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INFLUENCE OF SALT STRESS ON GROWTH, LEAF PIGMENTATION, IONIC DISTRIBUTION AND METABOLITES ACCUMULATION OF TALINUM TRIANGULARE (JACQ.) WILLD. LEAFY VEGETABLES AS AFFECTED BY DIFFERENT CASSAVA PEEL LEVELS

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Abbreviations: Ascorbic acid-ASA; calcium-Ca; chlorophyll-CHL; leave dry weight-LDW; leave fresh weight-LFW; leaf relative water content-LRWC; leave yield-LY; magnesium-Mg; nitrogen-N; number of leaf-NL; organic carbon-C; phosphorus-P; plant height-PH; potassium-K; sodium-Na; soluble proteins-SP; stem diameter-SD; sulfate-S; total flavonoids content-TFC; total leaf area-TLA; total phenolic-TP; total soluble sugars-TSS; total tannin content-TTC; week after sowing-WAS.

Abstract

Talinum triangulare Jacq. (Water leaf) is a leafy vegetable eaten in most countries in Africa in preparation of soups to enrich the starchy main dishes due to its tastes, medicinal uses such as antiinflammation, anti-fungal, anti-bacterial properties. Impact of four levels of cassava peel (0, 9, 12 and 15 t ha^{-1}) in water leaf in terms of leaf pigment, nutrients, matabolites, antioxidant compounds, minerals, growth and yield under salt stress (0, 50, 100 and 200 mM NaCl) were investigated. Results show that salinity decreased growth parameters, total photosynthetic pigments, ascorbic acid and yield components accompanied by increases in sodium content, carbohydrate, soluble proteins, tannins total phenolic content and flavonoid in the leaf of water leaf. On the other hand, all applied treatments cassava peel caused significant increases in most parameters under investigation as growth parameters, minerals content, all components of photosynthetic pigments, and leaf yield, metabolites. It is obvious that cassava peel treatment at 15 t ha^{-1} was the most pronounced treatment either under non saline conditions or under salt stress conditions. Increased osmolyte and metabolite accumulation, and redox components in CP supplemented plants regulated the NaCl tolerance by further strengthening the antioxidant mechanisms. The increasing CP supplementation regulates salt tolerance in T. triangulare through modulations in the metabolism of antioxidants, osmolytes and metabolites.

Keywords: *cassava peel, leaf pigmentation, metabolites, salt stress, Talinum triangure.*

1. INTRODUCTION

Water leaf (*Talinum triangulare Jacq.*) is a plant to the family *Taliniaceae* and commonly found in humid tropics. It is an erect glabrous perennial herb (80-100 cm tall), usually strongly branched; roots are swollen and fleshy. The leaves are alternate, simple, almost sessile and succulent (Oluwole *et al.,* 2018). Water leaf is eaten as vegetables many countries in Africa especially in preparations of slightly shiny soups and stews to support the starchy main dish. In Cameroon, where it is used in the preparation of Eru and as treatment for measles but in Asia (India), it is used for treating diabetes. It is also used in treating common diseases such as contusion, inflammations and tumors; decoctions are used for painful eyes and to aid recovery from blows and falls (Aja *et al.,* 2010; Ezekwe *et al.,* 2013). Water leaf has been made into drugs such as tonic from its roots. It is often used as an ornamental plant or edging plants in gardens. Water leaf shows a whole range of medicinal properties such as anti-inflammation, anti-fungal and anti-bacterial properties (Oluwole *et al.,* 2018). Water leaf grows better during raining season but slows down considerably during the dry season. They are mostly propagated through stem cuttings (10-15cm) but seed germination is inevitable (Ayoola *et al.,* 2009).

Climate change is a monumental factor which affects all living organisms (plants and animals). Thus, one of its effects is seen in salt stress faced by the plants. Salinity is one of the major abiotic stressors which limits crop production and poses a serious threat to global food security. Approximately, 20% percent of the arable land and 50% of total irrigated land have varying levels of salinity (Abogadallah, 2010). Salinity stress induces a multitude of adverse effects on plants including morphological, physiological, biochemical, and molecular changes. It affects plant growth and development by creating osmotic stress, causing specific ions (Na^+ and Cl^-) toxicity, stomatal closure, and reducing the rate of photosynthesis (Munns and Tester, 2008). All these physiological changes in plant under salinity aggravate overproduction of reactive oxygen species (ROS) that interferes normal cellular metabolism and results in oxidative damage by oxidizing proteins, lipids and DNA and other cellular macromolecules (Gill and Tuteja, 2010). Plants have an excellent network of ROS detoxification system including, either non-enzymatic through protein, carbohydrate, ascorbic acid (AsA), β carotene and carotenoids, phenolic compounds and flavonoids or through enzymatic antioxidants, such as superoxide dismutase, peroxidase, catalase, and peroxidase (Gill and Tuteja, 2010). Salinity tolerance mechanisms in plants are remarkably varied among the species and even within different accessions of a species. Various factors such as biological, environmental, biochemical, physiological, ecological and evolutionary processes, and salinity are involved in the quantitative and qualitative improvement of natural antioxidants in this vegetable crop (Selmar and Kleinwachter, 2013).

