



**THE INFLUENCE OF SALTS STRESS ON SOME GROWTH
MORPHOLOGY OF (MELON PUMPKIN) *Cucurbita maxima* Duchesne
GROWN IN-VITRO**

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ABSTRACT

The influence of different salts (KH_2PO_4 , CaCl_2 and MgSO_4) stress on some growth morphology of (Melon Pumpkin) *Cucurbita maxima* were studied in-vitro in the laboratory. Melon pumpkin seeds were surface sterilized and then sown into petric dishes packed with cotton wool. Growth parameters were determined two weeks after germination using standard procedures. The morphological studied included seedling height, leaf area, root length, percentage germination

and moisture content. Salts salinity has deleterious effects on many food crops especially in morphology of the plants. It was observed that with the increase in salinity levels plants growth was adversely affected which showed the melon pumpkin was highly sensitive to salinity and increased level of salinity has lethal effects on it resulted reduction in growth parameters. This results is of great economic significance to abiotic stresses like water stress, salt stress, metal stress etc., can cause an increase in the active principle contents in medicinal plants and this will be helpful for the cultivation of this plant in salt affected areas.

KEYWORDS: *Cucurbita maxima*, morphology, salt stress, parameters.

INTRODUCTION

Salinity is one of the most important abiotic stresses limiting crop production in arid and semiarid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching^[1,2]. Salinity affects many morphological, physiological and biochemical processes, including seed germination, plant growth, water and nutrient uptake^[3]. The

growth and development of the plant are the internal processes that are under the control of the environment. Temperature, moisture, radiation, nutrients and gases can either enhance or retard the growth and development of the plant. Some times these factors may act as stress leading to injury and in extreme cases the death of the plant ^[4]. Salinity effects are more conspicuous in arid and semiarid regions, where limited rainfall, high evapotranspiration and high temperature associated with poor water and soil management contributes to the salinity problem and is also of great importance to the agricultural production in these regions. Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern ^[5]. Every year more and more land becomes nonproductive owing to salt accumulation. Salinization plays a major role in soil degradation. It affects 19.5% of irrigated land and 2.1% of dry land agriculture in the world. In Nigeria, out of 9.38 million ha of salt-affected soils, 3.88 million ha are alkali soil and 5.5 million ha (including coastal lands) are saline soils ^[6].

Cucurbita maxima (melon pumpkin) is a gourd-like squash of the genus *Cucurbita* and the family Cucurbitaceae (which also includes gourds). It commonly refers to cultivars of any one of the species *Cucurbita pepo*, *Cucurbita mixta*, *Cucurbita maxima*, and *Cucurbita moschata*, and is native to North America. They typically have a thick, orange, green or yellow shell, creased from the stem to the bottom, containing the seeds and pulp ^[7]. It generally weighs 4–8 kg capable of reaching a weight of 34 kg. The pumpkin varies greatly in shape, ranging from oblate to oblong. The rib is smooth and usually lightly ribbed. Although pumpkins are usually orange or yellow, some fruits are dark green, pale green, orange-yellow, white, red and gray ^[8]. Pumpkins are monoecious, having both male and female flowers on the same plant. The female flower is distinguished by the small ovary at the base of the petals. These bright and colourful flowers have extremely short life spans and may only open for as short a time as one day. The colour of pumpkins is derived from the orange pigments abundant in them. The main nutrients are lutein and both alpha and beta carotene, the latter of which generates vitamin A in the body ^[7]. *Cucurbita maxima* are very versatile in their uses for cooking. Most parts of the pumpkin are edible, including the fleshy shell, the seeds, the leaves, and even the flowers. In the United States and Canada, pumpkin is a popular Halloween and Thanksgiving staple. Homemade pumpkin purée can serve the same purpose ^[9]. This study was carried out to assess and compare the different salt tolerance of *Cucurbita maxima* during germination and early seedling growth.

MATERIALS AND METHODS

Collection of Seeds

Matured dry seeds of *Cucurbita maxima* were obtained from Akwa Ibom State Agricultural Development Project (AKADEP) Uyo Zone and the viable seeds were used for the study.



Matured ripe fruit/seeds of melon pumpkin (*Cucurbita maxima*)

Salt Preparation/Procedures

- (i) One hundred milliliters (100ml) of distilled water was added to 1 gm of KH_2PO_4 to give 1% solution of KH_2PO_4 .
- (ii) One milliliter (1ml) of 1% KH_2PO_4 was added to 9 ml of distilled water to form 0.1% solution of KH_2PO_4 .
- (iii) One milliliter (1ml) of 0.1% KH_2PO_4 was added to 9 ml of distilled water to form 0.01% solution of KH_2PO_4 .
- (iv) One milliliter (1ml) of 0.01% KH_2PO_4 was added to 9 ml of distilled water to form 0.001% solution of KH_2PO_4 .
- (v) One milliliter (1ml) of 0.001% KH_2PO_4 was added to 9 ml of distilled water to form 0.0001% solution of KH_2PO_4 .

