

Effects of Monopotassium-phosphate, Nano-calcium fertilizer, Acetyl salicylic acid and Glycinebetaine application on growth and production of tomato (*Solanum lycopersicum*) crop under salt stress

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Abstract. Salinity problem is increasingly affecting tomato production in Lebanon leading to economic losses. The study investigated the potential effects of nano-Calcium (LITHOVIT®), monopotassium-phosphate (MKP: 0-52-34) fertilizers, Acetyl salicylic acid (Aspirin) and the osmoregulator glycinebetaine (GB) on salt tolerance of potted determinate tomato (variety Sila) plants in open-field. Salt stress was induced by irrigation solutions of EC = 2, 4, 6, 8 and 10 mS cm⁻¹ and MKP (2, 3 and 3.5 g L⁻¹), Aspirin (50, 75 and 100 mg L⁻¹), LITHOVIT® (0.5, 0.75 and 1 g L⁻¹) and GB (4.5, 6 and 7.5 g L⁻¹) were applied through foliar application or fertigation. Comparisons between treated and non-treated plants at each salinity level (control) showed that LITHOVIT® decreased the salinity-induced reductions in stem diameter, leaf area and chlorophyll content. Medium concentrations of LITHOVIT® and Aspirin improved stem diameter and all products except Glycinebetaine improved flower number compared to control. Root dry weight and Root Mass Fraction were mostly enhanced in MKP and Aspirin-treated plants. Best improvement in plant yield (76%) was obtained with low concentrations of MKP and LITHOVIT® at EC = 8 mS cm⁻¹ due to improvement in fruit number rather than fruit weight. Consequently, LITHOVIT® and MKP showed superior effects under salt stress compared to Aspirin and Glycinebetaine.

Key words: osmoregulator, foliar fertilizers, salt-tolerance, fruit yield, fruit quality.

INTRODUCTION

Salinity is a major problem affecting plant growth and development (Aly et al., 2003). Tomato is a moderately sensitive crop to salinity (Maksimovic & Ilin, 2012) and severe salt stress could alter plant physiology causing reductions in growth, photosynthesis, respiration and metabolic accumulation (Ebrahim, 2005). This is mainly due to ion toxicity caused by sodium (Na⁺) accumulation in soils and plants (Munns, 2002) which affects water potential and availability (Franco et al., 2011) and interferes with nutrient uptake from roots (Chavarria & dos Santos, 2012). In addition, plant

production is reduced (Tantawy et al., 2013) by salinity-induced reductions in flowering capacity (Boamah et al., 2011), size and number of marketable fruits (del Amor et al., 2001). On the contrary, salinity has a stimulatory effect on fruit quality (Boamah et al., 2011). It increases sugars, acidity, pH (Cuartero & Fernández-Muñoz, 1999), total soluble solids (TSS) contents as well as titratable acidity (del Amor et al., 2001). One way to improve salt tolerance of plants is through the application of compatible solutes (Ben Ahmed et al., 2010; Saxena et al., 2013) such as the osmoregulator glycinebetaine (GB) (Sakamoto & Murata, 2002; Chen & Murata, 2011) which accumulates in the cytoplasmic compartments while ions are sequestered in the vacuole (Salisbury & Ross, 1992). GB maintains the osmotic balance (McCue & Hanson, 1992) and protects membrane functions from high concentrations of Na⁺ and chloride (Cl⁻) (Fariduddin et al., 2013). It has been reported to ameliorate net photosynthesis, increase stomatal conductance, decrease photorespiration and improve fruit set and yield on tomato (Mäkelä et al., 1999; Tantawy et al., 2009). On the other hand, potassium and phosphorus fertilization has been reported as a method to alleviate salinity effects on crops (Yurtseven et al., 2005; Tantawy et al., 2013; Afzal et al., 2015). In specific, on tomato monopotassium-phosphate (MKP) application could improve plant growth, fruit quality (Fan et al., 2011), chlorophyll content and dry matter accumulation (Chapagain & Wiesman, 2004). On the same crop, it was stated that Acetyl salicylic acid (Aspirin) that is an artificial analogue of salicylic acid (Senaratna et al., 2000) could have a potential role in counteracting salinity impacts through foliar application due to its ability to increase photosynthetic pigments, soluble compounds in fruits as well as leaf water content and membrane stability (Agamy et al., 2013). Moreover, nano-fertilizers are being increasingly used in agriculture (Froggett, 2009), however there were minor reports regarding their role under salt stress. Their importance resides in their ability to improve nutrient use efficiency causing higher crop productivity (Solanki et al., 2015). They could increase photosynthesis rate and dry matter production through foliar application (Hediat & Salama, 2012; Suriyaprabha et al., 2012; Tarafdar et al., 2012). Under salt-stress, Tantawy et al. (2014) found that nano-calcium (LITHOVIT) treatment improved tomato yield and fruit nutritional status.

