International Journal of Plant Science and Ecology
Vol. 6, No. 3, 2020, pp. 42-55
http://www.aiscience.org/journal/ijpse
ISSN: 2381-6996 (Print); ISSN: 2381-7003 (Online)

Impacts of Growth Regulators & Moringa Extract Pre-treatment on Germination, Growth & Pigments in Two Tomato Cultivars Under Salinity Stress

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Abstract

The aimed of this studies to explain the role of Ascorbic acid (ASA), Gibberellic Acid (GA3) & *Moringa oleifera* Leaf Extract (MLE) for alleviates the adversely effect of salinity stress on two cultivars (cv. Cobra-resistant and cv. Newton-sensitive) of tomato (*Lycopersicon esculentum,* L.) plant which collected from Al-Dakhil Agriculture and Trading Establishment, Al-Qassim City, KSA. The tomato seeds soaked in in ASA 0.75 mM; GA3 0.05 mM and Moringa Extract MLE 5% before germinated for 12 hours in the dark. The seeds planted in trays of cork contain 218 eyes for 14 days, seedlings plant transplanted to plastic containers containing a mixture of sand/peat-moss (1:2). The tomato seeds watering using distilled water until the true leaf appearance then transferred to a larger pots which containing the same mixture of soil as above. The experiment was carried out under greenhouse conditions with temperature 18°C±1°C (night) & 22°C±2°C (day) and relative humidity varied between 60-70%. The tomato plant irrigated with NaCl salinity (0.0, 50, 100, 150, 200 mM NaCl) alternative with Hoagland nutrient solution. Salinity treatments decreased seeds germination rate (%), shoot height and root depth; fresh and dry weights; water relation (succulence) and all Photosynthetic pigments content (chlorophyll a, chlorophyll b, carotenoids, and total pigment contents) while the dry matter content (%) increased significantly with increasing NaCl salinity. Whereas the seeds soaking before germination in ASA, GA3 & MLE get an increased seeds germination rate (%), shoot height and root depth; fresh and dry weights; water relation (succulence and dry matter contents) and all Photosynthetic pigments content (chlorophyll a, chlorophyll b, carotenoids, and total pigment contents) compared with control. The results indicated that the plant growth parameters increased significantly in the present of ASA, GA3 & MLE under non-saline and salinity stress thereby reduces the harmful effects of salinity and increases resistance to salinity more than in the absent of ASA, GA & MLE compared with control. The application of soaking tomato seeds before germination in GA3, MLE, ASA respectively get evidence to the tolerance effect of salinity stress.

Keywords

Growth Regulators, Ascorbic Acid, Gibberellic Acid, Moringa, Germination, Growth, Chloroplast Pigments, Tomato, Salinity Stress

Received: August 6, 2020 / Accepted: September 1, 2020 / Published online: September 24, 2020

1. Introduction

A major challenge towards world agriculture involves production of 70% more food crop for an additional 2.3 billion people by 2050 worldwide. Salinity is a major stress limiting the increase in the demand for food crops. More than 20% of cultivated land worldwide (~ about 45 hectares) is

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affected by salt stress and the amount is increasing day by day. Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity and eventually die). Majority of major crop species belong to this second category. Thus salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide [1]. Salinity is one of the main abiotic stresses which affects crops in the local demographic throughout the globe, as 800 million hectares of land are salt affected globally, accounting for approximately 6% of the entire acreage [2-3].

The salt promotes changes of functions of the cell membranes and the cell walls and may affect the water potential of the cytosol and cellular extensibility, and thus, may affect seed germination and seedling growth. The seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease. These salts interfere with seed germination and crop establishment. Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Salinity stress can affect seed germination through osmotic effects [4], so, salt tolerance plant which is an adaptive strategy of plants in response to salinity stress with a range of various mechanisms at the cellular, organelle and plant levels. This includes the regulation of osmoprotectant molecules, reactive oxygen species (ROS) scavenging, antioxidant defense system, osmotic balance and compartmentalization of toxic ions [3, 5].

Consequently, it is very important to study the mechanisms of salinity tolerance to determine which plants are able to enact physiological and biochemical mechanisms to cope with salinity stress. Moreover, the use of sustainable agricultural tools to improve plant growth and productivity even under abiotic stress can ensure the acquisition of high productive crops under expected climatic changes [6]. Plants grown under saline conditions accumulate high levels of salt, causing disturbances within the physiological and biochemical parameters that appear in ion toxicity, photosynthesis reduction, accumulation of free radicals, membrane instability and various metabolic disorders [7].

Tomato is an important crop throughout the world and is grown under a wide range of production systems. In the areas with optimal climate for tomato cultivation, salinity is a serious constraint [8]. As Most cultivated plants, tomato inclusive, are sensitive to salt stress [9]. However, Tomato is sensitive to moderate levels of salt in the soil. However, tomato production has been gradually extended into the more marginal lands, thus, exposing the crop to a greater risk of salt stress Tomato is a popular vegetable necessitating its improvement to fit in the environments with varying salinity [10]. In addition, earlier application of salt stress to tomato plants, such as immediately after flowering during periods of high temperature, frequently results in blossom-end rot (BER) [11].

Ascorbic acid and phenolic compounds serve as non-enzymatic antioxidants, which increase the antioxidant capacity of a cell to scavenge ROS produced under salinity stress [12]. Ascorbic acid (ASA - Vitamins C) is one of the universal non-enzymatic antioxidants having substantial potential of not only scavenging ROS, but also modulating a number of fundamental functions in plants both under stress and non-stress conditions. Most efficient role of ASA is to protect lipids and proteins against salinity - induced oxidative adversaries [13], enhancing plant growth, rate of photosynthesis, transpiration, and oxidative defense potential and photosynthetic pigments, it also has a role in cell division and cell enlargement, stomatal regulations, as well as acting as a co-factor for many enzymes [14]. Additionally, ascorbic acid protects metabolic processes against H_2O_2 and toxic derivatives of oxygen which affect various enzyme activities and decreases the damage caused through oxidative processes by functioning in synergy with other stabilizing membranes and antioxidants [15]. Gibberellic acid (GA3) is an endogenous plant growth regulator; it has many toxic effects including testicular toxicity. *Phoenix dactylifera* L. (date palm) is widely used in traditional medicine as anticancer, hepatoprotective, neuroprotective, nephroprotective, gastrointestinal protective and antimicrobial agent [16].