The same procedures were repeated for CaCl_2 and MgSO_4 and each group was presented in triplicate. Distilled water were served as a control in each case ^[10,11].

Planting: Fifty four (54) petric dishes were pad with cotton wool and divided into three groups. Five sterile seeds of *Cucurbita maxima* were placed in each petric dish *in-vitro*.

Determination of Growth Parameters: Percentage germination, shoot length, leaf area and moisture content were determined after 10-days of sowing.

Determination of Leaf Area

Leaf area (LA) was determined by multiplying leaf length by leaf width with the correction co-efficient (r) which is 0.72 as proposed by Hoyt and Brafield^[12], Umoh and Esenowo^[13].

$$LA = L \times W \times r$$

Where: L = Leaf length

W = Leaf width

r = Correction co-efficient (0.72)

Statistical analysis

Results are expressed as mean \pm Standard Error (S.E) of three replicates. Statistical significance between the different groups was determined by two-way Analysis of Variance (ANOVA). $P < 0.05$ was considered as statistically significant^[14].

RESULTS

Growth performance of the plants was estimated by measuring seedling height, leaf area, root length, and total moisture content at weeks of sowing. Under 1% of KH_2PO_4 , CaCl_2 and MgSO_4 stress plant growth was negatively affected, reducing moisture content to 82.0 ± 0.31 (1% of KH_2PO_4), 70.2 ± 0.02 (1% of CaCl_2) and 81.03 ± 0.33 (1% of MgSO_4) Table 4.1 - 4.3. Leaf area showed the highest value in the control plants whereas; under salt conditions it decreased gradually with the increase in salinity (Table 4.1, 4.2 and 4.3). Seedling height decreased by up to 2.45 ± 0.09 , 0.85 ± 0.60 and 2.20 ± 0.30 in 1% of KH_2PO_4 , CaCl_2 and MgSO_4 respectively when compared with 0.0001% of KH_2PO_4 , CaCl_2 and MgSO_4 which recorded 9.42 ± 0.32 , 11.8 ± 0.20 and 12.5 ± 0.20 respectively. Similarly, root length in KH_2PO_4 , CaCl_2 and MgSO_4 low salt stress (0.01%, 0.001% and 0.0001%) were stimulated above high salinity (1%) (Table 4.1). One hundred percent (100%) germination were recorded in all level of salinity (Table 4.2 – 4.3). Also increase in leaf area were recorded in KH_2PO_4 thus: 0% (4.10 ± 0.74), 1% (3.12 ± 0.43), 0.1% (6.67 ± 0.20), 0.01% (7.71 ± 0.31), 0.001% (8.80 ± 0.20) and 0.0001% (9.42 ± 0.32) (Table 4.1, Plate 4.1), CaCl_2 in 0% (12.4 ± 0.23), 1% (0.71 ± 0.52), 0.1% (5.70 ± 0.32), 0.01% (7.50 ± 0.40), 0.001% (8.0 ± 0.65) and 0.0001% (8.9 ± 0.36) (Table 4.2, Plate 4.2), MgSO_4 in 0% (5.16 ± 0.56), 1% (2.00 ± 0.63), 0.1% (5.96 ± 0.40), 0.01% (7.0 ± 0.74), 0.001% (8.83 ± 0.50) and 0.0001% (8.76 ± 0.63) (Table 4.3, Plate 4.3).

Table 1: Effects of Salinity (MgSO₄) on Growth Morphology of *Cucurbita maxima*

Conc. MgSO ₄ (%)	Seedling Height (cm)	Leaf Area (cm ²)	Root Length (cm)	Moisture Content (%)	Percentage Germination (%)
0	10.8 ± 3.11	5.16 ± 0.56	13.5 ± 0.40	87.8 ± 1.27	100.0 ± 0.00
1	2.20 ± 0.30	2.00 ± 0.63	7.16 ± 0.40	82.0 ± 0.31	100.0 ± 0.00
0.1	9.90 ± 0.20	5.96 ± 0.40	14.1 ± 0.30	88.3 ± 0.81	100.0 ± 0.00
0.01	10.3 ± 0.30	7.00 ± 0.74	16.0 ± 0.40	89.9 ± 0.92	100.0 ± 0.00
0.001	11.5 ± 0.24	8.83 ± 0.50	17.6 ± 0.70	90.0 ± 0.32	100.0 ± 0.00
0.0001	12.5 ± 0.20	8.76 ± 0.63	18.7 ± 0.70	89.7 ± 0.11	100.0 ± 0.00

