




PHYTOCHEMICAL AND NUTRITIONAL COMPOSITION OF THREE VARIETIES OF COWPEA (*VIGNA UNGUICULATA*) PRE-TREATED WITH SALICYLIC ACID UNDER ARSENIC STRESS

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ABSTRACT

Article History

Received: 10 February 2022

Revised: 22 March 2022

Accepted: 5 April 2022

Published: 20 April 2022

Keywords

Salicylic acid
Arsenic
Cowpea
Phytochemical
Nutrition
Varieties
Stress.

Abiotic stress is one of the major factors limiting production and utilization of cowpea in developing nation of the world. Salicylic acid serve to promote and regulate plant growth. The work was design to examine the impacts of Salicylic acid on phytochemicals and nutritional composition of cowpea under arsenic stress. Three varieties of clean seeds of cowpea each were soaked in 0,75 and 150mg/L of salicylic acid for five hours. They were air-dried and sown in pots of soil containing 0, 250 and 500mg/L of sodium arsenate in a completely randomized design and were replicated thrice. The seeds were harvested at maturity and analyzed for phytochemical composition (total phenolic and flavonoid), Proximate composition (Crude protein, fat, ash, moisture, crude fibre and carbohydrate) and elemental composition (As, Ca, K, P, Mo, Mg and Zn). Data were analysed using ANOVA at $\alpha 0.05$. By comparing treatments with the control, crude protein, fat, phosphorus, calcium, magnesium and molybdenum were by increased by 2 folds among the three cultivars, total phenolic, flavonoid were increased by 4 and 2 folds in ART98-12, 5 and 3 folds in Ife brown and ITOK-568-18 in 250mg/L sodium arsenate-treated soil with SA (150mg/L). The result shows that 150mg/L of salicylic acid could be appropriate concentration to alleviate arsenic stress in cowpea .

Contribution/Originality: This study contributes to mitigation of arsenic stress with the use new method of plant biotechnology. It originates from use of growth hormones to improve plant growth. The paper primary contribution is that 150mg/L salicylic acid could be appropriate concentration to improve cowpea resistance and ameliorate arsenic stress in cowpea.

1. INTRODUCTION

Cowpea (*Vigna unguiculata*) is one of Africa's oldest crops, and it's also a major crop in other nations, owing to its comparatively low cost of protein, which makes it a good substitute for animal protein, which is expensive in developing countries. Nigeria and Niger, in the African continent, produce over 66 percent of the world's cowpea. The seed contains a higher concentration of proteins, energy, minerals, and vitamins. Cowpea seeds have a low fat content and are high in protein (approximately 25%) [1]. When compared to starch from cereals, cowpea starch is continuously digested, which has been shown to be advantageous to human health. An vital nutrient is found in the grain of cowpea seed that helps to inhibit neural tube defects in unborn babies [2]. Arsenic is a metalloid that can be found in our environment in organic and inorganic forms, with the latter being the more dangerous. It occurs naturally in a variety of minerals, mainly including sulphur and other metals, as well as volcanoes in the

atmosphere. Arsenic is found in water, air, and soil as a result of human activities such as smelting, minning, and residues of agricultural pesticides, all of which serve as sources of pollution in our environment [3]. Based on the frequency of occurrence and toxicity, it is considered the most poisonous substance. Arsenic as an element is not water soluble, however its salts (arsenite and arsenate) have a wide spectrum of solubilities. In humans, arsenic toxicity has been linked to lung cancer, bladder cancer, and skin cancer [4]. Arsenic contamination of crops causes oxidative stress, which is caused by the generation of reactive oxygen species, which is mediated by a reduction in antioxidant enzymes that protect crops from harm. Crop consequences include nutritional imbalances, soluble sugar accumulation, withering, curling, necrosis of leaf blades, lower growth and productivity, and a decrease in number and area [5]. Because arsenic is chemically similar to phosphorus, a critical plant nutrient for the growth and production of cowpea, it can be used to replace phosphorus in plant nutrition. Furthermore, the agronomic requirements of cowpea, which include the use of pesticides and insecticides, are increasingly serious. As a result, environmental contamination of soil, water, and air would continue to rise. As a result, bioregulators such as salicylic acid, which plays an important role in promoting growth, are used. Alleviating role of kinetin in maize seedlings under arsenic stress through evaluation of physiological, biochemical and nutritional properties had been reported by Haijuan, et al. [6]. Moreover, heavy metals component of our locally consumed cowpea in major markets in Ibadan had been evaluated and arsenic content were found to be above tolerable standard stipulated by Food Agricultural Organisation [7]. Hence the present study; Phytochemical and Nutritional composition of three varieties of cowpea (*Vigna unguiculata*) pre-treated with salicylic acid under arsenic stress.

