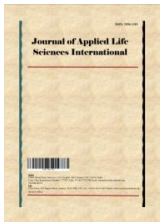

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Induce of Plant Growth Regulators with and without Bio-fertilizer for Enhances the Adverse Effect of Salinity on Germination, Growth and Photosynthetic Pigments

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Authors' contributions

This work was carried out in collaboration between both authors. Authors RAAB and HESAES designed the study, wrote the protocol, initiated the experiments, collected the data, performed the statistical analysis, managed the literature review and wrote the final draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

This study aimed to explain the induce of plant growth regulators (ascorbic acid - AsA & salicylic acid - SA) in the presence or absence of bio-fertilizer (Acadian extract –ACE) for alleviated the effect of salinity stress on two cultivars of lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal). The lettuce seeds for four cultivars (cv. Paris Island Cos (cv. Paris) S1, cv. Royal S2, cv. Nader S3 & cv. Marvilli S4) soaked in PGRs (AsA, SA & GSH – 0.5 mM) and Acadian extract (ACE - 1%) for 12 hours in the dark at 4°C, for test of lettuce seeds viability (germination rate %). Germination both cultivars (cv. Paris S1 & cv. Royal S2) in trays of cork contains 218 eye for 14 days, transplanted the seedlings plant to plastic containers each pot containing one plant was irrigated with using NaCl salinity concentrations (0.00, 50; 100; 150 mM) 1st group alternative with distilled water and 2nd group alternating with ACE (1%), until harvest after 84 days. The results of germination indicated that the PGRs (AsA & SA) with both cultivars (cv. Paris S1 & cv. Royal S2) gives best results more the other PGRs (GSH) & bio-fertilizer (ACE) for the other cultivars. The data explained that the leaf

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number and leaf area, fresh and dry weights for shoot decreased significantly with increasing salinity concentrations compared with control, whereas the growth increased significantly more in cv. Royal S2 than in cv. Paris S1, particularly with AsA in the absence of bio-fertilizer (-ACE) more than SA compared with control. Whilst, the shoot succulence increased significantly with salinity concentrations more with AsA than SA especially in the absence of bio-fertilizer (-ACE) compared with control. However, the shoot dry matter content % decreased for both cultivars with increasing NaCl salinity concentrations especially with AsA more than SA in the absence (-ACE) compared with control. The evident recorded a significantly increased the photosynthetic pigments (Chl. a, Chl. b, carotenoids and total pigments) of leaves lettuce plant for both cultivars (cv. Paris & cv. Royal) with increasing NaCl salinity, also the photosynthetic pigments increasing more in cv. Royal S2 than in cv. Paris S1 especially with AsA more than SA in the absence (-ACE) under saline or non-saline conditions compared with the control. The data provide strong support to the hypothesis that exogenous application of AsA individually reduces the harmful effects of salinity and increases resistance to salinity in lettuce plant for both cultivars.

Keywords: Salinity; ascorbic acid; salicylic acid; Lactuca sativa L.; germination; chlorophyll; water relations; photosynthetic pigments and bio-fertilizer.

ABBREVIATIONS

Plant growth regulators (PGRs), Ascorbic acid (AsA), Salicylic acid (SA), Paris Island Cos (cv. Paris), Glutathione (GSH), Acadian extract (ACE), Seaweed extracts (SWE), Ascophyllum nodosum extracts (ANE), Gibberellic acid (GA3), Dry matter content (DMC %) and Chlorophyll (Chl).

1. INTRODUCTION

Salinization is one of the main constraints for agriculture productivity worldwide [1]. This important abiotic stress has worsened in the last 20 years due to the increase in water demands in arid and semi-arid areas [2-4]. The predicted global warming will supposedly increase the severity and frequency of salinity in the coming days. Saline soils have been estimated to occupy more than 7% of the Earth's land surface [5-7].

Saudi Arabia needs sustained agricultural development to cope with the social and economic obligations that are the normal consequences of the continued high rates of population growth [8] and organic farming is an eco-friendly practice for sustainable agriculture, the most essential component of organic farming is bio-fertilizers and it is one of such strategies that not only ensures food safety but also an effective tool for desert development under less polluted environments and decreasing agricultural costs [9-10].

Khan et al. [11-12]; Asgher et al. [13] they reported that the plant growth regulators (PGRs) play an important role in plant developmental processes and regulation a wide range of biotic and abiotic stress responses to the plants tolerance. Ascorbic acid (AsA) is an organic compound belonging to the family of monosaccharide's, it is highly soluble in water and is one of the important non-enzymatic antioxidants and plays vital role in the growth and normal functioning of plants [14]. Salicylic acid (SA) is one of the endogenous PGRs and it plays a crucial role in the modulate various metabolic and physiological events during the entire lifespan of the plant such as seed germination, vegetative growth, respiration, transpiration, glycolysis, Krebs cycle, the alternative respiratory pathway, seed production and senescence [15-20].

In recent years, the use of natural seaweed (algae) as bio-fertilizer has allowed in organic farming practices toward sustainable agriculture, where the use of algae as bio-fertilizer is based on renewable source of energy which does not pollute the environment and increases the crop yield in comparison to the agrochemicals [21-23]. *Ascophyllum nodosum*, is considered one of the brown algal species that contains a wide range of bioactive compounds with different biological effects [24-25]. In particular, *A. nodosum* extracts contain approximately 42 – 70% polysaccharides, 1.2 – 12% protein, 1.2 – 4.8% lipids and 18 – 27% minerals [26]. In addition, *A. nodosum* was containing fucoxanthins, catechins hydroxybenzoic acid, coumaric acid, cinnamic acid, and caffeic acid [27]. Generally, crude extracts from this seaweed are rich in phenolic compounds than other seaweeds [28-30].

Liquefied seaweed extracts (SWE) is usually manufactured from *Ascophyllum nodosum*, the methods for liquefying seaweed are often proprietary, but in most cases, extracts are made by processes using water, alkalis or acids, or physically by disrupting the seaweed by low temperature milling to give a micronized suspension of fine particles [31-32]. Recently, attention of scientists has been paid to novel extraction methods, such as enzyme-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, and ultrasound assisted extraction, enabling improved extraction of biologically active compounds without their degradation [33].

Lettuce (*Lactuca sativa* L.): It is the most popular leafy vegetable and is the worlds most used salad crop [34]. Since lettuce is generally eaten raw, more nutrients are retained compared to other vegetables that are cooked or processed, such as potatoes [35], but some forms are also cooked [36]. Lettuce has acquired a folk reputation as an aphrodisiac, anodyne, carminative, diuretic, emollient, stimulate digestion, relieve inflammation, febrifuge, hypoglycaemic, hypnotic, narcotic, parasiticide and sedative [37]. The most of therapeutic properties of this plant is due to sesquiterpene lactones- lactucin, lactucopicrin the most bioactive compound of *Lactuca sativa* [38]. The objective of the present study was to evaluate the effects of exogenous application of PGRs (AsA & SA) on lettuce seeds before germinated for enhances the adverse effect of salinity stress on germination, growth, water relations and photosynthetic pigments for two cultivars of lettuce (*Lactuca sativa*, L.) plant in the presence or absence of bio-fertilizer Acadian extract (ACE).

2. MATERIALS AND METHODS

2.1 NaCl Salinity Concentrations

Prepared Molar solution (1 M) NaCl concentrations, from a molar solution, prepare different concentrations of NaCl (0.0, 50, 100 and 150 mM).

2.2 The Soil Used

The soil used for cultivated lettuce plant was the ratio between the peat- moss with agricultural perlite (agrolite) (3:1) then add sand, as a ration (2: 1–v: v), in each pot (diameter 16 cm and depth of 16 cm), completed by the same size in each pot using the ratio from the peat moss/soil sand (2:1- v: v).

