

Response of Salt-Stressed Wheat (*Triticum aestivum* L.) to Potassium Humate Treatment and Potassium Silicate Foliar Application

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THE PRESENT study was carried out to investigate the effect of high NaCl concentrations on some growth parameters and physiological processes of wheat (*Triticum aestivum* L.) plant. The results indicated that Gemeza.9 was the most sensitive cultivar to high salinity levels compared to all tested wheat cultivars. Moreover, salinity stress caused a reduction in the germination percentage by 65% and all growth parameters by 21%, 25% and 60% in the lengths of shoot, root and leaf area, also by 27% and 48% in fresh weights of shoot, root and 40% and 75% in dry weights of shoot and root. It also increased the activity of antioxidant enzymes (peroxidase and catalase), lipid peroxidation and ascorbic acid content. Salinity induced increase of osmolytes compounds such as total soluble proteins, carbohydrates and amino acids. Furthermore, all measured yield parameters, the percentage of grain's maturity and productivity were highly decreased. Accordingly, the role of potassium humate and potassium silicate either sole or combined in alleviating the toxic effect of salinity was also studied. Results showed that application of potassium humate as sole had a stimulatory effect higher than potassium silicate or their combination. It increased the germinating percentage by 128% at 200mM NaCl. In addition, potassium humate and potassium silicate increased the photosynthetic activity, decreased the activity antioxidant enzymes (Peroxidase and Catalase) as well as biosynthesis of MDA and ascorbic acid also they induced increase biosynthesis of proteins and carbohydrates in yielded grains. Accordingly, our study recommends the application of potassium humate as an organic fertilizer and potassium silicate as a foliar spray for improving the quality and quantity of the sensitive wheat cultivar Gemeza.9 cultivated in salty lands and increases its productivity.

Keywords: Salinity, Amelioration, Foliar application, Potassium Humate, Potassium Silicate.

Introduction

Wheat (*Triticum aestivum* L.) is the second most important food crop after rice (Malav et al., 2017). Wheat is considered as one of the most important edible crops in Egypt. It provides approximately one-fifth of the total caloric input of the world's population (FAO, 2010). In wheat grains, starch is present in large amounts (60-68%) where, it is considered the most important reserve polysaccharide of many types of cereals (Parker & Ring, 2001). Moreover, wheat grains contain protein (6-21%), fats (1.5- 2.0%), cellulose (2.0-2.5%), minerals (1.8%) and vitamins (Das, 2008 and Malav et al., 2017). Egypt remains the world's largest wheat importer. Egypt imported about 12 million tons of wheat in 2016/2017, this estimation about 1.3 million tons above the average for the last five years (FAO, 2016). Many efforts should be directed toward increasing cultivating wheat in Egypt to fill the gap between production and consumption (Kandil et al., 2016).

Soil salinity is a global problem that adversely affects 20 % of irrigated land and reduces crop yields (Qadir et al., 2014). It results in ion toxicity, osmotic and oxidative stress, nutritional imbalances, reduction of cell divisions and alterations in metabolic processes as photosynthesis, respiration, and disorganization of membranes of plants (Sen et al., 2002 and Hasanuzzaman et al., 2014). Many investigators have reported the reduction of plant growth due to salinity stress.

The addition of supplemental organic matters of different sources acts as an ameliorative agent against salinity stress (Walker & Bernal, 2008). Humic substances (humates, humic acids, and fulvic acids) make up the bulk of humus which resulted from organic soil decomposition and constituting 65-75% of organic matters (Shahryari et al., 2009). Potassium humate is an effective fertilizer that positively affects growth, yield and

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chemical constituents of wheat plant (Kandil et al., 2016). Potassium humate increases the rate of nutrient uptake, enhances plant biomass and reduces the soil compaction (Canellas et al., 2015).

On other hands, foliar spraying of plants with micronutrient elements increases plant's tolerance under salinity stress (Van Bockhaven et al., 2013). Between all the micronutrients assimilation by plants, silicon is considered as the same macronutrients concentration needed by plants (Ahmad et al., 2013). Silicon has the ability to ameliorate salt stress due to its high efficiency to increase relative water content, membrane stability index, antioxidant enzyme activity such as peroxidase and catalase and decline proline content so it's foliar application in the form of silicate liquid solutions can be used as a promising alternative to mitigate abiotic stress-induced damage in wheat (Sapre & Vakharia, 2017).

Therefore, the objective of this work was to detect the effect of salinity stress on growth and yield of wheat (*Triticum aestivum* L.) and assessing the role of potassium humate and potassium silicate on alleviating the deleterious effect of salinity.

Materials and Methods

Germination percentage

This experiment was carried out in small plastic pots (15 cm² diameter and 10 cm depth), each one was filled with 5 kg clay-sandy soil (2:1w/w), 10 grains were sown in each pot for each treatment and was replicated 3 times, the different concentrations of NaCl were prepared (0, 50, 100, 150, 200, 250, 300, 350, 400, 400 and 450 mM) for determination the lethal and sublethal level of NaCl. Pots were irrigated with NaCl solutions for 7 days. Gemeza.9 cultivar was chosen as the most sensitive one at 200 mM NaCl as sublethal level to be used in the present study.

To determine the germination percentage of wheat grains (cv. Gemeza.9) which treated with potassium humate, pots were divided into two groups then irrigated with NaCl concentrations (0-200mM); the second group was irrigated with NaCl concentrations and potassium humate (0.02 g/Pot, 1kg/fad) and left in the environmental conditions for 7 days.

Plant material, growth conditions, and treatments

Wheat grains (*Triticum aestivum* L., cv.Gemeza.9) were selected for uniformity in size and shape, washed several times with distilled water and grown in clay sandy soil (2:1 w/w) in plastic pots (40 cm² diameter, 45 cm depth).The experiments were carried out during the growing season of wheat i.e. from December to end of March. Pots were divided into four groups then irrigated with (80% field capacity). The seedlings were left to grow for 7 days under normal environmental conditions at $29 \pm 2^\circ\text{C}$ during the day and $12 \pm 2^\circ\text{C}$ during the night. On the 8th day, the first group was irrigated with NaCl (0-200 mM) while the second group was treated with the recommended dose of potassium humate (Pot.H) (0.2 g/Pot, 1kg/fad). Moreover, the third group was sprayed with 25% potassium silicate (Pot.Si) (250 mg/L SiO₂) according to the recommended dose. Finally, the fourth group was treated with a combination of them. Samples were collected after 30 days (seedling stage), 60 days (vegetative stage) and 120 days (yield stage) and subjected to the following experiments.

Photosynthetic activity and pigments

Photosynthetic activity (Fv/Fm) of dark-adapted leaves was measured with OS-30p chlorophyll fluorometer (Hudson, NH 03051 USA). The photosynthetic pigments, chlorophyll a (Chl.a), chlorophyll b (Chl.b) and carotenoids were assayed according to Arnon (1949) and expressed as mg g⁻¹dry weight.

