Effects of some plant growth stimulants on yield and yield components of wheat (*Triticum aestivum* L., Narin cultivar) under saline condition

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**Abstract**
In recent years, spreading environmental stresses such as salinity have had a significant impact on the quantitative and qualitative of agricultural products. In order to investigate the effects of using some plant growth stimulants to improve growth and yield of wheat in saline condition, this experiment was conducted with seven treatments including 1-control (application of chemical fertilizer based on soil test) 2-first treatment + soil application of 5 kg.ha⁻¹ of humic acid 3-first treatment + spraying amino acids in 5 gr.lit⁻¹ 4-first treatment + seed priming of Azotobacter with a concentration of 1% 5-first treatment + foliar application of fulvic acid in 5 gr.lit⁻¹ 6-first treatment + foliar applying seaweed in 5 gr.lit⁻¹ and 7-combined treatment (1+2+3+4+5+6) in a randomized complete blocks design in three replications and at two years (2017-2019) in Qom province. The biennial results showed highest seed, straw and biological yield of wheat were obtained in treatment seven, which had a significant difference with another stimulant and control and with 3194, 4993 and 8187 kg.ha⁻¹, respectively which was 35%, 25% and 30% higher than control. Also, the yield trait, such as plant height, cluster length and tillers plant⁻¹ were the highest in this treatment. It seems that the combined use of growth stimulants had a significant effects on yield and some yield traits of wheat in saline conditions and it is suggested to investigate the effects of these stimulants in combination with 50 and/or 70% out of the recommended fertilizers and as well as the time of applying these stimulants.

**Key words:** Wheat, Salinity, Sea weed, Amino acids, Fulvic acid, Calcium molar fraction

1. Introduction

Environmental stresses are among the factors that limit agriculture in harnessing the maximum potential of water, soil and plants for sustainable production. In the meantime, salinity is one of the most important of these tensions (Gheibi, 2018). In general, the presence of high ions in excess of the optimal levels required by plants in the soil or irrigation water causes salinity stress (Homayi, 2002). One of the main problems of agriculture in arid and semi-arid regions is salinity and the accumulation of the salts in the surface layer of the soil, which has reduced yield and area under cultivation (Jalili, 2007). It has been estimated that worldwide 20% of total cultivated and
33% of irrigated agricultural lands are afflicted by high salinity. Furthermore, the salinized areas are increasing at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices. It has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Jamil et al., 2011). In Iran, about 20% of the total land have different salinity levels (Momeni, 2010). This area is equivalent to 4.1 million hectares of irrigated lands. The most important effects of salinity stress on plant growth are osmotic stress, ion imbalance in the plant and ion toxicity (Homayi, 2002; Singroha et al., 2022). It can also be attributed to the negative effects of salinity on plant growth from the presence of chlorine and sodium ions, increased ethylene production by the plant, plasmolysis of cells, inhibition of photosynthesis and ultimately inhibition of seed germination and growth, flowering and fruiting was found (El-Bassiony et al., 2005).

According to the Iran’s location in hot and arid climates and as well as the high salinity and sodium levels of some soils in large areas of the country’s arable land, particular attention is required for studies and research aimed at reducing the effects of salinity and sodium on agricultural production and wheat in particular. Therefore we should look for new methods that can increase the production of plants. Also, low precipitation, particularly in recent years and uncontrolled groundwater harvesting have gradually lowered the level and quality of groundwater. On the other hand, due to limited water resources, the use of poor quality water is inevitable. Excessive salinity can reduce plant yield. Therefore, the use of methods that lead to an increased salt tolerance of the plant is very important.

Today, one of the new ways to deal with salinity stress is through the use of plant growth stimulants. In the study by GhaffariNejad et al., (2020) it was reported that plant growth stimulants have improved and increased the stability of plant production while increasing plant resistance to abiotic stress and plant quality.