2.3 Plant Growth Regulators: Ascorbic Acid (AsA -0.5 mM)

Ascorbic acid obtained from Sigma Chemical Co. UK, was initially dissolved in a little amount of distilled water and the final volume was reached, using distilled water. Salicylic Acid (SA - 0.5 mM): Salicylic acid; 2-hydroxybenzoic acid, obtained from Sigma Chemical Co. UK (*Polyoxyethylenesorbitan Monolaurate, Sigma Chemicals, UK*), were initially dissolved in dimethyl sulfoxide to obtained concentration of 0.5 mM (pH 6.0 - 6.5) then added 0.02% Tween 20 to help for distributed the SA in media [39]. Glutathione (GSH - 0.5 mM) obtained from Sigma Chemical Co. UK, was initially dissolved in a little amount of distilled water and the final concentration was 0.5 mM using distilled water. The concentration of PGRs (0.5 mM) was used in this experiments as reported in previous research workers.

2.4 Acadian Extract (ACE) Treatments: Preparation of *Ascophyllum nodosum* Extracts (ANE)

The commercial *Ascophyllum nodosum*; Acadian marine plant extract powder was purchased from Gulf Palace factory (Second industrial city in Riyadh). Stock solutions of 1% (w/v) ANE were prepared and stored at 4°C. The required volume of stock solution was mixed with distilled water.

2.5 Seed Viability (Germination Rates %)

Selected of the seeds intact, homogeneous in size and free from wrinkles for four lettuces cultivar, (*Lactuca sativa,* L. cv. Paris; cv. Royal; cv. Nader; cv. Marvilli) seeds used for cultivation at Taif City, Kingdom of Saudi Arabia. Then soaked the seeds for 12 hours in the dark and leaves in the refrigerator for dormancy the lettuce seeds soaked in distilled water, (10 seeds in each Petri dish) for every cultivar. Then the Petri dishes covered with Aluminum foil for germinated in dark for three days during this period watering the seeds one time a day by micropipette. After submerge the seeds (Germinated) counted the number of germinated seeds. Calculated the germinated seeds percentage for every cultivar of lettuce plant by the following equation:

$$Seed\ Germinated\ Rate\ (\%) = \frac{Total\ Number\ of\ Germinated\ Seeds \times 100}{Total\ Number\ of\ Seeds\ (50)} \quad (1)$$

2.6 Impact of NaCl Salinity on Germination

Germinated of lettuce (*Lactuca sativa*) seeds, for four cultivars, (1)- cv. Paris; (2)- cv. Royal; (3)- cv. Nader; (4)- cv. Marvilli, by different characteristics. The germinated seeds take 25 days for seedling stage, then the all of lettuce seedling plant for four cultivars transplanting into a plastic pot (16 cm diameter and 16 cm height) under greenhouse conditions, then treated for 14 days with NaCl salinity at different concentrations (50, 100, 150 mM) for tested the resistance to NaCl salinity.

2.7 Induce of Plant Growth Regulators (PGRs) on Germination Rates

Selected of the seeds intact, homogeneous in size and free from wrinkles for four lettuces cultivar, (*Lactuca sativa,* L. cv. Paris; cv. Royal; cv. Nader; cv. Marvilli) seeds. Then soaked the seeds for 12 hours in the dark and leaves in the refrigerator for dormancy the lettuce seeds as follow: (1)- 1st group, seeds soaked in distilled water (control). (2)- 2nd group, seed soaked in a solution of 0.5 mM ascorbic acid (AsA). (3)- 3rd group, seeds soaked in a solution of 0.5 mM salicylic acid (SA). (4)- 4th group, seeds soaked in a solution of 0.5 mM glutathione (GSH). (5)- 5th group, seeds soaked in a solution of 1% Acadian extract (ACE). Germinated the lettuce seeds from different four 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. After submerge the seeds (Germinated) counted the number of germinated seeds and calculated the germinated rates % for every cultivar of lettuce plant by the above equation (1).

Using both cultivars (cv. Paris and cv. Royal) for study, after soaking in different PGRs (AsA & SA) germination in 4 trays of cork (39 cm × 67 cm), which containing 218 tray diameter eyes (3 cm and depth 6.5 cm) 2 trays for each PGRs treatment. The seeds growing under greenhouse conditions at temperature of 14°C ± 2°C (night)/ 20°C ± 2°C (day), the relative humidity varied between 60-70% and day light from 11 to 12 h. The lettuce seeds watering with distilled water until the emergence of the 4th leaf then transplanted to a pot (diameter 21 cm and depth of 18 cm with perforated bottoms) which containing the sandy soil and peat moss with agricultural perlite (agrolite) as (2:1 - v: v).

2.8 Transplanting Seedling Plant and Irrigation System

Transplanting the lettuce plant from cork trays to plastic pots, each pot containing one plant, all pots were irrigated with 450 ml distilled water immediately after transplanting. Then the second irrigated started treatments for each group, the first one irrigated with using NaCl salinity concentrations alternating with distilled water, and the second one irrigated with using NaCl salinity concentrations alternating with bio-fertilizers (ACE - 1%) as shown in the Table 1. Used nutrient solution (Agroleaf power - N: P: K +Trace Elements - 20:20:20) as 1.5 gl^{-1}, produced by COMPO Epert GmbH (Germany), once every two weeks after the emergence of the 4th leaf for all experiments treatments.

2.9 Growth Parameters Determination

At 84 days after transplanting, a random sample (3 plants) was taken from each experimental unit to measure: The leaf area (cm^2/leaf) assessed using the leaf No. 5 from the lower, by a Portable Area Meter (Area Meter Model CI, 202) as shown in Fig. 1A & B. The shoot (leaves and stems) fresh and dry weights (g/plant) harvesting and placing samples fresh in oven for drying at 80°C for 24 h. Then reduce the temperature to 75°C for 72 h. until proven weight then was weighing on digital balance for dry weight.

2.10 Water Relations (Succulence and Dry Matter Contents %)

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

Succulence = Fresh Weight/Oven Dry Weight (2)

Dry Matter Content (%) = (Oven Dry Weight /Fresh Weight) x 100. (3)

2.11 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. [40]. The following equations were

used to determine the concentration of the pigments fractions as µg / ml.

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

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

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

2.12 Statistical Analysis

Statistical analyses were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Quantitative data were described using mean and standard error. Significance of the obtained results was judged at the 5% level. The used tests were as follow: - (1) Student t-test: For normally distributed quantitative variables, to compare between two studied groups. (2) F-test (ANOVA): For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (*LSD*) for pairwise comparisons [41-42].

3. RESULTS AND DISCUSSION

3.1 Germination Seeds

3.1.1 Seeds viability (germination rate %)

The lettuce seeds viability (germination rate %) for four cultivars (cv. Paris S1, cv. Royal S2, cv. Nader S3 & cv. Marvilli S4), occurred from the first day as shown in Fig. 2 & Table 2. The germination rates take a time (day) depending on the cultivar as follow; cv. Paris S1 and cv. Royal S2 reached to 100% after (6 days); while, cv. Nader S3 and cv. Marvilli S4 still germinated for (6 days) at 91% and 85%, respectively. The germination process comprises two distinct phases the first is imbibition, mainly dependent on the physical characteristics of the seeds and the second is a heterotrophic growth phase between imbibition's and emergence. So, the germination was a crucial stage in seedling establishment and plays a key role in crop production [43].

