Effect of Treatment with Humic Acid (HA) on the Growth of Salt-Stressed Sweet Basil Plants

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Abstract:

Research was carried out during the year 2024 in the village of Burj Al-Qasab – Latakia – Syria, by planting sweet basil seedlings (*Ocimum basilicum* L.) in plastic bags that were distributed according to a randomized complete design (R.C.D) with three replicates. This research aimed to study the effect of spraying humic acid on the leaves of sweet basil plants at concentrations (1, 3 and 5 g/L) before irrigation with salt solutions prepared from sodium chloride (NaCl) at concentrations (6, 12 and 18) dS/m two weeks later with a control without treatment with humic acid and sodium chloride salt. Measuring a set of morphological indicators (plant height (cm), number of branches (branch/plant), physiological indicators (total leaf surface area (cm^2), photosynthesis rate ($mg/cm^2/day$)), Biochemical indicators (chlorophyll content (µg/g fresh weight)) and proline content in leaves (µmol/g fresh weight). Treatment with salt stress led to negative effects that became more severe with increasing salinity concentration on all the morphological, physiological and biochemical characteristics studied, thus affecting the growth and development of the sweet basil plant.

Treatment with humic acid, especially at two concentrations (1 and 3 g/l), led to an increase in plant height, number of branches, total leaf surface area, photosynthesis rate, and chlorophyll content in the leaves compared to the control and the other concentrations. It also led to an improvement in the proline content in the leaves compared to the control and treatments. Salinity and high concentration of humic acid, thus positively affecting the growth of the basil plant, and this applies to most of the criteria and characteristics studied. The initial treatment with humic acid, especially at a concentration of (1 g/l), improved the studied indicators under salinity conditions, especially at a low salinity concentration (6 dS/m) compared to the control and the rest of the treatments. Thus, increasing the tolerance of sweet basil plants to salt stress. Therefore, it can be suggested to use foliar spray treatments with humic acid at concentrations (1 and 3 g/l) for the purpose of improving the growth of the sweet basil plant and increasing its tolerance to salt stress.

Keywords: Basil plant, humic, salt stress, plant height, chlorophyll content, proline content.

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تأثير المعاملة بحمض الهيوميك (HA) في نمو نباتات الريحان الحلو المجهدة بالملوحة

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الملخص:

نفذ البحث خلال عام 2024 في قرية برج القصب – اللاذقية – سورية، من خلال زراعة شتلات الريحان الحلو (*Ocimum basilicum* L.) في أكياس بلاستيكية تم توزيعها وفق التصميم العشوائية الكاملة (R.C.D) وبثلاث مكررات. هدف هذا البحث إلى دراسة تأثير رش حمض الهيوميك على أوراق نباتات الريحان الحلو بالتراكيز (1، 3 و5 غ/لتر) قبل الري بالمحاليل الملحية المحضرة من ملح كلوريد الصوديوم (Nacl) بالتراكيز (6، 12 و18) ديسي مول/سم بأسبوعين مع وجود شاهد دون المعاملة بحمض الهيوميك وملح كلوريد الصوديوم، تم قياس مجموعة من المؤشرات المورفولوجية (ارتفاع النبات (سم)، عدد الأفرع (فرع/نبات)، المؤشرات الفسيولوجية (مساحة مسطح الورقي الكلي (سم^2)، معدل التمثيل الضوئي (ملغ/سم^2/يوم))، المؤشرات البيوكيميائية (محتوى الكلوروفيل) (ميكروغرام/غ وزن رطب)) ومحتوى البرولين في الأوراق (ميكرومول/غ وزن طازج)). أظهرت النتائج أنّ المعاملة بالإجهاد الملحي أدت إلى تأثيرات سلبية تزداد حدتها مع زيادة تركيز الملوحة على جميع الخصائص المورفولوجية، الفسيولوجية والبيوكيميائية المدروسة وبالتالي التأثير على نمو وتطور نبات الريحان الحلو.