In the last 20 years, salinity was amplified in arid and semi-arid areas of the Mediterranean region (Cirillo et al., 2016). In Lebanon, this problem is dominant in cultivated areas of southern coasts where seawater intrusion was caused by excessive groundwater pumping (El Moujabber et al., 2004; Korfali & Jurdy, 2010). In addition, soil EC of more than 52% of open field sites in the semiarid northern areas are in the range of slightly saline to saline (Darwish et al., 2002) which has been limiting the type and production rate of crops grown there (El Moujabber et al., 2005). Tomato is one of the most important agricultural crops in Lebanon and is widely cultivated in regions subjected to salinity (Darwish, 1995). Based on all previous statements, the experiment was carried out in order to evaluate if the application of nano-Ca, MKP, GB and Acetyl salicylic acid (Aspirin) could be adopted as a strategy to mitigate negative salinity impacts in local cultivation of tomato crop.

MATERIALS AND METHODS

Plant material and treatments

In late-May, tomato seedlings were transplanted at the stage of 2–3 true leaves into plastic pots containing washed sandy clay soil with a Cation Exchange Capacity of 1 meq per 100 g. Seedlings were left to grow in open-field conditions under natural lighting (25–20) ± 2 °C day/night temperature with air relative humidity of 60%. Soil salinity (EC_e) was analyzed prior to transplantation in saturated paste extracts and it was of 0.17 mS cm⁻¹. Plants were subjected to five salinity levels (EC = 2, 4, 6, 8 and 10 mS cm⁻¹) and to the application of four various products with 3 different concentrations: LITHOVIT® (LITHO-Low: 0.5 g L⁻¹, LITHO-Med: 0.75 g L⁻¹ and LITHO-High: 1 g L⁻¹), ASPIRIN (ASP-Low: 50 mg L⁻¹, ASP-Med: 75 mg L⁻¹ and ASP-High: 100 mg L⁻¹), MKP (MKP-Low: 2 g L⁻¹, MKP-Med: 3 g L⁻¹ and MKP-High: 3.5 g L⁻¹) and GB (GB-Low: 4.5 g L⁻¹, GB-Med: 6 g L⁻¹ and GB-High: 7.5 g L⁻¹). LITHOVIT® is a natural mineral (based on limestone) containing a lot of CO₂ bonds and consisting of (Ca; Mg) CaCO₃ and other micronutrient (Bilal, 2010). LITHO and ASP were applied through foliar spray, MKP through fertigation while GB through both methods. GB, LITHO and MKP were dissolved in distilled water while ASP was mixed with ethanol at high temperature prior to dilution. Each product was applied 3 times during growth cycle (15, 29 and 43 DAT: Days after transplantation). Plants were irrigated with tap water (EC = 0.4 mS cm⁻¹) until 14 DAT and saline irrigation started at 19 DAT with an interval of 3 days between consecutive irrigations; NaCl solutions were prepared after dissolving in water tanks of 100 L the corresponding weight of salt needed to reach each level. A continuous monitoring of the EC of the saline solution was done using an EC meter. At each salinity level, enough drainage was allowed until obtaining an EC_{water drainage} = EC_{irrigation solution}. Adjustment of EC_{water drainage} was done using the corresponding saline solution. This was done to guarantee that the EC_{root zone} is stable at the set point and to ensure a steady state value of the EC during the entire growing period.

