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Influence of Salicylic acid on Proximate, Mineral element, Vitamin composition and Amino acid profile of *Vigna unguiculata*

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Abstract

Vigna unguiculata is a multifunctional crop widely cultivated worldwide for its nutritional value. But its growth and development are being limited by major constraints including the unavailability of improved seeds, poor soil fertility among others which ultimately reduce overall protein, vitamin content and nutrient bioavailabilty thereby making the crop less beneficial for both human diets and livestock. Salicylic acid, a plant growth bioregulator, has been shown to enhance the growth and development of plants. The present study aims to investigate the influence of salicylic acid on proximate, mineral element, vitamin composition and amino acid profile of *V. Unguiculata*. Two varieties of *V. unguiculata* seeds (Ife BPC and BBT-white) n=20 were surface sterilised and pre-treated with 0.5 and 1.0 µM of salicylic acid respectively. They were then sown (n=5) in a 5kg pot of soil in a completely randomised blocked design (CRBD) in a screen house. The plants were harvested at maturity, and proximate, mineral element, vitamin composition and the amino acid profile were evaluated. The results showed that crude protein, fat and fibre, potassium, calcium, phosphorus, zinc, magnesium and molybdenum vitamins (A, B1, B2, B3, B6 and B9) and amino acid profile were significantly increased in *V. Unguiculata* treated with 0.5 and 1.0 µM when compared with the control, while highest mean value was observed in plant treated with 1.0 µM of salicylic acid. Also, Ife-BPC exhibits a higher mean value than BBT-white in all the parameters examined. Therefore, this study suggests that salicylic acid at 1.0 µM could serve as a promising concentration to enhance the proximate, mineral element, vitamin composition and amino acid profile of *V. Unguiculata*.

Keywords: *V. unguiculata*; Salicylic Acid; Amino Acid; Proximate; Vitamins; Mineral Elements

1. Introduction

V. unguiculata holds significant economic value, particularly in developing countries, where it serves as a low-cost source of protein, vitamins, and minerals for millions Amonsou *et al* [1], Herniter *et al* [2]. Globally, production exceeds 9.77 million tons annually across 15.19 million hectares, with sub-Saharan Africa accounting for the majority, supporting smallholder farmers' livelihoods through income generation and food security Bulgari *et al* [3], Carvalho *et al* [4]. Beyond human consumption, *V.unguiculata* is used in livestocks such as animal fodder, manure, erosion biocontrol and nitrogen fixation thereby reducing fertilizer needs in intercropping systems. Its resilience to poor soils and low inputs makes it economically viable in marginal lands, contributing to poverty alleviation and sustainable agriculture in regions like Africa and South Asia, with market value projected to reach USD 12.32 billion by 2033. Costa *et al* [5] Coulibaly *et al* [6], Dadson *et al [*7]. Nutritionally, *V. unguiculata* seeds are rich in proteins (20–30%), carbohydrates (50–60%), and essential amino acids like lysine and tryptophan, alongside antioxidants such as flavonoids and phenolics that combat oxidative stress Daryanto *et al* [8], Anjum *et al* [9]. It also contains bioactive compounds like lectins and trypsin inhibitors, with enzymatic activities (e.g superoxide dismutase and catalase) enhancing cellular protection. du jardin *et al* [10], Egbadzor *et al* [11], Ehlers [12]. However, majority of our farmland

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are occupied by Industrial activities in addition to pollution from various sources which could be accountable for loss of soil nutrients although *V. unguiculata* is a nitrogen fixing crops, it stills required appreciable amount of phosphorus for its optimal growth which may be obtained from inorganic fertilizers, they may be cost-ineffective to some low-income farmers. Abel and Theolog [13], Adegbaju *et al* [14]. Although phosphorus can be obtained inform of fertilizers, some low-income farmers lack access to it due to cost implication, although organic fertilisers may become option but it produces little or no effects when other stringent factors are carefully considered (Gupta *et al* [15], Hall and Thiaw [16], Ayeni *et al* [17] and Battacharyya *et al*. [18]. Bioregulators are plant growth hormones which had been implicated in regulating various process in plant like flowering, cell expansion, gene regulation, nutrition, antioxidants, alleviation of biotic and abiotic stress and overall plant growth Adkins *et al* [19], Aloraya *et al* [20], Bautista-Baños [21], Avanza [22], Esan and Olaiya [23]. Salem Al [24] had reported the improvement in yield and biochemical characteristics of cowpea (*Vigna sisnensis* on application of different growth regulator. Mashkooe *et al* [25] had reported the significant increase in nitrogen and protein content of cowpea on application of liquid organic fertilizer and salicylic acid.Therefore, the aim of the present work is to investigate the influence of salicylic acid on the proximate, mineral element, vitamin composition and amino acid profile of *V. unguiculata.*

