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Effect of Foliar Anti-Salinity Application on Chemical Constituents of Neem Plants under Salinity Condition

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ABSTRACT

This study was conducted at a Privet Nursery in New Damietta City, Damietta Governorate, Egypt during the two consecutive seasons of 2017/2018 and 2018/2019 in split-plot design in three replicates to estimate the effect of salinity levels (control (tap water), 3600, 5400 and 7200 ppm) as main plot and foliar spraying with (distilled water, salicylic acid, proline, yeast extract and licorice roots extract) as sub plot as well as their combination treatments on chemical constituents of neem plants (*Azadirachta indica*). The obtained results revealed that with increasing salinity levels photosynthetic pigments, reducing and non-reducing sugars as well as mineral contents (N, P, K, Ca and Mg) of neem leaf were decreased but it not suffered to level 3600 ppm and recorded the highest values, while content of proline, total phenol and Na increased with increasing salinity levels to 7200 ppm. As for the effect of foliar application found that plant sprayed with proline followed by licorice extract resistance the salinity and recorded the highest values of all mentioned traits. Interaction treatments between foliar application and salinity treatments recorded highest values for all traits with proline under 3600 ppm except Na, proline and total phenol at 7200 ppm under foliar spray with proline at 0.2 g/l.

Keywords: Neem, salinity, proline, salicylic acid, licorice extract and yeast extract.

INTRODUCTION

Neem tree (*Azadirachta indica*) which belongs to Meliaceae family is a tropical evergreen tree native to India. Indian neem tree is the subject matter of numerous scientific studies concerning its utilization in medicine and agriculture (Koul and Wahab, 2004). The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled "Neem - a tree for solving global problems" (Biswas *et al*., 2002). All parts of the tree have been used medicinally and is now being used in pharmaceutical and cosmetics industries. Neem fruits, seeds, oil, leaves, bark and roots used as antiseptics, antimicrobials and treatment of inflammatory diseases (Brototi and Kaplay, 2011). The neem tree contains more bioactive compounds as azadirachtin, salannin, meliantriol, nimbin, nimbidin, nimbolides, gedunin, meliacin and valassin (Pankaj *et al*., 2011). In the main, these compounds belong to a general class of natural products called "triterpenes"; more specifically, "limonoids" (Washington, 1992).

The use of sea water in soilless culture is an interesting option to limit freshwater withdrawal for food production. Since, the rhythm of fresh water with drawal is faster than its regeneration with any additional negative imput into soils and the counts of available fresh water on less than 1% of the water on Earth and of such share an average of 70% is absorbed by the agricultural sector (Atzori *et al*., 2019). The high concentration of ionic elements in sea water is the main restricting factor in the utilization of sea water for irrigation (Xiao-Hua *et al*., 2009). There are several disadvantages when irrigation with saline water induced abiotic stress and toxic effects on plants which lead to gradually declined in photosynthesis (Manai *et al*., 2014) and decreased chlorophyll and NPK contents of the plant (Aydin *et al*., 2012) but increased the level of amino acids, particularly proline (Jouyban, 2012). Proline might play a sensitive role in protecting plants under saline conditions. The salinity tolerance depends on the interaction between salinity and other environmental factors. Under salt stress, crop management practices that improve plant resistance to salt stress are different in which employ new strategies to improve salt stress tolerance to the plants (Acosta-Motos *et al*., 2017).

Licorice (*Glycyrrhiza glabra*) belong to the family Leguminoseae which grows in Egypt. Using root extract in many practical studies for being a vegetarian alternative to extract natural growth regulators contribute to improve plant growth (Newall *et al*., 1996). It contains more than 100 various compounds including triterpene saponins (including glycyrrhizin) and phenolic compounds (Shibata, 2000; Shabani *et al*., 2009) as well as flavonoids, amino acid (Asparagin), monosaccharide, starch, different types of vitamins such as B1, B2, B3, B6, C, E, folic acid and many mineral compounds (P, K, Ca, Fe, Mn, Zn, Na and Si) (Fukai *et al*., 1998; Arystanova *et al*., 2001). Abd El-Hamied and El-Amary (2015) found that spraying pear with licorice root extract at (4 g/l.) improved leaf mineral contents (nitrogen, phosphorus and potassium).

Yeast (*Saccharomyces cervicisae*, L.) extract is one of the natural stimulators is characterized by its richness in proteins, large amount of vitamin B (Thiamin, riboflavin and pyridoxines) and amino acids. Also, yeasts are prolific producers of hormones, mineral elements and natural plant growth regulators namely cytokinin (Mahmoud, 2001). El-Sayed (2013) recorded that application of dry yeast on olive trees increased total chlorophyll contents and leaf mineral content (N, P, K, Fe, Zn and Mn) compared with control (without dry yeast) under drip irrigation system (5000 ppm saline of water).

Salicylic acid (SA) is a phenolic compound that produced in a large number of plants by root cells and plays a lot of roles in

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growth and development of plants as a quasi-hormonal substance (Khan *et al*., 2015). Application of SA enhanced the photosynthetic rate, maintained the stability of membranes and lowered the electrolyte leakage in salt-stressed plants resulted in decreasing uptake of Na^+ and Cl^- (El-Tayeb, 2005). EL Sayed *et al.* (2017) demonstrated that foliar spraying *Duranta plumieri* plants with salicylic acid at 250 and 500 ppm combined with irrigation with diluted sea water at three concentrations: 2000, 4000 and 6000 ppm significantly increased chlorophyll, carotenoids, proline, total carbohydrate, reducing and non-reducing sugars as well as mineral uptake (N, P, K and Mg) contents with increasing the salinity concentrations during the both seasons.

