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Comparative Effects of Salicylic and Giberellic Acid on Lipid Peroxidation and Antioxidant Potentials of Three Cultivars of *Vigna unguiculata* (L.) Walp under Heavy Metal Toxicity

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Cowpea is a commonest and affordable legume widely consumed in developing nation of the world. Salicylic (SA) and Gibberellic (GA) acids serve to play prominent roles in induction of growth and development in cowpea. However, heavy metal such as arsenic is one of the major pollutant commonly encountered and its exposure to plant often result in alteration in physiological and biochemical functions. Therefore the aim of this work was to examine the effects of Salicylic and Gibberellic acid on lipid peroxidation and antioxidant potentials of three cultivars of *Vigna unguiculata* under heavy metal toxicity. Seeds of three cowpea cultivars (Ife Brown, ITOK-768-18and ART 98-12) were sorted and each of the three cultivars were soaked in SA [0 control, (75, 150) mg/L} and GA [0 control, (75, 150) mg/L} respectively. The seeds (n=5) were planted in a completely randomised design in pots of soil containing each of 0, 250 and 500mg/L sodium arsenate. Vitamin C, otal flavonoid, phenolic, lipid peroxidation, superoxide dismutase, peroxidase, catalase activities in leaves were determined on day 90 using spectrophotometry The results show that in comparing treatment with the control among the three cultivars, superoxide dismutase, peroxidase and catalase, vitamin C, total flavonoid and phenolic were increased by (3, 4, 2, 12, 4 and 3 folds respectively), in Ife brown; (2, 5, 12, 12, 5 and 3 folds respectively), in ART 98-12; (4, 3, 14, 5 and 4 folds respectively), in ITOK-768-18 in the leaves of 250mg/L sodium arsenate-treated soil with SA (150mg/L). Lipid peroxidation were reduced by (5 and 3 folds respectively), in Ife brown and ITOK-768-18 in 250mg/L sodium arsenate-treated soil with SA (150mg/L) but was reduced by 3 folds in 250mg/L sodium arsenate-treated soil with SA (75mg/L) for ART98-12. Salicylic and gibberellic acids therefore reduced lipid peroxidation, enhanced antioxidant potentials of *Vigna unguiculata* to increase tolerance and promote growth during heavy metal toxicity.

Key words: SA, GA, heavy metal. arsenic, cowpea

*The cowpea is an annua*l herbaceous legume which utilise sandy soil and low rainfall for its growth, therefore in the dry regions all over Africa and other countries it serves as vital crop. (Maredia *et al*., ,2000). Higher amount of proteins, energy, minerals and vitamins are obtained from the seed. Countries that cultivate cowpea as a main food crop uses cowpea as supplements to their cereal diet. It is referred to as meat for the poor beacause of greater levels of protein in the seed and leaves (Odenda *et al.,* 2011).

Arsenic is an ordinary element which acts like a metal. It exist in our environment, occurring in various different forms such as inorganic or organic, with highest toxicity found in inorganic form. Arsenic exist in all the natural environment and great amount in the atmosphere. Natural sources, such as volcanoes form three quarter of arsenic in the atmosphere while the remaining are from synthetic sources. Manufacturing practices such as mining, smelting and coal-fired power plants are responsible for the arsenic presence in air, water and soils all of which account for the environmental contamination. Arsenic can occur in four oxidation states namely: +3 (arsine), 0 (arsenic metal), -3 (arsenite), and +5 (arsenate). Arsenite {As(III)} prevailed under reducing condition, while arsenate {As(V)} is more stable in oxidizing condition. Solubility in water does not take place in elemental arsenic (Jana and Choudhari, 1982). Based on the incidence of occurrence, toxicity, and the likelihood of contact with humans, arsenic has been regarded as the most harmful substance (ATSDR, 2003). Arsenic-induced reactive oxygen species (ROS) causes oxidative stress and inactivation of antioxidant enzymes necessary to protect crops against injury mediated by reactive oxygen species. The effects includes: rise in lipid peroxidation, wilting, curling, necrosis of leaf blade, reduced growth and productivity and so on (Chandrakar *et al*., 2016).

