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Study Effect of Supplemental Nutrition on Yield, Yield Components and Grain Protein Content of Wheat Cultivars under Drain Water Irrigation

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ABSTRACT

BACKGROUND: The macro and micronutrients play an important role in the crop nutrition and thus they are important for achieving higher yields, better growth and development of plants.

OBJECTIVES: This study aimed to investigate the effect of supplemental nutrition (chemical and organic fertilizer) on quality parameters and grain yield of wheat cultivars under irrigation condition with drain water of farms.

METHODS: This experiment was carried out as a split-split plot in a randomized complete block design with three replications. The treatments included irrigation at two levels of drain water and pure water irrigation in the main plot, fertilizer compounds at six levels, growth promotion and high potassium in sub-plot and sub-sub plot including wheat cultivars (Mehregan, Shoosh, and Chamran2).

RESULT: The results indicated that the traits of grain yield, harvest index, number of tillers, number of spikelet per spike, number of grain per spike were affected by fertilizer treatment and grain yield, biological yield, and harvest index were affected by irrigation. The number of grains per spike in irrigation with pure water had a higher average. The highest thousand grain weight and the number of tillers were obtained in Chamran2 cultivar. The highest grain protein content was achieved in Mehregan cultivar. The results of correlation analysis revealed that the number of tillers had a significant direct correlation with the variables of number of spikelet per spike, number of grains per spike, and grain yield. In examining the interaction of treatments, the highest grain yield was obtained in the application of humic acid seed inoculation and growth promotion of Shoosh wheat cultivar under drain water irrigation.

CONCLUSION: the highest amounts of grain yield were obtained approximately 6925 kg per hectare by applying humic and growth-promoting fertilizers in irrigation conditions with drain water of fields in Shoosh wheat cultivar.

KEYWORDS: *Correlation, Growth Promotion, Humic Acid, Proline, Seed weight.*

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1. BACKGROUND

Due to the adaptability of its genotypes to different environmental conditions and different aspects of its use, wheat with the scientific name of (*Triticum aestivum* L.) is one of the plants that is cultivated in large areas of the world with diverse climatic conditions (Saberi *et al*., 2013). Today, due to the prevailing conditions in dry areas (lack of organic matter, drought, salinity, and calcareous soils), increasing the wheat yield through increasing the area under cultivation is almost impossible. Iran is among the countries that will face physical water scarcity by 2025. This means that, even with the highest efficiency and productivity in water consumption, there will not be enough water to meet conventional needs (Zoski *et al*., 2013). Therefore, the use of unconventional water, especially agricultural water drain in arid and semi-arid regions, can be considered as strategies for water resources management (Rezaei and Shahidi, 2016). In investigating the effect of mixing saline and non-saline waters with different management on soil and plant, Xue and Ren (2017) demonstrated that conjunctive use of saline and non-saline water (mixture, periodic alternate use, and one-alternate use), in addition to soil remediation, increased plant density and crop yield. Sharma *et al*. (2000) also stated that the average wheat yield, when using only saline water of drainage, was 74% of potential yield, and if the first irrigation is replaced with non-saline water and the rest of the steps is irrigated with saline water drainage, wheat yields will increase by 84 percent. To reduce the effect of irrigation with drain water of farms, using fertilizers containing micronutrients (iron, zinc, manganese, copper, and boron) along with plant growth promotions such as amino acids and humic acids can play an effective role in improving yield and enhancing product quality. Due to its hormonal compounds, humic acid can be used to improve plant growth and increase resistance to favorable environmental factors (Soheili and Eftekharian Jahromi, 2015). Abid *et al*. (2016) reported that moderate consumption of nutrients increases wheat production under stress conditions in soils with a lack of macro and micronutrients. By assess the effect of irrigation with saline water on yield and its components in wheat inoculated with growth-promoting bacteria and humic acid, Chamani *et al*. (2012) reported interaction of seed inoculation with growth-promoting bacteria and humic acid intake during irrigation with saline water was significant on grain yield and spike yield. The highest grain and spike yield was obtained from seed inoculation treatment with Azotobacter and lack of humic acid consumption and salinity application of 75 mM. Selection of superior cultivars, attention to physiological characteristics, and use these indices in selection of compatible and high-yielding cultivars are the most essential indirect factors in the successful increase in wheat yield (Ahmadi *et al*., 2012). Different wheat cultivars exhibit different reactions to salinity stress and are placed in a wide range from tolerant to salinity sensitive cultivars (Farahudi and Khodarahmpour, 2015).

