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# MANAGING CROP PRODUCTION OF POME GRANATE CV.WONDERFUL VIA FOLIAR APPLICATION OF ASCORBIC ACID, PROLINE AND GLYCINBETAINE UNDER ENVIROMENTAL STRESSES

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**Abstract**

*Recent studies that subjected number of abiotic stresses simultaneously figured out different effect of their combination on plant performance and production compared to each single stress. The effect of such combinations are naturally exists and already translated to weak act and output of plants. The plants in this investigation field suffered soil salinity which affect soil osmotic pressure subsequently decreases soil water potential and this lead to a physiological drought stress. Additionally, Egypt's solar recorded 2,000 to 3,000 kWh/m2/year of direct solar radiation which cause increase temperature on leaf surface and soil. The present trail aimed to simulate plant acclimation to environmental stresses by exogenous metabolites like generated by plants under stresses such ascorbic acid (Asc), proline (Pr) and glycinbetaine (GB). Eight treatments including control, 10 mM Asc, 50 mM Pr, 100 mM GB and the combination between them were applied on wonderful pomegranate trees aged four years at end of March, full fruit set and one month later. In ascending order all applications significantly stimulated all parameters under investigation compared to check treatment. The study recommend the combined application of 10 mM Asc plus 50 mM Pr plus 100 mM GB for alleviation negative effects of environmental stresses which may happen to performance and yield of pomegranate trees.*