Proline is one of the most important amino acids produced and accumulated in the plant in response to salt stress (Marin *et al*., 2010). Exogenous foliar application of proline not only effectively regulates solute potential but also plays an important role in enhancing plant growth under stress environment (Ashraf and Foolad, 2007; Hoque *et al*., 2007). Alotaibi *et al*. (2019) indicated that chlorophyll, N and K contents of jojoba leaves gradually increased as proline levels increased from 10-20 mM under salt stress conditions, whereas Na+ and Cl- levels were decreased relative to the control. These results also were parallel with Abdelkader *et al*. (2019) who cleared that proline treatment at 200 ppm registered the highest values of total chlorophyll content and total carbohydrates percentage in rosemary plants under soil salinity stress.

The main objective of this study was to evaluate the role of proline, licorice root extract, salicylic acid and yeast as foliar application on counteracting the deleterious impact of different levels of salinity on chemical constituents of *Azadirachta indica* plants.

MATERIALS AND METHODS

This study was conducted during the two consecutive seasons of 2017/2018 and 2018/2019 at a Privet Nursery in New Damietta City, Damietta Governorate, Egypt, to estimate the effect of foliar spraying with distilled water, salicylic acid, proline, yeast extract and licorice roots extract under saline irrigation with diluted seawater and their combination treatments on chemical constituents of neem plants (*Azadirachta indica*).

The experimental was performed using split-plot design with 20 treatments in 3 replicates, four treatments of salinity and five anti-salinities as follows; salinity treatments were (control (tap water), 3600, 5400 and 7200 ppm) as main plot and anti-salinities were (Control "Distilled water", salicylic acid "0.2 g/l", proline "0.2 g/l", yeast extract "5 g/l" and licorice roots extract "5 g/l") as sub plot were used in the foliar way. Thus, the total number of pots required for each season was 60 pots.

Clayey soil was collected for the experiment from the upper layer (0-15 cm) of a cultivated field. The collected soil was air dried and crushed and homogeneously mixed with sand and peat moss as 2:1:1 before subjecting to different treatment. The physical and chemical properties of the soil textures used in the two seasons are shown in Table (1) according to Chapman and Pratt (1978).

Table 1. The physical and chemical properties of the soil used in both seasons.

Particle size distribution (%)				Textural class	EC dSm^{-1} (1:5)	pH	$CaCO_3$ (%)	Organic matter (%)	SP (%)
Coarse sand	Fine sand	Silt	Clay	Clayey	0.97	7.83	3.05	1.18	56.3

Anions meq/100g soil								Available (mg/kg)		
Ca^{++}	Mg^{++}	Na^+	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	N	P	K
1.03	0.78	2.90	0.26	-	1.22	2.70	1.05	48.05	5.95	17.53

Seeds of neem (*Azadirachta indica*) have been collected from private orchard in Ismailia, then, were soaked in water at 30°C for 24h to stimulate germination. The collected seeds were sown in perforated black pots 7 cm (2 seeds for each) filled in soil mixture of clay: peat moss: sand (2:1:1, respectively) by volume on the 18 October during both seasons. The pots were watered regularly. After 5 months on the 24 Mars, plants were transplanted singly to another pots (30 cm width and 30 cm depth) contained 5 Kg with prescribed medium. The seedlings were allowed to grow for two months for adaptation prior to being treated with salinity. Plants were grown under a natural condition of day length ranging from 10 to 12 h, mean day/night temperatures 35/22°C, and the relative humidity ranging from 55 to 67%. Two months after transplantation, the healthy seedlings were classified into 5 similar groups (36 seedlings for each). Each group was arranged into 4 treatments.

Treatments of salinity were prepared by dilution of sea water stock with fresh tap water to obtain the selected concentrations (3600, 5400 and 7200 ppm) and added about (500 ml/pots) at the mentioned scheduled irrigation intervals from start to end of experiment. Sea water was obtained from the Mediterranean Sea in New Damietta City, Damietta Governorate, Egypt. The irrigation treatments were supplied on every 3 days interval up to 3 months. The treatments were imposed in 20 July.

Thus, judicious use of special management practices to minimize the adverse effect of salinity on plant growth by using licorice extract, yeast extract, salicylic acid and proline as a foliar application represent an acceptable means in this study.

Salicylic acid and proline were obtained from El-Gomhoria Pharmaceuticals Medicinal Plants Production Company, Mansoura, Egypt. Salicylic acid was initially dissolved in a few drops of ethanol and the final volume was reached, using distilled water, and was added on the plant leaf surface.

Yeast extract: to prepare the solution, 5 g dry yeast +100 g sugar are dissolved in 500 ml warm distilled water at 35°C and was left for an hour to brewed. Thereafter, the media was frozen and thawed directly before using the next day and completed by 1000 ml distilled water.

Licorice roots extract: licorice roots were obtained from the market then sifted and the fine powder was (5 g) was mixed for 15 minutes with one liter of distilled water at 40°C in a mixer. Thereafter, the mixture was left for 24 hours to settle and filtered and completed by distilled water to one liter.

The solutions were sprayed with a hand sprayer in early morning on the leaves at the beginning of experiment for twice before applied the irrigation of treatments by diluted sea water and repeated after the irrigation treatments on every 15 days for 3 months. As for the control plants were also sprayed with distilled water.

