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Research Article

Comparison between Salicylic Acid and Pomegranate Peel Extract in Reducing the Deleterious Effect of Salinity Stress on Wheat Plant

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Abstract

Background and Objective: Salicylic acid act as a signal molecule or chemical messenger that induces the immune system in plants. Meanwhile, the antioxidant activity of pomegranate can be ascribed to the presence of some components like ascorbic acid and phenolic compounds. This study investigated the physiological role of salicylic acid or pomegranate peel extract on wheat plants. **Materials and Methods:** Two pot experiments were conducted at the greenhouse of National Research Centre, Dokki, Cairo, Egypt, during two winter seasons of 2018/2019 and 2019/2020. This study investigated the physiological role of salicylic acid at 100 and 200 mg L⁻¹ or pomegranate peel extract at 5 and 10% on wheat plants irrigated with diluted seawater (4000 ppm). **Result:** Results indicate that plant irrigation with diluted seawater significantly decreased vegetative growth parameters, photosynthetic pigments, IAA, grains yield and its components, carbohydrate content accompanied by significant increases in leaf osmolytes (total soluble sugar and proline), biochemical content of the yielded grains (phenolic content, flavonoids) and non-significant increases in lycopene and β-carotene. On the other hand, all applied treatments alleviated the deleterious effect of salinity via enhancing vegetative growth parameters, photosynthetic pigments, IAA, grains yield and its components, leaf osmolytes, carbohydrate content, phenolic content, flavonoids, lycopene and β-carotene of the yielded grains. **Conclusion:** It is obvious that the most optimum treatments were pomegranate peel extract at 10% followed by salicylic acid at 200 mg L⁻¹.

Key words: *Punica granatum* L., *Triticum aestivum* L., plant extract, phenolic acid, seawater

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity stress is one of the most serious defining factors for crop development and production in the arid and semiarid zones. The adverse effects of salinity stress on plant productivity include increases in ion toxicity, osmotic stress, water stress, oxidative stress, nutritional imbalances, alterations in metabolic processes, disorganization of membranes and reduction in cell division and expansion¹. Salt stress restricts plant growth by adversely influencing various aspects of physiology and biochemistry, such as photosynthesis, superoxide ion homeostasis, antioxidant responses, osmolyte accumulation and proline metabolism^{2,3}. Accumulation of salts in growth medium induces the formation of toxic reactive oxygen species including singlet oxygen, superoxide and hydroxyl radical. Reactive oxygen species react with cellular components such as nucleic acids, proteins and lipids of the cell membrane and others, it results in oxidative damage in the plant⁴. To overcome these reactive oxygen species, plants generate an antioxidant defence system that includes antioxidant compounds or antioxidant enzymes⁵.

One of the effective practices to mitigate the deleterious effects of salinity stresses and inducing salt tolerance in many crops under arid region conditions is the use of salicylic acid⁶ or natural extract from plant wastes as pomegranate peel.

Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological and biochemical processes in plants⁷. Salicylic acid acts as a signal molecule or chemical messenger that induces the immune system in plants⁸ and affects directly on specific enzymes function or may activate the genes responsible for protective mechanisms⁹. Salicylic acid signalling is not a simple linear route and salicylic acid may interact with several other stress-related compounds¹⁰. Its activity is essential for basal defence and systemic acquired resistance¹¹ and leads to the reprogramming of the expression of genes and the synthesis of proteins, affecting several metabolic processes¹⁰. Hassoon and Abduljabbar¹² mentioned that salicylic acid has many important physiological roles, such as stimulating the flowering, nutrient transfer, increasing the representation of CO₂ gas, controlling the movement of stomata, photo materials, gas exchange and protein synthesis. It plays an important role in increasing metabolic rates, which contributes to the energy saving of the plant through alternative pathways accompanied by a change in the level of nucleic and amino acids within the plant¹³. Foliar application of salicylic acid may play a significant role in plant water relations¹⁴, photosynthesis, growth rate and stomatal regulation¹⁵, as well as ion absorption and transport⁶, raising

indole acetic acid content and enhancing division and extension of root cells¹⁶. Exogenous application of salicylic acid can maintain the stability and integrity of the cell membrane¹⁷, increase the antioxidant activity¹⁸ and improve the photosynthetic capacity¹⁹. Babar *et al.*²⁰ concluded that foliar application of salicylic acid mitigated the salinity induced adverse effects on the fenugreek growth biomass, gas exchange attributes and chlorophyll contents. It increased antioxidant enzymes activity such as APX and PPO and reduced the harmful effects of reactive oxygen species caused by oxidative stress²¹. So, salicylic acid is used in plant cultivation as a potential growth regulator to regulate the plant resistance response to different environmental stresses, particularly salt stress^{6,7,22,23}.