Enzymes extractions and assays

A sample of 0.5 g fresh leaves was frozen and then homogenized in 8 ml of 50 mM cold phosphate buffer (pH 7.0) (Beauchamp & Fridovich, 1971). The homogenates were centrifuged at 4000 rpm at 4^o for 20 min. The supernatant was used as a raw extract for enzymatic assay. Activities of catalase [EC1.11.1.6] and peroxidase [EC1.11.1.7] were assayed according to Kato & Shimizu (1987). Enzyme activity was expressed in units of μM of the substrate converted per min. per gram fresh weight

Lipid MDA and ascorbic acid content

Lipid peroxidation level was measured by determining malondialdehyde (MDA) content (peroxidation product). MDA content was estimated according to Heath & Packer (1968) and calculated using the extinction coefficient

(155 mM⁻¹cm⁻¹) and expressed as μmolg^{-1} fresh weight. Ascorbic acid was estimated according to Oser (1979). Ascorbic acid content was calculated as mg g⁻¹ fresh weight by using a calibration curve.

Total soluble protein and carbohydrates

The phenol-sulfuric acid method has been used to estimate the total soluble carbohydrates using a calibration curve according to Dubois et al. (1956). Total soluble protein content was estimated quantitatively using phosphate buffer (pH 7) extract using a calibration curve by Bovine serum albumin (BSA) as a standard protein according to Bradford (1976). They are expressed as mg g⁻¹ dry weight.

Total amino acids and proline content

Amino acids content was assayed using ninhydrin and glycine as a standard according to Lee & Takahashi (1966). Proline was estimated according to Zungia et al. (1989) and Bates (1973) by using toluene as a blank. They are calculated as mg g⁻¹ dry weight.

Statistical analysis

The results were analyzed statistically using one-way (ANOVA) test to determine the degree of significance. The analysis was

carried out using COSTAT statistical program. In addition to correlation coefficient (R^2) were applied for investigating the significance of the relationships between the studied variables of plant the analysis was carried out by SPSS (v.6.12).

Results

Preliminary experiment

Data shown in Table 1 indicates that increasing NaCl concentrations decreased the germination percentage except at 50mM conc. the germination percentage has no effect compared with the control. As shown that Sakha.93 is the most tolerant cultivar while Gemeza.9 is the most sensitive one (Table 1).

Germinating percentage

Results in Table 2 indicate that treating wheat grains (cv.Gemeza.9) with high salinity level (200 mM NaCl) resulted in a reduction of germination percentages by 65% compared with the control. However, the germination percentage of all treated samples with potassium humate was increased and at high salinity level (200 mM NaCl) the percentage was increased by 128% compared with salt-stressed samples (Table 2).

TABLE 1. Effect of different concentrations of NaCl (0 - 450 mM) on the germination percentage of different 7-day-old *Triticum aestivum* L. cultivars grains grown in clay sandy soil (2:1w/w).

Conc. of NaCl (mM)	Germination percentage					
	Sakha. 93	Giza.168	Misr.1	Sods.12	Gemeza.11	Gemeza.9
0	99±1 ^g	99±1 ^g	99±1 ^f	99±1 ^f	99±1 ^g	99±1 ^e
50	99±1 ^g	99±1 ^g	99±1 ^f	99±1 ^f	99±1 ^g	99±1 ^e
100	98±1 ^g	95±3 ^g	85±3 ^e	95±3 ^f	85±3 ^f	70±3 ^d
150	75±3 ^f	85±3 ^f	70±2 ^d	65±3 ^e	75±3 ^e	60±2 ^c
200	65±3 ^e	70±3 ^e	50±2 ^c	45±2 ^d	50±2 ^d	35±2 ^b
250	50±2 ^d	50±2 ^d	45±2 ^c	35±2 ^c	40±2 ^c	00.0 ^a
300	45±2 ^d	35±2 ^c	35±1 ^b	25±1 ^b	35±2 ^b	00.0 ^a
350	35±2 ^c	25±1 ^b	00.0 ^a	00.0 ^a	00.0 ^a	00.0 ^a
400	20±1 ^b	00.0 ^a				
450	00.0 ^a	00.0 ^a	00.0 ^a	00.0 ^a	00.0 ^a	00.0 ^a
R ²	99.41%	97.99%	96.31%	96.81%	96.21%	95.94%
P-value	0.0001***					

Values are the mean of three replicates ± SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$)

TABLE 2. Effect of different concentrations of NaCl (0-200mM) on the germination percentage of 7-day-old *Triticum aestivum* L. (cv.Gemeza.9) grains grown in clay sandy soil (2:1w/w) after soil supplementation with potassium humate (Pot.H) (0.02 g/Pot, 1kg/fad).

Conc. of NaCl (mM)	Germination percentage	
	Non-treated	Pot.H (0.02 g/pot, 1kg/fad)
0	99±1 ^d	99±1 ^b
50	99±1 ^d	99±1 ^b
100	70±3 ^c	95±3 ^b
150	60±2 ^b	85±2 ^a
200	35±2 ^a	80±2 ^a
R ²	96.33 %	99.84 %
P-value	0.0001***	

Values are the mean of three replicates ± SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$).

Main experiment

Growth parameters

Table 3 indicates that at 200 mM NaCl, all the measured growth parameters of the seedlings decreased by 21%, 25% and 60% in the lengths of shoot, root and leaf area, respectively compared with the control. However, the reduction reached 27% and 48% in fresh weights of shoot, root and 40% and 75% in dry weights of shoot and root, respectively compared to control. Data presented in Table 3 also show that all growth parameters of

seedlings (the lengths of shoot, root and leaf area) and (fresh and dry weights of shoot and root) increased by treating with potassium humate as sole (15%, 27% and 90%) and (20%, 33%, 28% and 200%), respectively compared with control while application of potassium silicate only increased all of them (4%, 9% and 45%), (7%, 12%, 15% and 100%), respectively compared with control. Accordingly, the combination of potassium humate and silicate approximately has the same effect of potassium silicate (Table 3).

TABLE 3. Effect of different concentrations of NaCl (0-200mM) on some growth parameters of 30-day-old *Triticum aestivum* L. (cv.Gemeza.9) seedlings grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation with potassium humate (Pot.H) (0.2g/pot, 1kg/fad) and spraying using potassium silicate (Pot.Si) (250 mg/L SiO₂) as sole or combined.

Treatment	Length (cm/organ)		Fresh weight (g/organ)		Dry weight (g/organ)		Leaf area (cm ² /leaf)
	Shoot	Root	Shoot	Root	Shoot	Root	
Control	17.1±0.1 ^d	15.1±0.3 ^c	1.34±0.01 ^d	0.25±0.001 ^c	0.20±0.001 ^c	0.04±0.002 ^c	2.8±0.2 ^d
200 mM NaCl	13.4±0.1 ^a	11.3±0.4 ^a	0.97±0.05 ^a	0.13±0.005 ^a	0.12±0.005 ^{ab}	0.01±0.001 ^a	1.1±0.1 ^a
Pot.H (0.2g/pot, 1kg/fad)	19.8±0.1 ^e	18.4±0.2 ^b	1.97±0.01 ^f	0.32±0.001 ^b	0.29±0.001 ^d	0.06±0.002 ^e	4.3±0.3 ^f
Pot.H+200 mM NaCl	15.4±0.4 ^c	14.4±0.2 ^d	1.17±0.05 ^c	0.18±0.005 ^c	0.16±0.005 ^b	0.03±0.001 ^d	2.1±0.1 ^c
Pot.Si (250mg/L SiO ₂)	18.3±0.3 ^c	16.2±0.4 ^f	1.45±0.01 ^e	0.28±0.001 ^f	0.26±0.001 ^d	0.05±0.002 ^c	3.3±0.2 ^c
Pot.Si+200 mM NaCl	13.9±0.5 ^a	12.3±0.2 ^b	1.04±0.05 ^b	0.15±0.005 ^b	0.14±0.005 ^{ab}	0.02±0.001 ^b	1.6±0.1 ^b
Pot.H+Pot.Si	18.9±0.5 ^f	17.3±0.3 ^e	1.46±0.01 ^c	0.30±0.001 ^e	0.27±0.001 ^d	0.05±0.002 ^f	3.9±0.2 ^f
Pot.H+Pot.Si +200 mM NaCl	14.6±0.2 ^b	13.1±0.3 ^c	1.06±0.05 ^b	0.16±0.005 ^c	0.14±0.005 ^a	0.03±0.001 ^c	1.7±0.1 ^b
R ²	96.13 %	95.84 %	97.22 %	96.51 %	96.81%	99.21%	98.94%
P-value	0.0001***						

Values are the mean of three replicates ± SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$).