2. MATERIALS AND MEHODS

The present work was carried out in a screen at Institute of Agricultural Research and Training Ibadan Oyo State, Nigeria. Pot (5kg) were filled with soil and were arranged in a complete randomised design, replicated thrice with 15 treatments/ varieties of cowpea. Sodium arsenate (250 and 500mg/L) were later prepared. Aqueous sodium arsenate (500ml) prepared above was applied to each 5 kg of soil in the pot. It was left for 14 days for proper acclimatization. Cowpea seed varieties were obtained from Institute of Agricultural Research Training (Ife Brown and ART 98-12) while (ITO7K-568-18) was obtained from International Institute of Agricultural Research & Training.

2.1. Salicylic acid Preparation

Salicylic acid (75 and 150 mg/L) was prepared by using procedure of Coolbear [8] and Heydecker [9].

2.2. Planting of Seeds

Three different cowpea types (Ife brown, ART98-12, and ITO7K-568-18) were soaked in two different salicylic acid concentrations (75 and 150 mg/L, respectively). Other parts of the seeds that constitute the control were soaked in distilled water. They were stored at 37°C for 6 hours in the dark. The seeds were then drained of these solutions, rinsed three times with distilled water, and air-dried for roughly an hour. The seeds were placed in arsenic-stressed soil and left to germinate until they were ready to harvest.

2.3. Preparation of Seeds for Analysis

Seed from each of the varieties were crushed using laboratory blender.

2.4. Determination of Total Phenolic

Total phenolic compounds were estimated according to method reported by Singleton, et al. [10] using Folin-Ciocalteu reagent. Standard curve was plotted using gallic acid and the results were expressed as mg/GAE/g of the dry weight.

2.5. Determination of Total Flavonoid

The content of total flavonoids were determined by colorimetric method reported by Zhishen, et al. [11]. Flavonoid contents were expressed as mg/QUE/g/ dry weight.

2.6. Determination of Proximate Composition

Proximate composition is the routine analysis of a sample. However, salicylic acid are bioregulators which are involve in various processes that boost food production.

2.7. Procedures

Samples were analyzed chemically according to the official methods of analysis described by the Williams and George [12].

2.8. Determination of Mineral Elements and Arsenic Composition

Arsenic toxicity resulted in the loss of essential nutrients required for plant development and production. This is due to the inhibition of micronutrient and macronutrient uptake, most likely due to competitive binding with uptake carriers. However, SA facilitates the uptake of these nutrients through different mechanisms, such as using both the first and second line of defense, resulting in enhanced growth during stress.

2.9. Procedure

Mineral nutrients and arsenic content were determine according to the official methods of analysis described by the Williams and George [12]. The extracts obtained was analysed for magnessium, zinc, arsenic, molybdenum, phosphorus using Atomic Absorption Spectrophotometer while Flame photometer was used to determine potassium and calcium

2.10. 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 significant ($P \leq 0.05$) among the treatments.

3. RESULTS

The results of phytochemical composition of cowpea pre-treated with salicylic acid under arsenic stress is shown below in Figures 1-6. Cowpea exposure to arsenic significantly reduce the total phenolic and flavonoid contents when compared to the control but cowpea initially pre-treated with salicylic acid show increase in the total phenolic and flavonoid contents when exposed to arsenic. Moreover, nutritional composition of cowpea pre-treated with salicylic acid under arsenic stress is shown below in Tables 1-6. Cowpea exposure significantly reduce the nutritional and mineral compositions examined when compared to the control but cowpea initially pre-treated with salicylic acid show increase in the nutritional compositions.

