DOI: 10.17707/AgricultForest.65.1.02

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PERFORMANCE OF SALT-STRESSED TOMATO CROP AS AFFECTED BY NANO-CACO$_3$, GLYCINE BETAINE, MKP FERTILIZER AND ASPIRIN APPLICATION

SUMMARY

Salinity problem is a major abiotic stress affecting tomato growth. In Lebanon, the problem is rising in coastal zone and Northern (Baalback-Hermel belt) areas. The current work aimed to study the effect of Monopotassium-phosphate (MKP), Lithovit® (LITHO) (nano-CaCO$_3$), Glycine betaine (GB) and Aspirin (ASP) applied each in three concentrations (Low, Med and High) on tomato (*Solanum lycopersicum* L.) subjected to five salinity levels (EC=2,4,6,8 and 10 dS/m). Control treatments were those subjected to the five salinity levels with no products application. Results showed that increased salt stress reduced fresh weight of aboveground parts and roots while MKP-High improved fresh weight of aboveground parts at EC8 (by 44.6g) and EC10 (32.7g) and ASP-Med improved fresh weight of roots by 18g at EC10 compared to control. Root mass fraction was enhanced by Aspirin applied with all concentrations at EC2 and EC4 and by Lithovit at EC8. Dry matter accumulation in the aboveground parts was only improved by MKP at EC4, 6 and 10 and by Lithovit at EC6 and 8. Leaf area was reduced by 142.4g and cell electrolyte leakage was increased by 17% with increasing salinity. Lithovit enhanced leaf area with Lithovit-Med and total chlorophyll content with all concentrations at all ECs. Finally at EC4 total soluble solids increased following the application of Lithovit, MKP, ASP and GB with the highest concentrations, while Titratable acidity was increased only with GB-low. In conclusion, products' effects varied with EC level and applied dose.

Keywords: tomato, fertilizer, osmo-regulator, salt-tolerance.

INTRODUCTION

Salinity is one of the common factors causing significant reduction in crop yields and affecting plant growth (Hassan *et al*., 2015). It causes disturbance of water balance, closure of leaf stomata and inhibition of cell division (Zhang *et al*., 2016). It also reduces the production of leaf photo-assimilates due to stomatal

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Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

closure and the accumulation of Na^+ and Cl^- in leaves (Romero-Aranda and Syvertsen, 1996). It was reported earlier that salinity negatively affects plant growth parameters like plant height, leaf area and fresh weight as well as chemical contents such as N, P, and K (Tantawy *et al*., 2013). On the contrary, high salinity positively influenced tomato fruit quality (Boamah *et al*., 2011) by increasing sugars content and acidity (Cuartero and Fernàndez-Muñoz, 1999). This is due to the inhibition and prevention of water uptake and transport improving the concentration of soluble solids (Sakamoto *et al*., 1999; Li *et al*., 2001). Del amor *et al*. (2001) found a correlation between the improvement in fruit acidity and the accumulation of Na^+ and Cl^- due to salinity. Attempts are constantly done to find new methods to alleviate the negative impacts of salinity on plants especially that this problem is raisin in many regions of the world and also in Lebanon (Darwish *et al*., 2002).

Recently, nano-fertilizers showed a potential use as a pioneer in solving problems (Froggett, 2009). LITHOVIT® or nano-$CaCO_3$ is a CO_2 foliar fertilizer (Bilal, 2010) increasing CO_2 concentration and stimulating light saturated photosynthesis in C3 plants (Ainsworth and Rogers, 2007). There are a few reviews about the effect of nano-particles on plants (Tantawy *et al*., 2014) and minor reports on its efficiency under salt stress. On the other hand, the use of fertilizers rich in phosphorus and potassium was noted as beneficial in mitigating salinity effect on crops (Afzal *et al*., 2015) due to their contribution in ion homeostasis and osmotic balance (Perkins-Veazie and Robert, 2003). Acetylsalicylic acid or Aspirin which was previously stated to increase leaf water potential, membrane stability and soluble compounds (Agamy *et al*., 2013) could enhance tomato tolerance to salinity. Finally, the positive role of glycinebetaine (GB) against salinity which was reported on various crops, while on tomato salt-stressed plants its role is still leading to confusion due to contradictory reports upon this subject. GB being an osmolyte accumulated naturally in plants in stressful conditions, but not in tomato. It has a role in protecting photosynthetic apparatus from abiotic stress (Chaum and Kirdmanee, 2010) and in maintaining osmotic balance (McCue and Hanson, 1992).