Bio-fertilizer has been described by Ogbo and Odo (2011) as living or latent cells of efficient strains of microbes. It uses as soil amendment have also been recognized. International Federation of Organic Agriculture Movements (IFOAM, 2005) accepts the view that bio-fertilizer can be used as inputs for organic cropping. Agricultural waste and various other types of materials have been research on as carrier materials for bio-fertilizer production (Kannaiyan, 2002). Agricultural waste like cassava peel are of huge quantity in the developing countries. FAO, Food and Agricultural Organization of the United Nations data proves 230 million tons of cassava (*Manihot esculenta* Crantz) production geographically pointed to the developing countries. Although numerous research findings about utilization of the waste as animal feed is on record, but it can be easily observed that large quantity of it is found in heaps, around processing facility, deposited in locations such as water ways where it ends-up polluting and contaminating the atmosphere, underground including surface water bodies (Kalu *et al.,* 2009; Onwudike *et al.,* 2016). Since Cassava peel is abundant and practically of no economic value in several developing countries, then the need arises to put this waste into more productive and gainful utilization.

Thus, this study determine the effects of Cassava peel on the growth, yield and leaf pigmentation, ionic distribution, accumulation of metabolites and antioxidant compounds of *T. triangulare* under salt stress and the best organic manure that will be best suitable for the cultivation of *Talinum triangulare*.

2. Materials and methods

2.1. Site description

The field experiments were conducted during the 2020 and 2021 cropping seasons at University of Douala research farm (4°01N, 9°44 E, 13 m.a.s.l.), in the coastal region of Cameroon. The climate belongs to the equatorial domain of a particular type call Cameroonian characteristics by two seasons with a lengthy rainy season (at least 9 months), abundant rainfalls (about 3597 mm per year), high and stable temperatures (26.7 °C). The relative humidity remains high the whole year and near to 100%. The soil of the experimental site is classified as yellow ferralitic soil.

2.2. Plant growth conditions and treatment

Talinum triangulare Jacq. has been used as plant material. Seeds were provided by the breeding program of the TECHNISEM (SEMAGRI, Douala). The seeds were surface sterilized with 3% sodium hypochlorite for 20 min and washed four times with deionized water. The seeds were planted in cavity trays in the greenhouse of the Faculty of Science at University of Douala, Cameroon, on 4th June 2021 and transplanted when seedlings reached 8 cm in height into the prepared polythene bags containing 5 kg of sterilized soil. Each pots of seven litres capacity perforated at the bottom to allow unimpeded drainage. The pots were arranged in a complete randomized design with one plant per pot and four replicates per treatment. The plants were watered immediately after transplanting to avoid drought stress. All plants were fertilized daily with a modified nutrient solution (in g L^{-1}): 150 g $Ca(NO_3)_2$, 70 g KNO_3, 15 g Fe-EDTA, 0.14 g KH_2PO_4, 1.60 g K_2SO_4, 11 g $MgSO_4$, 2.5 g $CaSO_4$, 1.18 g $MnSO_4$, 0.16 g $ZnSO_4$, 3.10 g H_3BO_4, 0.17 g $CuSO_4$ and 0.08 g MoO_3 (Hoagland and Arnon, 1950). The pH of the nutrient solution was adjusted to 7.0 by adding HNO_3 0.1 mM. For the determination of agromorphological, physiological and biochemical responses to salt stress, of *Talinum triangulare Jacq.*was subjected to 0 (control), 50, 100 and 200 mM NaCl. Plants were watered with deionized water every morning.

The amendment in each case was applied 6 WAS with four fertilization rates (0, 9, 12 and 15 t h^{-1}) each of cassava peel (CP) and 250 kg h^{-1} of NPK (15 – 15 – 15). Fresh Cassava peel was obtained from Cassava processing facilities at various locations within Douala, Cameroon. The Cassava peel was dried at 50 °C, grinded coarsely in local mill to particles ranging 0.1 – 1.5 mm, the material was sterilized by putting it into an autoclave. Selected cassava manure chemical properties are shown in Table 2. Phytosanitary treatment has been realised with Emamectine bezoate (Insecticide) used before transplantation of plants; Thiophanate-methyl (fongicide).

2.3. Soil moisture content determination, cassava peel sampling and analysis

Soil samples were collected from representative spots on the experimental site from where soil was collected for potting using soil auger to a depth of 20 cm, the samples were made into a sample. A sub-sample was taken, air-dried, crushed and sieved with 2 mm mesh sieve after which physical and chemical analyses were carried out (Table 1). The following chemical analyses were done on the soil, tap water and cassava peel (Tables 1, 2 and 3). Organic carbon (C), was determined by the wet oxidation procedure (Walkley and Black, 1934) and total Nitrogen (N) by micro-Kjeldahl digestion method. Magnesium (Mg) was extracted using the Mehlich 3 method and determined by auto ANALYSER 5Technicon 2). The total and available soil phosphorus (P) were determined by the method of Okalebo et al (1993). Soil was measured potentionmetrically in 1:2.5 soil: water mixture. Calcium (Ca), potassium (K) and sodium (Na) were determined by aflame photometer (JENWAY) as described by Taffouo et al (2008). Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, SO_4^{2-}, NO_3^{-}, Cl^{-} content in water tap was determined by using colorimetric amperometric titration method (Taleisnik *et al.,* 1997) (Table 3). Electric conductivity and pH were determined by conductometer.