Application of biological stimulants, especially plant growth stimulants and non-biological growth (aminoclates and organic acids) in combination with chemical fertilizers, is one of the integrated plant nutrition strategies for sustainable management of agricultural systems (Keutgen and Pawelzik, 2008).

Tehrani (2015) announced the appropriate time for applying plant growth stimulants in wheat, was foliar spraying at the beginning of tillering and its completion stage. Although, using these substances in the short time before the onset of cold stress has less of an impact on plant resistance, but using these substances after the occurrence of stress, is more effective in reducing the effects of frostbite. Vasconcelos et al., (2009) reported that the consumption of humic acid in maize and soybeans under drought stress conditions increased the activity of the superoxide dismutase and ascorbate peroxidase enzymes, which play an important role in neutralizing free radicals in root cell membranes. The use of growth stimulants containing chelated amino acids with high and low nutrient consumption under drought stress conditions increased stress tolerance in tomatoes due to increased water use efficiency (Simon-Grove et al., 2016). Also, using of this growth stimulant higheer the proline content in plant which lead to less damage from drought stress. Similar positive results from the use of growth stimulants has reported by Khan et al., (2009); Pradikovic et al., (2011) and Ertani et al., (2013).

The advantages of using amino acid fertilizers could be included increasing the activity of beneficial soil organisms, uptake of micro and macronutrient nutrients, releasing stabilized mineral compounds in the soil, cation exchange capacity, plant’s root development, efficiency of plant synthesis and metabolism (protein and carbohydrates), ability to chelate trace elements such as iron and thus increase their absorption by the plant, plant’s resistance to salt and drought stress. Strengthening the root system, as well as is one of the benefits of using fertilizers with amino acids (Khalil et al., 2008).

Marosz (2009) reported that fulvic acid in rapeseed increased the chlorophyll content and photosynthesis intensity and decreased the permeability of the cell membrane. These changes indicated an increase in plant tolerance to drought stress when using fulvic acid. In wheat plants affected by salt stress, spraying with fulvic acid reduced stomatal elongation, which in turn reduced leaf opening and transpiration.

Seaweed extract stimulates plant growth and resistance to biotic and abiotic stresses. The use of biological and microbial substances is also being considered because of their ability to increase the absorption of nutrients. Because of the importance and role of various stressors
in the production of crops and orchards, the use of plant growth stimulants causes plants to withstand a wide variety of stressors. On the other hand, the effect of using growth stimulants varies depending on the environmental conditions, the type of plant, and even the variety.

According to the reports, the effects of using growth stimulants under normal conditions and some stresses have been mentioned, but so far reports of the effects of these substances on saline and field conditions have been limited. The aim of this study was to examine the effect of some growth stimulants on wheat yield under saline and field conditions.

