.00^{1a}) at 0.95 mg/L of endosulfan when compared with the control (5.16 ± 0.48^{2c} –5.90 ± 0.43^{1d}); superoxide dismutase increased from (11.12 ± 0.00^{3c} -12.31 ± 0.00^{2a}) at 0.19 mg/L , (11.16 ± 0.10^{2a} –12.66 ± 0.00^{3b}) at 0.48 mg/L and (11.05 ± 0.00^{2b} –12.39 ± 0.00^{3d}) at 0.95 mg/L of endosulfan when compared with the control (10.18 ± 0.62^{1c} –$10.14\pm0.51^{2c)}$.Catalase activity also increased from (0.26 ± 0.00^{1a} -0.49 ± 0.00^{3a}) at 0.19 mg/L, (0.34 ± 0.00^{2b} –0.72 ± 0.00^{3b}) at 0.48 mg/L and (0.42 ± 0.00^{2c} –0.76 ± 0.00^{3c}) at 0.95 mg/L of endosulfan when compared with the control (0.23 ± 0.05^{1b} –$0.42\pm0.03^{2b)}$; glutathione peroxidase increased from (8.12 ± 0.00^{2c} -10.12 ± 0.00^{2b}) at 0.19 mg/L, (9.32 ± 0.00^{2a} –9.32 ± 0.00^{2a}) at 0.48 mg/L and (9.36 ± 0.00^{2a} –9.42 ± 0.00^{2a}) at 0.95 mg/L of endosulfan when compared with the control (7.05 ± 0.46^{2b} –$8.02\pm0.56^{3a)}$; the glutathione reductase increased from (14.19 ± 0.00^{2b} -14.19 ± 0.00^{2b}) at 0.19 mg/L,(14.26 ± 0.00^{1c} –19.41 ± 0.00^{2c}) at 0.48 mg/L and (14.86 ± 0.00^{1d} –20.64 ± 0.00^{2d}) at 0.95 mg/L of endosulfan when compared with the control (13.80 ± 0.54^{1a} –$13.97\pm0.42^{1b)}$; the glutathione S transferase increased from (3.29 ± 0.00^{2d} -5.73 ± 0.00^{1b}) at 0.19 mg/L , (3.56 ± 0.00^{1a} –8.28 ± 0.00^{2d}) at 0.48 mg/L and (3.36 ± 0.00^{1b} –8.92 ± 0.00^{2c}) at 0.95 mg/L of endosulfan when compared with the control 2.78 ± 0.51^{3c} –$4.71\pm0.62^{1a)}$ respectively and

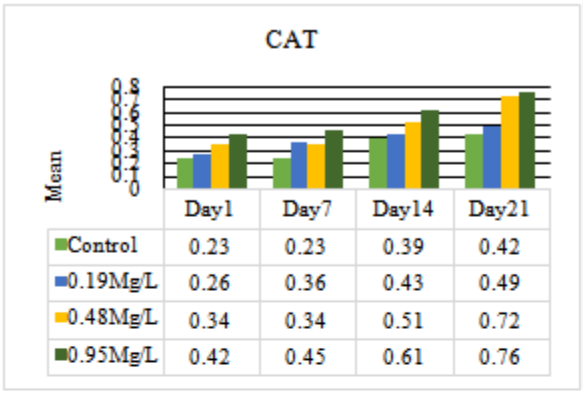
Table 1 Effect of sublethal concentrations of endosulfan on Oxidative stress parameters indices of *C.gariepinus at* day 1, 7,14 and 21