Table 1. Physical and chemical characteristics of soil used

Physio-chemical properties	Quantity
Clay %	14.21 ± 1.98
Coarse sand%	27.93 ± 2.76
Fine sand%	25.72 ± 2.58
Coarse silt %	26.02 ± 2.71
Fine silt %	6.31 ± 0.53
Total carbon %	0.77 ± 0.09
Total nitrogen %	0.33 ± 0.06
Ratio C/N %	2.33 ± 0.08
Moisture (%)	1.01 ± 0,12
Phosphorus (ppm)	4,82 ± 0.17
Potassium (g kg^{-1})	0.26 ± 0.08
Sodium (g kg^{-1})	2.8 ± 0.19
Calcium (g kg^{-1})	0.33 ± 0.08
Magnésium (g kg^{-1})	0.17 ± 0.07
pH	8.47 ± 0.62
EC dS/m	2.24 dS^{-1}

Table 2. Physical and chemical characteristics of cassava peel (CP)

Physical and chemical characteristics

Nutrient source	Fat (mg kg^{1})	Carbohydrate (mg kg^{-1})	Protein (mg kg^{1})	Moisture (%)	Porosiy (%)	P (%)	K (%)	N (%)
CP	3.1	64.63	8.2	0.016	8.03	1.23	0.88	3.11

Table 3. Chemical characteristics of irrigation water

Chemical characteristics

Irrigation Water	Ca^{2+} (mg g^{-1})	Mg^{2+} (mg g^{-1})	K^{+} (mg g^{-1})	HCO_3^{-} (mg g^{-1})	Na^{+} (mg g^{-1})	SO_4^{2-} (mg g^{-1})	Cl^{-} (mg g^{-1})	Ph	CE (dS m-1)
Tap water	233.2	116.8	23.4	61.7	438.1	518.8	26.1	7.31	1.34

2.4. Plant Measurements and leaf pigmentation

Seedlings were harvested 14 WAS by carefully removing and washing the soil particles from the roots, after which the plants parts were separated into shoots and roots (Metwally *et al.,* 2013). The tissues (leave and stem) were dried for 24 h at 105˚C (Taffouo *et al.,* 2008). The dry samples were weighted. Plant samples were harvested after 14 weeks of culture and under 10 weeks of water stress, plant were collected to determine agro morphological characters (number of leave per plant, stem diameter, total leaf area, leave fresh weight, leave dry weight, plant height, leave yield) in *Talinum triangulare Jacq.*

The relative water content (RWC) in leaf was recorded according to the formula as follows: RWC = (FFW - FDW)/ (TW - FDW) × 100, where FFW is fresh weight, FDW is dry weight, and TW is turgid weight and TLA (length × width × 0.80 × total number of leaves × 0.662) were calculated using the methodology described by Kumar et al (2002). Fresh leaves were extracted with acetone (80%) (Sigma-Aldrich Co. LLC) and placed for one hour at 5 ºC. The extractions were centrifuged for 15 min at 3,000 x *g*. Chlorophyll *a*, *b*, and carotenoids content were measured with a spectrophotometer

(Helios UVG1702E, England) at wave lengths 663, 647, and 470 nm, respectively. Chlorophyll was measured according to the method of Lichtenthaler and Wellburn (1983). The calculation was done according to the following equations:
Chlorophyll *a* = 12.70 × A663 – 2.79 × A647
Chlorophyll *b* = 20 × A647 – 4.62 × A663
Total chlorophyll = Chlorophyll *a* + Chlorophyll *b*
The contents of carotenoids were determined using a procedure previously described by Nagata and Yamashita (1992). The absorbance of extracts was measured at 453, 505, 645 and 663 nm and the contents of carotenoids (β-carotene and lycopene) were calculated according to the following equations, and further expressed in mg/100 g of fresh weight:
β- carotene (mg/100 mL) = 0.216 × *A*663 - 1.220 × *A* 645 - 0.304 × *A*505 + 0.452 × *A*453;
Lycopene (mg/100 mL) = - 0.0458 × *A*663 + 0.204 × *A*645 - 0.304 × *A*505 + 0.452 × *A*453;

2.5. Metabolites contents

For measurement of total soluble sugar (TSS), a modified phenolsulfuric assay was used (Dubois *et al*., 1956). Subsamples (100 mg) of dry leaves were placed in 50 mL centrifuge tubes. 20 mL of extracting solution (glacial acetic acid: methanol: water, 1:4:15 (v/v/v)) was added to the ground tissue and homogenized for 15 sec at 16000 rpm. The homogenate was centrifuged for 10 mn and the supernatant was decanted to a 125 ml Erlenmeyer flask. The residue was resuspended in 20 mL of extracting solution and centrifuged another 5 min. The supernatant was decanted, combined with the original extract, and made up to 100 mL with water. One mL of 5% (v/v) phenol solution and 5 mL of concentrated H_2SO_4 were added to 1 mL aliquots of SS (reconstituted with 1 mL water). The mixture was shaken, cooled to room temperature, and absorbance recorded at 490 nm wavelength with spectrophotometer (Pharmaspec UV-1700 model). The amount of TSS present in the extract was calculated using standard curve prepared from graded concentration of glucose.
Soluble protein content (SP) was determined by Bradford's method (1976). Briefly, appropriate volume (from 0 - 100 µl) of sample was aliquoted into a tube and the total volume was adjusted to 100 µl with distilled water. A 1 ml of Bradford working solution was added to each sample well. Then the mixture was thoroughly mixed by vortex mixer. After left for 2 min, the absorbance was read at 595 nm. The standard curve was established by replacing the sample portions in the tubes with proper serial dilutions of bovine serum albumin.
Total tannin content (TTC) estimation was done by using the FC technique described by (Shad *et al.*, 2012) with modifications. The plant extract (1 ml) was diluted with 49 ml of distilled water, 0.1 ml metaphosphoric acid, 1.7 ml 75% ethanol, 2.5 ml of FC, and 10 ml of (1.0 mol/ml) Na_2CO_3 in a 100 ml volumetric flask. The mixture was shaken properly and kept for 15 min at room temperature. The absorbance of sample mixtures and standard solutions were measured against the blank in a spectrophotometer (UV 2700, Shimadzu, Japan) at 680 nm. As a reference, tannic acid (TA) was used, and therefore the total tannin content in the plant extract was reported as equivalent of TA (mg TA/g DW) based on standard curve ($R2$ = 0.9972).
For estimation of ascorbic acid content (ASA), 1 g of frozen leaf tissues was homogenised in 5 mL of ice-cold 6% m-phosphoric acid (pH: 2.8) containing 1 mM EDTA (Gossett *et al.*, 1994). The homogenate was centrifuged at 20,000 × g for 15 min at 4˚C. The supernatant was filtered through a 30-µm syringe filter, and 50 µL of the filtrate was analyzed using an HPLC system (PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5-µm column (Spheri-5 RP-18; 220 × 4.6 mm; Brownlee) and UV detection at 245 nm with 1.0 mL/min water (pH: 2.2) as the mobile phase, run isocratically (Gahler *et al.*, 2003)
Total phenolic (TP) content of the extract was determined by the Folin Ciocalteu method (Marigo, 1973). Subsamples (1 g) of fresh leaves were ground at 4 °C in 3 mL of 0.1 N HCl. After incubation to 4 °C during 20 min, the homogenate was centrifuged at 6000 g during 40 min. The supernatant was collected, the pellet re-suspended in 3 mL of 0.1 N HCl and centrifuged as previously. The two supernatant 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 min and absorbance read at 720 nm wavelength with a spectrophotometer (Pharmaspec UV-1700 model). A standard curve was established using chlorogenic acid. TP content was expressed as mg g^{-1} fresh weight.
Total flavonoids content TFC content of crude extract was determined by the aluminium chloride colorimetric method (Chang *et al.*, 2002). 50 µL of crude extract (1 mg/mL ethanol) were 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 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were 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 min, and absorbance was recorded on spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a rutin calibration curve, and the result was expressed as g rutin equivalent per g dry weight.

