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# The Active Role of Calcium Chloride on Growth and Photosynthetic Pigments of Cowpea "*Vigna unguiculata* L. (Walp)" Under Salinity Stress Conditions

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**Abstract:** Salinity is considered a major problem in agriculture and crop production, therefore, a long time ago scientists focused on studying the impact of salts on the growth and productivity of various plants and how to resist salinity and overcome it. In this study, the effect of calcium chloride on growth and photosynthetic pigments of cowpea plants (*Vigna unguiculata* L.) under salinity stress which caused by sodium chloride were studied. Cowpea seeds were grown in plastic pots under a greenhouse to assess the morphological measurements and photosynthetic pigments at 50 days after sowing. Results proved that shoot and root length, leaves number, leaf area and fresh and dry weights of shoot and root were significantly decreased with increasing salt concentrations, except for the low concentration 20 mM NaCl where it non-significantly increased compared with the control plants. Addition of calcium chloride led to a significant increase in all growth characters compared to salt stressed plants. Highly significant increase in photosynthetic pigments contents in cowpea plants were obtained at 20 mM NaCl and then it significantly decreased in the higher concentrations of salt. When the salt stressed plants treated with calcium chloride, photosynthetic pigments contents were increased significantly.

**Key words:** Sodium chloride · Calcium chloride · Salinity stress · Growth · Cowpea

## INTRODUCTION

Soil salinity is considered a global problem particularly in arid and semi-arid regions and one of the most serious factors limiting crops growth as well as causing great losses for the production of most crops, because of the impact of salinity on the physiological and biochemical imbalances in plants which are grown under salt stress. The world growing population and the shortage of water resources for agriculture as well as the shortfall in the amount of irrigated lands with an increase in human needs for more food, led to the study of the soil areas that affected with salt in order to improve agriculture [1]. Therefore, plant responses to environments with high salt content became one of the most important topics which are interest to Agricultural Researchers in the field of agriculture and crops production, as the growth decline or the inability to originally growth of crops observed in these lands and as they in a close association with human food source. Thus scientists interested in studying the impact of salts on plants growth and damages they cause and studying the mechanism of plant resistance to salts including the use of plant growth regulators, mineral elements, plant hormones, fertilizers and antioxidants in order to overcome the negative effects of salinity and to improve crop production in saline environments or irrigated with water with relatively high salt content and develop new varieties of crop plants with high degree of resistance to salinity [2]. One of these mechanisms is using calcium, where the cellular juice "cytosol" in plant cells containing calcium in the range 10-20 nm under the normal conditions, while the concentration of calcium in the cell wall, vacuole, endoplasmic reticulum and mitochondria is 1-10 mM [3]. However, certain conditions

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such as stresses lead to an increase in the calcium level in cellular juice “cytosol” up to the level of 1µM which considered toxic if it continues for a longer time in the cytosol. Therefore, plants store excess calcium either in apoplast or in vacuole, or endoplasmic reticulum, so it was found that endoplasmic reticulum and vacuole can be used with the cell wall to raise the level of calcium under stress conditions as a defensive response [4]. Calcium also considered an essential nutrient that regulate the growth and development of the plant and improve its productivity and increase the biomass production [5]. Therefore, the importance of calcium in the development and growth of plants reflects its participation in many operations, as if considered an important element in the composition of the cell wall and the plasma membrane and the production of plant tissues and maintains the strength of herbaceous plants stem and it regulates the absorption of nutrients across the plasma membrane of the cell and plays an active role in metabolism by influence on the transfer of sugars and the formation of the nucleus and the mitochondria. It is believed that calcium works as a stimulant for some enzymes such as phospholipase, arginine kinase and adenosine tri-phosphatase etc. Calcium also plays a role in the elongation and division of plant cells. It exists as calcium pectate which interference in the installation of the middle lamella between the cell walls and has a role in the permeability of cell membranes and in the metabolism of nitrogen, in addition to his role in the equivalent of the negative charges of proteins and its presence is important in the formation of flowers [6].