3.1.2 Impact of NaCl concentration on germination rates (%)

The results indicated that the germination rate (%) decreased for four cultivars with increased salinity concentrations as shown in Fig. 3 & Table 3. The results indicated that the both cultivars (cv. Paris S1 & cv. Royal S2) more tolerance to NaCl salinity than the other both cultivars (cv. Nader S3 & cv. Marvilli S4). Osmotic potential caused by salinity stress which prevent water uptake by providing conditions for the entry of the ions that may be toxic to embryo or developing seedlings, thereby salinity caused a reduced germination of either direct toxic effects of salts or osmotic stress resulting in longer exposure of seedling to biotic and a biotic hazards [44-47]. Similar results obtained by Datta et al. [48]; Akbarimoghaddam et al. [49]; Naz et al. [50]; Nee et al. [51] they found the different NaCl concentrations exhibited significant reduction in germination as compared to their non-saline 2019) [52] they found the germination percentage was reduced significantly with the increasing exposure of salt in lettuce (*Lactuca sativa* L.). The observed decrease in germination percentage may be attributed to the decrease in osmotic potential, increasing toxic ions, changing the remobilization balance of seeds reservoirs, loss of viability at higher salinity level and reduced water imbibition's. In addition, high salinity delayed radical emergence and decreased germination percentage [51].

Table 1. Analysis and natural components of the acadian extract (*Ascophyllum nodosum*)

Components	Ratio v/w	Components	Ratio v/w
Algae extract %	100	S %	0.23
Organic matter %	10.58	Mg %	0.04
Carbohydrate %	7.0	Ca%	0.02
Amino acids %	0.1	Fe (ppm)	20-50
pH	8	Cu (ppm)	5-1
N %	0.7	Zn (ppm)	15.0-5.0
P %	1.5	Mn (ppm)	1.0-5.0
K %	6.0	B (ppm)	20-30

Colour: Brown. Natural growth regulators: Cytokinins, Gibberellins, Auxins, Betaines and other Carbohydrates: Alginic acid, Mannitol, Laminarin. As reported on the label

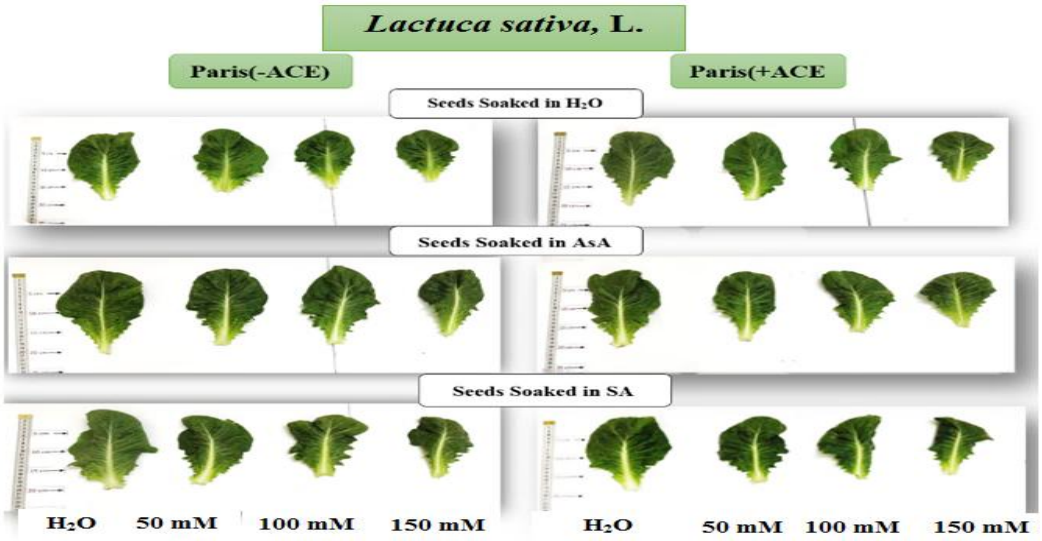

Fig. 1A. Impact application of plant growth regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE) On Leaf Area (cm^2/Leaf) of *Lactuca sativa* L, (cv. Paris) plant grown under salinity stress

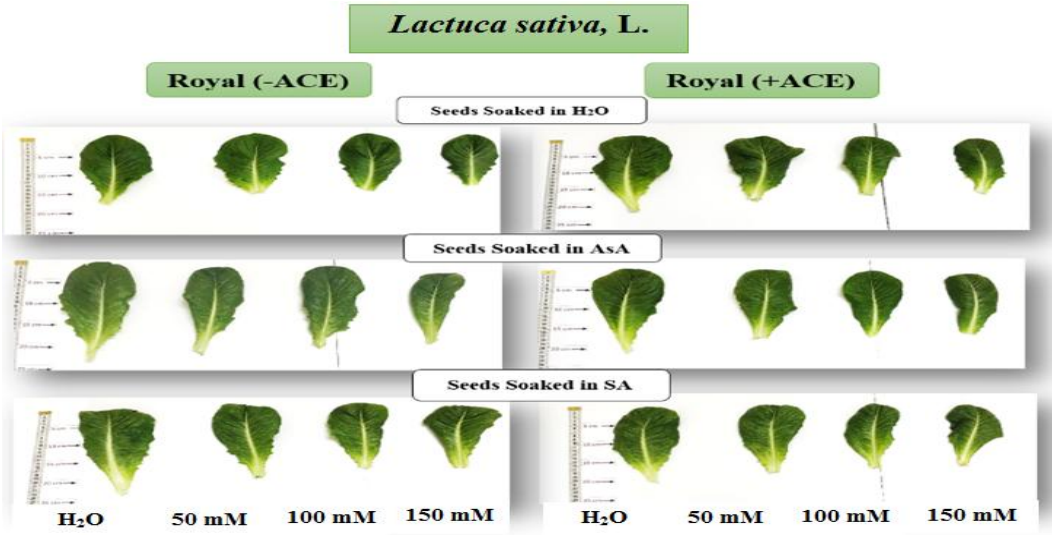

Fig. 1B. Impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) On Leaf Area (cm^2/Leaf) of *Lactuca sativa* L, (cv. Royal) plant grown under salinity stress

Table 2. Statistical analysis of interactive the seeds viability (germination rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Cultivars / Time/Days Statistical Analysis	Seeds Viability (Germination Rate %)			
	cv. Paris (S1)	cv. Royal (S2)	cv. Nader (S3)	cv. Marvilli (S4)
F	1485.720*	910.680*	1466.880*	327.600*
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.624	1.624	1.624	1.779

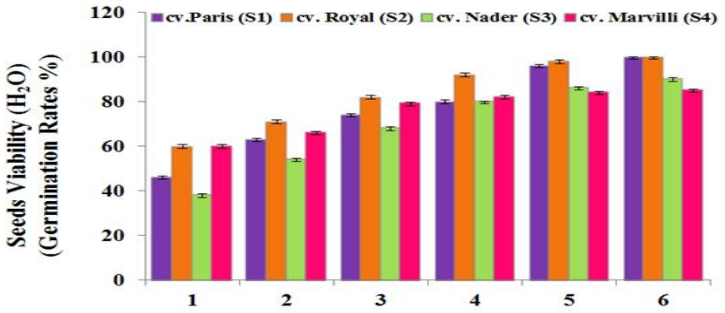

Fig. 2. The seeds viability (germination rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

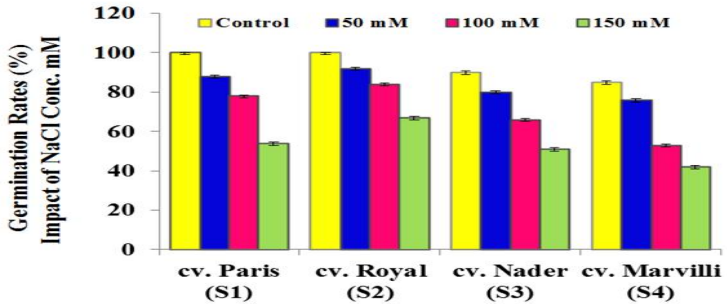

Fig. 3. Impact of NaCl concentrations on germination rate (%) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Table 3. Statistical analysis of interactive impact of NaCl concentrations on germination rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Cultivars / NaCl (mM) Statistical Analysis	Germination Rate (%)			
	cv. Paris (S1)	cv. Royal (S2)	cv. Nader (S3)	cv. Marvilli (S4)
F	1525.333*	795.667*	864.750*	1190.000*
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.633	1.633	1.886	1.886

