Effects of Exogenous Application of Glycine Betaine and Proline on Productivity of Valencia Orange Trees Grown in a Saline Soil

Sanaa, M. Mohamed and G.F. Abd El- Rahman
Department of Citrus, Horticulture Research Institute, Agricultural Research Center, Egypt

ABSTRACT

The natural resources of agricultural lands in Egypt are exposed to many problems due to the application of improper management as well as climatic changes. The exogenous application of Glycine betaine (GB) and proline is a convenient method for the induction of crop tolerance to various abiotic stresses.Olinda Valencia orange trees (*Citrus sinensis*) (C.*sinensis* L.) budded on Volkamer lemon rootstock (*Citrus Volkameriana*) Tan. andPasq. were grown in soil influenced by salinity (where EC was 3.64 ds/m) at El Bustan County, El Behera Governorate, Egypt were used under study. Trees were sprayed with proline and (GB) twice (at full bloom stage and two weeks later) during the two seasons (2020 and 2021). The experiment included seven treatments as follow: proline at (100, 200 and 300 ppm), Glycine betaine (GB) at (1000, 2000 and 3000 ppm), and untreated trees (salt stress). Results indicated that, exogenous application of proline at 300ppm and (GB) at 2000ppm increased the trees tolerance to the adverse effects of salinity and achieved the best results (increased the concentrations of leaf total chlorophyll, leaf mineral content) especially the ratio between K^+ and Na^+ (where, lower Na^+ is a good indicator of salt tolerance in plants), improved fruit quality and consequently increased the averages of yield for the two seasons than the control by 33.4% and 30.6%, respectively .Based on the economic study, it can be recommended to use proline at a rate of 300 ppm to give the highest net profit/fed. (**6986** L.E), followed by GB at 2000 ppm (**5720** L.E) in descending order, where these treatments mitigated the inhibitory effects of salinity and promoted plant growth, as well as increasing the yield of the final crop.

Key words: Valencia orange, salinity, proline, Glycine betaine, yield.

INTRODUCTION

Citrus is a salt sensitive crop which suffers from physiological disturbances and reduced Citrus is most frequently grown in semi-arid regions where many soils are affected by salt or exposed to a high salinisation growth even at low to medium salinities (Maas, 1992).Salinity stress depresses plant growth and development at different physiological levels. The mechanisms by which salt stress damage plants are still a discussing matter due to very complex nature of the salt stress in plants (zhu, 2001). Plants growing in saline soil generally come across with major drawbacks; the first is the increase in the osmotic stress due to high salt concentration of soil; the second is the increase in concentration in Na^+ and Cl^-, exhibition tissue accumulation of Na^+ and Cl^-, and inhibition of mineral nutrient uptake, Mesut, et al. (2010).

Many approaches have been adopted to find the best horticultural practices to solve this problem. The use of Glycine betaine and L-proline had beneficial effects that increase the tolerance of plants to the unfavorable environment. Glycine betaine (GB) "N, N, N-trimethylglycine" is an amphoteric compound that is electrically neutral over a wide range of physiological pH values. It is extremely soluble in water but includes a non-polar hydrocarbon moiety that consists of three methyl groups. The molecular features of GB allow it to interact with both hydrophilic and hydrophobic domains of macromolecules, such as enzymes and protein complexes(Kanu, et al. 2017). Foliar application of GB at 50 and 100 mM on Washington navel orange increased plant height, leaf area, leaf number, and total chlorophyll content in leaves, while they did not affect the branches number (Abdallah, et al.2017).In this concern, Denaxa et al. (2012) stated that exogenous GB application enhanced leaf Chl. a, b, and total Chl. content and showed better growth and lesser Na^+ accumulation in olive trees under salt stress.. Moreover, application of GB reduced the accumulation of Na^+ accompanied by an increased accumulation of K^+ which resulted in an increased K^+/Na^+ and Ca^{++}/Na^+, ratios of wheat under saline conditions (Raza, et al. 2006), thus, it showed its contribution to salt tolerance via its role in ion homeostasis. Also, Makela et al. (1998) reported that exogenous GB application caused a significant increase in growth and yield in greenhouse and field grown tomatoes. This improvement in growth and/or yield was linked to high endogenous GB level, improved water status of plants (Lopez, et al., 2002), increased photosynthetic capacity (Yang and Lu 2005).

Amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses and may alleviate salt stress injury on cell tissues, (Kavi Kishore, 2005). In this regard, Ferreira (2005) showed that 'Valencia late' (*Citrus sinensis*) is sensitive to salt stress and demonstrated that, in the presence of NaCl, the growth rate decreased and proline accumulated in cells. Moreover, application of exogenous L-proline provides the osmoprotection and also enhanced the plant growth under salinity stress (Peng, et al., 1996; Rhodes, et al., 2002; Sharma and Dietz, 2006).

Therefore, the objective of this investigation was to study the effect of exogenous application of glycine betaine and proline on alleviating the damaging effects of salinity stress as an environmental condition on Olinda Valencia orange trees.