In addition to the basic calcium roles it has secondary functions where is the molecule and the carrier messenger in the plants exposed to various stresses, including all the biotic and abiotic stresses, especially salinity stress which increases the plant's resistance to it [4]. It has been found that the optimal concentration of calcium ion plays an important role in various physiological processes of plants and causes an increase in plant resistance for stresses like salt, drought and heavy metals, it also improves the growth and the biochemical measurements of plants exposed to salinity stress [7]. Therefore, experiments were conducted on many agricultural crops to alleviate the salinity by adding calcium chloride to the irrigation water so as to loosen up the growth inhibition resulting from salinity and reduce the impact of the salt NaCl [8].

Among the most important crop plants, cowpea *Vigna unguiculata* (L.) Walp which is one of the major green food crops that contributes to achievement of food security and poverty alleviation in poor countries [9]. It is considered one of the most cultivated and consumed legumes in the continents of Asia and Africa and classified under the subfamily *Papilionoideae* in the family *Leguminosae* [10]. It has been noted that cowpea leaves are eaten as it contain a greater proportion of protein than the dry seeds, thus it can be a substitute for animal protein in areas of the third world, where crop production is more important than animal production and it have a medical value where it prevents digestion disorders and constipation as it contains non-soluble fiber and it controls blood sugar levels and has a role in prevention of heart disease and provides the body with protein and iron and increases the activity of the body and provide it with energy [11].

## MATERIALS AND METHODS

Cowpea *Vigna unguiculata* L. (Walp) (var. Clifornia Black Eye#5) seeds obtained from the Ministry of Agriculture, Riyadh, Saudi Arabia were washed several times and sterilized with Sodium Hypochlorite Solution (5%) for two minutes, then cultivated in 15cm diameter plastic pots filled with mixture of sand and peat moss (1:1). Five seeds were used in each pot on 2cm depth of approximately equal distances. Pots were kept under plastic greenhouse conditions and the temperature were adjusted to 20-25°C and irrigated 3 times a week with specific amounts of water, or sodium chloride solution alone, or a mixture of sodium chloride and calcium chloride solution. Pots were divided into 4 groups, each group with five replicates for each treatment as follows: 1$^{st}$ group was irrigated by 200ml of water to represent the control, the 2$^{nd}$ group was divided into 3 sub-groups and irrigated by 200ml of NaCl solution with the concentrations 20, 60 and 100 mM; 3$^{rd}$ group was divided into 3 sub-groups and irrigated by 100ml of NaCl solution with the previous concentrations+100ml of $CaCl_2$ solution with the concentration 2mM; and the 4$^{th}$ group was divided into 3 sub-groups and irrigated by 100ml of NaCl solution with the previous concentrations+100ml of $CaCl_2$ solution with the concentration 4mM. At the end of the experiment, growth parameters of the plants were assessed as well as some physiological measurements as photosynthetic pigments content of cowpea plants were estimated.

**Solutions Preparation:** Sodium chloride saline solution was prepared by dissolving 58.44 g of NaCl in 1L distilled water to obtain the concentration of 1000 mM and then the concentrations 20, 60 and 100 mM were prepared by dilution method from the stock solution. Calcium chloride solution was prepared by dissolving 110.99 g of $CaCl_2$ in 1L distilled water to obtain the concentration of 1000 mM and then dilution method was used to prepare the concentrations 2 and 4mM from the stock solution.

**Measurements of Growth:** Growth parameters were assessed at 50 days after sowing (DAS), these parameters included shoot and root length (cm), fresh and dry weights per plant (g), then samples were placed in the drying oven at 80°C until weights become stable to take the dry weight of plants (g) and then preserved in desiccator until the chemical analysis.

**Measurements of Leaf Area ($cm^2$) and Leaves Number:** The area of the third leaf for each plant was taken at 50 DAS (30 days of saline stress treatment) using leaf area METER CID, INC CI- 202 AREA.

**Chemical Analysis by Extraction and Estimation of Photosynthesis Pigments:** The method of Metzner *et al.* [12] was used in chlorophyll extraction by crushing 0.5 g of plant leaves in a mortar with 10 ml of acetone 80% for 5 minutes to extract pigments from the fresh leaves and then placed in a centrifuge for 5 minutes on the speed of 1000 rpm. The supernatant was taken into a tube and the total volume was adjusted to 25 ml with 80% acetone.