Measured indicators

Leaf number and stem diameter were evaluated at the date of destructive sampling (55 DAT). Plant height was measured twice; at 24 and 55 DAT. Number of trusses, flowers and fruits was counted on plants. Fruit set consisted of the ratio of fruit number on flower number and expressed in percentage. Fruit diameter was measured using a sliding caliper and weight of individual fruit (g) was recorded for determining plant yield (g per plant). Aboveground and underground parts of plants were separated and oven-dried at 100 °C until constant weight for measuring dry weights, consequently Root Mass Fraction (RMF) was calculated as follows: RMF (g/g) = root dry mass/total plant dry mass (Poorter et al., 2012). Chlorophyll content and cell electrolyte leakage were assessed as described by Levent Tuna et al. (2007) using the upper most expanded leaves (del Amor et al., 2001). On fruits, Total soluble solids (TSS) was measured using Euromex RF (360) refractometer and titratable acidity was assessed by titration with NaOH (Garner et al., 2005).

Statistical analysis

The experimental design consisted on a Complete Randomized Block Design (CRBD) with 2 factors: salinity (5 salinity levels) and product application (4 products x

3 concentrations) and 10 replicates. Data was subjected to analysis of variance which consisted on means \pm SE compared by Fisher's least-significant differences test (*LSD*) using STATISTICA 10 program. Repeated Measures ANOVA was used to evaluate the evolution of plant growth with time.

RESULTS AND DISCUSSION

Results of Factorial ANOVA (Table 1) showed that the separated effects of EC and Treatment were significant on averages of all parameters ($P < 0.05$) with the exception of those of EC on plant height, number of flowers and number of fruits. The 2-way interactive effects of EC and Treatment were significant on averages of all parameters except fruit weight, yield, dry weight of roots and root mass fraction. The non-interactive of Time, the 2way and 3way interactive effects of Time, EC and Treatment were significant on all parameters.

Table 1. Effects of the experimental factors (EC, Treatment and Time) and their interactions on the different measurements averages

	EC	Treatment	EC* Treatment	TIME* EC	TIME* Treatment	TIME*EC* Treatment
Plant height (cm)	NS	**	**	**	**	**
Leaf number	**	**	**	**	**	**
Number of clusters	**	**	**	**	**	**
Number of fruits	NS	**	**	**	**	**
Stem diameter (cm)	**	**	*	-	-	-
Number of flowers	NS	**	**	-	-	-
Fruit set (%)	**	**	*	-	-	-
AWF (g)	**	**	NS	-	-	-
ADF (cm)	**	**	*	-	-	-
Yield/plant (g)	**	**	NS	-	-	-
RMF (g/g)	**	**	NS	-	-	-

AWF: Average weight of individual fruit, ADF: Average diameter of fruit, DWR: Dry weight of roots; RMF: Root Mass Fraction.

Plant growth

In general, average plant height in salt-stressed plants was mostly enhanced at late stages of growth (55 DAT) mainly by MKP-Low and MKP-Med at EC4 and by MKP-Med and MKP-High at EC8, while it was the most improved by LITHO-Med and LITHO-High at EC10 compared to control (Fig. 1).

An improvement in average leaf number was observed at EC4 following MKP-Low and ASP-Med application, and at EC8 and EC10 with MKP-High and MKP-Low respectively (Fig. 2).