أدت المعاملة بحمض الهيوميك وخاصة بتركيزين (1 و3 غ/ل) إلى زيادة ارتفاع النبات، عدد الأفرع، مساحة مسطح الورقي الكلي، معدل التمثيل الضوئي ومحتوى الكلوروفيل في الأوراق مقارنة بالشاهد وباقي التراكيز، كما أدى إلى تحسن محتوى البرولين في الأوراق مقارنة بالشاهد ومعاملات الملوحة والتركيز المرتفع من حمض الهيوميك، وبالتالي التأثير بشكل ايجابي على نمو نبات الريحان، وهذا ينطبق على معظم المعايير والصفات المدروسة. أدت المعاملة الأولية بحمض الهيوميك وخاصة بتركيز (1غ/ل) إلى تحسين المؤشرات المدروسة تحت ظروف الملوحة وخاصة عند تركيز الملوحة المنخفض (6 ديسي مول/سم) مقارنة بالشاهد وباقي المعاملات، وبالتالي زيادة تحمل نبات الريحان الحلو للإجهاد الملحي. وبالتالي يمكن اقتراح استخدام معاملات الرش على الأوراق بحمض الهيوميك بتركيز (1 و3 غ/ل) لغرض تحسين نمو نبات الريحان الحلو وزيادة تحمله للإجهاد الملحي.

الكلمات المفتاحية: نبات الريحان، الهيوميك، الإجهاد الملحي، ارتفاع النبات، محتوى الكلوروفيل، محتوى البرولين.

*باحث – اختصاص محاصيل حقلية – كلية الهندسة الزراعية – جامعة تشرين

Introduction:

Basil (*Ocimum basilicum* L.) is a medicinal plant, belonging to the Labiatae family, grown worldwide as one of the most aromatic, commonly used and popular vegetables Hosseini Farahi and Norouzinejad (2016). Types of basils have extensive medicinal and industrial applications Asgharipour and Rafiei (2016). The active ingredients of this plant are appetizing and can also be used for the treatment of bloating, enhancement of digestive tract, and treatment of some heart problems and enlarged spleen. Moreover, basil is used in traditional medicine as an expectorant, diuretic, anti-flatulence, stomach pain aid, and a stimulant Silva *et al*. (2023).

The intensive agriculture using chemical fertilizers has caused deterioration and salinization of soils Lira-Saldivar *et al*. (2018), with NaCl being the predominant salt that is used for the studies to simulate saline soils. The salinity in soils reduces the growth and development of plants because of water stress and ionic toxicity due to the high concentration of specific ions, as well as a nutritional imbalance due to the high levels of Na and Cl that reduce the uptake of K^+, NO^- Safdar *et al*. (2019). Additionally, it increases the production of reactive oxygen species that damage macromolecules Lamz and González (2013).

One of the most widespread alternatives is the use of biostimulants for plant growth Yilmaz *et al*. (2023). In the last two decades several bio-stimulants have been used in agriculture, which have helped to reduce plant stress to adverse environmental conditions, increasing plant growth and development and also increasing agricultural output Shahrajabian and Sun (2024).

Calderín-García *et al*. (2012) reported that different doses of humates could ameliorate negative impacts in plants submitted to stress conditions. The positive effects of humic substances on plant development and growth highlight their assertive influence with ion transport, making absorption easier and improving membrane permeability. This effect improves metabolic processes such as respiration, protein synthesis and photosynthesis increasing or decreasing activity of various enzymes and hormones, reflected in growth indicators and some biochemical-physiological processes Sayarer *et al*. (2023).

In another study, Jamali *et al*. (2015) assessing effects of humic acid, compost and phosphorus on growth traits and some micronutrient uptake in basil medicinal plant, showed plants treated with humic acid compared to control have significantly more height, number of lateral branches and shoot dry weight. Moreover, Khaled and Fawy (2011) reported that different levels of humic acid increase basil growth and nutrient uptake under salinity stress, and ameliorate effects of the stress on basil.

Therefore, this research aims to improve the growth of sweet basil plants by foliar spraying with humic acid when exposed to salinity stress conditions.

Material and Methods:

Experimental Conditions and Treatments:

The experiment was carried out in Burj Al-Qasab- Latakia- Syria- 2024 a greenhouse with temperature 25±2 ºC, relative humidity 50% and 60% during day and night respectively, and 14h photoperiod throughout the experiment. Seeds of basil (O. *basilicum*) were sown in pots with a depth of 25 cm and a diameter of 20 cm filled with clay loam (pH of 7.5, 10% organic material, 1.30 dS/m, 50% field capacity). Seeds were tested for their vigor and sown in a depth of 1 cm. In each pot, four healthy plants were grown.

Humic acid (HA):

Humic acid treatments were at control (tap water) and three different concentrations of humic acid: 1, 3, and 5 g/l. The first spray was 45 days after seed sowing and 3 sprays as total were done with 7 days intervals. Humic acid consists of potassium hyomate percentage 86% in humic acid. Humic acid commercial product (Hammer) was obtained from Union for Agriculture and Development company (UAD) it contains 86% humic acid, 6% K O and 7% fulvic acid.