MATERIALS AND METHODS

Twelve years old trees of Olinda Valencia orange [(*Citrus sinensis*, L.)Osbeck] budded on Volkamer lemon rootstock (*Citrus Volkameriana*) Tan. andPasq. planted at 4x6m apart and grown in soil influenced by salinity in a private orchard, located at El Bustan County, El Behera Governorate, Egypt were selected for two seasons (2020 and 2021). The experiment area was irrigated by drip irrigation system. Trees were used according to vigor and number of flowers for data collection.

Exogenous application of proline and Glycine betaine (N, N, N-trimethylglycine) (GB) were used under study. The experiment involved seven treatments as follow:

1- Control (untreated)
2- Proline at 100 ppm (0.1 g /L).
3- Proline at 200 ppm (0.2 g /L).
4- Proline at 300 ppm (0.3 g /L).
5- Glycine betaine at 1000 ppm (1.0 g /L).
6- Glycine betaine at 2000 ppm(2.0 g /L).
7- Glycine betaine at 3000 ppm (3.0 g /L).

All treatments were applied as foliar spraying twice (full bloom stage and two weeks after full bloom). Triton B was used as a wetting agent added to all treatments at 0.05 %. Foliar sprays was done till runoff (6 L /tree). The following parameters of the studied treatments were carried out.

Leaf total chlorophyll:

Leaf total chlorophyll was determined according to the method mentioned by Moran and Porath, (1980).

Leaf proline content:

Leaf proline content was determined in fresh leaves according to the method described by Bates, *et al*., (1973).

Yield:

Number of fruits per tree at harvesting time (mid March) was done and the yield per tree (kg) and (Fadden (ton) were determined.

Fruit quality:

Ten fruits of Olinda Valencia orange were randomly taken in the two seasons for each replicate and the following determinations were carried out:

Total soluble solids (T.S.S %), total acidity (%), T.S.S/ acid ratio and juice weight percentage were determined in fruit juice according to (A.O.A.C, 1995). Peel thickness (mm) was measured by using a digital vernier caliper. Peel firmness was measured with Effegl, Pentrometer (11.1 mm diameter prop, Effegl, Alfonsing, Italy and expressed as Lb/inch2). Rind color measurement (Hue angle) was determined by using a Hunter colorimeter type (DP-9000) for the estimation of a, b and hue angle (h˚). In this system of color representation the values a*, and b* describe a uniform two dimensional color space, where a* is negative for green, and positive for red, and b* is negative for blue and positive for yellow. From a & b values, a/b were calculated Hue angle (hº= arc tan b*/a*) determines the red, yellow, green, blue, purple, or intermediate colors between adjacent pairs of these basic colors Hue angle (0˚= red-purple, 90˚ = yellow, 180˚=bluish-green, 270˚= blue), as described by (McGuire,1992).

Leaf mineral content:

Leaf samples were collected according to Jones and Embleton (1960) to determine leaf content of macro and micro elements on leaf dry weight basis. Total nitrogen (%) was determined using Microkjeldahl method according to Pregl (1945). Phosphorus (%) was determined according to Troug and Meyer, (1939). Potassium (%) was determined according to Brown and Lilliland, (1966). Sodium (%) was determined the method by Anderson *et al*., (1968). Iron, Manganese and Zinc (ppm) were determined by using atomic absorption according to Carter, (1993).

Soil analysis:

Soil samples were taken before starting the experiment. Soil physical and chemical properties were determined. Available nitrogen was determined according to Black (1982). Available phosphorus was determined spectro-photometrically as the method outline by Watanabe and Olsen,(1965).Available potassium was determined using flame-photometric (APHA, 1992). Soil reaction (pH) was measured in 1: 2.5 soil water extract using glass electrode pH meter Model (955), and electric conductivity (EC) was measured in 1:5 soil water extract using glass electrode conductivity meter Model Jenway 4310. Table (1) shows physical and chemical analyses of the soil.

Table (1):Physical and chemical analyses of the soil before starting the study.

Physical properties of soil (%)						
Coarse sand	Fine sand	silt	Clay	Texture	OM	$CaCo_3$
73.85	4	6.50	15.65	Sandy loam	0.28	1.8

Chemical properties of soil										
		Cationsmeq / L				Anions meq / L				
pH1:2.5	ECds/m	Ca^{++}	Na^{+}	Mg^{++}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$	SP (%)
8.82	3.64	10.6	9.50	15.69	0.61	-	7.40	23.0	6.00	28%

Available macro and micro nutrients (mg/kg) of soil					
N	P	K	Fe	Zn	Mn
17.5	12.00	58.50	3.51	0.45	2.00

Where SP = Saturation percentage.

Statistical analysis:

The experiment was designed in a completely randomized block design with three replicates for each treatment and each replicate was represented by two trees. The obtained data for the two seasons were subjected to analysis of variance according to Clark and Kempson, (1997), and the means were differentiated using Duncan multiple range test at 5% level (Duncan, 1955).