Chlorophyll *a*, *b* and carotenoids were estimated using spectrophotometer (UV/Visible Spectrophotometer - LKB-Biochrom 4050) at wave lengths 663, 644, 452.5 nm and a glass cell with optical path with 1 cm thickness, using following equations:

Chlorophyll (*a*) = 10.3 × O.D 633 – 0.918 × OD 644 = µg/ml.
Chlorophyll (*b*) = 19.7 × O.D 644 – 3.87 × OD 633 = µg/ml.
Carotenoids = 4.2 × OD 452.5 – (0.0264 Chlorophyll (*a*) + 0.426 Chlorophyll (*b*) = µg/ml.

where:
OD: optical density for chlorophyll extraction and carotenoids at the given wave length.

The pigments expressed as (µg/100g fresh weight) and three replicates were used for each treatment.

**Statistical Analysis:** All results were statistically analyzed using the (F. Test) (One-Way ANOVA) to calculate the least significant difference (LSD) and the difference between the means were compared at probability of 1% and 5% according to Snedecor and Cochran [13].

## RESULTS

**Growth Characters:** The different growth parameters were recorded (shoot and root length, leaves number, leaf area and fresh and dry weights of shoot and root ) for cowpea plants treated with NaCl in the presence or absence of $Ca^{+2}$ with both concentrations 2 and 4mM at 50 DAS. Data presented in Table 1 and Figures 1, 2, 3 and 4 showed that, the effect of the concentrations 20, 60 and 100 mM of the salt NaCl on the shoot and root length, leaves number, leaf area and fresh and dry weights of shoot and root; where growth characters non-significantly increased or significantly increased only with the low concentration 20 mM of NaCl in comparison with control and then a gradually decreased by increasing the salt concentration. Shoot length was decreased at 60 and 100 mM of NaCl by 21.74 and 53.62%, respectively, in comparison with control (non- salt stressed plants) (Table 1). Data also indicated that when salt-stressed plants treated with 2 and 4mM $CaCl_2$ the growth characters were increased and the increase in plants treated with 4mM was significant compared with unsalt-stressed plants (untreated with calcium chloride). The increase in the concentration of $CaCl_2$ up to 4mM resulted in an increase in growth character that that the concentration 2mM $CaCl_2$.

**Photosynthetic Pigments:** Results showed a significant increase in chlorophyll *a*, chlorophyll *b*, carotenoids contents and the total pigments (Table 2). This increase was highly significant at the low concentration of sodium chloride 20mM and then declined significantly at the concentrations of 60 and 100 mM NaCl. Also, when adding $CaCl_2$ to salt stressed plants with the concentrations 2 and 4mM the chlorophyll *a*, chlorophyll *b*, carotenoids contents and total pigments were increased in both concentrations compared to untreated plants with calcium chloride. The increase in salt stressed plants which treated with 4mM calcium was highly significant compared to control plants (untreated with calcium chloride). The increase in chlorophyll *a* for the salt stressed plants with the concentrations 20, 60 and 100mM of NaCl and treated with calcium concentration 4mM estimated by 15.49, 6.1 and 21.56%, respectively, compared to unsalted stressed plants (untreated with calcium chloride). Also the significant increase in chlorophyll *a*, chlorophyll *b*, carotenoids contents and total pigments in salt stressed plants with the concentration 100mM of NaCl when treated with 4mM of calcium estimated by 21.56, 27.51, 36.56 and 27.94%, respectively (Table 2 and Figures 5, 6, 7 and 8).

Table 1: Effect of different concentrations of NaCl and $CaCl_2$ on growth characters of cowpea plants