Stem diameter was reduced with increasing salinity levels (by 0.20 cm between EC2 and EC10), however it was enhanced by ASP-Med at EC4, EC8 and EC10 (by 0.18 cm, 0.29 cm, and 0.34 cm respectively), MKP-Low at EC8 (by 0.38 cm) and LTHO-Med at EC6 (by 0.29 cm) compared to control (Fig. 3).

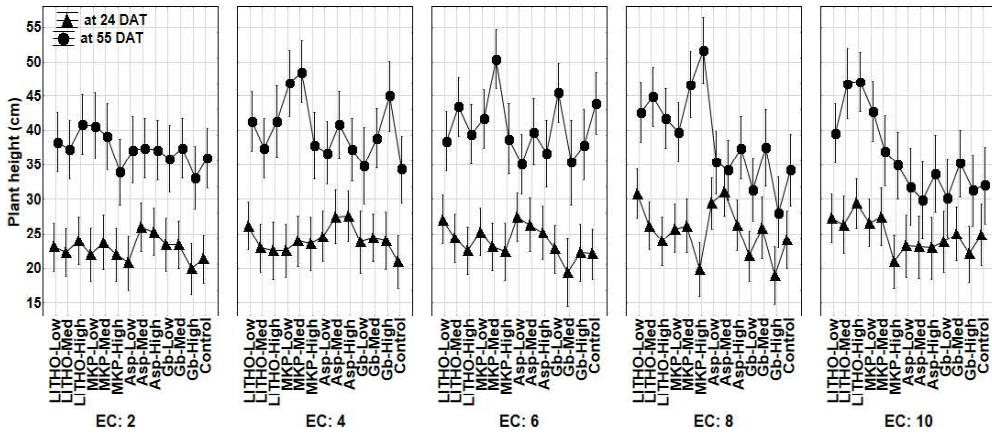


Figure 1. Averages (middle markers) and the 95% limits of confidence (± 2 xSE) (vertical bars) of the plant height.

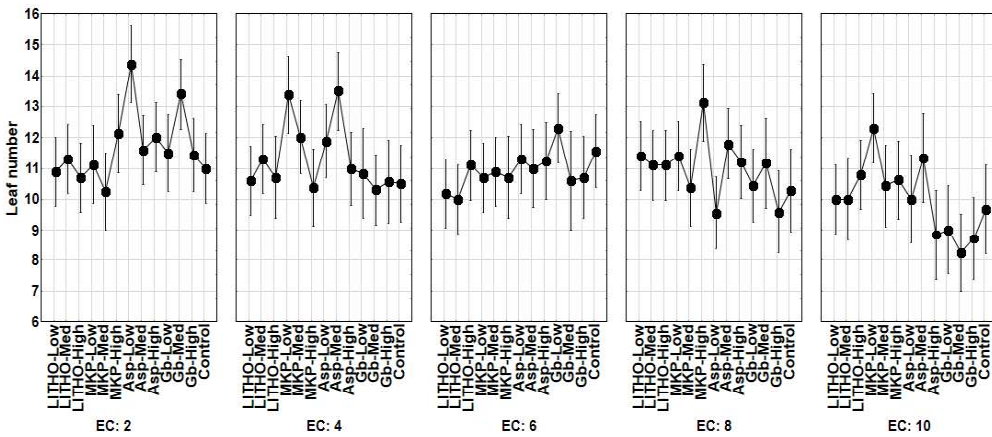


Figure 2. Averages (middle markers) and the 95% limits of confidence (± 2 xSE) (vertical bars) of the leaf number.