2. Materials and Methods

2.1. Plant Material

Two varieties of *V. Unguiculata* seeds were obtained from Institute of Agricultural Research Training [(Ife BPC (Red coat) and BBT-white (white coat)]

2.2. Preparation of Salicylic acid

This was carried out by using procedure of Esan and Olaiya [23] with slight modification**.**

2.3. Pre-treatment of *V. unguiculata* and Planting

Pre-treatment *of V.unguiculata* seeds were performed according to the methods of Ailenokhuoria and Orimadegun [26]. Two varieties of *V.unguiculata* (Ife BPC and BBT-white) were treated with Salicylic acid (0.5 and 1.0 µM respectively) while untreated seed serve as control. The seeds were sown n=5 in the pot of soil. The seeds were harvested at 80 days after planting and proximate, mineral elements, vitamins and amino acid composition were evaluated.

2.4. Procedure for determination of proximate composition of *V.unguiculata*

Samples were analyzed for proximate composition (crude protein, fat, fibre, moisture, ash and carbohydrate) according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C [27].

2.4.1. Determination of Mineral Elements

Mineral elements (sodium, potassium, calcium, phosphorus, zinc, molybdenum, magnesium) were determined according to the methods (A.O.A.C [27].

2.4.2. Determination of Amino acid Profile

Amino acid extraction and analysis were done according to the methods of Huda and Adel [28] with slight modification.

$$\%\ Amino\ Acid\ = \frac{Absorbance\ of\ sample\ X\ Gradient\ factor\ X\ Dilution\ Factor}{10{,}000}$$

2.4.3. Determination of Vitamins

Vitamin A, B1, B2, B3, B6 and B9 were determined using spectrophotometry methods of Uzoekwe [29]

2.4.4. Statistical analysis

The results were analysed using ANOVA [Statistical Analysis System (SAS) Version 9]. Means were compared using Duncan Multiple Range Test (DMRT) and data were considered significantly different at $P \leq 0.05$. The values are presented as Mean±standard error, with number of replicate (n=3). Graph prism version 5 was used to plot the graph.

3. Results

Table 1 Effects of Salicylic acid on amino acid profile of *V. unguiculata* (Ife-BPC)

SAMPLE	**Control**	**SA 0.5 µM**	**SA 1.0 µM**
Parameters (%)	VALUES	VALUES	VALUES
Lysine	5.71±0.02^{c}	5.96±0.03^{b}	6.23±0.04^{a}
Arginine	4.04±0.01^{c}	5.48±0.01^{b}	5.46±0.02^{a}
Asparagine	8.24±0.01^{c}	8.77±0.02^{b}	9.01±0.04^{a}
Threonine	3.32±0.02^{c}	4.03±0.00^{b}	4.28±0.02^{a}
Serine	4.27±0.02^{c}	4.56±0.01^{b}	4.89±0.03^{a}
Glutamine	4.54±0.01^{c}	4.81±0.02^{b}	5.01±0.02^{a}
Proline	4.25±0.02^{c}	4.58±0.01^{b}	4.75±0.03^{a}
Glycine	4.03±0.01^{c}	4.39±0.03^{b}	4.61±0.03^{a}
Alanine	4.35±0.04^{c}	4.83±0.00^{b}	5.03±0.04^{a}
Cysteine	2.57±0.00^{c}	2.92±0.01^{b}	3.05±0.02^{a}
Valine	4.06±0.02^{c}	4.36±0.03^{b}	4.75±0.03^{a}
Methonine	1.42±0.01^{c}	1.74±0.01^{b}	1.98±0.01^{a}
Isoleucine	3.23±0.01^{c}	3.83±0.01^{b}	4.07±0.02^{a}
Leucine	6.52±0.03^{c}	7.11±0.04^{b}	7.39±0.04^{a}
Tyrosine	4.09±0.02^{c}	4.42±0.01^{b}	4.75±0.04^{a}
Phenylalanine	4.72±0.01^{c}	5.41±0.02^{b}	5.83±0.03^{a}
Ornithine	0.31±0.01^{c}	0.49±0.01^{b}	0.62±0.01^{a}
Cystine	0.81±0.01c	1.11±0.02^{b}	1.41±0.01^{a}
Tryptophan	1.31±0.02^{c}	1.41±0.01^{b}	1.78±0.02^{a}