2. OBJECTIVES

Considering that growth and yield under the influence of biotic and abiotic factors such as irrigation water quality, salinity, drought, and plant nutrition management, this study aimed to investigate effect of supplemental nutrition (chemical and organic fertilizer) on quality parameters and grain yield of wheat cultivars under irrigation condition with drain water of farms.

3. MATERIALS AND METHODS

3.1. *Field and Treatments Information*

This experiment was conducted as a split-split plot in a randomized complete block design with three replications during two crop years of 2016-2017 and 2017-2018 in Ahvaz station of Agricultural and Natural Resources Research Center of Khuzestan province with geographical specifications of 31 degrees 20 minutes north latitude, 48 degrees 40 minutes east longitude, and height of 18 meters above sea level. In terms of climate, Ahvaz is one of the arid and semi-arid regions. According to the meteorological statistics of the last 10 years in synoptic station of Ahvaz Airport, the average annual rainfall in Ahvaz is 240.6 mm, the average temperature is 25.3 °C, the maximum temperature is 51.2 °C, the minimum temperature is -1 °C, and the average annual relative humidity is 45.7%. The factors examined including irrigation at two levels (I1: drain water and I2: pure water) in the main plot, compound fertilizer in six levels (F0: control (no fertilizer use), F1: seed inoculation with a liquid mixture of zinc, iron, nitrogen, phosphorus, and potassium, F2: seed inoculation and humic acid, F3: seed inoculation and humic acid and growth promotion, F4: seed inoculation and humic acid and growth promotion and high phosphorus, F5: seed inoculation and humic acid and growth promotion and high potassium) were conducted in a sub-plot, and the sub-sub plot consisted of three wheat cultivars (C1: Mehregan, C2: Shoosh, C3: Chamran 2).

3.2. *Farm Management*

To determine the chemical and physical properties of the test piece soil, a composite sample was prepared from the depth of 0-30 cm from the soil surface and sent to the laboratory. The soil test results were presented in Table 1. The seedbed preparation was in late October, and the date of planting was in November due to the climatic conditions of Khuzestan. In each plot, the number of six planting lines with a length of four meters and a width of 20 cm was considered. In each subplot, the planting lines were created by the seed machine at the plot level.

Table 1. Physical and chemical properties of the tested soil

Depth of sampling (cm)	Soil texture	The constituent particles of soil (%)			Organic carbon (%)	Available Phosphorus (mg. kg^{-1})	Available potassium (mg. kg^{-1})	pH	Electrical conductivity (ds.m^{-1})	SP (%)
		Sand	Clay	Slit						
0-30	Clay Loam	21	41.5	37.5	0.6	9.2	151	7.1	3.62	48

3.3. *Measured Traits*

The traits studied in this experiment included grain yield, biological yield, harvest index, number of fertile tillers, number of spikes, number of spikelet per spike, number of grains per spike, thousand grain weight, grain protein content, zinc and proline content.

3.4. *Statistical Analysis*

The analysis of variance and the calculation of correlation coefficients were performed using Minitab software, and the comparison of mean treatments was carried out using MSTAT-C software with LSD method at 5% level of probability.