Data are processed and expressed as mean ± Standard Error of three replicates

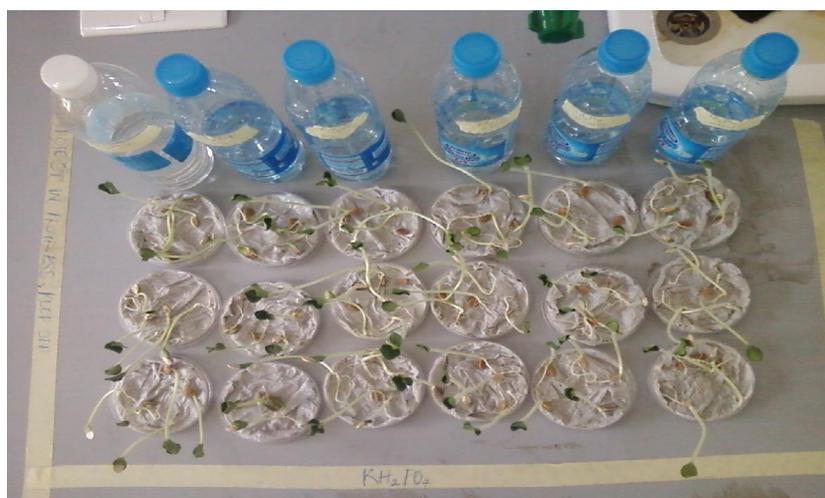


Plate 1: Experimental Set-up on Effect of KH₂PO₄ on *Cucurbita maxima* Growth. From Left to Right: 0%, 1%, 0.1%, 0.01%, 0.001% and 0.0001%.

Table 2: Effects of Salinity (CaCl₂) on Growth Morphology of *Cucurbita maxima*

Conc. CaCl ₂ (%)	Seedling Height (cm)	Leaf Area (cm ²)	Root Length (cm)	Moisture Content (%)	Percentage Germination (%)
0	8.43 ± 0.40	12.4 ± 0.23	15.8 ± 1.64	90.1 ± 0.51	100.0 ± 0.00
1	0.85 ± 0.60	0.71 ± 0.52	6.2 ± 1.31	70.2 ± 0.02	100.0 ± 0.00
0.1	9.6 ± 0.40	5.7 ± 0.32	15.5 ± 0.82	99.0 ± 0.78	100.0 ± 0.00
0.01	10.5 ± 0.30	7.5 ± 0.40	18.0 ± 1.22	84.4 ± 0.62	100.0 ± 0.00
0.001	10.9 ± 0.40	8.0 ± 0.65	19.6 ± 0.82	92.0 ± 0.42	100.0 ± 0.00
0.0001	11.8 ± 0.20	8.9 ± 0.36	19.7 ± 1.10	89.4 ± 0.48	100.0 ± 0.00

Data are processed and expressed as mean ± Standard Error of three replicates



Plate 2: Experimental Set-up on Effect of CaCl_2 on *Cucurbita maxima* Growth. From Left to Right: 0%, 1%, 0.1%, 0.01%, 0.001% and 0.0001%.

Table 3: Effects of Salinity (KH_2PO_4) on Growth Morphology of *Cucurbita maxima*

Conc. KH_2PO_4 (%)	Seedling Height (cm)	Leaf Area (cm^2)	Root Length (cm)	Moisture Content (%)	Percentage Germination (%)
0	6.1 + 0.22	4.1 + 0.74	13.4 + 0.99	87.5 + 0.68	100.0 + 0.00
1	2.5 + 0.09	3.1 + 0.43	7.8 + 0.24	81.03 + 0.33	100.0 + 0.00
0.1	9.2 + 0.28	6.7 + 0.20	14.6 + 1.25	90.7 + 0.92	100.0 + 0.00
0.01	10.34 + 0.42	7.7 + 0.31	17.7 + 0.21	90.0 + 0.00	100.0 + 0.00
0.001	11.8 + 0.22	8.8 + 0.20	19.1 + 0.54	89.1 + 0.78	100.0 + 0.00
0.0001	12.4 + 0.20	9.4 + 0.32	19.2 + 1.77	79.7 + 0.18	100.0 + 0.00

Data are processed and expressed as mean \pm Standard Error of three replicates



Plate 3: Experimental Set-up on Effect of MgSO_4 on *Cucurbita maxima* Growth. From Left to Right: 0%, 1%, 0.1%, 0.01%, 0.001% and 0.0001%.