After 90 days from treatments; Three plants were randomly chosen from each treatment in both seasons to determine the following parameters:

Photosynthetic pigments: Chlorophyll (a), chlorophyll (b), total chlorophyll and carotenoids contents were determined in fresh leaves after 90 days from treatments according to Moran (1982). Leaf pigment contents in mg/100 cm^2 concentrations were calculated according to following equations with some modification:

Chlorophyll a (µg/ml) = 12.25(A665) – 2.55(A647).
Chlorophyll b (µg/ml) = 20.13(A647) – 5.03(A665).
Total chlorophyll (µg/ml) = 17.906(A647) + 8.08(A665).
Carotenoid (µg/ml) = {1000(A470)-3.27(chl a) – 104 (chl b)}/227.

Proline content: Proline amount (mg/g) in dry leaves was assayed according to Bates *et al.* (1973).

Reducing and non-reducing sugars: Were determined in dry leaves (mg/g) according to Dubios *et al*. (1956).

Total phenols contents: Were determined in dry leaves (mg/g) according to Stabell *et al.* 1996) with modification as described by Li *et al*. (2007).

Total nitrogen and phosphorus percentages: It was determined in the digested dried leaves according to the method adapted by Jackson (1967).

Potassium, calcium, magnesium and sodium percentages: Were determined in the digested dried leaves using a flam photometer according to Black (1965).

All data were statistically analyzed according to the technique of analysis variance (ANOVA) and the least significant difference (L.S.D) at the 5% level method was used to compare the deference between the means of treatment values to the methods described by Gomez and Gomez, (1984). All statistical analyses were performed using analysis of variance technique by means of Computer Software CoSTATE.

RESULTS AND DISCUSSION

1. Photosynthetic pigments:

Data illustrated in Table (2) show the effect of different concentration of salinity; tap water, 3600, 5400 and 7200 ppm and foliar application with some resistance salinity (distilled water, salicylic acid, proline, yeast extract and licorice roots extract) as well as their interaction on photosynthetic pigments parameters expressed on (chlorophyll a, b, total and carotenoids) in leaves of neem during both seasons.

Photosynthetic pigments in fresh leaves were affected significantly under salt stress levels. The highest values were 2.735, 0.694, 3.429 and 0.678 for chlorophyll a, b, total and carotenoids, respectively in the first season; the same trend was true in the second one, which recorded at 3600 ppm comparing with the other treatments of salinity.

Concerning foliar application effects, data in Table (2) showed that all used treatments caused an increase in photosynthetic pigments over than the control. Too, the photosynthetic pigments indicated the highest mean values with foliar by proline followed by licorice root extract then decreased with salicylic and yeast. Therefore, application of proline recorded the highest significant photosynthetic pigments of (2.657 & 3.234) for chlorophyll a, (0.661 &0.776) for chlorophyll b, (3.317 & 4.010) for total chlorophyll and (0.669 & 0.725) for carotenoids, respectively in two seasons. While, control plants had the least significant photosynthetic pigments for both seasons, consecutively. The other treatments gave intermediate values with significant differences among themselves in the two seasons.

Table 2. Effect of salinity levels and some foliar application as well as their interaction on Photosynthetic pigments of neem leaves during 2017/2018 and 2018/2019 seasons.

Treatments		Chlorophyll a mg/100cm^2		Chlorophyll b mg/100cm^2		Total chlorophyll mg/100cm^2		Carotenoids mg/100cm^2	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Salinity levels									
Tap water		2.561	3.080	0.626	0.743	3.186	3.823	0.633	0.701
3600 ppm		2.735	3.319	0.694	0.804	3.429	4.122	0.678	0.740
5400 ppm		2.451	2.897	0.565	0.665	3.016	3.562	0.606	0.662
7200 ppm		2.158	2.722	0.507	0.620	2.665	3.342	0.585	0.644
LSD at 5%		0.010	0.024	0.006	0.005	0.014	0.022	0.005	0.001
Foliar application									
Control		2.069	2.689	0.487	0.604	2.556	3.293	0.574	0.630
Yeast		2.485	2.972	0.591	0.693	3.077	3.664	0.610	0.679
Salicylic		2.550	2.995	0.613	0.717	3.163	3.713	0.620	0.684
Licorice		2.621	3.132	0.639	0.749	3.259	3.881	0.656	0.718
Proline		2.657	3.234	0.661	0.776	3.317	4.010	0.669	0.725
LSD at 5%		0.010	0.019	0.005	0.004	0.012	0.020	0.003	0.005
Interaction									
Tap water	Control	2.027	2.694	0.458	0.603	2.485	3.297	0.570	0.657
	Yeast	2.661	3.045	0.632	0.736	3.293	3.781	0.628	0.690
	Salicylic	2.662	3.105	0.649	0.743	3.311	3.848	0.628	0.693
	Licorice	2.711	3.209	0.689	0.800	3.400	4.009	0.654	0.723
	Proline	2.741	3.347	0.702	0.833	3.442	4.180	0.687	0.742
3600 ppm	Control	2.648	2.997	0.612	0.714	3.260	3.711	0.620	0.685
	Yeast	2.669	3.150	0.654	0.757	3.324	3.907	0.629	0.694
	Salicylic	2.685	3.171	0.684	0.789	3.369	3.959	0.638	0.707
	Licorice	2.829	3.554	0.739	0.851	3.568	4.405	0.748	0.804
	Proline	2.844	3.721	0.782	0.908	3.625	4.630	0.758	0.808
5400 ppm	Control	1.921	2.671	0.445	0.586	2.366	3.258	0.565	0.605
	Yeast	2.517	2.932	0.578	0.667	3.094	3.599	0.614	0.672
	Salicylic	2.578	2.932	0.595	0.682	3.172	3.614	0.616	0.673
	Licorice	2.603	2.954	0.600	0.687	3.203	3.642	0.618	0.679
	Proline	2.638	2.997	0.609	0.702	3.247	3.698	0.619	0.682
7200 ppm	Control	1.679	2.394	0.432	0.513	2.111	2.907	0.540	0.571
	Yeast	2.094	2.760	0.502	0.610	2.595	3.370	0.570	0.658
	Salicylic	2.274	2.774	0.523	0.656	2.798	3.430	0.599	0.661
	Licorice	2.339	2.809	0.528	0.659	2.867	3.468	0.606	0.665
	Proline	2.405	2.872	0.551	0.662	2.955	3.534	0.610	0.667
LSD at 5%		0.019	0.037	0.010	0.008	0.024	0.040	0.007	0.009