Moringa oleifera is a medicinal plant used to treat numerous diseases. It is rich in allelochemicals [amino acids (Threonine, methionine and phenylalanine), fatty acids (Palmitic, oleic and linoleic), phenols (Gallic acid, p-coumaric acid and ferulic acid) flavonoids (Catechin, quercetin, kaempferol and niazimicin) and other bioactive compounds, vitamins (B, A, C, D and K), zeatin and essential macro (Potassium, magnesium and phosphorus) and microelements (Iron and zinc) [17]. So, this research aims to study the alleviated the impact of NaCl salinity on seed germination, growth, water relations and photosynthetic pigments contents traits: chlorophyll a, b, carotene, and total photosynthetic pigments, for two selected tomato (*Lycopersicon esculentum*, L.) cultivars cv. Cobra (resistant) and cv. Newton (sensitive) by soaking the seeds before germinations in growth regulators (ASA 0.75 mM; GA3 0.05 mM) and *Moringa oleifera* leaves extract (MLE-5%), which will help in producing more tomatoes Tolerance to salinity stress.

2. Materials and Methods

2.1. Tomato Plant Species and Culture Techniques

The plant used in this study is tomato *(Lycopersicon esculentum,* L.) seeds obtained from Al-Dakhil Agriculture and Trading Establishment, Al-Qassim City, Saudi Arabia for both cultivars (cv. Cobra and cv. Newton) which are resistant and sensitive respectively to salinity stress. Both cultivars are characterized by its earliness, high yield ability, uniform ripening and disease tolerance. Germination was undertaken in the greenhouse at Al Qassim city, Kingdom of Saudi Arabia.

2.1.1. The Soil Used

The soil used for cultivated tomato (*Lycopersicon esculentum*, L.) plant was the ratio between the sand and peat-moss (1:2 – v: v), added in each pot (diameter 16 cm and depth of 16 cm), by the same ratio of the soil of the volume.

2.1.2. Nutrient Solutions

The base nutrient solution used was similar to that applied by Hoagland and Arnon [18] the nutrient solution composed of: (2.5×10^{-7} M KNO_3, 5×10^{-4} M KH_2PO_4, 2.5×10^{-5} M Ca $(NO_3)_2$, 10^{-3} M $MgSO_4$). A supplementary solution the essential trace element was added to the nutrient solution and this contained 2.3×10^{-7} M H_2BO_4, 7×10^{-8} M $MnCl_2$, 7×10^{-9} M $ZnSO_4$, $7H_2O$, 2.5×10^{-7} $CuSO_4$, $5H_2O$, 6×10^{-9} M (NH_4) MoO_4 and 1.6×10^{-6} M Ferric Citrate. The solution was held at pH 6 throughout the experiment alternative with salinity different concentrations.

2.1.3. Application of NaCl by Irrigation System

Using a hand spray for irrigation system to improve the growth and avoid the accumulation of salts in pot. The irrigated system was applied twice a week (once every two days) by 400 ml different NaCl concentrations (50; 100; 150; 200 mM), alternative with water.

2.2. Growth Regulators

2.2.1. Ascorbic Acid (ASA)

Ascorbic acid **(**0.75 mM) obtained from *Sigma Chemical Company, UK,* was initially dissolved in a little amount of distilled water and the final volume was reached, using distilled water.

the seeds for 12 hours in the dark and leaves the tomato seeds as follow: (1) 1st group, seeds soaked in distilled water (control). (2) 2nd group, seeds soaked in a solution of ascorbic acid (ASA - 0.75 mM). (3) 3rd group, seeds soaked in a solution of gibberellic acid (GA3 - 0.05 mM). (4) 4th group, seeds soaked in Moringa Leaves Extract (MLE - 5%).

2.2.2. Gibberellic Acid (GA3)

Gibberellic Acid (0.05 mM) obtained from *Somatco Laboratory Chemicals Company, Saudi Arabia*, was initially dissolved in a little amount of distilled water and the final volume was reached, using distilled water.

2.3. Moringa oleifera Leaves Extract (MLE - 5%)

For preparation of *Moringa oleifera* leaves extract (MLE) take one kilogram (1 kg) from moringa leaves (air-dried under shade for two weeks and grounded to reach powder) then mixed with one litre of ethyl alcohol (80% aqueous) using a blender. The extract was purified by filtering twice through (Whatman No. 1) filter paper. After purification the extract was subjected to a rotary evaporator to fully evaporate the alcohol and get the crude extract. The concentrations 5% were prepared by take from the crude extract 5 ml and diluted with 95 ml distilled water for prepare MLE 5% 100 ml [19].

2.4. Seeds Viability Test

Tomato seeds viability tested for both cultivars, (cv. Cobra and cv. Newton) which are resistant and sensitive respectively to salinity stress by different characteristics used for cultivation at greenhouse. Plant growth experiments were carried out from October 2018. Soaking the tomato seeds for 12 hours in distilled water, then, the seeds germination in Petri dishes with a diameter (10 cm) on filter papers Whatman No.1 and moistened with distilled water. Germinated 100 seeds for each tomato (*Lycopersicon esculentum*, L.) cultivars (cv. Cobra and cv. Newton) allocated in 5 Petri dishes (20 seeds in each Petri dish) for every cultivar, then covered with Aluminum foil for germinated in dark for three days during this period watering the seeds one time a day by micropipette. After submerging the seeds (Germinated) counted the number of germinated seeds. Calculated the germinated seeds percentage for every cultivar of tomato plant by the following equation:

$$\text{Germination Rates \%} = \frac{\text{Total Number of Germinated seeds}}{\text{Total Number of Seeds (100)}} \times 100 \quad \textbf{(1)}$$

2.5. Impact of Different Growth Regulators (ASA & GA3) on Germination of Tomato Seeds

Selected tomato (*Lycopersicon esculentum,* L.) seeds for two cultivars (cv. Cobra and cv. Newton) intact, homogeneous in size and free from wrinkles. Then soaked

Germinated the tomato (*Lycopersicon esculentum,* L) seeds from different both cultivars under different treatments were at 20 - 24°C in Petri dishes with a diameter (10 cm) on filter papers Whatman No.1 and moistened with distilled water. Germinated 100 seeds for each cultivar of tomato then calculated the germinated seeds percentage for every cultivar by the above equation (1).

2.6. Transplanting Tomato (*Lycopersicon esculentum*, L.)

The tomato (*Lycopersicon esculentum,* L.) transplanted after germinated (15 days) in trays of cork (39 cm × 67 cm), which containing 218 tray diameter eyes (3cm and depth 6.5 cm). The tray eyes containing an equal amount of peat-moss only mixture thoroughly with water so distributed one seed in each eye tray and left the seeds to grow under greenhouse conditions at temperature of 18°C±1°C (night) 22°C±2°C (day) and relative humidity varied between 60 - 70%. The tomato seeds watering using distilled water until the true leaf appearance then transferred to another pots (diameter 16 cm and depth of 16 cm) which containing the sandy soil washed by diluted hydrochloric acid (1N HCl) and washed thoroughly with distilled water more five times. Used the same pots, and each pot containing the same volume of washing sandy soil and peat moss, (1: 2 - v: v), the sand culture technique and nutrient solution were similar to those adopted by Hoagland and Arnon [18].