Biochemical analysis

Photosynthetic activity and photosynthetic pigments

Salinity resulted in a negative effect on photosynthetic activity and photosynthetic pigments content (Fig.1). High salinity level (200 mM NaCl) caused a marked reduction in photosynthetic efficiency (30%) compared with the control and caused a high reduction in photosynthetic pigments (Chl.a and Chl.b) by (44% and 87%), respectively and increased carotenoids by 94% compared with control. Results in Fig.1 indicate that application of potassium humate and potassium silicate either sole or combined decreased the negative effects of salinity. However, application of potassium humate as sole was more efficient than potassium silicate or their combination whereas; it increased the photosynthetic activity by 25% and pigments content (Chl.a and Chl.b) by 50% and 414%, respectively compared with control. Accordingly, potassium silicate increased the photosynthetic activity by 13% and pigments contents (Chl.a and Chl.b) by 24% and 271%, respectively compared with control. However, potassium humate and potassium silicate separately or combined expressed similar effect in reducing carotenoids (18% and 15%), respectively compared with control.

Antioxidant enzymes, lipid peroxidation and ascorbic acid content

Results in Fig.2 show that the activity of both peroxidase and catalase enzymes were significantly enhanced in response to salinity stress. The percentages of increase were 55% and 60%, for peroxidase and catalase, respectively relative to the control. Similarly, ascorbic acid and the malondialdehyde (MDA) content were highly increased in salt-stressed seedlings by 57% and 215%, respectively compared with the control. Data in Fig.2 indicates that potassium humate, potassium silicate and their combination caused a significant reduction in peroxidase and catalase activities and the contents of ascorbic acid and MDA. However, application of potassium humate as sole was more efficient in reducing peroxidase and catalase activities by 32% and 25%, respectively. The content of ascorbic acid was decreased by 26% and MDA by 61% compared to control. Accordingly, potassium silicate reduced the activities of peroxidase and catalase enzymes by 22% and 12.5, respectively and the contents of ascorbic acid and MDA by 17% and 36%, respectively compared with control. The combination of potassium humate and potassium silicate treatments has the same effect of potassium silicate.

Total soluble proteins and carbohydrates

Data in Fig.3 indicates that high salinity level (200 mM NaCl) caused a significant increase in total soluble proteins and carbohydrates content, the increase reached 300% and 250%, respectively relative to the control. Application of potassium humate, potassium silicate and their combination increased the total soluble proteins and carbohydrates compared with control. However, potassium humate as sole was significantly decreased total soluble proteins and carbohydrates content by 38% and 35%, respectively while potassium silicate single and combined with potassium humate has the same effect in reducing both total soluble proteins and carbohydrates by 18% and 28%, respectively compared with untreated seedlings.

Total soluble amino acids and proline content

Data in Fig.4 shows that treatment with (200 mM NaCl) caused a highly significant increase in the total soluble amino acids by 160% and proline content by 200% relative to the control. However, potassium humate and potassium silicate and their combination approximately have the same effect in decreasing contents of total soluble amino acids and proline whereas; they decreased total soluble amino acids by 44% and 23%, respectively as well as decreased proline content with 87% and 86% compared with control.

Yield parameters

Table 4 shows that the salinity (200 mM NaCl) caused a significant decrease of all the measured yield parameters (spike length, the number of spikes/plant, and the number of spikelet/ plant, spike weight, the number of grains/spike, and weight of 1000 grains). As shown in Table 4 that salinity decreased the number of spikes/plant, the number of grains/spike, and weight of 1000 grains with 75%, 68%, and 64%, respectively compared with control. However, applications of potassium humate and potassium silicate as sole or combined increased all measured yield parameters. Accordingly, potassium humate was more efficient than potassium silicate and their combination in enhancing the productivity and maturity where, the percentage of maturity reached to 80% and weight of thousand grains increased by 155% compared with control. While potassium silicate increased the productivity (weight of 1000 grains) by 120% compared with control. On other hands, potassium humate increased weight of thousand grains by 65% while potassium silicate and the combination increased it by 14% and 17%, respectively relative with control (Table 4).