| Treatments NaCl/$CaCl_2$ mM/mM | | Shoot length (cm) | Root length (cm) | Leaves number | Leaf area | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | | 13.800 ± 1.923 | 24.800±1.483 | 4.600±1.517 | 17.890±2.245 | 1.316±0.129 | 0.248±0.015 | 0.370±0.051 | 0.120±0.058 |
| 20 | 0 | 14.800±1.789 | 25.400±2.702 | 4.800±1.483 | 19.057±4.132 | 1.532±0.189 | 0.276±0.050 | 0.410±0.042 | 0.156±0.038* |
| 20 | 2 | 16.200± 2.168 | 27.600±3.209* | 5.200±1.304 | 22.137±5.118 | 2.190±0.291** | 0.328±0.047 | 0.450±0.037 | 0.180±0.037 |
| 20 | 4 | 19.400±2.074** | 29.400±1.140** | 5.800±.0837 | 26.960±6.256** | 2.600±0.440** | 0.460±0.11** | 0.574±0.056** | 0.206±0.036** |
| 60 | 0 | 10.800±0.837** | 21.800±1.483** | 3.200±0.837* | 10.537±1.206** | 1.094±0.251 | 0.218±0.029 | 0.272±0.016* | 0.082±0.019* |
| 60 | 2 | 12.800± 0.837* | 23.400±1.140 | 4.200±0.837 | 13.727±0.421 | 1.562±0.388 | 0.236±0.027 | 0.304±0.011 | 0.102±0.019 |
| 60 | 4 | 15.800± 1.304** | 25.600±1.140** | 4.600±0.548* | 16.430±1.254* | 2.074±0.813** | 0.430±0.068** | 0.344±0.017 | 0.132±0.022** |
| 100 | 0 | 6.400±1.140** | 18.200±0.837** | 2.400±0.548** | 6.777±1.710** | 0.586±0.350** | 0.162±0.159** | 0.196±0.018** | 0.038±0.013** |
| 100 | 2 | 8.600±1.140** | 20.600±1.140* | 3.400±0.548 | 8.957±5.918 | 0.966±0.234 | 0.200±0.024 | 0.230±0.026 | 0.066±0.021 |
| 100 | 4 | 10.800±1.095** | 22.400±0.894** | 3.800±0.837** | 11.490±0.915* | 1.100±0.252* | 0.226±0.032* | 0.274±0.021 | 0.102±0.026** |
| LSD 0.05 | -- | 1.68 | 2.01 | 1.17 | 4.74 | 0.51 | 0.07 | 0.08 | 0.04 |
| LSD 0.01 | -- | 2.22 | 2.66 | 1.55 | 6.30 | 0.67 | 0.09 | 0.11 | 0.05 |

Each value is a mean of 5 replicates ± SE, * Significant difference, ** Highly Significant difference

Table 2: Effect of different concentrations of NaCl and $CaCl_2$ on photosynthetic pigments (µg/100g fresh weight) content of cowpea plants.

| Treatments NaCl/$CaCl_2$ mM/mM | | Chlorophyll *a* | Chlorophyll *b* | Carotenoids | Total pigments |
|---|---|---|---|---|---|
| 0 | | 746.73±15.233 | 455.63±13.435 | 403.59±13.891 | 1605.94±14.608 |
| 20 | 0 | 804.10±18.814** | 479.18±12.142* | 440.47±10.190** | 1723.75±40.998** |
| 20 | 2 | 845.42±9.511** | 512.97±15.389** | 464.56±13.112* | 1822.92±11.797** |
| 20 | 4 | 951.53±14.835** | 673.64±13.059** | 544.79±13.045** | 2169.95±37.350** |
| 60 | 0 | 654.85±14.042** | 330.50±11.441** | 281.43±13.611** | 1266.72±13.006** |
| 60 | 2 | 676.29±17.380* | 347.97±11.744** | 333.02±14.002** | 1287.26±15.154 |
| 60 | 4 | 697.04±12.351** | 375.74±13.537** | 366.39±13.250** | 1379.17±17.976** |
| 100 | 0 | 361.38±11.808** | 123.77±13.412** | 116.85±12.678** | 602.10±11.286** |
| 100 | 2 | 421.56±11.282** | 141.72±10.508 | 130.42±10.838 | 693.69±11.654** |
| 100 | 4 | 460.68±11.155** | 170.75±15.582** | 184.18±13.005** | 835.61±39.546** |
| LSD 0.05 | -- | 22.03 | 22.65 | 20.42 | 65.24 |
| LSD 0.01 | -- | 29.30 | 30.13 | 27.16 | 68.76 |

Each value is a mean of 3 replicates ± SE, * Significant difference, ** Highly Significant difference