2.6. Ions distribution

K, Ca, Na and Mg contents in the leaf tissue of the plants were evaluated in dry, ground, and digested samples in a CEM microwave oven (Abreu *et al.*, 1995). Potassium by flame photometry; magnesium, sodium and calcium by atomic absorption spectrometry (Malavolta *et al.*, 1997). Iron and zinc contents were determined by method reported in (Pauwels *et al.*, 1992). Leaf of water leaf was dry ashed at 450˚C for 2 hours and digested on heat cave with 10 ml HNO_3 1 M. The solution was filtrated and adjusted at 100 ml with HNO_3 at 1/100 and analyzed with an atomic absorption spectrophotometer (Rayleigh, WFX-100).

2.7. Experimental design and statistical analysis

The experiment was conducted as a factorial completely randomized design with four NaCl treatments and four levels of cassava peel in four replications. Data are presented in term of mean (±standard deviation). All data were statistically analysed using Statistica (version 9, Tulsa, OK, USA) and first subjected to analyses of variance (ANOVA). Statistical differences between treatment means were established using the Fisher LSD test at $p < 0.05$.

3. Results

3.1. Influence of cassava peel application on growth and yield characteristics under salinity stress

The changes in PH, NL, TLA, SD, LFW, LDW and LY of the *Talinum triangulare Jacq*. are presented in Table 4. The increase in NaCl doses in the nutrient solution reduced growth and yield characteristics. However, CP applications significantly enhanced the growth and yield components such as NL, HP, TLA, SD, LFW, LDW and TF under salt stress. The plants had a maximum PH, NL, TLA, SD, LFW, LDW and LY of 36.1 cm, 62.3, 56.1 cm^2, 3.34 cm, 52.2 g $plant^{-1}$, 18.2 g $plant^{-1}$ and 31.8 g kg^{-1} respectively with 15 t ha^{-1} CP and under non saline (0 mM NaCl). Salinity linearly decreased PH, NL, TLA, SD, LFW, LDW and LY, accounting for a 52.5%, 41.9%, 39.1%, 61%, 38%, 71.5% and 49.4% respectively from 0 to 200 mM NaCl without application of CP (Table 4).

3.2. Pigment synthesis and LRWC improved due to cassava peel supplementation under NaCl stress

LRWC, total chlorophyll, lycopene and *β*- carotene depended on NaCl treatments (Fig. 1). At higher NaCl concentration (200 mM NaCl), LRWC, total chlorophyll lycopene and *β*- carotene decreases significantly compared to control of 14.3%, 43.2%, 67.5% and 53.1% respectively (Fig. 1A, 1B, 1C and 1D). The maximum value of LRWC (90.4%), total chlorophyll (6.25 mg g^{-1} FW), lycopene (6.68 mg kg^{-1}) and *β*- carotene (9.76 mg kg^{-1}) were obtained at control. Contrary, the lowest content of LRWC (77.5%), total chlorophyll (3.55 mg g^{-1} FW), lycopene (2.17 mg kg^{-1}) and *β*- carotene (4.58 mg kg^{-1}) were detected at 200 Mm NaCl with non fertilizer (CP).