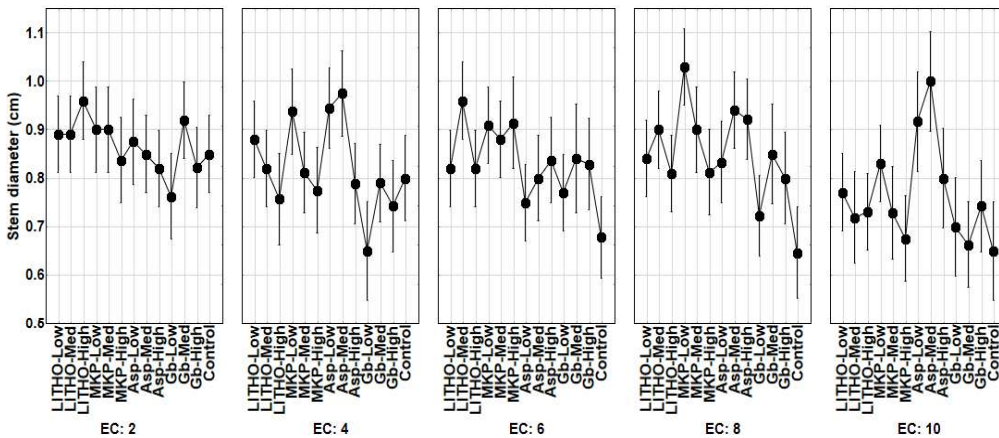


Figure 3. Averages (middle markers) and the 95% limits of confidence (± 2 xSE) (vertical bars) of the stem diameter.

Flowering, production and fruit quality

Salinity-induced reductions in the majority of flowering and productive indicators were not statistically significant; however they were observed at high salinity levels (EC8 and EC10) in control plants. Application of LITHOVIT®, MKP and ASP with low, medium and high doses increased the average number of trusses at EC8 while at EC10 improvement in this parameter was significant following LITHO-Low, LITHO-Med, LITHO-High and MKP-Low applications compared to control. Also, at those salinity levels, all products except GB decreased the reductions in average flower number per plant, with various significance depending on the application dose (Figs 4, 5).

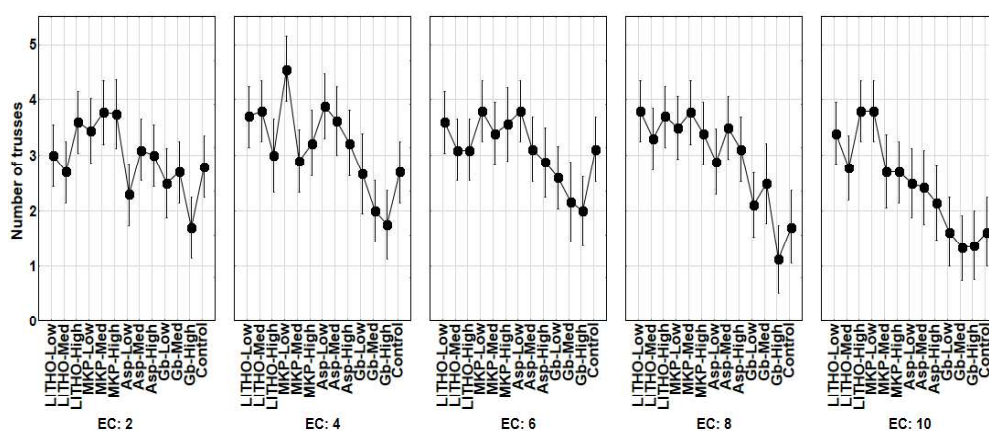


Figure 4. Averages (middle markers) and the 95% limits of confidence ($\pm 2 \times SE$) (vertical bars) of the number of trusses.