Therefore, the current study aimed to find the optimal solution to improve physiological responses of tomato plant to salinity together with the preservation of ameliorative effect of this abiotic stress on fruit quality. This was done through the application of LITHOVIT®, MKP, Aspirin and GB in various concentrations on salt-stressed tomato plants.

MATERIAL AND METHODS

Treatments

Tomato seedlings (determinate Var. Sila) of 3-4 leaves were transplanted in pots containing washed sandy clay soil during May. The date of transplantation was referred as initiation date for all practices. After transplantation, irrigation with sweet water was carried out till 14 DAT. LITHOVIT® (LITHO), Monopotassium-phosphate (MKP) (0-52-34), Aspirin

(ASP) and Glycinebetaine (GB) products were applied in 3 different concentrations: Low, Medium and High with respectively 0.5 g/L; 0.75 g/L and 1 g/L for LITHO, 2 g/L, 3 g/L and 3.5 g/L for MKP, 4.5 g/L, 6 g/L and 7.5 g/L for GB and 50 mg/L, 75 mg/L and 100 mg/L for ASP. Each treatment was applied 3 times starting at 15 DAT with an interval of 15 days between consecutive applications. LITHO and ASP were applied by foliar spray, MKP through fertigation and GB by both methods.

All products were dissolved in distilled water except for ASP (tablets of 100mg) that was mixed at high temperature with ethanol. Salinity was induced by saline irrigation which started at 19 DAT using different solution's ECs according to the corresponding treatment: 2, 4, 6, 8 and 10 dS/m. Saline irrigation was done continuously with a frequency of 3 days and a dose of 1 L per plant. Control consisted of tomato plants irrigated by all ECs, however not treated by the various products.

Physiological indicators

Six plants of each treatment were selected for measuring their fresh (aboveground and root parts) and dry weights. Fresh weight was measured first and dry weight was then assessed after oven-drying at 100^{o} C until constant weight. Consequently dry matter content was measured based on fresh and dry weights of plants parts. Root mass fraction and were measured based on dry weights of plant parts following the method of Poorter *et al*. (2012). Three tomato plants were selected from each treatment for measuring leaf area on their total number of leaves.

Cell electrolyte leakage was measured as described by Mumtaz Khan *et al*. (2013). Chlorophyll content test was performed as follows: 0.1 g of calcium carbonate was added to 1g of fresh leaves. The mix was macerated in 50 mL of acetone (80 %). The liquid phase was then transferred into small beakers and the remaining solution was macerated once more in acetone (80%) until full discoloration of leaves. The solution was subjected to centrifugation at 3000 rpm for 5 minutes. The absorbance was red on a spectrophotometer at the wavelengths: 663 nm and 645 nm. Finally, total chlorophyll was determined in µg/g (mg/L) according to Porra (2002).

Fruit quality

Total Soluble Solids (TSS) content was evaluated by Euromex RF (360) refractometer (Tigchelaar, 1986). Titratable acidity (TTA) in fruits was measured by titration of tomato juice (6g of tomato juice in 50 mL of distilled water) with 0.1M NaOH to pH=8.1 (Rangana, 1979).

Statistical analysis

Data was subjected to analysis of variance which consisted on means ±SE compared by Fisher's least-significant differences test (LSD) using STATISTICA 10 program.

RESULTS AND DISCUSSION

Physiological parameters

In general, from the probabilities associated with Fisher statistics for the different effects (Table 1), it was found that the separate effects of both EC and Treatments (product application) were statistically (P_{value}<0.05) significant on all parameters except for the non-interactive effect of EC on fresh weight of aboveground parts and dry matter of roots. Finally, the combined (interactive) effects of EC x Treatment was not statistically (P_{value}>0.05) significant on all parameters.

Table 1: ANOVA null hypothesis rejection probability for the effects of the experimental factors and their interactions on the different measurements averages

	F.W.A.P (g)	F.W.R (g)	D.M.A.P (%)	D.M.R (%)	RMF ($g.g^{-1}$)
EC	0.070	0.000	0.031	0.196	0.000
Treatment	0.000	0.000	0.000	0.000	0.000
EC*Treatment	0.177	0.091	0.734	0.089	0.317

F.W.A.P: Fresh Weight of Aboveground parts; F.W.R: Fresh Weight of Roots; D.M.A.P: Dry Matter of Aboveground parts; D.M.R: Dry Matter of Roots.