2.7. Determination of Growth Parameters

Harvest plant started from transplanting the seedling plants (after 14 days from germination), till 45 days. The determined all growth parameters by using three replicates for each treatment, three plants for each treatment were washed with distilled water, blotted thoroughly and then divided into root and shoot. The shoot and root fresh after weighing, dried at 80°C reweighed, fresh and dry weights (g/plant) of shoot and root every time harvesting and placing samples fresh in oven for drying at a temperature of 80°C for 72 h until proven weight then was weighing in the balance of digital for dry weight.

2.8. Water Relations (Succulence & Dry Matter Content %)

The percentage of the succulence content and dry matter content (DMC) were determined after drying the shoot and root samples in air – circulation oven at 75°C after constant weight, and calculated as the following equation:

$$\text{Succulence} = \text{Fresh Weight/Oven Dry Weight} \quad (2)$$

$$\text{Dry Matter Content (\%)} = \frac{\text{Oven Dry Weight}}{\text{Fresh Weight)}} \times 100 \quad (3)$$

2.9. Photosynthetic Pigment Analysis

The leaf No. 5 from the down was homogenized immediately a known fresh weight (0.5 g) in a mortar with 5-10 ml cold aqueous acetone (85%) then centrifuged. The pigment content of the extract obtained was measured *Spectrophotometrically* at wavelengths E 664; E 645; E 452 nm according to the method of Metzner *et al*. [20]. The following equations were used to determine the concentration of the pigments fractions as µg / ml.

$$\text{Chlorophyll a} = 10.3\ E_{664} - 0.918\ E_{645} \quad (4)$$

$$\text{Chlorophyll b} = 19.7\ E_{645} - 3.870\ E_{664} \quad (5)$$

$$\text{Carotenoids C} = 4.3\ E_{452} - (0.0264\ \text{Chl. a} + 0.426\ \text{Chl. b}) \quad (6)$$

2.10. Statistical Analysis

Statistical analysis of the data was fed to the computer and analyzed using IBM SPSS software package version 20.0. For normally distributed data, comparison between different groups was analyzed using *F*-test (*ANOVA*). To find the effect between stages, growth regulators (mM), Moringa Leaves Extract and NaCl ppm and their interactions two ways *ANOVA* was used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level [21-23].

3. Results and Discussion

3.1. Germination Seeds

3.1.1. Seeds Viability (Germination Rate %)

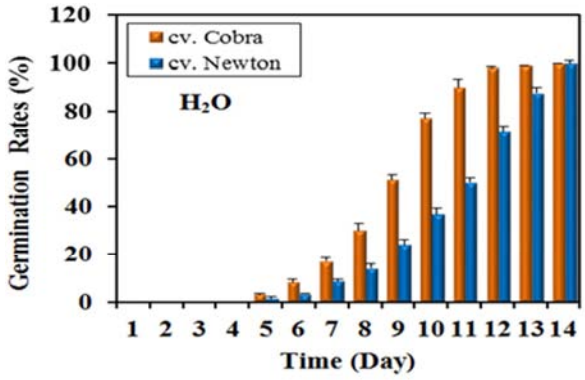

Figure 1. Seeds Viability (Germination Rate %) of Tomato (*Lycopersicon esculentum*, L.) seeds for both cultivars (cv. Cobra & cv. Newton).

The tomato seeds viability (germination rate %) for both cultivars (cv. Cobra & cv. Newton), occurred from the first day as shown in Figure 1 & Table 1. The germination rates take a time (day) 14 days for both cultivars. The results indicated that the tomato seeds viability of cv. Cobra and cv. Newton were 100% after 14 days. Seed germination is highly related not only to the seedlings survival rate but also

subsequent vegetative growth, so the soil salinity limits seed germination and plant growth, development and production by creates an osmotic potential around the outside of seeds, resulting in decreased water uptake during germination and an increase in the excessive uptake of ions, which causes the toxic effects of Na^+ and Cl^- ions to seeds [24-28].

Table 1. Statistical Analysis for Seeds Viability (Germination Rate %) of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton).

Statistical Analysis *(ANOVA)* / Germination Time (Day)	*Lycopersicon esculentum*, L.	
	cv. Cobra	cv. Newton
	Seeds Viability (Germination Rates GR%)	
	0.0 mM	0.0 mM
F	574.938*	657.314*
p	<0.001*	<0.001*
LSD	4.617	4.522

F: F for *ANOVA* test, Pairwise comparison bet. Each 2 groups were done using Post Hoc Test (LSD) p: p value for comparing between the studied Time for germination Means in the same column with Common letters are not significant (i.e. Means with Different letters are significant) *: Statistically significant ($P \leq 0.05$), Data was expressed using Mean ±SE.

3.1.2. Impact of NaCl Salinity Stress on Tomato Seeds Germination Rates %

The tomato seeds germination rate % under NaCl salinity Concentrations showed that the both tomato cultivars (cv. Cobra & cv. Newton) were more tolerant to NaCl salinity respectively as shown in Figure 2 & Table 2. The results indicated that the germination rates of tomato seeds for both cultivars (cv. Cobra & cv. Newton) decreased with increasing concentrations of NaCl salinity. There is an inverse relationship between the salinity concentrations and the germination rate (%), so the NaCl concentration increase, the Germination rate decreases in both cultivars. The germination rates % for cv. Cobra was: 90, 83, 65 & 55%, while in cv. Newton the germination rates Were: 89, 79, 60 & 50% with salinity concentration (50, 100, 150 & 200 mM NaCl) respectively, compared to control.

Table 2. Statistical Analysis for Impact of NaCl Different Concentration on Germination rates % of Tomato (*Lycopersicon esculentum*, L.) seeds for both cultivars (cv. Cobra & cv. Newton).

Statistical Analysis *(ANOVA)* / Germination Time (Day)	*Lycopersicon esculentum*, L.									
	cv. Cobra					cv. Newton				
	NaCl Concentrations (mM)									
	0.0	50	100	150	200	0.0	50	100	150	200
F	574.938*	574.088*	512.856*	675.234*	566.337*	657.314*	611.014*	599.497*	680.330*	370.437*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	4.617	3.998	4.193	3.108	2.839	4.522	4.121	3.424	2.555	2.839