4. RESULTS AND DISCUSSIONS

4.1. *Biological yield*

The results obtained from combined analysis of variance treatments in two years (Table 2) revealed that the effect of year and irrigation treatment were significant at 1% probability level and fertilizer and cultivar treatments at 5% probability level on biological yield. Among fertilizer treatments, F3 treatment (seed inoculation + humic acid + growth promotion) with an average of 1451.11 g/m2 had the highest effect on biological yield, and F2 treatment (seed inoculation + humic acid) with an average of 1235.833 g/m2 had the lowest effect on biological yield (Table 3). Using plant growth-promoting bacteria (PGPB) in saline conditions can lead to increased wheat yield. In a study that used plant growth-promoting bacteria with the ability to produce exopolysaccharide, the biological yield of inoculated wheat increased compared to un inoculated wheat. Moreover, shoot dry weight, root, and seed germination of inoculated seeds were increased, and there was a positive correlation between root system development and wheat yield (Upadhyay *et al*., 2011). Irrigation treatment with drain water and irrigation treatment with pure water had the highest and lowest effect on biological yield, respectively (Table 3). Thus, we can say that the use of drain water in irrigation can improve biological yield. According to the results of the study conducted by Shahidi and Miri (2018), salinity stress had a significant effect on biological yield. Of the cultivars, the highest biological yield belonged to Shoosh cultivar, and the lowest biological yield was related to Mehregan cultivar (Table 2). There was a significant correlation between biological yield and variable of harvest index (Table 4). Increase of harvest index indicated a positive effect of humic acid and growth-promoting bacteria on the production of cultivated material and transfer to reservoirs as well as surface production of biomass in irrigation with drain water of farms which ultimately led to increased harvest index. Yazdipour *et al*., (2006) reported that the higher the percentage of drain waters in irrigation, the higher the production efficiency as well.

4.2. *Grain Yield*

The combined analysis of variance results of treatments on grain yield indicated that year and fertilizer treatments at 1% probability level and irrigation treatments at 5% probability level had a statistically significant effect on grain yield (Table 2).

Table 2. Analysis of variance results of yield, yield components and qualitative parameters of wheat cultivars

S.O.V	df	Biological yield	Grain yield	Harvest index	Number of fertile tillers	Number of spikes
Year	1	8873963**	165724**	4264.44**	62.29**	319.79^{ns}
Repeat (Y)	4	189525	19764	96	4.39	15848.30
I	1	1155009**	46905*	921.11**	1.18^{ns}	18720.90^{ns}
I × Y	1	39501.11^{ns}	8804^{ns}	187.50^{ns}	4.16^{ns}	90.74^{ns}
Error I	4	30993	3478	69.63	3.69	9887.37
F	5	351310.40*	70105.31**	68.39^{ns}	2976**	1506.64^{ns}
F × Y	5	79452.62^{ns}	34859.66^{ns}	684.85**	14.55**	1506.64^{ns}
F × I	5	39321.31^{ns}	12200.51^{ns}	99.15^{ns}	2.01^{ns}	744.36^{ns}
F × I× Y	5	123526.51^{ns}	10531.12^{ns}	129.08^{ns}	2.93^{ns}	744.36^{ns}
Error II	40	133425.90	18753.17	158.87	3.64	1254.54
C	2	207032.11*	24989.93^{ns}	9.99^{ns}	0.36^{ns}	36.14^{ns}
C × Y	2	27944.39^{ns}	9390.69^{ns}	228.76*	4.06^{ns}	36.14^{ns}
C × I	2	55874.43^{ns}	8804.25^{ns}	20.31^{ns}	0.36^{ns}	204.19^{ns}
C ×Y ×I	2	42346.33^{ns}	6944.11^{ns}	284.07**	2.04^{ns}	204.19^{ns}
C × F	10	41902.70^{ns}	6895.32^{ns}	139.01*	0.89^{ns}	419.57^{ns}
C × F × Y	10	47221.80^{ns}	8201.61^{ns}	45.28^{ns}	0.69^{ns}	419.57^{ns}
C × I× F	10	15463.35^{ns}	5909.44^{ns}	64.37^{ns}	1.39^{ns}	228.95^{ns}
C × I× F× Y	10	39697.63^{ns}	4671.79^{ns}	7.41^{ns}	1.24^{ns}	228.95^{ns}
Error III	96	45263.53	8804.25	65.78	1.38	232.19
CV (%)	-	26.10	20.42	25.69	21.45	4.46