*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 groups; Means in the same column with Common letters are not significant (i.e. Means with Different letters are significant); *: Statistically significant at p ≤ 0.05; Data was expressed using Mean ± SE*

3.1.3 Impact of AsA - 0.5 mM on germination rates (%)

After soaking the lettuce seeds in AsA (0.5 mM) the germination rate (%) increased and reached to 100% after 3 days for both cultivars (cv. Paris S1 & cv. Royal S2), whereas, the other both cultivars (cv. Nader S3 & cv. Marvilli S4) still germinated for 6 days at 94% and 90% respectively as shown in Fig. 4 & Table 4. Ascorbic acid (AsA) is an essential compound for plants and plays important roles in many physiological processes such as regulates cell division and growth, [53-54] they found that exogenous AsA enhances α-amylase activity and increases endogenous gibberellic acid (GA3) accumulation of the seeds, and ultimately promotes embryo dormancy breaking of *Malus sieversii* seeds. However, there is other research demonstrating that a high dose AsA treatment can induce cell death in mesothelioma cells and suppress germination in wheat seeds. These opposite effects of AsA on seed germination suggest that the production of AsA in seed must be finely controlled or regulated [55].

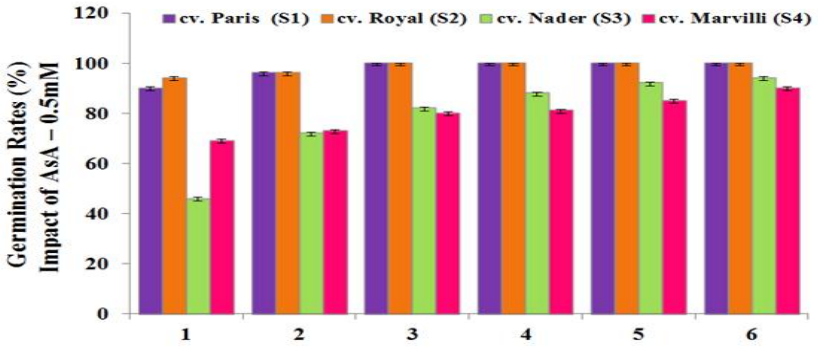

Fig. 4. Impact of ascorbic acid (AsA - 0.5 mM) on germination rate (%) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Table 4. Statistical analysis of interactive impact of ascorbic acid (AsA - 0.5 mM) on germination Rate (%) of Lettuce (*Lactuca sativa*, L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Cultivars / NaCl (mM) Statistical Analysis	Germination Rate (%)			
	cv. Paris (S1)	cv. Royal (S2)	cv. Nader (S3)	cv. Marvilli (S4)
F	150.0*	63.600*	973.200*	177.200*
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.027	1.027	1.779	1.779

3.1.4 Impact of SA - 0.5 mM on germination rates (%)

After soaking the lettuce seeds in SA (0.5 mM) the germination rate (%) increased and reached to 100% after 4 and 3 days for both cultivars (cv. Paris S1 & cv. Royal S2) respectively, whereas, the other both cultivars (cv. Nader S3 & cv. Marvilli S4) still germinated for 5 and 4 days at 90% and 89% respectively as shown in Fig. 5 & Table 5.

3.1.5 Impact of GSH - 0.5 mM on germination rates (%)

After soaking the lettuce seeds in GSH (0.5 mM) the germination rate (%) increased and reached to 100% after 6 and 5 days for both cultivars (cv. Paris S1 & cv. Royal S2) respectively, whereas, the other both cultivars (cv. Nader S3 & cv. Marvilli S4) still germinated for 5 days at 94% and 90% respectively as shown in Fig. 6 & Table 6.

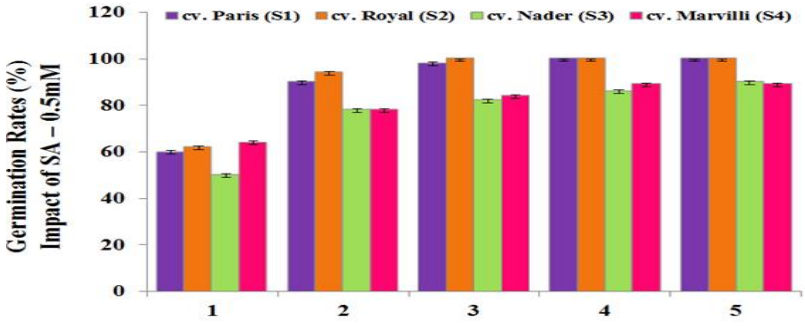

Fig. 5. Impact of salicylic acid (SA-0.5mM) on germination rate %) of lettuce (*Lactuca sativa,* L.) for four Cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Table 5. Statistical analysis of interactive impact of salicylic acid (SA - 0.5 mM) on germination Rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Cultivars / NaCl (mM) Statistical Analysis	Germination Rate (%)			
	cv. Paris (S1)	cv. Royal (S2)	cv. Nader (S3)	cv. Marvilli (S4)
F	1454.0*	2049.0*	753.600*	326.100*
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.409	1.151	1.819	1.819

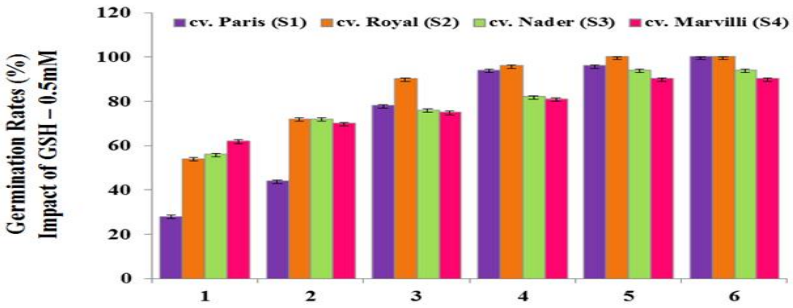

Fig. 6. Impact of glutathione (GSH - 0.5 mM) on germination rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Table 6. Statistical analysis of interactive impact of glutathione (GSH - 0.5 mM) on germination Rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Cultivars / NaCl (mM) Statistical Analysis	Germination Rate (%)			
	cv. Paris (S1)	cv. Royal (S2)	cv. Nader (S3)	cv. Marvilli (S4)
F	3304.320*	1552.800*	627.600*	375.600*
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.624	1.4525	1.779	1.779

3.1.6 Impact of bio-fertilizer (acadian extract - ACE 1%) on germination rates (%)

After soaking the lettuce seeds in bio-fertilizer (ACE) the germination rate (%) increased and reached to 100% after 5 days for both cultivars (cv. Paris S1 & cv. Royal S2), whereas, the other both cultivars (cv. Nader S3 & cv. Marvilli S4) still germinated for 5 days at 91% and 87% respectively as shown in Fig. 7 & Table 7. Using liquid bio-fertilizers have gained popularity because it's easy handling and application either on seeds or in soil [56]. Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as enhance early seed germination and establishment and seedling growth [57-59]. Since many phytohormones stimulate germination and root development, the increased plant growth and vigor after application of seaweeds may be through increased efficiency of nutrients and water uptake [60]. Thus, seaweed cultivation and its utilization is an economically successful approach in agricultural production [61-62].

3.2 Growth Parameters

From the germination results the data indicated that the PGRs (AsA & SA) with both cultivars (cv. Paris S1 & cv. Royal S2) gives best results more the other PGRs (GSH) & bio-fertilizer (ACE) for the other cultivars. After soaking lettuce seeds in PGRs (AsA & SA) transplanting both cultivars (cv. Paris S1 & cv. Royal S2), so the data has shown that the rate of growth increased with using AsA more than SA.