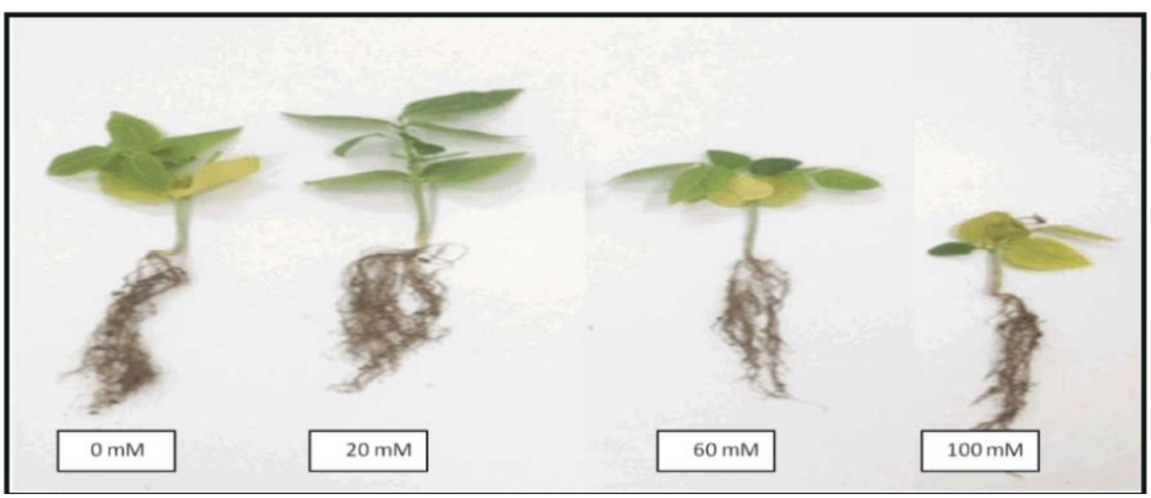


Fig. 1: Effect of different concentrations of NaCl on cowpea plants

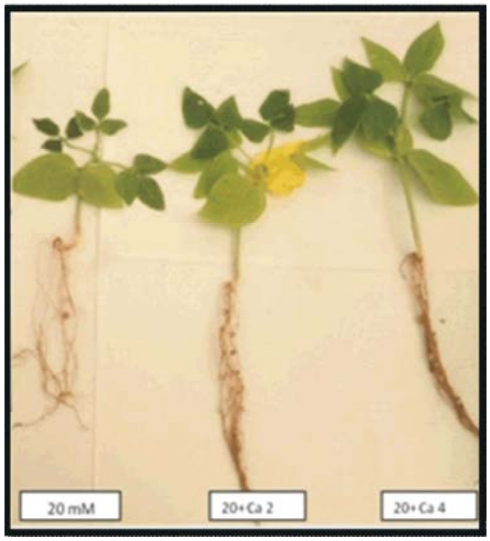


Fig. 2: Effect of different concentrations of $CaCl_2$ and 20 mM NaCl on cowpea plants


Fig. 3: Effect of different concentrations of $CaCl_2$ and 60 mM NaCl on cowpea plants

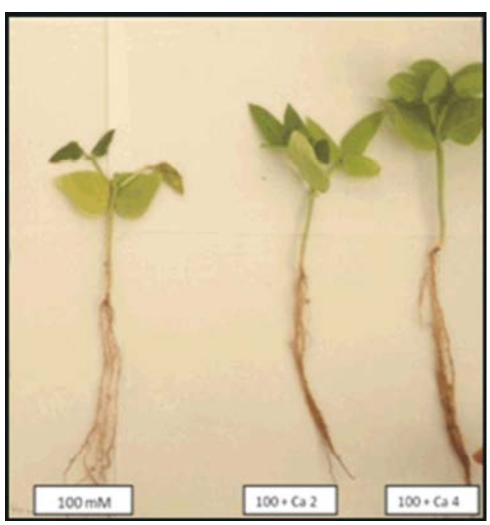


Fig. 4: Effect of different concentrations of $CaCl_2$ and 100 mM NaCl on cowpea plants