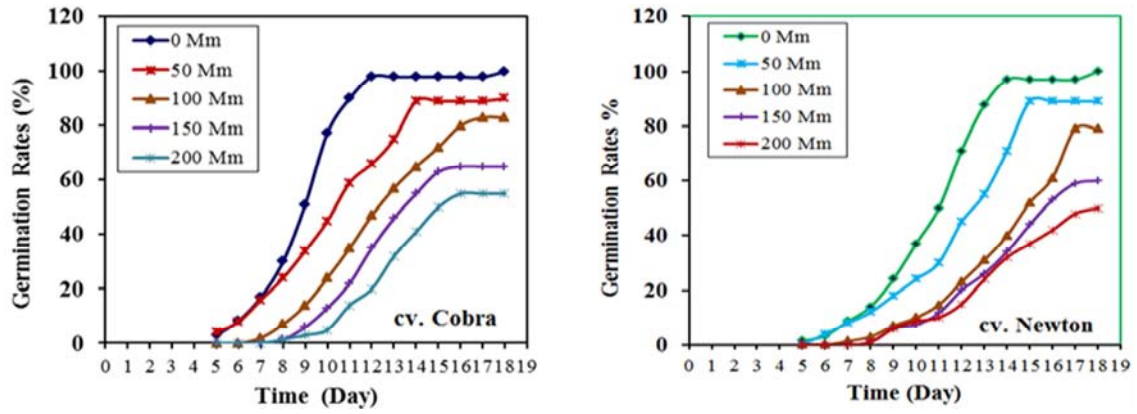

Figure 2. Impact of NaCl Salinity Different Concentrations on Germination rate % of Tomato (*Lycopersicon esculentum*, L.) seeds for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

3.1.3. Impact of Different Growth regulators (GRs) and Moringa Leaves Extracts (MLE) on Tomato Seeds Germination Rates (%)

a) Impact of H_2O on Tomato Seeds Germination Rates (%): After soaking the tomato seeds in water (H_2O) the germination rate (%) increased and reached to 100% after 14 days for both cultivars (cv. Cobra & cv. Newton) as shown in Figures 3 & Table 3.

b) Impact of ASA (0.75 mM) on Tomato Seeds Germination Rates (%): The germination rate (%) after soaking the tomato seeds in ASA - 0.75 mM, increased and reached to

100% after 9 & 11 days for both cultivars (cv. Cobra & cv. Newton) respectively as shown in Figure 3 & Table 3.

c) Impact of GA3 (0.05 mM) on Tomato Seeds Germination Rates (%): The germination rate (%) after soaking the tomato seeds in GA3- 0.05 mM, increased and reached to 100% after 7 and 8 days for both cultivars (cv. Cobra & cv. Newton) respectively, as shown in Figures 3 & Tables 3.

d) Impact of Moringa Leaves Extracts (MLE – 5%) on Tomato Seeds Germination Rates (%): The germination rate (%) after soaking the tomato seeds in MLE 5%, increased and reached to 100% after 7 and 8 days for both cultivars (cv. Cobra & cv. Newton) respectively, as shown in Figures 3 & Tables 3. So, all of this results it has been used both cultivars (cv. Cobra & cv. Newton), then transplanting at seedling stage after soaking seeds in ASA, GA3 and MLE. The data shown the germination rate increased has been in the present of GA3 and MLE more than ASA.

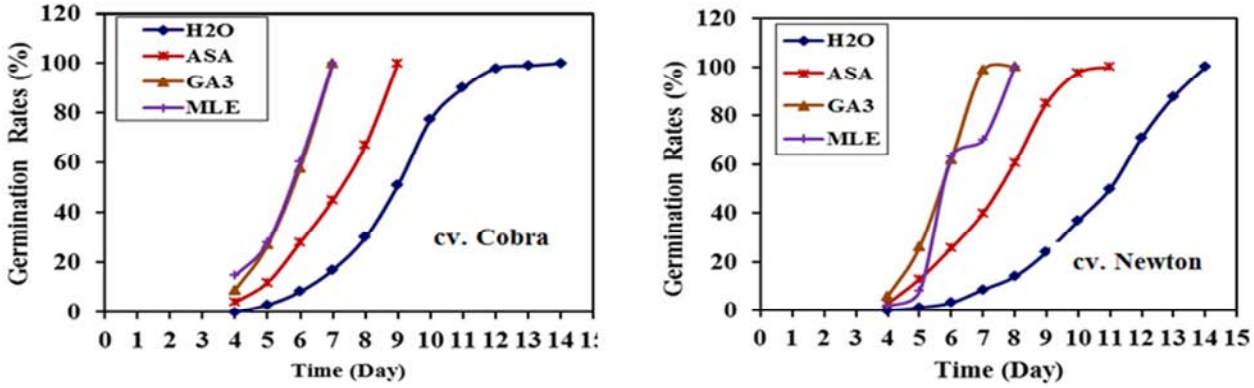

Figure 3. Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Germination rate % of Tomato (*Lycopersicon esculentum*, L.) seeds for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

Table 3. Statistical Analysis for Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Germination rate % and Viability Germination of Tomato (*Lycopersicon esculentum*, L.) seeds for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

Germination Time (Day) / Statistical Analysis (*ANOVA*)	*Lycopersicon esculentum*, L.							
	cv. Cobra				cv. Newton			
	Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE)							
	H_2O	ASA	GA_3	MLE	H_2O	ASA	GA_3	MLE
F	530.745*	1136.680*	1310.462*	1030.299*	411.223*	461.294*	792.659*	647.910*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	5.273	3.425	2.749	2.962	5.165	5.306	3.586	4.362

3.2. Growth Parameters

3.2.1. Shoot Height and Root Depth (Cm/Plant)

Regarding to the shoot height and root depth (cm/Plant) of tomato (*Lycopersicon esculentum,* L.) Plants for both cultivars (cv. Cobra & cv. Newton), tended to decreased with increasing NaCl salinity concentrations compared with control as shown in Figure 4 & Table 4 Shoot height and root depth tended to decrease after using growth regulators (ASA & GA3) and Moringa Leaf Extract (MLE) before planting compared with control. Shoot height and root depth of tomato plant for both cultivars (cv. Cobra & cv. Newton) gives the best results with using MLE, GA3, and ASA before germinating respectively but decreased compared to the control. The shoot height and root depth (cm/Plant) of tomato (*Lycopersicon esculentum,* L.) plants gives more effective results in cv. Newton (sensitive) more than Cobra (resistant). Overall, the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl and growth regulators (ASA & GA3) and MLE at 45 days indicated that the *F* test and *LSD* highly significant at $p \leq 0.001$. Also, the results reported by Tal and Shannon [29] they found that the salinity stress reduces rate of main stem elongation in tomato. It was also reported that moringa leaf extract (MLE) in tomato enhanced lateral vegetative shoot number and plant height compared to untreated control plants [30]. Salinity reduces the ability of plants to utilize water and causes a reduction in the growth and yield, and changes in the plant metabolic processes [31].

Moringa leaf extract (MLE) is considered one of the plant biostimulants, which are substances when applied as seed soaking and/or foliar spray positively modify plant growth and production with alterations in metabolic processes under normal or stress conditions [32-35]. Whereas, foliar application of moringa extract with low concentration contains sufficient amounts from stimulant substances encouraged increasing cell-division rate, cell-enlargement, strengthens plants, eventually produces more and higher yield, they illustrated also that application of MLE extract

may be induced cytokinin bio-synthesis in turned maximum number of photosynthetic active leaves and that is obvious from number and area of leaves per plant maintaining the chlorophylls in higher concentrations which reflect on plant yield [36-37].

