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Evaluation of sublethal phyto-toxic effects of herbicides using biochemical indices

D.F. Ogeleka[1,*], F.E. Okieimen[2], B.O. Peretiemo-Clarke[3]

[1*]Department of Chemistry, Federal University of Petroleum Resources, Effurun, Nigeria
[2]University of Benin, Geo-Environmental and Climate Change Adaptation Research Centre, Benin City, Nigeria
[3]Department of Chemistry, Delta State University, Abraka, Nigeria
***Corresponding author**: Email: dorysafam@yahoo.com

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Abstract**—** *The study assessed the biochemical alterations of three commonly used herbicides (Starforce®, Dragon® and Force Uron®) exposed to anon-target environmental receptor -Allium cepa L at sub-lethal levels. The oxidant activity - measured as malondialdehyde and the anti-defensive mechanism (superoxide dismutase and catalase were used to evaluate the deleterious effects of the test herbicides. At higher percentage of the test herbicides, there was elevated levels of MDA in the exposed Allium cepa Linn with respect to the control (P<0.05). This was followed bya decrease in the antioxidant activities (SOD and CAT) as concentrations increased (P<0.05).The study concluded that the test herbicides generated reactive oxygen species (ROS) resulting in elevated levels of lipid peroxidation, which may possibly havealtered the activities of SOD and CAT, thereby leading to oxidative stress in Allium cepa Linn at levels below and at the presumed safe limit (10% of EC_{50}) and this should be a concern to human who are the end users of this edible non-target plant.*

Keywords**— Allium cepa Linn, herbicides, oxidative stress, reactive oxygen species (ROS).**

I. INTRODUCTION

In the last few decades, more than eight(8) billion kilograms of different herbicides have been applied yearly to control weeds and increase crop yield. However, exposure to herbicide scan lead to perturbations in non-target plants, which may generate reactive oxygen species (ROS) resulting in oxidative stress manifested as alteration in lipid peroxidation (LPO)(Caverzan *et al*., 2014).Reactive oxygen species (ROS) are produced in both unstressed and stressed cells, however when there is an imbalance between oxidative and anti-oxidative mechanism in the specie, oxidative stress sets in. Plants have well developed defense systems against ROS, involving both limiting the formation of ROS as well as instituting its removal (Alscher *et al*., 2002). The defense systems (anti-oxidants) include enzymes such assuperoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which help in protecting cells from the damaging effects of ROS.

Overproduction and accumulation of ROS can be a threat to cells leading to peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition and ultimately leading to death of cells (Sharma *et al*., 2012; Gurvinder, 2019).Oxidative stress and alteration in oxidant/antioxidant status in living organisms have been considered as an important tool in assessing pesticide toxicity especially at sub-lethal levels (Mansour *et al*., 2017). Most of the perturbations caused by herbicide treatment in plants are related to ROS generation. The use of biomarkers for studying contaminant exposures and effects on living organisms has been identified as a useful environmental tool. Biomarkers are used in toxicity bioassay to give a supplementary and more accurate approach and picture, than the chemical analysis because of the need to integrate all

environmental variables and stressors. These are used to indicate the health status of organisms and to obtain early warning signs and responses (Ogeleka and Okieimen, 2019).

Starforce® is afluazifop-b-butyl herbicide(Butyl 2-[4-[5-(trifluoromethyl) pyridin–2-y] oxyphenoxylpropanoate) used to control perennial and annual grass weeds (Cieslik *et al*.,2017). It acts as an inhibitor of acetyl CoA carboxylase (Accase) that catalyzes the formation of matonyl-CoA during metabolism of lipids and/or of some secondary compounds. Dragon® is a paraquat dichloride herbicide(1,1-dimethyl-4,4-bipyridinium dichloride)used to control broad-leaved weeds and grasses. It is a quick acting, non-selective compound that destroys green plant tissue on contact and by translocation within the plant (Janaki *et al*., 2017). Similarly, Force Uron®, a diuron herbicide (3-(3,4-dicholophenyl)-1,1-dimethylurea) is a potent inhibitor of photosynthesis (Kumar *et al*., 2010).Fluazifop-P-butyl, paraquat dichloride and diuron herbicides have not only been associated with high degree of toxicity but also have been known to induce oxidative stress on living organisms. Some researchers have linked induction of ROS with fluazifop-p-butyl (Fayez *et al*., 2014; Liu *et al*., 2017).Several studies have also shown that Paraquat, a commonly used herbicide, induces oxidative stress via ROS generation (Somayajulu-Nitu *et al*., 2009; Moustaka *et al*., 2015; Orti *et al*., 2016). Diuron herbicide used in champagne vineyard was found to generate reactive oxygen species which induced a shift of the balance between pro-oxidative and anti-oxidative reactions leading to oxidative stress (Geoffroy *et al*., 2002).