RESULTSAND DISCUSSIONS

Leaf total chlorophyll content:

Analysis of the total chlorophyll content in plant leaves is an important approach to assess the health of the plant's internal system during photosynthesis and is an accurate and rapid method for detecting and quantifying tolerance of plants for stress (Li, et al., 2006). Data presented in Table 2 and Fig. (1) showed that, the leaf total chlorophyll content was positively affected by exogenous application of proline and Glycine betaine (GB) .The highest averages of the two seasons (2020 and 2021) were obtained by application of proline at 300 ppm (92.44µg/ cm^2) and(GB) at 2000 ppm (88.22µg/cm^2) in contrast with control treatment (salt stress) (82.39 µg/ cm^2) and the other treatments gave the intermediate values with slight fluctuations in this regard Fig. (1). These results are in line with those obtained by (Delfine, et al. 1999) who mentioned that, the reduction in photosynthesis under salt stress can be attributed to a decrease in chlorophyll content. In this concern, foliar application of GB increased the leaf chl. b and total leaf chlorophyll content under saline conditions on sunflower (Ashraf and Sultana 2000), and wheat (Raza, et al., 2006).

Table(2):Leaf total chlorophyll a,b (µg/ cm^2) content of OlindaValencia orange trees.

Treatments	Total chlorophyll a, b (µg/cm^2) 2020	2021
Control	83.33 d	81.45 cd
Proline at 100 ppm	90.35 b	80.58 d
Proline at 200 ppm	90.46 b	82.38 c
Proline at 300 ppm	93.45 a	91.42 a
GB at 1000 ppm	90.11 b	80.33 d
GB at 2000 ppm	92.29 a	84.14 b
GB at 3000 ppm	88.45 c	80.39 d

In each column, differences between all treatments means having a same letter(s) are not significantly different by Duncan's multiple range tests at the 5% level. Where, GB refers to Glycine betaine.

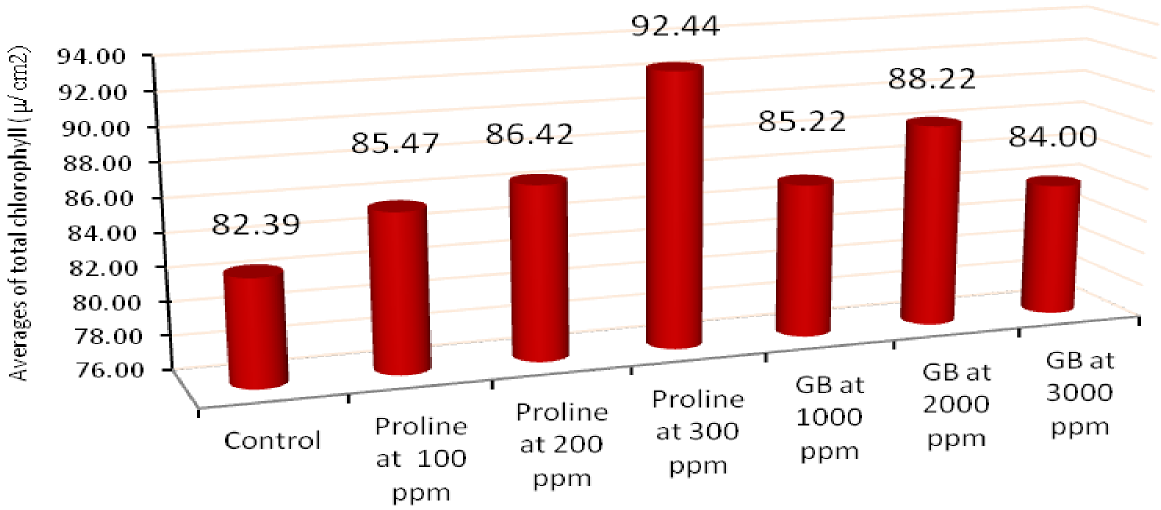

Fig. (1): Averages of leaf total chlorophyll content a, b (µg/ cm^2) of the two seasons (2020 and 2021). Where, GB refers to Glycine betaine.

Leaf proline content:

Numerous studies have indicated that, there is a positive relationship between plant stress and proline accumulation (Munns and Tester, 2008). Fig. (2) shows the averages of proline content of the two seasons (2020 and 2021). It could be noticed that proline content increased with control treatment (1.1 mg/ g FW) significantly followed by trees sprayed with GB at 3000 ppm (0.91 mg/g FW) and proline at 200 ppm (0.83 mg/g FW), while averages of proline content decreased by proline application at 300 ppm and reached to (0.53 mg/ g FW). These results are in harmony with those obtained by (Mohamed, et al., 2018) who found that, accumulation of proline in leaves of Valencia orange trees under salt stress. Proline, an amino acid, plays a very beneficial role in plants exposed to various stress conditions and it may play a protecttive role against the osmotic potential generated by salt (Hoque, et al., 2008). During stress, it acts as an excellent osmolyte, and plays three main roles, i.e. an antioxidant defense molecule, as a signaling molecule and a metal chelator (Shamsul, et al., 2012). In this concern, Kanu, et al., (2017) revealed that, proline is used for protein synthesis, has protective functions as an osmolyte, contributes to the maintenance of the redox balance, can regulate development and is a component of metabolic signaling networks controlling mitochondrial functions, stress relief and development in plants.