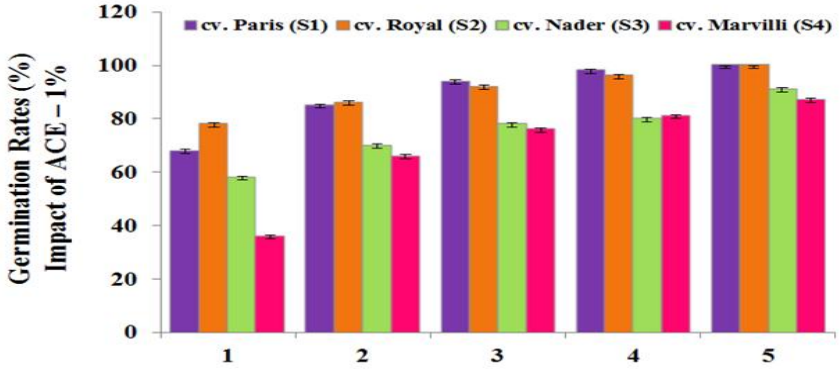

Fig. 7. Impact of bio-fertilizer (acadian extract - ACE 1%) on germination Rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Table 7. Statistical analysis of interactive impact of bio-fertilizer (acadian extract - ACE 1%) on Germination Rate %) of lettuce (*Lactuca sativa,* L.) for four cultivars (cv. Paris; cv. Royal; cv. Nader; cv. Marvilli)

Cultivars / NaCl (mM) Statistical Analysis	Germination Rate (%)			
	cv. Paris (S1)	cv. Royal (S2)	cv. Nader (S3)	cv. Marvilli (S4)
F	641.250*	280.500*	452.400*	1211.100*
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.627	1.627	1.819	1.819

3.2.1 Leaf number and leaf area

Overall, leaf number and leaf area in lettuce (*Lactuca sativa*, L.) plant tended to decreased highly significant at ($p \leq 0.001$), for both cultivars (cv. Paris & cv. Royal) with increasing NaCl salinity concentrations in the presence or absence of bio-fertilizer (ACE) compared with control as shown in Fig. 8 & Table 8. The impact of PGRs (AsA & SA) and bio-fertilizer (ACE) individually, the leaf number and leaf area increased highly significantly ($p \leq 0.001$) for both cultivars but decreased highly significantly ($p \leq 0.001$) with increase salinity concentrations compared with control. So, the results indicated that the PGRs (AsA & SA) more effective in the absence (-ACE) than in the presence (+ACE) of bio-fertilizer. Whereas, the effect of AsA in the absence (-ACE) of bio-fertilizer on the leaf number and leaf area tended to increased more highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity than SA compared with control. While, the leaf number and leaf area increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the presence of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress and PGRs (AsA & SA) in two cultivars in the presence or absence of bio-fertilizer (ACE) indicated that the *LSD* test highly significant at $P \leq 0.001$. The decrease of leaf numbers may be due to the accumulation of sodium chloride in the cell walls and cytoplasm of the older leaves. Also, Hussein and Alva [63] they found increased salinity by irrigation water decreased the plant growth, and biomass, while foliar application of AsA increased number of leaves and leaf area in millet plants grown under different salinity. So, the exogenous application of AsA can be enhance foliar growth which may contribute to increased plant biomass and yield. Similarly, Jerry et al. [64] they showed that foliar spraying of AsA at 100 mg/L increased yield and improved plant characteristics such as, number, fresh & dry weights and leaf area per plant. Saberi et al. [65]; Parvin et al. [66]; Parvin and Haque [67]; Youssef et al. [68] they found that salinity reduced the number of leaves and average leaf length plant, while SA significantly reduced the saline toxicity on number of leaves, average leaf length and size plant under different level of saline treatment.

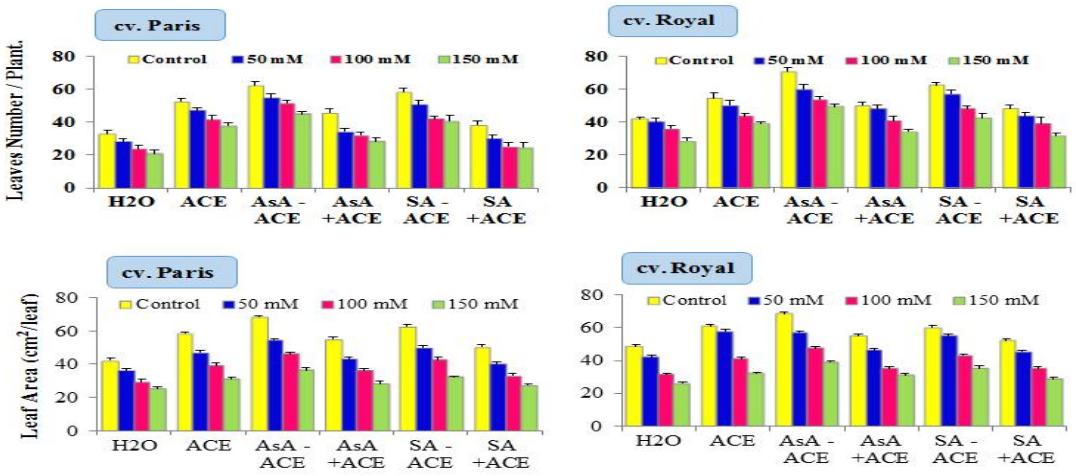

Fig. 8. Impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) On leaf number and leaf area (cm^2/Leaf) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Azzedine et al. [69]; Al-Amry and Mohammed [70] they reported that the application of AsA was effective to mitigate the adverse effect of salt stress on plant growth due to increase in plant height and leaf area and improved chlorophyll (Chl) and carotenoids contents under irrigation with saline water using NaCl this results agree with the results presented in this work. Zhang and Ervin, [71]; Taiz and Zeiger [72] they found that the use of *A. nodosum* extract increased leaf area responses can be related to the alterations in cytokinin production by plants, a hormone that drives the leaf development. Whereas, Pacheco et al. [73] noticed since *A. nodosum* extract can modifies its endogenous synthesis trends to reduce the leaf area per yarrow plant, due to decreases in the individual leaf area after the use of seaweed-based product. Recent studies showed that some algal extracts exert potent bio-stimulation of vegetative growth and yield of *Ficus carica* L. [74], rice and maize [75-76] *Zea maize* [77] plants *Brassica oleracea* [78]. The positive influence and stress amelioration by the exogenously supplementation of AsA on different plants under various abiotic stress conditions during different phases of development to improve stress tolerance in different types of crops [51-79].

3.2.2 Fresh and dry weight (g/plant)

Overall, shoot fresh and dry weight in lettuce (*Lactuca sativa*, L.) plant tended to decreased highly significant at ($p \leq 0.001$), for both cultivars (cv. Paris & cv. Royal) with increasing NaCl salinity concentrations in the presence or absence of bio-fertilizer (ACE) compared with control as shown in Fig. 9 & Table 9. The impact of PGRs (AsA & SA) and bio-fertilizer (ACE) individually, the shoot fresh and dry weight increased highly significantly ($p \leq 0.001$) for both cultivars but decreased highly significantly ($p \leq 0.001$) with increase salinity concentrations compared with control. So, the results indicated that the PGRs (AsA & SA) more effective in the absence (-ACE) than in the presence (+ACE) of bio-fertilizer. Whereas, the effect of AsA in the absence (-ACE) of bio-fertilizer on the shoot fresh and dry weight tended to increased more highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity than SA compared with control. While, the shoot fresh and dry weight increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the presence of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress and PGRs (AsA & SA) in two cultivars in the presence or absence of bio-fertilizer (ACE) indicated that the *LSD* test highly significant at $P \leq 0.001$.

