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## Seedling priming and foliar spray of sodium nitroprusside role in alleviating the adverse impact on wheat irrigated by different Mediterranean Sea salts dilutions

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This study investigates the best strategy of applying sodium nitroprusside, the nitric oxide donor, in concentration 150  $\mu$ M either seedling priming or foliar spray to mitigate the adverse effect exerted on wheat plants irrigated by diluted concentrations of Mediterranean sea salts. Pot experiment was carried out during seasons (2015/2016-2016/2017). It designed a two-factorial factors experiment in a layout of RCBD. Twelve treatments composed of four salinity levels as a main factor (Control, 2000 ppm, 4000 ppm, 6000 ppm), and three sub-factors; Control, which sprayed by Tween 20, sodium nitroprusside in conc. 150  $\mu$ M, used as priming (SNP-P) and foliar spraying (SNP-S). Results clearly indicated the adverse effect of increasing salinity on all studied growth parameters and yield. Meanwhile, biochemical components; total sugars, total soluble phenols, total free amino acid and free proline, antioxidant capacity and antioxidant enzymes activity were increased by salinity. Applying sodium nitroprusside (SNP), either seedling priming or foliar spray enhanced all the previous when compared with control untreated plants. Additionally, it enhanced chlorophyll b, which increases photo-capture efficiency under stress. This study recommends applying SNP-S on plants, which irrigated by 4000 ppm of Mediterranean Sea salts, which showed enhancing growth, yield, biochemical components, antioxidant capacity and antioxidant enzymes in wheat roots, leaves, stalks and spikes. In addition, there is no significant different between recommended treatment and plants irrigated by non-saline or 2000 ppm salinity level.

**Keywords:** Wheat, growth, chlorophylls, antioxidant capacity, antioxidant enzymes, Sea salts, biochemical components, yield, sodium nitroprusside, nitric oxide.

### INTRODUCTION

Wheat is one of the oldest and most important cereal crop in Egypt, which plays a special role in people's nutrition, trading, economy and country politically, Al-Naggar et al., (2015). Although wheat productivity in Egypt has increased during the past years, wheat production satisfies only 45% of its annual domestic demand. Egypt still is one of the largest countries that import wheat. Exploiting and increasing production is necessary

to reduce the gap between production and consumption of many crops like wheat, and this agreed by Al-Ashkar and El-Kafafi (2014). Nowadays, Classical breeding in Egypt was able to develop some bread wheat cultivars, such as Sakha 8 and Sakha 93 of higher salinity tolerance than other commercial cultivars. Sodium nitroprusside  $[\text{Fe}(\text{CN})_3\text{NO}]^- \text{Na}^+$  (SNP) has been known since 1850. It is neither a nitro compound nor a prusside but the trivial name has been

widely adopted. Under appropriate conditions, SNP can act as a donor of NO or of NO<sup>+</sup> and can deliver cyanide ion (Williams, 2004). Nitric oxide is an easily diffused bioactive and signal transmitting molecule that directly regulates many plant functions including germination, leaf expansion, root growth, stress physiology, and sequential cell death (Shamsul et al., 2010). This molecule also participates in the adaptation of plants to environmental stresses, working as the key signal carrier in defense response. Recent studies have shown that nitric oxide imparts synergistic effects with phyto-hormones in physiological regulation and signal transmission. Nitric oxide is known having multidiscipline in mitigate abiotic stress in plants. It reduced the adverse impact generated by oxidative stresses, enhancing the antioxidant enzymatic activities and antioxidant capacity to scavenger ROS species. Salinity problems and water scarcely are global issues, which attracts scientist's attention for seeking on alternatives that would counteract salinity and water shortage problems. Using seawater directly in agriculture, industry or human needs has several difficulties. In addition, reclamation of new land has obstacles. Thus, this article aimed to study the physiological, biochemical and growth response of wheat as a strategic crop in Egypt, which is irrigating by diluted seawater, and the study investigates the mitigating effect exerted by applying sodium nitroprusside, the nitric oxide donor either by seedling priming or by spraying it on plants.

## MATERIALS AND METHODS

This experiment was carried out and repeated during the two successive seasons of 2015/2016 and 2016/2017 at the greenhouse of Plant Physiology Division, Faculty of Agri., Cairo University. Sand culture experiment was designed in a two-factorial factors experiment in a layout of RCBD, and ebb and flow design. Pots of 10 cm diameter, filled with pre-washed sands. The experiment had 12 treatments composed of four salinity levels as a main factor (Control, 2000 ppm, 4000 ppm, 6000 ppm), and three treatments; Control, which sprayed by water and Tween 20. SNP-P; Priming by 150 µM sodium nitroprusside (SNP) and SNP-S; spraying by 150 µM SNP and Tween 20. Sakha93, an Egyptian wheat cultivar obtained from Agricultural Research Center, Giza, Egypt. Grains were vernalized and sowed at 15 November in each season. Some germinated grains were primed in 150 µM sodium nitroprusside for 12 hours prior

sowing. Sodium nitroprusside used in experiment obtained from Bayer Company. Mediterranean seawater salts were prepared by El Nasr Salines Co., which evaporating Mediterranean seawater that was withdrawn from the sea at depth of 25 km, in El-Hammam village in the North Coast of Egypt. Irrigated solution was a mixture of half-strength Hoagland ingredients (Hoagland and Arnon, 1950) and dilutions of Mediterranean seawater salts were undergone chemical analysis, in which its composition has shown in Table 1. Soil was sampled and undergone to analysis, which were randomly taken each season before cultivation, and were subjected for physical and chemical analysis according to Jackson (1967). The mean values for both seasons of the soil mechanical and chemical analysis were illustrated Table 2.

## Data recorded

In both two successive seasons, sampling was represented each treatment at different physiological ages; booting and anthesis, which were collected randomly at 15 Jan (60 days from sowing) and 1 March (105 days from sowing), respectively. Then the grains and straws were harvested at starting of May (165 days from sowing) of each year. Three plants were collected at each sampling time for studying the growth parameters and three other plants were sampled for chemical analysis. In addition, samples were collected for enzymatic assay. Plant growth parameters obtained are shoot height (cm) and root length (cm), shoot and root dry weights (g/plant), shoot: root dry weight ratio, No. of branches/plant, total leaves area and flag leaf area were determined. Yield and its parameters such as spike number, spike weight, spike height, spikelet number, straw weight, grain weight, grain number, 1000-grain weight were calculated. Chemical analysis; photosynthetic plant pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids) were determined using dimethylformamide according to Moran (1982). In ethanol extract, total sugars, total free amino acids, total soluble phenols and total antioxidant capacity in roots, leaves, stalk, spikes are determined are determined in ethanol extract, on fresh basis and expressed as mg/g F.w. Total sugars determination was carried out by using the phenol-sulphuric method according to Dubois et al., (1956). Total soluble phenols were estimated using the folin-Ciocalteu colorimetric method (Swain and Hillis (1959).

**Table 1. Chemical composition of Mediterranean Sea Salts performed by El-Nasir Saline Co.**

Moisture %	Insoluble solids %	Ca(HCO <sub>3</sub> ) <sub>2</sub> %	CaSO <sub>4</sub> %	MgSO <sub>4</sub> %	NaCl % (Wet)
6.300	0.260	0.097	0.625	0.345	90.232

**Table 2. Mechanical and chemical analysis of the soil experimental site**

Particle size distribution:	Sand %: 88.8	Silt %: 2.0	Clay % 9.2	Texture class: Sand			
<b>Chemical analysis</b>							
E.C dS /m 0.65	Soluble anions (meq/l)			Soluble cations (meq/l)			
pH 7.4	HCO <sup>-</sup> 1.60	Cl <sup>-</sup> 2.3	SO <sub>4</sub> <sup>-2</sup> 2.8	Na <sup>+</sup> 2.6	K <sup>+</sup> 0.80	Ca <sup>+2</sup> 2.20	Mg <sup>+2</sup> 1.10

The total free amino acids were determined using Ninhydrin reagent according to Moore and Stein (1954). Non-enzymatic antioxidant capacity was determined using phosphomolybdenum method by Prieto et al., (1999). Proline was determined by Ninhydrin reagents to Bates et al., (1973). Antioxidant enzymes activities were determined in crude enzyme extract by Chance and Maehly (1955).