Pomegranate (*Punica granatum* L.) peels have been used in folk medicine since ancient times because of their health benefits due to the presence of various useful compounds. The pomegranate fruit nutritional parameters are not limited to the edible part of the fruit, the vital role is played by the non-edible fractions of fruit and tree i.e. leaves, barks, buds, flower and peel. Although these parts are considered to be waste, they contain an enormous amount of nutritional value and biologically active compounds compared to the edible portion of the fruit²⁴. During the industrial processing of pomegranate, the large volume of waste is produced in the form of pomegranate peel as an inedible portion of the fruit which has a wide range of nutritional values and has a high potential of antioxidant and antifungal properties²⁵. Pomegranate peels are considered agro-waste, that act as a source of bioactive chemicals, antioxidants, nutrients. Secondary metabolites, numerous phytochemicals and also possesses antibacterial and antifungal activity²⁶. Aviram *et al.*²⁷ stated that the antioxidant activity of pomegranate can be ascribed to the presence of some components like ascorbic acid and phenolic compounds and have the highest antioxidant activity. Additionally, malic acid, fumaric acid, oxalic acid, succinic acid, citric acid and tartaric acid are among the major organic acids in pomegranate. Furthermore, the flavonoids of pomegranate are luteolin, kaempferol and Naringenin found in glycoside forms²⁷. Additionally, pomegranate peel is one of the most beneficial parts when compared to the seeds, leaf and flower due to its substantial amounts of bioactive ingredients such as polyphenolic and flavonoid compounds that can be attributed to the antioxidant index^{28,29}. This might be due to the high content of polyphenolic compounds found in peel (~73%) compared to the polyphenolic compounds from seeds³⁰ (~27%). Pomegranate peel methanolic extract has shown the highest antioxidant activity over different ranges of polar and nonpolar extracts³¹.

Wheat (*Triticum aestivum* L.) is a winter crop that belongs to the Poaceae, one of the most widely cultivated cereal crops in the world and in Egypt, it is widely cultivated in the Nile Delta region³².

This work aimed to investigate the physiological role of salicylic acid at 100 and 200 mg L⁻¹ or pomegranate peel extract at 5 and 10% on wheat plants irrigated with diluted seawater.

MATERIALS AND METHODS

Study area: Two pot experiments were conducted at the greenhouse of National Research Centre, Dokki, Cairo, Egypt, during two winter seasons of 2018/2019 and 2019/2020. Grains of wheat Sakha 94 were obtained from Agricultural Research Centre, Giza, Egypt.

Preparation of ethanolic extract of pomegranate peel: The fresh fruits of pomegranate fruit were collected from the local market and manually peeled. The collected peels were then rinsed with distilled water and dried in an oven by hot air (50°C) for 48hrs and powdered to get 60-mesh size using a mixing grinder and stored in airtight container. The powdered peels of pomegranate (10 g) were macerated with 100 mL of 80% ethanol and kept in a rotatory shaker for 24 hrs. The macerated material was strained through Whatman No 1 filter paper. The extract was concentrated at 40°C by using a rotary evaporator and then dried in an oven at 50°C for 48 hrs. Finally, dissolve the dried extract, with distilled water to obtain extract³³ at 5 and 10%.