Due to the fact that cowpea harbor pest both at pre and harvest stage of its production application of insecticide and pesticide is paramount. Also cowpea is being cultivated in Northern part of Nigeria where irrigation serve as substitute to rainfall, part of irrigated water may be contaminated with heavy metals predominantly arsenic based on the frequency of its occurrence.Amelioration of heavy metal toxicity to increase crop productivity has therefore been of major concern. The modulation of biological and cellular molecules by plant bioregulators (Salicylic and Gibberellic acids) for defense against ROS-induced damage may therefore serve as approach to mitigate arsenic toxicity. Salicylic and Gibberellic acids are plant bioregulators that normally partake in the control of biochemical processes, demonstrating defense against arsenic-mediated injuries in plants because ROS produced by arsenic toxicity cause hormonal imbalance in plants. Arpita (2014) found out that there is reduction in growth inhibition through pre-treatment of the seedling of mungbean with phytohormones (GA_3, IAA, Kinetin and macronutrients (KH_2PO4, $NaH_2PO_4.2H_2O$, $K_2SO_4.CaCl_2.2H_2O$, $MgSO_4.7H_2O$) under sodium arsenate toxicity. Ailenokhuoria and Omolekan (2019) has also reported on evaluation of heavy metals component of our locally consumed cowpea in major markets in Ibadan and arsenic content were found to be above tolerable standard stipulated by FAO. Hence the present study on. Comparative effects of salicylic and gibberellic acid on Lipid peroxidation and antioxidant potentials of three cultivars of *Vigna unguiculata* (L.) Walp under heavy metal toxicity.

MATERIALS AND METHODS

The present work was done in a screen at Institute of Agricultural Research and Training (I.A.R&T) Ibadan Oyo State, Nigeria. Five kilogrammes of pot were filled with soil and were arranged in triplicate in a complete randomised design with 15 treatments for each of the three cultivars of cowpea.

Preparation of 250 mg/L sodium arsenate

Sodium arsenate (1.45g) salt was dissolve in a small volume of distilled water and then transferred to a 1 liter volumetric flask and completed to the mark with distilled water to make 250mg/L

Preparation of 500 mg/L sodium arsenate

Sodium arsenate (2.90g) salt was dissolve in a small volume of distilled water and then transferred to a 1 liter volumetric flask and completed to the mark with distilled water to make 500mg/L

Application of sodium arsenate to the soil

Five hundred millilitres of aqueous sodium arsenate prepared above was applied to each 5 kg of soil in the pot. It was left for 14 days for proper equilibration of soil and sodium arsenate.

Plant materials

Three cultivars of cowpea seeds were obtained from Institute of Agricultural Research Training [(Ife Brown (Red coat) and ART 98-12 (white coat)] and International Institute of Agricultural Research & Training [ITO7K-568-18 (Red coat)].

Preparation of bioregulators

Bioregulators (Salicylic and Gibberellic acids) were prepared by using procedure of (Coolbear and Heydecker 1997).

Planting

Three different cultivars of cowpea (Ife brown, ART98-12 and ITO7K-568-18) were soaked in a plastic film with two concentration bioregulators (SA and GA) 75 and 150 mg/L respectively. Distilled water was used in place of bioregulators for other part of the seeds which represent the control. All were kept for 6hrs without light at room temperature. Thereafter, the solutions were decanted off, and seeds were rinsed twice with distilled water and air-dried for 1 hour. The seeds were sow under heavy metal stress, and allowed to germinate until maturity. The plants were harvested at maturity and were divided into leaf and seed for various physicochemical analysis: elemental composition, photosynthetic pigment, antioxidant enzyme activities, compatible solutes, phytochemicals, proximate analysis and growth parameters.

Determination of antioxidant enzymes

Sample preparation for enzyme assay

One gram of leaf were crushed in 10 ml solution containing 0.10M Potassium phosphate buffer, pH 7.5 and containing 0.50mM Ethylene diamine tetra acetic acid. The mixture formed were later centrifuged for 20 minutes at 1500 rpm and the filtrate were collected for enzymes assays.

Determination of catalase.

Procedure. Catalase activities was analysed based on Raghu et al. (2014) technique. The result was expressed as unit/mgprotein.

Principle. The principle of the test above was on the disappearance in absorbance detected at 240 nm as catalase seperate hydrogen peroxide.

Calculation

$$\text{Catalase activity} = \frac{A_{240}/\text{min} \times \text{ml of reaction} \times \text{dilution}}{0.0435 \times \text{ml of sample} \times \text{mg protein/mL}}$$

$$= \mu mH_2O_2/\text{min/mg protein}$$

Determination of superoxide dismutase.

Procedure. The SOD activity was determined by according to procedure of Shalini and Dubey (2003).