2. Materials and methods

This experiment was conducted in a two-year trial in the Qamrouddivision of Qom province, a relatively hot and dry area, central of Iran, 39°56912 East and 38°42304 North and classification of fine-loamy, mixed, thermal, typical haplocalcides, Soil Survey Staff, 2014). The experiment was carried out in a randomized complete blocks design with seven treatments including 1- control (application of chemical fertilizer based on soil test) 2- soil application of humic acid (first treatment + soil application of 5 kg of humic acid.ha⁻¹) 3- Amino acid spraying (first treatment + amino acid spraying with a concentration of 5 gr.lit⁻¹) 4- seed priming of azetobacter (first treatment + seed priming with a concentration of 1%) 5- Fulvic acid foliar application (first treatment + fulvic acid foliar application with a concentration of 5 gr.lit⁻¹) 6- seaweed foliar application (first treatment + seaweed foliar with a concentration of 5 gr.lit⁻¹) and 7- combined treatment (including the first treatment + treatments 6, 5, 4, 3, 2) in three replications. The Humic acid sample used contained 52.95% humic acid, the amino acid contained 36.79% free amino acid, the fulvic acid used contained 22.1% fulvic acid and the algae (sea weed extract) used contained 10% alginic acid. Also, for seed priming treatment, the amount of Azotobacter inoculation was used with a population of 10⁷ ml⁻¹ CFU in the amount of one percent (equal to one liter per 100 kg of seed). The inoculation solution was mixed with the seeds until the seed surface was completely wetted and impregnated with the material. It was then dried on clean plastic in shadow to fully adhere to the seed. The experiment was performed in soil with a salinity level of approximately 13 dS.m⁻¹ and water of approximately 8 dS.m⁻¹. Some physical and chemical properties of the soil including: pH in saturated paste through glass electrode, electrical conductivity of the saturated soil extract with electroconductivity meter, percentage of organic carbon (Walkley and Black, 1934), phosphorus in sodium bicarbonate (Olsen et al., 1954), soil texture by hydrometer method (Bouyoucos, 1951) and exchangeable potassium by neutral ammonium acetate method (Kenudsen and peterson, 1982) and absorbable trace elements by the method of DTPA (Lindsey and Norwell, 1978) and Calcium and magnesium indices and their ratio as well as SAR were determined. Some chemical properties of the water such as acidity, salinity, concentration of cations and anions were also measured using conventional methods in the laboratory (Brown et al., 1970) (Table 1 and 2).

Table 1. Results of the field soil test in the first and second year of the experiment (EC_w = 7.5dS.m⁻¹)

| Depth cm | Year | EC_w | pH | T.N.V | % | O.C | % | T.N.V | % | K | % | P | % | SP % |
|----------|------|------|----|-------|---|-----|---|-------|---|----|---|---|---|----|
| 0-30     | 1    | 7.5  | 7.5| 20.9  | 7.5| 13.5| 33| 1     | 0-30| 16.2| 0.51| 1.24| 3   | 320 |
| 0-30     | 2    | 7.8  | 7.5| 20.5  | 7.5| 8.5 | 33| 2     | 0-30| 16.2| 0.51| 1.24| 3   | 320 |

Table 2. Chemical results of irrigation water analysis (meq.lit⁻¹)

| SAR* | K⁺ | Na⁺ | Mg²⁺ | Ca²⁺ | SO₄²⁻ | CI | HCO₃⁻ | CO₃⁻ | pH | EC | Year |
|------|----|-----|------|------|-------|----|-------|------|----|----|------|
| 13.7 | 0.1| 50.2| 9.3  | 17.4 | 23.2  | 51.7| 2.1   | -    | 7.6| 7.5| 1    |
| 14.0 | 0.1| 52.2| 9.3  | 18.4 | 23.2  | 54.9| 2.2   | -    | 7.5| 7.8| 2    |

SAR* = Na⁺/√Ca²⁺+Mg²⁺/2
Before sowing, in all treatments a uniform amounts of triple superphosphate fertilizer (100 and 150 kg.ha\(^{-1}\) in the first and second year) and a quarter of nitrogen fertilizer (urea) were applied (in first and second year 300 and 250 kg.ha\(^{-1}\)). The remaining nitrogen were used in three times (end of tillering, middle of stem, start of clustering). A deficiency in trace elements was also eliminated through fertilization. The seed sowing, took place on fall of 2017 and 2019. Narin wheat cultivar seeds were planted in a density of 450 plants.m\(^{-1}\) in plots with a surface of 14 m\(^2\) (four line in each plots). Half a meter from the beginning and end of each line was taken as a margin. The irrigation method was based on the conventional method (Furrow) in the region and the water consumption were based on the water needs of wheat and five percent of leaching fraction excess (seven irrigations with atotal approximate volume of 7,000 m\(^3\).ha\(^{-1}\)).