$^{ns, *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

I: Irrigation; Y: Year; F: Fertilizer; C: Cultivar

Based on the results of comparison of the mean treatments, F3 treatment (seed inoculation + humic acid + growth promotion) with 606.97 gr.m^2 had the highest effect on grain yield, and control treatment with 490.31 gr.m^2 had the lowest effect on grain yield. The average grain yield in irrigation with drain water was 582.52 gr.m^2, and irrigation with pure water was 553.05 gr.m^2 respectively. So, it can be said that the use of drain water improved grain yield. Moreover, the highest grain yield belonged to Shoosh cultivar and the lowest belonged to Mehregan cultivar (Table 3). The correlation results showed that grain yield had a positive and significant correlation with the traits of the number of fertile tillers, the number of spikelet, and biological yield (Table 4). Although the use of seed inoculated fertilizer treatment and humic acid and growth promotion also had a positive effect on increasing grain yield and was able to cover the negative impact of irrigation with drain water, increasing grain yield under irrigation with drain water could be attributed to increased leaf area of wheat and ultimately increased light absorption and photosynthesis. According to the results of Shahidi and Miri (2018), salinity stress has a significant effect on grain yield.

Table 3. Results of comparison of mean yield, yield components of wheat cultivars

Treatment	Biological Yield (gr.m^{-2})	Grain yield (gr.m^{-2})	Harvest Index (%)	Number of Fertile Tillers	Number of Spikes
F_1	1402.50*	606.31	44.68	8.47	248.61
F_2	1235.83	562.56	46.21	8.44	234.25
F_3	1451.11	606.97	44.22	8.33	248.44
F_4	1293.11	551.94	44.44	9.11	236.67
F_5	1340.83	588.67	45.48	8.83	242.94
F_0	1241.75	490.31	42.16	6.53	235.72
LSD	80.43	25.92	2.71	0.37	58.00
I_1	1400.64	582.52	42.46	8.36	236.69
I_2	1254.39	553.05	46.59	8.21	245.51
LSD	56.63	19.92	1.61	0.29	43.09
C_1	1274.33	549.66	44.83	8.30	240.39
C_2	1381.56	586.88	44.11	8.20	241.13
C_3	1326.66	566.81	44.64	8.34	241.81
LSD	57.56	19.25	1.91	0.29	40.74

*The means were compared independently with the LSD test at the probability level of 1 and 5%.

F_0: Control; F_1: Seed Inoculation; F_2: Seed Inoculation + Humic Acid; F_3: Seed Inoculation + Humic Acid + Growth Promotion, F_4: Seed Inoculation + Humic Acid + Growth Promotion + High Phosphorus; F_5: Seed Inoculation + Humic Acid + Growth Promotion + High Potassium; I_1: Irrigation with Drain Water; I_2: Irrigation with Pure Water; C_1: Mehregan, C_2: Shoosh, C_3: Chamran 2.

4.3. *Harvest Index* (*HI*)

The combined analysis of variance results of treatments on harvest index in both years of experiment indicated that the effect of year and irrigation treatment and interaction of fertilizer × year and cultivar × year × irrigation at 1% probability level and interaction of fertilizer × cultivar and cultivar × year at 5% probability level was significant (Table 2). On the other hand, fertilizer treatments did not show significant effect on harvest index. Therefore, the application of different synthetic fertilizers did not affect the harvest index. The results related to the analysis of variance of cultivars showed that harvest index did not show significant differences among the cultivars used in this study (Table 2). Based on the results of mean comparison (Table 3), the highest and lowest harvest index with 46.21% and 42.16%, respectively, were related to F2 treatment (seed inoculation + humic acid) and control treatment (Table 3). Faiziasl and Velizadeh (2004) reported that the application of different levels of fertilizer had no significant effect on the wheat harvest index under dryland conditions. Ebrahimpur Nourabadi *et al.* (2007) reported that one of the ways to enhance wheat yield is to increase harvest index by reducing the number of tillers per plant. According to the correlation results, harvest index had a significant correlation with biological yield (Table 4). With the increase in the number of fertile tillers and an increase in the number and weight of seed, the ratio of grain yield to biological yield can be increased and enhance the harvest index.