Catalase activity, CAT (EC 1.11.1.6) was estimated using the method described by Sinha (1972). Peroxidase activity, POX (EC 1.11.1.7) was determined according to the method of Herzog and Fahimi (1973). Superoxide dismutase activity, SOD (EC 1.15.1.1) was assayed according to the method of Beauchamp and Fridovich (1971). Phenyl alanine lyase activity, PAL (EC: 4.3.1.5) was determined by Brueske (1980). Polyphenol oxidase activity, PPO (EC 1.14.18.1) was assayed by the method described by Galeazzi et al., (1981) and Liu et al., (2005). They expressed as U mg<sup>-1</sup>P min<sup>-1</sup>. Total soluble proteins were estimated according to Lowry-Folin as described by Dawson et al., (1986), expressed mg g<sup>-1</sup>F.w..

#### Statistics analysis:

Data collected were subjected to the proper statistical analysis of variance of combined two factorial factor design, RCBD layout, according to the procedures outlined by Snedecor and Cochran (1980). Combined data were analyzed, as both seasons (2015/2016-2016/2017) had same trend and homogenized. LSD at 5% level of significance was used to compare means of treatments. All statistical analysis was performed by using analysis of variance technique of (MStat-C, 1989) Computer software package.

## RESULTS AND DISCUSSION

The effect of irrigation using four dilutions of

Mediterranean seawater levels on wheat growth parameters at both booting and anthesis stages were studied in Table 3 and Table 4. Results showed a similar trend in all growth parameters,

which decreased with increasing salinity level in both growth stages except in root dry weight at booting stage and number of leaves in both stages, with respect of salinity effect. All these mentioned parameters had an inversely trend with increasing salinity levels in both stages except 2000 ppm, which showed a reverse significant trend, which showed an increase in all parameters in anthesis stage, and non-significant increase in total leaves area when compared with non-saline treatments. Our results confirms plant accommodates with increasing salinity until reaching 2000 ppm, which could considered having an encouraging-like effect and the same trend was found by Hanafy Ahmed et al., (2008). This evidence explains our results, which found a significant highest record of shoot/root ratio in plants treated by 2000 ppm. It is well known that increasing salinity was found to reduce shoot: root ratio as found by Ali Turan et al., (2010), which is in contradictory with our result. This point explains the dry matter partitioning was directed towards plant shoot rather than plant roots, which confirms that 2000 ppm has an encouraging effect when used in well-balanced salts combination, whereas, increasing salinity to 4000 and 6000 ppm significantly reduced shoot/root ratio. All mentioned parameters in plants irrigated by 6000 ppm recorded the lowest values at booting stage and significantly reduced at anthesis stage, except root dry weight, which had the highest significant record in plants irrigated by 4000 and 6000 ppm at booting stage. Then it significantly reduced at anthesis stage. Increasing root dry weight at first stage would to enhance plant absorption system to enhance plant tolerance to stress, then at the second stage, while dry matter directed towards shoot and floral growth.

**Table 3. Shoot height and root length (cm), flag leaf area, total leaves area (cm<sup>2</sup>) and No. of leaves of wheat at booting and anthesis stages subjected to four salinity levels (control, 2000, 4000, 6000 ppm) and treated by 150 µM sodium nitroprusside either seedling priming or foliar spray (Combined seasons 2015/2016-2016/2017).**

Growth stages Salinity Treatments	Booting				Mean	Anthesis				Mean	
	Tap Water	2000 ppm	4000 ppm	6000 ppm		Tap Water	2000 ppm	4000 ppm	6000 ppm		
<b>Shoot height (cm)</b>											
Control	45.83	47.00	46.33	39.25	44.60	55.92	59.75	52.00	39.92	51.90	
SNP (Priming)	49.50	45.60	44.84	42.75	45.67	53.68	55.58	46.05	45.73	50.26	
SNP (Spraying)	54.00	48.67	46.55	46.67	48.97	61.67	59.42	53.50	48.17	55.69	
Mean	49.78	47.09	45.91	42.89		57.09	58.25	50.52	44.61		
L.S.D. at 5%	A= 1.90		B=1.65		AB=3.31		A=2.70		B=2.34		AB=4.69
<b>Root length (cm)</b>											
Control	26.68	23.48	23.17	22.58	23.98	22.50	21.10	24.67	26.68	23.48	
SNP (Priming)	20.58	19.68	21.53	23.75	21.39	22.09	25.18	23.80	20.58	19.68	
SNP (Spraying)	27.67	28.01	24.90	25.33	26.48	24.56	23.50	21.88	27.67	28.01	
Mean	24.98	23.73	23.20	23.89		23.05	23.26	23.45	24.98		
L.S.D. at 5%	A= ns		B=1.85		AB=ns		A=1.88		B=ns		AB= 3.256
<b>No of leaves</b>											
Control	6.33	7.17	7.50	7.58	7.15	7.00	6.00	5.67	5.50	6.04	
SNP (Priming)	7.60	7.28	6.65	7.28	7.20	6.18	3.80	6.33	3.80	5.03	
SNP (Spraying)	7.00	8.33	7.67	8.67	7.92	6.58	5.25	5.83	5.33	5.75	
Mean	6.98	7.59	7.27	7.84		6.59	5.02	5.94	4.88		
L.S.D. at 5%	A=0.56		B=0.49		AB=0.97		A=0.67		B=0.58		AB=1.16
<b>Flag leaf area (cm<sup>2</sup>)</b>											
Control	10.87	7.14	7.68	8.73	8.60	24.38	13.45	11.60	6.49	13.98	
SNP (Priming)	26.23	14.20	17.46	13.51	17.85	11.60	18.32	9.57	16.68	14.04	
SNP (Spraying)	18.04	15.87	12.54	12.72	14.79	20.35	16.60	18.54	5.28	15.19	
Mean	18.38	12.40	12.56	11.65		18.78	16.12	13.23	9.48		
L.S.D. at 5%	A=3.601		B=3.119		AB=ns		A=4.14		B=ns		AB=7.17
<b>Total leaves area (cm<sup>2</sup>)</b>											
Control	53.29	51.81	51.11	48.56	51.19	68.26	61.11	57.08	66.53	63.25	
SNP (Priming)	100.09	81.76	68.75	73.66	81.06	51.11	73.82	61.39	74.93	65.31	
SNP (Spraying)	93.24	89.40	86.90	99.68	92.30	78.54	70.76	93.06	63.26	76.41	
Mean	82.21	74.32	68.92	73.97		65.97	68.56	70.51	68.24		
L.S.D. at 5%	A=ns		B=15.07		AB=ns		A=ns		B=3.86		AB=7.73

This result was similar to Puvanitha and Mahendran (2017) who found highest root dry weights at vegetative, reproductive, ripening stages of rice exposed to salinity. Bernstein et al., (2002) mentioned that root growth is usually less sensitive to salt stress than shoot growth, whereas an increased root/shoot ratio was often observed in saline treated plants.

Maggio et al., (2001); Omami and Hammes (2006) declared that lower root/ shoot ratio observed in salinized plants may have been functionally associated with the need of salt-stressed plants to restrict the uptake of toxic ions to the shoot while still maintaining high turgor and a positive growth rate. Additionally, they found that root /shoot ratio was higher in plants treated by drought stress more than salinity stress. The reduction induced by salinity in these results was attributed to the dual adversely effects, osmotic and ionic effects. The osmotic stress induced due to presence of salts in soil solution, which hinder plant roots ability to withdraw water effectively showing initial stress effect on plant in form of growth reduction. An experiment proved that soil salt concentration of 4 dSm<sup>-1</sup> or 40 mM NaCl has an osmotic pressure of about 0.2 MPa, which mentioned by Shabala and Munns (2012). This osmotic effect on roots reduces cell turgor, volume and elongation, subsequently cell division.

This reduction in turgor pressure increases the sensitivity to mechanical stimulation towards mechano sensitive ion channels. In addition, cells regain their original volume and turgor owing to osmotic adjustment within hours also confirmed by Shabala and Munns (2012), cell elongation rates are reduced and smaller leaves area, which is similar to our results. Additionally, Our results agreed by Omami and Hammes (2006); Abbas et al., (2018).