3.3. Cassava peel availability induces increasing of metabolites under salinity stress

Salinity stress significantly increased metabolites content and decreased ASA content in the leaves of *Talinum triangulare Jacq.* (Fig. 2). Application of NaCl from 0 to 200 mM without CP, significantly decreased ASA by 40.7% and increased TSS, TTC, TP and TFC by 31.1%, 77%, 45.2% and 26.4% respectively (Fig. 2). Metabolites (TSS, TTC, TP and TFC) content increased due to supplementation of CP and maximal accumulation was observed with higher dose (15 t ha^{-1}). Maximal content of 61.1 g kg^{-1} for TSS, 45.7 g kg^{-1} for SP, 30.5 mg kg^{-1} for TTC, 55.1 mg kg^{-1} for TP and for 886.5 mg kg^{-1} TFC was observed in 15 t ha^{-1} CP under the non 200 mM NaCl (Fig. 2A, 2B, 2C, 2E and 2F). Conversely, the highest content of ASA (394.2 mg kg^{-1}) was obtained at 15 t ha^{-1} CP and non saline condition (Fig. 2D).

3.4. Cassava peel application reduced Na accumulation and improved Mg, Ca, iron and K uptake

Salinity (from 0 to 200 mM NaCl) reduced the K, Mg, Ca and Iron content to 60.3%, 63.1%, 58.9% and 36.2% respectively and increased Na content to 492.4% (Table 5). The minerals (K, Mg, Ca and Iron) content was significantly increased by level of cassava peel. The maximum content of K, Mg, Ca and Iron (1903.1 mg kg^{-1}, 1895.1 mg kg^{-1}, 1676.3 mg kg^{-1} and 53.6 mg kg^{-1} respectively) were produced under non saline treatment and at 15 t ha^{-1} CP. Contrary, Na content significantly decreased with increasing CP. The highest value of Na (16.47 mg kg^{-1}) was obtained from the highest salt concentration (200 mM NaCl) under non fertilizer and the lowest content (1.72 mg kg^{-1}) was produced under non saline and at 15 t ha^{-1} CP (Table 5).

4. Discussion

4.1. Effect of cassava peel on growth, yield plant, leaf pigmentation and LRWC under salinity

The accumulation of Na and other toxic ions alter the physiological stability of plant cells leading to considerable damage to their structural and functional stability (Ahanger *et al.*, 2018). Effect of different rates of CP on some growth and yield parameters of water leaf (*Talinum triangulare Jacq*.) under saline soil are presented in Table 4 from which data revealed that in control soil, salinity condition gradually caused a significant reduction the growth and yield parameters as compared to the other treatments. Several researchers (Amirjani, 2010; Kargar and Kareh, 2017) demonstrated that accumulation of salts particularly in the root zone resulted in a reduction in plant growth and yield production. This phenomenon is attributed to the osmotic effects of salt in plants the uptake of some mineral nutrients dissolved in water is also restricted. They also illustrated that generally, soils contain some water-soluble salts by other irrigation ways. Plants may absorb essential nutrients in the form of soluble salts as added in chemical forms, but excessive accumulation strongly suppresses either plant growth or physical, chemical and biological degradation processes, causing a serious consequence to global natural resources such as compaction, inorganic/organic contamination and diminished microbial activity/ diversity). Excess of salt (Na^+ and Cl^-) can also, cause the increased expenditure of energy on maintenance respiration or ion transport, reduced energy for the translocation of carbohydrates and diversion of photosynthesis from growth to osmoregulation. Similarly, were noticed by several authors (Munns and Tester, 2008; Mannan *et al.*, 2013).

In present study increasing cassava peel imparted apparent enhancement in pigment synthesis and the photosynthetic efficiency and it was evident that cassava peel supplemented seedlings exhibited less decline due to NaCl treatment. Salinity stress reduces photosynthetic efficiency through its deleterious effects on the synthesis of chlorophylls and Rubisco protein (Iqbal *et al.*, 2015). Salinity stress may reduce the leaf chlorophyll content through an increase in the chlorophyllase activity, which affects the membrane stability and weakens the protein–pigment–lipid complex (Taffouo *et al.*, 2010). Alam et al (2015) report that a reduction in the carotenoids with increasing salinity was also observed in

Portulaca oleracea. Thus, under the prevailing experimental conditions the decrease in carotenoids contents may relate to the decrease in photosynthetic processes under salinity. A possible explanation would be that salinity may inhibit or upregulate the biosynthetic pathway of carotenoids via inhibition of the genes encoding enzymes related to β-carotene (Dumas *et al.,* 2003). Salt stress caused an inhibition in the expression of the gene encoded for lycopene β-cyclase, the enzyme that converts lycopene to beta carotene (Babu *et al.,* 2011).

4.2. Effect of cassava peel on metabolites compounds and ions accumulation under salt stress

The present results indicate the increase of NaCl increasing metabolites and decresasing ASA. Accumulation of osmolytes has been related with salinity tolerant mechanisms in water leaf. Hanafy Ahmed et al (2002) working on wheat mentioned that salinized plants accumulate soluble carbohydrate, amino acids, soluble phenols and proline for osmoregulation that allows the plants to maximize sufficient storage reserves to support basal metabolism under stressed environment.

Accumulation of osmolytes such as soluble sugars help to regulate osmotic stress in plant cells and leads to protection of biomolecules and membranes (Irannejad and Shahbazian, 2004). These data suggest that the degree of the cellular oxidative damage in plants exposed to abiotic stress is controlled by the ability to protect against oxidative agents (Ksouri *et al.,* 2007). Salinity stress, which enhances the accumulation of the higher phenolic and flavonoid compounds within the plant cell, may accelerate the capacity of the plant to cope with oxidative stress under excessive salt concentrations or adverse conditions (Wahid and Ghazanfar, 2006).The increased synthesis of TP, TF contents under saline conditions may reflect some kind of defense against stress conditions since salt stress was accompanied by increased production of reactive oxygen species (Singh *et al.,* 2015). Flavonoids are often induced by abiotic stress and have a role in plants protection (Grace and Logan, 2000). Similarly, an increase in condensed tannins was observed. It is generally accepted that phenolic compounds give plants a strong antioxidant activity (Jun Jin *et al.,* 2001).