In this study, the phyto-toxic effects of three commonly used herbicidesin Nigeria (Starforce®, Dragon® and Force Uron®) were evaluated on *Allium cepa Linn*at sublethal levels using an oxidant index (lipid peroxidation) and two anti-oxidants parameters(superoxide dismutase and catalase) as biomarkers.

II. MATERIALS AND METHODS

2.1 Test chemicals

Sublethal concentrations of the test herbicides (Starforce®, Dragon® and Force Uron®) was used in this assessment after a lethal evaluation (Okieimen *et al*., 2020). Sublethal concentrations of these three herbicides at2%, 5% and 10% of their respective EC_{50} values was used for this study (Table 1).

Table 1: EC_{50} and the concentrations used for this study

Chemical	Herbicide formulation (active ingredient)	Effective concentrations(EC_{50}) mg/L	2%	5%	10%
Starforce®	Fluazifop-p-butyl herbicide,	0.237	0.00474	0.01185	0.0237
Dragon®	Paraquat dichloride	0.042	0.00084	0.0021	0.0042
Force Uron®	Diuron	0.169	0.00338	0.00845	0.0169

2.2 Test species

Allium cepa has been commonly used in the laboratory to determine the toxicity of xenobiotics and environmental risks. The *Allium cepa* test is a simple, sensitive and rapid bio-tool that shows high sensitivity to toxic chemicals /substances. Roots have been found to the most vulnerable and reliable system to study the mechanism of herbicide interaction as a primary receptor (Igbal *et al*., 2019; Srivastava and Singh, 2020).

2.3 Bioassay for biochemical indices in *Allium cepa L*

Onion bulbs (*Allium cepa L*) of the purple variety, with an average weight and length of 73.00 ± 0.24g and 6.30 ± 0.07 respectively were obtained from vendors inWarri, Delta state, Nigeria. The bioassay was conducted using the Organization for Economic Co-operation and Development (OECD) protocol #208 for root growth inhibition (OECD, 2003) before the biochemical evaluation. At the end of 48 hours when the mitotic activity was presumed optimal, the root tips were excised from each bulb and used for the analysis. Homogenates of the onions were prepared by homogenizing 0.5 g of the onion root tips in ice-cold phosphate buffer at pH 7.2. The homogenates were centrifuged at 4000 rpm for 10 minutes and the supernatant used for the biochemical analysis.

2.4 Lipid peroxidation assay

Peroxidation was estimated using the method of Hodges *et al*., (1999) based on malondialdehyde assay. MDA, a product of lipid peroxidation and a biomarker for oxidative stress, when heated with 2-thiobarbituric under acidic conditions forms a pink coloured product which has a maximum absorbance at 532nm. Malondialdehyde content was expressed as mmol/MDA wet root.

2.5 Antioxidant enzymes (superoxide dismutase and catalase)

The activity of SOD in *Allium cepa Linn* was estimated spectrophotometrically using the method of Misra and Fridovich (1972). The assay of SOD is an indirect method based on the inhibitory effect of SOD in the initial rate of epinephrine (adrenaline) auto-oxidation. SOD was expressed in unit/mg onion root.The activity of CAT was determined by the method of Hasanuzzaman *et al*., (2011). It was based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2) after the addition of the material containing the enzyme and was expressed asunit/mg onion root.

2.6 Statistical Analysis

Values of the enzymological results were expressed as mean ± standard deviation. These were statistically analyzed for significant differences between treated and control groups using Student's t test in analysis of variance (ANOVA), where P values ≤ 0.05 were considered statistically significant.

III. RESULTS

The results for the biochemical indicators (MDA, SOD and CAT) are presented in Table 2 and Figures 1-3. The results in Table 2 indicated that there was increase in the values for MDA (lipid peroxidation) while a decrease was observed in SOD and CAT activities as the concentrations of the test herbicides increases. However, there was significant difference between the various activities in some of the treatment groups and the control.