DISCUSSION

The analysis of variance showed a significant effects ($p < 0.05$) on all traits due to salinity stress. Similar decreases in growth parameters were found in *Withania somnifera* under salt stress^[15] and in *Salvadora persica* under saline conditions^[16]. *Cucurbita maxima* plants act as an absorber of the salts and may be incapable of coping with it, leading to the leaves eventually suffering from the toxic effects and resulting in reduced leaf growth, as reported in *Withania somnifera*^[15]. Due to abiotic stress from salts, the plant tries to cope with the situation by decreasing its leaf area, seedling height hence, conserving energy. High KH_2PO_4 , CaCl_2 and MgSO_4 concentrations in the growth medium of plants generate primary effects that negatively affect plant growth and development. Primary effects are ionic toxicity and osmotic stress. Ionic toxicity occurs because high concentrations of the salts in the cytoplasm of cells disturb several biochemical and physiological processes, and osmotic stress is induced by the lowering of the water potential causing turgor reduction and cellular water loss. Secondary effects of salt stress include inhibition of mineral uptake, membrane dysfunction and generation of reactive oxygen species in the cells^[17,18,19,20].

The salt solutions evidently imposed osmotic stressing *Cucurbita maxima* concentrations of 0.1% and 1% higher. Even though the RGR was not significantly reduced at 0.1%, the leaves were smaller and thicker and the stems and roots shorter at this salinity. These morphological changes were probably caused by the reductions in turgor pressure within the cells that restricted cell expansion. Ionic toxicity of salt generally occurs at concentrations in the cytoplasm exceeding 1% where most enzymes start to become inhibited^[21]. Ionic toxicity, due to salt accumulation in the cytoplasm, did not seem to be of major importance for *Cucurbita maxima*, as the difference in concentration in the tissues between plants grown at 0.01% and above, where RGR was not affected, and 1% where RGR was strongly reduced, was only minor. Rather, disturbance of the mineral acquisition resulting in very high ratios in the tissues were presumably a main factor responsible for the salt injury, as has also commonly been found for both terrestrial and aquatic plants^[22,23,24,25]. salts competes with mineral for uptake into cells, particularly when the external concentrations of minerals are substantially higher than that of ions, and the ability to maintain Na^+/K^+ homeostasis in the cells is crucial for the salt tolerance of plants. Our results indicate that *Cucurbita maxima* lacks mechanisms to maintain homeostasis as the high concentrations of salts in the growth solution created a deficiency of K^+ , as has also been shown in other studies^[18,26,27]. Compartmentalization of Na^+ and Cl^- into the vacuole, and the accumulation of organic

solutes, such as sugars, and amino acids, that do not inhibit metabolic processes, in the cytoplasm, is a common mechanism of maintaining intercellular homeostasis. Proline is one of the so-called compatible compounds that are commonly found in high concentrations when plants are exposed to salt stress ^[17,25,28,29].

Increasing salinity levels causes a significant decrease in vegetative growth of *Cucurbita maxima*, in which the morphological changes are probably caused by the reductions in turgor pressure within the cells that restricted cell expansion ^[30]; consequently, the expansion of leaf area is reduced. Reduction in plant growth has also been attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of ionic imbalance and decrease in many metabolic activities ^[31]. It appears that the decreased root and shoot fresh weight is due to the reduced water absorption, which in-turn causes a reduction in the amount of water in plant tissue ^[32]. Moradi and Ismail ^[33] found that the cause of decrease in production of rice plant in saline conditions created by irrigation water salinity increased energy costs, reduced carbon capture and photosynthesis per unit leaf area. The results show that all growth characteristics in treatments 3 dS m⁻¹ compared with control plants had a slight increase, the increase in plant growth and production of shoot and root indicates the significance and necessity of having a suitable level of absorbing water and minerals in stress conditions, which are accompanied by reduction of lateral roots ^[34].

CONCLUSION

From the results of this investigation, it is clear that, the three salts (KH₂PO₄, CaCl₂ and MgSO₄) treatment caused significant growth stress on *Cucurbita maxima* though it reduces the plant growth. This result is of great economic significance to abiotic stresses like water stress, salt stress, metal stress etc., can cause an increase in the active principle contents in medicinal plants and this will be helpful for the cultivation of this plant in salt affected areas. Based on this research the following recommendations are therefore necessary; physiochemical properties of garden soil should be carried out before planting, proximate composition should be carried out on crops planted on saline soil, phytochemical properties of plants sown on saline soil should be investigated, comparative studies should be carried out on other crops on saline soil, control measures should be worked out to neutralise saline soil.

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