Increasing in salinity level (from EC2 to EC10) has significantly reduced fresh weight of aboveground parts (Figure 1a) by 41 g (77 g at EC2 compared to 36 g at EC10 in control plants). However, the application of MPK-High improved this parameter compared to control at all EC levels; with a significant difference at EC4 (by 63.2 g) and EC8 (44.6 g) and a slight difference at EC2 (16 g), EC6 (18.2 g) and EC10 (32.7 g). In addition, at EC4, MKP application (MKP-Low, MKP-Med and MKP-High) has enhanced fresh weight of roots (Figure 1b) by 38 % while at EC10, Asp-Med application has significantly enhanced it by 69 %, and at EC6, MKP-Low and ASP-High application has slightly increased this trait compared to control (respectively 26.1 g and 26.7 g compared to 16.3 g).

Root mass fraction (Figure 1c) was slightly improved by Asp-Med at EC2 and EC4 (0.43 $g.g^{-1}$ and 0.37 $g.g^{-1}$ compared to 0.25 $g.g^{-1}$ and 0.22 $g.g^{-1}$ in control at EC2 and EC4 respectively) and by Lithovit® at EC8 with all the applied concentrations (0.44 $g.g^{-1}$, 0.38 $g.g^{-1}$ and 0.46 $g.g^{-1}$ respectively at Litho Low, Med and High compared to 0.35 $g.g^{-1}$ in control). Concerning dry matter accumulation in plants (Figure 1d), there was no significant difference in the percentage of dry matter accumulated in aboveground plant parts when comparing between the various treatments and control at all EC levels with the exception of MKP-Med at EC8 (16.9 % compared to 9 % in control). On the contrary, dry matter in roots was affected variously by different treatments; it increased significantly compared to control at EC2 and EC6 with MKP-Med (by 15.3 % and 15.7 % respectively), at EC4 and EC10 with MKP-High (by 32.3 % and 11% respectively) and at EC8 with Litho-Low (by 19.9 %).