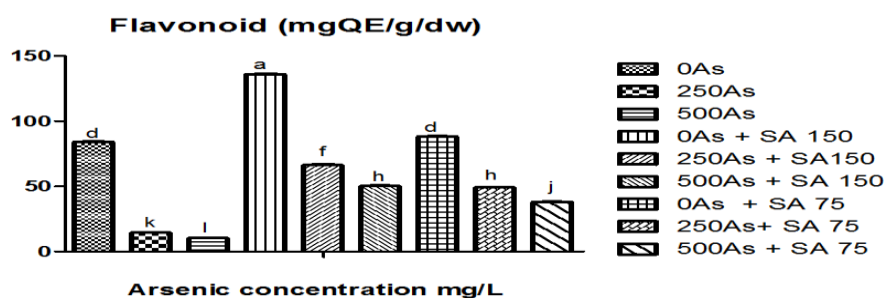
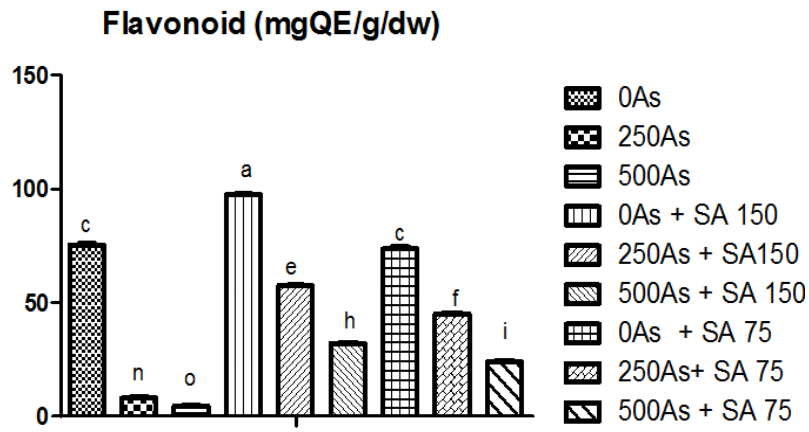


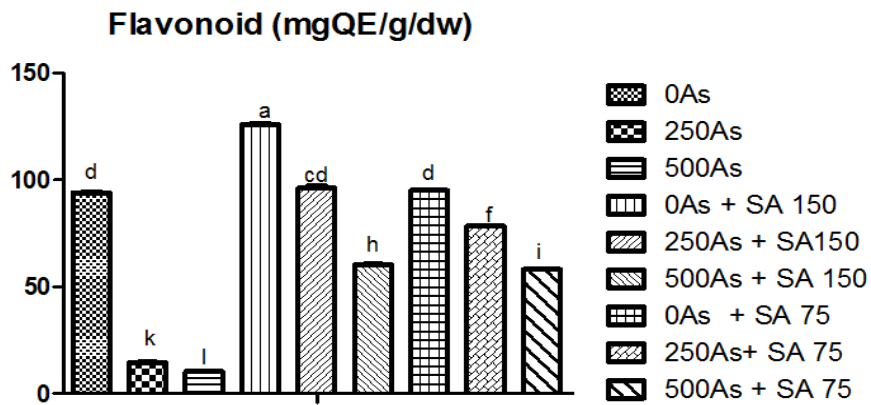
Figure 1. Effect of salicylic acid on flavonoid contents of cowpea (Ife brown) under arsenic stress.

Note: Means with the same letter are significantly different from each other.



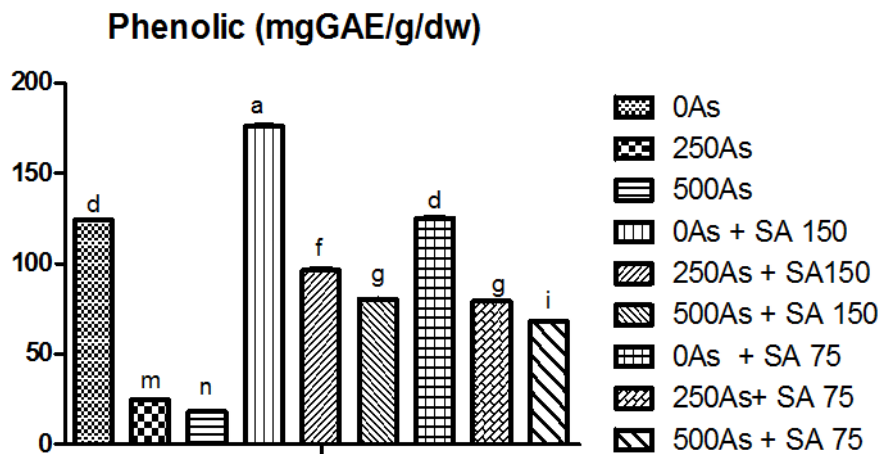
Arsenic concentration mg/L

Figure 2. Effect of salicylic acid on flavonoid contents of cowpea (ART 98-12) under arsenic stress.
 Note: Means with the same letter are significantly different from each other.



Arsenic concentration mg/L

Figure 3. Effect of salicylic acid on flavonoid contents of cowpea (ITOK-568-18) under arsenic stress.
 Note: Means with the same letter are significantly different from each other.



Arsenic concentration mg/L

Figure 4. Effect of salicylic acid on phenolic contents of cowpea (Ife brown) under arsenic stress.
 Note: Means with the same letter are significantly different from each other.

