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# Effect of Irrigation with Magnetized Water and Foliar Spraying with Humic Acid on Seedling Growth of Squash Plant (Cucurbita pepo L.) Under Salinity Stress

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**Keywords:**
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**ABSTRACT**

The experiment was conducted to investigate the effect of magnetic water (MW 14500 Gauss) and humic acid (HA 30 ppm) on the germination and growth of squash plants (Cucurbita pepo L.) exposed to salinity stress. Salinity stress was imposed by irrigation with saline water at concentrations of 1700, 2700, and 3700 ppm in addition to a control (tap water). The results showed that with increasing salinity stress, all vegetative growth parameters, seed germination ratio, and photosynthetic pigments decreased. On the other hand, foliar application of HA and irrigation with MW had a favorable impact on increasing vegetative traits and photosynthetic pigments. These results give a positive indication of the use of HA and MW to ameliorate the negative effects of salinity on squash plants.

# تاثير الري بالماء المغنظ والرش الورقي بحمض الهيوميك علي نمو بادرات نبات الكوسة(Cucurbita pepo L) تحت الإجهاد الملحي

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**الكلمات المفتاحية:**
الإجهاد الملحي
الماء المغنظ
نبات الكوسة
حمض الهيوميك.

**الملخص**

أجريت التجربة لمعرفة تأثير الماء المغناطيسيmagnetic water (14500Mw جاوس) وحامض الهيوميك humic acid (HA30 جزء من المليون) على إنبات ونمو نبات الكوسة (*Cucurbita pepo* L) المعرض للإجهاد الملحي. تم فرض الإجهاد الملحي عن طريق الري بالمياه المالحة بتركيزات 1700 و2700 و3700 جزء في المليون، بالإضافة إلى الضابط (ماء الصنبور). أظهرت النتائج مع زيادة الإجهاد الملحي تنخفض جميع مؤشرات النمو الخضري ونسبة إنبات البذور وصبغات البناء الضوئي. ومن ناحية أخرى، كان للرش الورقي بحمض الهيوميك والري بالماء المغنظ تأثير إيجابي على زيادة الصفات الخضرية وصبغات البناء الضوئي. هذه النتائج تعطي إشارة إيجابية لاستخدام HA وMW لتخفيف التأثيرات السلبية للملوحة على نباتات الكوسة.

## 1. Introduction

Salinity is a major abiotic stress, reducing the growth and yield of a wide range of crops worldwide [1]. Approximately 20% of irrigated and 2% of non-irrigated agricultural lands are under salt stress globally [2]. The harmful influence of salinity is attributed to its impact on osmotic stress, ion toxicity, nutritional disorders, and the production of reactive oxygen species (ROS), which induce complex interactions at the morphological, physiological, biochemical, and molecular levels [3]. These processes damage DNA, proteins, chlorophyll, and cell permeability, and cause inhibition of photosynthesis, metabolic toxicity, inhibition of potassium (K) uptake, reduced germination rate, and ultimately cell death [4].

Salt stress affects all growth stages of a plant, with seed germination and seedling growth stages being more sensitive for most plant species [5]. At these stages, salt stress is expressed through the reduction in germination percentage, delayed

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germination rate, and inhibited tissue elongation. Salt stress further affects the ultrastructure of root cells and severely inhibits root growth during early seedling growth [6].

Squash (Cucurbita pepo L.) is an important vegetable crop. Its fruits are very low in calories (19 kcal/100 g), have high moisture content (94.8 g), and large edible portions (94%), and contain significant amounts of fiber (0.8 g) [7]. Squash is moderately tolerant to salinity; however, high salinity causes a reduction in both yield and quality, reducing fresh weight, and the number and area of the leaves [8][9].

Efforts have been made to control salinity and other stresses by various technological means, including the application of organic matter such as humic acid (HA) to alleviate the inhibitory effects of soil salinity by improving the physical and chemical properties of soils, increasing soil water holding capacity, and enhancing nutrient uptake during plant growth [10]. HA increases membrane permeability and facilitates the transport of essential elements within plant roots [11], oxygen uptake, respiration, photosynthesis, phosphate uptake, root cell elongation, hormone regulation, and the uptake of mineral elements [12]. Additionally, HA is known to accumulate toxic heavy metals [13], stimulate seed germination [14], induce levels of endogenous proline, and decrease membrane leakage, which are considered indicators of better adaptation to saline conditions [15].

Hartwigsen and Evans [16] found that adding HA to the substrate increased the fresh weight of squash plant roots and the number of leaves. Another study demonstrated that adding HA increased the fresh and dry weight of squash fruits and the number of female flowers [17]. HA can stimulate shoot and root growth and improve resistance to environmental stress in plants [18].