Table 4. Results of Pearson correlation test for yield and yield components of wheat cultivars

Traits	Number of Fertile Tillers	Number of Spikelet per Spike	Number of Grains per Spike	Thousand grain Weight	Grain Yield	Biological Yield
Number of Spikelet per Spike	0.64**					
Number of Grains per Spike	0.59**	0.40 *				
Thousand Grain Weight	0.19	0.33 *	0.01			
Grain Yield	0.46**	0.47**	0.31	0.09		
Biological Yield	0.21	0.28	0.24	0.05	0.71**	
Harvest Index	0.11	-0.02	-0.08	-0.06	0.11	-0.55**

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

4.4. *Number of Fertile Tillers*

The results of combined analysis of variance (Table 2) showed that the effect of year and fertilizer treatment and the effect of fertilizer × year were statistically significant at 1% probability level on the number of fertile tillers. According to the mean comparison table (Table 3) among treatments, F4 (seed inoculation + humic acid + growth promotion + high phosphorus) had the greatest effect on the number of fertile tillers. The control treatment (F0) had the lowest effect on this variable so that the mean number of fertile tillers in this treatment was 6.53. By examining the humic acid, Akhtar *et al*. (2014) reported that humic acid significantly increased yield and yield components of wheat compared to control treatment and, in general, humic acid had a significant effect in the production of favorable crop in wheat. Irrigation treatment with drain water and irrigation treatment with pure water had the highest and lowest effect on the number of fertile tillers, respectively. The highest number of fertile tillers belonged to Chamran 2 and Mehregan cultivars, and the lowest number of fertile tillers related to Shoosh cultivar (Table 3). As well as, the correlation results represented that the number of fertile tillers had a direct and significant correlation with the variable of the number of spikelet, the number of grain per spike, and grain yield (Table 4). In other words, by increasing the number of fertile tillers, the number of spikelet and the number of grains per spike increase, which eventually leads to an increase in grain yield.

4.5. *Number of spikes per unit area*

The results obtained from the two-year combined analysis of variance table (Table 2) showed that the effect of none of the treatments on the number of spikes per square meter was significant. Based on the results of a comparison of the mean number of spikes per square meter (Table 3), no significant difference was observed between fertilizer control treatment and other treatments. The results of mean comparison exhibited no significant difference in the number of spikes per square meter in two types of irrigation. Although the mean of this variable in irrigation with pure water was higher than that of irrigation with drain water, these means have no statistically significant differences with each other (Table 3). According to the results of the study carried out by Yazdipour *et al*. (2006), the use of drain water had no significant effect on the number of spikes per square meter. Furthermore, there was no significant difference in the mean number of spikes per square meter among cultivars. Although the mean number of spikes per square meter in Chamran 2 cultivar was higher than Mehregan and Shoosh cultivars, these means have no statistically significant differences with each other (Table 3).

4.6. *Number of spikelet per spike*

The results obtained from the two-year combined analysis of variance table (Table 5) showed that the effect of year and fertilizer treatment on the number of spikelet per spike was significant at 1% probability level. And this trait was influenced by the difference of cultivars at the 5% probability level. Based on the results of mean comparison (Table 6), the number of spikelet had the highest value in fertilizer treatment F2 (seed inoculation + humic acid) and had the lowest value in control treatment with 17.63. The results of the study performed by Sabzevari (2010) showed that the effect of foliar application of humic acid on the number of spikelet during growth was significant. The results of mean comparison also represented no difference between the mean numbers of spikelet in two types of irrigation (Table 6). Afiooni and Mahlooji (2007) examined the effect of irrigation water salinity on wheat yield and reported that the effect of irrigation water salinity on the number of spikelet did not show a significant difference with the control. As well as, the highest and lowest number of spikelet belonged to Shoosh and Chamran 2 cultivars, respectively (Table 6). Based on the results of the Pearson correlation, the number of spikelet per spike had a significant correlation with the number of fertile tillers, number of grains per spike, 1000-kernel weight, and grain yield (Table 4). Changes in the number of fertile tillers cause a change in the number of spikelet per spike and will ultimately affect grain yield by influencing the number and weight of the grain.