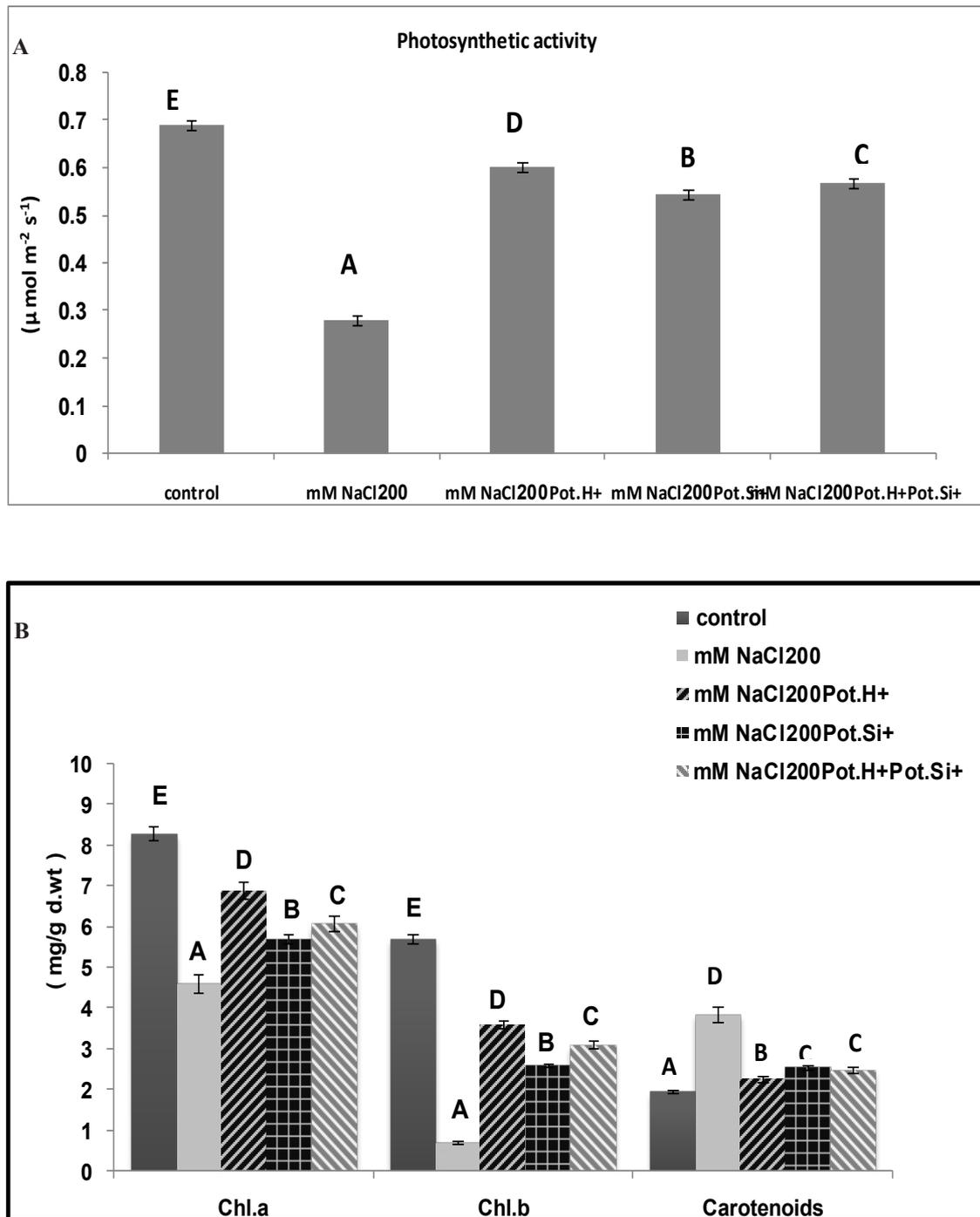


Fig.1. Effect of different concentrations of NaCl (0-200mM) on photosynthetic activity (Fv/Fm) (A) and photosynthetic pigments (Chl.a, Chl.b and carotenoids) (B) of 30-day-old *Triticum aestivum* L. (cv.Gemeza.9) seedlings grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation with potassium humate (Pot.H) (0.2g/pot,1kg/fad) and spraying using potassium silicate (Pot.Si) (250 mg/L SiO₂) as sole or combined. Values are the mean of three replicates \pm SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$)

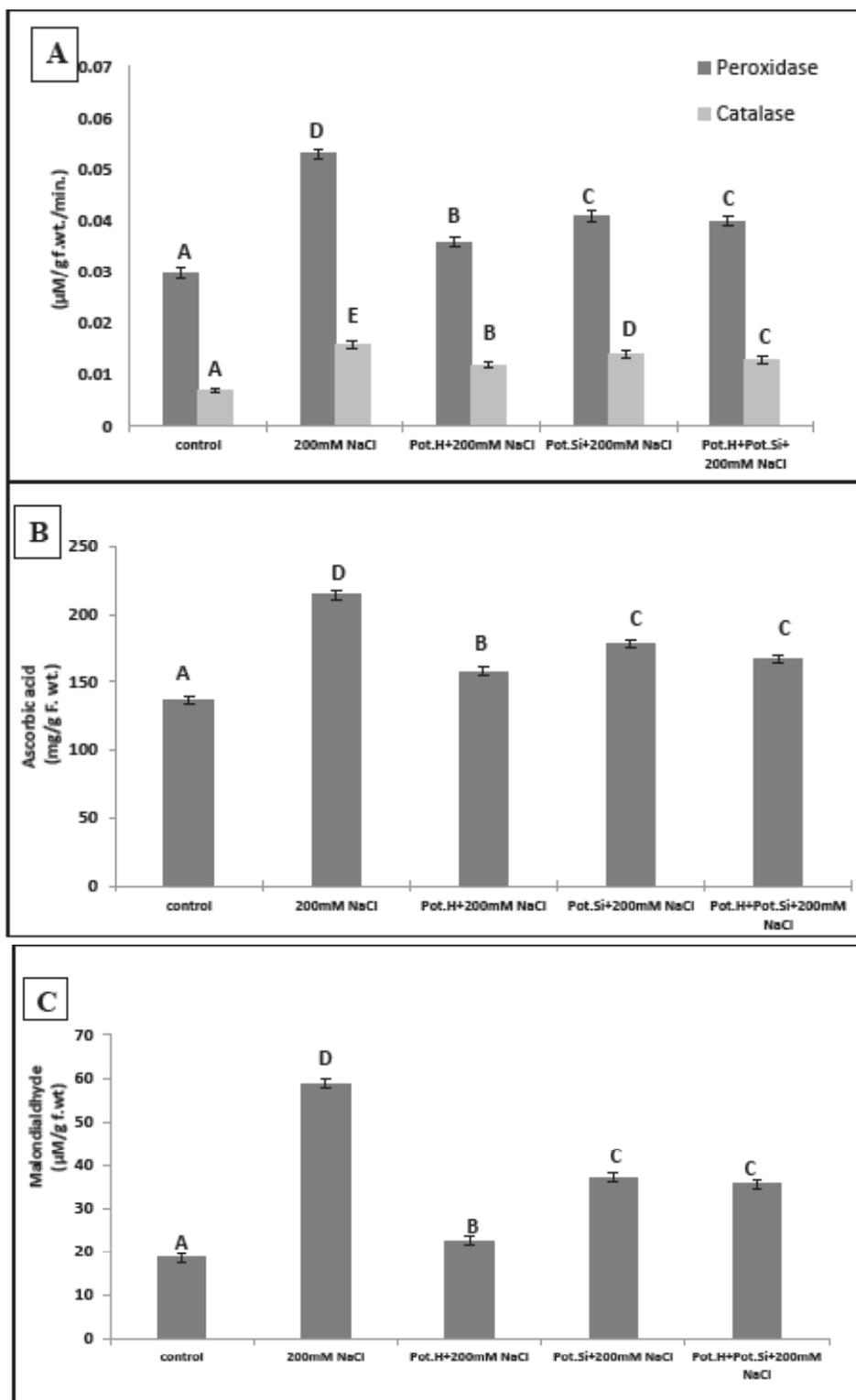


Fig.2. Effect of different concentrations of NaCl (0-200mM) on peroxidase and catalase enzyme (A) and ascorbic acid (B) and MDA (C) contents of 30-day-old *Triticum aestivum* L. (cv.Gemeza.9) seedlings grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation with potassium humate (Pot.H) (0.2g/pot,1kg/fad) and spraying using potassium silicate (Pot.Si) (250 mg/L SiO_2) as sole or combined. Values are the mean of three replicates \pm SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$)