Magnetic water (MW) technology is an environmentally friendly and cost-effective technique recently used in agricultural fields to improve crop growth and yield. MW is generated by treating normal water with a magnetic field, resulting in the rearrangement of the water structure into a new hexagonal form [19]. Some studies have shown that the application of magnetic fields increases the number of flowers, earliness, and total fruit yield of strawberries and tomatoes [20][21], nutrient uptake [22], seed germination percentage, and speed of emergence [23].

El Sayed [24] indicated that MW treatment had a significant positive effect on plant growth parameters (i.e., height, leaf area, leaf, stem, root fresh weight, and dry weight) of broad bean (Vicia faba L.) seedlings, significantly increased the number of leaves per plant, and the fresh and dry weight of pear seedlings [25]. MW increases plant metabolism in terms of photosynthesis and water uptake [26].

Therefore, the present study aimed to investigate the efficiency of foliar HA with MW applications (as a combination treatment) on the alleviation of the adverse effects of salt stress on seed germination, vegetative growth, and photosynthetic pigments under laboratory conditions.

## 2. Materials and Methods

### 2.1 Plant Material

Two laboratory experiments were conducted in a completely randomized design (CRD) with three replicates at the Horticulture Department, Faculty of Agriculture, Benghazi University, Benghazi, Libya, in 2022. The aim was to evaluate the response of squash plants (Sakata variety) to different levels of saline water (both magnetized and non-magnetized, with a magnetic field of 14,500 Gauss or 1.45 Tesla from Delta Water, Egypt) and foliar spraying with 30 ppm of humic acid.

### 2.2. Experimental Procedures

2.2.1. First Experiment:

Germination tests of squash seeds were carried out under laboratory conditions. The seeds were germinated in covered, sterilized, disposable Petri dishes containing moistened Whatman filter paper (three Petri dishes with 10 seeds each, ($n_{=3}$). The Petri dishes were irrigated daily with 5 mL of the treatments: tap water (control), 30 ppm HA+MW, 1700, 2700, and 3700 ppm NaCl, with and without 30 ppm HA+MW, every 24 hours for a period of 5 days. The filter papers were changed every 48 hours to avoid salt accumulation.

2.2.2. Second Experiment:

Fifty seeds per treatment (control and treatments) were sown in germination pots containing a mixture of clay and sand (2:1 v/v) at 25±3°C. Each pot was irrigated with equal amounts of tap water for one week. In the second week, the seedlings were divided into three groups, each consisting of ten replicates, which were irrigated with tap water for two weeks. Each group was further divided into three subgroups, which were irrigated with different levels of saline water (magnetized and non-magnetized) and foliar application of HA as follows:

- **Group I: Tap Water**
  - Subgroups: Control (tap water) and foliar application of 30 ppm HA+MW
- **Group II: Saline Treatment**
  - Subgroups: 1700 ppm, 2700 ppm, and 3700 ppm NaCl
- **Group III: Magnetic Treatments with Foliar Application of 30 ppm HA**
  - Subgroups: 1700 ppm saline water + MW+HA, 2700 ppm saline water + MW+HA, 3700 ppm saline water + MW+HA

All plants were fertilized with Kristalon (NPK 19:19:19).

### 2.3 The data recorded

Germination % and morphological parameters**:** shoot height (cm), root length (cm), type of flower, number of leaves/plant, leaves area/plant $cm^2$ ( using leaf area-leaf weight relationship [27], fresh and dry weights of the plant.

The salt tolerance index (STI) is the ratio of the plant dry weight (DW) of the control treatment and the plant dry weight of the salt treatments. The STI was calculated from the following relation[28]:

$$STI\% = \frac{\text{Total DW salt stress X100}}{\text{Total DW control}} \qquad \textbf{Equation 1}$$

Chlorophyll a, chlorophyll b, and carotenoids were determined for plant growth using the spectrophotometric method as recommended by Arnon [29] .

### 2.4 Statistical Analysis

The obtained results were subjected to a two-way analysis of variance (ANOVA). The means of treatments were compared using the Least Significant Difference (LSD) test at the 0.05 level.