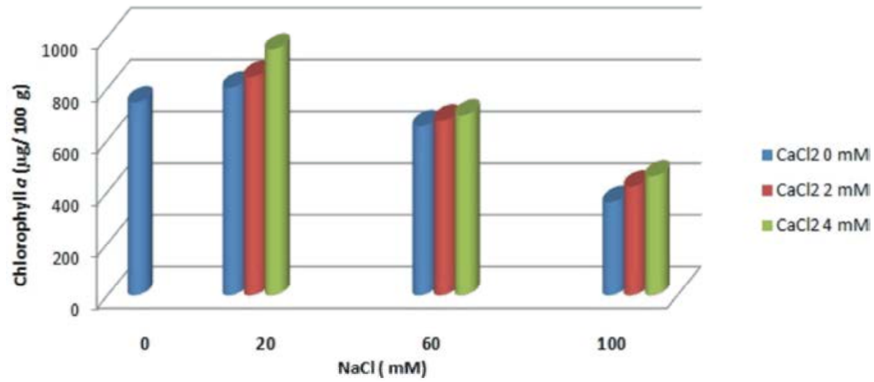


Fig. 5: Effect of different concentrations of NaCl and $CaCl_2$ on chlorophyll *a* content of cowpea plants

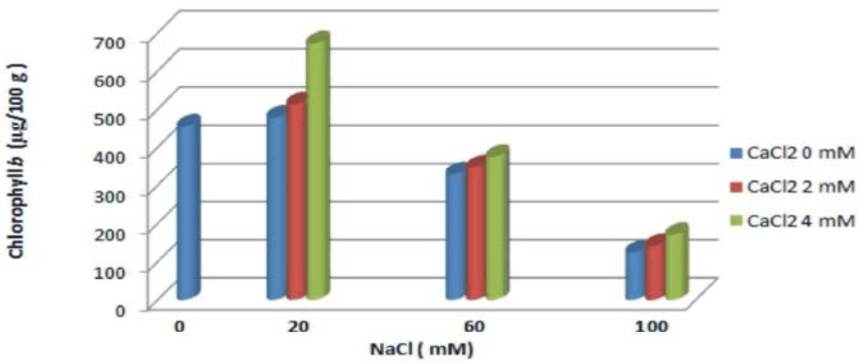


Fig. 6: Effect of different concentrations of NaCl and $CaCl_2$ on chlorophyll *b* content of cowpea plants

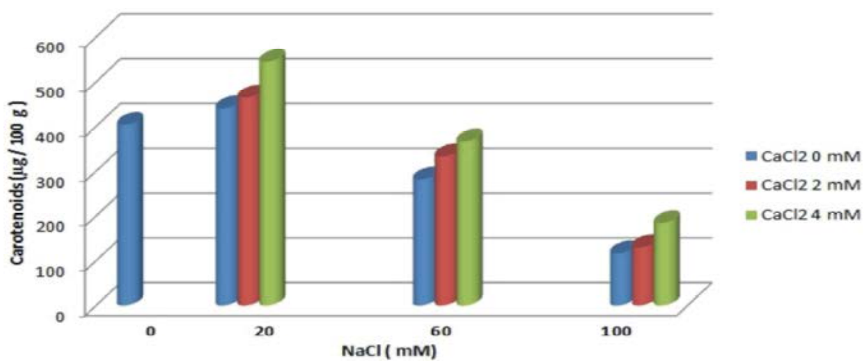


Fig. 7: Effect of different concentrations of NaCl and $CaCl_2$ on carotenoids content of cowpea plants

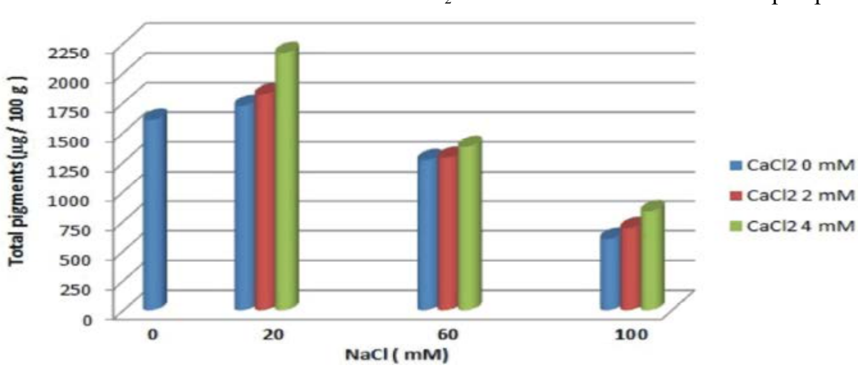


Fig. 8: Effect of different concentrations of NaCl and $CaCl_2$ on total pigments content of cowpea plants

