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Effects of Mycorrhizal Biofertilizer on the Biochemical Constituent, Nutrient Uptake, Chlorophyll Content, Growth and Agronomic Parameters of American Yam Bean (*Pachyrhizus erosus* L.) under Saline Condition

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Authors' contributions

This work was carried out in collaboration among all authors. Author NAE wrote the protocol, conducted the experiment, wrote the first draft and came out with the final draft, author ESS, MEK and EWC took part in the lab analyses, preparing and editing the manuscript and author TVD corrected the final draft, made some technical inputs and supervised the editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Salt stress negatively impacts plant physiology, metabolism and productivity.The effects of mycorrhizal biofertilizer on the biochemical constituent, nutrient uptake, chlorophyll content, growth and agronomic parameters of American Yam bean (*Pachyrhizus erosus* L.) under saline conditions were investigated. Seeds were planted in polythene bags previously filled with sand, 50 g of Biofertilizer and supplied with a nutrient solution for six weeks in a completely randomized design in a greenhouse. Plants were subjected to NaCl concentrations (0, 50, 100 and 200 mM) with 0 as control. The concentrations 0 and 50 mM NaCl were tested on the farm for five months. Plots were

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arranged in a randomized block design. The number of flowers per plant, flowering time, number of pods per plant, yield and harvest index were evaluated. The results in the greenhouse showed that the stem height, leaf area, the number of leaves, noose diameter, dry biomass and chlorophyll content decreased significantly (P<0.01) from 100 mM NaCl. Mg, Ca and K significantly (P<0.001) decreased in plant organs with the supply of intake doses of NaCl in the culture medium. The different metabolites (Total soluble proteins, Proline, Total free amino acids, Total soluble carbohydrates and Total phenolic contents) increased significantly (P< 0.001) from 50 mM NaCl. The findings indicate that the accumulation of AMF positively influenced the growth and agronomic parameters of American yam bean under salinity.Their use alleviated the toxicity of NaCl, improved crop productivity and could be encouraged for better development of salinity affected areas.

Keywords: Pachyrhizus erosus; salinity; growth parameters; metabolites; biofertilizer; yield.

1. INTRODUCTION

Salinity is an abiotic stress that reduces plant growth and productivity in the world [1,2,3,4]. The high soil salinity is due to saline parent bed rocks, mineral degradation, invasion of sea water in coastal regions and irrigation culture [3]. High concentrations of salt in the soil have a negative impact on stomatal closure, osmotic balance, nutrient uptake, chlorophyll content, plant metabolism and productivity [5]. Additionally, it also causes an imbalance in water potential, cellular dehydration, inhibition of intracellular enzyme activities, cell division and expansion [6,3]. Combating the negative effect of salt on plant crops is a big challenge such that the use of arbuscular mycorrhizal biofertilizer to alleviate salinity stress on plant growth and production has received great attention [7].

From the work done by [6,8,3], salinity stress increases the sodium (Na^+) content in plant partitioning while decreasing potassium (K), calcium (Ca) and Magnesium (Mg) content and negatively impacts the photosynthetic activities. Other workers [9,10,11] showed that arbuscular mycorrhizal fungi (AMF) in the presence of salinity decreesed the Na^+ content and increased K, Ca, Mg, Na/K ratio and photosynthesis.

To cope with soil salinity plants have developed some protective mechanisms some of which include the accumulation of certain osmolytes (Proline (PRO), soluble carbohydrates (CH), total free amino acids (FAA), proteins (PR), total phenol (TP) and flavonoids (FLA) content) [12,8,3]. Proline acts as a mediator of osmotic adjustment stabilizing the deleterious effect of salt in the vacuole [13]. PR is involved in osmotic adjustment; they are stored as nitrogen under salt-stress and re-used at the end of stress [14]. It has been proven that salt-tolerant plants store more soluble carbohydrates (CH) under salt stress conditions [15]. The production and accumulation of free amino acids (FAA) by plant tissues during salt stress is an adaptive response according to [6]. Total phenol (TP) acts as a defense mechanism against biotic and abiotic stress [8] and flavonoids (FLA) are the main subgroup of polyphenols with a wide array of biological functions including lipid peroxidation inhibition. Their accumulation during stress could be considered as a cellular adaptive mechanism for scavenging oxygen free radicals [18]. In the salinity stress conditions, AMF has been reported to modulate the biosynthesis of certain osmoprotectants such as proline, soluble carbohydrates, proteins and total free amino acids content and the result is to improve and protect photosynthetic activity [16,11].

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that contribute to the improvement of growth in several plant species under saline conditions [17]. This is mainly related to a combination of biochemical, physiological, and nutritional factors [11]. Among the mechanisms involved in salinity tolerance in AMF inoculated plants, are its enhancement of water absorption capacity, nutrient uptake, the accumulation of osmoregulators like proline and sugars [16]. AMF plays an important role in membrane stability and stimulates the plant to produce its own defence enhance the photosynthetic pigments and maintain the osmotic and ionic balance of the cell [35].

American yam bean is an economically important cash crop with nutritional and medicinal properties; and has great potentials as a food crop. It is a great source of different vitamins and minerals. It is an excellent source of vitamin C, fibre, folic acid, beta-carotene, potassium, iron, and calcium [19]. Due to its low fat and calories content, yam bean helps to control cholesterol levels and so reduces the risk of having heart

problems such as heart attack and hypertension. It acts as a powerful antioxidant and anti-inflammatory [19]. The crop is not widely known in some countries like Cameroon due to a lack of information as regards its production to boost food security. Therefore, the work aims to study the effects of mycorrhizal biofertilizer on the biochemical constituent, non-enzymatic antioxidants, nutrients uptake, chlorophyll content, growth, and agronomic parameters of American Yam bean (*Pachyrhizus erosus* L.) under saline conditions.