Salt stress has been affecting the nutritional value of many edible plants. In the present study, salt stress increased significantly the uptake of Na in the leaves, while K, Ca, iron and Mg were reduced drastically. The reduction of these elements may be directly linked to excessive Na+ absorption by the roots as reported by Benito et al (2014). However, sufficient K, Ca, and Mg are required to meet basic metabolic processes such as intracellular K homeostasis, which is essential for optimal functioning of the photosynthetic machinery and maintenance of stomatal opening (Shabala and Pottosin, 2014). Our results agree with those conducted by (Parvez *et al.,* 2020) on *Chenopodium quinoa* (genotype A7) and *Cichorium spinosum* in saline conditions, respectively, where it was reported that higher transport of K and Ca into new shoots and leaves contributed to mitigating ion toxicity in leaf cells. Increased cassava peel supplementation resulted in significant increase in the uptake of K, Mg, Ca and Iron accompanied by reduced Na accumulation. The application of manure increases soil fertility. Macro and micronutrients become more available, and the soil microorganism population becomes more abundant (Sutrisno and Yusnawan, 2018). Burhan and AL-Taey (2018) mentioned that the addition of organic matter declines the sodium percentage because organic matter stores a part of sodium in the form of a Na-Organic compound.

Table 4. Effects of cassava peel rates on growth and yield characters of *Talinum triangulare Jacq.* under salt stress (14 WAS)

		Growth and yield parameters						
Treatment (mM NaCl)	CP (t ha^{-1})	HP (cm)	NL	TLA (cm^2)	SD (cm)	LFW (g plant^{-1})	LDW (g plant^{-1})	LY (g kg^{-1})
Control	0	26.3±0.02g	54.2±0.71b	46.3±2.24d	2.51±0.12l	46.3±0.12d	13.7±0.91j	23.9±0.91h
	NPK	29.8±0.02g	56.2±0.71b	48.3±2.24c	2.61±0.12l	48.6±0.12c	15.5±0.91j	25.3±0.91h
	9	27.3±0.03g	55.1±0.82b	47.3±3.01d	2.87±0.16l	47.4±0.16d	14.3±0.08j	24.1±0.08h
	12	31.7±0.02g	58.9±0.81a	51.5±3.21c	2.93±0.23l	49.2±0.23c	16.5±0.07i	27.9±0.07g
	15	36.1±0.04f	62.3±0.93a	56.1±3.33b	3.34±0.41l	52.2±0.41c	18.2±0.11i	31.8±0.11g
50	0	23.5±0.03h	48.8±0.88c	40.6±2.67e	2.06±0.21l	41.5±0.21e	10.6±0.12k	19.4±0.12i
	NPK	25.8±0.02h	50.2±0.71c	42.3±2.24e	2.36±0.12l	43.6±0.12d	11.3±0.91j	20.5±0.91i
	9	24.6±0.06h	49.6±0.76c	41.3±2.34e	2.22±0.32l	42.4±0.32e	12.1±0.09j	20.1±0.09i
	12	27.4±0.04g	52.7±0.67c	44.4±2.88d	2.53±0.28l	44.5±0.28d	13.8±0.13j	23.6±0.13h
	15	31.2±0.05g	56.6±0.59b	48.9±3.45c	2.92±0.25l	46.5±0.25d	15.4±0.07j	26.4±0.07g
100	0	17.9±0.02i	40.5±0.62e	35.3±3.12f	1.59±0.17m	36.5±0.17f	7.4±0.11k	16.8±0.11i
	NPK	18.8±0.02i	42.2±0.71e	36.7±2.24f	1.86±0.12lm	37.6±0.12e	7.9±0.91k	17.9±0.91i
	9	18.3±0.07i	41.2±0.73e	36.3±2.88f	1.48±0.44m	37.1±0.44e	7.8±0.09k	17.2±0.09i
	12	20.3±0.07i	44.6±0.75d	39.9±4.01e	1.75±0.59m	39.5±0.59e	8.7±0.14k	19.7±0.14i
	15	23.8±0.03h	48.1±0.82c	42.7±3.45e	1.95±038m	41.5±038e	10.1±0.06k	22.4±0.06h
200	0	12.5±0.06j	31.5±0.78g	28.2±2.66g	0.98±0.58m	28.7±0.58g	3.9±0.05l	12.1±0.05j
	NPK	13.8±0.02j	33.2±0.71f	29.8±2.24g	1.15±0.12m	29.6±0.12g	5.3±0.91l	13.8±0.91j
	9	13.7±0.7j	32.6±0.84f	29.3±2.33g	1.07±0.62m	31.4±0.62g	4.8±0.11l	13.2±0.11j
	12	15.5±0.03j	34.4±0.79f	31.4±3.04g	1.27±0.49m	33.5±0.49f	5.7±0.12c	14.7±0.12j
	15	18.7±0.04i	37.1±0.76e	34.5±3.23f	1.58±0.76m	35.1±0.76f	6.3±0.11k	16.1±0.11i
Two way ANOVA results								
Salt stress (SS)		**	**	**	*	*	*	*
Cassava peel (CP)		*	*	*	*	*	*	*
Interaction CP x SS		*	*	**	*	*	*	*

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different ($p < 0.05$).