Salt stress treatments(S):

Irrigation with NaCl solutions (0, 6, 12, 18 dS/m) was carried out during active vegetative growth (before flowering), at a rate of three irrigations every week. S_0: Plants were irrigated with water only.

Data recorded Growth characters:

1. **Plant Height (cm):** was measured for each experimental treatment, starting from the soil surface level to the growing top, before the plants entered the inflorescence formation stage, that is, about 6 weeks after transplanting.
2. **Number of branches/ plant** (branch/plant)**:** the number of branches on the plant branch/plant
3. **Plant leaf area (PLA) (cm^2):**
   From the following equation:
   PLA (cm^2/plant) = sum of the area of all leaves of a plant.
   LAI = leaf area of the plant (cm^2) / area occupied by the plant on the ground (cm^2)
   The leaf area was measured by the gravimetric method according to Vivekanandan *et al*. (1972).
4. **Net Photosynthesis Rate (mg/cm^2/day):**
   It is calculated from the following equation Williams (1946):
   $$(NPR = \frac{(Log\ eL2\ - Log\ eL1)(W2 - W1)}{(T2 - T1)(L2 - L1)})$$
   NPR: net photosynthetic production, mg/cm2/day, L2 and L1: leaf area (cm2) at the beginning and end of the measurement period, respectively, W2 and W1: plant dry weight at the beginning and end of the measurement period, respectively, T2 and T1: number of days between the two phases (At the beginning of the active vegetative growth phase and the end of this phase, i.e. at 30 and 60 days from transplanting).
5. **Determination of Chlorophyll content in leaves (µg/g):**
   Chlorophyll content determination Pigments were extracted from the leaves. Th e extraction of leaf pigments was performed with 80% acetone, and the absorbance at 663 and 645 nm was measured with an Amersham spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). The total chlorophyll quantities were calculated according to the method of Arnon (1949). Total carotenoid content was measured at 470 nm. The pigment concentrations were expressed as µg g^{-1} fresh weight (FW).
6. **Determination of proline content in leaves (µmol/g):**
   The proline content was determined according to Bates *et al*. (1973). Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3% sulfosalicylic acid at 4 °C. Th e extract was fi ltered with Whatman No. 2 fi lter paper. In a test tube, 2 mL of fi ltrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed and incubated at 100 °C for 1 h. Th e reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as µmol proline g^{-1} FW.

Statistical Analysis:

Statistical analyses were performed by the analysis of variance (ANOVA) with Tukey. All data are presented as means ± standard deviation (SD) of three replicates. Differences at $P < 0.05, 0.01$ were considered to be significant.

Results and Discussion:

1. **Effect of treatment with humic and salt stress on plant height (cm):**

The data in Figure (1) indicate that there are significant differences at the level (P<0.05) between the studied treatments in terms of the height of sweet basil plants (cm).

Salt stress led to a decrease in plant height, and this decrease increased with increasing concentration of added salt.

While treatment with humic acid at two concentrations (1 and 3 g/l) increased plant height compared to the control

Treatment with humic acid at a concentration of 1 g/l and salinity of 6 dS/m also outperformed all other parameters and the control.

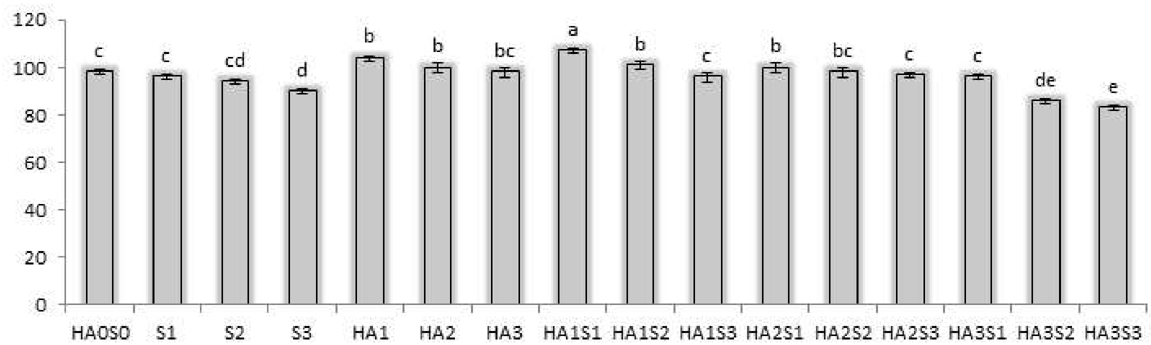

Figure (1): Effect of humic on plant height under salt stress.

All data refer to averages plus standard error (means ± SE), n=3, and different letters (a, b, c... to show the significant differences between the averages for each indicator at each treatment (P<0.05), ANOVA-Tukey test.