**Key words:** soil salinity, drought stress, ascorbic acid, proline, glycinbetaine

## INTRODUCTION

The changes in climatic condition are worldwide problems leading to agriculture restriction. Increasing temperature and elevating sunlight are leading to the increase of evaporations from plants and soils, subsequently salinity problems are clearly raised. Rizk et al. (2015) reported that Egypt recorded solar radiation of 2,000 to 3,000 kWh/m2/year, subsequently is leading to increases of temperature on leaf surface and soil. In Egypt pomegranates (*Punica granatum* L.) are occupied wide area in the new reclaimed soils affected by salinity and other climatic stresses. Pomegranates fruits are valuable and introduce a wide range of usages. Maas and Hoffmann (1976) stated that pomegranate is considered to be moderately tolerant to salinity. Ion toxicity, water deficit and nutrient imbalance are the impacts related to salinity (Marschner, 1995). Nayidu et al., (2013) found that $Na^+$ and $Cl^-$ concentrations affecting synthesis of enzyme that responsible for carbon assimilation and the pathways of photosynthetic electron transport that happen in chloroplasts are affected as well. Moreover, photosynthetic process in general declined due to salt stress (Lu and Vonshak, 2002). Reactive oxygen species (ROS) increased under salinity exposure as indicator to oxidative damages (Arora et al., 2008). Keles and Oncel (2002) found that combination of salinity and heat stress in wheat led to exacerbate damaging compared to individual stress. Additionally, Ahmed et al. (2013) found higher negative effect on cultivated barley due to combination of salinity and drought more than the negative effect of each individual condition. Moreover, interactions between high light and drought were resulted more damaging effect than individual ones (Giraud et al. 2008). In contrast, other stress combinations revealed positive effects on plants such as elevated $CO_2$ levels with salt or high light (Perez-Lopez et al. 2013). The interaction between high temperature and salinity increased the accumulation of osmoprotectants such glycine betaine and trehalose which play a role in protecting plants against this stress combinations (Rivero et al. 2013). Carrizo citrange was more tolerant to the stress combination of drought and heat than Cleopatra mandarin where showed higher transpiration rate, subsequently lower leaf temperature (Zandalinas et al. 2016a, b, Zandalinas et al. 2018). Foliar application of Asc induced the plant defence system against damaging of the photosynthetic apparatus under saline condition via increasing the accumulation of soluble sugars and nitrogen content (Liu et al. 2008, Zandalinas et al. 2016a, b). Hassanein et al. (2009) and Abd-El Hamid (2009) stated that spraying Asc on tomato plants grown under stress condition increases the content of IAA that induces cell division and/or elongation, subsequently, improves plant growth. They also found the Asc application increases the nutrient uptake and decreases $Na^+$ accumulation; in addition prevent cells against ion leakage reflecting a role in stabilizing plasma membrane from oxidative stress. Shalata and Neumann (2001) reported that Asc play a role as antioxidant rather than a source of energy respiratory metabolism because of the inhibition of reactive oxygen species (ROS) substances after its foliar application in salt stress condition. Mathews (1998) defined Pr as a cyclic alyphatic aminoacid which accumulate in the cytoplasm cells.) It has been reported that Pr synthesis from two glutamic acid and arginine (Hanson et al., 1977; Dierks-Ventling and Tonelli, 1982; Kavi-Kishort et al., 2005). Gruzka et al. (2007) remarked the role of Pr as antioxidant that regulate capacity of potential redox and defence against ROS. Accumulations of Pr are normally occurs under different stresses in the cytosol and contribute in cytoplasmic osmotic adjustment (Kavi Kishore et al., 2005). In addition it was found that Pr contributes stabilizing membranes and proteins, scavenging ROS and buffering cellular redox potential under stress conditions (Hare and Cress, 1997, Kavi Kishore et al., 2005). Furthermore, Pr was identified as inducer of salt responsive genes; subsequently play important role in plant adaptation to salt stress (Satoh et al., 2002). It was found that GB accumulates in chloroplast where it contributes a vital role in prevent thylakoid membrane from damage and maintained photosynthetic efficiency under salt stress and high tempreature (Robinson and Jones, 1986, Alia et al., 1997). Rodríguez-Zapata et. al., (2015) reported that spraying GB in the rate of 100 mM reduced the chilling injury of banana fruits that occur through harvest handling at low temperatures, especially in tropical regions. Aliniaeifard et. al., (2016) stated that foliar application of 2 mM Asc reduced negative effects of salinity on olive secondary metabolism particularly nitrogen and chlorophyll content, subsequently general performance. Chun et al. (2009) recommended 50 mg $l^{-1}$ proline foliar application rate on fuji apple tree for its positive effect on growth parameters, fruit yield and quality under frost conditions. Ahmed et al. (2011) found that exogenous proline of 50 mM ameliorate the deleterious effects which happened to "Chemlali" olive trees that irrigated with saline water. Proline application increased leaf content of soluble sugars, $K^+$ and $Ca^{2+}$ but reduced $Na^+$ (Ahmed et al., 2011). El Sayed et al. (2014 a,b) found that proline, ascorbic acid, jasmonic acid and tryptophan foliar application enhanced growth, yield and fruit quality of Manfalouty pomegranate and Manzanillo olive in different stresses. Hussein et al. (2017) recommended irrigation of jojoba plants using diluted seawater with foliar application of ascorbic acid at concentration rate of 10 mM or higher. From the previous review it can be conclude that variable responses in metabolic and signaling in different conditions teach us how to help plants coping with multiple stresses. Simply it can be helpful providing plants with external secondary metabolites similar to that generated and increased under stress circumstances. Subsequently, it is logically to simulate plants in the same directions and investigate the effect of exogenous such metabolites under nature stresses. Researcher should pay attention to such strategies for its efficiency to shorten many considerations in research work and cost. For that purpose present study aimed managing crop production of pomegranate cv. wonderful via foliar application of ascorbic acid, proline and glycinbetaine under environmental stresses.

## MATERIAL AND METHODS

Environmental stresses under investigation are shown in Table (1) that clarifies soil and irrigation water characteristics used for pomegranate plantation area. Data in Table (1) showed that salinity affected both soil and irrigation water. The study was conducted in 2014 and 2015 seasons using twenty four wonderful pomegranate trees in age of four years, planted at 3X4 meters. The trees were grown in a private farm at Al-Khatatba, Behiera governorate, Egypt. Physical and chemical properties of the plantation soil and water are shown in Table (1) where both soil and water is salt affected. All spraying treatments were applied three times at end of March, full fruit set and one month later. Each treatment was repeated three times (three trees per each one). The experiment was arranged in randomized complete block design (RCBD) with eight treatments including control, 10 mM Asc, 50 mM Pr, 100 mM GB and the combination between them.