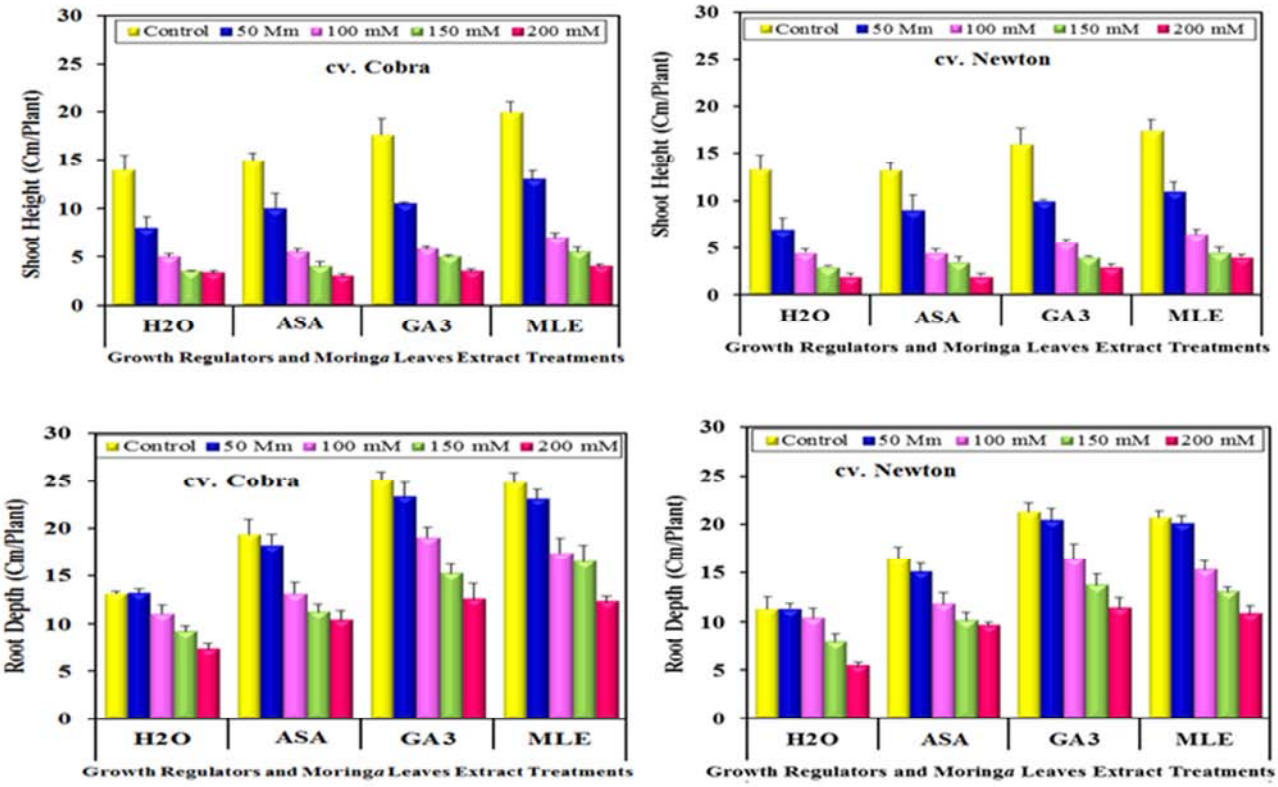

Figure 4. Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Shoot Height, Root Depth (Cm/Plant), of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

Table 4. Statistical Analysis For Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Shoot Height and Root Depth (Cm/Plant) Of Tomato (*Lycopersicon esculentum*, L.) for Both Cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

NaCl Conc. (mM) / Statistical Analysis *(ANOVA)*	*Lycopersicon esculentum*, L.							
	cv. Cobra				cv. Newton			
	Growth Regulators and Moringa Leaves Extract Treatments							
	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot Height (cm/Plant)								
F	40.808*	68.106*	30.040*	63.966*	27.414*	29.727*	50.282*	59.349*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	2.198	1.900	3.308	2.600	2.724	2.664	2.375	2.308
Root Depth (cm/Plant)								
F	18.838*	12.187*	17.657*	16.959*	8.970*	10.524*	12.420*	33.979*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.002*	<0.001*	<0.001*	<0.001*
LSD	1.865	3.706	3.976	3.941	4.489	2.955	3.792	2.352

3.2.2. Fresh and Dry Weight (g/Plant)

Overall, shoot and root fresh and dry weight of tomato plant for both cultivars (cv. Cobra & cv. Newton) decreased significantly ($p<0.001$) with increasing NaCl salinity concentrations, however, the shoot and root fresh and dry weight at 150 and 200 mM NaCl showed dramatically depression compared with control as shown in Figure 5 & Tables 5. Whereas, the statistical analysis indicated that the shoot and root fresh and dry weight of tomato plants for both cultivars (cv. Cobra & cv. Newton) increased significantly at $p\leq0.001$ in the present of ASA (0.75 mM), GA3 (0.05 mM) and MLE (5%) under saline and non- saline conditions. MLE, GA3 then AsA tended to increasing shoot and root fresh and dry weight under saline and non-saline conditions at (45 Days) respectively more than control. Overall, the two ways analysis of variance (ANOVA) between different concentrations of NaCl and Growth regulators (ASA & GA3) and MLE at 45 days indicated that the F test and LSD highly significant at $p\leq0.001$. Significant reductions in fresh and dry weight of tomato shoots were reported in response to salinity stress [38]. The effect of mild salinity stress (70 mM) to maize plant decreased shoot, root length and fresh, dry weight, this reduction in growth might affect assimilate production and their translocation to the reproductive organs reported by

Yasmeen et al. [39] & Ali et al. [40].