Parameter		Concentration(µg/l) Exposure Duration (Days)			
	Mg/L	DAY 1	DAY 7	DAY 14	DAY 21
LPO	Control	5.16 ± 0.48^{2c}	5.86 ± 0.48^{2c}	5.87 ± 0.40^{1d}	5.90 ± 0.43^{1d}
	0.19	6.32 ± 0.00^{2b}	6.42 ± 0.00^{2b}	6.54 ± 0.03^{1b}	6.64 ± 0.00^{3c}
	0.48	6.26 ± 0.00^{3d}	6.36 ± 0.00^{3d}	6.41 ± 0.00^{2c}	6.74 ± 0.00^{1b}
	0.95	6.32 ± 0.00^{2a}	6.42 ± 0.00^{2a}	6.66 ± 0.00^{3a}	7.12 ± 0.00^{1a}
SOD	Control	10.18 ± 0.62^{1c}	10.28 ± 0.62^{1c}	10.36 ± 0.92^{3d}	10.14 ± 0.51^{2c}
	0.19	11.12 ± 0.00^{3c}	11.19 ± 0.00^{3c}	12.21 ± 0.00^{1c}	12.31 ± 0.00^{2a}
	0.48	11.16 ± 0.10^{2a}	11.36 ± 0.10^{2a}	11.44 ± 0.02^{1b}	12.66 ± 0.00^{3b}
	0.95	11.05 ± 0.00^{2b}	11.12 ± 0.00^{2b}	11.22 ± 0.00^{1a}	12.39 ± 0.00^{3d}
CAT	Control	0.23 ± 0.05^{1b}	0.23 ± 0.05^{1b}	0.39 ± 0.12^{3c}	0.42 ± 0.03^{2b}
	0.19	0.26 ± 0.00^{1a}	0.36 ± 0.00^{1a}	0.43 ± 0.00^{2b}	0.49 ± 0.00^{3a}
	0.48	0.34 ± 0.00^{2b}	0.34 ± 0.00^{2b}	0.51 ± 0.00^{1a}	0.72 ± 0.00^{3b}
	0.95	0.42 ± 0.00^{2c}	0.45 ± 0.00^{2c}	0.61 ± 0.00^{1a}	0.76 ± 0.00^{3c}
GPX	Control	7.05 ± 0.46^{2b}	7.35 ± 0.46^{2b}	7.42 ± 0.98^{1d}	8.02 ± 0.56^{3a}
	0.19	8.12 ± 0.00^{2c}	9.12 ± 0.00^{2c}	9.42 ± 0.00^{1c}	10.12 ± 0.00^{2b}
	0.48	9.36 ± 0.00^{2a}	9.46 ± 0.00^{2a}	9.54 ± 0.00^{1b}	10.32 ± 0.00^{3c}
	0.95	9.32 ± 0.00^{2a}	9.36 ± 0.00^{2a}	9.41 ± 0.00^{1a}	9.42 ± 0.00^{2a}
GR	Control	13.80 ± 0.54^{1a}	13.88 ± 0.54^{1a}	13.92 ± 0.56^{2a}	13.97 ± 0.42^{1b}
	0.19	14.19 ± 0.00^{2b}	14.21 ± 0.00^{2b}	16.33 ± 0.00^{3b}	14.19 ± 0.00^{2b}
	0.48	14.26 ± 0.00^{1c}	14.28 ± 0.00^{1c}	18.94 ± 0.00^{3c}	19.41 ± 0.00^{2c}
	0.95	14.86 ± 0.00^{1d}	14.96 ± 0.00^{1d}	20.36 ± 0.00^{3c}	20.64 ± 0.00^{2d}
GST	Control	2.78 ± 0.51^{3c}	2.88 ± 0.51^{3c}	3.08 ± 0.57^{2a}	4.71 ± 0.62^{1a}
	0.19	3.29 ± 0.00^{2d}	3.50 ± 0.00^{2d}	5.64 ± 0.00^{3b}	5.73 ± 0.00^{1b}
	0.48	3.56 ± 0.00^{1a}	3.66 ± 0.00^{1a}	8.24 ± 0.00^{3c}	8.28 ± 0.00^{2d}
	0.95	3.36 ± 0.00^{1b}	3.86 ± 0.00^{1b}	8.36 ± 0.00^{3d}	8.92 ± 0.00^{2c}

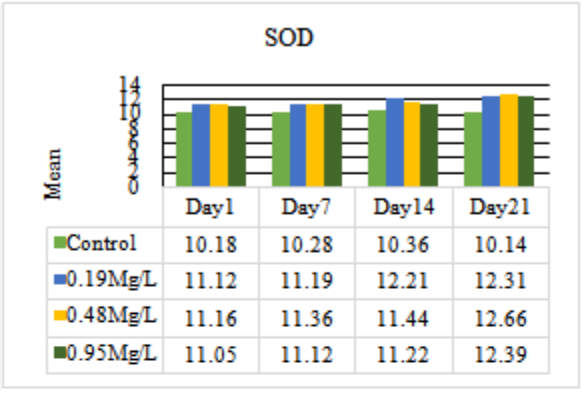
Different alphabetic letters show significant difference ($p<0.05$) among endosulfan Concentrations within the rows while different numeric superscripts indicate significant difference among durations of exposure within the horizontal as determined by Duncan's Multiple Range.



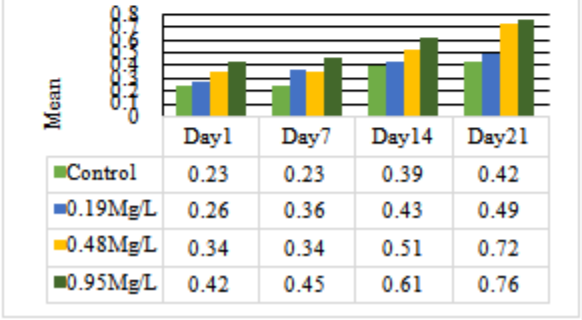




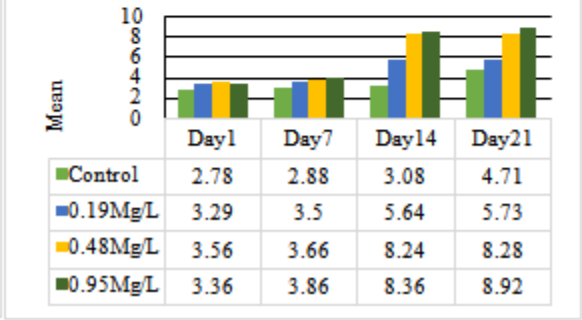
	Day1	Day7	Day14	Day21
Control	0.23	0.23	0.39	0.42
0.19Mg/L	0.26	0.36	0.43	0.49
0.48Mg/L	0.34	0.34	0.51	0.72
0.95Mg/L	0.42	0.45	0.61	0.76