Shabala and Munns (2012) who declared under moderate salinity stress, an inhibition of lateral shoot development becomes apparent over weeks, and over months. During this time, a number of older leaves may die. However, production of younger leaves continues. All these changes in plant growth are responses to the osmotic effect of the salt, and are similar to drought responses. This confirms our results in reducing number of leaves between two stages; booting and anthesis stage.

Concerning the effect of applying 150 µM sodium nitroprusside, either foliar spray or seedling priming on plant growth parameters, resulted revealed that all parameters were increased in plants treated by sodium nitroprusside by both methods when compared with control.

**Table 4. Shoot and root dry weights (g/plant) and shoot: root dry weight ratio of wheat at booting and anthesis stages subjected to four salinity levels (control, 2000, 4000, 6000 ppm) and treated by 150 µM sodium nitroprusside either by seedling priming or foliar spray (combined seasons 2015/2016-2016/2017).**

Growth stages Salinity Treatments	Booting				Mean	Anthesis				Mean
	Tap Water	2000 ppm	4000 ppm	6000 ppm		Tap Water	2000 ppm	4000 ppm	6000 ppm	
<b>Shoot dry weight (g)</b>										
Control	0.570	0.612	0.578	0.468	0.557	1.398	1.562	1.119	0.604	1.171
SNP (Priming)	0.643	0.677	0.725	0.583	0.657	1.301	1.465	1.133	0.999	1.224
SNP (Spraying)	0.708	0.741	0.685	0.630	0.691	1.706	1.449	1.451	0.866	1.368
Mean	0.641	0.677	0.663	0.560		1.468	1.492	1.234	0.823	
L.S.D. at 5%	A=0.06	B=0.05		AB=ns		A= 0.15		B=0.13	AB= 0.26	
<b>Root dry weight (g)</b>										
Control	0.447	0.390	0.368	0.327	0.383	0.529	0.505	0.459	0.365	0.465
SNP (Priming)	0.175	0.185	0.266	0.275	0.225	0.292	0.295	0.236	0.196	0.255
SNP (Spraying)	0.442	0.305	0.455	0.539	0.435	0.471	0.626	0.576	0.530	0.551
Mean	0.355	0.293	0.363	0.380		0.431	0.475	0.424	0.363	
L.S.D. at 5%	A= 0.05	B= 0.04		AB= 0.08		A= 0.07		B= 0.06	AB= ns	
<b>Shoot / Root dry weight</b>										
Control	1.28	1.57	1.57	1.43	1.45	2.64	3.09	2.44	1.66	2.52
SNP (Priming)	3.68	3.67	2.73	2.12	2.92	4.45	4.98	4.79	5.10	4.81
SNP (Spraying)	1.60	2.43	1.51	1.17	1.59	3.62	2.32	2.52	1.63	2.48
Mean	1.81	2.31	1.83	1.47		3.41	3.14	2.91	2.26	
L.S.D. at 5%	A=0.356	B=0.310		AB=0.616		A=0.57		B=0.49	AB=ns	



Plant treated with foliar spray of 150  $\mu\text{M}$  SNP found to be superior on primed seedling plants in all parameters except shoot: root dry weight ratio. In details, plants which foliar sprayed by 150  $\mu\text{M}$  SNP, were significantly recorded the highest shoot and root length, shoot and root dry weights, number of leaves as well as flag leaf area and total leaves area in booting stage. In anthesis stage, a similar trend observed between both treatments, foliar spray of SNP significantly increased all parameters except root length and flag leaf area. Seedling priming plants recorded the significant records in shoot : root dry weight ratio. These results confirmed that sodium nitroprusside has a promoting role on growth parameters, whatever, applied as foliar sprayed or seedling primed, whereas the former was found to be superior at concentration of 150  $\mu\text{M}$ . Our result was approved by Qasim et al., (2017) who declared the reasons beyond promoting effect is improving seed vigor and germination and early establishment of seedlings with better growth. Nitric oxide (NO) is known to have a promoting role on plant growth and molecules associated with many biochemical and physiological processes under biotic and abiotic stresses. NO was classified as a phyto-hormone that might function as a gaseous endogenous or exogenous plant growth regulator. Nitric oxide regulates root organogenesis, hypocotyl growth, defense responses, stomatal movement, apoptosis, hypersensitive responses, growth and development, and phyto-alexin production.

Shamsul et al., (2010) added that nitric oxide found to have effect on plant growth parameters through multiple ways, preserving and increasing the chlorophyll content, photosynthesis and it was found auxin indole acetic acid (IAA) and NO might share some common steps in the signal transduction pathway because both elicit the same responses in plants. Evidence in rapidly growing pea seedlings showed lower concentrations of NO increased the rate of leaf expansion. NO also activated the growth of root segments of maize comparable to that by indole acetic acid. Although SNP (0.1 mM) inhibited growth of hypocotyls in potato, lettuce, and Arabidopsis, it induced root development in cucumber. The effect of NO on plant growth was found to be concentration dependent. Treating maize seedlings with lower concentration of SNP promoted root growth whereas higher concentration was inhibitory.

The combinational effect of both salinity levels and sodium nitroprusside applied in both ways

showed that a fluctuate trends among studied parameters. It was concluded that plants treated by foliar spray of 150  $\mu\text{M}$  SNP and irrigated by 4000 ppm recorded an increase in all parameters when compared with either control plants irrigated by 4000 ppm or saline untreated plants in both growth stages. Finally, plants, which treated by 4000 ppm and foliar spray 150  $\mu\text{M}$  SNP showed the highest significant records in total leaves area among all other treatments. These results indicating that promoting effect initiated by foliar spray of sodium nitroprusside applied in concentration of 150  $\mu\text{M}$  exploited wheat growth under irrigation using diluted Mediterranean Sea salt in concentration of 4000 ppm.

#### **Chemical components:**

Chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b and carotenoids were studied in Table 5. Results showed a similar trend in all studied plant pigments in both growth stages, with respect of salinity effect. It was found that total chlorophyll, chlorophyll a and b as well as carotenoids concentration had an inversely trend with increasing salinity, with respect the effect of salinity, except under 2000 ppm which showed a reversely significantly increasing trend in all pigments concentrations in both growth stages. These results were resembled to those found in growth parameters. All mentioned plant pigments showed a significant reduction under 6000 ppm in both growth stages, whereas it showed significantly highest records in plants irrigated by 2000 ppm. These results were similar to Hanafy Ahmed et al., (2008) who found the same trend at same salinity level on wheat. Salinity effect on chlorophyll, photo system were studied by many investigators, Downton et al., (1985) found some morphological changes and functional consequences in stressed spinach plants like thicker leaves and chlorophyll per unit area. Richardson and McCree (1985) mentioned that salinity stress has same inhibitory effect on photosynthesis like drought by closing stomata and inhibiting  $\text{CO}_2$  fixation process and inhibiting electron transport through photosynthesis. Giardi et al., (1997) mentioned that electron transport measurements indicated a reduction was found in electron transport in photosystem II and across electron transport chain and fatty acid and lipid composition of thylakoid membranes, which influence photosynthesis process, are affected by salinity stress. Masojidek et al., (1991) mentioned that PSII polypeptides damage might occur due to generation of ROS species and consequently

resulting in degradation of D<sub>1</sub> protein, which can be deduced, from disappearance of PSII core chlorophyll proteins.

Concern the effect of sodium nitroprusside on pigments concentrations, data showed that total chlorophyll, chlorophyll a and b as well as carotenoids concentrations were significantly recorded the highest and the lowest values in both stages in plants foliar sprayed by SNP and control plants, respectively. Applying 150 µM sodium nitroprusside in form of foliar spraying was significantly superior to seedling priming in recording pigments concentrations in both stage. Several studies indicated a vital relationship between NO and chlorophyll and photosynthesis in general, mainly under stress. Mehar and Khan (2014) study confirmed chlorophyll completely reverting after being decayed due to Cd stress when treated by NO donor for 7 days. Addition study performed by Bonab et al., (2015) who approved the protective role of nitric oxide, mentioning an important feature for NO. NO has a dual role as a powerful oxidant and an effective antioxidant, and this vital role depends on concentration and site of action. The protective role is stated as being interacts with lipid hydroperoxyl radicals or high activation of superoxide, which both increase lipid peroxidation as well as promoting stomatal closure. Besides, reducing the ROS damaging effect. This preventing the chlorophyll disintegration and injury to membranes like thylakoids membrane, by preventing the increase in thiobarbituric acid reactive substances content, in addition maintaining the balance in the PS II complex proteins. Studied mentioned by Shamsul et al., (2010) who demonstrated that NO donors (SNP) have been found to enhance chlorophyll concentration in potato, lettuce, and Arabidopsis, and mentioned its protective and preserving role is attributed on its NO effects on iron availability. They mentioned that NO treatment increased the chlorophyll concentrations in maize leaves up to the control level.