2. MATERIALS AND METHODS

2.1 Study Area and Plant Material

The study was performed in a greenhouse and field conditions of the Faculty of Science, The University of Bamenda located in Bambili-Cameroon. The seeds of American yam bean (*Pachyrhizus erosus*) and AMF biofertilizer (*Gigaspora margarita* + *Acaulospora tuberculata*) used were provided by the Institute of Agronomic Research and Development (IRAD) breeding program of Cameroon.

2.2 Plant Growth Conditions and Salt Treatments

After a viability test, the seeds of the American yam bean were sterilized with 3% of sodium hypochlorite for 10 minutes, washed five times with demineralized water and transplanted into 3 L polythene bags filled with 3 kg of sterilized sand. The plants were arranged in a complete randomized block design, with one plant each and five replications per treatment. They were daily enriched with a modified nutrient solution. (in g/L) made of 150 g $Ca(NO3)_2$, 70 g KNO_3, 15 g Fe–EDTA, 0.14 g $KH2PO_4$, 1.60 g $K2SO_4$, 11 g $MgSO_4$, 2.5 g $CaSO_4$, 1.18 g $MnSO_4$, 0.16 g $ZnSO_4$, 3.10 g $H3BO_4$, 0.17 g $CuSO_4$ and 0.08 g MOO_3 [20]. The pH of the nutrient solution was adjusted to 7.0 by adding HNO_3 0.1 mM. Plants were subjected to different salt concentrations (0, 50, 100 and 200 mM NaCl) in the culture medium for six weeks to determine the physiological and biochemical responses of cultivars to salt stress. The average day and night temperatures in the greenhouse were between 26 and 20 °C respectively during the growth period with an average relative air humidity of 69.5%. Parameters were evaluated under greenhouse conditions: stem height, leaf area, number of leaves, noose diameter, dry biomass of roots and shoots, chlorophyll (a+b) content, metabolites (proline, soluble carbohydrates, total phenol and flavonoids content) and mineral (Na, K, Ca and Mg contents of roots and shoots).

2.3 Growth Parameters

The leaf area, stem height, number of leaves, noose diameter and dry weight were recorded after six weeks. The stem height was determined by measuring with a ruler. The number of leaves was obtained by counting. The noose diameter was obtained with a vernier calliper, roots and shoots were dried separately at 60 °C for 72 hours and their dry biomasses were determined. Leaf area was calculated using the [21] formula: surface area (cm^2) = 1/3 (Length × Width).

2.4 Mineral Distribution

The sodium, potassium, calcium and magnesium in plant partitions were determined. 2 g of dried organs were separately reduced to ashes by heating at 550 °C for 4 hours and thoroughly mixed with 250 mL of deionized water. The filtrate was analysed with an atomic absorption spectrophotometer (Rayleigh WFX-100) method [22].

2.5 Chlorophyll Content

The chlorophyll content was determined using the method described by Arnon [23]. After six weeks 0.80 g sample of fresh leaves were crushed and extracted with 80% of alkaline acetone (v/v). The filtrate was analyzed using a spectrophotometer (Pharmaspec model UV-1700) at 645 and 663 nm.

2.6 Osmolytes

2.6.1 Proline content

PRO was estimated using the method described by Bates [24]. 0.5 g of fresh leaves were weighed and put inside a flask. 10 mL of 3% aqueous sulphosalicylic acid was poured into the same flask. The mixture was homogenized, and then filtered with a Whatman N^o 1 filter paper. 2 mL of filtered solution was poured into a test tube, and then 2 mL of glacial acetic acid and ninhydrin acid were respectively added into the same tube. The test tube was heated in a warm bath for 1 hr. The reaction was stopped by placing the test tube in an ice bath. 4 mL of toluene was added to the test tube and stirred. The toluene layer was separated at room temperature, the mixture a purple colour and the absorbance of the purple

mixture was read at 520 nm by spectrophotometer UV (Pharmaspec model UV-1700). At 520 nm, the absorbance was recorded and the concentration of PRO was determined using a standard curve as µg/g FW.

2.6.2 Total soluble carbohydrate content

CH was obtained using the phenol-sulphuric acid Dubois [25] method. The fresh leaves (1 g) were ground in 5 mL of 80% ethanol and filtered with the Whatman N^{o} 1 filter paper. The collected extracts were diluted by deionized water to 50 mL. 1 mL of each sample was poured in a test tube, then 1 mL of phenol solution and 5 mL of sulphuric acid was added. The mixture was then swirled. The absorbance was read at 490 nm using a spectrophotometer (Pharmaspec UV-1700 model). The quantity of CH was deduced from the glucose standard curve.

2.6.3 Total soluble protein content

PR was evaluated using the method described by Bradford [26]. The standard protein used was the bovine serum albumin (BSA). 0.1 g of fresh leaves were ground and mixed with 4 mL of an already prepared sodium-phosphate buffer, pH 7.2. The mixture was then centrifuged at 13 000 rpm for 4.5 mins at 4 °C. 1 mL of the supernatant was poured into a tube containing 5 mL of the Bradford reagent. The mixture was shaken and incubated in the dark for 15 mins. The absorbance of the resulting blue complex was read at 595 nm with a spectrophotometer UV (PG instruments T60). The standard curve was obtained using BSA 1 mg/mL.

2.6.4 Total free amino acids content

FAA content was determined by using the ninhydrin method described by Yemm and Cocking [27]. Fresh leaves (1 g) were ground in 5 mL of ethanol 80%, amino acids were then extracted using reflux technique in boiling ethanol for 30 mins. After decanting, the supernatant was filtered using Whatman N°1 filter paper. The filtrate was collected and the residue was used to repeat the extraction. The two filtrates were mixed and the raw extract of amino acid content was measured using the ninhydrin method. The absorbance of the purplish-blue complex was read at 570 nm wavelength. The standard curve was established using 0.1 mg/mL of glycine.