Means with same letters are not significantly different from each other at P≤0.05

Table 2 Effects of Salicylic acid on amino acid profile of *V. unguiculata* (BBT white)

SAMPLE	**Control**	**SA 0.5 µM**	**SA 1.0 µM**
Parameters (%)	VALUES	VALUES	VALUES
Lysine	4.51±0.02^{c}	4.96±0.03^{b}	5.13±0.04^{a}
Arginine	3.04±0.01^{c}	3.48±0.01^{b}	4.33±0.02^{a}
Asparagine	7.19±0.01^{c}	7.77±0.02^{b}	8.31±0.04^{a}
Threonine	3.02±0.02^{c}	3.53±0.00^{b}	3.88±0.02^{a}
Serine	3.05±0.02^{c}	3.42±0.01^{b}	3.89±0.03^{a}
Glutamine	3.51±0.01^{c}	3.93±0.02^{b}	4.11±0.02^{a}
Proline	3.26±0.02^{c}	3.58±0.01^{b}	3.75±0.03^{a}
Glycine	3.01±0.01^{c}	3.39±0.03^{b}	3.61±0.03^{a}
Alanine	3.75±0.04^{c}	3.83±0.00^{b}	4.03±0.04^{a}
Cysteine	2.27±0.00^{c}	2.52±0.01^{b}	2.85±0.02^{a}

Valine	3.57±0.02^{c}	3.86±0.03^{b}	4.05±0.03^{a}
Methonine	1.22±0.01^{c}	1.55±0.01^{b}	1.78±0.01^{a}
Isoleucine	2.93±0.01^{c}	3.23±0.01^{b}	3.57±0.02^{a}
Leucine	5.02±0.03^{c}	5.71±0.04^{b}	6.09±0.04^{a}
Tyrosine	3.59±0.02^{c}	4.02±0.01^{b}	4.45±0.04^{a}
Phenylalanine	3.72±0.01^{c}	4.41±0.02^{b}	4.83±0.03^{a}
Ornithine	0.25±0.01^{c}	0.39±0.01^{b}	0.55±0.01^{a}
Cystine	0.71±0.01c	1.01±0.02^{b}	1.38±0.01^{a}
Tryptophan	1.01±0.02^{c}	1.29±0.01^{b}	1.56±0.02^{a}

Means with same letters are not significantly different from each other at P≤0.05

Table 3 Effects of Salicylic acid on Vitamin A, B1, B2, B3, B6 and B9 of *V. unguiculata* (Ife-BPC)