Meanwhile, foliar sprayed plants and irrigated by 4000 ppm showed the highest significant chlorophyll concentration in booting stage. In addition, they recorded the highest values at anthesis stage. These plants were significantly highest when comparing with control plants in both stages. Results indicated that plants irrigated by 2000 ppm showed the lowest significantly and lowest records of chlorophyll a/b in booting and anthesis stages, respectively, with respect to salinity effect only. Additionally, foliar sprayed

plants were recorded the lowest significant chlorophyll a/b ratio in both growth stages, when concerning the effect of SNP. Similarly, the combinational effect of both treatments, which foliar sprayed plants and irrigated by 2000 ppm were recorded the lowest chlorophyll a/b in both booting and anthesis stages. Reducing chlorophyll a/b ratio indicated increasing the concentration of chlorophyll b relative to concentration of chlorophyll a, which was performed by discussed previously treatments. Previous studied investigating changes in chlorophyll b concentration and effect on photosynthesis in general through its effect on photosystems I and photosystem II as well as electron transport chain, the vital step in photosynthesis. Xu et al., (2001) added that chlorophyll b could serve as the major pigment in functional photosystem II complexes of cyanobacteria. They demonstrated that chlorophyll b replaces part of chlorophyll a in the PS II core. Moreover, the energy absorbed by chlorophyll b can be used efficiently by the reaction centers and can cause QA reduction. Additionally, the vital role of electron supply to photosystems is mainly provided by PSII in the photosynthesis process, which eventually affecting all the photosynthesis process. Previous studies investigated the effect of different stress.

on chlorophyll b concentration and PSII. Zhang et al., (2016) mentioned that content of chlorophyll increased in studied plants grown under low light stress, which markedly returns to an increase in chlorophyll b. Congming and Zhang (1995) speculated the decrease in photosynthesis under water stress could be associated with the perturbations of the biochemical processes. In particular, PSII has been shown to be very sensitive to water stress. They added that water stress resulted in damage to the oxygen-evolving complex of PSII, which is confirmed by Toivonen and Vidaver (1988) and to the PSII reaction centers too. Reduction in electron supply produced by water splitting in PSII will counteract in a reduction in electron supply, which influence photosynthesis process. Masojidek et al., (1991); Giardi et al., (1997) confirmed a reduction in measurements of electron transport in PSII due to reduction in water splitting driving electrons which inhibits photosynthesis under salinity stress. Zhang et al., (2014) speculated that evidences proves that reducing chlorophyll a/b has a clue on adapting an economic adapting strategy under different situations.

Table 5. Plant pigments, chl. a, b, total chl., ch.a/b and carotenoids (mg/g F.w.) of wheat leaves at booting and anthesis stages subjected to four salinity levels (control, 2000, 4000, 6000 ppm) and treated by 150 µM sodium nitroprusside either by seedling priming or foliar spray (combined seasons 2015/2016-2016/2017).

Growth stages Salinity Treatments	Booting				Mean	Anthesis				Mean
	Tap Water	2000 ppm	4000 ppm	6000 ppm		Tap Water	2000 ppm	4000 ppm	600 ppm	
<b>Chlorophyll a (mg/g F.w.)</b>										
Control	0.582	0.821	0.657	0.414	0.619	0.669	0.485	0.655	0.521	0.583
SNP (Priming)	0.590	0.980	0.607	0.565	0.685	0.561	1.032	0.679	0.642	0.729
SNP (Spraying)	1.017	1.038	0.991	0.781	0.957	1.119	1.152	0.999	0.640	0.978
Mean	0.729	0.947	0.752	0.587		0.783	0.890	0.778	0.601	
L.S.D. at 5%	A=0.09		B=0.08	AB=0.15		A=0.07		B=0.06	AB=0.13	
<b>Chlorophyll b (mg/g F.w.)</b>										
Control	0.198	0.360	0.262	0.143	0.241	0.254	0.183	0.249	0.181	0.217
SNP (Priming)	0.198	0.463	0.241	0.198	0.275	0.179	0.504	0.226	0.229	0.285
SNP (Spraying)	0.490	0.537	0.465	0.350	0.461	0.612	0.656	0.441	0.325	0.508
Mean	0.295	0.453	0.323	0.231		0.348	0.447	0.306	0.245	
L.S.D. at 5%	A=0.06		B=0.05	AB=ns		A=0.05		B=0.05	AB=0.09	
<b>Total chlorophyll (mg/g F.w.)</b>										
Control	0.779	1.181	0.919	0.558	0.859	0.924	0.667	0.904	0.702	0.799
SNP (Priming)	0.788	1.443	0.847	0.763	0.960	0.740	1.536	0.906	0.871	1.013
SNP (Spraying)	1.507	1.575	1.457	1.131	1.417	1.731	1.808	1.440	0.965	1.486
Mean	1.024	1.400	1.074	0.817		1.132	1.337	1.083	0.846	
L.S.D. at 5%	A=0.138		B=0.119	AB=0.239		A=0.114		B=0.099	AB=0.198	
<b>Carotenoids (mg/g F.w.)</b>										
Control	0.305	0.475	0.370	0.234	0.346	0.368	0.307	0.335	0.271	0.320
SNP (Priming)	0.311	0.592	0.369	0.353	0.406	0.306	0.642	0.377	0.393	0.430
SNP (Spraying)	0.628	0.674	0.615	0.448	0.591	0.755	0.797	0.592	0.377	0.630
Mean	0.415	0.581	0.452	0.345		0.477	0.582	0.435	0.347	
L.S.D. at 5%	A=0.06		B=0.05	AB=0.104		A=0.05		B=0.05	AB=0.09	
<b>Chlorophyll a/b (mg/g F.w.)</b>										
Control	2.981	2.436	2.668	2.819	2.726	2.753	2.577	2.695	2.915	2.735
SNP (Priming)	3.040	2.184	2.565	2.848	2.659	3.128	2.108	3.669	2.791	2.924
SNP (Spraying)	2.178	2.033	2.198	2.283	2.173	1.876	1.770	2.270	2.048	1.991
Mean	2.733	2.218	2.477	2.650		2.586	2.152	2.878	2.585	
L.S.D. at 5%	A=0.24		B=0.203	AB=ns		A=ns		B=0.45	AB=ns	



They mentioned two cultivars of *Physocarpus* augmented the synthesis of Chl.b that does not exhibit the property of reaction center, in order to capture more light under a low light intensity.

In addition, they added that this is an economic strategy in adaption of low light intensity. In addition, the Chl.b increase could also help with the absorption of blue-violet light under low light, and this is an adaptive mechanism to low light stress to improve growth of the plants. This declared the influence of stress; low light intensity, water stresses and salt stresses on chlorophyll content and photosystems. Another studies reticulated the vital role of NO on PSII and photosynthesis process. Shamsul et al., (2010) stated Nitric oxide and its donors such as sodium nitroprusside is recognized to differentially regulate the photosynthetic rate. They mentioned that nitric oxide is able to influence the photosynthetic electron transport chain directly. They mentioned that studies proved that PS II is an important site for NO action within PS II complex; important binding sites of NO are the non-heme iron between QA and QB binding sites, YD, Tyr residue of D2 protein, and manganese (Mn) cluster of water-oxidizing complex. NO donor SNAP does not modify the maximal quantum efficiency (Fv/Fm) but inhibits the linear electron transport rate and light-induced pH formation (DpH) across thylakoid membrane, and decreased the rate of ATP synthesis. Moreover, NO donor has also been found to slow down the electron transfer between the primary and the secondary quinone electron acceptor *in vivo*, in a concentration-dependent manner. These evidences proves that reducing chlorophyll a/b has a clue on adapting an economic adapting strategy under different situations. Our treatments, which reduced chlorophyll a/b ratio, were noticed in plants irrigated by diluted Mediterranean Sea water in conc. 2000 ppm and foliar sprayed by 150  $\mu$ M SNP.