2.6.5 Flavonoids content

FLA of crude extract was determined by the aluminium chloride colorimetric method [28]. 50 µL of crude extract (1 mg/mL ethanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 minutes of incubation, and the mixture was allowed to stand for 6 minutes. Then, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 mins, and absorbance was recorded on a spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a standard curve, and the result was expressed in (mg/g DW).

2.6.7 Total phenolic content

TP was determined using the folin-ciocalteu method [29]. 1 g of fresh leaves was ground at 4 °C for 20 minutes in 3 mL of 0.1 NHCl, the homogenate was centrifuged at 6000 g for 40 minutes. The pellet was re-suspended in 3 mL of 0.1 NHCl and centrifuged as previously described. The two supernatants are mixed and constitute the crude extract of soluble phenol. The reaction mixture containing 15 µL of extract 100 µL folin-ciocalteu reagents, 0.5 mL of 20% Na_2CO_3 was incubated at 40 °C for 20 minutes and absorbance read at 720 nm wavelength with a spectrophotometer (Pharmaspec UV-1700 model). The TP (mg/g FW) content was determined through a standard curve established by using chlorogenic acid.

2.7 Yield Components

The field experiment was performed at the University of Bamenda agricultural research farm located in Bambili-Cameroon. Bambili (5^{o} 60' 33" North and longitude 10^{o} 15' 21" East, Elevation 1444 m) is found in Mezam division of the North West region of Cameroon. The work was carried out from January 2019 to October 2021, Average rain fall, and temperatures are 854 mm/year and 30 °C and relative humidity is nearest to 84%. Prevailing winds carry the tropical monsoon. Table 1 shows the physico-chemical properties of the soil taken from 0-20 cm depth of the experimental site in Bambili. The plots were arranged in a randomized complete block design within a split-plot layout with two main treatments 0 and 50 mM NaCl with three replications and 0

Table 1. Physico-chemical properties of the soil taken from 0-20 cm depth of the experiemental site in Bambili, Cameroon

Properties	Values	Properties	Values
Fine sand (%)	17.66±1.52	Ratio C/N	2.68±0.03
Coarse sand (%)	16.33±0.57	Exchangeable cations (cmol + kg^{-1})	0.2±0.04
Fine silt (%)	15.16±0.76	Cation Exchange capacity (cmol + kg^{-1})	11.23±0.25
Coarse silt (%)	15.66±1.15	Phosphoros (ppm)	63.73±0.25
Clay (%)	36.66±1.52	Potassium (g kg^{-1})	0.01±0.01
Moisture content (%)	14.37±2.37	Calcium (g kg^{-1})	2.22±0.07
porosity (%)	38.33±1.52	Magnesium (g kg^{-1})	1.35±0.14
pH water	5.45±0.2	Sodium (g kg^{-1})	0.04±0.01
pH kcl	4.93±0.05	Sulfur (g kg^{-1})	3.56±0.03
Organic carbon (%)	5.04±0.05	Iron (g kg^{-1})	117.97±6.90
Organic mater (%)	8.69±0.20	Conductivity (mS/cm)	0.08±0.01
Nitrogen (%)	1.88±0.07		

as a control. Plots were 4×1 m surface and intra spacing was 1.5 m and inside the plots the cultivars were 0.50 m. Yield datas were collected from eighteen plants per repetition for each variant of the experiment.

The agronomic parameters assessed were the flowering time, number of flowers per plant, number of pods/plant, yield and harvest index. The number of flowers was determined by counting flowers every week for each treatment until the emergence of the first pods. The number of pods per plant was determined every week for each treatment until harvest time. The flowering time was gotten by noting the date of first appearance of flower for each treatment. The yield was obtained Yield (t/h) = Total production (tonne)/surface (hectare). The harvest index (HI) was calculated.
HI = (WT/(WT+Biomass (shoot and root)) x 100 [30]. Where; HI = Harvest Index, WT = Weight of tubers.

2.8 Statistical Analysis

The experiment was performed using a completely randomized design. All data were presented in terms of mean (± standard deviation), statistically analysed using Graph pad Prism version 5.01 and subjected to analysis of variance (ANOVA). Statistical differences between treatment means were established using the Fisher Least Significant Difference (LSD) at P < 0.05. Probability level using Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

The various growth parameters (stem height, number of leaves, noose diameter, leaf area and dry biomass) were generally influenced by intake doses of NaCl and decreased significantly (p<0.05) in the culture medium from 100 to 200 mM of NaCl (Table 2). The treatment with AMF significantly improved growth parameters in all concentrations compared to the treatment without AMF. These results are supported by those of Abdel Latef [31]; Kosovà et al. [32]; Ndouma et al. [8] and Nouck et al. [3]. They showed that salinity disrupts physiological processes (imbalance in water potential, cellular dehydration, inhibition of intracellular enzyme activities, ionic toxicity, cell division and expansion) negatively affecting the growth and yield thereby contributing to their reduction. For plants exposed to AMF, it was shown that AMF alleviates the negative effects of salt stress by enhancing nutrient uptake, photosynthetic activities and water absorption; consequently contributing to the improvement of the growth of several plant species under saline conditions Evelin et al. [17]. The roots and shoots dry weight decreased in the culture medium for both treatments. (Table 2). These results corroborate those of Meguekam et al. [18]; Nouck et al. [33]; Hand et al. [6] and Ndouma et al. [8]. They showed that the reduction of photosynthetic capacity and water uptake of a plant under salinity can reduce the plant dry biomass. Applications of AMF have been shown to reduce the negative effects of NaCl and our results are in line with Kaya et al. [34] and Alqarawi et al. [9]. According to them, AMF provides an adequate supply of mineral nutrients, particularly

phosphorous. In this study, AMF improved root, shoot and ratio dry weight and growth parameters in general. It is proof of enhanced tolerance to salt stress, compared with the non-mycorrhizal plants.