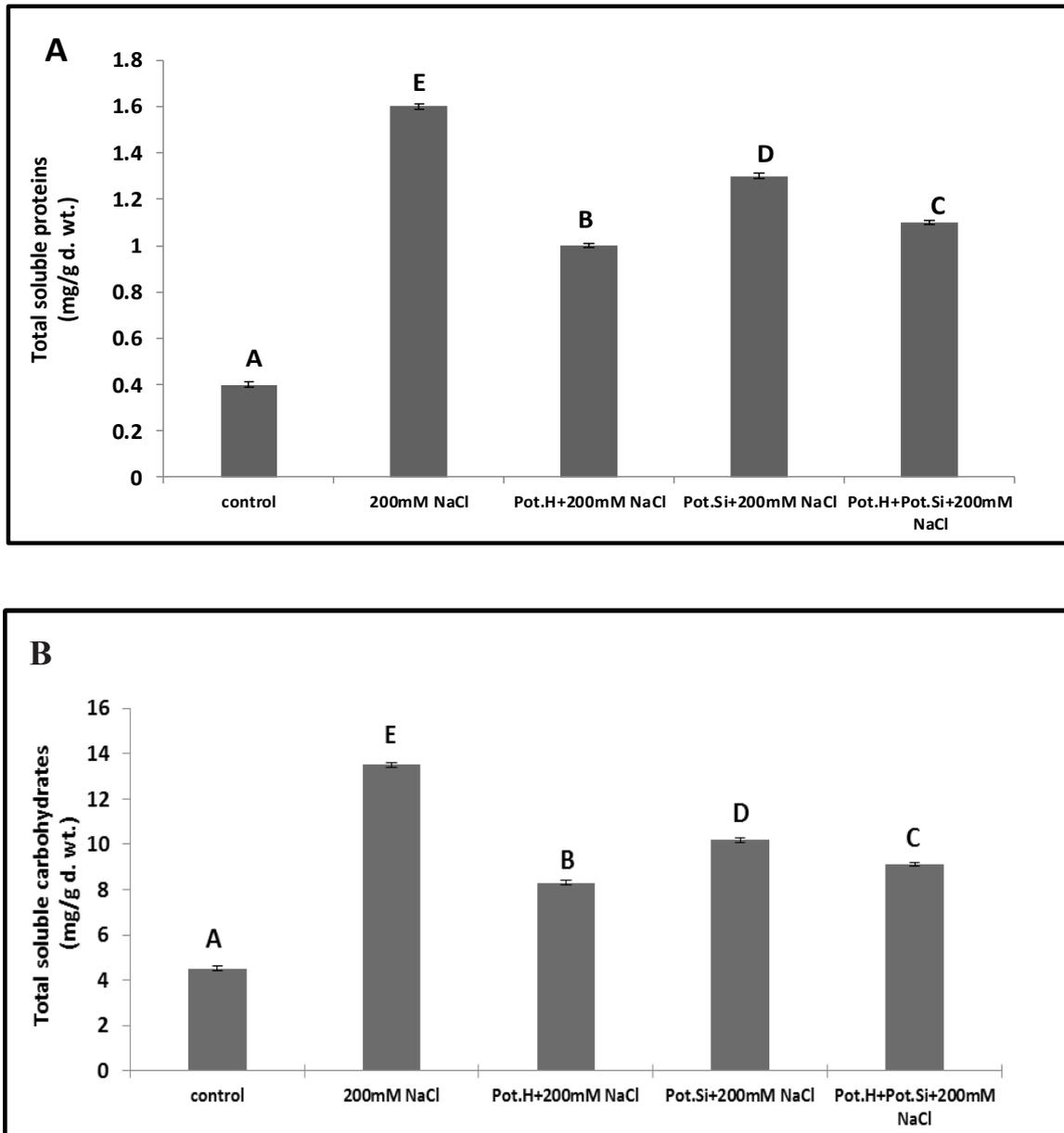


Fig.3. Effect of different concentrations of NaCl (0-200mM) on total soluble proteins (A) and carbohydrates (B) of 30-day-old *Triticum aestivum* L. (cv.Gemeza.9) seedlings grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation with potassium humate (Pot.H) (0.2g/pot,1kg/fad) and spraying using potassium silicate (Pot.Si) (250 mg/L SiO₂) as sole or combined. Values are the mean of three replicates ± SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$)

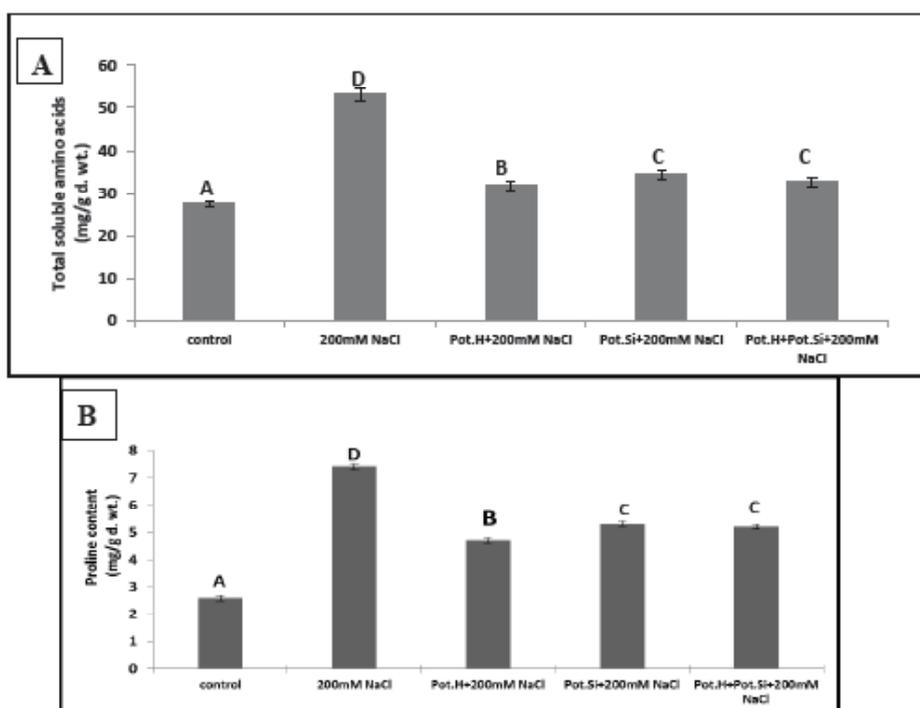


Fig.4. Effect of different concentrations of NaCl (0-200mM) on total soluble amino acids (A) and proline (B) content of 30-day-old *Triticum aestivum* L. (cv.Gemeza.9) seedlings grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation with potassium humate (Pot.H) (0.2g/pot,1kg/fad) and spraying using potassium silicate (Pot.Si) (250 mg/L SiO₂) as sole or combined. Values are the mean of three replicates \pm SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$)

TABLE 4. Effect of different concentrations of NaCl (0-200mM) on some yield parameters of 120-day-old *Triticum aestivum* L. (cv.Gemeza.9) grains grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation with potassium humate (Pot.H) (0.2g/pot, 1kg/fad) and spraying using potassium silicate (Pot.Si) (250 mg/L SiO₂) as sole or combined.