In Table 6 and Table 7, total sugars, total soluble phenols, total free amino acids, free proline in both growth stages; booting and anthesis are shown in leaves and roots, spikes and stalks of wheat plants, which subjected to studied treatments. A similar trend was observed in all studied organic components with respect the effect of salinity stress, in which total sugars, total soluble phenols, and total free amino acids and proline were showed to increase with increasing salinity levels in both growth stages, in leaves and roots.

In respect to the effect of salinity, total sugars

and total free amino acids in Table 6 showed a significant increase in both roots and leaves at booting stage, which counteract the salinity increase. Plants irrigated by 4000 and 6000 ppm showed the highest significant records when compared with other treatments. Whereas, at anthesis stage, the significant increase in their concentrations were observed in both roots and leaves, which counteract salinity increase until 4000 ppm, however, a significant reduction was noticed at 6000 ppm in leaves.

The similar trend was observed in total soluble phenols and proline concentration, which showed in Table 7.

Total soluble phenol concentration was increased with increasing salinity until 4000 ppm and a reduction was observed a 6000 ppm irrigated plants when compared with control, in both roots and leaves, at two growth stages. Proline concentration showed non-significant increase in leaves, while it significantly increased in roots with salinity, at both growth stages. Plant accommodates the salinity effect with responding to two phases; osmotic and ionic stress; the reduction and internal injury phases, respectively as mentioned by Shabala and Munns (2012). They added that after stress existence, cells regain their turgor owing to osmotic adjustment, through accumulating unused assimilates, changing its metabolism diverting them from structural growth process to pool of soluble carbohydrates, phenols and free amino acids as well as accumulating proline which stated by Esfandiari et al., (2008), Neseim et al., (2014) and Zeid et al., (2014). Dajic (2006) added the mechanisms by which salinity inhibits growth through disturbed photosynthesis and decline in turgor of expanding tissues and insufficient osmoregulation and disturbance in mineral supply to the shoot. These evidences are similar to our results. It was found that sugars were significantly recorded highest values in plant spikes at anthesis stage, which irrigated by 2000 ppm. This point would a standing step for grain accumulation. Meanwhile, it observed to be reduced in free amino acids, soluble phenols and proline. This indicating the encouraging trend found in this treatment as discussed in growth parameters. Focusing to organic components accumulation in stalk in respect of salinity effect, total free amino acids, soluble phenols, proline concentrations were increased with increasing salinity. On the other hand, total sugars were recorded a significant reduction in stalk.

**Table 6. Total soluble sugars and total free amino acids in roots, leaves, spikes and stalks of wheat plant at booting and anthesis stages subjected to four salinity levels (control, 2000, 4000, 6000 ppm) and 150 µM sodium nitroprusside either seedling priming or foliar spray (combined seasons 2015/2016-2016/2017).**

Total sugars (mg/g F.w)										
Salinity Treatments	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean
	Leaves in booting stage					Leaves in anthesis stage				
Control	12.72	9.96	10.26	9.02	10.49	8.72	20.78	17.31	13.73	15.14
SNP (Priming)	4.87	5.61	12.48	12.09	8.76	15.40	35.35	30.58	15.38	24.18
SNP (Spraying)	10.58	11.39	12.47	14.19	12.16	10.16	12.78	28.20	22.60	18.43
Mean	9.39	8.99	11.74	11.77		11.43	22.97	25.36	17.24	
L.S.D. at 5%	A=1.80		B= 1.56		AB= 3.11	A=0.52		B= 0.45		AB= 0.89
	Roots at booting					Roots at anthesis				
Control	2.52	3.54	5.46	6.46	4.49	2.85	1.87	2.84	2.96	2.63
SNP (Priming)	3.16	3.15	2.82	3.20	3.08	2.74	4.24	2.85	3.40	3.31
SNP (Spraying)	2.97	4.00	2.98	2.55	3.12	2.67	2.75	2.63	2.52	2.64
Mean	2.88	3.56	3.75	4.07		2.76	2.95	2.77	2.96	
L.S.D. at 5%	A=0.51		B=0.44		AB=0.88	A=0.17		B=0.15		AB=0.30
	Spike at anthesis					Stalk at anthesis				
Control	17.92	10.23	14.73	11.56	13.61	18.42	11.51	12.36	5.70	12.00
SNP (Priming)	7.92	16.70	11.80	6.80	10.80	12.13	24.45	18.18	8.49	15.81
SNP (Spraying)	13.98	12.63	8.46	12.00	11.77	18.53	10.38	5.75	5.68	10.08
Mean	13.27	13.18	11.66	10.12		16.36	15.45	12.10	6.62	
L.S.D. at 5%	A=0.29		B=0.25		AB=0.51	A=0.59		B=0.51		AB=1.01
Total free amino acids (mg/g F.w)										
	Leaves in booting stage					Leaves in anthesis stage				
Control	7.73	7.45	11.88	15.79	10.71	23.99	29.89	33.20	35.73	30.70
SNP (Priming)	5.45	8.77	6.87	5.48	6.64	35.25	35.22	26.47	34.74	32.92
SNP (Spraying)	5.92	7.06	7.55	4.12	6.16	29.60	34.58	55.13	33.68	38.25
Mean	6.37	7.76	8.76	8.46		29.61	33.23	38.27	34.71	
L.S.D.	A=1.50		B= 1.30		AB= 2.60	A=2.70		B= 2.35		AB=4.70
	Roots at booting					Roots at anthesis				
Control	1.34	1.78	2.57	2.10	1.95	1.27	1.56	1.94	2.15	1.73
SNP (Priming)	1.64	2.47	2.46	2.74	2.33	2.86	2.72	2.89	2.88	2.84
SNP (Spraying)	1.69	2.24	2.15	1.14	1.81	1.56	1.22	1.92	2.53	1.81
Mean	1.56	2.17	2.39	1.99		1.90	1.84	2.25	2.52	
L.S.D.	A=0.29		B= 0.25		AB=0.51	A=0.31		B= 0.27		AB= 0.53
	Spike at anthesis					Stalk at anthesis				
Control	12.47	15.90	22.02	20.55	17.73	8.79	11.63	15.69	10.29	11.60
SNP (Priming)	13.74	21.16	19.95	16.71	17.89	12.70	16.44	21.02	15.20	16.34
SNP (Spraying)	15.99	11.61	28.19	20.03	18.95	15.59	14.08	13.08	10.49	13.31
Mean	14.07	16.22	23.38	19.09		12.36	14.05	16.60	11.99	
L.S.D.	A=1.95		B= ns		AB= 3.37	A=1.02		B= 0.88		AB= 1.76

Concerning the effect of sodium nitroprusside, results indicated that plants treated by 150  $\mu\text{M}$  SNP either foliar sprayed or seedling priming found having superior trend when compared with control in accumulating, managing and manipulating the organic constituents. Study indicated that foliar sprayed plants had better trend than those seedling primed. Regarding sugar accumulation, spikes in foliar sprayed plants found to be significantly accumulated sugars than seedling priming plants, at the anthesis stage. In addition, sugars accumulation was reduced in other plant parts; stalk, leaves and roots, which referring to the treatment effect on managing sugar metabolism in plants. The same trend found in total soluble phenols and proline. Whereas, total free amino acids followed the same trend, except leaves at anthesis stage, which recorded the highest significant values. Findings suggested that NO alleviates abiotic stress through different metabolism, and antioxidant capacity modulation, which is reported to be one of the most important pathways as mentioned by Babri-Bonab et al., (2018). Proline is a compatible solute that accumulates in great quantities under osmotic stress and participates in osmoregulation and osmoprotection. The authors added that proline accumulation is assumed because of increasing synthesis together with a reduction in the degradation. They concluded that the activity of P5CS, the key enzyme in proline synthesis, increased with the time of osmotic stress, suggesting that glutamic acid was converted to proline in wheat shoots. In addition, they found that both 0.2 and 2 mM SNP increased the activity of P5CS; pyrroline-5- carboxylate, and consequently increased proline concentration. This result was agreed by Huai-Fu and Chang-Xia (2012). López-Carrión et al., (2008) studied the activity of both P2C5 and PDH; pyruvate dehydrogenase, enzymes, the proline synthesizing and degradation enzymes, respectively under salts stress and nitric oxide donor. They resulted that salinity does not change the activity of P2C5, while it inhibited the activity of PHD enzyme. Whereas, plants treated with 100 mM NaCl besides 0.25 or 0.5 mM SNP showed a lower proline content was due to the stronger PDH activity. The proline degradation seems to gain beneficial effect in the response to stress, given that the degradation of proline to glutamate generates reducing equivalents that support mitochondrial oxidative phosphorylation. Furthermore, the PDH activity has capable of consuming  $\text{O}_2$  and perhaps could reduce the

oxidizing power of the cell and in turn possibly generate ROS. Additionally, NO appears to be capable of mitigating damage associated with salinity stress by reducing oxidative stress and inducing proline degradation, mechanisms that permit the plant to adapt with greater facility under these conditions.