Chlorophyll (a+b) content in the leaves of *P erosus* decrease significantly (p <0.001) from 100 mM NaCl for both treatments (Fig. 1) which is in accordance with Nouck et al. [33]; Hand et al. [6] and Ndouma et al. [8]. They reported that the decrease of chlorophyll content under salinity is due to the photoinhibitory damage caused by the activation of chlorophyll degradation by chlorophyllase and the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments. Higher chlorophyll content in leaves of *P. erosus* inoculated with mycorrhizal under saline conditions has been observed in this work according to Hashem et al. [10] Scagel and Bryla [11], AMF inoculation maintains the activity of photosynthetase under salinity conditions and increases photosynthetic activities. We can suggest that there is less salt interference with chlorophyll synthesis in the presence of AMF (Fig. 1).

The results obtained with minerals showed that Na increased significantly (p<0.01) with intakes doses of NaCl in the culture medium in both shoots and roots while other minerals (K, Ca and Mg) decreased significantly (p<0.01) at the same concentrations in both treatments (Table 3). According to Nouck et al. [33] and Hand et al. [6], Na in plants increased with increased salinity due to imbalance in the K/Na transporters and high salt concentration in the soil. Additionally, it can be caused by the loss of osmotic potential of root medium and the lack of nutritional ions. Plants treated with NaCl in combination with AMF showed less effect on ionic balance and mitigate the deficiency of K, Ca and Mg and also increases the K/Na ratio (Table 3). The results are in accordance with Beltrano et al. [35] and Alqarawi et al. [9]. They reported that AMF symbiosis plays a key role in higher K accumulation and hence higher K/Na ratio in mycorrhizal plants. AMF would attenuate the detrimental effects of salinity and enhance the absorption of K, Ca and Mg so as to reinstate ionic balance.

The biochemical constituents (PRO, CH, FAA and PR) of *P. erosus* in the leaves increased significantly (p<0.001) with increased intake doses of NaCl in the culture medium for both treatments (Fig. 2). Increasing proline with salt concentration in this study have been in line with works of Nouck et al. [3]; Hand et al. [6] and Ndouma et al. [8]. They showed that build-up of PRO is a mechanism of stress tolerance because its accumulation contributes to the acquisition of tolerance by maintaining turgor in cells of many species responsible for osmotic adjustment in tolerant plants grown under saline conditions. Several results reported by many authors showed that PRO concentration increased in AMF plants than in non-AMF plants under salinity Evelin et al. [17]; Abdel Latef and chaoxing [36] and Scagel and Bryla [11].

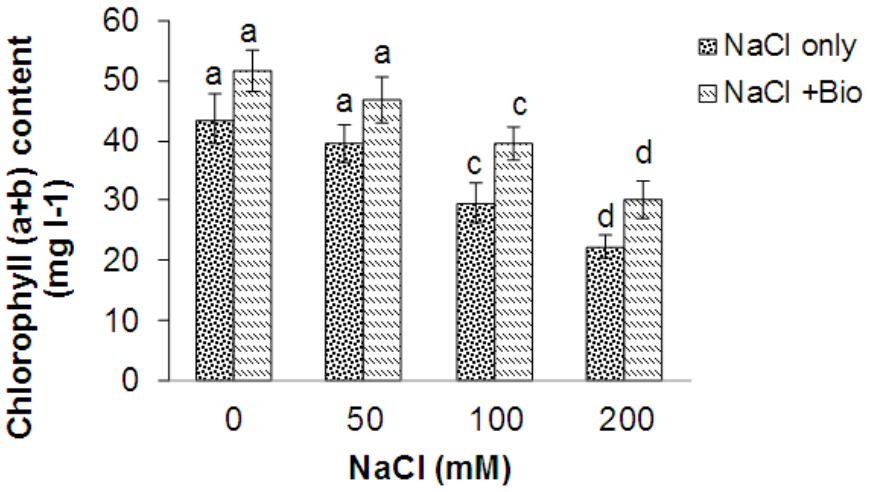

Fig. 1. Effects of arbuscular mycorrhizal biofertilizer application on chlorophyll (a+b) at different salt concentrations. Mean results of five replications ± SD

Table 2. Effects of arbuscular mycorrhizal biofertilizer application on growth and dry biomass (g) at different salt concentrations

		Concentrations	Plant dry weight (g plant-1)							
Cultivar	**Treatments**	**(mM NaCl)**	**RDW**	**SDW**	**TDW**	**RDW/SDW**	**LA**	**ST**	**NL**	**ND**
		0	2.3±0.03a	3.62±0.07a	5.92±0.08a	0.63a	7.45±0.04a	17.42±1.36a	6.2±0.44a	1.36±0.05a
		50	2.1±0.04a	3.16±0.04a	5.26±0.06a	0.66a	6.01±0.06b	15.03±0.27a	5.2±0.44a	1.23±0.01a
	NaCl	100	1.79±0.06c	2.52±0.1c	4.32±0.07c	0.71c	5.48±0.07c	11.70±0.42c	4.0±0.70c	0.62±0.06c
		200	0.67±0.1d	1.41±0.04d	2.10±0.10d	0.47d	4.15±0.07d	8.63±0.06d	2.8±0.83d	0.48±0.05d
P. erosus		0	2.71±0.11e	4.62±0.33e	7.33±0.26e	0.58e	9.16±0.40e	24.03±0.91e	8.6±0.54e	1.98±0.05e
		50	2.53±0.05f	4.29±0.12e	6.82±0.12f	0.59e	7.17±0.07a	21.94±1.79f	6.8±0.44f	1.68±0.04f
	NaCl + Bio	100	2.35±0.08a	3.65±0.15a	6.01±0.17g	0.64g	6.86±0.08g	16.20±1.02a	5.2±0.44a	1.37±0.03a
		200	1.51±0.12c	2.35±0.22c	3.86±0.2h	0.64g	5.9±0.05cg	12.01±0.89c	3.8±0.44h	0.92±0.04h
						Two way ANOVA results				
	NaCl		*	*	*	*	*	**	*	*
	Biofertilizer		*	*	**	*	*	**	*	*
	Biofertilizer × NaCl		*	*	*	*	*	*	*	ns