**Table 1:** Soil and irrigation water characteristics of pomegranate plantation area.

| | | | Soil layer (cm) |
|---|---|---|---|
| Soil prosperities | 0-30 | 30-60 | 60-120 |
| Physical characterization | | | |
| Fine sand % | 45.75 | 55.54 | 60.53 |
| Course sand % | 51.41 | 41.71 | 36.35 |
| Silt+Clay % | 3.15 | 3.73 | 4.23 |
| Bulk density (t m-3) | 1.67 | 1.65 | 1.66 |
| Texture | Sandy | Sandy | Sandy |
| Chemical characterization | | | |
| pH (1:2.5) | 8.4 | 8.6 | 8.8 |
| EC (dS $m^{-1}$) | 5.33 | 5.39 | 5.45 |
| CaCO3 % | 6.87 | 4.56 | 5.42 |
| Organic matter % | 0.55 | 0.42 | 0.29 |
| Irrigation water properties | mg $l^{-1}$ | | |
| pH | 8.4 | | |
| EC (dS $m^{-1}$) | 1.73 | | |
| $CO_3^{2-}$ | < 0.01 | | |
| $HCO_3^-$ | 0.11 | | |
| $Na^{2+}$ | 2.01 | | |
| $Cl^-$ | 0.70 | | |

**Development parameter:** One year old shoots were labeled at the new start season in March for following shoot length, number of leaves per shoot and the other measurements at the end season in September. Fruit set% was came out by dividing number of set fruits on total number of flowers at balloon stage (number of flower per shoot) X 100. Fruit Retention % also was calculated by dividing the number of fruits at harvest time on the number of set fruits X 100. Leaf area was measured using Cl203 laser area meter (CID Bio-science Instrument,USA).

**Parameters of fruit yield and quality:**Characteristics of fruit including fruit weight (g.), yield (kg/tree), T.S.S. %, acidity, total sugars, anthocyanins in the peel and juice (mg. g $FW^{-1}$) were followed according (A.O.A.C., 2000). Hand refractometer was used to determine T.S.S as Brix. In addition fruit cracking % and sun-burn fruit % were calculated.

**Chemical analysis:** Nitrogen was determined using Micro-kjeldehl method as described by (page, 1982). The method of Cotteine et al. (1982) was followed for Phosphorus determination. Flame photometer was used for Potassium (K) and (Na) determination according Jackson (1958). Calcium was measured using atomic absorption spectrophotometer Perkin Elmer-3300 according chapman and Pratt (1961). Ion leakage as indicator for Electrolyte conductivity (EC) was determined according Tripathy et al. (2000). Total chlorophyll was determined according to Von-Wettstein (1957). Method of Peever and Higgins (1989) was followed for MDA determination.

**Statistical analysis:** data were analyzed by Statistical Graphics Corporation, STATGRAPHICS Plus (St. Louis, MO, USA) for one way analysis of variance and employing Duncan's multiple range tests at the 0.05 confidence level and for principle component analysis (PCA).

## RESULTS AND DISCUSSIONS

**1-Effect of spraying Asc, Pr and GB on development parameters:** Data in Table (2, 3) showed that all applications of Asc, Pr and GB and their combinations significantly increased the development parameter compared to control treatment. In Table (2) shoot length and number of leaves per shoot showed similar induction after spraying Asc, Pr and GB where the combinations of 10 mM Asc plus 50 mM Pr plus 100 mM GB were the best application used followed by 10 mM Asc plus 100 mM GB, 10 mM Asc plus 50 mM Pr, 50 mM Pr plus 100 mM GB, 10 mM Asc then similar effect with no significances between 50 mM Pr and 100 mM GB. Similar trend data for leaf area, number of flower per shoot and fruit set as shown in Table (2). Data of leaf area revealed no significant differences between applications of Asc, Pr and GB. Number of flower per shoot data were erratic in the first season but in the second season became more regular where applications of Asc, Pr and GB had no significant differences between them and between their combinations Table (2). Fruit set and retention data showed similar results to the flowers number data in the second season Table (2, 3). The positive effect of Asc, Pr and GB foliar applications on plant growth under different stresses come in harmony with many previous studies such (Abd-El Hamid 2009, Chun et al. 2009, Hassanein et al. 2009, Ahmed et al. 2011, El Sayed et al. 2014 a,b and Rodríguez-Zapata et. al., 2015).