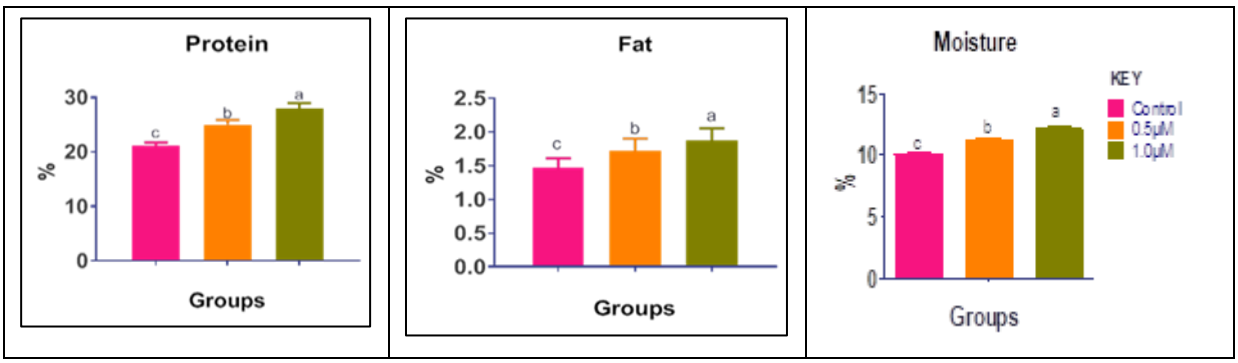
Treatment	Vitamin A (mg/ml)	Vitamin B1 (mg/ml)	Vitamin B2 (mg/ml)	VitaminB3 (mg/ml)	Vitamin B6 (mg/ml)	Vitamin B9 (mg/ml)
Control	0.002±0.000^{c}	0.885±0.08^{c}	0.219±0.01^{c}	2.103±0.02^{c}	0.316±0.04^{c}	0.629±0.08^{c}
0.5 µM SA	0.010±0.0001^{b}	0.925±0.10^{b}	0.251±0.02^{b}	2.141±0.01^{b}	0.343±0.03^{b}	0.657±0.15^{b}
1.0 µM SA	0.060±0.0001^{a}	0.951±0.15^{a}	0.282±0.05^{a}	2.198±0.07^{a}	0.375±0.05^{a}	0.675±0.21^{a}

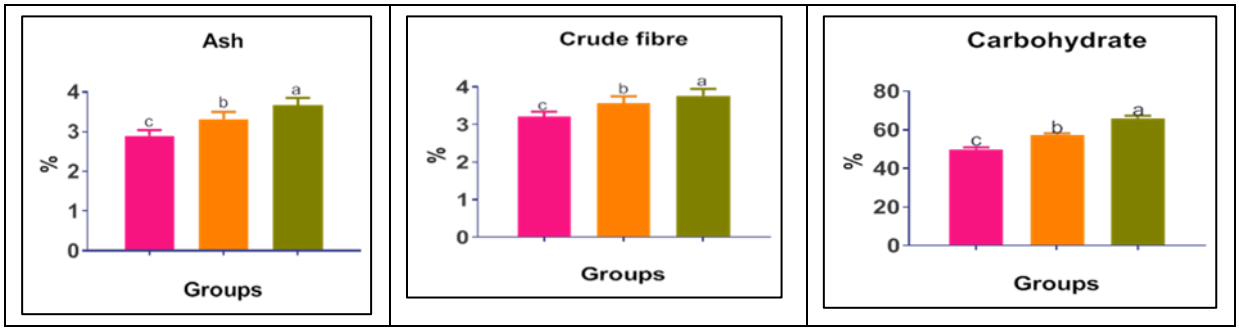
Mean with the same letters are not significantly different at (P≤0.05)

Table 4 Effects of Salicylic acid on Vitamin A, B1, B2, B3, B6 and B9 of *V. unguiculata* (BB white)

Treatment	Vitamin A (mg/ml)	Vitamin B1 (mg/ml)	Vitamin B2 (mg/ml)	VitaminB3 (mg/ml)	Vitamin B6 (mg/ml)	Vitamin B9 (mg/ml)
Control	0.004±0.000^{c}	0.905±0.10^{c}	0.238±0.02^{c}	2.141±0.04^{c}	0.321±0.03^{c}	0.642±0.11^{c}
0.5µM SA	0.030±0.0001^{b}	0.956±0.13^{b}	0.286±0.04^{b}	2.201±0.03^{b}	0.364±0.02^{b}	0.671±0.19^{b}
1.0 µM SA	0.080±0.0001^{a}	0.985±0.21^{a}	0.301±0.06^{a}	2.254±0.05^{a}	0.392±0.06^{a}	0.693±0.24^{a}