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different ($p < 0.05$). (= $p < 0.05$ and $p < 0.01$).*

Table 3. Effects of arbuscular mycorrhizal biofertilizer application on mineral uptake (µg g^{-1} MS) after four weeks at different salt concentrate

Cultivar	Treatement		Concentration	Mineral uptake (µg g-1 MS)				
			mM NaCl	Na^+	K^+	Ca^{2+}	Mg^{2+}	K^+/Na^+
		Roots	0	1.75±0.01a	10.69±0.69a	17.66±1.87a	19.94±2.81a	6.10a
			50	2.52±0.02b	5.15±0.20b	12.95±1.06b	16.44±1.72b	1.98b
	NaCl only		100	4.38±0.05c	3.05±0.04c	6.18±0.08c	10.51±1.38c	0.69c
			200	6.74±0.20d	1.64±0.01d	3.22±0.03d	6.38±1.60d	0.24d
		shoots	0	2.12±0.08b	28.91±2.60e	41.13±3.51e	52.39±3.24e	14.10e
			50	5.34±0.18c	23.74±2.44f	33.01±2.09f	42.19±3.14f	4.44f
			100	9.42±0.83g	19.28±1.92g	26.58±2.60g	32.38±2.98g	2.04b
P. erosus			200	18.39±1.56h	14.48±1.71h	20.61±1.70h	24.06±2.59h	0.78c
		Roots	0	1.93±0.04a	12.69±2.32ah	19.49±1.77ai	22.36±1.07i	6.57a
			50	3.09±0.06j	7.92±2.03j	15.44±1.61a	19.56±0.99a	2.56j
	NaCl + Bio		100	6.17±0.02d	4.23±0.06b	10.01±0.05k	14.59±0.81b	0.68c
			200	8.41±0.30g	2.05±0.07d	6.22±0.02c	9.36±1.04c	0.24d
		shoots	0	2.52±0.04b	31.8±2.73m	50.53±4.10m	57.17±3.14m	12.61e
			50	7.52±0.08d	26.31±2.12e	40.65±3.74e	49.66±3.56e	3.49f
			100	13.98±1.33o	21.01±1.55f	30.53±2.90f	37.50±2.75o	1.50b
			200	22.77±1.69p	17.03±2.20g	24.52±2.61g	29.38±2.27p	0.74c
					Two way ANOVA results			
	NaCl			**	**	**	**	*
	Biofertilizer			*	*	*	**	*
	Biofertilizer × NaCl			*	*	*	*	*

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different. ($p < 0.05$). (= $p < 0.05$ and $p < 0.01$).*