Experimental procedures: Wheat grains were sorted for symmetry by selecting those of similar colour and size. In plastic pots filled with around 7 kg clay soil, ten uniform wheat grains were sowed on November, 25th, along a central row at 30 mm depth. The soil was blended with yellow sand in a 3:1 (v/v) ratio to minimize compaction and promote drainage. Fertilizers were applied as recommended dose. The seedlings were reduced to 5 seedlings per pot ten days after seeding. The experimental design was a six-replicate complete randomized block design. Salicylic acid at 100 and 200 mg L⁻¹ and pomegranate peel extract at 5 and 10% were sprayed on plants at 20 DAS and 30 DAS. The salinity of the seawater was lowered to 4000 mg L⁻¹. From 30 DAS until the completion of the experiment, the pots were watered with equal volumes of the salt level at 4000 mg L⁻¹. Plant specimens were taken 45 days after planting for evaluation of growth characteristics such as shoot height, shoot, root fresh and dry weight and

root fresh and dry weight. Plant samples were taken for chemical analysis as photosynthetic pigments, indole acetic acid (IAA). After drying of plant samples, total soluble sugars (TSS) and proline were determined. At harvest: Plant height, spike length, spike weight, spikelet's number/spike, grains weight/plant, 1000-grains weight and biological yield/plant were determined. Some nutritional values of the yielded grains were determined as carbohydrate, DPPH, total phenolic content, flavonoids, lycopene and β -carotene.

Measurements: Photosynthetic pigments were determined using the method described by Lichtenthaler and Buschmann³⁴. Indole acetic acid content (IAA) was analyzed by the method of Gusmiaty *et al.*³⁵. Total soluble sugars (TSS) were extracted and analyzed by the method of Homme^{36,37}. Proline was assayed³⁸. Determination total carbohydrate was analyzed³⁹. The free radical scavenging activity (DPPH) of plant extracts was measured according to Gyamfi *et al.*⁴⁰. Total phenolic content was determined⁴¹. Flavonoid content was determined by the aluminium chloride colourimetric method⁴². Lycopene and β -carotene were determined⁴³.

Statistical analysis: The data were statistically analyzed on a complete randomized design system (CRBD) with four replicates. A combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability⁴⁴.

RESULTS

Vegetative growth parameters: Table 1 show that wheat plants irrigated with diluted seawater suffer from significant decreases in all vegetative growth parameters under investigation relative to those irrigated with tap water. Since shoot and root dry weight significantly decreased due to salinity by 21.05 and 33.33%, respectively relative to control. Regarding irrigation with either tap water or diluted seawater, it was noted that salicylic acid at 100 and 200 mg L⁻¹ and pomegranate peel extract at 5 and 10% caused significant increases in all vegetative growth parameters. The most pronounced treatments were pomegranate peel extract at 10% followed by salicylic acid at 200 mg L⁻¹. Since the maximum significant value in shoot dry weight under irrigation with tap water was 0.74 and 0.70 g that was achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively. Similarly, the maximum significant value in shoot dry weight under irrigation with diluted seawater was 0.64 and 0.55 g that

Table 1: Impact of salicylic acid and P extract on vegetative growth parameters of wheat plant irrigated with diluted seawater

Salinity	Materials	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight(g)	Root dry weight (g)
0	Control	41.33 ^f	1.79 ^e	0.38 ^e	0.34 ^{ef}	0.09 ^{de}
	Salicylic acid at 100 ppm	49.33 ^d	2.28 ^{bc}	0.55 ^{cd}	0.44 ^d	0.11 ^{bc}
	Salicylic acid at 200 ppm	54.00 ^b	2.53 ^a	0.70 ^a	0.57 ^b	0.14 ^a
	P extract at 5%	51.67 ^c	2.29 ^{bc}	0.59 ^{bc}	0.50 ^c	0.12 ^b
	P extract at 10%	57.00 ^a	2.66 ^a	0.74 ^a	0.62 ^a	0.14 ^a
4000 (mg L ⁻¹)	Control	32.33 ^g	1.20 ^f	0.30 ^f	0.27 ^g	0.06 ^f
	Salicylic acid at 100 ppm	42.67 ^f	1.88 ^{de}	0.50 ^d	0.32 ^{ef}	0.09 ^e
	Salicylic acid at 200 ppm	45.67 ^e	2.23 ^c	0.55 ^{cd}	0.43 ^d	0.10 ^{cd}
	P extract at 5%	45.33 ^e	2.11 ^{cd}	0.54 ^{cd}	0.36 ^e	0.09 ^e
	P extract at 10%	46.33 ^e	2.50 ^{ab}	0.64 ^b	0.48 ^c	0.11 ^b

Means with different letters were significantly different at the 0.05 level according to Duncan's multiple range tests

Table 2: Impact of salicylic acid and P extract on photosynthetic pigments ($\mu\text{g g}^{-1}$ fresh leaf tissues) of wheat plant irrigated with diluted seawater