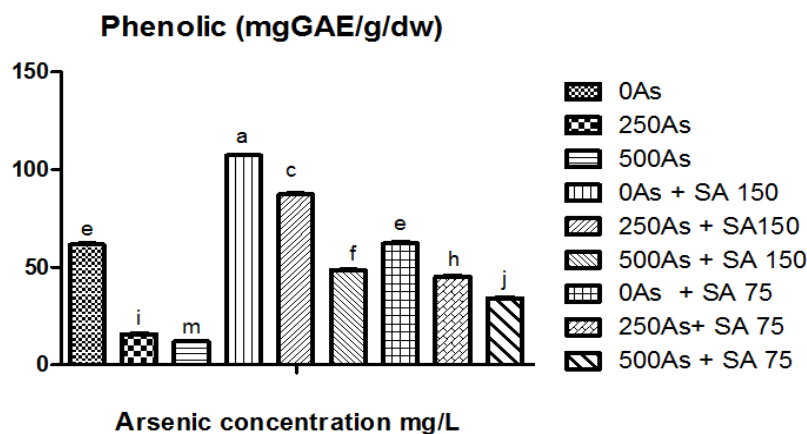


Figure 5. Effect of salicylic acid on phenolic contents of cowpea (ART 98-12) under arsenic stress.
Note: Means with the same letter are significantly different from each other.

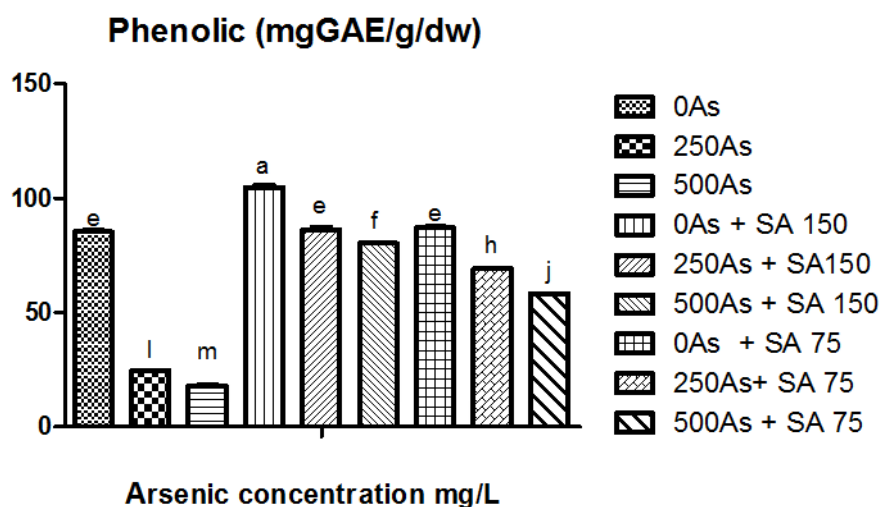


Figure 6. Effect of salicylic acid on phenolic contents of cowpea (ITOK-568-18) under arsenic stress.
Note: Means with the same letter are significantly different from each other.

4. DISCUSSION

The flavonoid and phenolic content of cowpea exposed to 250 and 500 mg/L sodium arsenate significantly decreased in comparison to the control plant in all three varieties studied, as shown in Figures 1-6. This is because the rate of production of reactive oxygen species exceeded the rate of production of antioxidant due to higher levels of arsenic toxicity. However, SA (150 and 75mg/L) significantly increased flavonoid and phenolic of sodium arsenate exposed plants in all the three varieties respectively. Therefore, application of SA (150 and 75mg/L) which show significantly rise in their level was as a result of the increasing in the action of these phytochemicals via chelation with reactive oxygen species generated from arsenic-induced toxicity and render them to non-toxic form. They also acts by altering activities of biosynthetic enzyme of reactive oxygen species.

The result in Tables 1-3 shows that crude protein, fat, ash, moisture, crude fibre and carbohydrate contents of plant exposed to 250 and 500 mg/L sodium arsenate significantly reduce when compare to control plant in all the varieties, this might be due to distortion in activities of proteases (responsible for hydrolysis of protein), α and β amylase (starch degrading enzyme), acid invertase (production of glucose and fructose from sucrose). However, SA (75 and 150mg/L) significantly increase their level in all the varieties respectively. This also in according with the work of Vibhuti, et al. [13] which reported that [14] observed reduction in the ratio of reducing/non-reducing sugars in the *Oryza sativa* L under arsenic-induced toxicity.