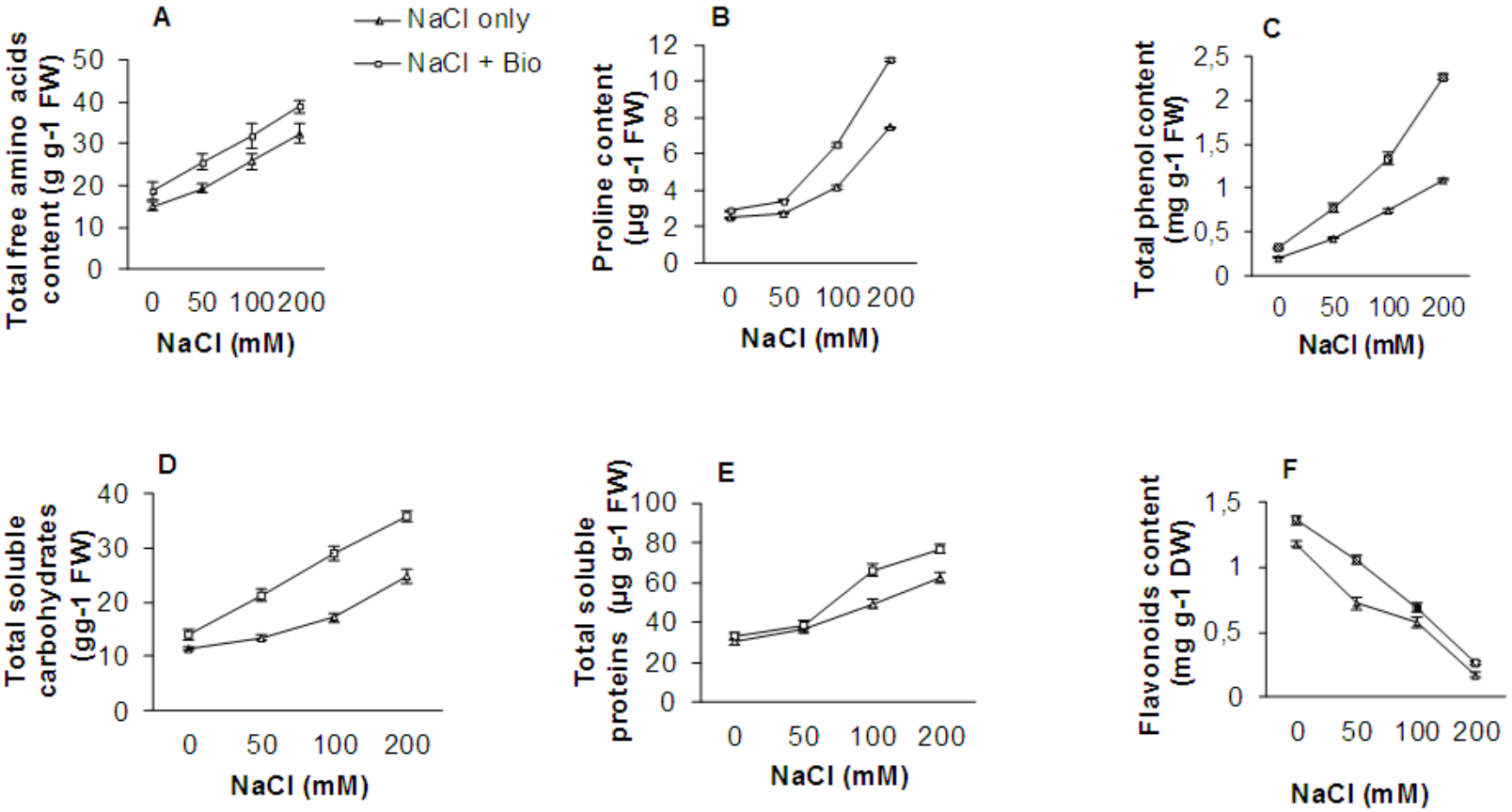

Fig. 2. Effects of arbuscular mycorrhizal biofertilizer application on metabolites at different salt concentrations. A: FAA; B: PRO; C: TP; D: CH; E: PR and F: FLA. Bars are means (n= 5) ± SD

Table 4. Changes in yield components measured as flowering time; number of flowers per plant; number of pods per plant; yield and Harvest index after addition of 0 and 50 mM NaCl and arbuscular mycorrhizal biofertilizer application

			Yield components				
Cultivar	**Treatments**	**Concentrations**	**Flowering time**	**Number flowers**	**Number of pods**	**Yield**	**Harvest**
		(mM NaCl)	**(Week)**	**per plant**	**per plant**	**(t/hectare)**	**index (%)**
		0	25.2±1.09a	16.6±0.44a	13.2±0.83a	10.54±0.75a	0.52±0.05a
	NaCl only	50	21.4±1.94b	10.4±0.54b	7.33±0.17b	7,28±0.68b	0.43±0.02b
P. erosus		0	20.2±1.48b	18.6±1.14c	16.08±0.45c	15.21±1.25c	0.71±0.06c
	NaCl + Bio	50	17.6±0.54d	14.2±0.83d	12.2±0.44a	13.15±1.05d	0.63±0.05d
				Two way ANOVA Result			
	NaCl		*	*	*	**	*
	Biofertilizer		**	**	**	***	*
	Biofertilizerer × NaCl		*	*	*	*	*

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different. ($p < 0.05$). (* = $p < 0.05$; ** = $p < 0.01$ and. *** = $p < 0.001$).

The same observations were made in this study suggesting that proline accumulation in plants may be a responsive strategy in less salt-tolerant species. In addition this accumulation contributed to improve and protect photosynthetic activities Abdel Latef and chaoxing [36] and Scagel and Bryla [11]. Total soluble carbohydrates, total free amino-acids and proteins increased with increase in salinity. According to Khosravinejad et al. [15] and Nouck et al. [33] regulatory osmotic adjustments of the salt stress was due to the accumulation of CH, FAA and PR in plant tissues. These osmoprotectants were accumulated more in AMF than non-AMF plant under salinity (Fig. 2). This can be due to the beneficial role of AMF in enhancing the stress tolerance by contributing to maintenance of cellular water content, nutrient uptake, the photosynthetic pigments and maintains the osmotic and ionic balance of the cell Abdel Latef and chaoxing [36] and Scagel and Bryla [11]. Total phenol increased significantly (p<0.001) with concentration of salt. This result correlates with those of Ndouma et al. [8] and Nouck et al. [3]. According to these authors, high accumulation of phenol in plants is physiologically important in overcoming salinity-induced oxidative stress Meguekam et al. [18]. The flavonoids results are contrary to phenol content, flavonoids decreased with increasing salinity. This can be explained by the fact that, the biosynthesis of flavonoids, especially flavonol is stimulated by the changes of the cellular redox homeostasis as MYB (myeloblastosis) transcription factors, involved in flavonol biosynthesis and regulated by varied cellular redox potentials. Our result is in line with those of Hand et al. [6] in *Capsicum annuum*. According to him, the inhibition effects of salt stress on flavonoid is due to the reduction of functions like enzymatic activities caused by the detrimental effects of salinity on polyphenols which contribute to reduce scavenging free radicals or detoxify ROS in the plants which are of primary significance in plants suffering from severe stress conditions. The non-enzymatic antioxidants (FLA and TP) produced in American yam bean that received AMF increased significantly (p <0.001). Hashem et al. [37] reported that AMF enhanced the accumulation of non-enzymatic antioxidants which assisted to counter the effects of stress on the plants.