Salinity	Materials	Chlorophyll A	Chlorophyll B	Carotenoid	Total photosynthetic pigments
0	Control	1144 ^e	670 ^c	228 ^{de}	2042 ^e
	Salicylic acid at 100 ppm	1368 ^c	768 ^a	264 ^b	2400 ^c
	Salicylic acid at 200 ppm	2638 ^a	689 ^b	313 ^a	3640 ^a
	P extract at 5%	2233 ^b	585 ^d	272 ^b	3091 ^b
	P extract at 10%	2681 ^a	667 ^c	304 ^a	3652 ^a
4000 (mg L ⁻¹)	Control	857 ^g	411 ^g	174 ^f	1442 ^h
	Salicylic acid at 100 ppm	1222 ^{de}	450 ^e	214 ^e	1887 ^f
	Salicylic acid at 200 ppm	1390 ^c	578 ^d	246 ^c	2216 ^d
	P extract at 5%	1265 ^d	470 ^e	226 ^{de}	1962 ^{ef}
	P extract at 10%	1444 ^c	586 ^d	240 ^{cd}	2271 ^d

Means with different letters were significantly different at the 0.05 level according to Duncan's multiple range tests

achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively.

Photosynthetic pigments: Table 2 show that wheat plants irrigated with diluted seawater suffer from significant decreases in all components of photosynthetic pigments relative to those irrigated with tap water. The decrease in total photosynthetic pigments due to salinity was 29.38% relative to control.

On the other hand, it was noted that salicylic acid at 100 and 200 mg L⁻¹ and pomegranate peel extract at 5 and 10% caused significant increases in total photosynthetic pigments. The most pronounced treatments were pomegranate peel extract at 10% followed by salicylic acid at 200 mg L⁻¹. Since the maximum significant value in total photosynthetic pigments under irrigation with tap water was 3652 and 3640 ($\mu\text{g g}^{-1}$ fresh leaf tissues) that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively. Likewise, the maximum significant value in total photosynthetic pigments under irrigation with diluted seawater was 2271 and 2216 ($\mu\text{g g}^{-1}$ fresh leaf tissues) that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively. In other words, pomegranate peel extract at 10% significantly increased the total photosynthetic pigment of wheat plants irrigated with tap water by 78.84% and those irrigated with

diluted seawater by 57.49% relative to corresponding controls. Likewise, salicylic acid at 200 mg L⁻¹ significantly increased the total photosynthetic pigment of wheat plants irrigated with tap water by 78.25% and those irrigated with diluted seawater by 53.67% relative to corresponding controls.

IAA and osmolytes: IAA was significantly decreased by salinity stress accompanied by significant increases in total soluble sugars and proline contents relative to control in Table 3. On the other hand, it was noted that salicylic acid at 100 and 200 mg L⁻¹ and pomegranate peel extract at 5 and 10% caused significant increases in IAA, total soluble sugars and proline contents relative to corresponding controls. The most pronounced treatments were pomegranate peel extract at 10% followed by salicylic acid at 200 mg L⁻¹ either under irrigation with tap water or diluted seawater. Since, under irrigation with tap water, the maximum significant value in IAA was 51.76 and 44.55 ($\mu\text{g 100 g}^{-1}$ fresh weight fresh leaf tissues), total soluble sugar was 1526.33 and 1428.0 ($\mu\text{g g}^{-1}$ dry weight), that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively. Similarly, under irrigation with diluted seawater, the maximum significant value in IAA was 43.19 and 40.24 ($\mu\text{g 100 g}^{-1}$ fresh weight fresh leaf tissues), total soluble sugar was 1620.0 and 1571.0 ($\mu\text{g g}^{-1}$ dry weight), that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively.