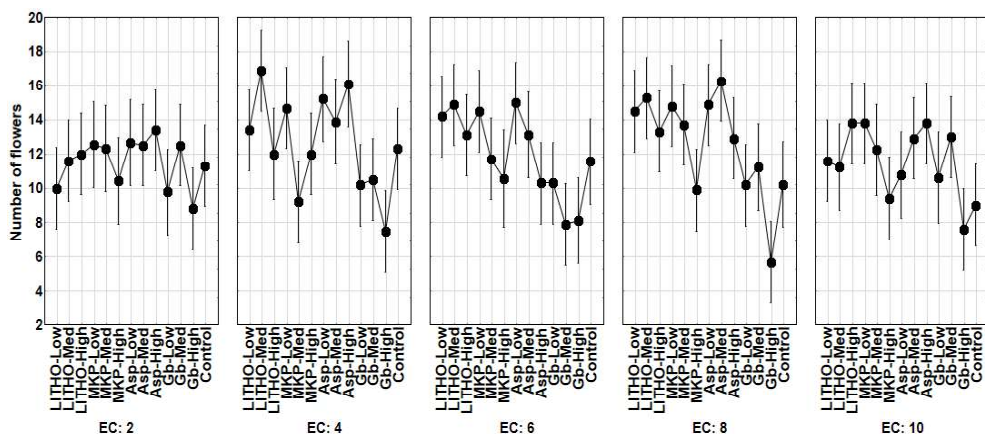


Figure 5. Averages (middle markers) and the 95% limits of confidence ($\pm 2 \times SE$) (vertical bars) of the number of flowers.

Fruit set was the highest in LITHO-treated plants compared to other treatments. When comparing with control, significant increase in fruit set was observed at EC2 and EC8 with LITHO-Low and at EC2 and EC10 with MKP-Med and MKP-Low respectively. Moreover, although none of the products have shown a significant effect

on average values of individual weight and diameter of fruits, however MKP and LITHO enhanced average fruit number and average yield while ASP and GB did not. In specific, MKP-Low increased fruit number by 4 at EC4 and EC6 and by 6 at EC8 and EC10, while MKP-Med increased it by 4 at EC2 and EC6 compared to control. Also, at EC6 number of fruits was increased by 4 following LITHO application with various doses, and by 6 and 5 fruits with LITHO-Low and LITHO-Med respectively at EC8 (Fig. 6).

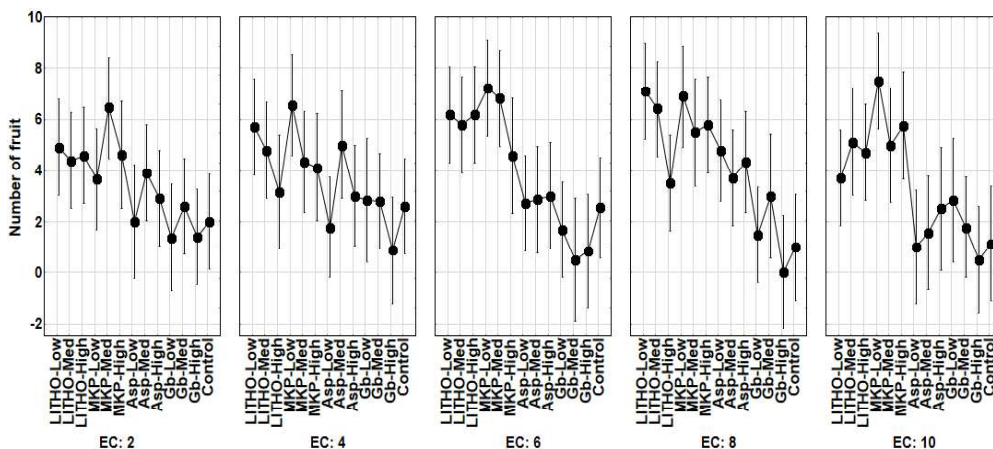


Figure 6. Averages (middle markers) and the 95% limits of confidence ($\pm 2 \times SE$) (vertical bars) of the number of fruits.