## DISCUSSION

Salinity considers a global problem, therefore, a long time ago scientists interested in studying the impact of salts on the growth and productivity of various plants, especially economic plants to meet food requirements that increase with population increase. So this research plans to study the effect of salinity stress on the growth of cowpea plants and attempt to resist the negative and harmful effects of it by adding $CaCl_2$ to mitigate these effects. This experiment was conducted in plastic pots to clarify the effect of sodium chloride in the presence or absence of $CaCl_2$ with the concentrations 2 and 4mM on some morphological characteristics and some physiological processes at 50 DAS. This study showed that treating cowpea plants with the low concentrations 20mM of the sodium chloride led to an increase in shoot and root length, leaves number, leaf area and fresh and dry weights of shoot and root, while growth characters were decreased significantly or highly significant in treated plants with the medium and the high concentrations 60 and 100mM compared to control plants (0 mM NaCl). These results are consistent with Farag [14] on cowpea plant, Keling and Zhujun [15] on *Brassica campestris*, Celik and Atak [16] on tobacco plants *Nicotiana tabacum* L., Turan *et al.* [17] on corn and Rahimi and Bioglarifard [18] on a strawberry plants. Patel *et al.* [19] showed that salinity led to a significant decline in the length of cowpea plants (*Vigna unguiculata*) and the root length with increasing salt concentration and when rice plants (*Oryza sativa* ) treated with 0, 25, 50, 100 and 200mM of NaCl both the fresh and dry weights as well as leaf area decreased [20]. Querioz *et al.* [21] found that treating soybean (*Glycine max*) plants with the concentrations of 50, 100 and 200mM NaCl led to a lack of growth characters and the root was less affected than the shoot, while leaf area was decreased significantly compared to the control plants. Jaarsma *et al.* [22] also noted that treating potato plants (*Solanum tuberosum*) with the concentrations 0, 60 and 180 mM of NaCl for 21 days reduced characters, especially in the high concentration 180mM.

The decline in the various growth parameters in cowpea plants can be explained on the basis of photosynthesis deficiency where plants close the stomata to maintain water quantity in the leaves and therefore diminish the entry of $CO_2$ and then diminish photosynthesis rate [23], which leads directly or indirectly to reduced the amount of photosynthesis products that reach the growth areas [24]. Chookhampaeng [25] found that the low concentration of NaCl 50mM did not affect the growth rates of pepper seedlings (*Capsicum annuum* L.), while high concentrations 100 and 200mM led to a significant decrease in growth characters. The lack of growth characters in the treated plants with the high concentrations of NaCl may be due to a lack of water pressure and a lack of nutrition elements as the high concentration of NaCl worked on the lack of absorption of $K^+$, $Mg^{+2}$ and $Ca^{+2}$, also $Na^+$ competes with $K^+$ and replace it in the cell [18]. Tabatabaei [26] reported that the lack of leaf area due to the accumulation of $Na^+$ and $Cl^-$ ions in the leaf, leading to a failure in stretching of leaf cells. The lack of growth parameters also can be explained by the accumulation of abscisic acid (ABA), one of the inhibitory growth regulators [27], as this acid has a positive role to push the seedlings to resist salinity through closing the stomata as less transpiration process and increasing the water content in the plant [28].