Table 3: Impact of salicylic acid and P extract on IAA and some osmolytes of wheat plant irrigated with diluted seawater

Salinity	Materials	IAA ($\mu\text{g}/100\text{ g}$ fresh weight)	Total soluble sugars ($\mu\text{g g}^{-1}$ dry weight)	Proline ($\text{mg}/100\text{ g}$ dry weight)
0	Control	27.03 ^f	1265.00 ^h	43.29
	Salicylic acid at 100 ppm	33.74 ^e	1310.33 ^g	56.61 ^h
	Salicylic acid at 200 ppm	44.55 ^b	1428.00 ^e	70.66 ^f
	P extract at 5%	36.53 ^d	1401.33 ^{ef}	72.17 ^{ef}
	P extract at 10%	51.76 ^a	1526.33 ^c	80.30 ^c
4000 (mg L^{-1})	Control	18.76 ^h	1387.33 ^f	63.02 ^g
	Salicylic acid at 100 ppm	30.21 ^f	1497.67 ^d	73.17 ^e
	Salicylic acid at 200 ppm	40.24 ^c	1571.00 ^b	84.25 ^b
	P extract at 5%	32.19 ^{ef}	1520.67 ^{cd}	77.50 ^d
	P extract at 10%	43.19 ^b	1620.00 ^a	86.67 ^a

Means with different letters were significantly different at 0.05 level according to Duncan's multiple range tests

Table 4: Impact of salicylic acid and P extract on grains yield and yield components of wheat plant irrigated with diluted seawater

Salinity	Materials	Plant height (cm)	Spike length (cm)	Spike weight (g)	Spikelet number /spike	Grains weight /plant (g)	1000 grains weight (g)	Biological yield (g)
0	Control	54.67 ^f	9.33 ^{de}	2.71 ^f	8.67 ^{de}	2.18 ^f	28.13 ^g	4.12 ^g
	Salicylic acid at 100 ppm	63.30 ^{cd}	10.00 ^{cd}	3.32 ^{cd}	9.33 ^{cd}	2.72 ^d	33.92 ^d	4.76 ^{de}
	Salicylic acid at 200 ppm	70.50 ^a	11.33 ^{bc}	3.95 ^b	11.00 ^b	3.26 ^b	37.59 ^b	5.44 ^b
	P extract at 5%	65.13 ^{bc}	11.00 ^{bc}	3.55 ^c	11.00 ^b	2.86 ^c	35.48 ^c	5.05 ^c
	P extract at 10%	67.50 ^b	13.00 ^a	4.27 ^a	12.67 ^a	3.50 ^a	38.72 ^a	5.99 ^a
4000 (mg L^{-1})	Control	42.33 ^g	8.33 ^e	2.38 ^g	8.00 ^e	1.82 ^h	24.28 ^h	3.65 ^h
	Salicylic acid at 100 ppm	57.33 ^e	9.33 ^{de}	3.01 ^e	9.33 ^{cd}	2.01 ^g	30.80 ^f	4.43 ^f
	Salicylic acid at 200 ppm	59.17 ^d	10.33 ^{bcd}	3.56 ^c	10.33 ^{bc}	2.42 ^e	33.66 ^d	4.71 ^e
	P extract at 5%	59.00 ^e	11.00 ^{bc}	3.08 ^{de}	10.33 ^{bc}	2.21 ^f	32.06 ^e	4.52 ^{ef}
	P extract at 10%	61.67 ^d	11.67 ^{ab}	3.25 ^{de}	10.33 ^{bc}	2.65 ^d	34.21 ^d	4.98 ^{cd}

Means with different letters were significantly different at the 0.05 level according to Duncan's multiple range tests

Table 5: Impact of salicylic acid and P extract on some chemical composition of the yielded grains of wheat plant irrigated with diluted seawater

Salinity	Materials	Carbohydrate (%)	DPPH (%)	(mg/100 g dry weight)			
				Total phenolic content	Flavonoids	Lycopene	β -carotene
0	Control	42.74 ^a	35.30 ⁱ	60.43 ^h	13.25 ^h	0.408	0.379
	Salicylic acid at 100 ppm	43.04 ^d	43.15 ^h	73.08 ^g	16.87 ^g	0.436	0.412
	Salicylic acid at 200 ppm	43.90 ^b	48.66 ^f	98.63 ^e	18.50 ^f	0.432	0.432
	P extract at 5%	43.38 ^c	44.10 ^f	87.92 ^f	17.40 ^g	0.467	0.481
	P extract at 10%	44.24 ^a	50.05 ^e	113.98 ^c	19.51 ^e	0.535	0.538
4000 (mg L^{-1})	Control	41.82 ^g	43.24 ^h	84.47 ^f	19.24 ^{ef}	0.491	0.461
	Salicylic acid at 100 ppm	41.98 ^g	51.00 ^d	118.42 ^b	23.32 ^d	0.603	0.569
	Salicylic acid at 200 ppm	42.74 ^a	58.50 ^c	130.97 ^a	28.13 ^b	0.678	0.582
	P extract at 5%	42.07 ^f	62.07 ^b	106.49 ^d	25.80 ^c	0.583	0.589
	P extract at 10%	42.86 ^e	65.10 ^a	132.23 ^a	31.52 ^a	0.671	0.633