**, * significant at 1 and 5% probability levels, respectively, NS not significant

Table 5. Effects of cassava peel rates on leaf minerals of *Talinum triangulare Jacq.* under salt stress (14 WAS)

Leaf chemicals and nutritional components						
Treatment (mM NaCl)	CP (t ha^{-1})	Na (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	K (mg kg^{-1})	Iron (mg kg^{-1})
Control	0	2.78±3.2n	1098.2±7.7c	1325.3±2.4b	1241.6±0.12b	45.6±0.12g
	NPK	2.36±2.9n	1131.2±7.5c	1334.3±2.4b	1253.6±0.12b	47.5±0.11g
	9	2.57±2.3n	1124.1±6.82c	1329.3±3.1b	1248.4±0.16b	46.8±0.16g
	12	2.13±2.2n	1338.2±6.8b	1566.5±3.21a	1582.2±0.23a	49.2±0.23f
	15	1.72±2.4n	1676.3±6.9a	1895.1±3.33a	1903.2±0.41a	53.6±0.41f
50	0	4.85±1.8m	879.8±5.8d	1081.6±2.7c	1012.5±0.22c	40.3±0.21h
	NPK	4.42±1.2m	888.2±5.7d	1088.3±2.4c	1018.6±0.12c	42.1±0.12h
	9	4.67±1.6m	883.6±6.7d	1085.3±2.34c	1016.6±0.32c	41.9±0.32h
	12	3.43±1.4n	902.7±6.6c	1385.4±2.8c	1315.5±0.8b	43.7±0.28g
	15	3.14±1.5n	931.6±5.9c	1688.9±3.45a	1617.5±0.25a	46.4±0.25g
100	0	9.76±2.2l	604.5±5.6d	807.3±3.12d	743.5±0.17d	34.2±0.17i
	NPK	9.18±2.1l	609.9±5.7d	811.8±2.24d	747.6±0.12d	35.8±0.12i
	9	9.45±1.7l	607.2±5.7d	809.3±2.88d	745.1±0.44d	35.1±0.44i
	12	7.53±1.7m	624.6±6.5d	822.9±4.01d	776.5±0.59d	38.7±0.59h
	15	6.72±2.3m	654.1±5.8d	851.7±3.45d	798.5±038d	41.6±038h
200	0	16.47±1.6k	451.5±5.8e	489.2±2.66e	492.7±0.58e	29.1±0.58j
	NPK	15.83±1.2k	456.2±5.7e	494.3±2.24e	498.6±0.12e	30.9±0.12j
	9	16.15±1.7k	454.6±5.84e	492.1±2.33e	495.4±0.62e	30.1±0.62j
	12	13.64±2.3k	478.4±5.9e	514.4±3.04d	522.5±0.49d	33.4±0.49i
	15	11.76±1.9l	503.1±5.6d	535.5±3.23d	551.2±0.76d	36.3±0.76i
	Two way ANOVA results					
Salt stress (SS)		**	*	*	*	*
Cassava peel (CP)		*	*	*	*	*
Interaction CP x SS		*	*	*	*	*

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different ($p < 0.05$).
**, * significant at 1 and 5% probability levels, respectively, NS not significant

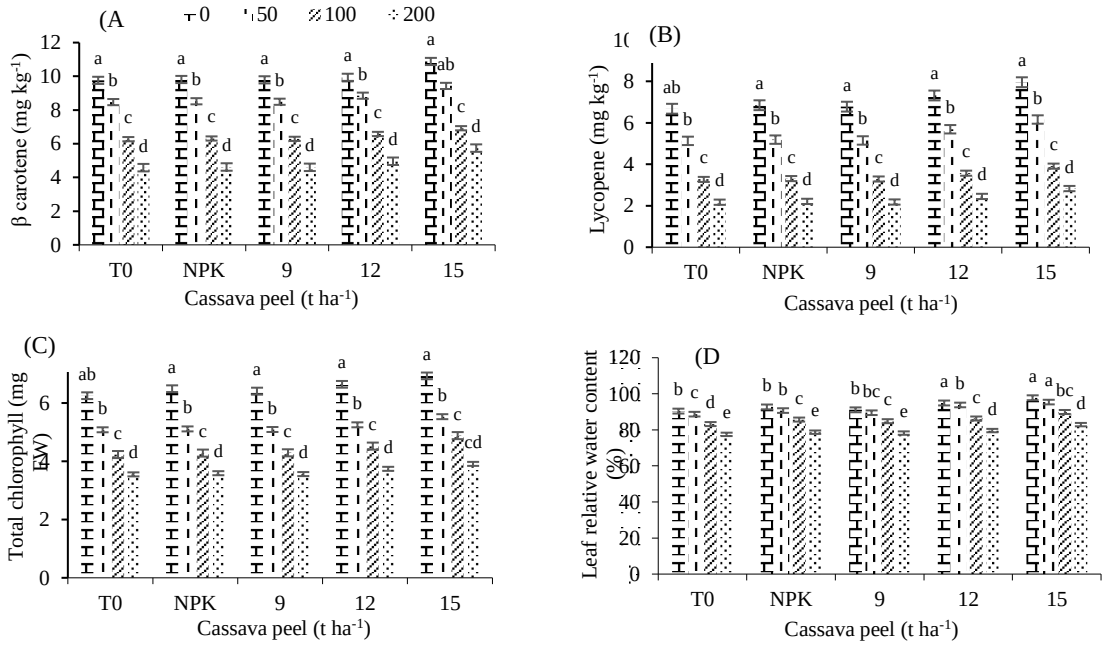

Fig. 1. Effects of cassava peel rates on leaf pigmentation and leaf relative water of water leaf (*Talinum triangulare Jacq.*) under salt stress (50, 100 and 200 mM NaCl) at 14 WAS. β carotene (A), lycopene (B), total chlorophyll (C) and LRWC (D). Bars are means (n=5) ± SD. Means followed by different letter are significantly different ($p < 0.05$).