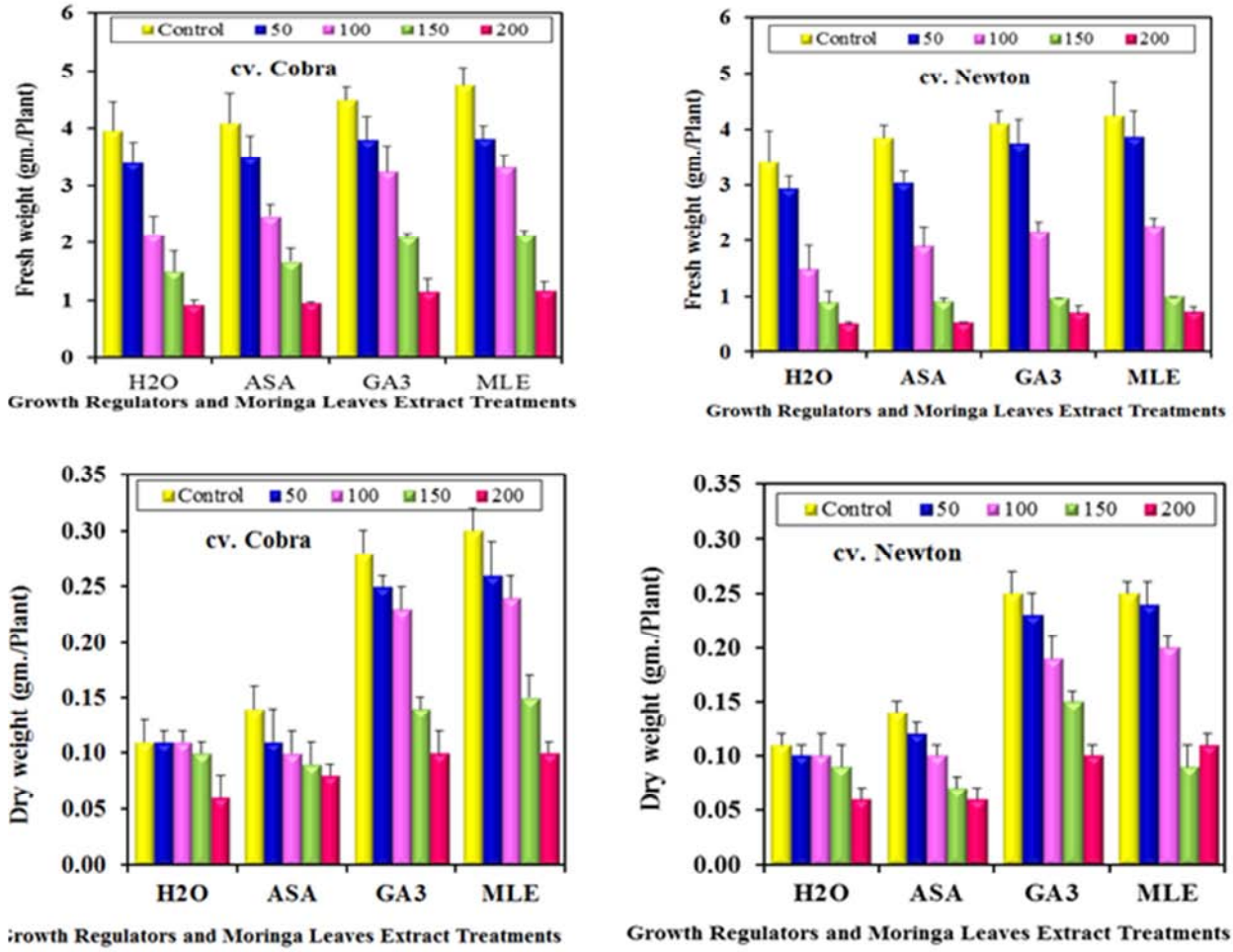

Figure 5. Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Fresh and Dry Weights (gm. /Plant) of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

Table 5. Statistical Analysis for Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Fresh and Dry Weights (gm./Plant) of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

NaCl Conc. (mM) / Statistical Analysis (*ANOVA*)	*Lycopersicon esculentum*, L.							
	cv. Cobra				cv. Newton			
	Growth Regulators and Moringa Leaves Extract Treatments							
	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Fresh weight (gm./Plant)								
F	13.882*	16.934*	19.355*	44.975*	14.085*	43.967*	43.247*	21.421*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.0779	0.9846	0.9594	0.6636	1.0733	0.6707	0.7495	1.1034
Dry weight ((gm./Plant)								
F	3.205	1.371	22.953*	6.351*	1.568	9.683*	12.728*	17.094*
p	<0.062	<0.311	<0.001*	<0.008*	<0.257	<0.002*	<0.001*	<0.001*
LSD	0.038	0.063	0.051	0.102	0.049	0.034	0.053	0.056

3.3. Water Relations

3.3.1. Succulence Content (Fresh Weight/Oven Dry Weight)

Regarding the succulence content (Fresh weight/Oven Dry weight) of Tomato (*Lycopersicon esculentum*, L.) plant for both cultivars (cv. Cobra & cv. Newton), tended to decrease with increasing NaCl salinity concentrations compared to control as shown in Figure 6 & Table 6. The concentration of 200 mM NaCl gives the lowest value of succulence. The best result shown with using ASA, more than GA3 & MLE for both cultivars (cv. Cobra & cv. Newton) compared to control. Overall, the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl and growth regulators (ASA & GA3) and MLE at 45 days indicated that the *F* test and *LSD* highly significant at $p \leq 0.001$