Some agronomic properties of wheat such as height, number of tillers.plant\(^{-1}\),cluster length, number of seed. cluster\(^{-1}\)and 1000-seed weight were measured. Seed and straw yields were also measured after omitting 0.5 m from the top and bottom of each plot and two side rows and then converting them to hectare. In addition, ten plants from each plot were sampled at the time of clustering (Alley, 1993) and transferred to the laboratory, and then the concentrations of phosphorous, potassium, calcium, magnesium, iron, zinc, copper and manganese were measured. The significance test of all variables was performed using analysis of variance and F test and the means were compared using Duncan’s multiple range test. Statistical analysis were performed using SAS software (9.1) (SAS, 2009).

3. Results and Discussion

3.1 Yield and yield components

Based on the results of the Biennial analysis of variances experimental data (Table 3), consumption of all growth stimulants, had a significant effects (at 5% of Duncan’s test) on seedand straw yield, 1000-seed weight, height, number of seeds per cluster,number of tillers.plant\(^{-1}\) of the Narin variety compared to the control (non-use of stimulants). Accordingly, the combined treatment (treatment seven) achieved the highest grain, straw and biological yield. The grain yield from the treatment of humic acid and fulvic acid was also in the next statistical class (Table 4). According to the comparison table of the mean values (Table 4), the highest mean values of 1000-seed weight, height, number of seeds per cluster, number of tillers.plant\(^{-1}\)were obtained in the amino acid, combined, seed priming, combined and combined treatments, respectively.

Table 3. Results of the analysis of variance of the effect of growth stimulants on grain yield, straw and some agronomic characteristics of Narin wheat (biennial combined analysis)

| tillers per plant | Cluster length | seeds per Cluster | Height | 1000-seed weight | Biological yield | Straw yield | Seed yield | Degrees of freedom | Source of variation |
|-------------------|----------------|------------------|--------|-----------------|-----------------|-------------|------------|-------------------|-------------------|
| 3.9" 0.05 | 7.4" 0.01 | 3405" 3.6 | 15352" 20.8" | 46" 0.1 | 6531737" 307533 | 11600412" 132877 | 148096 61030 | 1 | Year |
| 0.85" 0.32 | 1.53" 0.9 | 28 21 | 40.3" 14.4" | 12.9" 1724158" 55158" | 1345725" 568266" | 336334" | 6 | Stimulant |
| 0.18" 0.65 | 11.6 16 | 7.2 | 0.047 | 310971" 120374 | 56416 | 24 | Total Error |
| 18.1 9.1 | 10.3 2.9 | 2.9 1.5 | 8.6 8.5 | 9.1 | - | C.V. |

** And * were significant in 1% and 5% of Duncan test, respectively.
The studies by Latif and Mohamed (2016) have shown that foliar application of humic acid could be able to increase the photosynthetic activity of the plant and the Rubisco enzyme and thus increase the salinity tolerance of the plant. In the present study, soil application of humic acid had a significant effect on growth and yield in comparison with the control (nutrient uptake based on soil test) (Table 4). Also, it has shown that the use of humic acid had reduced the electrical conductivity of the soil, which could be lead to the plant’s tolerance to saline conditions (Mohamed, 2012). It is therefore suggested that future studies consider foliar application of concentrations greater than 0.5% of these stimulants, or the method of sowing or soil application of these substances.

The results of studies by some researchers have shown that the consumption of humic acid in the soil has a positive effect on ascorbate peroxidase (Kaya et al., 2018). This enzyme plays an important role in the inhibition of hydrogen peroxide radicals and reactive oxygen species (ROS). Hence, in the present study, it appears that the consumption of humic acid in the soil increases the activity of this enzyme and ultimately increases the plant’s tolerance to salinity. In a recent experiment, however, this treatment was followed by a combined treatment of dry matter and grain yield, which showed a positive effect of the interaction of other growth stimulants besides the soil consumption of humic acid on the yield and its components in canola. According to research, the intake of some amino acids, including the simultaneous administration of methionine and proline or glutamic acid with methionine and tryptophan in the form of foliar applications, increases the yield of plant dry matter and the