Agronomic parameters decreased in the field at 50 mM NaCl compared with control in all treatments. Flowering time and number of flowers per plant significantly decreased in the presence of NaCl for both treatments (Table 4). These results are in line with those of Nouck et al. [33] on *Lycopersicum esculentum* L. According to him, salinity might have reduced the flowering time and the number of flowers per plant due to loss of photosynthetic capacity, which is a limiting factor to the supply of carbohydrate for plants growth. A good response was obtained with plant treated with AMF confirming the beneficial role of AMF in enhancing the stress tolerance by contributing to maintenance of cellular water content. The same results were seemed in the number of pods per plant and the root yield. These results are supported by those of Nouck et al. [33] who mentioned that salinity might have reduced the production of crop by overturning water and nutritional balance of plant and loss of photosynthetic capacity. The latter is limiting factor to the supply of carbohydrate for plant grow. The number of pods per plant and the root yield were more in AMF than non-AMF plants under salinity (Table 4). These results corroborate with those of Evelin and Kapoor [1] Beltrano et al. [35] and Scagel and Bryla [11]. They showed that AMF enhanced the stress tolerance by contributing to maintenance of cellular water content, improving productivity. Harvest index increased with the addition of biofertilizers compared to the treatment with NaCl only, this could be attributed to the production of phytohormones by biofertilizers.

4. CONCLUSION

At the end, the results revealed that American yam bean was negatively affected by NaCl stress in the culture medium. The growth parameters, the dry biomass, the mineral uptake, Chlorophyll (a+b) decreased with increasing intake doses of NaCl from 100 mM NaCl while Na and osmolytes increased from 50 mM NaCl. AMF enhanced the accumulation of all the study parameters than non AMF plant under salinity. Agronomic parameters were improved from 50 mM NaCl in the treatment with AMF than treatment with NaCl only in the field. The AMF appeared to alleviate the impacts of salinity on the American yam bean resulting to significant improvements. The high accumulation of osmolytes with salinity doses could be added as indicators of early identification and osmotic adjustment ability for salt-tolerant plants in salt stress conditions. The American yam bean could be cultivated in the soil with moderate salinity. Using AMF as an alternative way of decreasing the NaCl stress in

plants will be more beneficial as it maintains the soil fertility and the yield.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Evelin H, Giri B, Kapoor R. Contribution of Glomus intraradices inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. Mycorrhiza. 2012;22: 203–217.
   DOI:10.1007/ s00572- 011- 0392- 0.
2. Augé RM, Toler HD, Saxton AM. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza. 2015;25:13–24.
   DOI: 10.1007/s00572- 014- 0585- 4.
3. Nouck AE, Hand MJ, Numfor EN, Ekwel SS, Ndouma MC, Wiraghan SE, Taffouo VD. Growth, mineral uptake, chlorophyll content, biochemical constituents and non-enzymatic antioxidant compounds of white pepper (*Piper nigrum* L.) grown under saline conditions. Inter J Biol Chem Sci. 2021; 15(4):1457-1468.
   DOI: 10.4314/ijbcs.v15i4.13.
4. Kinsou E, Amoussa A M, Mensah ACG, Kpinkoun JK, Komlan FA, Ahissou H, Lagnika L, Gandonou C B. Effet de la salinité sur la floraison, la fructification et la qualité nutritionnelle des fruits du cultivar local Akikon de tomate (*Lycopersicon esculentum* Mill.) du Bénin. Int. J. Biol. Chem. Sci. 2021;15(2):737-749.
   DOI:https://dx.doi.org/10.4314/ijbcs.v15i 2.27.
5. Abdel-Fattah GM, Hassan Rabie G, Shaaban Lamis D, Metwally D. The Impact of the Arbuscular Mycorrhizal Fungi on Growth and Physiological Parameters of Cowpea Plants Grown under Salt Stress Conditions. Int J Appl Sci Biotechnol. 2016;4(3);372-379.
   DOI: 10.3126/ijasbt.v4i3.15775.
6. Hand MJ, Taffouo VD, Nouck AE, Nyemene KPJ, Tonfack LB, Meguekam TL, Youmbi E. Effects of Salt Stress on Plant Growth, Nutrient Partitioning, Chlorophyll Content, Leaf Relative Water Content, Accumulation of Osmolytes and Antioxidant Compounds in Pepper (*Capsicum annuum* L.) Cultivars. Not Bot Horti Agrobo. 2017;45(2):481-490.
   DOI:10.15835/nbha45210928.
7. Elhindi KM, Sharaf El-Din A, Elgorban AM. The impact of arbuscular mycorrhizal fungi in mitigating salt- induced adverse effects in sweet basil (*Ocimum basilicum* L.). Saudi J Biol Sci; 2016.
   DOI:10.1016/j.sjbs.2016.02.010.
8. Ndouma MC, Nouck AE, Titah MA, Ndjouondo GP, Ekwel SS, Fotso, Taffouo VD. Growth parameters, mineral distribution, chlorophyll content, biochemical constituents and non-enzymatic antioxidant compounds of white yam (*Dioscorea rotundata* (L) var. gana) grown under salinity stress. GSC Biol Phar Sci. 2020;12(03):139-149.
   DOI: 10.30574/gscbps.2020.12.3.0286.
9. Alqarawi AA, Abd_Allah EF, Hashem A. Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. J Plant Interact. 2014;9(1): 802–810.
   Avilable :https://doi.org/10.1080/17429145. 2014.949886.
10. Hashem A, Abd_Allah EF, Alqarawi AA, Egamberdieva D. Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill by arbuscular mycorrhizal fungi. Saudi J Bio Sci. 2016;23:39–47.
    Available:
    https://doi.org/101016/j.sjbs.2015.11.007.
11. Scagel C F, Bryla D R. Salt exclusion and mycorrhizal symbiosis increase tolerance to nacl and CaCl2 salinity in 'Siam Queen' basil. Hortic Sci, 2017. 52(2):278–287.
    Available:https://doi.org/10.21273/ HORTSCI11256-16.
12. Kolupaev YE, Yastreb TO, E AI. Oboznyi IA, Ryabchun IN, Kirichenko VV.

Constitutive and cold-induced resistance of rye and wheat seedlings to oxidative stress. Russ J Plant Physiol. 2016; 63(3): 326-337.
DOI:10.1134/S1021443716030067.