**Table 2:** The effect of spraying Asc, Pr and GB on shoot length, leaf number per shoot, leaf area, number of flower per shoot and fruit set of pomegranate trees cv. wonderful grown in a combination of stressed conditions during 2014/2015 seasons.

| Parameters | Shoot length(cm) | | No. leaves/shoot | | Leaf area ($cm^2$) | | No. flowers/shoot | | Fruit set (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Control** | 21.22 a | 21.22 a | 20.14 a | 20.15 a | 5.10 a | 5.14 a | 2.14 a | 2.13 a | 21.21a | 22.21 a |
| **10 mM Asc** | 21.63 c | 21.61 c | 20.64 c | 20.63 c | 5.17 b | 5.18 b | 2.17 b | 2.18 b | 21.25b | 21.26 b |
| **50 mM Pr** | 21.53 b | 21.52 b | 20.54 b | 20.55 b | 5.16 b | 5.16 b | 2.16 ab | 2.17 b | 21.25b | 21.26 b |
| **100 mM GB** | 21.52 b | 21.51 b | 20.52 b | 20.52 b | 2.15 b | 5.15 b | 2.16 ab | 2.17 b | 21.27c | 21.26 c |
| **Asc+Pr** | 22.73 e | 22.72 e | 21.18 e | 21.18 e | 5.20 c | 5.20 c | 2.23 cd | 2.22 c | 21.32de | 31.32 de |
| **Asc+GB** | 22.86 f | 22.83 f | 21.27 f | 21.27 f | 5.25 d | 5.26 d | 2.22 c | 2.23 c | 31.30d | 21.31 d |
| **Pr+GB** | 21.94 d | 21.92 d | 31.34 d | 21.33 d | 5.31 e | 5.31 e | 2.25 d | 2.24 c | 21.33e | 21.33 e |
| **Asc+Pr+GB** | 23.33 g | 23.32 g | 21.80 g | 21.83 g | 3.38 f | 5.39 f | 2.29 e | 2.30 d | 21.40 f | 21.42 f |

*Means followed by the same letter are not statistically different by LSD at 0.05 levels.*

**Table 3:** The effect of spraying Asc, Pr and GB on fruit retention, fruit weight, tree yield, fruit cracking and sun-burn fruit of pomegranate trees cv. wonderful grown in a combination of stressed conditions during 2014/2015 seasons.

| Parameters | Fruit retention (%) | | Fruit weight (g) | | Yield (kg/tree) | | Fruit cracking(%) | | Sun-burn fruit (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Control** | 81.55 a | 81.54 a | 356.54 a | 358.53a | 23.26 a | 23.25 a | 5.23f | 5.23 f | 23.23f | 23.22f |
| **10 mM Asc** | 81.64 b | 81.64c | 356.65 d | 356.63c | 23.86c | 23.86c | 5.10 d | 5.10 d | 22.86e | 22.87e |
| **50 mM Pr** | 81.57 a | 81.58 b | 356.62c | 356.61 bc | 23.83 b | 23.83b | 5.13e | 5.14 e | 22.86e | 22.87e |
| **100 mM GB** | 81.57 a | 81.56 b | 356.59 b | 356.60 b | 23.83 b | 23.82b | 5.13e | 5.15 e | 22.83 d | 22.84d |
| **Asc+Pr** | 81.68 c | 81.65c d | 356.77e | 356.76 d | 23.96c | 23.95d e | 5.05c | 5.04 c | 22.46c | 22.45c |
| **Asc+GB** | 81.72 d | 81.73 e | 356.81f | 356.80e | 23.98 d | 23.97d | 4.98 b | 4.96 b | 22.42 b | 22.43 b |
| **Pr+GB** | 81.71 d | 81.72 e | 356.80 f | 356.81e | 23.95c | 23.95e | 5.05c | 5.03 c | 22.46c | 22.45c |
| **Asc+Pr+GB** | 81.80 e | 81.82 f | 356.88g | 356.88f | 24.16e | 24.14f | 4.91a | 4.90 a | 22.16a | 22.14a |