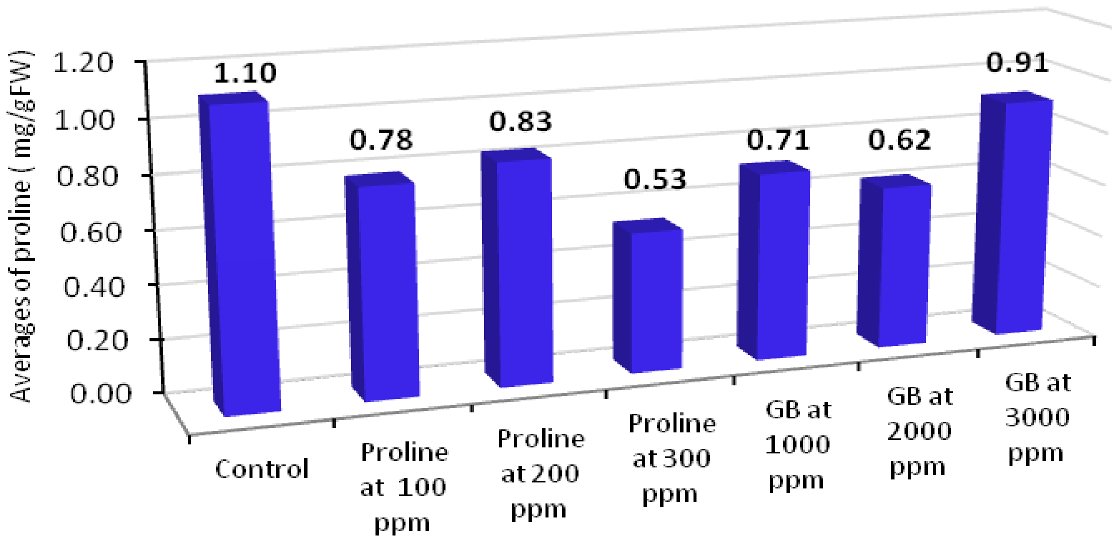

Fig.(2): Averages of leaf proline content (mg/ g FW) of the two seasons(2020 and 2021). Where, GB refers to Glycine betaine.

Yield:

It is evident from the data obtained in Table (3) that spraying trees with proline and glycine betaine (GB) had significant promotion on the number of fruit per tree, the average of fruit weight and the final yield (ton/fed) compared with control treatment in both experimental seasons. Maximum yield (ton / fed.)wasachieved with trees treated byprolineat 300 ppm (10.53, 10.63 ton / fed) and glycine betaine at 2000 ppm (10.30, 10.42 ton/fed) as compared with control treatment (8.35, 7.56 ton/fed) during the two seasons, (2020 and 2021), respectively. While, the other treatments gave intermediate values Table (3).Moreover, Fig. (3) shows the averages of yield over control (%) for the two seasons (2020 and 2021) and revealed that, all treatments increased the yield of Olinda orange trees from 16.8% to 33.4% over control treatment (salt stress). In more details, trees sprayed with prolineat 300 ppm, witnessed an increase in the yield by (33.4%) over control, followed by trees sprayed with glycine betaine at 2000 ppm (30.6%) , while, trees that received the application of glycine betaine at 3000 ppm showed an increase in yield by (22.0%) over control (salt stress).

Exogenous applications of GB and proline to plants, before, during, or after stress exposure, have been shown to increase the internal levels of these compounds and generally enhance plant growth and the productivity under stress conditions (Kanu, et al 2017). Moreover, these compounds allow cells to retain water and help in avoiding disturbances in their normal functions when exposed to abiotic stresses(Yancey et al. 1982). Consequently, all tested GB and proline concentrations increased final crop yield of Olinda orange trees. The positive effect of exogenous GB and proline on fruit yield and quality under variable conditions are in agreements with many previous studies (Abdallah et al., 2017), on navel orange, (Roussos et al., 2010), on olive and (Hamza and Shalan, 2020) on mango.

Table (3)Effect of proline and Glycine betaine foliar sprays on yield (ton/fed.) of Olinda Valencia orange trees.

Treatments	Fruit weight(gm)		Fruit No./tree		Yield/kgtree		Yield ton/fed.	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	165.6e	166.0d	288.3bc	260.0e	47.74d	43.17e	8.35d	7.56e
Proline at 100 ppm	184.4c	188.0b	281.7c	296.7bcd	51.95c	55.82c	9.04c	9.77c
Proline at 200 ppm	175.6d	183.3c	300.0abc	288.3cd	53.16bc	52.82d	9.30bc	9.24d
Proline at 300 ppm	191.1b	193.0a	316.7a	316.7a	60.49a	61.10a	10.53a	10.63a
GB at 1000ppm	188.9bc	196.0a	290.0bc	285.0d	54.73b	55.88c	9.58b	9.74c
GB at 2000ppm	193.3b	196.3a	305.0ab	303.3b	58.82a	59.52ab	10.30a	10.42ab
GB at 3000ppm	200.0a	192.7a	263.3d	300.0bc	52.64bc	57.74bc	9.21bc	10.10bc

In each column, differences between all treatments means having a same letter(s) are not significantly different by Duncan's multiple range tests at the 5% level. Where, GB refers to Glycine betaine.