The result was expressed in unit/mg protein

Principle. Superoxide dismutase capability to obstruct the autoxidation of adrenaline at pH 0.99 by superoxide ability is the basis for this reaction. (Dhindsa, 1981).

Calculation

$$\text{Rise in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

Where A_0 = absorbance after 0 seconds

A_3 = absorbance after 150 seconds

$$\% \text{ Inhibition} = \frac{\text{Rise in absorbance of substrate} \times 100}{\text{Rise in absorbance of blank}}$$

A unit of SOD activity is the amount of SOD necessary to produce 50% inhibition of the oxidation of adrenaline.

Determination of peroxidase

Procedure. Peroxidase activity was determine according to the method of (Raghu *et al.,* 2014).

Principle. The method is by using pyrogallol as hydrogen donor. The rate of reaction is estimated by measurement of an increase in absorbance at 420 nm due to decomposition of hydrogen peroxide. One unit results to the decomposition of 1µM of H_2O_2 under the specified conditions (Raghu *et al.,* 2014). The result was expressed in abs/min

Lipid peroxidation

Malondialdehyde (MDA) contents were estimated based on Dhinsa *et al.* (1981) technique with slight modification. The result was expressed in nmol/g/fw

Determination of phytochemicals

Phytochemicals (total flavonoid and phenolic) are regarded as secondary defencen on enzymic antioxidants and a great removal of singlet oxygen. Vitamin C plays role in eliminating Hydrogen peroxide produced from reactive oxygen species generated from arsenic toxicity. Salicylic acid act by increasing formation of specific phytochelatin to chelate with metalloids and then reduce arsenic toxicity.

Procedure

Determination of total phenolic

Total phenolic contents were estimated by use of folin- ciocalteau method (Singleton *et al.,* 1999). The results were expressed as mg/GAE/g/fresh weight.

Determination of total flavonoid

The total flavonoid contents were determined by colorimetric method of Zhishen *et al.* (1999). Flavonoid content were expressed as mg/QUE/g/fresh weight.

Determination of Vitamin C

Vitamin C was determine according to the method of Sulladmath *et al*. (2012). The result was expressed as mg/g/fresh weight.

Statistical analysis

The data were analysed using Statistical analytical for science (SAS). Anova was used for separation of means. Duncan multiple range test was used to determine the level of 5% significant among the groups of treatment.

RESULTS AND DISCUSSION

The result in Figures 1-9 shows that SOD, POD and CAT activities of cowpea exposed to 250 and 500 mg/L arsenate significantly reduce with respect to the control plant respectively in all three cultivars, this was as a result of the fact that the rate of generation of oxidant surpasses the rate of production of antioxidants caused by higher level of arsenic. However, SA, GA (150, 75 mg/L) significantly increase the level of SOD, POD and CAT in all the three cultivars respectively. The enzymatic components associated with defense against reactive oxygen species generated by arsenic toxicity include SOD, catalase, peroxidase and ascorbate/ glutathione. Superoxide dismutation dissuades two superoxide radicals ($O_2^{\cdot -}$) in water and O_2. and it is an important constituent of the antioxidant protective system in plants. Catalase is one of the key enzymes involved in the elimination of toxic peroxides and is universally present as oxidoreductase that decomposes hydrogen peroxide in water and molecular oxygen. (Lin and Kan, 2000). Application of SA and GA was able to upregulate the activities of these enzymic antioxidants therefore able to mitigate against arsenic toxicity and protect plant from injury. Arpital (2014) had reported that pre-treatment of mungbean seedling with GA_3, KH_2PO_4 and $NaH_2PO_4.2H_2O$ which helped in reduction of oxidative stress to some extent as observed by increasing seedling growth and lowered antioxidant enzyme actions and lessening proline accumulation. Chandra *et al.* (2011) had also reported the alleviating effects of Indole acetic acid on effect of toxic heavy metal (Pb, Cr) on wheat plant due to stimulatory effect of antioxidant enzyme like Superoxide dismutase, Catalase and Glutathione reductase.

Moreover, the result in Figures 10-12 shows that Lipid peroxidation of cowpea exposed to 250 and 500 mg/L arsenate significantly increase with respect to the water treated control plant respectively in all three cultivars which is due to the oxidation of polyunsaturated fatty acid of the membrane which then results in Lipid-peroxidised products hence loss of function of membrane. However, SA, GA (150, 75 mg/L) significantly reduce the level of lipid peroxidation in all the three cultivars.