Table 2: Mean values ±standard deviation of the enzyme analysis for Allium cepa L

Test chemical	% of EC_{50}	Concentration, mg/L	MDA (mmol/MDA)	SOD (unit/mgprotein)	CAT (unit/mgprotein)
		Control	0.89 ± 0.24^a	41.05 ± 5.00^a	23.38 ± 1.99^a
Starforce®	2	0.00474	0.95 ± 0.15^a	39.88 ± 3.91^a	15.46 ± 0.60^b
	5	0.01185	1.44 ± 0.07^b	28.32 ± 1.30^a	14.04 ± 0.45^b
	10	0.0237	1.88 ± 0.26^b	16.88 ± 0.01^b	12.45 ± 0.37^b
Dragon®	2	0.00084	1.26 ± 0.14^a	32.34 ± 0.10^a	15.78 ± 0.76^b
	5	0.0021	1.63 ± 0.18^b	16.89 ± 0.01^b	14.78 ± 0.15^b
	10	0.0042	2.38 ± 0.22^b	16.85 ± 0.03^b	14.02 ± 0.60^b
Force Uron®	2	0.00338	1.25 ± 0.11^a	38.65 ± 1.88^a	15.12 ± 0.09^b
	5	0.00845	1.48 ± 0.13^b	35.23 ± 1.23^a	14.58 ± 0.31^b
	10	0.0169	1.82 ± 0.14^b	29.44 ± 4.22^a	12.87 ± 1.34^b

Values are means± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (P<0.05)

3.1 Malondialdehyde activities

The values of malondialdehyde increased with respect to the control for all the test chemicals with increasing concentrations (Figure 1). Although MDA values increased in all concentrations, they were not statistically significant only in 2% of the EC_{50}. The effects induced by the test herbicide on lipid peroxidation expressed as MDA was higher at 10% of the $EC_{50}$and decreased down as the concentration reduces from 5% to 2%, which implied that as you progress further from the safe limit of 10% of the EC_{50}, the effect of the test herbicides with respect to oxidative stress reduces.

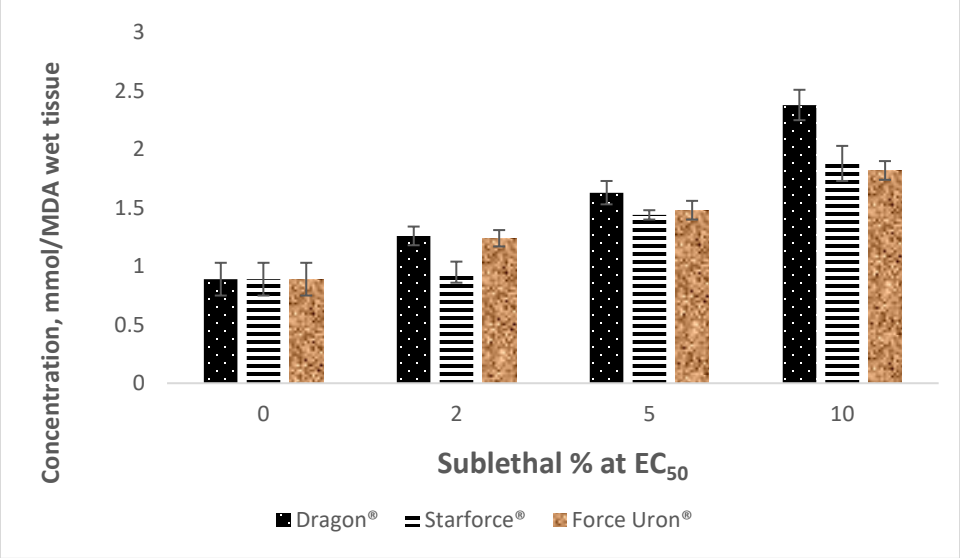

Fig.1: Malondialdehyde concentrations(mean ± SE) inAllium cepa L exposed to sublethal levels of test herbicides

3.2 Superoxide dismutase activities

Superoxide dismutase activities decreased with respect to control for the test herbicide, however, the decrease in SOD activities for some concentrations were not significant. The effect SOD induced by the herbicide was more at the lowest concentration of 2% of the EC_{50} and decreased down through 5% to 10%, which implied that as you move up away from the safe limit, the more reduced the levels of SOD and the weaker the anti-oxidant defense mechanism (Figure 2).