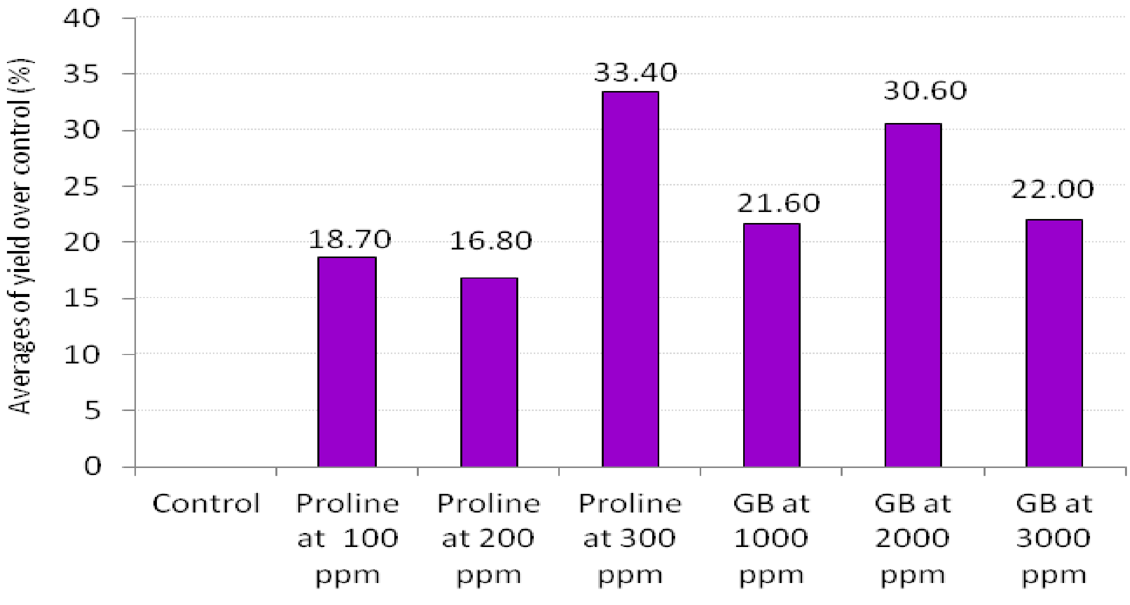

Fig. 3: Averages of yield over control (%) for the two seasons (2020 and 2021).
Where, GB refers to Glycine betaine.

Fruit quality:

Abiotic stress factors significantly cause reductions in crop production and deteriorate the crop quality, which eventually results in the depletion of food source. Data presented in (Tables 4, 5) indicated that, Olinda Valencia orange fruits under salt stress (control) had low content of juice weight percentage, an increase in T.S.S/acid ratio and the peel of fruits was thick with pale color. Exogenous application of glycine betaine and proline improved the internal and external quality of Olinda orange fruits. In more details, the results revealed that, trees treated by proline at 300 ppm and glycine betaine at 2000 ppm achieved maximum juice weight percentage (57.03, 53.5 %) and (56.3, 50.6%) as compared with the treesunder salt stress (47.5, 41.8 %) during the two seasons, respectively. Meanwhile, the other treatments recorded the intermediate values in this regard Table (4).As for fruit T.S.S and acidity percentage, it could be noticed that there were slight fluctuations among all treatments and the differences among other treatments didn't show any obvious trend during the two seasons.TSS/acid ratio is one of the important characteristics for citrus ripening and exportation. The results indicated that, spraying the trees with proline or glycine betaine improved TSS/Acid ratio under the soil affected by salinity conditions (Table 4). Concerning peel thickness it could be seen that, fruits of the all treatments had thinner fruit peel than the control treatment (salt stress). As for peel firmness, data indicated that there were no significant differences between all treatments in the first season, (2020) and the differences between all treatments were low to be significant in the second season, (2021).Regarding the peel lightness and color, it could be noticed that, fruits treated by glycine betaine or proline treatments had more lightness and had good rind color as compared with untreated fruits, so it seemed to be more attractive and had higher quality (Table 5).

The foregoing results go along with those finding by (Abdallah et al., 2017), on navel orange, (Seif et al., 2020) on grape and (Hamza and Shalan, 2020) on mango, who reported that, application of exogenous glycine betaine and proline as a foliar spray enhanced vegetative growth parameters, yield and fruit quality under salt stress which reflects an increase of the capability of trees to tolerate the harmful effect of soil salinity.

Table (4): Effect of proline and Glycine betaine foliar spraying on internal fruit quality of Olinda Valencia orange trees.