Means with different letters were significantly different at the 0.05 level according to Duncan's multiple range test

Grains yield and yield components: Salinity stress significantly decreased plant height, spike weight, grains weight/plant, 1000 grains weight and biological yield as compared to control in Table 4. Since salinity decreased grains weight/plant by 16.51% relative to control. On the other hand, all applied treatments significantly increased grains yield and its components in wheat plants irrigated with tap water or diluted seawater. It was obvious that pomegranate peel extract at 10% was the most pronounced treatment followed by salicylic acid at 200 mg L^{-1} . Since the maximum significant value in grains weight/plant under irrigation with tap water was 3.50 and 3.26 g that was achieved by applying pomegranate peel extract at 10% and salicylic acid

at 200 mg L^{-1} , respectively. Likewise, the maximum significant value in grains weight/plant under irrigation with diluted seawater was 2.65 and 2.42 g that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L^{-1} , respectively. Where, 10% pomegranate peel extracts increased grains weight/plant by 37.64% due to irrigation with tap water and by 40.89% due to irrigation with diluted seawater. 200 mg L^{-1} salicylic acid increased grains weight/plant by 33.63% due to irrigation with tap water and by 38.63% due to irrigation with diluted seawater.

Biochemical composition of the yielded grains: Table 5 show that salinity stress significantly decreased carbohydrate

content of the yielded grains accompanied by significant increases in DPPH and flavonoid relative to control. Additionally, non-significant increases were observed in lycopene and β -carotene relative to control. Meanwhile, all applied treatments significantly increased carbohydrate content, DPPH, phenolic and flavonoid contents in the yielded grains due to irrigation with tap water or diluted seawater. It was obvious that pomegranate peel extract at 10% was the most pronounced treatment followed by salicylic acid at 200 mg L⁻¹.

Since, under irrigation with tap water, the maximum significant value in carbohydrate was 44.24 and 43.90%, DPPH was 50.05 and 48.66%, which that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively. Similarly, under irrigation with diluted seawater, the maximum significant value in carbohydrate was 42.86 and 42.74%, DPPH was 65.10 and 58.50%, that achieved by applying pomegranate peel extract at 10% and salicylic acid at 200 mg L⁻¹, respectively.

DISCUSSION

Salinity stress significantly reduced vegetative growth parameters of a wheat plants as shown in Table 1. This reduction may be attributed to the high concentration of salts led to decrease water absorption and disturbing the uptake and translocation of nutritional ions^{13,45}. Furthermore, salinity stress limits plant growth and yield by adversely affecting various physiological and biochemical processes, such as photosynthetic capacity, chlorophyll contents, antioxidant activity, nitrogen metabolism, ion homeostasis and oxidative damage⁴⁶⁻⁴⁸.

Salinity stress significantly reduced photosynthetic pigments of the wheat leaf as shown in Table 2. Since, salinity around the root zone creates hindrance in the photosynthetic process including photosynthetic pigments, stomatal functioning, gaseous exchange attributes, structure and function of the thylakoid membrane, electron transport and enzyme activities⁴⁹. The decrease in chlorophyll content of salt-affected plants may be attributed to 1. The adverse effect of ions of various salts on chlorophyll biosynthesis^{2,49}. The possible oxidation of chlorophyll and other chloroplast pigments coupled with the instability of the pigment-protein complex⁵⁰. The formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation⁴⁷.

Regarding IAA, it was noted that salinity stress significantly decreased IAA contents of wheat leaves (Table 3). Environmental stresses reduced IAA may be due to

the decrease in enzyme activity that engages in IAA synthesis or increases in enzymes that participate in its breakdown⁵¹.