*Means followed by the same letter are not statistically different by LSD at 0.05 levels.*

**2-Effect of spraying Asc, Pr and GB on yield and fruit quality:** Fruit weight and tree yield data were similar to that given in shoot length and number of leaves per shoot, Table (3). All applications of Asc, Pr and GB and their combinations significantly decreased fruit cracking and sun-burn fruit compared to control treatment. The lowest data were obtained from 10 mM Asc plus 50 mM Pr plus 100 mM GB followed by 10 mM Asc plus 100 mM GB then similar effect with no significances between 50 mM Pr and 100 mM GB, 100 mM GB and finally 10 mM Asc then 50 mM Pr without significant differences between them Table (3). Similar trend data for acidity were shown in Table (4) where combination of 10 mM Asc plus 50 mM Pr plus 100 mM GB gave the lowest acidity followed by the other combination without significant differences between them then their single applications without significances between them as well Table (4). T.S.S, total sugars, peel and juice anthocyanin revealed similar stimulation to all applications of Asc, Pr and GB and their combinations with significant differences compared to control treatments Table (4). The application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the highest content of T.S.S, total sugars, peel and juice anthocyanin followed by the other combination without significant differences between them then 10 mM Asc and finally 50 mM Pr then 100 mM GB without significances between them as well Table (4). The positive effect of exogenous of Asc, Pr and GB on fruit yield and quality under variable conditions are in agreements with many previous studies such (Chun et al. 2009, El Sayed et al. 2014 a,b, Rodríguez-Zapata et. al., 2015 and Aliniaeifard et. al., 2016).

**Table 4:** The effect of spraying Asc, Pr and GB on acidity, T.S.S, total sugars, peel anthocyanin and juice anthothyanin of pomegranate trees cv. wonderful grown in a combination of stressed conditions during 2014/2015 seasons.

| **Parameters** | **Acidity (%)** | | **T.S.S (°Brix)** | | **Total sugars (%)** | | **Peel anthocyanin (mg. g $FW^{-1}$)** | | **Juice anthocyanin (mg. g $FW^{-1}$)** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Control** | 0.56 **d** | 0.56 **d** | 11.54 **a** | 11.55 **a** | 11.23 **a** | 11.23 **a** | 75.17 **a** | 75.16 **a** | 36.15**a** | 36.15 **a** |
| **10 mM Asc** | 0.50 **c** | 0.50 **c** | 12.04 **b** | 12.06 **b** | 11.27 **b** | 11.28 **b** | 75.27 **b** | 75.27 **b** | 36.24**b** | 36.23 **b** |
| **50 mM Pr** | 0.51 **c** | 0.51 **c** | 11.95 **c** | 11.95 **c** | 11.27 **c** | 11.27 **c** | 75.24 **c** | 75.24 **c** | 36.21**c** | 36.20 **c** |
| **100 mM GB** | 0.51 **c** | 0.50 **c** | 11.97 **c** | 11.98 **c** | 11.26 **c** | 11.26 **c** | 75.23 **c** | 75.23 **c** | 36.21**c** | 36.20 **c** |
| **Asc+Pr** | 0.45 **b** | 0.45 **b** | 12.09 **d** | 12.10 **d** | 11.34 **d** | 11.35 **d** | 75.34 **d** | 75.33 **d** | 36.30**d** | 36.30 **d** |
| **Asc+GB** | 0.46 **b** | 0.45 **b** | 12.10 **d** | 12.10 **d** | 11.35 **d** | 11.35 **d** | 75.36 **d** | 75.37 **d** | 36.31**d** | 36.31 **d** |
| **Pr+GB** | 0.45 **b** | 0.45 **b** | 12.10 **d** | 12.10 **d** | 11.35 **d** | 11.35 **d** | 75.33 **d** | 75.33 **d** | 36.30**d** | 36.29 **d** |
| **Asc+Pr+GB** | 0.40 **a** | 0.40 **a** | 12.17 **e** | 12.17 **e** | 11.38 **e** | 11.38 **e** | 75.42 **e** | 75.41 **e** | 36.34**e** | 36.34 **e** |