### Table 4. Comparison of the mean values effects of growth stimulants on grain yield, straw and some agronomic characteristics of Narin wheat (combined biennial analysis)

| Stimulant source | Biological yield | Straw yield | Seed yield | Tillers plant | Cluster length cm | Seeds cluster | Height cm | 1000- seed weight gr |
|------------------|------------------|-------------|------------|---------------|------------------|--------------|-----------|----------------------|
| Control          | 6337bc           | 3982bc      | 2355c      | 1.7c          | 8.1b             | 31.2b        | 87.5d     | 375f                 |
| Amino acid       | 5940c            | 3581c       | 2359c      | 2.3bc         | 8.7abc           | 32.6b        | 90c       | 43g                  |
| Sea weed         | 6377bc           | 3906c       | 2471c      | 2.4bc         | 8.7bc            | 30.6b        | 91.2bc    | 379f                 |
| Seed weed        | 6211c            | 3730c       | 2472bc     | 2.5bc         | 8.9bc            | 37.4bc       | 94.3bc    | 40.6c                |
| Humic acid       | 7187bc           | 4354bc      | 2833bc     | 2.7bc         | 8.9bc            | 32.9bc       | 92.5bc    | 40.5c                |
| Fulvic acid      | 6393c            | 3896c       | 2497c      | 2.2bc         | 9.2b             | 33.7abc      | 90.2bc    | 40.1c                |
| Combined         | 8187c            | 4993c       | 3194c      | 2.8c          | 9.7c             | 32.9bc       | 94.8c     | 41.2bc               |

** And * were significant in 1% and 5% of Duncan test, respectively.
tolerance to the salt content of tomatoes (Alfosea-Simón, 2020). In the present study, the foliar application of amino acids had no significant influence on the grain yield and the dry plant mass. Given that research has shown that only some amino acids affect plant salt tolerance, the percentage of amino acids that affect tolerance in the sample used in the experiment is likely to be low and likely could not have a significant effect.

Concentration and uptake of some nutrient elements

The results of experiment showed that applying stimulants has different effects on concentration and uptake of K, P, Na, Ca, Mg and as well as Fe, Zn, Mn and Cu. Contemplation of the element concentration showed that except of Ca, others in combined treatment (treatment 7), were not in first statistical level inverse in control, which was due to the dilution effect and concentration effect (Marschner, 1995). The uptake of these elements, was unlike due to the plant biomass.

In saline condition, Na could substitute Ca in root cell membrane and disturb the function of selectivity and integrity of root cells. Therefore, awareness of Ca situation and its ratio vis-a-vis another ions (especially Na+) is very important. According to the results of trial, calcium molar fraction (ratio of calcium to total cations) of the treatments showed better correlation to the grain, straw and biological yield than K/Na ratio (table 5). This result is in agree with Cramer et al., (1987) which suggested this indice instead of K/Na selectivity coefficient.

Table 5. The effect of growth stimulants on the concentration of some nutrients in wheat (Narin variety) at the end of stalking and the beginning of clustering