In addition, the result in Tables 1-3 shows that vitamin C, total flavonoid and phenolic of cowpea exposed to 250 and 500 mg/L arsenate significantly reduce with respect to control plant respectively in all three cultivars whereas SA, GA (150, 75 mg/L) significantly increase their level in all the three cultivars respectively. Vitamin C, total flavonoid and phenolic are non-enzymic antioxidant that act in addition to enzymic antioxidant to scavenge reactive oxygen species generated from arsenic toxicity. Vitamin C is a significant redox buffer that also serves as a cofactor for enzymes involved in photosynthesis, hormone production, and the regeneration of other antioxidants.. It is a critical substrate for the detoxification of reactive oxygen

species. It also serve as hydrogen peroxide eliminator while total flavonoid and phenolic act as phytochelatin to chelate the metal and renders them to non- toxic forms. Amit *et al*. (2015) also reported that pretreatment of rice with salicylic acid also enhanced the level of Ascobate, GSH/GSSG and total non-protein thiol pon arsenate exposure in all treatments than corresponding arsenate exposed plants.

Table 1: Effects of SA and GA on Vitamin C, Total flavonoid and phenolic contents of cowpea(Ife brown) exposed to sodium arsenate

Arsenic (mg/L)	Bioregulators (mg/L)		Vitamin C (mg/g/fw)	Flavonoid (mg QE/g/fw)	Phenolic (mg GAE/g/fw)
0		0	24.00 ± 0.57^{i}	74.00±0.58^{d}	92.33 ±1.45^{d}
250		0	6.70 ± 0.15^{g}	9.17± 0.44^{m}	19.30 ± 0.15^{m}
500		0	2.56 ± 0.23^{g}	5.47±0.15^{n}	14.46 ± 0.15^{n}
0	GA_3 150		31.66 ± 1.01^{e}	94.00±0.58^{a}	104.00± 0.58^{b}
250	$GA_3$150		72.50 ± 1.32^{b}	53.50±2.78^{f}	69.16 ± 0.44^{g}
500	$GA_3$150		42.17 ± 0.44^{d}	38.10±0.12^{h}	50.06 ± 0.62^{h}
0	$GA_3$75		28.17 ± 0.44^{ef}	78.17±0.44^{c}	78.16 ±0.44^{e}
250	$GA_3$75		63.50 ± 0.76^{c}	35.43±0.54^{i}	55.43±0.54^{i}
500	$GA_3$75		38.50± 2.56^{d}	25.60±0.31^{k}	35.60±0.31^{l}
0	SA 150		41.50± 0.58^{d}	86.00±0.18^{b}	136.00 ±0.58^{a}
250	SA 150		83.67 ± 1.86^{b}	46.33±0.88^{g}	76.33±0.88^{f}
500	SA150		41.83± 4.47^{e}	30.27±0.37^{j}	60.26 ± 0.37^{h}
0	SA 75		33.00 ± 1.04^{e}	65.17±0.44^{e}	95.16±0.44^{c}
250	SA 75		68.17 ± 0.93^{b}	29.23 0.39^{j}	59.23 ± 0.39^{h}
500	SA 75		32.50± 1.443^{e}	22.13±0.19^{l}	48.13±0.185^{k}

Means with different letter are significantly different from each other

Table 2: Effects of SA and GA on Vitamin C , Total flavonoid and phenolic contents of cowpea (ART 98-12) exposed to sodium arsenate

Arsenic (mg/L)	Bioregulators (mg/L)		Vitamin C (mg/g/fw)	Flavonoid (mgQE/g/fw)	Phenolic (mgGAE/g/fw)
0		0	19.40 ± 0.21 ^{de}	55.43±0.54^{c}	75.60 ±0.59^{c}
250		0	10.93 ± 8.53 ^{ef}	6.37±0.32^{n}	15.87 ±0.12^{l}
500		0	3.67 ± 0.88^{f}	3.90±0.06^{o}	11.93 ± 0.08^{m}
0	GA_3 150		25.83 ± 2.92^{de}	70.30±0.65^{a}	90.30 ±0.65^{b}
250	$GA_3$150		48.83 ±9.44^{abc}	41.00±0.58^{f}	51.83 ± 0.44^{f}
500	$GA_3$150		26.00 ±10.26^{de}	27.23±0.39^{i}	37.23 ±0.39^{i}
0	$GA_3$75		25.00 ±2.52 ^{de}	52.00±0.58^{d}	62.00 ±0.57^{d}
250	$GA_3$75		50.33 ±3.82 ^{ab}	34.00±0.58^{h}	38.00 ±0.57^{i}
500	$GA_3$75		30.33 ± 2.85^{d}	16.10±0.21^{l}	29.10 ±0.21^{k}
0	SA 150		33.33 ± 4.42 ^{cd}	57.63±0.33^{b}	107.30 ±0.45^{a}
250	SA 150		53.17 ± 4.11 ^{a}	37.50±0.76^{g}	107.30±0.45^{a}
500	SA150		35.00 ± 2.84 ^{bcd}	22.00±0.58^{k}	48.50 ± 0.29^{f}
0	SA 75		30.83 ± 4.08 ^{d}	43.67±0.88^{e}	63.17±0.60^{d}
250	SA 75		52.50 ± 3.78 ^{a}	25.17±0.44^{j}	45.17±0.44^{h}
500	SA 75		31.17 ± 3.42^{d}	14.00±0.58^{m}	34.00±0.57^{j}