Several researchers mentioned the role of nitric oxide in sugar metabolism. Bonab et al., (2015) found that application of 50  $\mu\text{M}$  SNP enhanced the reducing sugars content in non-saline and saline conditions. They added that soluble carbohydrate content observed to increase in response to 50 and 100 mM NaCl salinity. While 50  $\mu\text{M}$  SNP enhanced the carbohydrate content in non-saline condition, however, it reduced carbohydrate content in saline condition.

Sun et al., (2011) declared that declared that treating with 10  $\mu\text{mol L}^{-1}$  NO promoted the transformation from fructose and glucose to sucrose by improving SPS; sugar phosphate phosphatase activity, and delayed the decomposition of sucrose, during peach storage. This possible regulation for sugar metabolism in peach fruits attributed to role of nitric oxide.

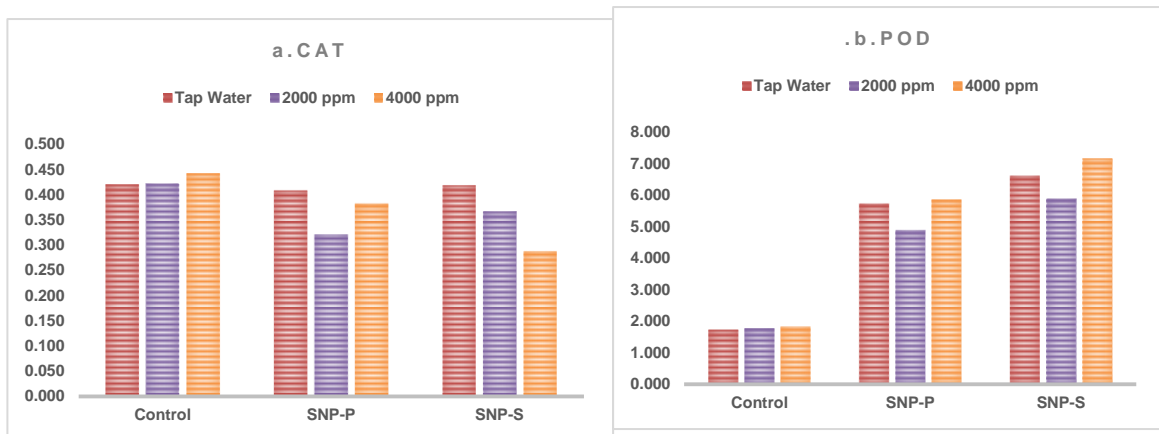
The specific activity of Catalase, peroxidase, super dismutase, polyphenol oxidase, phenyl alanine lyase and total soluble protein showed different responses to studied treatments in figures 1, 2 and 3. In respect to salinity effect, the specific activity of both catalase and peroxidase are found to be opposite to each other in their reponse to salinity. Results indicated increase in the specific activity of peroxidase, in plants that irrigated by 4000 ppm, however, a reduction found in plants irrigated by 2000 ppm. The same trend found in poly phenol oxidase specific activity. This result confirms the encouraging and promoting effect of 2000 ppm. On the other hand, catalase and phenyl alanine lyase did not show any change in theirs specific activity, in response to salinity. SOD was found to be the highest specific activity in plants irrigated by 2000 ppm and then a reduction was observed at 4000 ppm. The similar results found by Haihua et al., (2002) who found an increase in SOD activity in plants treated by 150 mM NaCl treatment, but at 300 mM NaCl decreased rapidly. Total soluble protein was increased significantly with increasing salinity stress. This result is similar to Babri-Bonab et al., (2018).

Results in Figures 4 and 5, revealed that antioxidant capacity was significantly increase in wheat roots and leaves at anthesis stages, on the

other hand, they were significantly reduced in spikes and stalks in 4000 and 6000 ppm plants. On the other hand, 2000 ppm treated plants showed a significant increase in antioxidant capacity in roots, leaves, spikes and stalks at anthesis stage.

Concerning the sodium nitroprusside effect on enzyme specific activity, sodium nitroprusside was found to have a promoting effect whether applied in foliar spray or seedling priming on specific activity of peroxidase, super dismutase, polyphenol oxidase and phenyl alanine lyase and total soluble protein, and antioxidant capacity as well, when compared with control. On the other hand, a reverse trend was observed in catalase specific activity. This result was similar to Wu et al., (2012). Focusing on sodium nitroprusside treatment, foliar sprayed plants showed an increase in enzymes specific activity when compared with seedling primed plants. Moreover, an increase observed in total soluble protein in sodium nitroprusside treated plants, either foliar sprayed or seedling priming, which is similar to study of Babri-Bonab et al.,(2018). These enzymes have vital role in oxidative stress and detoxify its effect. It develops as a result of overproduction of reactive oxygen species (ROS) and accompanies virtually all biotic and abiotic stresses; salinity or drought and so on. Shabala and Munns (2012) added that major sites of 'electron leakage' are including photosystem I and photosystem II in addition to mitochondrial complexes I and III. And peroxisomes is

considered on of ROS species source. Karuppanapandian et al., (2011) added that ROS species productions are controlled by various enzymatic and non-enzymatic antioxidant defense systems. Enzymatic antioxidant defense systems, including CAT, APX, POX, SOD, MDHAR, DHAR and GR and non-enzymatic antioxidant defense systems, including ascorbate, glutathione, carotenoids, phenolic compounds, proline, glycine betain, sugar, and polyamines. Superoxide dismutase, as a metalloenzyme, is the first enzyme of the detoxification processes, which catalyzes  $O_2^{\cdot-}$  to  $H_2O_2$  and  $O_2$ , it increased under salinity stress to increase plant tolerance. CAT is also important in the removal of  $H_2O_2$  generated in peroxisomes during the  $\beta$ -oxidation of fatty acids, photorespiration, and purine catabolism. POX also decomposes indole-3- acetic acid (IAA) and has a role in the biosynthesis of lignin and defense against biotic stresses by consuming  $H_2O_2$  in the cytosol, vacuole, and cell wall as well as in extracellular space. Phenolic compounds, the secondary metabolites including flavonoids, tannins, anthocyanin, hydroxycinnamate esters, and lignin, are abundant in plant tissues. Many secondary metabolites play widely important role similar as defensive agents against pathogens to general protection against oxidative stress using as electron donors for free radical scavenging. Phenylalanine ammonia lyase (PAL) activity is one of the main enzymes in the synthesis of phenolic compounds.