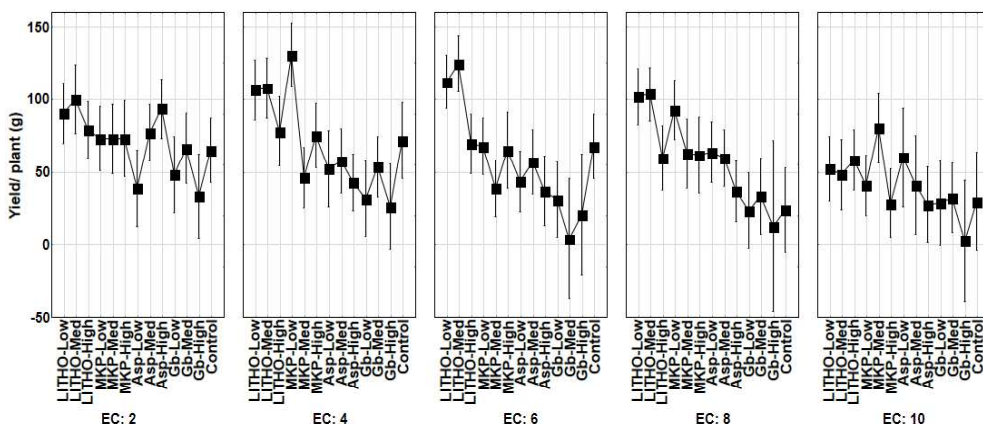


Figure 7. Averages (middle markers) and the 95% limits of confidence ($\pm 2 \times SE$) (vertical bars) of yield/ plant.

In addition, compared to control, improvements in yield by around 45% and 74% were observed at EC4 and EC8 respectively with MKP-Low and by 39% and 76% with LITHO-Low and by 46% and 76% with LITHO-Med at EC6 and EC8 respectively (Fig. 7). Fruit quality was improved with increasing salinity where TSS and titratable acidity contents were the highest in control fruits. Various investigated products did not affect fruit quality except for MKP positive effect on TSS content which was greatly improved mainly with MKP-Med under more severe salt stress (9.7% and 10.2% in

treated plants compared to 8.2% and 8.95% in non-treated plants at EC8 and EC10 respectively) (figures not included).

Physiological indicators

Increasing salinity levels had negatively influenced dry weight of aboveground plant parts; however dry weights were in general higher with LITHO (Med) and MKP (Low, Med and High) treatments mainly at EC6, 8 and 10 mS cm⁻¹. On the other hand, dry weight of roots and consequently RMF were the highest in ASP-Low treated plants at EC2, 4 and 6 and in MKP-Low and LITHO-Med treated plants at EC8 and EC10 respectively (Fig. 8).

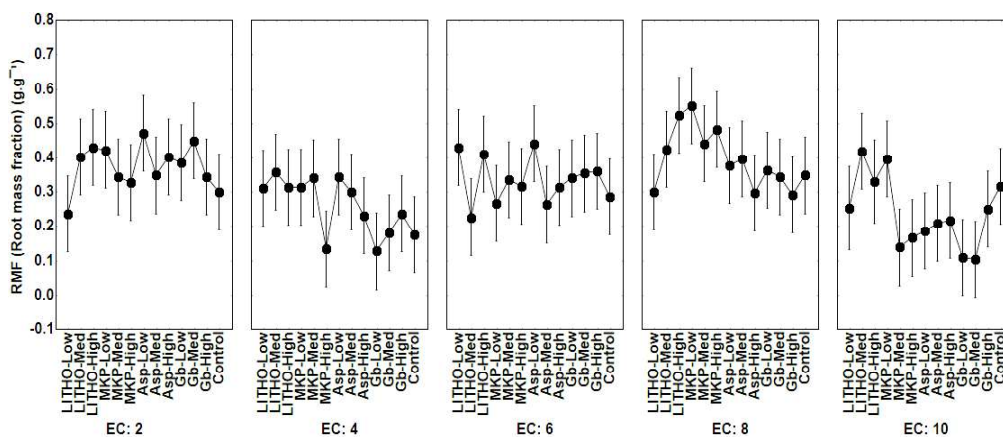


Figure 8. Averages (middle markers) and the 95% limits of confidence ($\pm 2 \times SE$) (vertical bars) of root mass fraction.