Results indicated that treatment of cowpea plants with the concentrations 2 and 4mM of $Ca^{+2}$ led to an increase in all growth characters, particularly at the concentration of 4mM compared to unsalt stressed plants with this element. These findings are consistent with Manivannan *et al.* [29] on mungbean (*Vigna radiate* L.), Jafari *et al.* [30] on sorghum (*Sorghum bicolor*), Rab and Haq [31] on tomato and Al-Whaibi *et al.* [32] on faba bean (*Vicia faba*). The effect of $Ca^{+2}$ on increasing growth characters is due to several reasons including that $Ca^{+2}$ contributes in the composition of the cell wall and the cell membrane, therefore it keeps the balance and stability of membranes through the contacting of types of protein and lipids on the surface of the membrane [33]. As well as affecting the pH in the cell which prevents the solvent exit out of the cytoplasm and works on increasing of the shoot length [34, 35]. Calcium helps in raising the level of plants to withstand saline stress, through many mechanisms including the stabilization of cell membranes and walls and organization of sodium and reduce the toxic effects of sodium chloride through facilitating the transfer of $Na^+$/ $K^+$. And as a result of the high rate of salinity, it causes an increase of calcium in vacuole which is shifted from apoplast and the compartments within cells [36]. Also, calcium chloride plays an important role in promoting bearing plants to salt stress by improving the defense system of anti-oxidation and the accumulation of osmolyte which lowers osmosis and adjusts the ion balance [37].

The current study demonstrated that the content of the total pigments of photosynthesis significantly increased in treated plants with the low concentration 20mM of NaCl, but it's greatly decreased significantly whenever the salt concentration increased. These findings are consistent with El-Sayed [38] on faba bean (*Vicia faba*), Amirijani [20] on rice (*Oryza sativa*), Silva *et al.* [39] on *Jatropha curcase* L. plants, Ozturk *et al.* [40] on pea (*Pisum sativum*), Nasseri *et al.* [41] on *Trigonella foenum graceum* plants, Ratnakar and Rai [42] on spinach (*Spinacia oleracea*) and Meloni *et al.* [43] on cotton plants. Scientists explain the decline in the total pigments of photosynthesis whenever the salt concentration increase to the accumulation of $Na^+$ and $Cl^-$ ions which hindering the process of chlorophyll synthesis by influencing the activity of some enzymes containing $Fe^{+3}$ [39, 44]. While, Mane *et al.* [45] stated that the high concentration 300mM of NaCl led to a large significant decrease in photosynthesis pigments due to damage in photosynthetic electron transport chain for the severe shortage of water uptake. The lack of water absorbed led to a shortage in the water pressure of leaves, causing a shortage of leaves efficiency of electron transport and energy production, which inhibits cyclic and noncyclic photophosphorylation [46, 47]. Chartzoulakis [48] also found that the photosynthesis rate in cucumber leaves which are salt stressed decreased by 90% compared to control plants and this was attributed to stomata close and the lack of $K^+$ content [49] which leads to a lack of the $CO_2$ fixation in the photosynthesis.

Salinity stress can lead to an increase in the free radical in chloroplasts and the demolition of chlorophyll through increasing the reactive oxygen species (ROS) producing the lack of both photosynthesis and growth [50]. The addition of $Ca^{+2}$ to the salt stressed plants affects most of the physiological processes within the plant and our data showed that the addition of $Ca^{+2}$ worked on increasing the total content of photosynthesis pigments compared to unsalted stressed plants (non-treatment with $Ca^{+2}$ element). Mozafari *et al.* [7] found that the treatment of *Descurainia sophia* plant with the concentration 25mM of NaCl led to a significant increase in chlorophyll *a* and the total pigments, while high concentrations 75 and 100mM led to a decrease of these two components, but when adding $CaCl_2$ with the concentration 5mM the chlorophyll *a* was increased significantly and it is probably due to the $Ca^{+2}$ work on protecting the chloroplasts wall and helps the activity of photosynthesis enzymes as reported by Yeo *et al.* [51] on rice and Siddiqui *et al.* [52] on faba bean (*Vicia faba*) stressed with the cadmium element, where it was found that there was an increase in the content of chlorophyll *a* and chlorophyll *b* when the plant treated with each of $Ca^{+2}$ and $K^+$. This result is in agreement with those reported by Khan *et al.* [37] on mustard plant. So, it was find that the calcium participates in the regulatory mechanisms that activate the plants to adapt to adverse environmental conditions such as salt stress and thus can serve as a treatment for the physiological processes to increase the resistance of plant bearing [53].

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