**Figure 1. a. Catalase, b. Peroxidase (  $Umg^{-1}Pmin^{-1}$ ) in wheat flag leaf at booting stage subjected to three salinity levels (control, 2000, 4000 ppm) and 150  $\mu M$  sodium nitroprusside either seedling priming or foliar spray.**

**Table 7. Total soluble phenols and free proline in roots, leaves, spikes and stalks of wheat plant at booting and anthesis stages subjected to four salinity levels (control, 2000, 4000, 6000 ppm) and 150  $\mu$ M sodium nitroprusside either seedling priming or foliar spray (Combined seasons 2015/2016-2016/2017).**

Total soluble phenols (mg/g F.w)										
Salinity Treatments	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean
	Leaves in booting stage					Leaves in anthesis stage				
Control	0.70	0.98	0.88	0.73	0.82	0.91	2.50	1.70	0.90	1.50
SNP (Priming)	1.04	0.90	0.80	0.73	0.87	1.62	1.96	2.80	0.86	1.81
SNP (Spraying)	0.84	0.79	1.06	0.88	0.89	0.76	1.22	1.73	1.38	1.27
Mean	0.86	0.89	0.91	0.78		1.10	1.89	2.08	1.05	
L.S.D. at 5%	A=ns		B=ns		AB=0.23	A=0.02		B= 0.02		AB=0.04
	Roots at booting					Roots at anthesis				
Control	0.33	0.43	0.29	0.33	0.34	0.25	0.23	0.29	0.28	0.26
SNP (Priming)	0.77	0.80	0.76	0.76	0.77	0.60	0.42	0.44	0.43	0.47
SNP (Spraying)	0.73	0.74	0.95	0.68	0.78	0.27	0.25	0.23	0.29	0.26
Mean	0.61	0.66	0.67	0.59		0.37	0.30	0.32	0.34	
L.S.D. at 5%	A=ns		B= 0.07		AB= 0.14	A=ns		B= 0.02		AB= 0.04
	Spike at anthesis					Stalk at anthesis				
Control	0.69	0.45	0.81	0.80	0.69	0.66	0.81	1.23	0.71	0.85
SNP (Priming)	0.49	0.91	0.80	0.51	0.68	1.20	0.86	1.10	0.74	0.97
SNP (Spraying)	0.56	0.59	0.51	0.84	0.63	0.94	1.11	0.82	0.64	0.88
Mean	0.58	0.65	0.71	0.72		<b>0.93</b>	<b>0.93</b>	<b>1.05</b>	<b>0.70</b>	
L.S.D. at 5%	A=ns		B=ns		AB=ns	A=0.02		B= 0.02		AB= 0.04
Total Proline (mg/g F.w)										
	Leaves in booting stage					Leaves in anthesis stage				
Control	2.53	2.75	3.12	4.04	3.11	6.06	9.41	11.11	12.58	9.79
SNP (Priming)	0.30	0.59	0.55	0.44	0.47	0.54	1.12	0.79	0.65	0.77
SNP (Spraying)	0.44	0.46	0.54	0.23	0.42	0.68	0.70	0.93	1.05	0.84
Mean	1.09	1.26	1.40	1.57		2.42	3.74	4.28	4.76	
L.S.D. at 5%	A=ns		B=0.32		AB=0.64	A=ns		B= 2.34		AB= ns
	Roots at booting					Roots at anthesis				
Control	0.25	0.32	0.44	0.36	0.34	0.71	1.06	1.30	1.73	1.20
SNP (Priming)	0.35	0.50	0.49	0.64	0.49	0.74	0.95	0.69	0.65	0.76
SNP (Spraying)	0.29	0.38	0.39	0.44	0.37	0.46	0.53	0.45	0.42	0.46
Mean	0.29	0.40	0.44	0.48		0.64	0.85	0.81	0.93	
L.S.D. at 5%	A=0.10		B= 0.09		AB= ns	A= 0.17		B= 0.14		AB= 0.29
	Spike at anthesis					Stalk at anthesis				
Control	1.12	3.42	9.38	2.93	4.21	3.28	1.64	6.16	4.83	3.98
SNP (Priming)	3.76	5.55	5.31	4.52	4.78	3.48	4.75	4.35	3.76	4.08
SNP (Spraying)	4.30	6.49	7.96	5.34	6.02	3.39	2.03	2.81	1.68	2.48
Mean	3.06	5.15	7.55	4.26		3.38	2.81	4.44	3.42	
L.S.D. at 5%	A=0.11		B= 0.09		AB= 0.19	A=0.21		B= 0.19		AB= 0.37



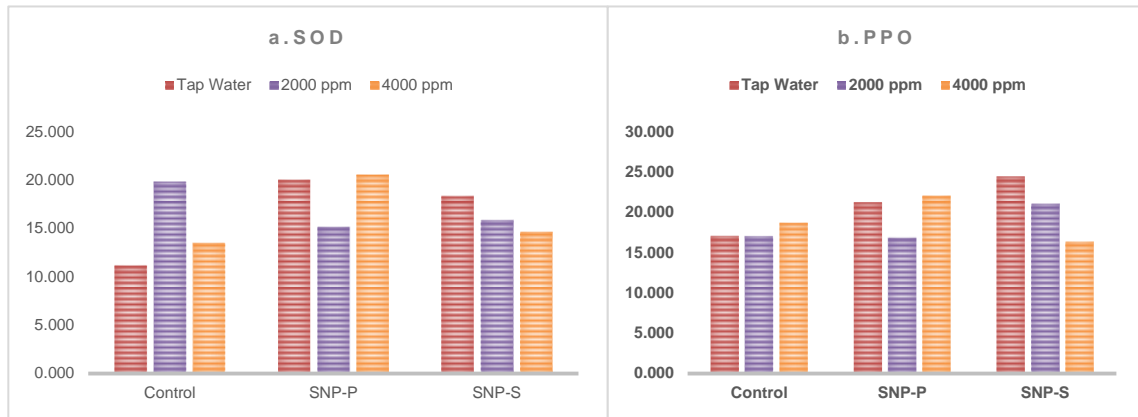


Figure 2. a. Super Dismutase and poly phenol oxidase ( Umg<sup>-1</sup>Pmin<sup>-1</sup>) in wheat flag leaf at booting stage subjected to three salinity levels (control, 2000, 4000 ppm) and 150 μM sodium nitroprusside either seedling priming or foliar spray.

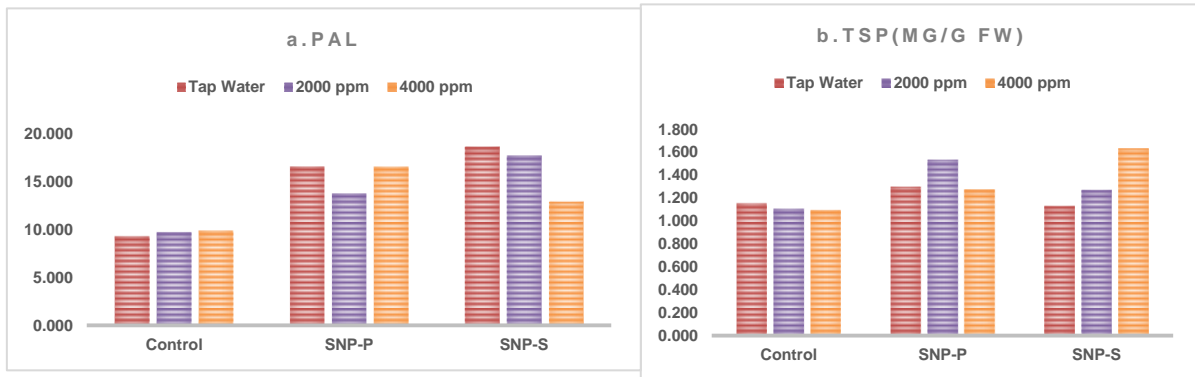


Figure 3. a. Phenyl alanine lyase ( Umg<sup>-1</sup>Pmin<sup>-1</sup>) and total soluble protein (mg/g F.w.) in wheat flag leaf at booting stage subjected to three salinity levels (control, 2000, 4000 ppm) and 150 μM sodium nitroprusside either seedling priming or foliar spray.