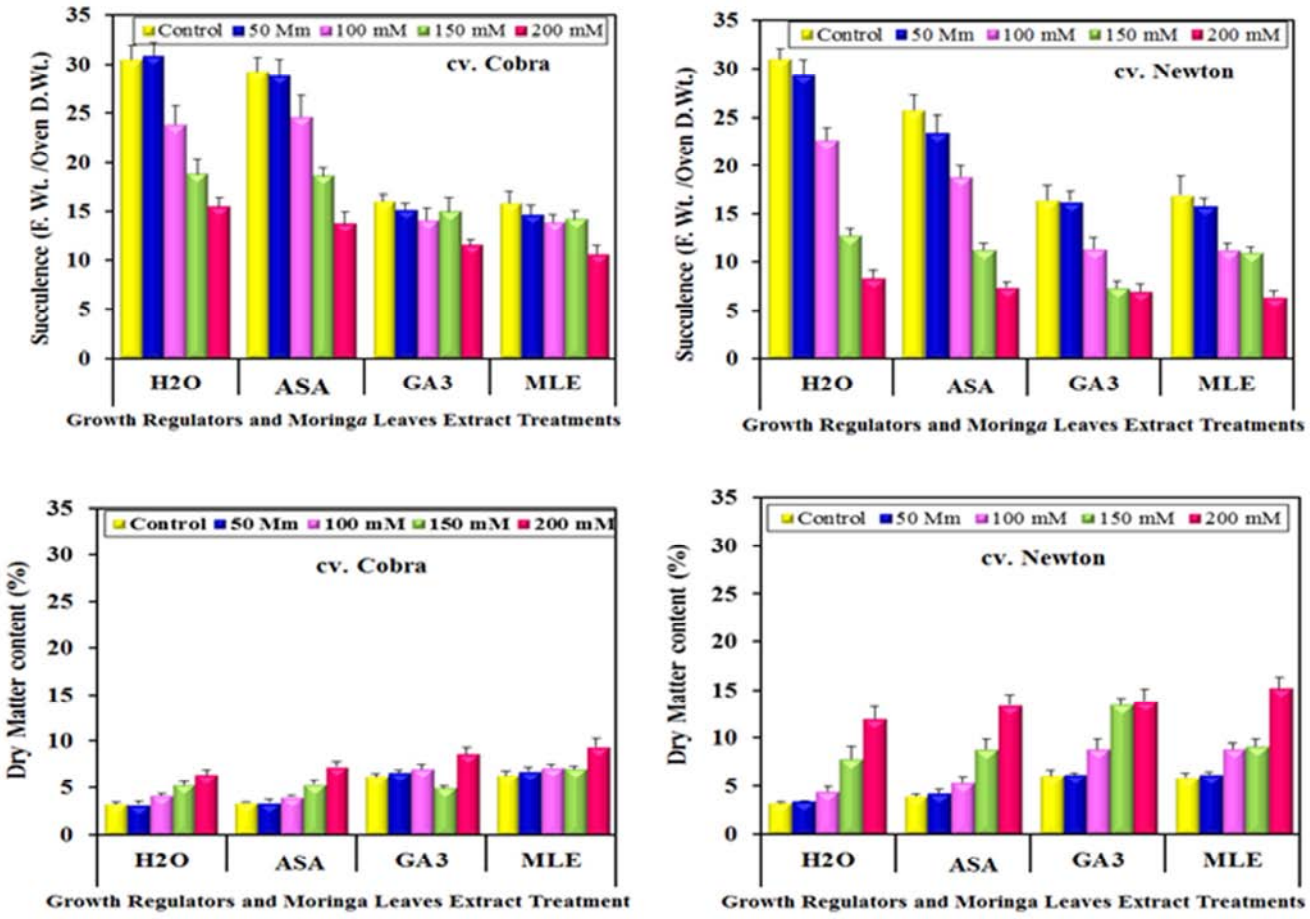

Figure 6. Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Succulence (F. Wt./ D. Wt.) and Dry matter Contents (D. wt. / F. Wt. X 100) of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

Table 6. Statistical Analysis for Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Succulence (F. Wt. / D. Wt.) and Dry matter Contents (D. wt. / F. Wt. X 100) of Tomato (*Lycopersicon esculentum*. L) For both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

NaCl Conc. (mM) / Statistical Analysis (*ANOVA*)	*Lycopersicon esculentum*, L.							
	cv. Cobra				cv. Newton			
	Growth Regulators and Moringa Leaves Extract Treatments							
	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Succulence (F. Wt. /Oven D. Wt.)								
F	22.266*	19.818*	3.411	4.171*	78.102*	39.359*	19.346*	16.393*
p	<0.001*	<0.001*	< 0.053	<0.030*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	4.606	4.779	2.988	3.051	3.584	3.933	3.301	3.300
Dry Matter Content (%)								
F	19.458*	19.974*	10.402*	5.954*	19.441*	30.366*	21.339*	31.674*
p	<0.001*	<0.001*	<0.001*	<0.010*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.990	1.163	1.316	1.582	2.677	2.308	2.612	2.105

3.3.2. Dry Matter Contents (DMC %), (Oven Dry Weight /Fresh Weight) x 100

Overall, Dry matter content (%) of tomato plant for both cultivars (cv. Cobra & cv. Newton) increased significantly ($P \leq 0.001$) with increasing NaCl salinity concentrations as shown in Figures 6 & Table 6. Moringa Leaves Extract (MLE) gives the best increase, then GA3 & ASA compared to control. The statistical analysis indicated that Dry matter content (%) of tomato plants for both cultivars (cv. Cobra & cv. Newton) increased significantly at $p \leq 0.001$ in the present of ASA (0.75 mM), GA3 (0.05 mM) and MLE (5%) under saline and non- saline conditions. Dry matter content (%) of tomato (*Lycopersicon esculentum,* L.) gives more effective results in cv. Newton (sensitive) more than Cobra (resistant). Overall, the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl and growth regulators (ASA & GA3) and MLE at 45 days indicated that the *F* test and *LSD* highly significant at $p \leq 0.001$. It has been reported that tomato plants become more succulent with increasing

salinity, due to enhanced water accumulation [41]. The succulence ensures independence from limited or unpredictable water supply after the growth phase [42].

3.4. Photosynthetic Pigments Contents (mg/g Leaf Fresh Weight)

The chlorophyll a, chlorophyll b, carotenoids, and total pigment contents of leaves tomato plant increased progressively with increasing NaCl salinity at 45 days, also the photosynthetic pigments increased in the present of MLE,

ASA & GA3 respectively. Overall, the chlorophyll a, chlorophyll b, carotenoids, and total pigment contents of tomato plant leaves decreased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations at 45 days as shown in Figures 7 & Table 7. The NaCl salinity induced to decrease significantly ($p \leq 0.001$) the photosynthetic pigment contents in tomato leaves. Whereas, in the present of MLE, ASA & GA3 induced to increase the content of photosynthetic pigments in leaves of tomato plant respectively compared with control. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl salinity and growth regulators (ASA & GA3) and MLE at growth stage (45 days) indicated that the *F* test and LSD highly significant at $p \leq 0.001$. From the results shown that the decreased highly significantly in chlorophyll a, chlorophyll b and carotenoids under salt stress which is an agree with a previous results by Turan *et al*. [43] on *P. vulgaris* L. and Taffouo *et al*. [44] on *Vigna subterranean* L.

The decrease in chlorophyll contents under salt stressed plants has been considered as a typical symptom of oxidative stress [45], and was attributed to the inhibition of chlorophyll synthesis, photosynthetic pigment contents together with the activation of its degradation by the enzyme chlorophyllase [46]. Reduction of chlorophyll contents, as a result of either slow synthesis or fast breakdown, indicated that there was a photoprotection mechanism through reducing light absorbance by decreasing chlorophyll contents [47].

Carotenoids, being antioxidants, have the potential to detoxify the plants from the effects of reactive oxygen species [48]. Carotenoids are known to function as collectors of light energy for photosynthesis and as quenchers of triplet chlorophyll and O_2. Moreover, they dissipate excess energy via the xanthophyll cycle and can act as powerful chloroplast membrane stabilizers that partition between light-harvesting complexes (LHCs) and the lipid phase of thylakoid membranes, reducing membrane fluidity and susceptibility to lipid peroxidation [49]. The decrease of carotenoid contents indicated that the protection by carotenoid was not one of the most important mechanisms under salt stress. Soil salinity condition decreased significantly chlorophyll a, b and carotenoids, the change in leaf chlorophyll content might be due to degradation of chlorophyll or reduction in biosynthesis under salinity stress condition, it is also showed that salinity stress break down of chloroplast including plastid envelop thylakoids effect of toxic Na^+ on photosynthesis apparatus or salt induced oxidative damage [50-51].

Ascorbic acid (ASA) causes positive effect in protection and stabilization of photosynthetic pigments and chloroplast from oxidative damage [52-53]. Thomas and Howarth [37], they illustrated also that application of MLE extract may be induced cytokinin bio-synthesis in turned maximum number of photosynthetic active leaves and that is obvious from number and area of leaves per plant maintaining the chlorophylls in higher concentrations which reflect on plant yield. Foliar applications of moringa with low concentration contain sufficient amounts from stimulant substances encouraged increasing cell-division rate, cell-enlargement, strengthens plants, eventually produces more and higher yield [36]. Different categories of bio-stimulants have been shown to enhance shoot growth, as quantified by plant height, root depth, shoot and root fresh & dry weight, succulence, dry matter [54].