The negative effect of salinity on plant height was pointed out by Zakharin (2001), who showed that high concentrations of salinity inhibit enzymatic activity and stop the elongation of growing shoot cells, which leads to shortening of the plant, in addition to not increasing the size of the meristematic cells. Preventing their transformation into adult parenchyma cells, which causes weakness in the overall growth of the plant and the formation of leaves of small size and area.

Kamel (1989) mentioned that the decreasing of plant height by salinity might be due to that salinity decreased cell division of plant or inhibited the meristematic activity and elongation

Sallam (2010) mentioned that beneficial effects of humic acid on plant growth could be referred to its acting as source of plant growth hormones. In addition, physiological mechanisms through which humic substances exert their effects may depend on hormones and, in particular, on the presence of auxin.

2. Effect of treatment with humic and salt stress on Number of branches/ plant (branches/ plant):

The data in Figure (2) indicate that there are significant differences at the level (P<0.05) between the studied treatments in terms of the Number of branches/ plant of sweet basil plants Salt stress led to a decrease in Number of branches/ plant, and this decrease increased with increasing concentration of added salt.

While treatment with humic acid at two concentrations (1 and 3 g/l) increased Number of branches/ plant compared to the control

Treatment with humic acid at a concentration of 1 g/l and salinity of 6 dS/m also outperformed all other parameters and the control.

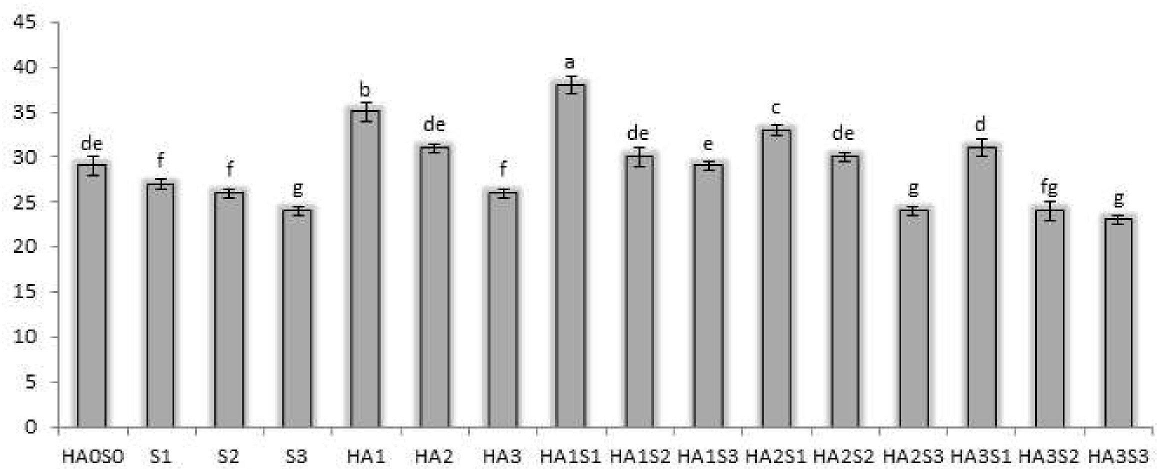

Figure (2): Effect of humic on number of branches/ plant under salt stress.

In plant growth under salty conditions, plants cause many physiological and morphological disorders. The main inhibitory effect of salinity is the osmotic effect and ionic toxicity, and it also negatively affects the number of plant branches Trivellini *et al*. (2014).

HA used for plant nutrition, enhance development, root and plant growth as well as yield due to its action on physiological and metabolic procedures (Eyheraguibel *et al*., 2008). Moreover, Said-Al Ahl *et al*. (2016) reported that spraying by HA recorded the best results of plant height, number of branches and seed yield compared to control .

Furthermore, Safaei *et al*. (2014) pointed out that different rates of humic acid imposed a significant effect on seed weight, seed and biological yields of black cumin plants compared to control.

Talaat and Balbaa (2010) on sweet basil Found an increase of branches/plant when treated by HA compared to control plant (untreated) .

3. Effect of treatment with humic and salt stress on total leaf surface area (cm2/plant):

The data in Figure (3) indicate that there are significant differences at the level (P<0.05) between the studied treatments in terms of the total leaf surface area of sweet basil plants.

Salt stress led to a decrease in total leaf surface area, and this decrease increased with increasing concentration of added salt.

While treatment with humic acid at two concentrations (1 and 3 g/l) increased total leaf surface area compared to the control

Treatment with humic acid at a concentration of 1 g/l and salinity of 6 dS/m also outperformed all other parameters and the control.