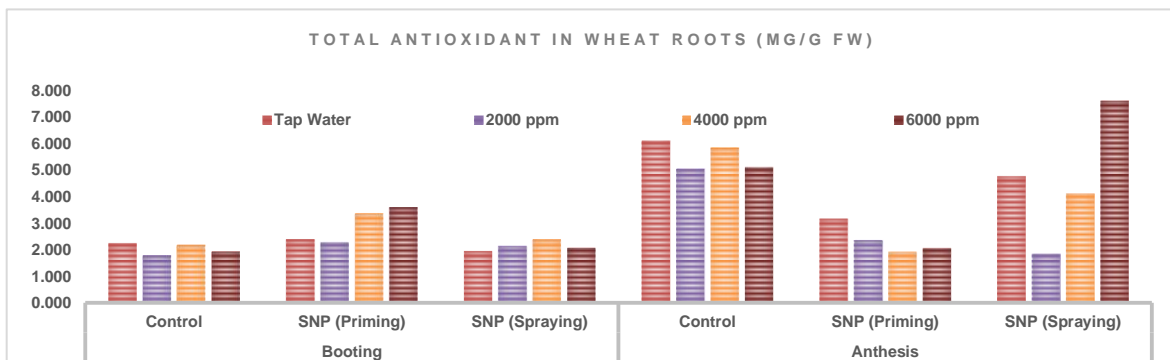
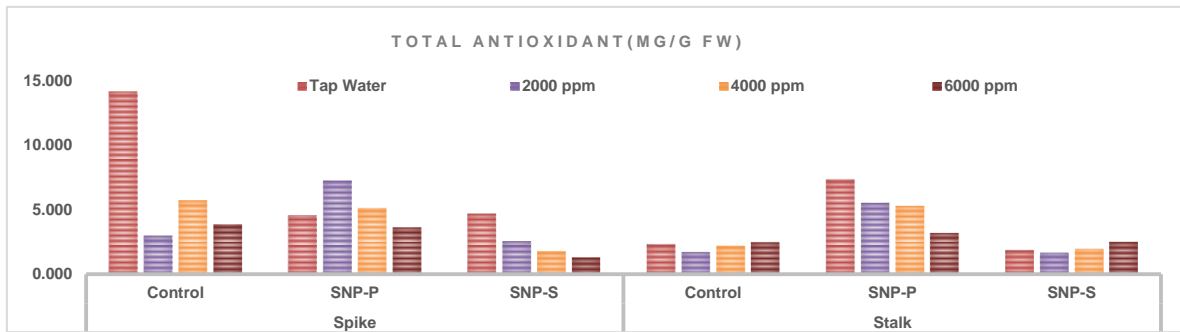


Figure 4. Total antioxidant capacity (mg/g F.w.) in roots of wheat plant at booting and anthesis stages subjected to three salinity levels (control, 2000, 4000 ppm) and 150 μM sodium nitroprusside either seedling priming or foliar spray.



**Figure 5. Total antioxidant capacity (mg/g F.W.) in spikes and stalks of wheat plant at anthesis stage subjected to three salinity levels (control, 2000, 4000 ppm) and 150  $\mu$ M sodium nitroprusside either seedling priming or foliar spray**

Poly Phenol oxidases (PPO) activities, another important enzyme which plays important role for oxidation of phenolic compounds, was changed under NaCl induced stress conditions. Results found that foliar sprayed plants and irrigated by 4000 ppm, found to have highest peroxidase specific activity and total soluble protein. On the other hand, catalase specific activity showed lowest records. Additionally, SOD mutase specific activity was increased in seedling-primed plants and irrigated by 4000 ppm. Nitric oxide has a dual protective role, involves its detoxify ability to oxidative stresses, together it showed changes in chlorophyll and Malondialdehyde (MDA) contents and plasma membrane permeability, which confirmed that SNP could markedly alleviate oxidative damage to wheat (*T. aestivum* L.) leaves induced by NaCl treatment as mentioned by Shamsul et al., (2010). NO significantly enhanced activities of SOD and CAT, both of which separately contributed to the delay of  $O_2$  and  $H_2O_2$  accumulation in wheat leaves under salt stress. These results therefore suggest that NO could strongly protect wheat leaves from oxidative damage caused by salt stress. Sánchez-Romera et al., (2018) mentioned processes that nitric oxide (NO) is involved in stomatal movement regulation and cross talk with ABA under stresses, it contributes in regulation of photosynthesis and mitochondrial functionality, respiration process through enzymatic regulation, gravitropism and floral development. Qasim et al., (2017) resulted that sodium nitroprusside increased antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD)

and catalase (CAT) and the contents of Ascorbic acid, Proline and total phenolic content (TPC) in the salt stressed wheat plants. In Table 8, yield and yield components showed a gradual significant reduction in all studied yield components with increasing salinity levels; spike height and weight, no of grains and spikelet per plant, grain and straw weight per plant. The same trend found on 1000-grain weight in Table 9. This result was similar and confirmed by Qasim et al., (2017) who performed a similar study on four wheat cultivars; Sahar-06, Punjab-11, Millat-11 and Galaxy-13, in Pakistan, and irrigated them using half strength Hoagland solution and 150 mM NaCl. Results in Table 9 indicated a significant increase in 1000-grains weight in foliar sprayed plants, with respect the effect of sodium nitroprusside, this result was similar to Qasim et al., (2017) who mentioned that SNP alleviating the adverse effect of salinity. Finally, yield components in foliar sprayed plants and irrigated by 4000 ppm recorded the highest values; No of grains and spikelet per plant, grain and straw weight per plant except in 1000-grains weight, which showed non-significant difference with all other plants irrigated by difference salinity levels. our results were in harmony with Kausar et al., (2013) who found that foliar spray by sodium nitroprusside significantly increased yield per plant, number of seeds per plant, and 100-seed weight of wheat under non-stressed conditions, while NO enhanced grain yield per plant under saline conditions.

**Table 8. Spike height (cm), Spike weight (g), Straw weight per plant(g), No. of spikelet per plant, Grain weight per plant(g), No. of grains per plant of wheat plant as subjected to four salinity levels (control, 2000, 4000, 6000 ppm) and 150  $\mu$ M sodium nitroprusside either seedling priming or foliar spray (Combined seasons2015/2016-2016/2017).**

Salinity Treatments	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean
	Spike height (cm)					Spike Weight (g)				
Control	14.25	13.14	10.29	6.81	11.12	2.32	1.77	1.01	0.44	1.38
SNP (Priming)	13.46	12.03	11.56	7.96	11.25	2.47	1.99	1.31	0.62	1.60
SNP (Spraying)	16.15	13.93	12.35	12.35	13.70	2.97	2.40	1.00	0.42	1.70
Mean	14.62	13.04	11.40	9.04		2.59	2.05	1.11	0.49	
L.S.D at 5%	A= 1.79		B= 1.55		AB=ns	A=0.29	B= 0.25		AB= ns	
	No of spikelet per plant					Grain weight per plant (g/plant)				
Control	10.25	10.25	11.00	11.54	10.76	0.61	0.40	0.29	0.52	0.46
SNP (Priming)	15.00	10.38	11.42	10.50	11.82	1.71	0.67	0.33	0.51	0.81
SNP (Spraying)	10.46	9.75	13.33	13.50	11.76	0.71	0.58	0.52	0.16	0.49
Mean	11.90	10.13	11.92	11.85		1.01	0.55	0.38	0.40	
L.S.D	A= 1.33		B= ns		AB=2.30	A= 0.23	B= 0.20		AB= 0.40	
	Straw weight per plant (g/plant)					No. of grains per plant (g/plant)				
Control	1.40	1.27	1.06	0.53	1.06	21.83	18.30	16.39	20.17	19.17
SNP (Priming)	1.55	0.55	0.57	0.41	0.77	49.10	19.42	15.67	10.84	23.76
SNP (Spraying)	0.94	0.93	0.78	0.65	0.82	22.22	23.08	21.00	13.86	20.04
Mean	1.29	0.92	0.80	0.53		31.05	20.27	17.68	14.96	
L.S.D	A= 0.15		B= 0.13		AB= 0.26	A= 4.33	B= 3.75		AB= 7.49	

**Table 9. 1000-grains weight (g) wheat plant affected by four salinity levels (control, 2000, 4000, 6000 ppm) and 150  $\mu$ M sodium nitroprusside either seedling priming or foliar spray. (Combined seasons2015/2016-2016/2017).**

Salinity Treatments	Tap Water	2000 ppm	4000 ppm	6000 ppm	Mean
	1000-grain (g)				
Control	28.35	21.97	17.54	19.73	21.90
SNP (Priming)	25.47	21.43	20.33	7.42	18.66
SNP (Spraying)	32.29	24.74	22.71	12.17	22.98
Mean	28.70	22.71	20.19	13.11	
L.S.D at 5%	A= 3.21		B= 2.78		AB=5.55

## CONCLUSION

This study recommends the application of foliar spraying of SNP-S in conc. 150- $\mu$ M on plants, which irrigated by 4000 ppm of Mediterranean Sea salts. This treatment showed enhancing growth, yield, biochemical components, antioxidant capacity and antioxidant enzymes in wheat roots, leaves, stalks and spikes. In addition, there is no significant different between recommended treatment and plants irrigated by non-saline or 2000 ppm salinity level.

## CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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with these terms.

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