In addition, the result in Tables 4-6 shows that phosphorus, potassium, calcium, zinc, magnesium and molybdenum of plant exposed to 250 and 500 mg/L sodium arsenate significantly reduce while arsenic contents of plant significantly increase when compare to control plant in all varieties, this might be due to higher accumulation

from the soil and also through competitive binding to the uptake carrier by arsenic as they belong to the same group in the periodic table. However, SA (75 and 150mg/L) significantly increase these mineral nutrients and reduce arsenic contents in all the three varieties respectively probably through increasing antioxidant activities. SA facilitate the uptake of this mineral nutrients in all the varieties which were observed by significant increase in the level of these mineral nutrients phosphorus, potassium, calcium and magnesium, zinc, molybdenum are primary, secondary and micronutrients require by plants for growth and developments. Arsenic toxicity inhibit the uptake of these nutrient especially replacement of phosphorus which is require for development of energy [ATP] of cell membrane in cowpea for its optimum growth and production. Also since arsenic is analogue of phosphorus, they tend to have affinity for the same transporter for their uptake thereby replacing phosphorus. It had been reported by Vibhuti, et al. [13] that Gusman, et al. [15] find out that arsenic may overtuned the uptake of other minor and major nutrient by competing with nutrient ions for binding to uptake carriers. It was also reported by Amit, et al. [16] that SA pre- treated plants accumulated 16 and 17% less arsenic in shoot upon exposure to 25 and 50 μ M AsV respectively. The rice plants accumulated significant amount of arsenic upon exposure to AsV in dose-dependent manner.

5. CONCLUSION

From the foregoing, arsenic is regarded as hazardous and carcinogenic chemical that occur due to natural and human activities in nature and therefore continuous exposure of plants to arsenic would increase its movement along the food chain resulting in loss of crop productivity as well as arsenic-induced illness d chain, resulting in crop productivity loss and arsenic-induced illness. Plant growth hormone may play a function in reducing the harmful effects of arsenic. As a result, for plants under arsenic stress, the application of salicylic acid, an endogenous regulator that has been demonstrated to enhance growth and development, is critical. Three varieties of cowpea seeds pre-treated with salicylic acid showed varied stimulatory effects against arsenic stress via increases in phytochemical components, mineral nutrients, and proximate composition in the current study. Hence, there is possibility of optimal growth and productivity during arsenic stress in cowpea through application of salicylic which will be of great benefits to agricultural producers.

Table 1. Effect of salicylic acid on proximate composition of cowpea (Ife brown) exposed to arsenic stress.

Arsenic (mg/L)	Bioregulator (mg/L)	Crude Protein %	Crude Fat %	Moisture %	Ash %	Crude fibre %	Carbohydrate %
0	0	23.30±0.06 ^a	1.60±0.06 ^b	9.23±0.08 ^{de}	3.33±0.03 ^{bc}	3.93±0.09 ^{bc}	58.30±0.10 ^c
250	0	18.53±0.12 ^c	0.57±0.03 ^g	7.17±0.09 ^h	3.03±0.03 ^f	3.13±0.03 ^{ef}	51.07±0.14 ^g
500	0	16.03±3.28 ^d	0.20 ±0.06 ^h	6.53±0.09 ⁱ	2.27±0.03 ^g	2.67±0.09 ⁱ	46.57±0.12 ^h
0	SA 150	24.07±0.24 ^a	1.83±0.03 ^a	9.83±0.07 ^a	3.33 ±0.04 ^{bc}	3.67±0.09 ^{de}	60.53±0.12 ^a
250	SA 150	23.80±0.06 ^a	1.73±0.03 ^{ab}	9.77±0.03 ^a	3.77±0.09 ^a	4.23±0.09 ^a	59.50±0.15 ^b
500	SA 150	24.20±0.06 ^a	1.40±0.06 ^c	9.17±0.03 ^e	3.40±0.06 ^{bc}	3.63±0.03 ^{de}	58.43±0.03 ^c
0	SA 75	22.33±0.09 ^{ab}	1.23±0.03 ^{de}	9.57±0.03 ^b	3.40±0.12 ^{bc}	3.33±0.09 ^{gh}	59.23±0.09 ^b
250	SA 75	22.77±0.09 ^{ab}	1.33±0.03 ^{cd}	9.37±0.03 ^{cd}	3.27±0.03 ^{cd}	3.77±0.09 ^{cd}	57.03±0.15 ^d
500	SA75	23.43±0.09 ^a	1.17±0.03 ^e	9.20±0.06 ^{de}	3.53±0.12 ^{ab}	3.53±0.03 ^{ef}	55.13±0.03 ^e

Note: Means with the same letter are significantly different from each other.

Letter indicates significance difference between and among the groups (control and treatments) according to Duncan multiple range test.

Table 2. Effect of salicylic acid on proximate composition of cowpea (ART 98-12) exposed to arsenic stress.