Treatments	Internal fruit quality							
	Juice weight (%)		T.S.S (%)		Acidity (%)		T.S.S /acid ratio	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	47.5c	41.8d	10.2 d	11.3 ab	0.84 f	0.93b	12.1 a	12.2 ab
Proline at 100 ppm	55.5ab	49.3bc	10.2 d	11.0 bc	1.2 ab	0.87 c	8.5 d	12.7 a
Proline at 200 ppm	56.1ab	49.7bc	10.2 d	11.0 bc	1.1 cd	1.01a	9.2 cd	11.0 de
Proline at 300 ppm	57.03a	53.5a	10.5bc	11.7 a	1.2 bc	1.00 a	9.1 cd	11.8 bc
GB at 1000 ppm	54.6b	48.8bc	10.7 b	11.0 bc	1.09 d	0.99 a	9.9 bc	11.2 cd
GB at 2000 ppm	56.3ab	50.6b	10.3 cd	11.0 bc	0.91 e	1.02 a	10.4 b	10.8de
GB at 3000 ppm	57.5a	47.9c	12.2 a	10.8 c	1.2 a	1.05 a	10.0 bc	10.3 e

In each column, differences between all treatments means having a same letter(s) are not significantly different by Duncan's multiple range tests at the 5% level. Where, GB refers to Glycine betaine.

Table (5): Effect of proline and Glycine betaine foliar spraying on peel quality of OlindaValencia orange trees.

Treatments	External fruit quality (Peel quality)							
	Peel thickness (mm)		Peel firmness		Peel lightness		Peel color (Hue angle)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	5.07 a	4.82 a	20.4a	20.7ab	67.3e	65.77e	68.7e	66.6c
Proline at 100 ppm	4.79 ab	4.12 c	20.9a	21.8ab	69.8c	66.56d	70.55d	68.9d
Proline at 200 ppm	5.04 ab	4.03 c	21.6a	21.9ab	68.1de	68.29c	70.8cd	69.9c
Proline at 300 ppm	4.33 d	4.17 c	21.1a	21.4ab	70.39b	69.62b	72.94a	68.8d
GB at 1000ppm	4.55 bc	4.14 c	21.9a	22.4a	68.61d	66.74d	71.17bc	72.7b
GB at 2000ppm	4.42 cd	4.49 b	21.8a	21.9ab	72.0a	71.04a	72.7ab	74.0a
GB at 3000ppm	4.83 ab	4.10 c	21.6a	20.2b	68.9cd	69.08b	69.74d	73.0b

In each column, differences between all treatments means having a same letter(s) are not significantly different by Duncan's multiple range tests at the 5% level. Where, GB refers to Glycine betaine.

Leaf mineral content:

Results obtained in (Tables 6 & 7) disclosed that, trees sprayed with both proline and glycine betaine treatments significantly alleviated the effect of salt stress compared with untreated trees and improved the uptake of mineral nutrients that was reflected on promoting plant growth and crop yield. The highest significant values of N, P, K, Fe, Zn and Mn content were obtained by application of proline at 300 ppm and GB at 2000ppm while the application of other tested treatments resulted in intermediate values with slight fluctuations in this regard and the lowest significant values was committed with the control (salt stress) in both seasons.

Table (6): Effect of proline and Glycine betaine foliar spraying on leaf macro elements content of Olinda Valencia orange trees.

Treatments	N^+ (%)		P^+ (%)		K^+(%)		Na^+(%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	2.23 d	2.27 d	0.10 a	0.12 a	0.83 d	0.95 c	0.28 a	0.29 a
Proline at 100 ppm	2.34 c	2.41ab	0.11 a	0.12 a	0.96 bc	0.99ab	0.22 ab	0.25 ab
Proline at 200 ppm	2.32 c	2.39 bc	0.11 a	0.13 a	0.93 c	0.97 bc	0.23 ab	0.24 ab
Proline at 300 ppm	2.44 a	2.46 a	0.14 a	0.16 a	1.06 a	1.04 a	0.16 d	0.16 d
GB at 1000ppm	2.37 bc	2.40ab	0.12 a	0.13 a	1.02 a	0.94 c	0.19 bc	0.20 bc
GB at 2000ppm	2.41 ab	2.44 ab	0.13 a	0.15 a	1.03 a	1.05 a	0.16 cd	0.18 cd
GB at 3000ppm	2.38 bc	2.36 c	0.12 a	0.14 a	1.01 ab	1.02 ab	0.21 bc	0.23 ab

In each column, differences between all treatments means having a same letter(s) are not significantly different by Duncan's multiple range tests at the 5% level. Where, GB refers to Glycine betaine.

Table (7): Effect of proline and Glycine betaine foliar spraying on leaf micro elements content ofOlinda Valencia orange trees.

Treatments	Fe ppm		Zn ppm		Mn ppm	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	75 f	71 g	22.36 e	18.35 f	24.68 f	26.52 e
proline at 100 ppm	96 d	88 e	39.33 d	42.51 c	42.59 b	29.56 d
proline at 200 ppm	98 cd	93 d	44.34 c	34.65 e	39.53 c	31.49 cd
proline at 300 ppm	107 a	118 a	58.36 a	51.39 a	46.38 a	40.47 a
GB at 1000ppm	90 e	85 f	46.55 c	37.59 d	37.45 d	33.58 bc
GB at 2000ppm	102 b	111 b	55.52 b	49.68 a	39.62 c	35.74 b
GB at 3000ppm	99 c	101 c	38.55 d	47.51 b	35.51 e	34.52 b

In each column, differences between all treatments means having a same letter(s) are not significantly different by Duncan's multiple range tests at the 5% level. Where, GB refers to Glycine betaine.