Table 7. Statistical Analysis for Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Photosynthetic Pigments (mg/g leaf F. Wt.), of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

NaCl Conc. (mM) / Statistical Analysis (*ANOVA*)	*Lycopersicon esculentum*, L.							
	cv. Cobra				cv. Newton			
	Growth Regulators and Moringa Leaves Extract Treatments							
	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Chlorophyll a (mg/g leaf F. Wt.)								
F	240.225*	21.525*	27.965*	99.126*	196.080*	54.846*	173.578*	109.789*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.036	0.131	0.123	0.064	0.041	0.082	0.046	0.061
Chlorophyll b (mg/g leaf F. Wt.)								
F	12.104*	14.224*	18.225*	15.706*	11.418*	10.738*	6.883*	5.100*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.006*	<0.017*
LSD	0.043	0.044	0.0364	0.034	0.057	0.052	0.061	0.060
Carotenoids (mg/g leaf F. Wt.)								
F	20.357*	18.536*	13.891*	12.692*	8.906*	10.380*	11.391*	12.692*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.002*	<0.001*	<0.001*	<0.001*

NaCl Conc. (mM) / Statistical Analysis (*ANOVA*)	*Lycopersicon esculentum*, L.							
	cv. Cobra				cv. Newton			
	Growth Regulators and Moringa Leaves Extract Treatments							
	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
LSD	0.030	0.030	0.039	0.042	0.046	0.041	0.046	0.0415
Total pigments (mg/g leaf F. Wt.)								
F	255.179*	28.641*	52.039*	84.447*	95.988*	51.006*	127.197*	98.640*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.018	0.055	0.044	0.032	0.031	0.042	0.027	0.031

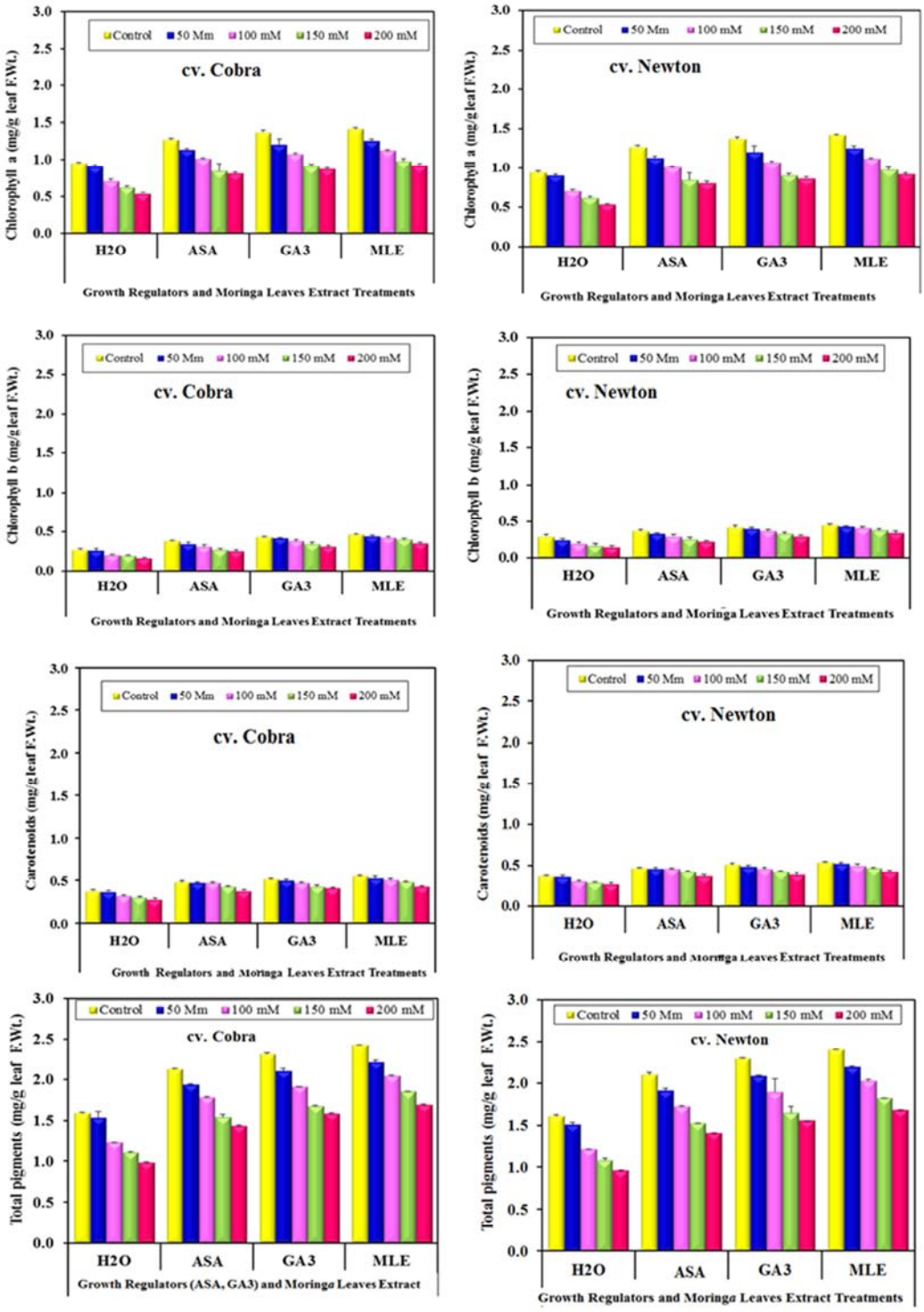

Figure 7. Impact of Growth Regulators (ASA & GA3) and Moringa Leaf Extract (MLE) on Photosynthetic Pigments as Chlorophyll a, b, Carotenoids and Total Pigments (mg/g leaf F. Wt.), of Tomato (*Lycopersicon esculentum*, L.) for both cultivars (cv. Cobra & cv. Newton) Under Salinity Stress.

4. Conclusion

In conclusion, generally, salinity stress reduced tomato plant growth for both cultivars, but when the seeds soaked before germination in GRs (ASA- 0.75 mM, GA3-0.05mM) & MLE (5%), the tomato plant resistance the harmful salinity effect and produced high productivity. The more effective on tomato seeds was GA3, MLE & ASA respectively compared with control. So, we recommended using GA3, MLE and ASA, by soaking the seeds of tomato plant before germination for tolerance the salinity stress and improve agriculture for produce new strain adapted to salinity stress.

Abbreviations

Growth regulators (GRs), Ascorbic Acid (ASA), Gibberellic Acid (GA3), Moringa Leaf Extract (MLE), Dry Matter Content (DMC) and Chlorophyll (Chl.).

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