## 3. Results and Discussion

### 3.1. Growth Parameters

3.1.1. Effect of Salinity Stress

Table 1 shows that salinity stress adversely affected various vegetative growth attributes of squash plants, including seed germination and all tested growth parameters, except for shoot height, which did not show a significant reduction compared to control plants. Increased salinity from 1700 to 3700 ppm caused a steady and significant reduction in growth parameters. These results are consistent with findings from various studies [30][31]. The reduced growth parameters of squash plants may be due to the high concentration of $Na^+$ and $Cl^-$ ions in the external solution, which are taken up by the roots at high rates, leading to excessive accumulation in the tissue. These ions may inhibit the uptake of other essential ions (such as $K^+$), increase growth inhibitors (e.g., abscisic acid), and decrease growth promoters (e.g., indole-3-acetic acid and gibberellins) [32]. Therefore, the interactions between nutrients in salt-stressed plants may have significant consequences for reduced growth.

3.1.2. Effect of Humic Acid and Magnetic Treatments

The application of humic acid (HA) and magnetized water (MW) led to significant improvements in most growth parameters, especially leaf area, which increased to 252.49 cm² compared to 198.70 cm² in control plants. Foliar application of HA with MW under salinity stress (1700, 2700, 3700 ppm) generally caused a significant increase in all estimated growth parameters compared to untreated values (Table 1). Based on the obtained results, the salt tolerance index in plants treated with salinity stress (1700, 2700, 3700 ppm) showed inhibition rates of 78.94%, 72.93%, and 69.92%, respectively. However, squash seedlings treated with HA and MW showed a better salt tolerance index under salinity stress compared to untreated seedlings, with a higher improvement (98.12%) at 1700 ppm (Table 1). Previous studies have reported increased growth parameters in plants subjected to salt stress as a result of HA and MW treatments [32]. This may be due to HA, which contains organic acids and micronutrients, contributing to plant growth, as well as its potassium content, which plays a vital role in regulating stomatal opening and closing and facilitating CO2 entry [33].

Conversely, the positive effects of magnetic treatments may be attributed to the role of the magnetic field in enhancing water properties by regulating charges and altering the properties of water molecules within a magnetic field, resulting in the dissociation of hydrogen bonds [34]. Additionally, changes in intracellular levels of $Ca^{2+}$ and other ionic currents across cellular membranes can alter osmotic pressure and affect cellular water absorption [35], biostimulation during initial growth stages, activation of protein formation, and enzyme activity [36], as well as changes in biochemical and physiological processes within seeds and plants, which positively affect development and productivity [37].

**Table 1:** Effect of salinity stress, humic acid and magnetic water treatments on growth parameters of squash plants

| Treatments | Type of flower ♂ | Type of flower ♀ | Germination (%) | Shoot height (cm) | Root length (cm) | Number of leaves/ plant | leaves area/plant ($cm^2$) | Fresh weight (g) | Dry weight (g) | STI (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **C** | 6.00 | 3.33 | 77.65 | 1.63 | 33.00 | 6.33 | 198.70 | 21.65 | 2.66 | - |
| **CHM** | 6.66 | 3.66 | 91.61 | 2.37 | 35.00 | 7.00 | 252.49 | 25.11 | 2.95 | - |
| **S1** | 5.33 | 2.33 | 62.66 | 1.23 | 24.33 | 5.66 | 195.45 | 15.00 | 2.10 | 78.94 |
| **S2** | 5.66 | 2.00 | 52.66 | 1.30 | 25.20 | 5.33 | 161.01 | 13.70 | 1.94 | 72.93 |
| **S3** | 6.00 | 0.66 | 49.65 | 1.06 | 23.30 | 5.33 | 158.62 | 12.61 | 1.86 | 69.92 |
| **M+H+S1** | 6.00 | 3.66 | 72.00 | 2.73 | 29.67 | 6.66 | 228.69 | 21.69 | 2.61 | 98.12 |
| **M+H+S2** | 5.66 | 3.00 | 69.00 | 2.46 | 32.50 | 6.33 | 223.68 | 18.40 | 2.41 | 90.60 |
| **M+H+S3** | 5.00 | 2.66 | 63.66 | 2.26 | 27.16 | 6.00 | 209.34 | 15.00 | 1.95 | 73.30 |
| **LSD** | 0.67 | 0.66 | 11 | 0.93 | 0.87 | 0.66 | 18.3 | 1.99 | 0.075 | |

LSD 0.05 (least significant different at 0.05 level), C (control), H (humic acid), M (magnetic water), CHM (HA and MW), S1 (1000 ppm) , S2 (2000 ppm), S3 (3000 ppm) and M+H+S1,2,3 (MW+HA+S1,2,3)