| Treatment       | Cu (mg kg⁻¹ of dry matter) | Mn (mg kg⁻¹ of dry matter) | Zn (mg kg⁻¹ of dry matter) | Fe (mg kg⁻¹ of dry matter) | Calcium molar fraction | K/Na (mg kg⁻¹ of dry matter) | Mg (mg kg⁻¹ of dry matter) | Ca (mg kg⁻¹ of dry matter) | Na (mg kg⁻¹ of dry matter) | K (mg kg⁻¹ of dry matter) |
|-----------------|----------------------------|-----------------------------|-----------------------------|----------------------------|------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Control         | 3.9b                       | 1.93b                       | 0.82b                       | 4.5b                       | 0.094b                 | 32.04b                      | 0.03b                       | 0.278b                      | 0.084b                      | 2.56b                       |
| Amino acid      | 4.2b                       | 0.47d                       | 2.4a                        | 6.01d                      | 0.092b                 | 22.3b                      | 0.021d                      | 0.223d                      | 0.093a                      | 2.80b                       |
| Sea weed        | 2.8a                       | 0.47d                       | 2.7b                        | 3.5f                       | 0.097d                 | 25.6b                      | 0.027d                      | 0.203d                      | 0.07a                       | 1.79a                       |
| Seed prime      | 4.47c                      | 0.59f                       | 4.6c                        | 6.8a                       | 0.101c                 | 16.3d                      | 0.025c                      | 0.255c                      | 0.12a                       | 2.1b                        |
| Humic acid      | 3.5d                       | 1.23b                       | 1.0d                        | 16.3a                      | 0.102c                 | 21.7c                      | 0.048c                      | 0.262e                      | 0.099b                      | 2.16b                       |
| Fulvic acid     | 4.4a                       | 1.33b                       | 1.37c                       | 7.1c                       | 0.105b                 | 24.7c                      | 0.025c                      | 0.267bc                     | 0.087b                      | 2.16b                       |
| Combined        | 3.9c                       | 1.93b                       | 0.82b                       | 4.5b                       | 0.094b                 | 21.3c                      | 0.022d                      | 0.282a                      | 0.099b                      | 2.12b                       |

Calcium molar fraction*:
In each column, values with similar letters did not differ significantly at the 5% level of Duncan test

Table 6. The effect of growth stimulants on the uptake of some nutrients by Narin wheat plant at the end of stalking and the beginning of clustering

| Treatment       | Cu (gr ha⁻¹) | Mn (kg ha⁻¹) | Zn (kg ha⁻¹) | Fe (kg ha⁻¹) | Mg (kg ha⁻¹) | Ca (kg ha⁻¹) | K (kg ha⁻¹)  |
|-----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Control         | 10.4b        | 5.1a         | 2.1d         | 11.9d        | 0.8bc        | 7.4c         | 55.2c        |
| Amino acid      | 13.3b        | 1.48c        | 7.8b         | 19.2e        | 0.67d        | 7.1ed        | 66.3b        |
| Sea weed        | 8.9a         | 1.48c        | 8.7b         | 11.0d        | 0.84b        | 6.43d        | 65.9b        |
| Seed prime      | 13.0b        | 1.7c         | 13.5a        | 19.7c        | 0.72ed       | 7.4e         | 60.5e        |
| Humic acid      | 12.7bc       | 4.5a         | 3.65c        | 59.4a        | 1.75a        | 9.6b         | 75.99a       |
| Fulvic acid     | 11.5cd       | 3.5b         | 3.6c         | 18.7c        | 0.67d         | 7.02ed       | 54.6c        |
| Combined        | 14.8b        | 3.7b         | 9.3b         | 22.9b        | 0.84b        | 10.62a       | 78.07a       |

In each column, values with similar letters did not differ significantly at the 5% level of Duncan test.
Based on the results of the biennial experiment, the combined use of growth stimulants had a significant effect on yield and some yield traits and as well as uptake of some nutrients and calcium molar fraction of wheat in saline conditions compared to the control (no stimulant consumption), which indicates the effect of these substances on increasing wheat tolerance in saline conditions. The highest grain and straw yield was obtained in the combined treatment. Therefore it is suggested to investigate the effects of these stimulants in combine with 50 and/or 70% out of the recommended fertilizers and as well as the time of applying these stimulants.

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**Authors’ contributions**

MHM and FN conceptualization and Methodology designed and conducted the experiment, collected the data. MHM wrote the manuscript. ARJ supervised the experiment. ARJ helped us to solve the problems during the experiment. MHM analyzed the data curation. All authors read and approved the final version of manuscript.

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**Ethics approval and consent to participate**

Not applicable.

**Conflict of Interest**

Authors declare that they have no conflict of interest.

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