Furthermore, although increasing salinity between EC2 and EC10 decreased average leaf area by 142.4 cm², and did not affect total chlorophyll content. LITHOVIT® application induced amelioration in both parameters with all application doses. It also decreased the injury level in cells of plant leaves that was reflected by lower percentages of cell electrolyte leakage in LITHO-Low at EC2, 4, 6 and 8 mS cm⁻¹ (70%, 78.9%, 78.9% and 84.2% compared to 73.3%, 80%, 82.4% and 87.5% in control plants).

Increasing salinity did not reduce plant height and leaf number which contradicted earlier findings (Oztekin & Tuzel, 2011; Parvin et al., 2015). On the contrary, a similar decline in stem diameter under salt stress was observed by Saberi et al. (2011) and could be attributed to the reduction in water potential leading to turgor in expanding tissues (Zhu, 2002). High salinity has negatively affected fruiting and physiological indicators causing yield reductions due to decline in fruit number confirming the results of del Amor et al. (2001). MKP application enhanced plant growth and yield confirming the results of Kaya et al. (2001) and this was due to the presence of K⁺ playing a major role in osmotic adjustment, enzyme activation, cell turgor (Marschner, 1995) and lowering Na⁺ uptake (Plaut et al. 2013). It improved the fertilization mechanism and ovary development causing fruit set (Satti et al., 1994). Lithovit® application has also shown a positive effect on plant height and stem diameter. This ameliorative effect was

observed by Sabina (2013) on *Koeleruteria paniculata* when tested as fertilizer and on tomato yields by Tantawy et al. (2014). In fact, LITHOVIT acts as a long term CO₂ reservoir supplying plants with CO₂ (Kumar, 2011).

Moreover, since Calcium was applied in a micronization form as CaCO₃ and CaO, plants could absorb enough Ca. In addition, Lithovit contains a lot of micronutrients such as Mg, Fe and Mn which contribute to the nutritional status of the plant (Bilal, 2010) and may reflect on its response to salinity compared to control. It is well known that salinity can disturb plant uptake of nutrients (Chavarria & dos Santos, 2012), hence providing these nutrients through foliar application would compensate for this disturbance. Mg is essential for chlorophyll formation and its foliar application may result in higher chlorophyll content hence better plant growth. In fact, LITHOVIT® has enhanced leaf area and chlorophyll content due to its stimulatory impact on photosynthetic rate caused by atmospheric CO₂ amelioration (del Amor, 2013).

Furthermore, the limited positive effect of Aspirin on leaf number and stem diameter could be related to enhanced root biomass, thus the higher water and nutrient absorption. The effect of this product was less studied on tomato salt-stressed plants. However, according to Sun et al. (1994) in stress conditions the positive activity of Acetyl salicylic acid is mainly to maintain the function of cellular membranes and to prevent lethal stress load. Finally, although some early studies have pointed out the efficiency of Glycinebetaine under salt-stress, however it had no evident effect in this experiment. Similar findings were reported by Heuer (2003) where GB was ineffective due to its inhibitory effect on ion accumulation in plant cells.

CONCLUSIONS

In conclusion, under the tested conditions, Aspirin effect was the most significant at rooting stage, while LITHOVIT® was the most beneficial at leaf formation, flowering and fruiting. MKP application improved plant performance at all stages of growth and production. Consequently, different combinations of treatments could be applied at different stages of plant growth under salt stress; mainly Aspirin (50 mg L⁻¹) and MKP (2 g L⁻¹) at early growth stages and MKP (2 to 3 g L⁻¹) and LITHOVIT® (0.75 g L⁻¹) at later growth stages. On the contrary, Glycinebetaine that had no effect under the current experimental conditions could be tested in upcoming studies with different application doses and timing.

ACKNOWLEDGEMENTS. Authors deeply thank the TRIBODyn AG Company for providing the LITHOVIT product and ensuring its shipping to Lebanon.

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