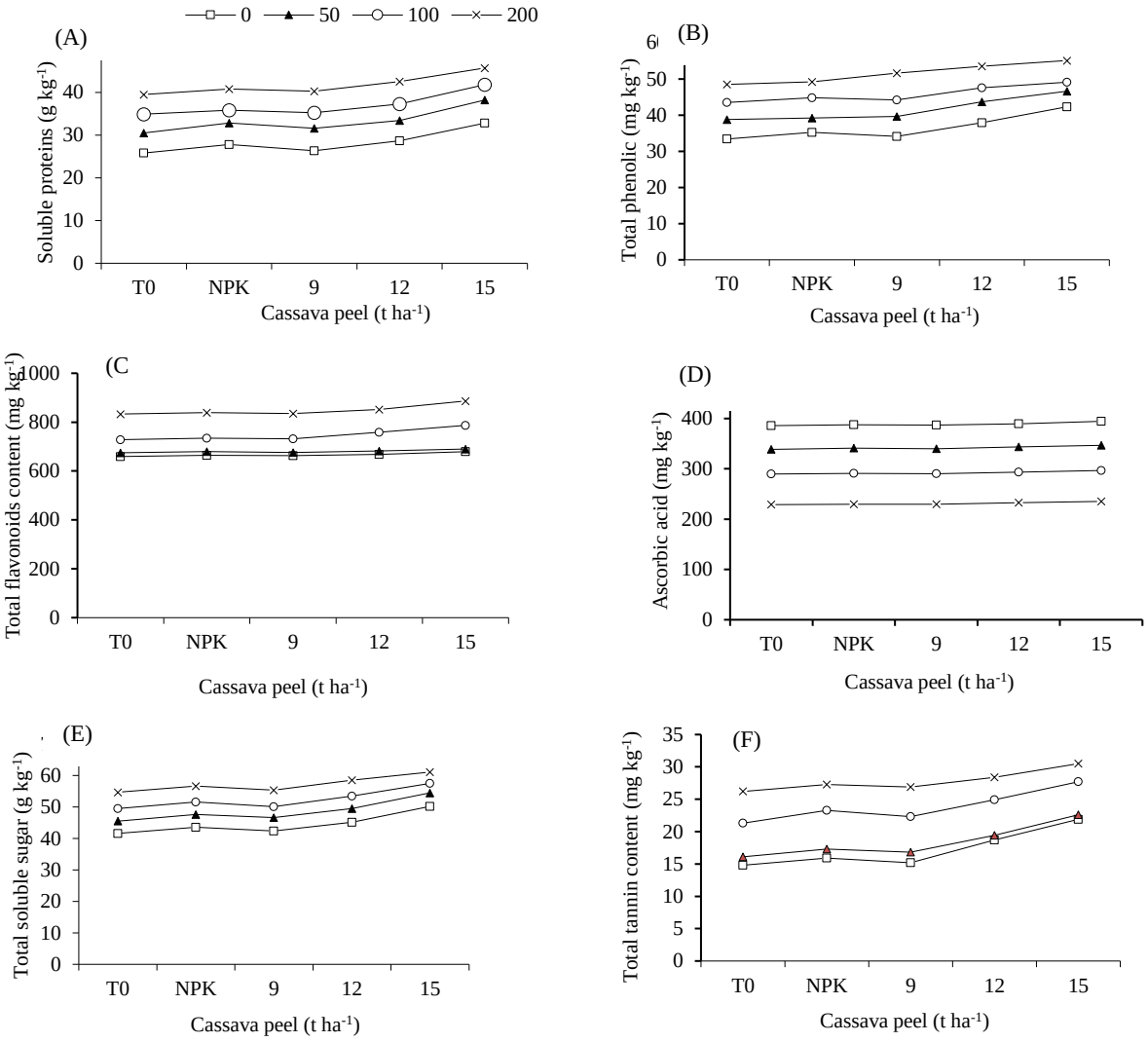

Fig. 2. Effects of cassava peel rates on metabolites content and ascorbic acid of water leaf (*Talinum triangulare Jacq.*) under salt stress (50, 100 and 200 mM NaCl) at 14 WAS. Soluble proteins (A), Total phenolic (B), Total flavonoids content (C), Ascorbic acid (D), Total soluble sugar (E) and Total tannin content (F). Bars are means (n=5) ± SD. Means followed by different letter are significantly different ($p < 0.05$).

Conclusion

The present study showed that *Talinum triangulare Jacq.* cultivated under salt stress could be contributed to a high nutritional quality of the final product in terms of nutrients, minerals, vitamins and antioxidant profiles. 15 t ha^{-1} level of cassava peel gave better chemical characteristics, growth and yield response in case of water leaf. Application of CP protects the growth and metabolism of water leaf seedlings through upregulation of the antioxidant system, osmolyte and secondary metabolite accumulation. Cassava peel prevented ill effects of salinity on photosynthetic functioning. CP levels proved beneficial in ameliorating the salinity triggered oxidative damage to significant extent. It is recommended that the use of these organic fertilizers in kitchen gardens and field is economically important where it not only replenishes soil organic matter but also gives the sustainable production. However, further research is needed underfield conditions to collect waste materials like cassava peels from the industries and homes to know the effect of various organic waste used as fertilizer on other plants especially vegetables and to evaluate their effects on plants when applied in larger quantities.

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