As for leaf Na^+ content (Table, 6) it is clear that, the uptake of Na^+ by control treatment was more pronounced (0.28 and 0.29%) during the two seasons (2020 and 2021), respectively as a result of salt stress, however, application of all treatments significantly reduced accumulation of Na^+ in leaves and there were high significant between them, wherelower Na^+ uptake is a good indicator of salt tolerance in plants. Results presented in (Fig. 4) indicated averages of K^+/Na^+ ratio during the two seasons and it is noticed that, trees treated by proline at 300 ppmachieved the highest value (6.56) followed by trees treated by GB at 2000ppm (6.12) while control treatment scored the lowest ratio (3.18). It is obvious that, increasing the absorption of K^+ shows the ability of trees to combat the salt stress that will strongly depend upon Na^+. These results are in harmony with those obtained by Binzel et al., (1987) who found that, salt tolerance is typically characterized by enhanced exclusion of Na^+ and increased absorption of K^+ to maintain optimum K^+/Na^+ ratio in plant shoots. Also, application of GB may have a role in Na^+/K^+ discrimination, which substantially or partially contributes to salt tolerance. In this concern, Raza*et al*. (2006) noted accumulation of Na^+ in the shoots and roots of wheat due to salt stress, while K^+ and Ca^{++} accumulation was decreased. In this concern, Roy, *et al*. (1993) reported that when L-proline was applied exogenously at a lower concentration, it enhanced the adverse effects of salinity in rice.

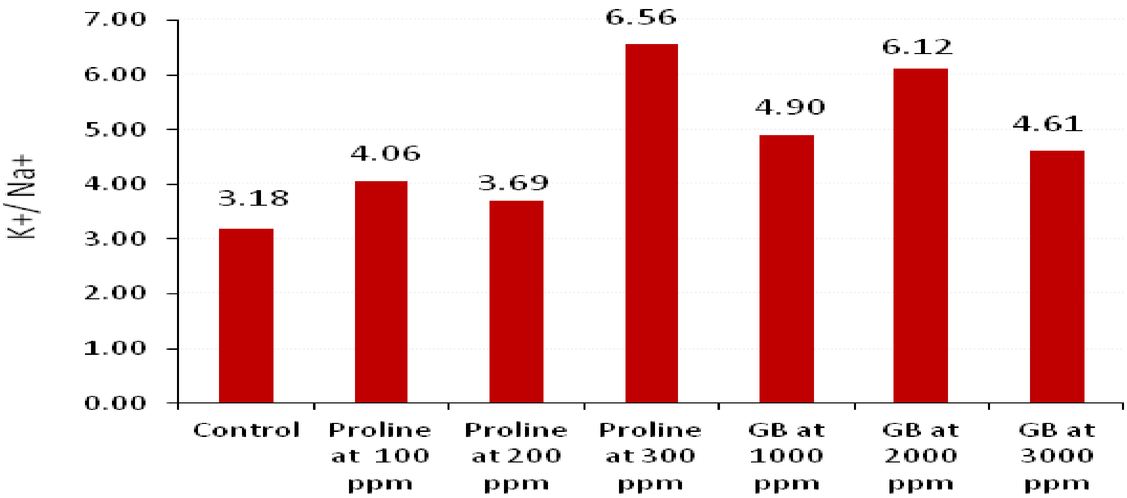

Fig. (4): Averages the ratio between leaf K^+ and Na^+ content duringthe two seasons (2020 and 2021). Where, GB refers to Glycine betaine.

Economic study:

As for the economic study of yield production (Table 8) reveals the main economic criteria and cost of each product(proline and glycine betaine) used under study (L.E/fed.), cost of labor and spraying motor (L.E/fed.), averages yield (ton/fed) for two seasons (2020 & 2021), price of yield over control (L.E) and net profit (L.E/fed.) for each treatment.

Other expenses such as the costs of supervision and royalties were not taken into consideration in this study. In more details unit price of proline was (650 L.E/kg), glycine betaine was (350 L.E/kg) and taking into account of both proline and glycine betaine were sprayed at two times under study. The study also revealed that, the price of Olinda Valencia orange fruits were(3 L.E/kg) and the cost of labor that were used per treatment as well as the spraying motor and thus the total costs were calculated. Also averages yield (ton/fed.) for the first and second seasons and yield over control were calculated and finally the net profit (L.E/ fed.) for yield over control was determined.

From this economic study it could be realized that, application of proline at 300 ppm was the best treatment for giving the highest net profit / fed. (**6986** L.E) followed in descending order by using of GB at 2000ppm(**5720** L.E), GB at 1000ppm(**4040** L.E) and so on.

CONCLUSION:

In conclusion, the results presented in this study clearly showed, that the growth and productivity of Olinda Valencia orange trees inhibited under salt stress conditions. Exogenous applications of Glycine betaine (GB) and were proline at different concentrations were used as foliar spraying twice (full bloom stage and two weeks after full bloom). Based on the economic study it could be recommended that, application of proline at 300 ppm was the best treatment for resulting in the highest net profit/fed. (**6986** L.E) followed in descending order by using GB at 2000ppm (**5720** L.E).

