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Case Study.

Nutrient uptake in pepper (*Capsicum annuum* L.) grown under salt stress.

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The determination of some macro and micro nutrients in pepper plants treated with varying concentrations of sodium chloride (NaCl) ranging from 0 (control) to 1000 mM NaCl was carried out. Treatments involved the daily application of 100 ml each of the concentrations to about 2.5 kg top soil in polyethylene bags containing *Capsicum annuum* plants after a stabilization period of four weeks. Sodium chloride treatments commenced at four weeks after planting for a period of one week followed by a break of one week and then for another period of one week. *C. annum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field. The salinity treatment significantly (P< 0.05) affected the uptake of sodium, potassium and nitrogen in components of the crop. The stem recorded the highest uptake of nutrients followed by the leaves, the roots, and then the fruits. Micro nutrients were taken up in trace quantities and the levels of iron were greater than those of chromium and zinc.

Key words: Salinity, Salt stress, Nutrients uptake, *Capsicum annuum.*

INTRODUCTION

Abiotic stresses are the principal threat to plant growth and crop productivity all over the world (Khan *et al*., 2009). There are a number of abiotic stresses common in nature such as salinity, drought, heavy metals, extreme temperatures, moisture, light, mineral deficiencies or toxicity, pH and pollutants which can diminish plant yield (Ozturk *et al.,* 2009). Out of all these abiotic stress, salinity can be disastrous as it affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. Salinity is one of the two major environmental factors that currently reduce plant productivity worldwide (Jamil, *et al.,* 2006). The other is drought. Salinity stress in some respects resembles drought stress (Sohan *et al.,* 1999). High levels of salinity in the soil can upset the nutrient balance in the plant or interfere with the uptake of some nutrients (Blaylock, 1994) and this nutritional disorder may result from the effect of salinity on nutrient availability, competitive uptake, transport and partitioning within the plant (Kaya and Higgs, 2003; Omami, 2005).

Capsicum annuum is of great economic value in West Africa and in Nigeria in particular. *Capsicum annuum* and *C. frutescens* are third among the cultivated vegetables utilized as a spice (Nwachukwu *et al*., 2007). It is also an important vegetable in Turkey, Pakistan and China, as well as the Mediterranean and Latin America. It contains an alkaloid a digestive stimulant that is used in ointment for the relief of arthritic and neuropathic pains (Nwachukwu *et al.,* 2007). *C. annuum* is eaten raw in salads (Remison, 2005). Capsaicin, the pungent constituents of capsicum pods is used in the manufacture of ginger ale and ginger beer (Remison, 2005). Paprika, a mild red spice from peppers, is derived from *C. annuum* and is used primarily in the flavouring of garnishes, pickles, meats, barbecue sauces, ketchup, cheese, snack food, salads and sausages (Bosland, 1996). There is indication that more than one-third of the world's agricultural land faces the problem of high salinity (Turkmen *et al.,* 2008). Salt causes both ionic and osmotic stress on plants (Dubey, 1997; Yeo, 1998). Osmotic effects of salts on plants are a result of lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil water potential, this condition interferes with the plant's ability to extract water from the soil (Sohan *et al.,* 1999). There is limited literature (Achakzai *et al*., 2010; Achakzai, 2007) on the effects of salinity on the nutrient uptake in vegetable crops and to our knowledge, none on *C. annuum* in Nigeria. The objectives of this study are to determine the effects of salinity on the nutrient content and uptake in pepper (*Capsicum annuum*) grown under salt stress.

MATERIAL AND METHODS

The experiment was conducted in the Department of Plant Biology and Biotechnology in the University of Benin, Benin City, Nigeria. Benin City is located in latitude 6^0 23' N and longitude 5^0 37' in Edo State, Nigeria. It is located in the rain forest region of Nigeria. Seeds of *C. annuum* (Ata Rodo) were obtained from Nigerian Institute for Horticultural Research (NIHORT), Ibadan.

Salinity was created by preparing 0 (control), 10, 50, 100, 250, 500 and 1000 mM (NaCl) sodium chloride solutions with distilled water. Polyethylene bags were three quarter filled with top soil or about 2.5 kg soil obtained from the vicinity of the experimental plot. The soil type was sandy to loamy with substantial quantity of humus with pH 5.63, Sand 64 %, Silt 24.38 %, Clay 11.16 % and Carbon 3.87 %. The treatments were therefore, 0 mM NaCl/kg soil, 4, 20, 40, 100, 200, and 400 mM NaCl/kg soil. A nursery was raised and four weeks after, at three leaf stage, the seedlings were transplanted into the polyethylene bags. The bags were placed in a screen house to prevent interference of rain.