Treatments	Spike length (cm)	No.of spikes /plant	No.of Spikelet /spike	No.of grains /spike	Spike weight (g)	Weight of 1000 grains (g)	Percentage of maturity
Control	9.20 \pm 0.03 ^d	4.0 \pm 0.3 ^d	40 \pm 1.5 ^c	37.33 \pm 1.5 ^c	1.9 \pm 0.1 ^c	28.4 \pm 1 ^c	93.3 \pm 1.5 ^{ef}
200 mM NaCl	7.40 \pm 0.01 ^a	1.0 \pm 0.3 ^a	29 \pm 1.2 ^a	12.00 \pm 0.6 ^a	0.7 \pm 0.1 ^a	10.1 \pm 1 ^a	41.3 \pm 2.5 ^a
Pot.H (0.2g/pot, 1kg/fad)	11.4 \pm 0.03 ^f	6.0 \pm 0.3 ^f	50 \pm 1.2 ^c	48.00 \pm 1.2 ^e	2.8 \pm 0.1 ^d	46.9 \pm 1 ^d	96.0 \pm 1.2 ^e
Pot.H+200 mM NaCl	9.10 \pm 0.03 ^d	3.7 \pm 0.3 ^c	36 \pm 1.1 ^d	29.00 \pm 0.6 ^d	1.3 \pm 0.1 ^{bc}	25.8 \pm 1 ^{bc}	80.0 \pm 0.3 ^d
Pot.Si (250mg/L SiO ₂)	10.8 \pm 0.04 ^e	4.3 \pm 0.3 ^d	42 \pm 1.2 ^b	40.00 \pm 1.2 ^f	2.1 \pm 0.1 ^d	32.4 \pm 1 ^d	95.2 \pm 1.2 ^e
Pot.Si+200 mM NaCl	8.12 \pm 0.03 ^b	3.0 \pm 0.3 ^b	32 \pm 1.4 ^{ab}	22.00 \pm 0.5 ^b	1.1 \pm 0.1 ^{ab}	22.2 \pm 1 ^b	68.7 \pm 0.5 ^b
Pot.H +Pot.Si	11.05 \pm 0.03 ^e	5.2 \pm 0.3 ^c	44 \pm 1.2 ^d	42.00 \pm 1.2 ^f	2.4 \pm 0.1 ^d	33.7 \pm 1 ^d	95.4 \pm 1.2 ^{ef}
Pot.H+Pot.Si+200 mM NaCl	8.45 \pm 0.03 ^c	3.2 \pm 0.3 ^b	33 \pm 1.2 ^{ab}	25.00 \pm 0.6 ^c	1.2 \pm 0.1 ^{abc}	24.8 \pm 1 ^{bc}	75.5 \pm 0.5 ^c
R ²	97.81 %	96.52 %	96.32 %	99.52 %	96.57 %	98.24 %	99.54 %
P-value				0.0001***			

Values are the mean of three replicates \pm SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$).

Total soluble proteins and carbohydrates of the produced grains

The results in Fig. 5 show that high salinity level at 200 mM caused a significant decrease in the total soluble protein and carbohydrates of produced grains and this decrease was represented by 216% and 183%, respectively compared with control. Accordingly, application of potassium humate and potassium

silicate single or combined were significant increased the content of total soluble protein and carbohydrates in the yielded grains. Results indicated that addition of potassium humate as sole increased the content of total soluble proteins and carbohydrates by 100% and 188%, respectively while potassium silicate and the combined treatments increased them by 40% and 118%, respectively compared with control.

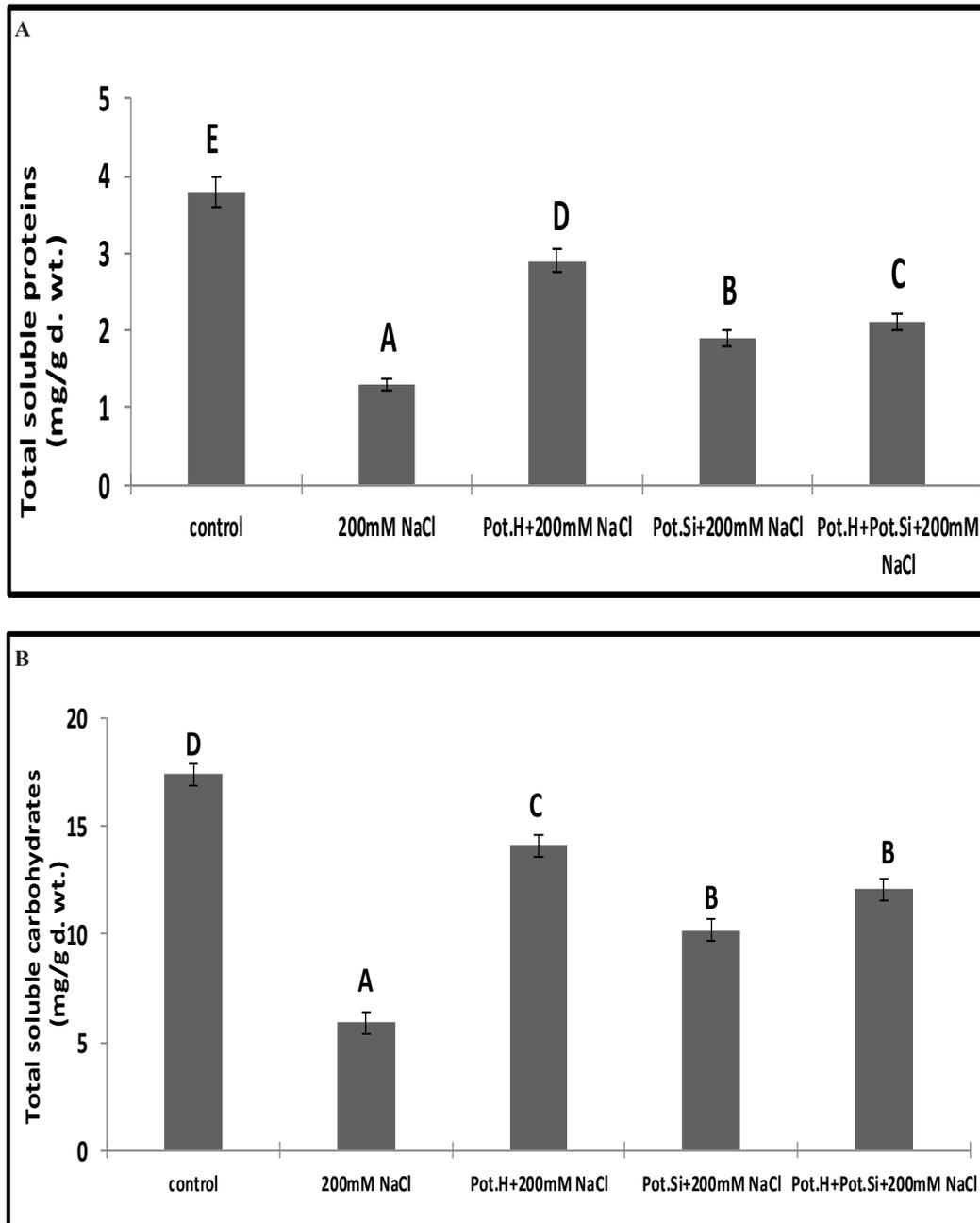


Fig.5. Effect of different concentrations of NaCl (0-200mM) on total soluble proteins (A) and carbohydrates (B) of 120-day-old *Triticum aestivum* L. (cv.Gemeza.9) grains grown in clay sandy soil (2:1w/w), in response to soil irrigation at (80% field capacity) followed by soil supplementation by (0.2g/pot,1kg/fad) potassium humate and spraying with (250 mg/L SiO_2) potassium silicate as sole or combined. Values are the mean of three replicates \pm SD, Values within the same column for each factor designed by the same letter are insignificant ($p \leq 0.05$), Values with different letters are significant ($p \leq 0.05$).

Discussion

Germination and plant growth

Seed germination is the initial step in plant life where, it has an influential role in yield (Sanchez et al., 2014). Wheat seedlings are more sensitive to salinity during germination than during emergence and early seedlings growth (Aflaki et al., 2017). In the present study, Gemeza.9 couldn't tolerate high NaCl level (200 mM) and its germination percentage was reduced (Table 1 and 2) also, all growth parameters were decreased in response to salinity stress (Table 3). Salinity delays or prevents seed germination and seedling establishment due to its effect on water absorption by reducing water potential (osmotic stress) that reduce water uptake by plants (Jeannette et al., 2002). High accumulation of salts in soils and intercellular spaces of the cells induces damage of the embryo (Rahman et al., 2008). Accordingly, the reduction of growth may be attributed to osmotic imbalance and ion toxicity under continuous exposure of the plant to saline conditions leading to changing in their morphology and anatomy (Singh, 2016). Similar observations were reported by Aflaki et al. (2017) in wheat.