	Day1	Day7	Day14	Day21
Control	10.18	10.28	10.36	10.14
0.19Mg/L	11.12	11.19	12.21	12.31
0.48Mg/L	11.16	11.36	11.44	12.66
0.95Mg/L	11.05	11.12	11.22	12.39



	Day1	Day7	Day14	Day21
Control	0.23	0.23	0.39	0.42
0.19Mg/L	0.26	0.36	0.43	0.49
0.48Mg/L	0.34	0.34	0.51	0.72
0.95Mg/L	0.42	0.45	0.61	0.76



	Day1	Day7	Day14	Day21
Control	2.78	2.88	3.08	4.71
0.19Mg/L	3.29	3.5	5.64	5.73
0.48Mg/L	3.56	3.66	8.24	8.28
0.95Mg/L	3.36	3.86	8.36	8.92

IV. Discussion

Biodegradation of xenobiotics releases free radicals which induce the antioxidant enzymatic systems to neutralize the toxic effects of the highly reactive species and safe the integrity of the cellular biomolecule [16,17,18]. When the level of the radicals overwhelms the activities of the antioxidants, cellular oxidative stress sets in[19].In this present study we show that endosulfan breakdown in the juvenile Clarias gariepinus probably release reactive oxygen species (ROS) scavenged by cellular antioxidants of the fish,with elevation of enzymatic activities to nullify the harmful effect of the excessive free radicals produced which is dependent on concentration and time of exposure. It was reported that lethality of foreign compounds to organisms is directly related to the concentrations, sex, developmental stages and exposure periods [20].

The increase in LPO indicates an increase in level of free radicals produced by endosulfan.This result agrees with previous reports of increase LPO in zebrafish exposed to deltamethrin, atrazine and roundup [21,22,23]. All acetylcholinesterase organophosphorus chemicals produce very reactive and non- selective hydroxyl radicals which attack most biomolecules [24].

There was a significant increase ($p<0.05$) in GPX activity in the catfish compared to the control group. GPX uses reduced glutathione GSH to convert hydroperoxides to water neutralizing its harmful effect to the cell[25]. Contrary to this result,[26,27] reported respectively decreased GPx activity in rainbow trout (Oncorhynchus mykiss) exposed to carbosulfan, and Clariasgariepinus exposed to fenthion respectively. [28]observed an increased GPX activity in the liver and skin of exposed adult green toad.

GR is an antioxidant enzyme that recycle oxidized glutathione GSSG to reduced formGSH which is non enzymatic antioxidant critical in controlling the lethal effects of induced free radicals by foreign chemicals in cells[27,29].The present study indicated concentration and time dependent significant increase in the GR.Increase in the activity of GR indicates indirect increase in GSH levels suggesting elevated response by cells against endosulfan – induced free radical imbalance in the Catfish [30].

There was a significant increase ($p<0.5$) in the GST values in the liver tissues of catfish exposed to the endosulfan at different concentrations and time. GST enzymes are involved in destroying the toxic effect of the foreign chemicals introduced to the cells from the environment, solubilizing the chemicals in water and subsequently detoxifying it thereby protecting the cells. The elevated level of GST activities observed in this study is similar to the reports of [31,27] on freshwater cyprinid crucian and African Catfish exposed to endosulfan

and fenthion respectively. Exposure of Rana esculenta to methoxychlor [32]and green toad to deltamethrin [28]also increased the GST activities.

CAT activity also increased significantly in a concentration and time dependent manner. The enzyme catalyses the breakdown of hydrogen peroxide to water and oxygen within the cells. It helps the cell to develop tolerance against the stress induced in the presence of free radicals in the cells [33]. The increase observed in the present study is contrary[34,35,36] but agree with[28] who observed increase CAT activities in the liver and skin of exposed adult green toad.

In this work a significant increase in the SOD activities was observed. SOD converts highly reactive superoxide radicals to the less reactive hydrogen peroxide and oxygen in nearly all cells[30]. This indicates that exposure to endosulfan caused increased production of superoxide ions in the catfish cells leading to increased SOD activities dependent on concentration and time. This result agrees with the report of [37,28]. They worked with Chinese Bufo bufo gargarizans tadpole exposed to spirotetramat in 4 days and green toad Bufotes viridis exposed to deltamethrin, respectively.

V. conclusion

In this work exposure of African Catfish to different concentrations of endosulfan with time increased the LPO and the activities of the enzymatic antioxidants which includes GPX, GR, GST, CAT and SOD in the treatment groups compared with the control. This result suggests that endosulfan can induce production of highly reactive species that may subsequently lead to oxidative stress when there is an imbalance in the oxidants and antioxidants ratio.This means that the use of endosulfan require regulation to prevent lethality to lives within and near the environment.

Acknowledgement

The authors wish to thank the Tertiary Education Trust Fund (TETFund) Nigeria, and the management of Enugu State University of Science and Technology (ESUT) for the grant received and the office space provided for the research.

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