More than two seedlings were placed in each bag and at 5 leaf stage, thinned to two seedlings per bag for uniformity. The experimental design was completely randomized block design with seven treatments replicated three times. Treatments were applied to young plants at four weeks after transplanting. One hundred milliliters (100 ml) of each concentration was applied every day for four times with an interval of one week and four times subsequently.

Plants were harvested three months after planting and fresh and dry weights of the components (roots, stems, leaves and fruits) of the crop were taken. Dried samples were ground in a mortar and the fine powder was used for the determination of N, P, K, Ca, Mg and Na. Three micro elements namely;Fe, Zn and Cr were also analyzed.

Nitrogen was determined by the Kjedahl method of the Association of Official Analytical Chemist (AOAC,1975). Phosphorus was determined by titrimetric method. Magnesium was determined using the technicon auto analyzer tool as described by Thomas *et al.,* (1987). K, Ca, and Na were determined using the gallenkamp digital flame analyzer. Micro elements were determined using spectrophotometer (BEL spectronic 70 – electro colorimeter). Care was taken to complete all readings within 30 minutes. Nutrient uptake was calculated as the product of dry weight of the component and concentration (mg/l) of the nutrient.

Duncan Multiple Range Test was used to analyze the data obtained using the SPSS version 16.0 software (Ogbeibu, 2005)

RESULTS

In the root of *C. annuum* salinity treatment significantly ($p<0.05$) promoted the uptake of Na (0.67±0.145 mg/l) and K (0.34±0.023 mg/l) when compared to the control treatment (0.11±0.030 mg/l Na and 0.26±0.089 mg/l K). Salinity had no significant effect ($P>0.05$) on the uptake of the other mineral nutrients (Table 1). Also, in the roots of *C. annuum*, the uptake of the mineral elements was in order of Ca>N>Mg>K>P>Na (1.40±0.346, 1.03±0.265, 0.29±0.065, 0.26±0.089, 0.12±0.299 and 0.11±0.030 mg/l, respectively) in the control treatment. The control treatment had the least uptake value for Na, Mg, K and N (Table 1). The treatment with 4 mM NaCl/kg soil had the highest amount of nitrogen uptake and the treatment with 40 mM NaCl/kg soil had the highest phosphorus uptake.

Results of the mineral uptake in the stem subjected to NaCl treatments of *C. annuum* were shown in Table 2. The order of uptake of nutrients in the stem of the control treatment was Ca>N>K>Mg>Na>P (2.34±0.65, 1.73±0.50, 0.68±0.271, 0.28±0.061, 0.23±0.080, 0.18±0.052 mg/l of corresponding elements). The control treatment had the least uptake value of N, K, Ca, Mg and Na when compared to the treatments with NaCl (Table 2). The salinity treatments had no significant effect on the uptake of phosphorus in the treatment with 40 and 100 mM NaCl/kg soil respectively. The uptake value of phosphorus was 0.23±0.055 and 0.14±0.022 mg/l while the control had a value of 0.18±0.052 mg/l.

Data presented in Table 3 shows that in the leaves of *C. annuum*, the order of the uptake of the nutrient elements in the control treatment studied was Ca>N>K>Mg>Na>P (1.36 ± 0.0297, 1.19 ± 0.225, 0.36±0.141, 0.24 ± 0.028, 0.13±0.041, 0.12 ± 0.021 mg/l respectively). The control treatment had the least uptake value of nitrogen, phosphorus, potassium, calcium, magnesium and sodium. Though not significant, the treatment with 100 mM NaCl/kg soil had the least uptake value of phosphorus

In the fruits of the *C. annuum* studied (Table 4) the treatment with 4 mM NaCl/kg soil had the least uptake value of nitrogen, phosphorus, potassium, calcium, magnesium and sodium. The uptake of these nutrients among the treatments was in order of 4<20<40<100 mM NaCl/kg soil. Irrespective of this order, the uptake of the mineral nutrients studied did not vary significantly ($P>0.05$) among the treatments in *C. annuum* fruits.