In respect to interaction effect, data in Table (2) cleared that 3600 ppm salinity during both seasons, combined with foliar application of proline recorded higher significant photosynthetic pigments in comparison to the other treatments. Such treatments recorded (2.844 & 3.721) for chlorophyll a, (0.782 & 0.908) for chlorophyll b, (3.625 & 4.630) for total chlorophyll and (0.758 & 0.808) for carotenoids, respectively in two seasons units, respectively. On the other hand, control plants had lower significant photosynthetic pigments of (2.027 & 2.694) for chlorophyll a, (0.458 & 0.603) for chlorophyll b, (2.485 & 3.297) for total chlorophyll and (0.570 & 0.657) for carotenoids, respectively in two seasons units. From the results, it is noticed that under the same salinity level, foliar application by proline improved photosynthetic pigments of neem as compared unsprayed plants.

In general, The decrease of these pigments values under salt stress is considered to be a result of accelerated degradation and the inhibited synthesis and/or fast plastid breakdown of that pigment (Chen, 2014). Rapid maturing of leaves is stated to be another reason for the decrease of Chl content under salinity (Yeo *et al*., 1991). So, reduction of Chl a and total Chl content in neem leaves may be one of the causes of less photosynthetic product and low biomass production of stressed neem seedlings. Also, it may be referred to that salt stress negatively affect cell water content and metabolic processes, leading to minimize cell size, that lead to concentration of the green pigments in small area. The results are in coincidence with those obtained by Ashour and Abdel Wahab (2017) on *Jatropha integerrima*, Rahneshan *et al.* (2018) on *Pistacia vera* L. and Jahan *et al.* (2018) on neem.

As for the effect of proline, these results may be owing to the important roles of proline in the biochemical processes, which positively reflected on chlorophyll pigments (Hoque *et al*., 2007). These results are in harmony with those found by Khalil *et al.* (2017) on jatropha and Butt *et al.* (2016) on two chilli genotypes. These results also were parallel with Al Mayahi and Fayadh (2015) on *Cordia myxa* L. and Ibrahim *et al.* (2019) on *Ocimum basilicum*, L.

2. Proline content:

The tabulated results in Table (3) cleared that proline content in the leaves of neem significantly raised with increasing salinity levels at 7200 ppm over than control or 3600 ppm. Such treatments resulted in 3.56, 1.97, 1.51 and 1.23 mg/g D.W, consecutive as affected by 7200, 5400, 3600 and tap water, respectively, in the first season. The same trend was true in the second one

Regarding to the effect of foliar application of some resistance salinity, data at the same Table indicated that the sprayed plant with proline had higher significant proline content as 2.93 and 2.94, respectively over than other treatments compared with untreated plants during both seasons.

In respect to interaction effect, data in Table (3) showed that 7200 ppm salinity level combined with foliar application of proline gave higher significant proline content of (5.70 and 5.74), respectively, in two seasons against (0.60 and 0.61) for control which resulted in lower significant proline content in comparison to the other treatment.

This result may be referred to that salinity elevates antioxidant enzymes and proline content as a stress response to deal with increased levels of reactive oxygen species (Ruhan *et al.*, 2004). Additionally, Lehman *et al.* (2010) revealed that proline concentrations of cells, tissues and plant organs are regulated by interplay of biosynthesis and degradation as well as intercellular transport processes. Among the proline transport proteins characterized so for, both general amino acid permeates and selective compatible solute transport were identified, reflecting the versatile role of proline under stress and non-stress situations. Also, the increased proline may lead to a reduction in stress induced cellular acidification and may also act as a hydroxyl radical and singlet oxygen scavenger. Additionally, the accumulation of high proline concentrations in the cytoplasm under stress conditions without interrupting cell structure and metabolism may be due to its zwitterion nature (Goyal and Asthir, 2010), it is thought to be involved in osmotic adjustment of stressed tissues. This may assist plants in their adaptation to salinity stress. It has also been reported that hyper accumulation of Proline is one of the positive indicators for the salinity resistance of plants, whereas other researchers affirm that it appeared to be a symptom of salt stress (Jimenez-Bremont *et al.*, 2006). These results agree with those of Ashour and Abdel Wahab (2017) on *Jatropha integerrima*, Rahneshan *et al.* (2018) on *Pistacia vera* L. and Jahan *et al.* (2018) on neem.

Increasing of proline content in the plant may be due to that the exogenous proline in plant tissues (Ali *et al.*, 2008). Nevertheless, the effectiveness of applied proline depends on plant species and growth stage, rate of application and its concentration (Ashraf and Foolad, 2007). This result is in agreement with those of Ben Ahmed *et al.* (2010) on olive, Khan *et al.* (2015) on Okra and Ibrahim *et al.* (2019) on *Ocimum basilicum*, L.