13. Khalil1 C, Abdelmajid H, Mohammed E H, Houssein B E. Growth and proline content in NaCl stressed plants of annual medic species. Inter J Adv Res Biol Sci. 2016;3(9):82-90.
DOI: 10.22192/ijarbs.
14. Singh P, Singh U, Eggum BO, Kumar KA, Andrews DJ. Nutritional evaluation of high protein genotypes of pearl millet (*Pennisetum americanum* (L) Leeke). J. Sci. Food Agric. 1987;38(1): 41-48.
Available:https://doi.org/10.1002/jsfa.2740380108.
15. Khosravinejad F, Heydari R, Farboodnia T. Effect of salinity on organic solutes contents in barley. Pak J Biol Sci, 2009;12(2):158-62.
DOI: 10.3923/pjbs.2009.158.162.
16. Yang X, Liang Z, Wen X, Lu C. Genetic engineering of the biosynthesis of glycine betaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. Plant Mol Biol. 2008;66:73–86.
DOI: 10.1007/s11103-007-9253-9.
17. Evelin H, Kapoor R, Giri B. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot, 2009;104: 1263–1280.
Available:https://doi.org/10.1093/aob/mcp251.
18. Meguekam TL, Taffouo VD, Grigore MN, Zamfirache MM, Youmbi E, Amougou A. Differential responses of growth,chlorophyll content, lipid perodxidation and accumulation of compatible solutes to salt stress in peanut (*Arachis hypogaea* L.) cultivars. Afr. J. Biotechnol, 2014;13(50):4577-4587.DOI: 10.5897/AJB2014.14248.
Udemezue J C, Onwuneme N A. Economic Analysis of American Yam Bean and Implications for Food Security in Nigeria, Mod Appl Bioequiv Availab. 2017;2(4):MABB.MS.ID.555592
DOI: 10.19080/MABB.2017.02.555592.
20. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. University of California, College of Agriculture, Berkley. 1950; 1-34.
21. Taillièz, Ballo Koffi. Une méthode de mesure de la surface foliaire du palmier á huile. Oléagineux, 1992; 47(8-9): 537-553.
22. Pauwels J M, Van Ranst E, Verloo M, Mvondo Z A. Analysis Methods of Major Plants Elements. Pedology Laboratory Manual: Methods of plants and Soil Analysis. Stock Management Equipment of Worms and Chemical Equipment. AGCD, Agriculture Publications : Brussels. 1992:1-28.
23. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenylodase in *Beta vulgaris*. Plant Physiol. 1949; 24 (1): 1-15.
DOI: 10.1104/pp.24.1.1.
24. Bates L, Waldren RP, Teare I D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39 (2):205-207.
DOI:doi.org/10.1007/BF00018060.
25. Dubois M, Gilles K A, Hamil J K, Rebers P A, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chemistry. 1956; 28(3): 350-356.
DOI :doi.org/10.1021/ac60111a017.
26. Bradford M M. 1976. A rapid and sensitive method for quantitation of microgram of protein utilizing the principle of protein- dye binding. Anal Biochem. 1976;72(1-2):248-254.
DOI:doi.org/10.1016/0003-2697(76)90527-3.
27. Yemm EW, Cocking EC. The determination of amino acids with ninhydrin. *The Analyst*. 1955; 80: 209-213.
DOI: 10.1039/an9558000209.
28. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food. Drug. Anal. 2002;10(3):178-182.
DOI: 10.38212/2224-6614.2748.
29. Marigo G. On a fractionation method and estimation of the phenolic compounds in plant. Analysis. 1973; 2(2): 106-110.
30. Bijalwan A, Manmohan J R D. Productivity of Wheat (*Triticum Aestivum*) as Intercropin *Grewia Optiva* Based Traditional Agroforestry System along Altitudinal Gradient and Aspectin MidHills of Garhwal Himalaya, India. Americ J Environ Prot. 2014;5(2): 89-94.
DOI:10.12691/env-2-5-3.
31. Abdel Latef A A H. Ameliorative effect of calcium chloride on growth, antioxidant enzymes, protein patterns and some

metabolic activities of canola (Brassica napus L.) under seawater stress. J Plant Nutri. 2011; 34:1303–1320.
Available:https://doi.org/10.1080/01904167

32. Kosová K, Prášil I T, Vítámvás P. Protein Contribution to Plant Salinity Response and Tolerance Acquisition. Inter J Mol Sci. 2013;14(4):6757-6789.
DOI: 10.3390/ijms14046757.
PMID: 23531537. PMCID: PMC3645664.
33. Nouck AE, Taffouo VD, Tsoata E, Dibong SD, Nguemezi S T, Gouado I, Youmbi E. Growth, Biochemical Constituents, Micronutrient Uptake and Yield Response of Six Tomato (*Lycopersicum esculentum* L.). *J. Agro*. 2016;**15** (2):58-67.
DOI: 10.3923/ ja.2016.
34. Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna A L, Cullu M A. The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. Sci Hort. 2009;121:1-6.
Available:https://doi.org/10.1016/j.scienta.2009 .01.001.
35. Beltrano J, Ruscitti M, Arango M C and Ronco M. Effects of arbuscular mycorrhizal inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. J Soil Sci Plant Nutr. 2013;13(1): 123-141.
Available: http://dx.doi.org/10.4067/S0718-95162013005000012.
36. Abdel Latef A A, Chaoxing H Does the inoculation with *Glomus mosseae* improve salt tolerance in pepper plants?. 2014; 33 (3): 644-653. J Plant Growth
37. Hashem A, Alqarawi A A, Radhakrishnan R, Al-Arjani A F, Aldehaish H A, Egamberdieva D, Allah E F A. Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. Saudi J Biol Sci. 2018;25(6):1102–1114.
Available:https://doi.org/10.1016/j.sjbs.2018.03.009.Regulat.

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