Table 1: Mineral uptake in the root of *Capsicum annuum*

+ Treatments NaCl (mM)/kg soil	Mineral elements (Macro) mg/l					
	N	P	K	Ca	Mg	Na
0 (Control)	1.03 ± 0.265	0.12 ± 0.299[b]	0.26 ± 0.089	1.40 ± 0.346	0.29 ± 0.065	0.11 ± 0.030
4	1.83 ± 0.297	0.10 ± 0.219[a]	0.87 ± 0.084	2.36 ± 0.460	0.38 ± 0.068	0.39 ± 0.123
20	1.31 ± 0.236	0.074 ± 0.011 [a]	0.58 ± 0.169	1.57 ± 0.242	0.52 ± 0.120	0.41 ± 0.057
40	1.54 ± 0.117	0.093 ± 0.025 [a]	0.65 ± 0.074	2.23 ± 0.424	0.43 ± 0.053	0.68 ± 0.086
100	1.12 ± 0.228	0.058 ± 0.011 [a]	0.34 ± 0.023	2.01 ± 0.703	0.40 ± 0.158	0.67 ± 0.145
Level of Significance	NS	NS	*	NS	NS	*

* Values are significant at P<0.05
NS = Values not significant
+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Table 2:Mineral uptake in the stem of *Capsicum annuum*

+ Treatments NaCl (mM)/kg soil	Mineral elements (Macro) mg/l					
	N	P	K	Ca	Mg	Na
0 (Control)	1.73 ± 0.500	0.18 ± 0.052	0.68 ± 0.271	2.34 ± 0.635	0.28 ± 0.061	0.23 ± 0.080
4	2.33 ± 0.150	0.11 ± 0.010	1.00 ± 0.120	2.93 ± 0.240	0.55 ± 0.102	0.55 ± 0.279
20	2.50 ± 0.730	0.42 ± 0.036	1.11 ± 0.351	10.02 ± 0.958	0.78 ± 0.237	0.57 ± 0.160
40	3.90 ± 0.660	0.23 ± 0.055	1.62 ± 0.130	5.38 ± 1.280	1.36 ± 0.650	1.39 ± 0.180
100	3.09 ± 0.537	0.14 ± 0.022	0.71 ± 0.124	3.91 ± 1.088	0.76 ± 0.284	1.70 ± 0.300
Level of significance	*	NS	*	*	*	*

* Values are significant at P<0.05
NS = Values not significant
+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Table 3:Mineral nutrient uptake in the leaves of *Capsicum annuum*

+ Treatments NaCl (mM)/kg soil	Mineral elements (Macro) mg/l					
	N	P	K	Ca	Mg	Na
0 (Control)	1.19 ± 0.225	0.12 ± 0.021	0.36 ± 0.141	1.36 ± 0.0297	0.24 ± 0.028	0.13 ± 0.041

Table 3 continues

4	4.67 ± 3.037	0.19 ± 0.124	1.84 ± 1.184	5.98 ± 4.312	1.20 ± 0.788	0.70 ± 0.474
20	2.07 ± 0.714	0.10 ± 0.027	0.96 ± 0.333	2.93 ± 0.882	0.53 ± 0.183	0.45 ± 0.219
40	2.21 ± 0.933	0.10 ± 0.0497	1.10 ± 0.497	2.37 ± 1.074	0.44 ± 0.127	0.35 ± 0.104
100	1.54 ± 0.322	0.062 ± 0.014	0.57 ± 0.101	2.11 ± 0.804	0.53 ± 0.212	0.62 ± 0.032
Level of significance	NS	NS	NS	NS	NS	NS

* Values are significant at P<0.05
NS = Values not significant
\+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Table 4: Mineral nutrient uptake in the fruits of *Capsicum annuum*

+ Treatments NaCl (mM)/kg soil	Mineral elements (Macro) mg/l					
	N	P	K	Ca	Mg	Na
0 (Control)	NA	NA	NA	NA	NA	NA
4	0.18 ± 0.177	0.01 ± 0.008	0.08 ± 0.083	0.16 ± 0.160	0.04 ± 0.038	0.04 ± 0.037
20	0.50 ± 0.504	0.03 ± 0.028	0.19 ± 0.189	0.48 ± 0.480	0.12 ± 0.117	0.07 ± 0.075
40	1.80 ± 1.017	0.10 ± 0.067	0.92 ± 0.529	1.96 ± 0.949	0.44 ± 0.205	0.26 ± 0.145
100	1.48 ± 0.751	0.06 ± 0.030	0.68 ± 0.344	1.49 ± 0.747	0.32 ± 0.168	0.35 ± 0.204
Level of significance	NS	NS	NS	NS	NS	NS