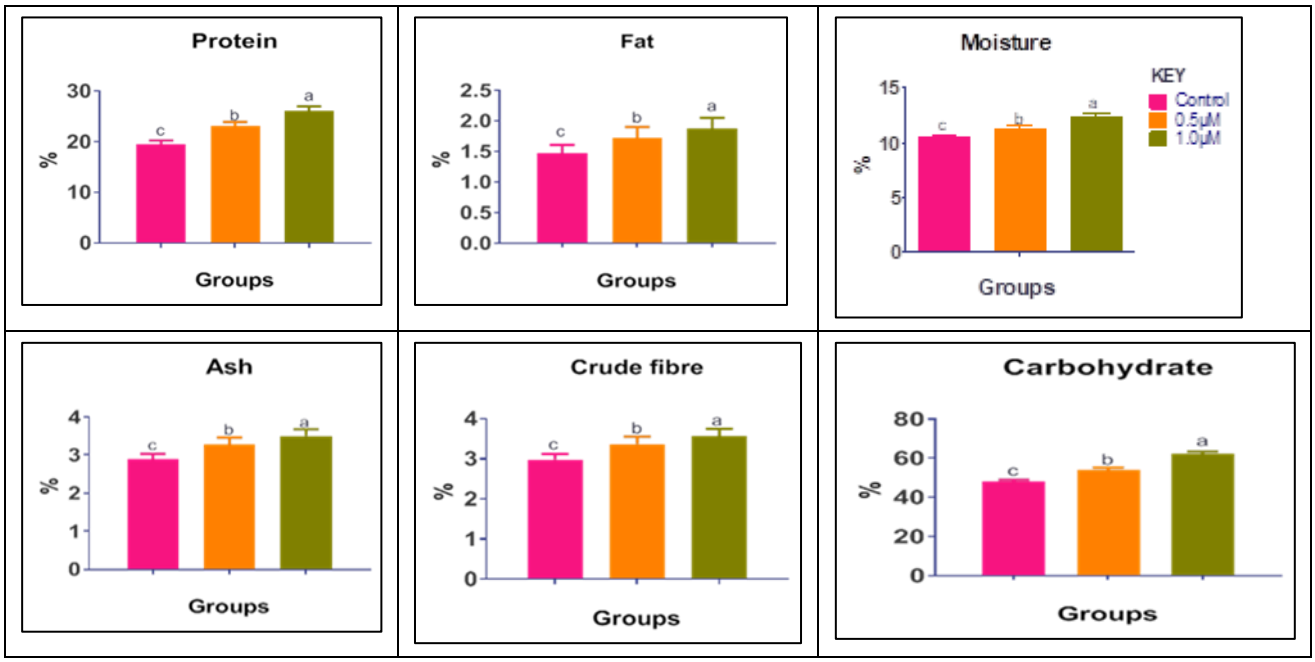
Mean with the same letters are not significantly different at (P≤0.05)





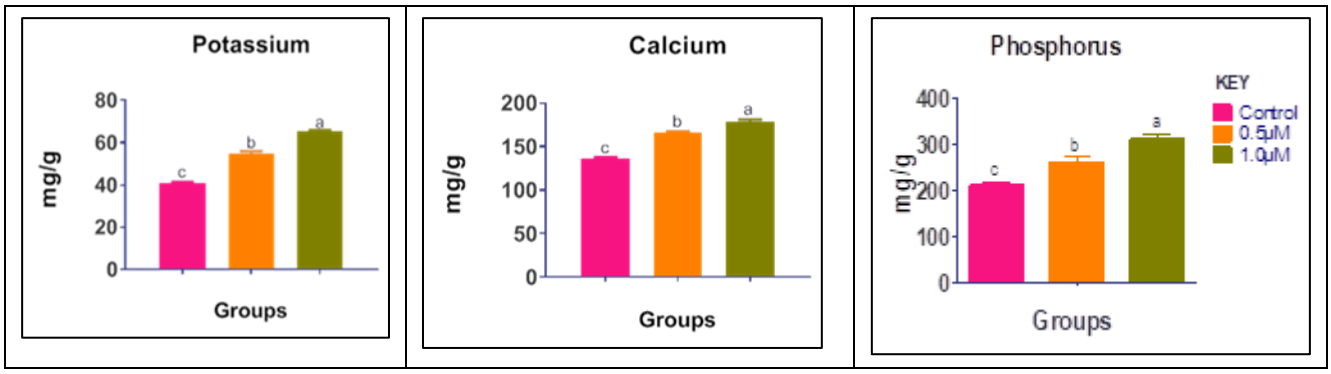
Means with same letters are not significantly different from each other at P≤0.05