As shown in our results, potassium humate had a marked effect against salinity stress. it increased the germination percentage (Table 2) and all measured growth parameters (Table 3). This ameliorative effect may be attributed to the entrance of humic substances into the cells carrying both micronutrients and water. They also increase water permeability of plant membranes and water holding capacity (Kumar & Singh, 2017). Many observations were documented about the high efficiency of humic substances and their effects on the plant growth (Chen & Aviad, 1990; Nardi et al., 2007; Cesco et al., 2002 and Rady, 2012). Yang et al. (2004) reported that humate substances can indirectly and directly affect the physiological processes of plant growth by providing minerals uptake and biochemical substances, and carry trace elements and growth regulators for enhancing the plant's growth.

The effective role of potassium silicate in increasing the growth parameters was attributed to increasing water contents in wheat (Ali et al., 2012) and photosynthetic efficiency which leads to increasing the plant's growth (Pati et al., 2017). It was hypothesized that the silicon decreases

of uptake Na^+ by plants under salt stress and the increase of K^+/Na^+ ratio also prevented Na^+ translocation in leaves and/or specific deposition in roots (Guntzer et al., 2011 and Abbas et al., 2015). Similar observation was reported by Ibrahim et al. (2016) in wheat.

Photosynthetic parameters

Photosynthetic process is very sensitive under stress conditions including salinity. According to data shown in Fig.1 which indicated that salinity stress reduced the photosynthetic efficiency (Fig.1A) and photosynthetic pigments (Chl.a and Chl.b) (Fig. 1B) could be attributed to stomatal closure (Bacelar et al., 2006) and/or down-regulation of CO_2 fixation. In addition increasing H_2O_2 induced by oxidative stress increase the photo-oxidation of photosystem antennae under salinity stress (Yamazaki et al., 2003 and Kafi, 2009). The reduction of Chl.a and Chl.b may be due to an increase of chlorophyllase enzyme which converts chlorophyll into chlorophyllide and phytol (Morita et al., 2009 and Lin et al., 2016) and/or damaging of photosynthetic apparatus through block of pigment-protein complexes (proteins of PSII) which synthesized in the chloroplasts (Nishiyama et al., 2011). More or less similar results were reported by Hidri et al. (2016). The results showed also that carotenoids were highly increased under salinity stress. This may be due to adaptation of the plant with high salt stress through stimulation of antioxidant defense systems (Liu et al., 2009 and He et al., 2009). Carotenoids are considered as one of the most important non-enzymatic antioxidant which induced decrease the accumulation of ROS under salt stress (Mittler et al., 2004) also, they induce abscisic acid hormone that promotes stomatal closure when exposed to stress (Leung & Giraudat, 1998). More or less similar data were reported by Liu et al. (2009).

The present results revealed high efficiency of potassium humate to ameliorate the deleterious effect of salinity stress by increasing chlorophyll A, chlorophyll B and carotenoids contents (Fig.1). This amelioration effect may be attributed to high efficiency of humic acid in increasing chlorophyll-A and chlorophyll-B content, stimulating the enzymes related to photosynthetic process, reducing electrolyte leakage and increasing the uptake of magnesium and iron which are required for chlorophyll biosynthesis (El-Ghamry et al., 2009 and Arafat

et al., 2011). Applying silicon to wheat improved photosynthetic activity. Tuna et al. (2008) found that silicon increases chlorophyll pigments under normal and salinity stress conditions. Applying silicon to saline soils resulted in reducing the amount of Na⁺ taken up by plants leading to maintaining photosynthesis process (Wang & Han, 2007). Similarly, Rios et al. (2014) found an increase in chlorophyll pigments in wheat leaves when silicon was applied. In soybean, silicon increased of photosynthetic enzymes leading to a high increase in photosynthetic pigments (Shen et al., 2010).

Antioxidant defense system

Data represented in Fig. 2 indicated that salinity stress caused a significant increase of peroxidase and catalase activities as well as malondialdehyde (MDA) and ascorbic acid contents. This increase may be due to the synthesis of reactive oxygen species (ROS) (Baxter et al., 2014 and Dietz et al., 2016). Antioxidant enzymes such as peroxidase and catalase are scavenging enzymes which regulate hydrogen peroxide (H₂O₂) content, thus inhibiting the production of OH⁻ radicals which produced from photorespiration and β-oxidation of fatty acids in organelles during metabolic processes (Rout & Shaw, 2001 and Bhutta, 2011). Damaging of membranes fatty acids could produce small hydrocarbon fragments including MDA which is an important sign of membrane system injury (Masood et al., 2006 and Moussa, 2008). Ascorbic acid is an important water-soluble antioxidant and cell moderator in physiological processes including photoprotection (Wolucka, 2005 and Khan & Ashraf, 2008) where, it can rapidly react with radical intermediates of an autoxidation chain and stop it (Khan, 2006). Latef (2014) reported that salt stress induced oxidative stress by increasing malondialdehyde (MDA) content and activity of peroxidase. Vaidyanathan et al. (2003) investigated the effect of different levels of salinity (100–300 mM) on rice (*Oryza sativa* L.) cultivar and they showed higher activities of the ROS scavenging enzymes.

Our results show clearly that potassium humate caused a significant inhibition of the activity of catalase and peroxidase and reduced MDA and ascorbic acid contents in salt-stressed plants. This amelioration effect could be attributed to protection of cell membranes from lipid peroxidation through increasing of metallic ions uptake and cell which is related to

the activity of both hydrophilic and hydrophobic sites on the surface of humic substances (Haghighi et al., 2010). Humic acids were the most effective fraction during the production of O₂⁻ through regulation of the xanthine /xanthine oxidase system which is the important key role in increasing antioxidant defense compounds (Vaughan & Ord, 1982; Cheng et al., 1995; Moussa, 2008 and Berbaraa & García, 2014).

Application of silicon decreased activities of antioxidant enzymes under salinity stress; this is associated with decreasing of electrolyte leakage, ROS synthesis and H₂O₂ contents (Siddiqui et al., 2014 and Shahid et al., 2015). More or fewer data were reported by Abbas et al. (2015) that foliar application of silicon (150 mgL⁻¹), decreased lipid peroxidation and increased antioxidant enzymes activities in both leaves and root of okra under salt stress. Similar studies showed that silicon-mediated enhancement in antioxidant enzyme activities and decreased in oxidative stress in salt-stressed tomato plants grown in sand culture (Li et al., 2015).

Total soluble proteins, carbohydrates, amino acids and proline content

Figure 3 and Fig.4 indicated that salinity stress caused highly significant increases in all the assessed organic compounds (total soluble sugars proteins, amino acids and proline content). This increase may be due to osmotic adjustment. These compatible solutes enhance the tolerance of plants and increasing their adaptation to severe stresses (Fahad et al., 2015). Proline has also been considered as a carbon and nitrogen source for rapid recovery from stress, a stabilizer for membranes and some macromolecules, a free radical scavenger (Jain et al., 2002) and a protective agent for cytosolic enzymes and cellular organelles (Yazici et al., 2007).