The reduction of the plant organs dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl^- and Na^+ [80-82]. The results agree with these results by Yildirim et al. [83]; Ekinci et al. [84]; Hniličková et al. [85] they reported that salinity conditions could adversely affect the shoot and

root fresh and dry weights of lettuce. The exogenous application of SA can act on the hormonal action stimulating plant growth and development and the induction of plant defense responses under stressful conditions [86-89].

The utilization of seaweed extracts (*Ascophyllum nodosum*) to stimulate germination, growth seedlings, enhancing flowering, improve crop performance and yield, increase biomass and quality (value) and elevated resistance to biotic and abiotic stress and its chemical constituents under high salinity conditions [31,90-92].

3.3 Water Relations

3.3.1 Succulence (fresh weight/ oven dry weight)

Overall, the shoot succulence (F. Wt. / Oven D. Wt.) in lettuce (*Lactuca sativa*, L.) plant tended to increased highly significant at ($p \leq 0.001$), for both cultivars (cv. Paris & cv. Royal) with increasing NaCl salinity concentrations in the presence or absence of bio-fertilizer (ACE) compared with control as shown in Fig. 10 & Table 10. The impact of PGRs (AsA & SA) and bio-fertilizer (ACE) individually, the shoot succulence increased highly significantly ($p \leq 0.001$) for both cultivars with increase salinity concentrations compared with control. So, the results indicated that the PGRs (AsA & SA) more effective in the absence (-ACE) of bio-fertilizer than in the presence (+ACE) of bio-fertilizer. Whereas, the effect of AsA in the absence (-ACE) of bio-fertilizer on shoot succulence tended to increased more highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity than SA compared with control. While, the all of this results it has been found the shoot succulence increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the presence of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress and PGRs (AsA & SA) in two cultivars in the presence or absence of bio-fertilizer (ACE) indicated that the *LSD* test highly significant at $P \leq 0.001$.

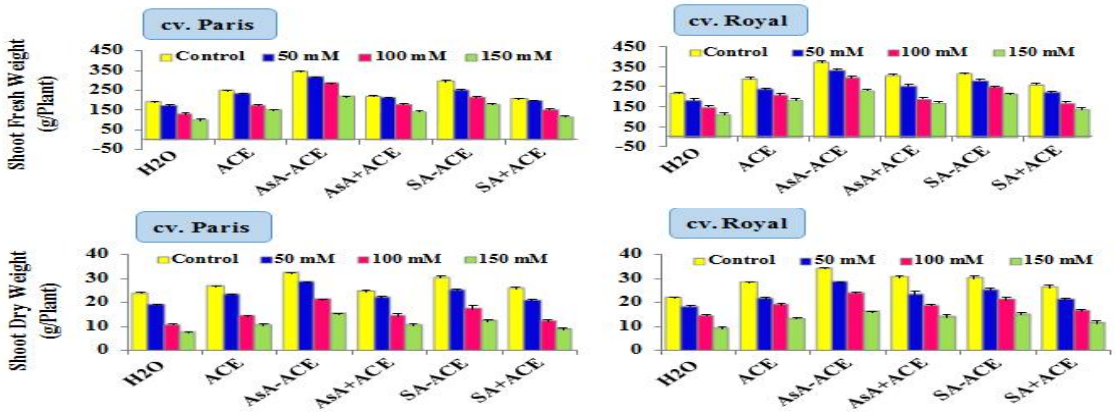

Fig. 9. Impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) on shoot fresh and dry weight (g/Plant) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

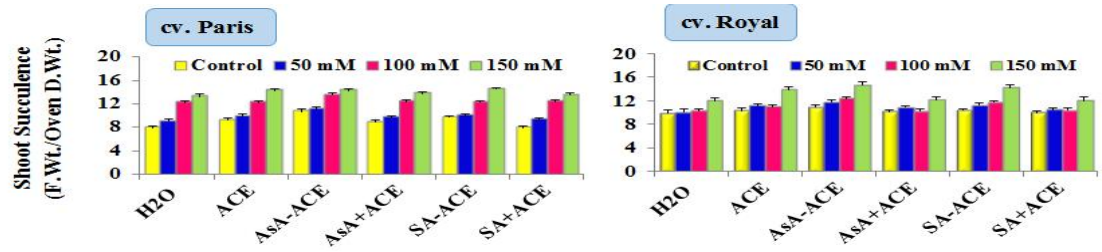

Fig. 10. Impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) on shoot water relations (succulence - F. Wt. / Oven D. Wt.) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Table 8. Statistical analysis of interactive impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) On leaf number and leaf area (cm^2/Leaf) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Statistical Analysis *ANOVA*	Lettuce (*Lactuca sativa* L.)											
	cv. Paris						cv. Royal					
	Application of Growth Regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE)											
	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE
	Leaf Number (No. / Plant)											
F	4.011	8.495*	7.248*	7.889*	10.048*	6.703*	5.475*	10.941*	10.096*	10.588*	16.498*	7.772*
p	0.052	0.007*	0.011*	0.009*	0.004*	0.014*	0.024*	0.003*	0.004*	0.004*	0.001*	0.009*
LSD	8.626	7.365	7.777	8.695	7.719	7.946	8.311	6.866	7.777	6.800	7.057	8.329
	Leaf Area (cm^2/Leaf)											
F	66.985*	182.901*	281.116*	132.143*	130.799*	23.593*	74.283*	176.708*	179.351*	184.103*	158.106*	145.870*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	2.991	2.829	2.636	3.204	3.627	.937	3.860	3.081	3.095	2.608	3.499	2.822

*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 groups, means in the same column with Common letters are not significant (i.e. Means with Different letters are significant), #: Statistically significant with H_2O, @: Statistically significant with AsA, ♦: Statistically significant for comparing between with water and with Acadian, *: Statistically significant at p ≤ 0.05, data was expressed using Mean ± SE*

Table 9. Statistical analysis of interactive impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) on shoot fresh and dry weight (g/Plant) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Statistical Analysis *ANOVA*	Lettuce (*Lactuca sativa* L.)											
	cv. Paris						cv. Royal					
	Application of Growth Regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE)											
	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE
	Shoot Fresh Weight (g/Plant)											
F	45.342*	64.738*	29.377*	54.220*	52.210*	113.869*	90.022*	69.264*	97.761*	36.349*	61.745*	43.088*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	18.899	22.431	44.508	19.957	28.582	13.101	21.843	31.131	33.561	38.917	36.385	35.788
	Shoot Dry Weight (g/Plant)											
F	140.340*	129.836*	168.108*	112.588*	208.242*	210.745*	140.691*	182.975*	48.128*	70.365*	64.637*	85.524*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.769	2.547	2.368	2.243	2.266	1.571	2.069	2.143	4.905	3.076	3.921	2.875

Table 10. Statistical analysis of interactive impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) on shoot water relations (Succulence - F. Wt. / Oven D. Wt.) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Statistical Analysis *ANOVA*	Lettuce (*Lactuca sativa* L.)											
	cv. Paris						cv. Royal					
	Application of Growth Regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE)											
	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE
	Shoot Succulence (F. Wt. / Oven D. Wt.)											
F	3.550	5.693*	5.034*	3.451	6.693*	5.177*	5.712*	10.325*	11.157*	5.298*	14.491*	3.638
p	<0.001*	0.002*	<0.001*	<0.001*	0.002*	<0.001*	<0.001*	0.004*	0.003*	<0.001*	0.001*	<0.001*
LSD	0.524	0.757	0.765	0.615	0.822	0.443	1.079	1.148	1.227	1.061	1.060	1.307