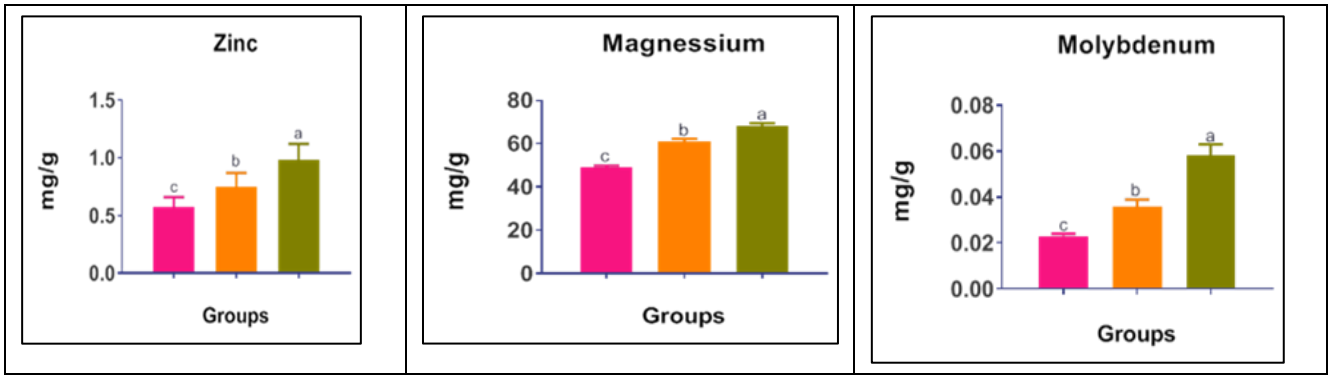
Figure 1 Effects of Salicylic acid on Proximate composition of *V.unguicuata* (Ife-BPC)



Means with same letters are not significantly different from each other at P≤0.05

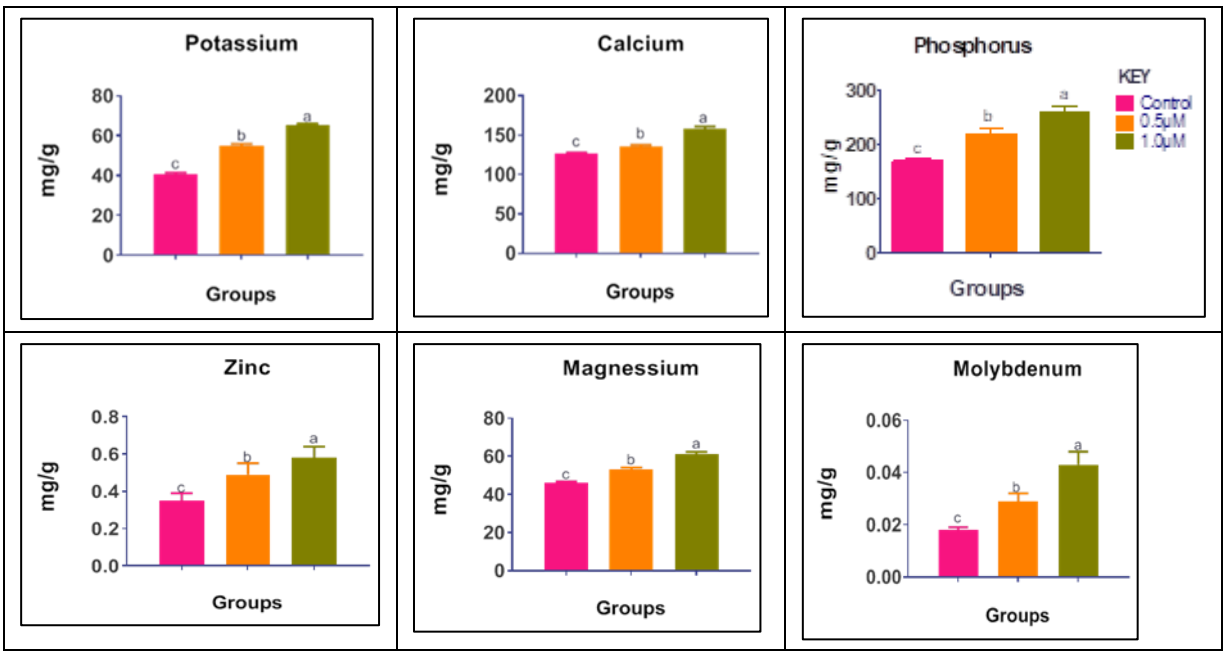
Figure 2 Effects of Salicylic acid on Proximate composition of *V.unguiculata* (BBT-White)