4.7. *Number of grains per spike*

Based on the results of combined analysis of variance concerning the number of grains per spike, the effect of year on the number of grains per spike at the 1% probability level was significant. Among the treatments, fertilizer treatments, and the interaction of year × cultivar on the number of grains per spike at 5% probability level were significant (Table 5). Based on the results of mean comparison (Table 6), the number of grains per spike in control treatment and F4 (seed inoculation + humic acid + growth promotion + high phosphorus) treatment had the lowest and highest values with 35.92 and 42.31 respectively.

Table 5. Analysis of variance results of yield components and qualitative parameters

S.O.V	df	Number of spikelet per spike	Number of grains per spike	Thousand grain weight	Grain protein content	Zinc content	Proline content
Year	1	188.90 **	30411.89**	0.37 ns	0.03 ns	0.01 ns	0.193 ns
Repeat (Y)	4	31.42	3417.27	172.45	0.53	0.96	0.084
I	1	4.74 ns	41.78 ns	2.89ns	0.33 ns	8.19 ns	2.465 **
I × Y	1	0.16 ns	16.11 ns	1.33 ns	0.13 ns	2.90 ns	0.210 ns
Error I	4	7.52	75.20	8.49	0.66	8.99	0.115
F	5	18.51**	229.85 *	32.06 ns	3.69 **	3.85 *	0.023 ns
F × Y	5	2.77 ns	155.81 ns	72.71 ns	5.78 **	3.85 *	0.023 ns
F × I	5	2.45 ns	38.74 ns	25.01 ns	0.51 ns	1.26 ns	0.010 ns
F × I× Y	5	2.85 ns	12.46 ns	10.28 ns	1.36 *	1.26 ns	0.010 ns
Error II	40	4	74.79	34.28	0.48	1.53	0.015
C	2	9.14 *	2.34 ns	3.24 ns	0.25 ns	0.36 ns	0.001 ns
C × Y	2	2.89 ns	44.64 *	2.88 ns	0.90 ns	0.36 ns	0.001 ns
C × I	2	0.28 ns	2.92 ns	16.79 ns	0.01 ns	0.29 ns	0.001 ns
C ×Y ×I	2	0.34 ns	33.42 ns	2.74 ns	0.03 ns	0.29 ns	0.001 ns
C × F	10	3.65 ns	15.45 ns	6.94 ns	0.30 ns	0.85 ns	0.002 ns
C × F × Y	10	0.42 ns	15.56 ns	11.28 ns	0.27 ns	0.85 ns	0.002 ns
C × I× F	10	2.06 ns	22.98 ns	3.46 ns	0.60 ns	0.25 ns	0.001 ns
C × I× F× Y	10	1.56 ns	10.69 ns	4.36 ns	0.45 ns	0.25 ns	0.001 ns
Error III	96	2.21	13.37	6.13	0.38	0.71	0.001
CV (%)		11.09	39.50	10.98	6.49	5.26	8.45

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.
I: Irrigation; Y: Year; F: Fertilizer; C: Cultivar

The increase in the number of grains per spike can be attributed to the increase in vegetative and reproductive growth and, ultimately, to accelerate the proper pollination of wheat as well as to the increase in photosynthetic production of wheat due to increasing its available resources because of moisture in the field. Foliar application of phosphorus fertilizer also increased the number of grains per spike. Garcia *et al*. (2003) observed that the number of grains per spike had a significant role in grain yield, especially under drought stress conditions. Observations of mean comparison suggested that there was no statistical difference between the mean numbers of grains per spike in the two types of irrigation. Although the mean number of grains per spike in irrigation with pure water was higher than in irrigation with drain water, these means had no significant differences. Despite the lack of significant differences in the number of grains per spike among cultivars, the values of this parameter were higher in Mehregan cultivar compared to Shoosh and Chamran 2 cultivars (Table 6). The number of grains per spike had a significant correlation with the variables of the number of fertile tillers and the number of spikelet (Table 4). The more the number of spikelet and the number of tillers, the number of grains will also increase.