Means with different letter are significantly different from each other

Table 3: Effects of SA and GA on Vitamin C, Total flavonoid and phenolic contents of cowpea (ART 98-12) exposed to sodium arsenate

Arsenic (mg/L)	Bioregulators (mg/L)	Vitamin C (mg/g/FW)	Flavonoid (mgQE/g/fw)	Phenolic (mg GAE/g/fw)
0	0	23.27 ± 0.50^{bc}	74.00±0.58^{c}	72.33±1.45^{e}
250	0	12.13 ± 9.43^{cd}	9.17±0.44^{j}	19.30± 0.15^{k}
500	0	4.87 ± 0.94^{d}	5.47±0.15^{k}	14.47±0.15^{l}
0	$GA_3$150	26.30 ± 2.65^{bc}	95.00±0.58^{a}	104.00±0.58^{a}
250	$GA_3$150	51.67 ± 10.84^{a}	53.50±2.78^{e}	69.17 ±0.44^{f}
500	$GA_3$150	26.00 ± 10.27^{bc}	26.43±0.27^{i}	50.07± 0.62^{i}
0	$GA_3$75	26.63 ± 1.73^{bc}	78.17±0.44^{b}	88.17±0.45^{b}
250	$GA_3$75	60.17 ± 2.46^{a}	35.43±0.55^{g}	55.43±0.55^{h}
500	$GA_3$75	24.83 ± 3.09^{bc}	25.60±0.31^{i}	48.00±0.58^{j}
0	SA 150	33.00 ± 3.52^{b}	96.00±0.58^{a}	86.00±0.58^{c}
250	SA 150	67.00 ± 5.51^{a}	46.33±0.88^{f}	76.33±0.88^{d}
500	SA150	34.43 ± 5.74^{b}	30.27±0.37^{h}	60.27 ±0.37^{g}
0	SA 75	32.47 ± 3.30^{b}	65.17±0.44^{d}	85.17±0.44^{c}
250	SA 75	67.50 ± 3.75^{a}	29.23±0.39^{h}	59.23±0.39^{g}
500	SA 75	34.00 ± 4.04^{b}	52.13±0.19^{e}	48.13±0.19^{j}