Arsenic (mg/L)	Bioregulator (mg/L)	Crude Protein %	Crude Fat %	Moisture %	Ash%	Crude Fibre%	Carbohydrate %
0	0	22.50±0.06 ^d	1.47±0.03 ^{bc}	9.20±0.06 ^e	3.20±0.06 ^{ef}	3.40±0.06 ^{bc}	58.13±0.03 ^b
250	0	14.60±0.06 ^h	0.70± 0.06 ^e	6.80±0.06 ⁱ	2.20± 0.06 ^h	2.57±0.03 ^d	50.80±0.12 ⁱ
500	0	11.77±0.09 ⁱ	0.17±0.03 ^g	6.10±0.06 ^j	1.94±0.06 ⁱ	2.03±0.03 ^e	45.23±0.09 ^j
0	SA 150	24.07±0.24 ^a	1.83±0.03 ^a	9.87 ±0.03 ^a	3.60±0.06 ^{ab}	3.67±0.09 ^b	60.03±0.12 ^a
250	SA 150	23.80±0.06 ^{ab}	1.73±0.03 ^{ab}	9.53±0.03 ^c	3.77±0.09 ^a	4.23±0.09 ^a	59.40±0.12 ^b
500	SA 150	24.20±0.06 ^a	1.17±0.03 ^d	9.17±0.03 ^{ef}	3.13±0.03 ^{cd}	3.53±0.03 ^b	55.13±0.03 ^{cd}
0	SA75	22.33±0.09 ^d	1.23±0.03 ^{cd}	9.70± 0.06 ^b	3.30±0.06 ^{cd}	3.33±0.09 ^c	59.23±0.09 ^b
250	SA75	22.43±0.09 ^d	1.57±0.03 ^b	9.27±0.08 ^{de}	3.43±0.09 ^{bc}	3.96±0.09 ^a	59.40±0.12 ^b
500	SA75	22.90±0.56 ^{cd}	1.17±0.03 ^d	9.20±0.06 ^e	3.13±0.03 ^{ef}	3.53±0.03 ^b	55.13±0.03 ^h

Note: Means with the same letter are significantly different from each other.

Letters indicates significance difference between and among the groups (control and treatment) according to Duncan multiple range test

Table 3. Effect of salicylic acid on proximate composition of cowpea (ITOK-568-18) exposed to arsenic stress

Arsenic (mg/L)	Bioregulator (mg/L)	Crude Protein %	Crude Fat %	Moisture %	Ash %	Crude fibre %	Carbohydrate %
0	0	22.00±0.06 ^{ab}	1.60±0.06 ^{de}	9.33±0.03 ^{de}	3.47±0.09 ^{cd}	3.53±0.03 ^{de}	58.60±0.06 ^e
250	0	19.70±0.06 ^b	0.90±0.06 ^h	8.03±0.12 ^h	3.07±0.03 ^g	3.37±0.03 ^f	54.77±0.49 ^h
500	0	16.03±3.28 ^c	0.20±0.06 ⁱ	7.50±0.06 ⁱ	2.90±0.06 ^h	2.73±0.09 ^g	46.57±0.12 ⁱ
0	SA 150	24.07±0.09 ^a	1.86±0.03 ^a	9.77±0.03 ^a	3.63±0.03 ^{bc}	3.60±0.06 ^{de}	60.06±0.09 ^a
250	SA 150	23.10±0.06 ^a	1.77±0.07 ^{ab}	9.57±0.09 ^{ab}	3.80±0.06 ^{ab}	4.00±0.06 ^a	59.96±0.18 ^b
500	SA 150	23.80±0.06 ^a	1.70±0.06 ^{bc}	9.50±0.06 ^{bc}	3.57±0.03 ^e	3.40±0.06 ^f	59.83±0.03 ^b
0	SA75	23.80±0.06 ^a	1.70±0.06 ^{bc}	9.57±0.03 ^{ab}	3.57±0.03 ^{bc}	3.40±0.06 ^f	59.80±0.06 ^b
250	SA75	22.23±0.09 ^{ab}	1.47±0.03 ^f	9.33±0.09 ^{de}	3.23±0.03 ^f	3.53±0.03 ^{de}	59.13±0.03 ^{bc}
500	SA75	22.77±0.03 ^a	1.63±0.03 ^{cd}	9.20±0.06 ^{ef}	3.23±0.03 ^f	3.53±0.03 ^{de}	59.03±0.03 ^{bc}

Note: Means with the same letter are significantly different from each other.
Letter indicates significance difference between and among the groups (control and treatments) according to Duncan multiple range test.