3. Reducing and non-reducing sugars contents:

Data depicted in Table (3) significantly decreased reduced and non-reduced sugar with increasing salinity levels. The values of such traits in decreasing order were 0.424, 0.516, 0.321 and 0.230 mg/g of reducing sugar and 1.281, 1.484, 1.053 and 0.947 mg/g of non-reducing sugar for tap water, 3600, 5400 and 7200 ppm, respectively, in the first season. the same trend was true in the second one.

As for the effect of different foliar application, data in Table (3) indicated that all treatments of foliar application resulted in higher reducing and non-reducing sugars. The highest values noticed with application of proline followed by licorice, salicylic and finally yeast, on the opposite unsprayed plants in two seasons recorded lower values of reducing and non-reducing sugars. The other treatments gave intermediate reducing and non-reducing sugars with significant differences among themselves in the two seasons.

Regarding interaction effect, the presented results in Table (3) showed that salinized plants at 3600 ppm and sprayed by proline had higher significant reducing and non-reducing sugars in their leaves of (0.671 & 0.678 reduced sugar) and (1.697 & 1.711 non-reduced sugar) for both seasons, respectively. While, 7200 ppm plants without treating by any spray had lower significant reducing and non-reducing sugars in their leaves of (0.159 & 0.169 reduced sugar) and (0.677 & 0.689 non-reduced sugar) for the two seasons, respectively. The other interaction treatments resulted in intermediate reducing and non-reducing sugars with significant differences among themselves in the most cases in the two seasons.

This can be attributed to the reduced chlorophyll content, nutritional imbalance due to the specific toxic effects of salinity, hyperosmotic stress and reduced photosynthesis. In this study, though reduced chlorophyll content caused decreases in sugar content but ultimately the decreasing sugar content may had positive effects in tolerance mechanism against salt stress. Also, excessive Na^+ and Cl^- concentrations in saline water affect adversely water and essential nutrients uptake, gas exchange

leading to decreases in intracellular CO_2 and photosynthetic activity (Parida *et al.*, 2002, Munns and Tester, 2008 and Abdallah *et al.*, 2016), all those decreased sugar accumulations. These results confirm with those of El-Beltagi *et al.* (2017) on cotton, Rahneshan *et al.* (2018) on *Pistacia vera* L. and Jahan *et al.* (2018) on neem.

Table 3. Effect of salinity levels and some foliar application as well as their interaction on reduced and non-reduced sugar, proline and total phenols contents of neem leaves during 2017/2018 and 2018/2019 seasons.

Treatments		Reduced sugar (mg/g)		Non-reduced sugar (mg/g)		Proline (mg/g)		Total phenols (mg/g)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Salinity levels									
Tap water		0.424	0.431	1.281	1.343	1.23	1.25	3.38	3.41
3600 ppm		0.516	0.521	1.484	1.503	1.51	1.52	4.01	4.04
5400 ppm		0.321	0.327	1.053	1.076	1.97	1.98	4.53	4.54
7200 ppm		0.230	0.239	0.947	0.957	3.56	3.60	4.85	4.92
LSD at 5%		0.01	0.03	0.03	0.01	0.02	0.08	0.06	0.05
Foliar application									
Control		0.257	0.262	0.948	0.970	0.89	0.90	2.87	2.83
Yeast		0.364	0.370	1.154	1.190	2.00	2.02	4.24	4.29
Salicylic		0.372	0.379	1.189	1.229	2.07	2.09	4.50	4.54
Licorice		0.411	0.417	1.309	1.324	2.46	2.48	4.58	4.63
Proline		0.458	0.468	1.357	1.387	2.93	2.94	4.77	4.84
LSD at 5%		0.03	0.03	0.03	0.03	0.06	0.04	0.07	0.07
Interaction									
	Control	0.232	0.236	0.970	0.978	0.60	0.61	2.18	2.20
	Yeast	0.445	0.451	1.213	1.314	1.22	1.24	3.21	3.25
Tap water	Salicylic	0.454	0.462	1.233	1.364	1.34	1.36	3.79	3.84
	Licorice	0.488	0.490	1.460	1.509	1.45	1.47	3.81	3.85
	Proline	0.501	0.514	1.530	1.551	1.57	1.59	3.90	3.93
	Control	0.438	0.440	1.192	1.240	0.87	0.88	2.99	2.97
	Yeast	0.459	0.467	1.389	1.399	1.59	1.59	4.00	4.04
3600 ppm	Salicylic	0.470	0.480	1.450	1.479	1.66	1.67	4.16	4.20
	Licorice	0.539	0.540	1.690	1.686	1.66	1.69	4.40	4.43
	Proline	0.671	0.678	1.697	1.711	1.78	1.78	4.50	4.57
	Control	0.201	0.205	0.954	0.972	0.89	0.89	3.15	2.99
	Yeast	0.315	0.321	1.040	1.053	1.89	1.90	4.61	4.66
5400 ppm	Salicylic	0.318	0.323	1.051	1.055	1.94	1.95	4.82	4.87
	Licorice	0.366	0.376	1.059	1.062	2.45	2.49	4.91	4.95
	Proline	0.404	0.412	1.164	1.240	2.65	2.67	5.14	5.20
	Control	0.159	0.169	0.677	0.689	1.21	1.24	3.17	3.18
	Yeast	0.239	0.243	0.974	0.993	3.28	3.35	5.15	5.21
7200 ppm	Salicylic	0.244	0.249	1.021	1.015	3.34	3.38	5.21	5.27
	Licorice	0.250	0.262	1.028	1.040	4.26	4.29	5.21	5.29
	Proline	0.256	0.270	1.036	1.045	5.70	5.74	5.54	5.66
LSD at 5%		0.06	0.06	0.07	0.06	0.11	0.07	0.13	0.15

The effectiveness of applied proline depends on plant species and developmental stage, as well as a rate of application and its concentration (Ashraf and foolad, 2007). Proline play important roles in enzymatic activates, ions and water balance and increasing photosynthetic rate, leading to enhancing in sugar accumulation (Hoque *et al*., 2007).