Means with different letter are significantly different from each other

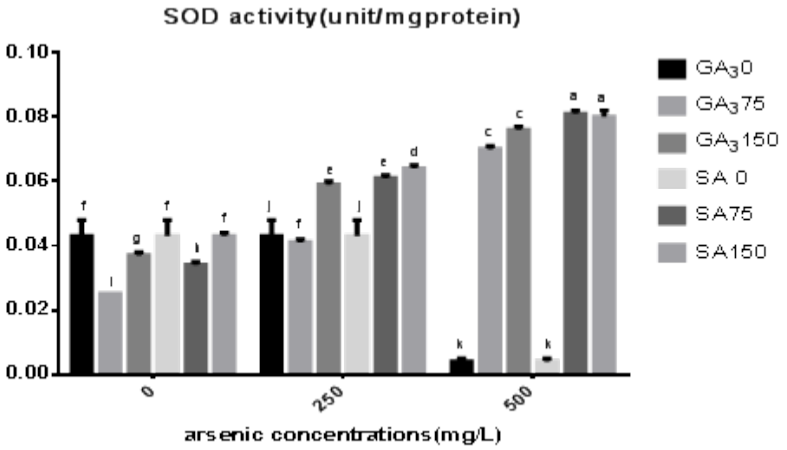

Figure 1: SOD of cowpea (Ife brown) exposed to arsenic toxicity

SOD: Superoxide Dismutase, GA_3 : Gibberellic acid, SA : Salicylic acid

Means with the same letter are significantly different from each other

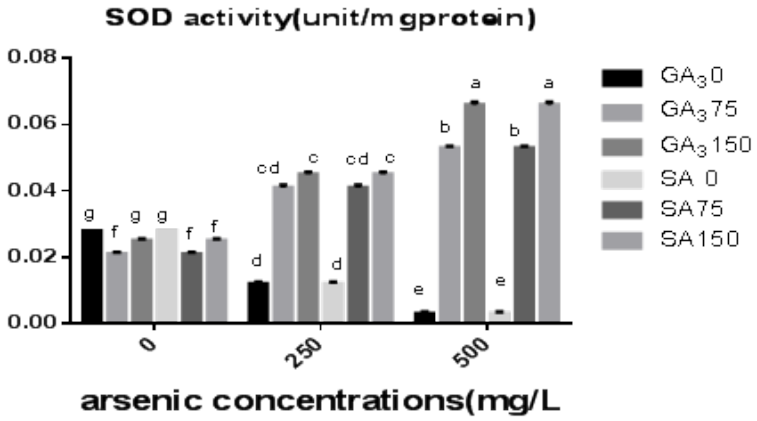

Figure 2: SOD of cowpea (ART 98-12) exposed to arsenic toxicity

SOD: Superoxide Dismutase, GA_3 : Gibberellic acid, SA : Salicylic acid

Means with the same letter are significantly different from each other

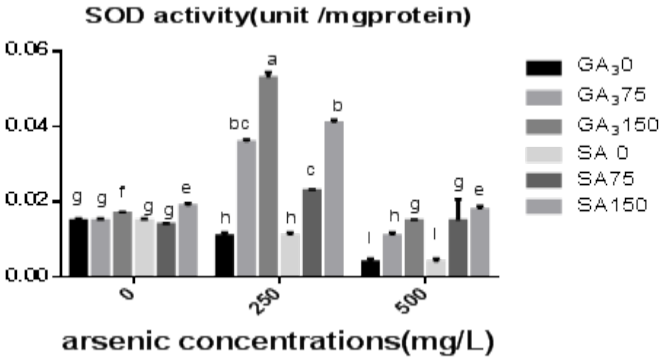

Figure 3: SOD of cowpea (ITOK-568-18) exposed to arsenic toxicity
SOD: Superoxide Dismutase, GA_3 : Gibberellic acid, SA : Salicylic acid
Means with the same letter are significantly different from each other

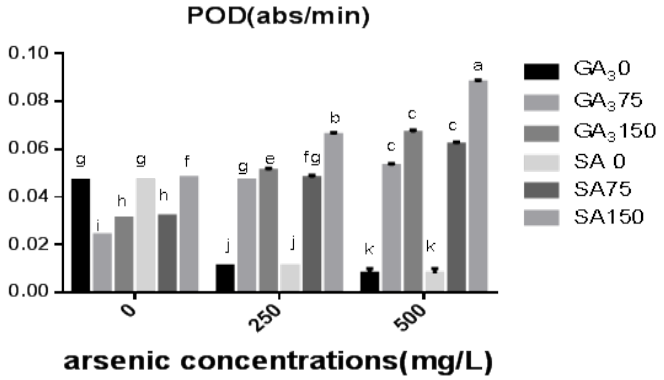

Figure 4: POD of cowpea (Ife brown) exposed to arsenic toxicity
POD: Peroxidase, GA_3 : Gibberellic acid, SA : Salicylic acid
Means with the same letter are significantly different from each other

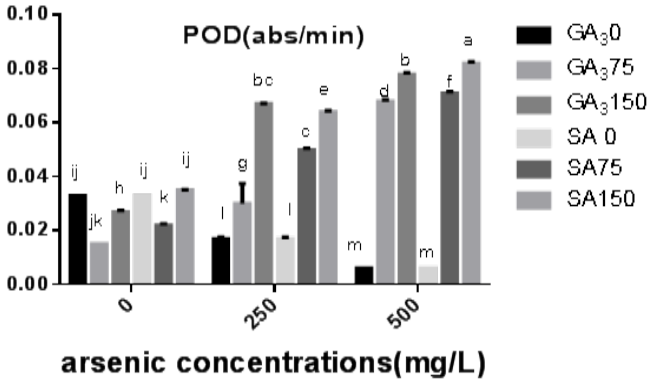

Figure 5: POD of cowpea (ART 98-12) exposed to arsenic toxicity
POD: Peroxidase, GA_3 : Gibberellic acid , SA : Salicylic acid
Means with the same letter are significantly different from each other