Significant increases in total soluble sugar and proline concentrations were observed in response to salinity as shown in Table 3. Since osmotic adjustment activates the production of a variety of compatible solutes such as soluble sugars⁵² and proline^{4,52} to maintain cell turgor and play several protective roles under unfavourable conditions⁵³. These increases in the two major organic osmolytes contents (proline and TSS) might help plants to regulate the osmotic potential of cells which led to improved water absorbance and translocation under salinity stress⁵⁴. As well, proline is involved in the protection of cellular structures, different enzymes from oxidative damages and acts as a scavenger of free radical⁵⁵.

The reductions in grains yield and yield components of the wheat plants under salinity stress might be through reductions in growth (Table 1) and photosynthetic pigments (Table 2). Since, salinity stress caused 1. Damage in chloroplast membranes and the antagonistic effects of sodium ion on magnesium ion absorption⁵⁶. Reduction in photosynthetic capacity via production of reactive oxygen species⁴⁶. Reduced the output of photosynthesis and diminished activities of Calvin cycle enzymes⁵⁷.

Regarding the biochemical composition of the yielded grains under salinity stress (Table 5), the reduction in photosynthetic pigments in leaves (Table 1) under the effect of salinity stress might have led to decreased levels of photo-assimilates in the leaves, mainly reflected total carbohydrates in the yielded grains⁵⁸. Plant secondary metabolites, specifically carotenoids and phenolics (phenolic acids and flavonoids), are stress-inducible, have important roles in plant growth and contribute to the nutritional value of vegetable and fruit crops⁵⁹. They are referred to as antioxidants because they can prevent, quench and scavenge the generation of ROS and attenuate oxidative damage⁶⁰. Likewise, the increases in antioxidant molecules like polyphenols and flavonoids under salinity help to minimize the negative effects of salt stress by removing free radicals, which enhances the tolerance against salt stress⁶¹. These increases could be due to an increase in enzymatic activities in plants which in turn support the cells to stabilize their structure and function by interacting with macromolecules^{62,63}. The accumulation of phenolic compounds in response to abiotic stress would be attributed to the activation of phenylalanine ammonia lyase⁶⁴ (PAL). Abdallah *et al.*⁶⁵ suggest that the potential antioxidant properties of carotenoids and flavonoids and their related key genes may be efficiently involved in the restriction of salt-induced oxidative damages. The activation of carotenoids synthetic pathway might be a

common mechanism for salt tolerance of glycophytes plants, considering that the regulation of their biosynthetic pathways leads to improved salinity tolerance in many transgenic plants including *Arabidopsis*, tomato tobacco and sweet potato⁶⁶⁻⁶⁹. Juan *et al.*⁷⁰ and Coesel *et al.*⁷¹ mentioned that most salt-resistant cultivars display a greater synthesis of carotenoids which suggests a potential protective role of these compounds against salinity-related impairment of plant fitness. In addition, Agius *et al.*⁷² and Krauss *et al.*⁷³ have reported that moderate salinity enhances lycopene and β -carotene in fresh tomato fruit. Likewise, according to De Pascale *et al.*⁷⁴, the total carotenoid and lycopene concentrations in tomato fruit are enhanced by moderate salinity but decrease as the level of salinity exceeds a threshold value. A possible explanation would be that salinity may inhibit or up-regulate the biosynthetic pathway of carotenoids via inhibition of the genes encoding enzymes related to lycopene and β -carotene⁷⁵.

Foliar applied salicylic acid enhanced vegetative growth by increasing fresh and dry biomass (Table 1). The increase in growth biomass in response to salicylic acid under salinity stress may be due to the protective role of salicylic acid on membranes, induction of antioxidant response and increased mineral uptake^{6,47}. Salicylic acid modulates the oxidative effects of stress that cause cell death and acts as a growth signal in cell resistance⁷⁶. Salicylic acid was involved in processes such as enhancement of nutrient uptake⁷⁷, induction of root formation and increased cell division in the apical region of the root meristem and eventually, it results from increment of plant growth⁷⁸.

Exogenously applied salicylic acid significantly enhanced net photosynthetic rate (Table 2) which could be due to improving the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or by chlorophyll biosynthesis⁷⁹. Salicylic acid-induced improvement in net CO₂ assimilation rate agreed with findings of earlier researchers where foliar applied salicylic acid enhanced photosynthetic capacity in various crops, for example, sunflower⁸⁰ and wheat¹⁵. Also, Emamveridian *et al.*⁸¹ stated that salicylic acid can regulate plant photosynthetic events by impacting different parameters including stomatal closure and influencing the structure of chloroplasts and enzymes involved in photosynthesis.