* Values are significant at P<0.05
NA = Not Analyzed
NS = Values not significant
\+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Micro nutrient uptake

Salinity treatments promoted the uptake of iron and zinc by the roots of *C. annuum* subjected to the treatments (Table 5). The treatments with 100 mM NaCl/Kg soil had uptake values of iron and zinc as 0.06 ± 0.026 and 0.03 ± 0.006 mg/l while the control treatment has a value of 0.03 ± 0.009 and 0.02 ± 0.005 mg/l respectively. Chromium was taken up in trace amounts in all components of the crop.

The uptake of the micro nutrients in iron, zinc and chromium was significantly promoted by the salinity treatments in the stems, however, were taken up in trace amounts (Table 6). The uptake value of iron, zinc and chromium in the treatment with 100 mM NaCl/kg soil was 0.08 ± 0.037, 0.07 ± 0.008 and 0.006 ± 0.0024 mg/l as against 0.024 ± 0.007, 0.030 ± 0.007 and 0.002 ± 0.0005 mg/l in the control treatment.

The uptake values of the various micro elements studied did not vary significantly among the treatments in the leaves (Table 7). The control treatment had the least uptake value of iron and zinc. The uptake value of iron and zinc in the treatments with 4 and 100 mM NaCl/kg

soil was 0.14 ± 0.100, 0.03 ± 0.005 and 0.10 ± 0.077, 0.04 ± 0.016 mg/l respectively. The uptake value of chromium was the same 0.00 ± 0.001, in the control treatment and the treatments with 20, 40, and 100 mM NaCl/kg soil.

In the fruit of *C. annuum*, the treatment with 100 mM NaCl/kg soil had the highest amount of iron, zinc and chromium when compared to the other salinity treatments. The treatment with 100 mM NaCl/kg soil had an uptake value of iron, zinc and chromium as 0.03 ± 0.005, 0.05 ± 0.018 and 0.00 ± 0.001 mg/l respectively as against 0.01 ± 0.000, 0.01 ± 0.000 and 0.00 ± 0.000 mg/l in the treatment with 10 mM NaCl/kg soil (Table 8).

Table 5:Micro nutrient uptake in the root of *C. annuum* grown under salinity stress

+ Treatments NaCl (mM)/kg soil	Micro nutrient uptake (mg/l)		
	Fe (%)	Zn (%)	Cr (%)
0 (Control)	0.00442±5.667	0.00375±0.873	0.000203±0.053
4	0.00824±7.363	0.00449±8.257	0.000285±0.206
20	0.00892±6.383	0.00288±1.510	0.000285±0.373
40	0.00555±4.933	0.00296±2.203	0.000312±0.438
100	0.00669±15.069	0.00305±1.737	0.000280±0.228
Level of significance	*	*	*

* Values are significant at P<0.05

+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Table 6:Micro nutrient uptake in the stem of *C. annuum* grown under salinity stress

+ Treatments NaCl (mM)/kg soil	Micro nutrient uptake(mg/l)		
	Fe (%)	Zn (%)	Cr (%)
0 (Control)	0.00285±5.697	0.00331±2.304	0.000189±0.071
4	0.00484±16.423	0.00392±1.513	0.000229±0.187
20	0.00674±11.654	0.00418±5.450	0.000235±0.175
40	0.00285±1.305	0.00458±2.40	0.000256±0.40
100	0.00390±9.990	0.00340±2.613	0.000277±0.574
Level of Significance	*	*	*

* Values are significant at P<0.05

+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Table 7:Micro nutrient uptake in the in the leaves of *C. annuum* grown under salinity stress

+ Treatments NaCl (mM)/kg soil	Micro nutrient uptake(mg/l)		
	Fe (%)	Zn (%)	Cr (%)
0 (Control)	0.00277±5.370	0.00523±22.241	0.000277±0.385
4	0.00518±4.494	0.00518±6.582	0.000269±0.282
20	0.00574±9.931	0.00392±2.617	0.000219±0.334
40	0.00344±4.457	0.00392±0.3997	0.000306±0.145
100	0.00304±2.897	0.00436±4.615	0.000354±0.808
Level of Significance	*	*	*