Table 6. Results of comparison of mean yield components and qualitative parameters of wheat cultivars

Treatment	Number of Spikelet per Spike	Number of Grains per Spike	Thousand Grain Weight (g)	Grain Protein Content (%)	Zinc Content (%)	Proline Content (%)
F_1	19.28*	38.67	38.78	12.27	11.15	0.587
F_2	19.56	37.97	38.86	12.41	11.33	0.627
F_3	18.86	40.44	37.56	12.49	11.13	0.586
F_4	19.31	42.31	37.81	12.51	11.32	0.603
F_5	19.47	40.97	36.56	12.43	10.92	0.589
F_0	17.63	35.22	36.92	12.27	10.49	0.551
LSD	0.47	3.65	0.96	0.17	2.66	0.12
I_1	19.16	38.82	37.86	12.33	11.04	0.58
I_2	18.87	39.70	37.62	12.25	11.12	0.44
LSD	1.42	2.190	2.240	1.230	2.314	0.098
C_1	19.11	39.37	37.79	12.34	11.02	0.50
C_2	19.31	39.31	37.51	12.31	11.08	0.51
C_3	18.62	39.05	37.93	12.22	11.16	0.51
LSD	0.35	2.59	0.69	0.13	1.86	0.08

*The means were compared independently with the LSD test at the probability level of 1 and 5%.

F_0: Control; F_1: Seed Inoculation; F_2: Seed Inoculation + Humic Acid; F_3: Seed Inoculation + Humic Acid + Growth Promotion, F_4: Seed Inoculation + Humic Acid + Growth Promotion + High Phosphorus; F_5: Seed Inoculation + Humic Acid + Growth Promotion + High Potassium; I_1: Irrigation with Drain Water; I_2: Irrigation with Pure Water; C_1: Mehregan, C_2: Shoosh, C_3: Chamran 2.

4.8. *Thousand Grain Weight*

The results of the combined analysis of variance (Table 5) demonstrated that none of the treatments and their interaction with each other had a significant effect on 1000- grain weight. The observations of mean comparison in Table 6 also indicate that there is no significant difference in 1000- grain weight among the treatments under study. Although the mean of 1000- grain weight in F2 treatment (seed inoculation + humic acid) was higher than other treatments, we could not say that this mean had a significant difference with the control mean. Moreover, although the mean of 1000- grain weight in irrigation with drain water was higher than that of irrigation with pure water and also it was higher in Chamran 2 cultivar than Mehregan and Shoosh cultivars, these means had no significant differences (Table 6). Thousand grain weights showed a significant correlation with the number of spikelets (Table 4). By evaluating some wheat genotypes under salinity in the field conditions, Saberi *et al*. (2013) stated that cultivars and lines had significant differences in terms of the number of days to spike emergence, the number of days to physiological maturity, plant height, peduncle length, thousand kernel weight, and grain yield at 1% probability level. In this experiment, among the yield components, grain weight had the most roles in grain yield under salinity conditions.

4.9. *Grain Protein Content*

The results of combined analysis of variance (Table 5) represented that the effect of fertilizer treatment and the effect of fertilizer × year at 1% probability level and the effect of fertilizer × irrigation × year at 5% probability level on protein content were significant. According to the results of mean comparison (Table 6), F4 treatment (seed inoculation + humic acid + growth promotion + high phosphorus) with 12.51% had the highest effect on grain protein, and F0 (control) treatment with 11.67% had the lowest effect on grain protein. F4, F3, F5, F2, and F1 treatments had the highest effect on protein content, respectively. In an experiment, the effect of foliar application of humic acid and nitrogen on wheat was evaluated, and the results showed that grain protein increased in the treatments under study. By increasing the activity of rubisco enzyme, humic acid also increased the photosynthetic activity of the plant and thus increased the yield (Delfino *et al*., 2005). According to the results of this experiment, irrigation with drain water and pure water treatments had the highest and lowest effect on protein content with 12.33% and 12.25%, respectively. As well as, the highest protein content belonged to Mehregan cultivar with 12.34% and the lowest protein content related to Chamran 2 cultivar with 12.22% (Table 6). Based on the results of a study conducted by Shahidi and Amiri (2018), increasing salinity stress leads to an increasing effect on protein content. The investigation of the correlation results revealed that protein content had a significant correlation with all variables except 1000-grain weight and harvest index (Table 4). The increase in protein in the abovementioned treatment was probably due to an increase in the amount of nitrogen available to the plant, which increased the protein content in the seed (Jorfi, 2014).