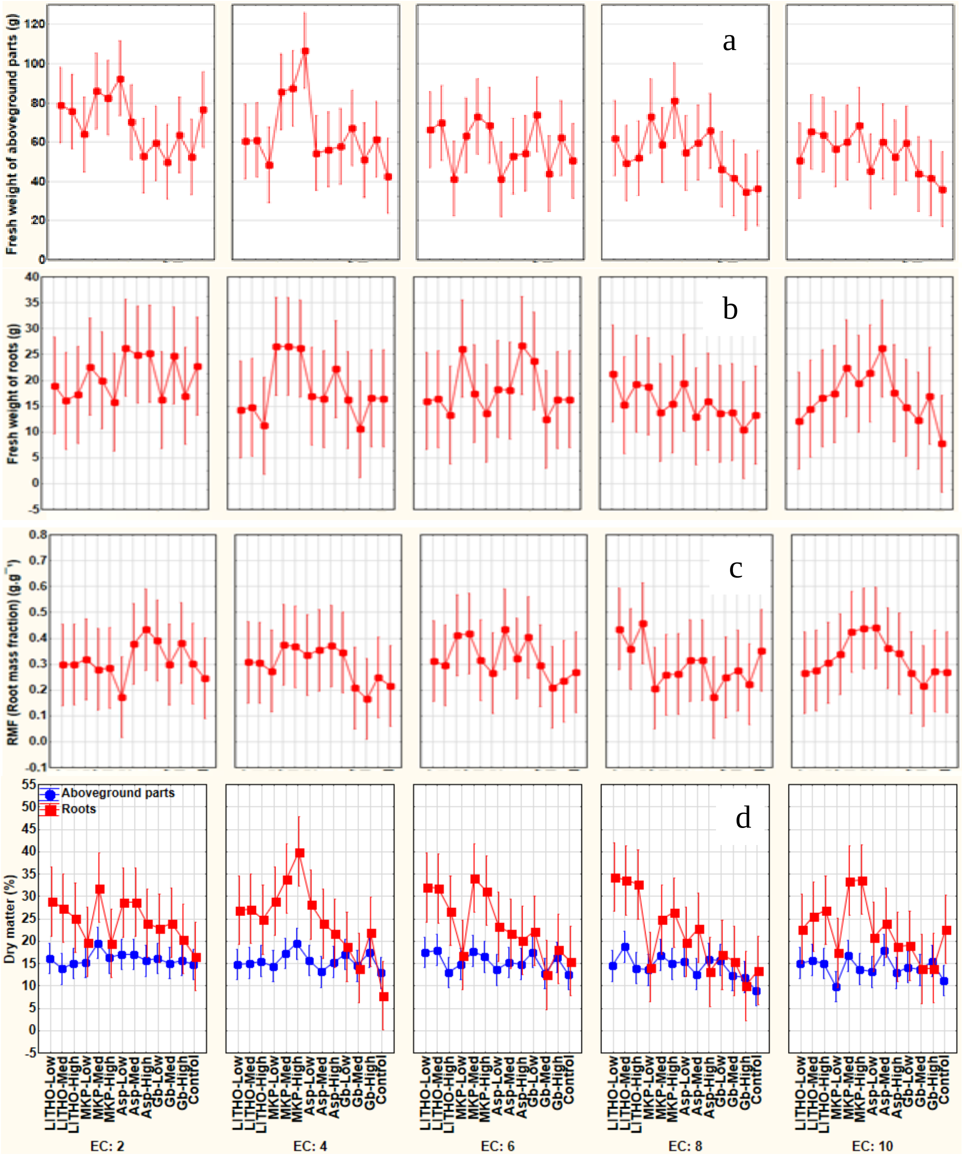

Figure 1: Averages (middle markers) and the 95% limits of confidence (±2 x standard Error: SE) (vertical bars) of various tested parameters

Leaf area was reduced by 142.4 g and cell electrolyte leakage was increased by 17% with increasing salinity level from 2 to 10 dS/m. However, Lithovit-Med enhanced leaf area at EC2 (by 50%), EC4 (46%), EC6 (44%), EC8 (65%) and EC10 (68%) compared to control. Lithovit was also beneficial with the 3 applied doses on total chlorophyll content at all ECs with the best improvement obtained with Litho-High compared to control (by 29%, 51%, 41%, 39% and 26% respectively at EC2, 4, 6, 8 and 10).

Fruit quality

Total soluble solids increased at EC4 following the application of Lithovit, MKP, ASP and GB with high concentrations by 10%, 6%, 5% and 16% and Titratable acidity was increased by 15% following GB low application compared to control.

The reduction in fresh weight of plant parts (aboveground parts and roots) caused by salinity could be attributed to its inhibitory effect on cell expansion and division as well as stomatal closure (Flowers, 2004) which mitigates the ion flux to the shoot (Hasegawa *et al.*, 2000). According to Läuchli and Epstein (1990), under salinity stress, the reduction in shoot growth is related to leaf area decline and stunted shoots resulting in an inhibition in photosynthetic activity, reduction in energy production and protein synthesis other physiological changes (Cramer and Nowak, 1992). In fact, ion imbalances caused by salinity prevent K^+ and Ca^{2+} uptake thus reducing root cell growth and root tips expansion (Larcher, 1980). The inhibition in tomato growth has been also reported as one of the most reliable indicators under salt-stress (Cruz *et al.*, 1990); significant reductions in fresh weight of tomato shoots were observed earlier (Bolarin *et al*., 1993).

Therefore, the beneficial effect of monopotassium phosphate application was due to the presence of both potassium and phosphorus elements. In fact, improving the potassium nutritional status and phosphorus content might have minimized the oxidative cell damage. This was possible by reducing both ROS (reactive oxygen species) and NADPH oxidase formation (Shin and Schachtman, 2004) that were previously stimulated by increasing salt-stress. On other solanaceous crops several studies stated the positive effect of K in mitigating salinity (Kaya and Higgs, 2003; Rubio *et al.*, 2009, Sajyan *et al*., 2018). This was translated in the current study by an improvement in fresh weight of plant parts and in dry matter of roots especially at EC4. In addition, LITHOVIT® application improved dry matter percentage, chlorophyll content and leaf area in roots compared to control especially at EC8,. Actually, LITHOVIT® is rich in Ca in a micronized form ($CaCO_3$), CO_2 and Mg (Bilal, 2010) which counteracted the negative impacts of salinity especially on leaf area and total chlorophyll content. Its application improved the atmospheric CO_2 (del Amor, 2013) and Mg an essential element for chlorophyll formation (Bilal, 2010) which could explain the improvement in photosynthetic activity. Furthermore, improvement of root mass fraction by Aspirin application at EC2, 4 and 10 and total soluble solids at EC4 could be related to the product role in maintaining cellular membrane function by preventing lethal stress load (Sun *et al.*, 1994) and by enhancing the activity of antioxidant enzymes (He *et al*., 2002). Finally, Glycine betaine was the least effective among all products and did not improved salt-tolerance of tomato crop which confirmed the findings of Heuer (2003) who has attributed the non-effect of GB to its inhibitory effect on ion accumulation in plant cells. It seemed that the applied concentrations (4.5, 6 and 7.5 g/L) were too high and glycine betaine should be applied in lower rates.

CONCLUSIONS

Under salinity stress, LITHOVIT® and MKP were more beneficial more than Aspirin and GB products. It seemed that improving ion uptake (K, P, Ca, Mg and others) have better reduced the salinity-caused effects compared to the use of an osmoprotectant (GB) or aspirin (acetyl salicylic acid).

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