4. Total Phenols contents:

As illustrated in Table (3), data show that accumulation of total phenol content increased with increasing salinity levels in leaves of neem plants. The highest phenol content was found with 7200 ppm of salinity stress as (4.85 and 4.92), in two seasons, respectively, which was statistically different to other stressed plants. While, the lowest phenol content recorded with control as (3.38 and 3.41) in the 1st and 2nd, respectively.

As for effect of different foliar application with resistance salinity comparing with the untreated plant (spray with distilled water), data in Table (3) revealed that all foliar application increased phenol content. The highest values are recorded with application of proline as (4.77 and 4.84) against the untreated plant as (2.87 and 2.83), respectively, during both seasons.

Concerning the effect of interaction salinity and some foliar application, data at the same Table indicated that all foliar application increased total phenols contents under all salinity levels. Meaning, with increasing salinity levels under all foliar application, phenol content significantly increased and recorded the highest values under 7200 ppm with foliar application of proline as (5.54 and 5.66) comparing with the untreated plant (2.18 and 2.20) during the 2017/2018 and 2018/2019 seasons of the experiment.

Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups. These compounds are also powerful chain breaking antioxidants and play a vital role in the defense against reactive oxygen species (ROS) (Sreenivasulu *et al.*, 2000). In this study, may be the increased levels of phenols at elevated levels of salinity induced accumulation of secondary metabolites to tolerate higher levels of salinity stress and aroused adverse conditions. Similarly, Ashour and Abdel Wahab (2017) on *Jatropha integerrima*, Rahneshan *et al.* (2018) on *Pistacia vera* L. and Jahan *et al.* (2018) on neem.

As mentioned before, increasing of phenol content in the plant may be due to that the exogenous proline in plant tissues (Ali *et al.*, 2008). Nevertheless, the effectiveness of

applied proline depends on plant species and growth stage, rate of application and its concentration (Ashraf and Foolad, 2007).

5. N, P and K percentages:

Data of N, P and K percentages are presented in Table (4). From the results in such Table revealed that with increasing all levels of salinity decreased significantly N, P and K concentrations in relative to the other treatments specially level of 3600 ppm, which gave the highest significant N, P and K%. Meanwhile, 7200 ppm had more adverse effect on such elements than other treatment 3600 and 5400 ppm during both seasons. Plants salinized with 3600 ppm recorded the highest mean values of (2.06, 0.312 & 1.85) and (2.18, 0.323 & 1.99) for N, P and K during first and second seasons, respectively.

Table 4. Effect of salinity levels and some foliar application as well as their interaction on N, P and K percentages of neem leaves during 2017/2018 and 2018/2019 seasons.

Treatments		N%		P%		K%	
		1st	2nd	1st	2nd	1st	2nd
Salinity levels							
Tap water		1.83	1.95	0.225	0.237	1.74	1.79
3600 ppm		2.06	2.18	0.312	0.323	1.85	1.99
5400 ppm		1.61	1.73	0.160	0.171	1.53	1.61
7200 ppm		1.46	1.53	0.129	0.145	1.48	1.57
LSD at 5%		0.03	0.02	0.003	0.005	0.04	0.04
Foliar application							
Control		1.46	1.55	0.131	0.142	1.38	1.44
Yeast		1.70	1.82	0.170	0.178	1.67	1.76
Salicylic		1.75	1.84	0.195	0.207	1.70	1.78
Licorice		1.84	1.96	0.258	0.275	1.75	1.86
Proline		1.96	2.07	0.281	0.294	1.78	1.88
LSD at 5%		0.03	0.03	0.004	0.005	0.04	0.03
Interaction							
Tap water	Control	1.39	1.51	0.117	0.128	1.41	1.45
	Yeast	1.79	1.91	0.193	0.203	1.73	1.81
	Salicylic	1.88	1.96	0.201	0.208	1.81	1.85
	Licorice	1.98	2.11	0.303	0.328	1.88	1.90
	Proline	2.10	2.25	0.312	0.318	1.89	1.95
3600 ppm	Control	1.77	1.89	0.187	0.193	1.72	1.79
	Yeast	1.90	2.07	0.211	0.217	1.81	1.89
	Salicylic	1.96	2.08	0.273	0.287	1.83	1.94
	Licorice	2.18	2.31	0.407	0.422	1.91	2.17
	Proline	2.47	2.53	0.483	0.497	2.00	2.17
5400 ppm	Control	1.38	1.47	0.112	0.123	1.20	1.28
	Yeast	1.64	1.73	0.147	0.153	1.60	1.68
	Salicylic	1.65	1.75	0.171	0.186	1.61	1.69
	Licorice	1.67	1.82	0.183	0.197	1.62	1.69
	Proline	1.72	1.88	0.187	0.197	1.64	1.71
7200 ppm	Control	1.28	1.33	0.107	0.122	1.19	1.24
	Yeast	1.46	1.55	0.127	0.139	1.52	1.64
	Salicylic	1.50	1.57	0.133	0.148	1.55	1.65
	Licorice	1.51	1.59	0.137	0.151	1.57	1.66
	Proline	1.54	1.61	0.142	0.165	1.59	1.67
LSD at 5%		0.06	0.06	0.008	0.009	0.07	0.07