Table (8).Economic study for using proline and glycine betaine applications on yield of Olinda Valencia orange trees.

Treatments	Total Q. of each treat/. fed.	Keg price (L.E)	Cost of each treat./fed (L.E)	No.labor/year	Labor &S.M. fees(L.E)	Labor &S.M. Cost(L.E)	Total costtreat./fed. (L.E)	Averageyield for twoseason (ton/fed.)	Yield overcontrol Ton/fed.	Yield over control price(L.E)	Net profit/ Fed.(L.E)
Control								7.960	-----		
Proline at 100 ppm /600 l.	60gm.	650	39 x 2time **(78)**	(2+S.M.) x 2time	100, 120	**640**	**718**	9.410	1.450	**4350**	**3632**
Proline at 200 ppm /600 l.	120 gm.	650	78x 2time **(156)**	(2+S.M.) x 2time	100, 120	**640**	**796**	9.270	1.310	**3930**	**3134**
Proline at 300 ppm /600 l.	180 gm.	650	117x 2time **(234)**	(2+S.M.) x 2time	100, 120	**640**	**874**	10.580	**2.620**	**7860**	**6986**
GB at 1000ppm /600 l.	600 gm.	350	210x 2time **(420)**	(2+S.M.) x 2time	100, 120	**640**	**1060**	9.660	1.700	**5100**	**4040**
GB at 2000ppm /600 l.	1200gm.	350	420x 2time **(840)**	(2+S.M.) x 2time	100, 120	**640**	**1480**	10.360	**2.400**	**7200**	**5720**
GB at 3000ppm /600 l.	1800gm.	350	630x 2time **(1260)**	(2+S.M.) x 2time	100, 120	**640**	**1900**	9.660	1.700	**5100**	**3200**

Where: (S.M.) refers to Spraying Motor., two times refer to (full bloom stage and two weeks after full bloom)andGB refers to Glycine betaine.

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تأثيرات الإضافة الخارجية بالجليسين بيتائين والبُرولين على إنتاجية أشجار البرتقال الفالنشيا النامية فى تربة ملحية

سناء مصطفي محمد وجمال فرج عبدالرحمن

***قسم بحوث الموالح، معهد بحوث البساتين، مركز البحوث الزراعية – مصر**

تتعرض الموارد الطبيعية للأراضى الزراعية فى مصر للعديد من المشاكل بسبب تطبيق الإدارة الغير سليمة لهذه الموارد وأيضا بسبب التغيرات المناخية. وتُعد الإضافة الخارجية للجليسين بيتائين والبُرولين طريقة مناسبة لتحفيز تحمل المحاصيل لمختلف الإجهادات البيئية الغير حيوية. هذا وقد تم إجراء التجربة على أشجار البرتقال الصيفى صنف أوليندا مطعومة علي أصل ليمون الفولكاماريانا ونامية في تربة متأثرة بالأملاح حيث كانت نسبة الملوحة (EC 3.64 ds/m) فى مزرعة خاصة بمنطقة البستان – محافظة البحيرة – مصر.

أُجريت الدراسة برش الأشجار بالبُرولين والجليسين بيتائين مرتين (فى مرحلة الإزهار الكامل وبعدها بأسبوعين) خلال الموسمين (2020, 2021). وإشتملت التجربة علي سبعة معاملات على النحو التالى:

البُرولين بتركيز (100، 200، 300 جزء فى المليون)، والجليسين بيتائين بتركيز (1000،2000، 3000 جزء فى المليون)، بالإضافة للأشجار الغير معاملة (الكنترول المتأثر بالإجهاد الملحي).

أشارت النتائج إلى أن رش الأشجار بالبُرولين بتركيز 300 جزء في المليون والجليسين بيتائين بتركيز 2000 جزء في المليون زادا من تحمل الأشجار للآثار الضارة للملوحة وأيضا حققا افضل النتائج (مثل زيادة تركيزات الكلوروفيل الكلى بالأوراق , والمحتوي المعدني للاوراق وخاصة النسبة بين الصوديوم والبوتاسيوم (حيث يُعتبر انخفاض نسبة الصوديوم إلى البوتاسيوم مؤشر جيد علي تحمل النباتات للأملاح)، هذا بالإضافة إلى تحسين جودة الثمار وبالتالي زيادة معدلات المحصول بنسبة4.33 ٪، ٣٠,٦٪ للموسمين مقارنة بالكنترول علي التوالي.

وبناءاً على الدراسة الإقتصادية يمكن التوصية بإستخدام البُرولين بمعدل 300 جزء في المليون لإعطاء أعلي صافي ربح للفدان (6986 جنية) ويليها بترتيب تنازلى الجليسين بيتائين بمعدل 2000 جزء فى المليون (5720 جنية)، حيث خففتا هاتين المعاملاتين من الآثار السيئة للإجهاد الملحى وساعدت على نمو النبات، بالإضافة إلى زيادة محصلة المحصول النهائى.

الكلمات الكشافة: برتقال الفالنشيا، الملوحة، البُرولين،الجليسين بيتائين، المحصول.