Potassium humate increased biosynthesis of organic compounds and this might be directly related to their role in increasing plant tolerance against abiotic stress and the activity of immune plant system (Hanafy et al., 2010). This is attributed to accelerating of plant energy metabolism through enhancing photosynthesis process and formation of starch (Mady, 2009). Humate substances increase uptake of oxygen for synthesis of protein enzymes which activate the formation of both carrier and structural proteins (Pettit & Robert, 2004). This is in agreement

with the findings of Hanafy et al. (2010) and El-Bassiony et al. (2010). Moreover, Aydin et al. (2012) studied the effect of humic acid in the alleviation of salinity stress in *Phaseolus vulgaris* L. and concluded that humic acid had significantly recovered the harmful effect of salinity and decreased amount of all organic compounds contents in bean plants.

Potassium silicate application caused an increase in biosynthesis of all determined organic osmolytes. Abbas et al. (2015) reported that foliar application of silicon increased proline, glycinebetaine, and total free amino acids in both shoots and roots of salt-stressed okra plant. Reduction in proline contents with silicon application under stress has been observed in many plant species such as soybean (Lee et al., 2010), maize (Moussa, 2006). Shekari et al. (2015) observed that addition of silicon increased soluble carbohydrates content in plants grown under salt stress where, they act as a protective osmolyte; also they reported that application of silicon under saline condition could be a better strategy for maintaining the crop productivity.

Yield parameters

Data in Table 4 indicated that salinity stress caused inhibition in all measured yield parameters. This inhibition may be due to formation of spikes during emergence and their abortion at later stages (Nicolas et al., 1994 and Chaabane et al., 2011). Potassium humate and potassium silicate increased the productivity and maturity of produced grains where, all measured yield growth parameters expressed highly significant increase as a result of their pronounced roles in enhancing physiological processes and growth of seedling where, these effects reflected on growth of yielded grains in stressed plant. Hemida et al. (2017) reported that potassium humate significantly improved the physical and chemical properties of soil. These effects were positively reflected on plant growth and productivity in *Phaseolus vulgaris* under salinity stress.

Hellal et al. (2012) and Kardoni et al. (2013) reported that silicon application increased pod yield, seed number per plant, chlorophyll contents, and yield of faba bean (*Vicia faba* L.) under salt stress. Similarly, application of potassium silicate increased plant weight, ear length, seed yield, and chlorophyll content in wheat under salt stress (Bybordi, 2014).

Total soluble proteins and carbohydrates of produced grains

Yield is a measure of the activity of processes contributing to deposition of starch in the grains, and protein percentage (Jenner, 1991). Data represented in Fig. 5 indicate that salinity stress caused a significant reduction in total soluble proteins and carbohydrates of grains where, salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as K^+ , N , and Ca^{2+} (Hu & Schmidhalter, 2005). Nitrogen-deficiency during early reproductive development severely limits crop yield (Dolferus et al., 2011). Moderate reduction in nitrogen, reduces sink protein capacity (Hirel et al., 2007 and Yang & Zhang, 2010).

Accordingly, potassium humate and potassium silicate had pronounced roles in enhancing physiological processes and growth of stressed seedlings where, these effects reflected on growth of yielded grains. Furthermore, potassium humate resulted in a remarkable increase in total soluble proteins and carbohydrates contents of grains and this may be due to increase of photosynthesis process which leads to formation of starch and/or increase N^+ , P^+ and K^+ uptake and increase the ratios of K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ which are necessary for plant growth (EL-Bassiony et al., 2010 and El-Hefny, 2010). On other hands, potassium silicate enhances the increase of grain's proteins and carbohydrates as a result of increasing the photosynthetic process and/or increasing the uptake and translocation of mineral elements in plants N^+ , P^+ and K^+ and Ca^{2+} , which they are important in increasing biosynthesis of proteins and carbohydrates (Hellal et al., 2012).

Conclusion

Salinity is one of the major limitations to wheat production worldwide. The present study demonstrates the effect of salinity stress on wheat at all growth stages. There are several strategies to increase wheat production in the salt-affected areas. One of the most important strategies for amending soil is supplemental it by organic materials that play a key role in the enhancement of plant growth and maintaining the crop yields. It can be concluded that, applying potassium humate as sole had a pronounced role in regulating the biochemical and physiological processes of wheat plant. Enhancement of wheat growth and productivity could be achieved by potassium humate treatment through inhibition

of antioxidant enzymes activity and reducing oxidation status under salinity stress. On other hands, foliar application of potassium silicate to wheat plant may help to overcome the deleterious effects of salinity and improve the adaptation capability of wheat to the saline environment where potassium silicate showed more beneficial effects on its growth and yield production. Finally, it was concluded that, applying potassium humate as an organic fertilizer as sole was more effective than potassium silicate or their combination on ameliorating the toxic effect of salinity stress.

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استجابة نبات القمح المجهد ملحيا للمعاملة بهيومات البوتاسيوم والرش الورقي بسليكات البوتاسيوم

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تعتبر الملوحة واحده من أهم أنواع الإجهاد الغير حيوي التي تقلل من إنتاجه العديد من النباتات ومن ضمنها نبات القمح. استهدف العمل في هذا البحث دراسة التغيرات التي تطرأ على النمو، المحصول، التركيب البيوكيميائي (محتوي الكربوهيدرات والبروتين) وبعض الأنشطة الفسيولوجية لنبات القمح تحت تأثير المعاملة بمستوي عالي من كلوريد الصوديوم (200 ملي مول). بناء على نتيجة التجربه المبدأيه تم اختيار صنف جميزه9- لإجراء الدراسة، حيث أظهرت النتيجة أن صنف جميزه9- كان أكثر الأصناف حساسيه للإجهاد الملحي عند تركيز 200ملي مول من كلوريد صوديوم. وقد أظهرت النتائج أن المستوي العالي للملوحة (200 ملي مول) سبب انخفاضا معنويا في النمو بنسبه 65% وإنتاجيه المحصول. كما تسبب الإجهاد الملحي في زياده نشاط إنزيمات مضادات الأوكسده (البيروكسيداز والكتاليز) وحمض الاسكوربيك. زياده محتوى المالدونديالدهيد (ناتج أكسده دهون خلايا النبات). وزياده مركبات ضبط الإسموزيه (الكربوهيرات و البروتين الكلي الذائب، الأحماض الأمينييه وحمض البرولين). ايضا انخفض محتوى الحبوب من الكربوهيدرات والبروتين الكلي. عندالمعامله المنفرده أو المتحدده بهيومات البوتاسيوم أوالرّش الورقي بسليكات البوتاسيوم لنبات القمح المجهد ملحيا تم تقليل التأثيرات الضاره للملوحة وكانت نتيجة ذلك حدوث زياده معنويه في النمو، محتوى الكربوهيدرات والبروتين الكلي للحبوب المنتجه مقارنة بالنباتات الغيرمعامله بهيومات البوتاسيوم او بسليكات البوتاسيوم. تقليل نشاط انزيمات مضادات الاكسده (البيروكسيداز و الكتاليز) وكذلك حمض الاسكوربيك مقارنة بالنباتات المجهد ملحيا. اشارت النتائج إلى أن معاملة النبات بهيومات البوتاسيوم منفردا كان له تأثير افضل من استخدام الرش الورقي بسليكات البوتاسيوم منفردا أو متحدا معه في تقليل التأثير الضار للإجهاد الملحي على نبات القمح. بناء على ما سبق توصي هذه الدراسة باستخدام هيومات البوتاسيوم والرّش الورقي بسليكات البوتاسيوم لزياده كميته وجوده المحصول لنبات القمح المجهد ملحيا.