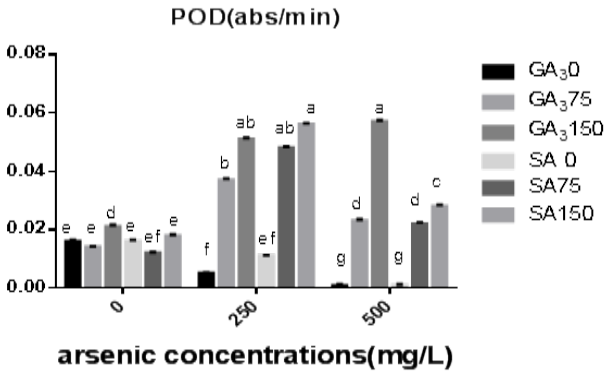

Figure 6: POD of cowpea (ITOK-568-18) exposed to arsenic toxicity
POD: Peroxidase, GA_3 : Gibberellic acid, SA : Salicylic acid
Means with the same letter are significantly different from each other

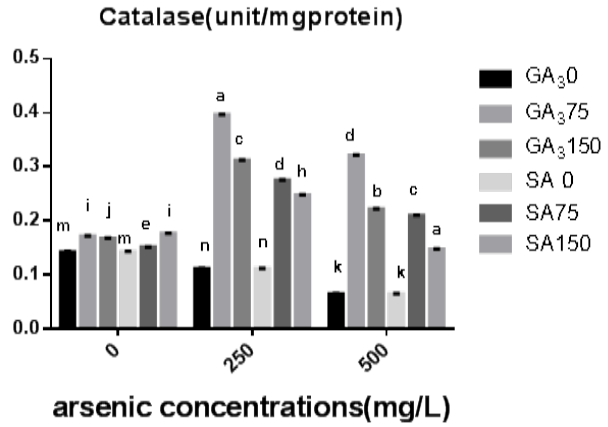

Figure 7: Catalase of cowpea (Ife brown) exposed to arsenic toxicity
GA_3 : Gibberellic acid, SA : Salicylic acid
Means with the same letter are significantly different from each other

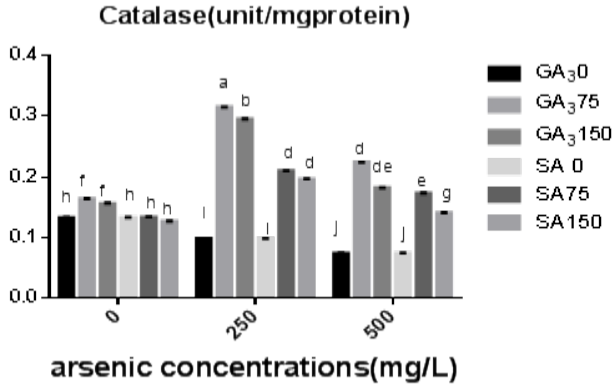

Figure 8: Catalase of cowpea (ART98-12) exposed to arsenic toxicity
SOD: Superoxide Dismutase, GA_3 : Gibberellic acid, SA : Salicylic acid
Means with the same letter are significantly different from each other

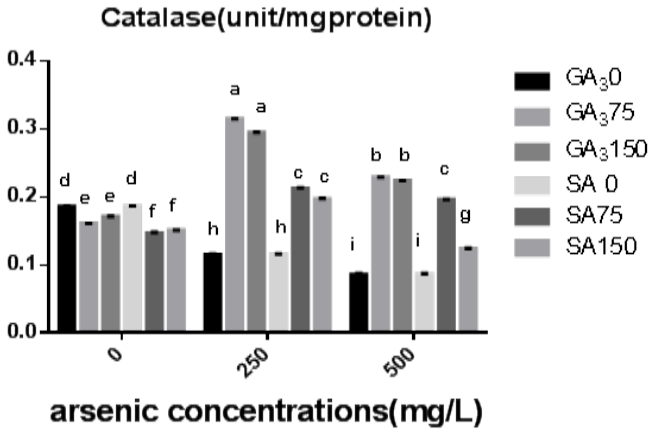

Figure 9: Catalase of cowpea (ITOK-568-18) exposed to arsenic toxicity
GA3 : Gibberellic acid , SA : Salicylic acid
Means with the same letter are significantly different from each other