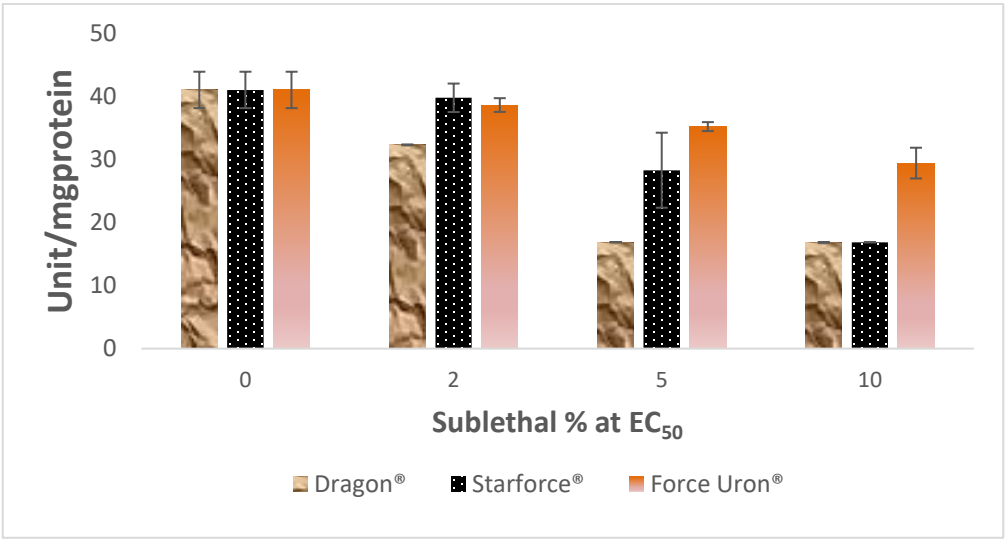

Fig.2: Superoxide dismutase concentrations (mean ± SE) in Allium cepa L exposed to sublethal levels of test herbicides

3.3 Catalase activities

Catalase activities decreased appreciably in the test herbicides for all concentrations with respect to the control.Thus, it can be seen that the effect on CAT induced by the test herbicides was more prominent at the lowest concentration of 2% of the $EC_{50}$and decreased down to 5% and finally 10%, which implied that as you more up away from the safe limit, the more reduced the levels of CAT and more vulnerable (exposed) the specie to oxidative stress from toxicants like the test herbicides (Figure 3).

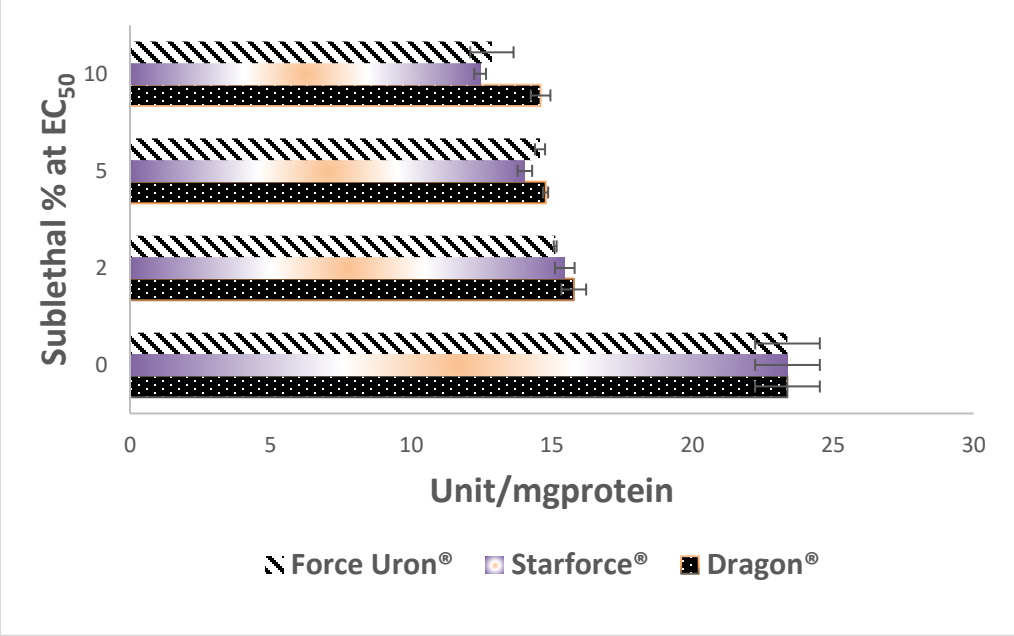

Fig.3: Catalase concentrations (mean ± SE) in Allium cepa L exposed to sublethal levels of test herbicides