Table 11. Statistical analysis of interactive impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) on shoot water relations (dry matter content %) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Statistical Analysis *ANOVA*	Lettuce (*Lactuca sativa* L.)											
	cv. Paris						cv. Royal					
	Application of Growth Regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE)											
	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE
	Shoot Dry Matter Content (%)											
F	3.686	6.046*	4.423*	4.698*	6.897*	6.675*	5.595*	9.955*	10.390*	2.666	15.288*	3.919
p	<0.001*	0.019*	<0.001*	<0.001*	0.013*	<0.001*	<0.001*	0.004*	0.004*	<0.001*	0.001*	<0.001*
LSD	1.066	1.078	0.904	1.013	1.174	0.811	1.093	0.840	0.736	1.163	0.670	1.128

Table 12. Statistical analysis of interactive impact application of plant growth regulators (AsA & SA) in the presence or absence of bio-fertilizer (ACE) on chloroplast pigments contents (chlorophyll a, chlorophyll b, carotenoids and total pigments as mg/g leaf F. Wt.) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Statistical Analysis *ANOVA*	Lettuce (*Lactuca sativa* L.)											
	cv. Paris						cv. Royal					
	Application of Growth Regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE)											
	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE	H_2O	ACE	AsA -ACE	AsA +ACE	SA -ACE	SA +ACE
	Chlorophyll a Contents (mg/g Leaf F.Wt.)											
F	594.226*	7436.536*	4120.463*	2750.340*	4982.227*	3060.045*	1.737	2353.620*	5950.267*	1742.287*	2077.291*	2402.980*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.237	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.066	0.058	0.081	0.065	0.071	0.071	1.604	0.070	0.065	0.064	0.081	0.062
	Chlorophyll b Contents (mg/g Leaf F.Wt.)											
F	175.305*	1728.098*	1309.473*	1051.942*	3435.171*	1892.507*	143.750*	252.645*	1432.699*	909.629*	2195.250*	1606.090*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.050	0.045	0.050	0.060	0.034	0.039	0.061	0.116	0.076	0.072	0.039	0.045
	Carotenoids Contents (mg/g Leaf F.Wt.)											
F	146.537*	299.641*	464.357*	644.162*	99.146*	368.524*	88.835*	190.102*	801.702*	955.097*	497.354*	350.983*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.089	0.071	0.058	0.056	0.095	0.059	0.064	0.079	0.067	0.051	0.062	0.077
	Total Pigments (mg/g Leaf F.Wt.)											
F	384.809*	8539.730*	10110.904*	4109.925*	3652.557*	3700.862*	841.789*	2378.151*	4805.452*	1656.329*	4293.365*	7070.658*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.139	0.087	0.083	0.105	0.101	0.110	0.098	0.128	0.140	0.152	0.105	0.074

Salinity, in particular, is considered one of the main environmental factors that affect plant growth and metabolism, leading to severe damage, turgor loss and severe inhibition of growth [93-96]. The data presented by Hirt and Shinozaki [97] they found that the effect of salt stress on plant depends on four responses: dehydration of the cells through the low water potential, nutritional imbalance caused by the interference of saline ions with essential nutrients in both uptake and translocation processes, toxicity due to the high accumulation of Na and Cl in the cytoplasm as well as the production of activated oxygen species during salt stress, so the salinity stress is a major problem to reduce the production of different crops.

3.3.2 Dry matter contents (DMC %)

Overall, the shoot dry matter content % in lettuce (*Lactuca sativa*, L.) plant tended to decreased highly significant at ($p \leq 0.001$), for both cultivars (cv. Paris & cv. Royal) with increasing NaCl salinity concentrations in the presence or absence of bio-fertilizer (ACE) compared with control as shown in Fig. 11 & Table 11. The impact of PGRs (AsA & SA) and bio-fertilizer (ACE) individually, the shoot dry matter contents (DMC %) decreased highly significantly ($p \leq 0.001$) for both cultivars with increase salinity concentration compared with control. So the results indicated that the PGRs (AsA & SA) more effective in the presence (+ACE) of bio-fertilizer than in the absence (-ACE) of bio-fertilizer. The presence of PGRs (AsA & SA) or ACE the DMC% was decreased, this results it might be because the antioxidant increasing in cells, the antioxidant able to remove the all free radicals (undesirable) and reducing the absorption of water. Whereas, the effect of AsA in the absence (-ACE) of bio-fertilizer on the DMC% tended to decreased more highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity than SA compared with control. While, the all of this results it has been found that the DMC% decreased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially with AsA in the absence (-ACE) more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress and PGRs (AsA & SA) in two cultivars in the presence or absence of bio-fertilizer (ACE) indicated that the *LSD* test highly significant at $P \leq 0.001$.

Increasing in NaCl salinity concentration tended to reduce the absorption of water leading to a drop in water content, the inhibitory effect of NaCl on growth parameters could be attributed to the osmotic effect of NaCl salinity, in addition, the changes in water status under NaCl stress may cause a reduction in meristem activity as well as cell elongation [98-99]. Also, the results obtained by Chookhampaeng [100]; El-Abagy et al. [101] the low level of salinity treatment (50 mM NaCl) had no deleterious effects on vegetative growth parameters, but at higher concentration of NaCl (100 and 200 mM), growth parameters were drastically reduced in lettuce, salt stress negatively affects plant growth and production of dry matter.

3.4 Chlorophyll a, b, Carotenoids and Total Pigment Contents (mg/g Leaf Fresh Weight)

Overall, the chlorophyll a, b, carotenoids and total pigment contents in lettuce leaves increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations for both cultivars (cv. Paris & cv. Royal) in the presence or absence of bio-fertilizer (ACE) compared with control as shown in Fig. 12 & Table 12. The impact of PGRs (AsA & SA) and bio-fertilizer (ACE) individually, the chlorophyll a, b, carotenoids and total pigment contents increased highly significantly ($p \leq 0.001$) for both cultivars with increase salinity concentrations compared with control. So the results indicated that the PGRs (AsA & SA) more effective in the absence (-ACE) of bio-fertilizer than in the presence (+ACE) of bio-fertilizer. Whereas, the effect of AsA in the absence (-ACE) of bio-fertilizer on the chlorophyll a, b, carotenoids and total pigment contents tended to increased more highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity than SA compared with control. So, the all of this results it has been found the chlorophyll a, b, carotenoids and total pigment contents increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially with AsA in the absence (-ACE) more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress and PGRs (AsA & SA) in two cultivars in the presence or absence of bio-fertilizer (ACE) indicated that the *LSD* test highly significant at $P \leq 0.001$.

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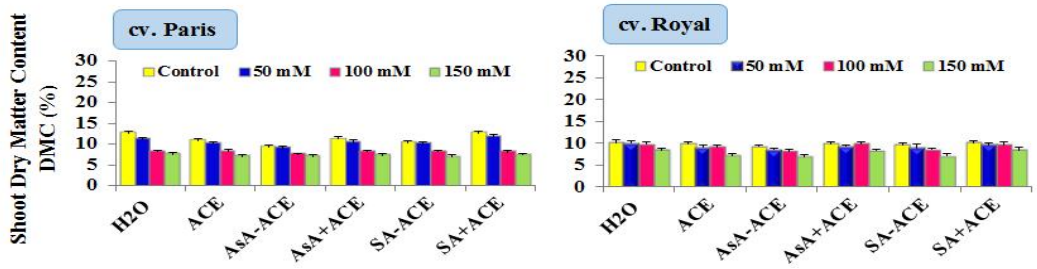

Fig. 11. Impact application of plant growth regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE) on shoot water relations (Dry matter content %) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