### 3.2. Photosynthetic Pigments:

3.2.1. Effect of Salinity Stress

Data presented in Table 2 reveal that salinity stress negatively impacts the levels of chlorophyll a, chlorophyll b, carotenoids, total chlorophylls, and total photosynthetic pigments in leaves. Generally, pigment concentrations decreased significantly with increasing salinity levels from 1700 to 3700 ppm compared to the control. These results are consistent with previous studies [31][38]. The lowest values were observed in seedlings irrigated with the highest salinity level (3700 ppm), showing significant reductions in chlorophyll a (0.202 μg/g), chlorophyll b (0.128 μg/g), carotenoids (0.105 μg/g), total chlorophylls (0.330 μg/g), and total pigments (0.435 μg/g), compared to control values of 0.329 μg/g, 0.299 μg/g, 0.202 μg/g, 0.628 μg/g, and 0.830 μg/g, respectively. The decrease in chlorophyll and carotenoid concentrations in salinized plants may be attributed to increased activity of chlorophyll-degrading enzymes and ion accumulation in leaves, which adversely affects chlorophyll concentration [39].

**Table 2:** Effect of salinity stress, humic acid and magnetic water treatments on photosynthetic pigments of squash plants.

| Treatments | Chl *a*. (μg/g) | Chl *b*. (μg/g) | Car. (μg/g) | Total chlorophylls | Total pigments |
|---|---|---|---|---|---|
| **C** | 0.329 | 0.299 | 0.202 | 0.628 | 0.830 |
| **CHM.** | 0.431 | 0.537 | 0.298 | 0.968 | 1.266 |
| **S1** | 0.214 | 0.123 | 0.145 | 0.337 | 0.482 |
| **S2** | 0.205 | 0.105 | 0.121 | 0.310 | 0.431 |
| **S3** | 0.182 | 0.098 | 0.085 | 0.280 | 0.365 |
| **M+H+S1** | 0.370 | 0.198 | 0.170 | 0.568 | 0.738 |
| **M+H+S2** | 0.355 | 0.188 | 0.155 | 0.543 | 0.698 |
| **M+H+S3** | 0.302 | 0.161 | 0.147 | 0.463 | 0.610 |
| **LSD** | 0.036 | 0.004 | 0.005 | 0.066 | 0.067 |

LSD 0.05 (least significant different at 0.05 level), C (control), H (humic acid), M (magnetic water), CHM (HA and MW), S1 (1000 ppm) , S2 (2000 ppm), S3 (3000 ppm) and M+H+S1,2,3 (MW+HA+S1,2,3)

3.2.2. Effect of Humic Acid and Magnetic Treatments

Data from Table 2 reveal that the application of (HA) and magnetized water (MW) positively influenced the photosynthetic pigments in leaves. Chlorophyll, a key photosynthetic pigment, serves as an indicator of plant health and plays a role in stress response mechanisms. Foliar application of HA and irrigation with MW resulted in significant increases in all photosynthetic pigments compared to control values (Table 2).

Salinity, however, significantly decreased the content of pigments (chlorophyll a, chlorophyll b, and carotenoids). The combined application of HA and MW mitigated these harmful effects, improving pigment content. Specifically, total chlorophylls increased from 0.337 to 0.568, 0.310 to 0.543, and 0.280 to 0.463, while total pigments increased from 0.480 to 0.738, 0.431 to 0.698, and 0.365 to 0.610 for salinity levels of 1700, 2700, and 3700 ppm, respectively. These findings are consistent with Hamid and Shamsullah [40], who reported that magnetically treated water and HA significantly increased leaf number and chlorophyll content.

HA enhances chlorophyll content by promoting nitrogen uptake, improving membrane stability, and thereby increasing nutrient absorption, which is directly linked to chlorophyll synthesis [41]. Additionally, HA influences the overproduction of reactive oxygen species (ROS), the replacement of potassium (K+) by sodium (Na+) ions, and the upregulation of chlorophyllase activity [42]. This aligns with previous studies showing that HA application under salinity stress increased total chlorophylls in maize [3], tomato [41], strawberry [41], and squash [27].

Furthermore, irrigation with MW can improve photosynthetic machinery and growth by modifying nutrient uptake and enhancing the paramagnetic properties of chloroplasts. This results in increased ion mobility and absorption, boosting photosynthesis. MW has been shown to increase chlorophyll and carotenoid levels in leaves, likely due to proline and GA3, which are triggered by Mg2+ accumulation for chlorophyll synthesis [43] and K+ for increasing chloroplast numbers. The effect may be due to MW's ability to enhance enzyme and hormone activation, promoting chlorophyll production [44]. These results are consistent with findings in cotton, wheat, pepper, soybean, and cowpeas [45].

## 4. Conclusion

Application of HA with MW (combination treatment) improved plant stress-defiance responses resulting in better plant performance under stress. Thus, the application of two factors may provide a useful amendment to reduce the adverse effects of salinity stress on squash (*Cucurbita pepo* L) plants irrigated with saline water.

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