The significant increases in IAA are due to salicylic acid application (Table 3) because of its role in the regulation of other hormone pathways⁸². Regarding osmolytes, Misra and Saxena² mentioned that biosynthesis and accumulation of compatible solutes may be due to alterations in the activities of enzymes involved in their biosynthesis and degradation

and contribute to osmotic adjustment at the cellular level, protect membrane integrity and consequently, mediate the damage induced by salt. The increase in proline content by salicylic acid is reported by Shakirova¹⁶ in wheat seedlings. Also, increasing soluble sugars and polysaccharide contents by application of salicylic acid is reported by Yuan *et al.*⁸³ which act as an osmoprotectant agent, elevating plant tolerance under different stresses. The salicylic acid-induced grains yield (Table 4) might be due to the certain physiological role of salicylic acid such as absorption of ions, enhancing flowering⁸⁴ and photosynthesis processes⁸⁵ which could directly or indirectly regulate the yield.

Regarding the biochemical composition of the yielded grains (Table 5), exogenous application of salicylic acid has been reported to be effective in inducing secondary metabolite formation like total phenolic content and total flavonoid content⁸⁶. Application of appropriate concentrations of salicylic acid reduces the harmful effects of oxidative stress by improving antioxidant capacity as well as the synthesis of protective compounds in plants⁹. Phenolic content is a suitable indicator for assessing environmental stress tolerance and improving plant metabolism through antioxidant protection⁸⁷. Khalil *et al.*⁸⁸ showed that total polyphenol content in *Thymus vulgaris* L. plants increased compared to control under salicylic acid as well as drought stress. Flavonoid contents increased under saline conditions may reflect some kind of defence against stress conditions since salt stress was accompanied by increased production of reactive oxygen species⁸⁹. The salicylic acid-treated plants also showed more antioxidant activity measured in terms of DPPH free radical scavenging activity. This higher free radical scavenging activity of the plants may be due to the presence of a high levels of phenolic and flavonoids. Some earlier workers also reported such positive correlation between antioxidant activity and phenolic content^{90,91}. Bakry *et al.*⁹² stated that spray of peanut with salicylic acid significantly increased total phenolic, antioxidant and plant pigments. Khan *et al.*⁸² and Koo *et al.*⁹³ concluded the impact of salicylic acid on plants' accumulation of osmolytes, regulation of mineral nutrition uptake, enhanced reactive oxygen species scavenging activity, enhanced secondary metabolite production, regulation of other hormone pathways.

Earlier research has shown that pomegranate peel extracts contain free radical scavenging and antioxidant properties^{94,95}. Pomegranate peel is a rich source of bioactive compounds and contains a variety of secondary metabolites²⁶. Antioxidant activity of pomegranate fruit can be ascribed to the presence of some components like ascorbic acid and phenolic compounds flavonoids and tannins²⁷. The

pomegranate peels are a powerful source of natural antioxidants such as polyphenolic and flavonoid compounds that can be attributed to the antioxidant index²⁹. It is worth mentioning that it was reported that a high concentration of the extract may induce allelopathic activity in tomato plants⁹⁶. Mercy *et al.*⁹⁷ mentioned that the fruit peel powder extract act as a natural fertilizer and fruit peel powder extract increased the growth of plants and yield.

Pomegranate fruit peel has K, N, Ca, P, Mg, Na and it has micronutrients like B, Fe, Zn, Cu, Mn⁹⁸. Application of fruit peel powder (banana, pomegranate and orange) into the soil leads to improve growth and yield of okra in sandy regosols compared to recommended inorganic fertilizer⁹⁹.

CONCLUSION

All applied treatments alleviated the deleterious effect of salinity via enhancing vegetative growth parameters, photosynthetic pigments, IAA, grains yield and its components, leaf osmolytes, carbohydrate content, phenolic content, flavonoids, lycopene and β -carotene of the yielded grains.

SIGNIFICANCE STATEMENT

This study discovered the most optimum treatments were pomegranate peel extract at 10% followed by salicylic acid at 200 mg L⁻¹. This treatment can be used for further purposes.

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