Table 4. Effect of salicylic acid on mineral elements of cowpea (Ife brown) exposed to arsenic stress.

Arsenic (mg/L)	Bioregulator (mg/L)	As(mg/g)	P(mg/g)	K(mg/g)	Ca(mg/g)	Zn (mg/g)	Mg(mg/g)	Mo(mg/g)
0	0	0.053±0.0033 ^{ghi}	275.00 ±2.89 ^d	46.67 ±1.67 ^c	171.66±1.67 ^{bc}	0.57 ±0.033 ^{cde}	54.33 ±2.33 ^{cd}	0.019±0.001 ^f
250	0	0.170±0.0033 ^b	192.33 ±1.45 ^g	19.33 ±0.67 ^f	121.66 ±1.67 ^h	0.17±0.270 ^g	33.33±1.67 ^h	0.011±0.000 ^h
500	0	0.208±0.0033 ^a	164.00±1.00 ^h	15.00±1.16 ^f	101.66 ±1.66 ⁱ	0.36±0.033 ^{efg}	23.33 ±1.67 ⁱ	0.005±0.000 ⁱ
0	SA 150	0.006±0.0033 ^{hi}	303.33±4.41 ^a	61.67±1.67 ^a	183.33±1.66 ^a	0.90±0.000 ^a	71.00 ±2.08 ^a	0.044±0.002 ^a
250	SA 150	0.013±0.0033 ^{ghi}	296.37 ±1.67 ^b	46.67 ±1.67 ^c	168.33±1.66 ^{cd}	0.57±0.033 ^{cd}	60.00 ±5.00 ^a	0.031 ±0.001 ^c
500	SA 150	0.017±0.0033 ^{gh}	276.66 ±1.67 ^d	43.33 ±1.67 ^c	163.33 ±1.67 ^d	0.43±0.033 ^{de}	43.33 ±1.67 ^b	0.027±0.001 ^d
0	SA 75	0.007±0.0033 ^{hi}	295.00 ±0.00 ^b	55.00 ±5.00 ^b	176.66 ±4.41 ^b	0.83±0.033 ^{ab}	65.00±5.00 ^{ab}	0.034±0.001 ^b
250	SA 75	0.030±0.0057 ^{de}	266.67 ±1.67 ^e	35.00 ±2.87 ^{de}	156.66 ±1.66 ^e	0.37±0.033 ^{ef}	40.02±0.89 ^{ef}	0.025±0.001 ^e
500	SA75	0.037±0.0033 ^d	255.00 ±2.86 ^f	31.67±1.67 ^e	150.00 ±2.87 ^f	0.27±0.033 ^{fg}	33.33 ±1.67 ^{fg}	0.017±0.000 ^g

Note:
Ars; arsenic, P; Phosphorus, K; Potassium, Ca; Calcium.
SA; Salicylic acid, Mo; Molybdenum, Mg; Magnesium, Zn; Zinc.
Means with the same letter are significantly different from each other.
Letter indicates significance difference between and among the groups (control and treatments) according to Duncan multiple range test.

Table 5. Effect of salicylic acid on mineral elements of cowpea (ART 98-12) exposed to arsenic stress.

Artenic mg/L	Bioregulator (mg/L)	As (mg/g)	P(mg/g)	K(mg/g)	Ca(mg/g)	Zn(mg/g)	Mg(mg/g)	Mo(mg/g)
0	0	0.061 ± 0.0033 ^{gh}	266.67±1.67 ^e	48.67±0.881 ^d	162.67±1.46 ^{bc}	0.53±0.033 ^{de}	56.67±0.88 ^{ab}	0.0030±0.00057 ^{cd}
250	0	0.183 ± 0.0057 ^b	180.00±2.85 ⁱ	22.00±1.15 ^g	126.67±1.67 ^f	0.23±0.033 ^g	23.33±0.88 ^g	0.0013±0.00033 ^f
500	0	0.226 ± 0.0088 ^a	153.33±1.67 ^j	16.67±0.88 ^h	106.67±1.67 ^g	0.089±0.008 ^h	22.00±1.15 ^g	0.0010±0.00000 ^f
0	SA 150	0.010±0.0000 ^{gh}	293.33±1.67 ^a	66.67±1.67 ^a	181.67±1.67 ^a	0.86±0.033 ^a	69.67±1.45 ^a	0.0053±0.00033 ^a
250	SA 150	0.003±0.0033 ^{def}	274.33±2.33 ^{cd}	42.00±0.58 ^e	168.33±1.67 ^{bc}	0.63±0.067 ^{cd}	53.33±1.67 ^c	0.0057±0.00033 ^a
500	SA 150	0.017±0.0033 ^{efgh}	263.33±1.67 ^{ef}	41.33±0.88 ^e	158.33±1.67 ^c	0.43±0.033 ^{ef}	36.67±1.67 ^e	0.0023±0.00033 ^{de}
0	SA 75	0.007±0.0033 ^{gh}	281.67±1.67 ^b	53.33±1.67 ^c	168.33±1.67 ^b	0.77±0.033 ^{ab}	58.33±1.67 ^b	0.0040±0.00057 ^{bc}
250	SA 75	0.010±0.0057 ^{gh}	260.33±1.45 ^f	35.33±0.88 ^f	153.33±1.67 ^d	0.43±0.033 ^{ef}	43.33±1.67 ^d	0.0030±0.00057 ^{cd}
500	SA75	0.020±0.0033 ^{efg}	241.67±1.67 ^h	31.67±1.67 ^f	153.33±1.67 ^d	0.23±0.033 ^g	36.67±1.67 ^e	0.0017±0.00033 ^{ef}