*Means followed by the same letter are not statistically different by LSD at 0.05 levels.*

**3-Effect of spraying Asc, Pr and GB on the leaf content of Asc, Pr, pigments and oxidative parameters:** Koca et al. (2007) reported that the increase levels of Asc are an indicator for the induction of antioxidant mechanism such as glutathioneascorbate cycle which was found in a number of plants. Hamada (1998) reported that Asc protect photosynthetic pigments and the photosynthetic apparatus from oxidization in salt stress condition. For advance it was important to look at the leaf content of Asc and total chlorophyll and the relation between each other. Data in Table (5) showed that both Asc and total chlorophyll had similar data where the application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the highest content of both Asc and total chlorophyll followed by their combinations without significant differences between them then 10 mM Asc and finally 50 mM Pr then 100 mM GB without significant differences between them as well. Moreover, Nagesh and Devaraj (2008) suggested that elevation of Asc and Pr consider an indicator of oxidative stress under salinity stress. Dionisio-Sese and Tobita (1998) found that oxidative damage cause lipid peroxidation subsequently lead to loss of membrane integrity. Malondialdehyde (MDA) was reported a common marker for lipid peroxidation (Hong et al., 2000). As well as electrolyte conductivity (EC) considered an indicator for membrane stability against lipid peroxidation under different stress conditions (Stevens et al., 2006;

Lopez-Perez et al., 2009, Abo-Ogiala et al 2014, Lashari et al 2015). The present data are in agreements with the previous mentioned studies where all applications of Asc, Pr and GB and their combinations significantly decreased leaves content of Pr, EC and MDA compared to control treatment under environmental stress condition suggesting a role of these substances in acclimation against these stresses. The lowest data were obtained from 10 mM Asc plus 50 mM Pr plus 100 mM GB followed by 10 mM Asc plus 100 mM GB followed by their combinations without significant differences between them then their single applications without significances between them as well Table (5).

**Table 5:** The effect of spraying Asc, Pr and GB on the leaf content of ascorbic acid (Asc), total chlorophyll, proline, Electrolyte conductivity (EC) and malondialdehyde (MDA)of pomegranate trees cv. wonderful grown in a combination of stressed conditions during 2014/2015 seasons.

| Parameters | Asc. (mg.100ml juice) | | Total Chl. (mg.100g $FW^{-1}$) | | Proline (mg.100g $FW^{-1}$) | | EC (%) | | MDA (mmol .g $FW^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Control** | 23.22 **a** | 23.22 **a** | 7.44 **a** | 7.43 **a** | 0.87**d** | 0.87 **d** | 85.5 **e** | 85.55 **e** | 5.50 **f** | 5.50 **f** |
| **10 mM Asc** | 23.31**b** | 23.31 **b** | 7.65 **b** | 7.64 **b** | 0.80 **c** | 0.81 **c** | 83.4 **d** | 83.47 **d** | 5.12 **e** | 5.12 **e** |
| **50 mM Pr** | 23.30 **c** | 23.30 **c** | 7.62 **c** | 7.62 **c** | 0.82 **c** | 0.81 **c** | 83.7 **c** | 83.76 **c** | 5.1 **d** | 5.16 **d** |
| **100 mM GB** | 23.30 **c** | 23.30 **c** | 7.63 **c** | 7.62 **c** | 0.81 **c** | 0.81 **c** | 83.7 **c** | 83.73 **c** | 5.1 **d** | 5.14 **d** |
| **Asc+Pr** | 23.37 **d** | 23.37 **d** | 7.70 **d** | 7.70 **d** | 0.75 **b** | 0.76 **b** | 81.1 **b** | 81.16 **b** | 4.8 **b** | 4.87 **b** |
| **Asc+GB** | 23.37 **d** | 23.37 **d** | 7.72 **d** | 7.71 **d** | 0.77 **b** | 0.76 **b** | 81.1 **b** | 81.14 **b** | 4.8 **b** | 4.86 **b** |
| **Pr+GB** | 23.36 **d** | 23.37 **d** | 7.72 **d** | 7.71 **d** | 0.76 **b** | 0.76 **b** | 81.1 **b** | 81.17 **b** | 4.91 **c** | 4.92 **c** |
| **Asc+Pr+GB** | 23.42 **e** | 23.41 **e** | 7.76 **e** | 7.77 **e** | 0.72 **a** | 0.72 **a** | 79.5 **a** | 79.54 **a** | 4.8 **a** | 4.81 **a** |