Means with same letters are not significantly different from each other at P≤0.05

Figure 3 Effects of Salicylic acid on Mineral elements of *V. unguiculata* (Ife-BPC)



Means with same letters are not significantly different from each other at P≤0.05

Figure 4 Effects of Salicylic acid on Mineral elements of *V. unguiculata* (BBT white)

4. Discussion

The present study demonstrates the influence of salicylic acid on proximate, mineral element, vitamin composition and amino acid profile of the two varieties of *Vigna unguiculata*. Figures 1-4 and Table 1-4 showed that there is significant increase in the level of proximate composition (crude protein, fat, fibre, ash, moisture, and carbohydrate), mineral elements (calcium, phosphorus, zinc, potassium, magnessium, and molybdenum), vitamin contents (A, B1, B2, B3, B6 and B9) and amino acid profile of *Vigna unguiculata* seed treated with salicylic acid as compared with control. The highest mean value was observed in the *V. unguiculata* treated with 1.0 µM of salicylic acid in the two varieties examined. Meanwhile, Ife BPC showed increased mean value than BBT-white in all the parameters examined. This might be appropriate and effective concentration to elicit the effects observed. Moreover, salicylic acid facilitate increase in uptake of mineral elements from the soil, upregulating antioxidant potential of plant and improving plant response to some major biotic and abiotic stress that limits plant growth thereby enabling plant to build organic molecules like carbohydrate, proteins, vitamins and amino acids and needed by cells of plant. This was also known in compliance with the work of Salem [24] who reported that Indole acetic acid (IAA), and Gibberellic acid (GA) improved the yield, proximate and biochemical properties of cowpea. Ailenokhuoria and Orimadegun [26] had reported the foliar application of Indole acetic acid and kinetin cause significant increase in the growth and biochemical parameters of Pigeon pea. Ali *et al* [30] had also reported that Seaweed extract-based biostimulants enhanced the amino acid and

mineral uptake in cowpea. Gharib *et al* [31] had also reported that there is increased in crude protein, mineral nutrients on application of ascorbic acid and gallic acid to *Vigna unguiculata* plants. This suggests the natural way of optimizing the nutrient value of crops hence could possibly be explored by low-resource farmers and commercial farming as its application pose no risk to plants, animals, humans or any other parts of ecosystem.

5. Conclusion

Salicylic acid is an endogenous plant bioregulator that participates in the control of some biochemical processes in plants such as photosynthesis, growth, nitrate metabolism, flowering and ethylene production. In the present study, two varieties of *V. unguiculata* seeds pre-treated with salicylic acid at (0.5 and 1,0 μM) shown varying effects in improving the nutrition and growth of *V. Unguiculata* through increase in proximate, mineral elements, vitamins and amino acid compositions and therefore may serve as an alternative to use of fertilizers application which could ultimately benefits low-resource farmers, agro-allied industries, researchers and other relevant stakeholders. Therefore, application of salicylic acid at 1.0 μM could serve as promising concentration to improve the nutritional composition *of V. unguiculata.*

Recommendations

From the results of this study, it could be recommended that

- The appropriate concentration of salicylic acid to improve the nutritional composition of *V. unguiculata* or any other crops is 0.5 and 1.0 μM.
- Being a bioregulator, it is safe as it poses no risk or residual toxic effects in plant, human and entire ecosystem.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Competing Interests

Authors have declared that no competing interests.

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