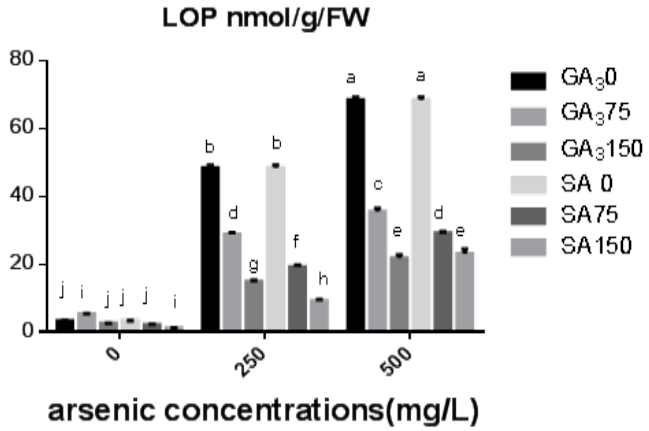

Figure 10: Lipid peroxidation of cowpea (Ife brown) exposed to arsenic toxicity
GA3 : Gibberellic acid , SA : Salicylic acid
Means with the same letter are significantly different from each other

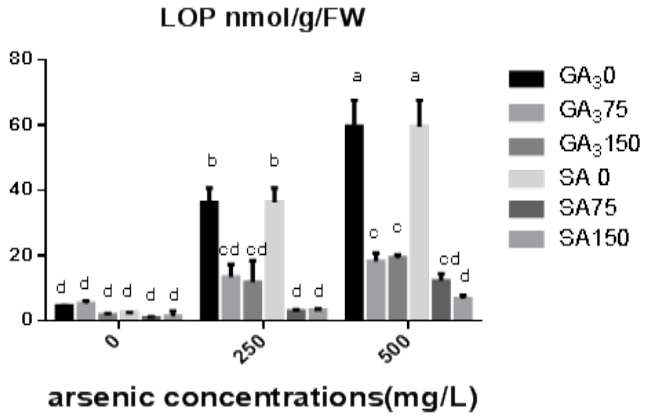

Figure 11: Lipid peroxidation of cowpea (ART 98-12) exposed to arsenic toxicity
GA3 : Gibberellic acid , SA : Salicylic acid
Means with the same letter are significantly different from each other

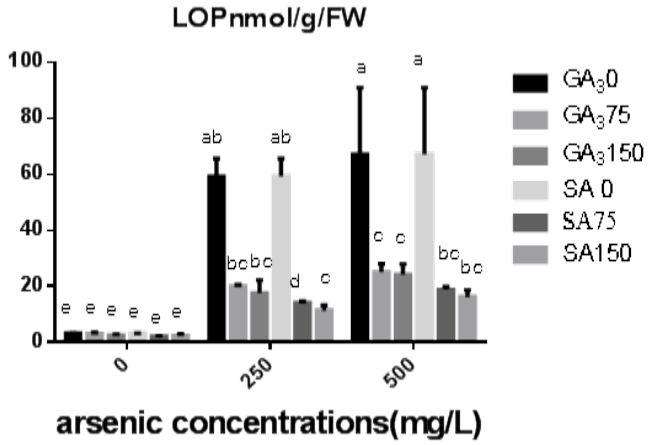

Figure 12: Lipid peroxidation of cowpea (ITOK-568-18) exposed to arsenic toxicity
Means with the same letter are significantly different from each other

CONCLUSIONS

Arsenic is a carcinogenic chemical that is found in our environment due to pollution from both natural and human source. However, continuous plant contamination with arsenic increases its movement along food chain which is` health hazard. Bioregulators such as salicylic and gibberellic acid have been shown to regulate some biochemical processes in plants and therefore serve as a potential modulator of antioxidant enzymes to mitigate arsenic toxicity. In the present study, three cultivars of cowpea seed pre-treated with salicylic acid and gibberellic acid show varying modulatory effects against arsenic toxicity through increase in the level of both antioxidant enzymes (SOD, POD and catalase) and non enzymic antioxidant (vitamin C, flavonoid and phenolic) and therefore promote growth during heavy metal toxicity. Hence there is possibility of optimal growth and productivity during heavy metal stress in cowpea through application of salicylic and gibberellic acids and this would be of great value to agricultural producers.

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CONFLICTS OF INTEREST

The authors declare that they do not have any conflict of interest for publishing this research.

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