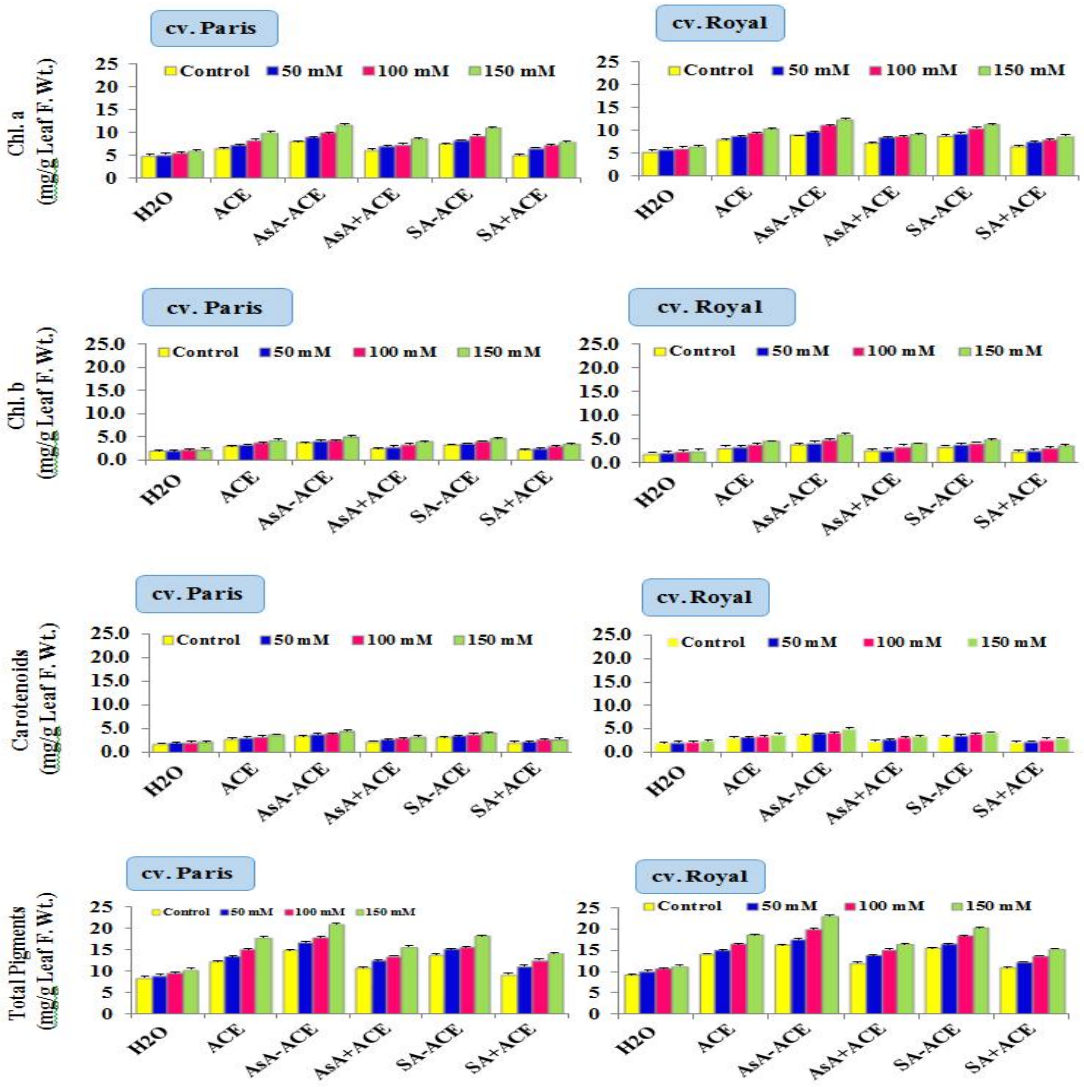

Fig. 12. Impact application of plant growth regulators (AsA & SA) in the presence or absence of Bio-fertilizer (ACE) on chloroplast pigments contents (chlorophyll a, chlorophyll b, carotenoids and Total Pigments as mg/g Leaf F. Wt.) of *Lactuca sativa* L, for both (cv. Paris & cv. Royal) plant grown under salinity stress

Babar et al. [102] they found that a marked reduction in photosynthetic pigments including chlorophyll a and chlorophyll b for both varieties of fenugreek, while, reduction in chlorophyll contents was mitigated by the foliar application of SA. Similarly, these results reinforce the results obtained by researchers where salt-induced reduction in the chlorophyll contents is alleviated by the foliar application of SA in crops such as tomato [103-104]. This alleviation of salt-induced harmful effect depends on type of plant species as well as concentration and mode of application of salicylic acid.

Lawlor and Cornic [105]; Kusvuran et al. [106]; Saha et al. [107]; Nazarbeygi et al. [108]; Garrido et al. [109] they observed a linear decrease in the levels of total Chl, (Chl a, & Chl b) under increasing NaCl concentrations. Whereas, the results obtained by Al-Erwy et al. [110] they found that the level of salinity (20% of seawater) increase chlorophyll a & b concentration in wheat plant.

In case using foliar application of AsA they showed significant improvement in chlorophyll a, b and total chlorophyll in non-saline as well as salinity treated plants. So, the results may be ascorbic acid (AsA) is involved in protecting the photosynthetic apparatus from oxidative damage induced by salt stress and induces chlorophyll synthesis and It was also reported that AsA stimulates the synthesis of IAA and GA3 and depresses ABA formation, which shields the chloroplast, resulting in increased production of photosynthetic pigments [111]. Likewise, Noreen et al. [112]; Youssef et al. [113] they found that increasing SA levels had a positive effect of all physiological compositions (chlorophyll a & b, carotenoids, total carbohydrate) as well, increased all yield components under salinity, while Yanik et al. [114] they found applying SA at high concentrations reduced the total chlorophyll content in rye plant. Ma et al. [89]; Thomson et al. [115] they expected may be probably the positive effect of SA on photosynthetic pigments could be attributed to its stimulatory effects on RuBisCO activity and the rate of photosynthesis and modifying the activity of some of the important enzymes.

The results obtained Kumari et al. [116]; Kaoaua et al. [117]; Vishnupriya and Flora [118] they found that SWE, irrespective of application methods, could increase photosynthetic pigments (chlorophylls and carotenoids) content result from work of the photosynthetic apparatus, in which the chlorophyll molecule occupies a key place. However, there is a close relationship between chlorophyll synthesis and the applied dose of SWE, where lower doses would be the most effective in promoting increases in chlorophyll content, the method of application is also referred as crucial factor to trigger increases in the chlorophyll content [119-120].

Plant growth regulators (PGRs) mainly differ from fertilizers in several points: (1) they alter and manage the cell division, (2) control of root and shoot elongation, and (3) initiation of flowering and other metabolic functions. While, fertilizers clearly supply nutrients needed for normal plant growth [121]. Other workers differentiate between biostimulants and bio-fertilizers by their direct hormonal effects (biostimulants) [122], indirect effects on nutrient availability (bio-fertilizers) [123]. Ascorbic acid (Vitamin C), regulates a number various physiological and biochemical processes and induces cell elongation and cell division [124-125], and it is a key antioxidant molecule for sustained photosynthesis and photosynthetic pigments [126-127]. Furthermore, AsA protects lipids and proteins and improves tolerance against various abiotic stresses and induces plant growth [128-129]. Azooz et al. [130] showed that application of ascorbic acid through seed soaking enhanced plants growth by increased germination percentage, root and shoot fresh and dry weights, chlorophyll content and higher accumulation osmolytes, this results agree with this studies results.

4. CONCLUSION

Generally, this study concluded that the impact of 3 PGRs (AsA, SA & GSH - 0.5 mM) and ACE 1% on germination of 4 cultivars to obtained the best treatment on all cultivars. The PGRs in the absence of bio-fertilizer (-ACE) resulted an increased the germination in both cultivars (cv. Paris & cv. Royal), the leaf number and leaf area, fresh and dry weights for both cultivars of lettuce plant increased by mitigate the impact of salinity led to increase the plant metabolism. Consequently, the AsA tended to improvement the growth parameters and increased the amount of chloroplast pigments in the absence of bio-fertilizer (-ACE) more than SA compared with control. Finally using PGRs gives effective results for reduced salinity stress especially in the absence of bio-fertilizer (ACE).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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