For foliar application effects, data in Table (4) suggested that application of various forms of foliar application which resistance salinity as (proline, licorice, salicylic and yeast) caused significant increase in N, P and K% over than untreated plants. It is noticed from the results that N, P and K% was depended on the exogenous concentrations of proline resulted in higher significant N, P and K % of (1.96 and 2.07% N), (0.281 and 0.294% P) and (1.78 and 1.88% K) in the two seasons, respectively. In the same line licorice root extract followed proline in higher values of N, P and K concentration. On the other hand, untreated plants recorded lower significant values regard N, P and K % of (1.46 and 1.55% N), (0.131 and 0.142% P) and (1.38 and 1.44% K) for both seasons, respectively.

Regarding interaction effects, data in Table (4) indicated that the different combination between salinity levels and forms of foliar application had different significant effects on leaf N, P and K% in both seasons. Whereas, salinized plants by 3600 ppm and sprayed with proline recorded the highest significant N, P and K%. Such treatment resulted in (2.47 and 2.53% N), (0.483 and 0.497% P) and (2.00 and 2.17% K) for both seasons, respectively. Contrast application of 7200 without spraying by any foliar forms gave lower significant N, P and K % of (1.28 and 1.33% N), (0.107 and 0.122% P) and (1.19 and 1.24% K) in the two seasons, respectively.

The harmful effect of salinity on NPK% may be owing to that elevated Na^+ and Cl^- concentration in the soil effect the absorption of many essential nutrients (e.g N, P, K, Ca, Mg) (Iqbal *et al*., 2015), this occurs through competitive interactions effecting ionic selectivity of cell membranes (Stoeva and Kaymakanova, 2008). Also, an increase in salinity in soil water reduces water uptake, water use efficiency and relative water content (Howladar, 2014) and inhibits of K, Ca and NO_3 uptake by plant roots (Howladar and Rady, 2012). Likewise, Ruhan *et al.* (2004) mentioned that under saline stress most plants are unable to discriminate between K^+ and Na^+ and accumulate high levels of Na^+ to the detriment of K^+ leading to loss of function of K^+. Similarly, Nouman *et al.* (2012) on *Moringa oleifera*, Ali *et al.* (2013) on jojoba and Ashour and Abdel Wahab (2017) on *Jatropha integerrima.*

As for the enhancing by proline, these results may be due to that proline has important roles in enhancing water uptake and might regulate mineral nutrients uptake (Ali *et al.*, 2008). While, as for the effect of licorice may be due to its role in increasing of endogenous hormones like GA_3 in treated plants which increased the metabolic processes role and its effect on mineral content in tissue (Thanaa *et al.*, 2016 on almond and on onion by Ghaloom and Faraj, 2012). Similar findings were found by Talat *et al.* (2013) on wheat, Butt *et al.* (2016) on two chilli genotypes and Alotaibi *et al.* (2019) on jojoba.

6. Ca, Mg and Na percentages:

Results in Table (5), revealed the effect of salinity level stress on Ca, Mg and Na% of neem. Its clear from the data that with increasing salinity levels, all of Ca, Mg increased at 3600 ppm then decreased with increasing salinity levels, whereas, Na% significantly increased. So, applied salinity levels over 3600 ppm decreased both of Ca and Mg but applied 7200 ppm salinity gave the highest values of Na% followed by 5200 then 3600 ppm and decreased with the control treatments. The higher significant Ca, Mg inidacted with 3600 ppm and Na% with 7200 ppm were (3.46 & 3.49% Ca), (0.69 & 0.79% Mg) and (0.61 & 0.70% Na), respectively, during two seasons of the experiment.

As for the effect of foliar application by different resistance salinity as (proline, licorice, salicylic and yeast), data at the same Table indicated that Ca, Mg and Na% of neem significant increased with all treatment used during both seasons. Application of proline alleviated the harmful effect of salinity on Ca, Mg and Na% of neem and improve its concentration due its positive effect and recorded the highest values comparing with the untreated plants during both seasons of the experiments.

The interaction effect between salinity levels and foliar application on Ca, Mg and Na% of neem at the same presented

in Table (5). It could be observed that using different forms of resistance salinity under any level of salinity leads to decrease concentration of Ca and Mg but increase concentration of Na% comparing to the untreated plants. The highest mean values were recorded with 3600 and 7200 ppm salinity and foliar application by proline during both seasons, respectively, for Ca, Mg and Na comparing with control, which recorded the lowest values.

Table 5. Effect of salinity levels and some foliar application as well as their interaction on Ca, Mg and Na percentages of neem leaves during 2017/2018 and 2018/2019 seasons.