4.10. *Zinc Content*

The results of the combined analysis of variance (Table 5) indicated that the effect of fertilizer and fertilizer × year at 5% probability level on grain zinc content was significant. According to the mean comparison table (Table 6) among treatments, F2 treatment (seed inoculation + humic acid) with 11.331% had the highest effect on grain zinc content, and control treatment with 10.499% had the lowest effect on grain zinc content. The crucial benefits of humic acid include increased absorption rate of phosphorus, nitrogen, and potassium and chelating ability of various nutrients such as sodium, potassium, magnesium, zinc, calcium, iron, copper, and other elements to overcome nutrient deficiencies (Ghorbani *et al*., 2010). The results of mean comparison of irrigation treatments on grain zinc content showed that irrigation treatment with pure water and irrigation with drain water with 11.124 and 11.046% had the highest and lowest effect on grain zinc content, respectively. Besides, the highest grain zinc content belonged to Chamran 2 cultivar, and the lowest grain zinc content belonged to Mehregan cultivar (Table 6).

Malakouti and Lotfollahi (1999), in the result of their study, pointed out that the application of micronutrients increased the concentration of the element in leaves and also enhanced grain yield.

4.11. *Proline Content*

Based on the results of the combined analysis of variance (Table 5), the effect of irrigation treatment at 1% probability level on grain proline content was significant. According to the mean comparison table (Table 6) among treatments, F2 treatment (seed inoculation + humic acid) with 0.627 had the highest effect on grain proline content, and control treatment with 0.555 had the lowest effect on grain proline content. The results of comparison of mean irrigation treatments on grain proline content (Table 6) indicated that irrigation treatment with drain water and irrigation treatment with pure water had the highest and lowest effect on grain proline content, respectively. Moreover, the highest and lowest grain proline contents were obtained for Shoosh and Mehregan cultivars, respectively. There are several mechanisms for maintaining turgor in plants under saline stress, one of which is proline accumulation. Accumulation of proline in the plant exposed to salinity, in addition to osmotic adjustment, assists in the absorption of water from the root environment and plays an essential role in maintaining intracellular turgor pressure. The results of the experiment performed by Karimian and Ataei Barazandeh (2013) exhibited that, with increasing salinity stress in the plant, proline concentration in wheat grain increased. Indeed, proline accumulation reduces damage to the membrane under stress conditions. Due to the higher proline concentration in the control treatment, it can be acknowledged that, due to the lack of use of fertilizers tested in the control treatment, salinity stress was felt in the plant. The mode of action of humic acid on plant growth is attributed to its direct and indirect effects and its effect on the membrane and, thus, improved nutrient transport, increased protein synthesis, and photosynthesis.

5. CONCLUSIONS

The results of this study demonstrated that the highest amounts of grain yield were obtained approximately 6925 kg per hectare by applying humic and growth-promoting fertilizers in irrigation conditions with drain water of fields in Shoosh wheat cultivar. Considering the high levels of salinity of soil and water in Khuzestan province, carrying out this method leads to optimal use of drain water, which will be a great step to achieve sustainable agriculture.

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AUTHORS' CONTRIBUTION

Sardar Pasha conducted an experiment and collected the data. Mojtaba Alavi Fazel and Alireza Jafarnejadi oversee how data is tested and analyzed. Shahram Lack and Mani Moqaddam have been involved in presenting and analyzing the results. All authors have evaluated the final version of the article.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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