IV. DISCUSSION

Plants activate antioxidant defense mechanisms under stresses, which helps in the maintenance of the structural integrity of the cell components and presumably alleviates oxidative damage. Several antioxidant enzymes contribute to plant defense such as superoxide dismutases, catalase, glutathione peroxidase and non-enzymatic antioxidants. (ascorbic acid, glutathione and phenolics) (Caverzan *et al*., 2016).

Recently, lipid peroxidation (LPO)has been gaining attention as a potential toxicological hazard and several pesticides including herbicides have been shown to stimulate peroxidation of cellular membranes. In this assessment, there was significant increase in the level of MDA of *Allium cepa Linn* roots exposed to the test herbicides with respect to the controland this increment was concentration dependent. Elevated level of lipid peroxidation may be an indication that the herbicides at higher concentrations generated ROS which could possibly led to oxidative stress in the exposed *Allium cepa Linn* (Nohatto *et al*., 2016). These observations are in accordance with reports by several researchers (Çavuşoğlu *et al*., 2011; Nohatto *et al*., 2016; Singh and Roy, 2017; Radwan *et al*., 2019; Srivastava and Singh, 2020).

The enzyme superoxide dismutase plays a significant role in defense against reactive oxygen species (ROS)in living organisms bycatalyzing the dismutation of most reactive and dangerous free radicals, superoxide radicals (O^{2-}) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) that would be less reactive. In this assessment, the activity of SOD decreased with respect to the control, which could possibly be as a result of the failure of SOD to scavenge the rapidly generated ROS. Liu *et al*., (2012), exposed maize and rice roots to chloracetanilide herbicide and found that the activities of superoxide dismutase, peroxidase and catalase were lower after the exposure while a lower activity of SOD was also corroborated byStajner *et al*., (2003) and Kumar *et al*., (2010).

Catalase activity is also an important indicator to evaluate herbicide-induced oxidative stress. Catalase catalyzes the decomposition of two hydrogen peroxide (H_2O_2) molecules to water (H_2O) and oxygen (O_2).In this appraisal, catalase activities were reduced as a result of exposure of *Allium cepa Linn* roots to the test herbicides.The reduction of CAT activity might be as aresult of inhibition of the enzyme synthesis or probably the herbicides caused stress conditions which changed the assembly of the enzyme subunits (Abedi and Pakniyat, 2010). Alterations in enzymatic antioxidants such as reduction in catalase activity have been documented (Stajner *et al*., 2003; Fakhari *et al*., 2020).

In addition, the increased in the levels of MDA which was used to assess the LPO may possibly be associated with decreased activity of scavenging enzymes such as catalase and superoxide dismutase. A decrease in the activities of these enzymes can lead to excessive availability of superoxides and peroxy radicals resulting in the initiation and propagation of LPO. Hence as you move up away from the safe limit of 10% of the EC_{50} values, the more reduced the levels of the anti-oxidant defense mechanisms (SOD and CAT) and thus enhanced probability of oxidative stress, which some species may likely not be able to overcome and may possible result to sub-cellular alterations that could have long term deleterious effects.

V. CONCLUSION

This study revealed that the test herbicides activated the enzymatic defense systems due to imbalance between production of ROS and scavengy tendency of the antioxidants leading to perturbation in *Allium cepa Linn* mechanism. This action therefore altered the status of lipid peroxidation (increase) and the enzymatic antioxidants – SOD and CAT (decrease). Herbicides exposure therefore can cause toxic effects such as alteration in the oxidants and antioxidants concentrations in *Allium cepa Linn* even at levels beyond the presumed safe limit (10% of EC_{50}). This should be of considerable public health and safety concerns to human who are the consumers of this edible non-target plant.

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