Note:

Ars; arsenic, P: Phosphorus, K: Potassium, Ca: Calcium.

SA: Salicylic acid, Mo: Molybdenum, Mg: Magnesium, Zn: Zinc.

Means with the same letter are significantly different from each other.

Letter indicates significance difference between and among the groups (control and treatments) according to Duncan multiple range test.

Table 6. Effect of salicylic acid on mineral elements of cowpea (ITOK-568-18) exposed to arsenic stress.

Artenic (mg/L)	Bioregulator (mg/L)	As (mg/g)	P(mg/g)	K(mg/g)	Ca(mg/g)	Zn(mg/g)	Mg(mg/g)	Mo(mg/g)
0	0	0.061± 0.0033 ^{gh}	266.67±1.67 ^e	48.67±0.881 ^d	162.67±1.46 ^{bc}	0.53±0.033 ^{de}	56.67±0.88 ^{ab}	0.0030±0.00057 ^{cd}
250	0	0.183± 0.0057 ^b	180.00±2.85 ⁱ	22.00±1.15 ^g	126.67±1.67 ^f	0.23±0.033 ^g	23.33±0.88 ^g	0.0013±0.00033 ^f
500	0	0.226 ± 0.0088 ^a	153.33±1.67 ^j	16.67±0.88 ^h	106.67±1.67 ^g	0.089±0.008 ^h	22.00±1.15 ^g	0.0010±0.00000 ^f
0	SA 150	0.010±0.0000 ^{gh}	293.33±1.67 ^a	66.67±1.67 ^a	181.67±1.67 ^a	0.86±0.033 ^a	69.67±1.45 ^a	0.0053±0.00033 ^a
250	SA 150	0.003±0.0033 ^{def}	274.33±2.33 ^{cd}	42.00±0.58 ^e	168.33±1.67 ^{bc}	0.63±0.067 ^{cd}	53.33±1.67 ^c	0.0057±0.00033 ^a
500	SA 150	0.017±0.0033 ^{efgh}	263.33±1.67 ^{ef}	41.33±0.88 ^e	158.33±1.67 ^c	0.43±0.033 ^{ef}	36.67±1.67 ^e	0.0023±0.00033 ^{de}
0	SA 75	0.007±0.0033 ^{gh}	281.67±1.67 ^b	53.33±1.67 ^c	168.33±1.67 ^b	0.77±0.033 ^{ab}	58.33±1.67 ^b	0.0040±0.00057 ^{bc}
250	SA 75	0.010±0.0057 ^{gh}	260.33±1.45 ^f	35.33±0.88 ^f	153.33±1.67 ^d	0.43±0.033 ^{ef}	43.33±1.67 ^d	0.0030±0.00057 ^{cd}
500	SA75	0.020±0.0033 ^{efg}	241.67±1.67 ^h	31.67±1.67 ^f	153.33±1.67 ^d	0.23±0.033 ^g	36.67±1.67 ^e	0.0017±0.00033 ^{ef}

Note:

Ars; arsenic, P: Phosphorus, K: Potassium, Ca: Calcium.

SA: Salicylic acid, Mo: Molybdenum, Mg: Magnesium, Zn: Zinc.

Means with the same letter are significantly different from each other.

Letter indicates significance difference between and among the groups (control and treatments) according to Duncan multiple range test.

Funding: This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: Both authors contributed equally to the conception and design of the study.

Acknowledgement: The authors are grateful to the Institute of Agricultural Research & Training and International Institute of Tropical Agriculture both at Ibadan, Oyo State Nigeria for providing cowpea seed used for the work.

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