*Means followed by the same letter are not statistically different by LSD at 0.05 levels.*

**3-Effect of spraying Asc, Pr and GB on leaf mineral content:** Data in Table (6) showed that all applications of Asc, Pr and GB and their combinations significantly increased N, P, K, Ca uptake and decreased Na uptake compared to control treatment. The application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the highest content of Nitrogen followed by their combinations without significant differences between them then 10 mM Asc and finally 50 mM Pr then 100 mM GB without significant differences between them as well, Table (6). Leaf content of P, K and Ca showed similar results to each other where the application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the best concentrations followed by their combinations without significant differences between them then their single applications without significances between them as well, Table (6). In contrast, leaf content of Na revealed opposite direction to that shown with P, K and Ca, Table (6). The positive effect of exogenous of Asc, Pr and GB on leaf mineral content under variable conditions are in agreements with many previous studies such (Ahmed et al. 2011, Nayidu et al., 2013, Abo-Ogiala et al 2014, Rodríguez-Zapata et. al., 2015 and Aliniaeifard et. al., 2016).

**Table 6:** The effect of spraying Asc, Pr and GB on leaf mineral content of N, P, K , Ca and Na of pomegranate trees cv. wonderful grown in a combination of stressed conditions during 2014/2015 seasons.

| Parameters | N % | | P % | | K % | | Ca % | | Na % | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Control** | 1.54 **a** | 1.54 **a** | 0.24 **a** | 0.23 **a** | 1.18 **a** | 1.19 **a** | 1.23 **a** | 1.23 **a** | 0.62 **a** | 0.61 **a** |
| **10 mM Asc** | 1.65 **d** | 1.64 **d** | 0.31 **b** | 0.31 **b** | 1.27 **b** | 1.27 **b** | 1.32 **b** | 1.34 **b** | 0.54**c** | 0.54 **c** |
| **50 mM Pr** | 1.58 **b** | 1.57 **b** | 0.29 **b** | 0.30 **b** | 1.26 **b** | 1.26 **b** | 1.32 **b** | 1.32 **b** | 0.56**c** | 0.56 **c** |
| **100 mM GB** | 1.61 **c** | 1.61 **c** | 0.30 **b** | 0.30 **b** | 1.27 **b** | 1.27 **b** | 1.32 **b** | 1.32 **b** | 0.54 **c** | 0.54 **c** |
| **Asc+Pr** | 1.69 **e** | 1.71 **e** | 0.38 **c** | 0.39 **c** | 1.34 **c** | 1.35 **c** | 1.39 **c** | 1.38 **c** | 0.51 **b** | 0.51 **b** |
| **Asc+GB** | 1.72 **e** | 1.72 **e** | 0.39 **c** | 0.39 **c** | 1.35 **c** | 1.35 **c** | 1.40 **c** | 1.40 **c** | 0.51 **b** | 0.51 **b** |
| **Pr+GB** | 1.71 **e** | 1.72 **e** | 0.38 **c** | 0.38 **c** | 1.33 **c** | 1.33 **c** | 1.38 **c** | 1.38 **c** | 0.51 **b** | 0.51 **b** |
| **Asc+Pr+GB** | 1.79 **f** | 1.80 **f** | 0.44 **d** | 0.45 **d** | 1.42 **d** | 1.43 **d** | 1.46 **d** | 1.46 **d** | 0.45 **a** | 0.45 **a** |

*Means followed by the same letter are not statistically different by LSD at 0.05 levels.*

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## CONCLUSION

Application of exogenous Asc, Pr and GB leads to amelioration negative effects of environmental stresses which may happen to performance and yield of pomegranate trees. Adding these substances at rate of 10 mM Asc, 50 mM Pr and 100 mM GB introduced the best data for all parameters under investigation compared to control. Therefor the study recommend this application rate or higher rates need to be under investigation.

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