Treatments		Ca%		Mg%		Na%	
		1st	2nd	1st	2nd	1st	2nd
Salinity levels							
Tap water		2.95	3.05	0.55	0.64	0.28	0.36
3600 ppm		3.46	3.49	0.69	0.79	0.34	0.43
5400 ppm		2.73	2.81	0.42	0.53	0.47	0.56
7200 ppm		2.59	2.65	0.36	0.48	0.61	0.70
LSD at 5%		0.03	0.03	0.02	0.03	0.02	0.02
Foliar application							
Control		2.44	2.49	0.29	0.40	0.23	0.32
Yeast		2.89	2.94	0.51	0.62	0.43	0.52
Salicylic		2.93	3.02	0.54	0.65	0.46	0.55
Licorice		2.99	3.06	0.59	0.69	0.48	0.57
Proline		3.42	3.49	0.61	0.71	0.53	0.62
LSD at 5%		0.04	0.04	0.02	0.03	0.02	0.03
Interaction							
Tap water	Control	2.53	2.62	0.33	0.43	0.21	0.30
	Yeast	2.93	3.02	0.52	0.62	0.26	0.35
	Salicylic	2.99	3.17	0.55	0.64	0.30	0.37
	Licorice	3.09	3.19	0.68	0.75	0.31	0.39
	Proline	3.19	3.25	0.69	0.78	0.32	0.40
3600 ppm	Control	2.6	2.67	0.35	0.47	0.22	0.32
	Yeast	3.21	3.23	0.71	0.8	0.33	0.43
	Salicylic	3.28	3.29	0.75	0.87	0.35	0.44
	Licorice	3.32	3.36	0.81	0.9	0.39	0.46
	Proline	4.87	4.9	0.84	0.92	0.42	0.50
5400 ppm	Control	2.41	2.43	0.24	0.35	0.23	0.33
	Yeast	2.8	2.83	0.43	0.55	0.47	0.53
	Salicylic	2.81	2.89	0.45	0.56	0.49	0.59
	Licorice	2.82	2.91	0.47	0.57	0.51	0.61
	Proline	2.83	2.97	0.49	0.6	0.64	0.73
7200 ppm	Control	2.2	2.24	0.22	0.34	0.25	0.34
	Yeast	2.63	2.69	0.37	0.49	0.66	0.75
	Salicylic	2.64	2.71	0.39	0.52	0.67	0.77
	Licorice	2.72	2.79	0.4	0.53	0.70	0.82
	Proline	2.78	2.82	0.42	0.54	0.74	0.83
LSD at 5%		0.08	0.07	0.04	0.07	0.04	0.05

This finding may be due to the excessive Ca, Na, Cl and Mg ions in the soil from application salinity. Also, Na^+ acts on the activation of a wide range of enzymes in plants, as involved in membrane osmosis and can also replace K^+ in some osmotic and metabolic functions. Cl^- plays an important role in photosynthesis, enzyme activation, osmotic regulation and cell division (Ashari and Gholami, 2010). Similar findings were found by Nouman *et al.* (2012) on *Moringa oleifera*, Ashour and Abdel Wahab (2017) on *Jatropha integerrima*, Rahneshan *et al.* (2018) on *Pistacia vera* L. and Jahan *et al.* (2018) on neem.

Application of proline induces high levels of antioxidants, accumulation of certain organic osmolytes and reducing the toxic ions (Na^+ and Cl^-) but increase water uptake and might regulate mineral nutrients uptake (Ali *et al.*, 2008). These results matched with those of Khan *et al.* (2015) on Okra and Butt *et al.* (2016) on two chilli genotypes.

CONCLUSION

The findings of this study show valuable information regarding plant pigments and chemical constituents performance of important medicinal tree species in different saline treatments, which may be useful to introduce neem plantation in the saline affected areas in presence of some anti-salinity as proline which gave good and highest values of all traits followed by licorice extract. However, based on the findings of the study it can be advocated that on-farm investigation should be conducted in real field conditions of saline prone area and spray with proline to confirm the performance of neem.

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تأثير الرش ببعض المواد المقاومه للملوحه على المحتوى الكيميائى لنباتات النيم تحت ظروف الملوحه

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أجريت هذه الدراسه خلال الموسمين المتتاليين 2017-2018 و 2018-2019 في مشتل خاص بمدينه دمياط الجديدة ، محافظه دمياط ، مصر. في تصميم قطع منشقة مرة واحدة في 3 مكررات لدراسه تأثير مستويات الملوحه (معاملة المقارنة (ماء الصنبور)، 3600، 5400 و 7200 جزء في المليون) كقطاعات رئيسيه و بعض مواد الرش (ماء مقطر، حمض السالسليك، برولين، مستخلص خميره و مستخلص عرق السوس) في القطاعات المنشقه و التفاعل بينهم على التركيب الكيميائي لنبات النيم. أوضحت النتائج تحت الدراسه الى انه بزياده مستويات الملوحه أدى الى نقص معنوي فى محتوى النبات من (صبغات التمثيل الضوئى ،السكريات المختزلة و الغير مختزلة وكذلك المحتوى المعدني (النيتروجين، الفوسفور، البوتاسيوم، الكالسيوم والماغنسيوم) لأوراق النيم، ولم يعاني النبات مع مستوي ملوحة 3600 جزء في المليون بينما ازداد محتوى النبات من الصوديوم و الفينول والبرولين مع مستوى 7200 جزء في المليون. بالنسبه للمواد المستخدمه لمقاومه الملوحه في صوره رش ورقي يعتبر البرولين يليها مستخلص عرق السوس افضلها في المقاومه و سجل لجميع الصفات السابقه اعلى معدل. التفاعل المشترك بين المعاملات بالرش والملوحه أوضح أن افضل نتيجه سجلت عند 3600 جزء في المليون لجميع الصفات ماعدا الصوديوم و الفينول و البرولين التي سجلت عند اعلى مستوى ملوحه 7200 جزء في المليون والرش بالبرولين بتركيز 2. جرام / لتر.