* Values are significant at P<0.05

+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

Table 8:Micro nutrient uptake in the fruits of *C. annuum* grown under salinity stress

+ Treatments NaCl (mM)/kg soil	Micro nutrient uptake (mg/l)		
	Fe (%)	Zn (%)	Cr (%)
0 (Control)	NA	NA	NA
4	0.003417±0.000	0.00288±0.000	0.000192±0
20	0.003426±0.000	0.00340±0.000	0.000240±0
40	0.002091±2.085	0.00305±2.307	0.000365±1.333
100	0.002281±1.455	0.00405±9.155	0.000284±0.12
Level of Significance	*	*	*

* Values are significant at P<0.05

NA = Not Analyzed

+ *C. annuum* plants treated with 200 and 400 mM NaCl/kg soil all died within the first two weeks in the field.

DISCUSSION

The results of this experiment corroborate earlier reports of Blaylock (1994), Kaya and Higgs (2003) and Turkmen *et al.* (2008) that salinity causes nutritional disorders in plants. For instance, high salt levels in the soil can upset the nutrient balance in the soil or interfere with the uptake of some nutrients. The uptake of Na and K in roots and several nutrients in the stem were significantly affected by the salinity treatments when compared to the control and the association observed here was an increased uptake of the mineral nutrients.

According to Omami (2005) high salt uptake competes with the uptake of other nutrient ions K^+, Ca^{2+}, N and P resulting in nutritional disorders. The results obtained in this experiments support earlier findings in the changes of some plant organs for all the nutrients except phosphorus which was not found sensitive to salt stress. However, the effect of salinity on the mineral nutrient uptake was not uniform in all components of the plants (see Table 1 – 4) and agrees with the report by Omami (2005). Salinity stress caused changes in the pattern of the dry matter accumulation and partitioning to different plant parts of *Amaranth*. This may be one of the salt tolerance strategies in crops (Omami, 2005). The value of the uptake of sodium in the treatment with 50 mM NaCl/kg soil in the leaves of *C. annuum* obtained in this experiment varies from the result obtained by Gunes *et al.,* (1999) using the same salinity treatment, the same component and species. They reported that the salinity treatment (50 mM, NaCl) reduced the uptake of sodium in the leaves while in this experiment sodium was 0.45 ± 0.219 mg/l as against 0.13 ± 0.041 mg/l in the control treatment. The difference in results may be due to the fact the *C. annuum* (Ata Rodo) variety used for this experiment is more salt tolerant than the variety used by Gunes *et al.,* (1999). Omami had attributed superior salt tolerance of the *Amaranth* genotype, *A. cruentus* compared to *A. tricolon* to higher water use efficiency.

This fact has been buttressed by Aktas *et al.,* (2006) that the ability of plants to maintain lower level of sodium within is one of the key mechanisms contributing to expression of high salt tolerance. Also according to Aktas *et al.,* (2006) increasing supply of NaCl impairs the uptake of potassium and calcium by roots, however, the result of the uptake of potassium and calcium under increasing supply of NaCl showed not significant reduction in the uptake value of potassium and calcium. The ability of plant genotypes to maintain high levels of K and Ca is also a mechanism that contributes to the expression of high salt tolerance (Aktas *et al.,* 2006).

The effects of salinity on the micro nutrients in the components of the crop are also in agreement with the earlier report by Omami (2005) that salinity had inhibitory as well as stimulatory effect on the uptake of some micro nutrients. Although, Gunes *et al.,* (1999) had reported that salinity inhibits the uptake of zinc. Our results indicate that salinity enhanced zinc uptake in all components of the plant. The results obtained in this study vary slightly from the results obtained by Achakzai *et al.*, (2010). They reported that salinity impacted negatively on the uptake of iron and promoted the uptake of zinc however in this study although salinity had an effect on these two elements. The uptake of iron was not negatively impacted but zinc was increased in all the components of the crop. The difference in the results may be due to the crop employed. Achakzai *et al.*, (2010) had worked on sunflower at early vegetative stage.

In general, salinity treatment affected the uptake of sodium, potassium and nitrogen in components of *C. annuum*. The stem recorded the highest uptake of nutrient followed by the leaves, the roots and the fruits. Micronutrients, iron, chromium and zinc were taken up in trace quantities.

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