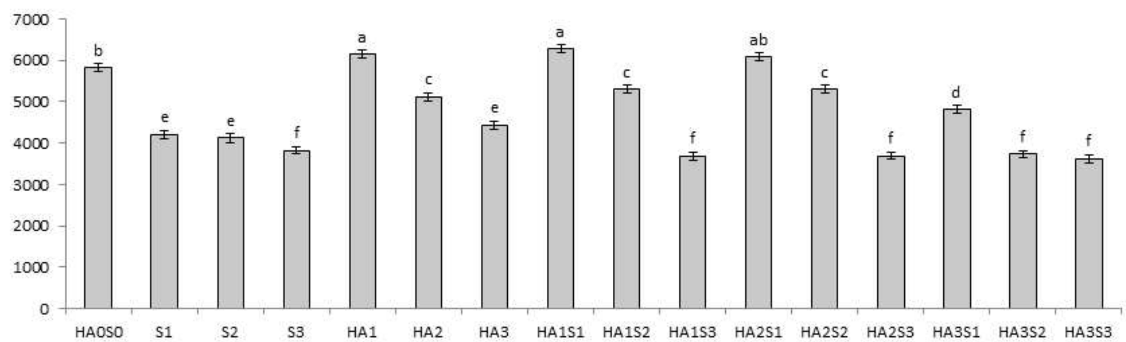

Figure (3): Effect of humic on total leaf surface area under salt stress.

Salt stress affects growth, morphology, and anatomical structure of leaves and reduces their area Delavari *et al*. (2014). Some studies revealed that humates stimulate plants' growth because they have analogous effects with plant hormones like auxins. Other pointed out that are because of humates contain free amino acids, polysaccharides, carbohydrates, inorganic elements, humic substances, beneficial microorganisms, and soluble humus that act positively in the total metabolism of the plants. However, the use of these constituents induces an increase in photosynthetic pigments such as chlorophyll and other physiological and morphometric characteristics in plants Calderín-García *et al*. (2013).

4. Effect of treatment with humic and salt stress on the net photosynthesis rate (mg/cm2/day):

The data in Figure (4) indicate that there are significant differences at the level ($P<0.05$) between the studied treatments in terms of the net photosynthesis rate of sweet basil plants.
Salt stress led to a decrease in net photosynthesis rate, and this decrease increased with increasing concentration of added salt.
While treatment with humic acid at two concentrations (1 and 3 g/l) increased net photosynthesis rate compared to the control
Treatment with humic acid at a concentration of 1 g/l and salinity of 6 dS/m also outperformed all other parameters and the control.

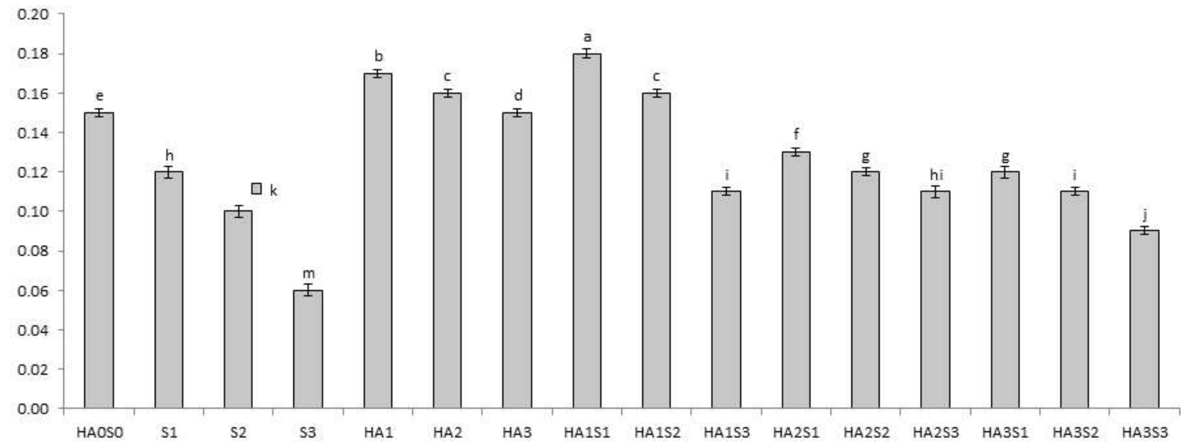

Figure (4): Effect of humic on net photosynthesis rate under salt stress.

Salt stress often induces ROS accumulation, leading top membrane lipid peroxidation and cell death Zhao *et al*. (2021). The salinity influences photosynthesis at the stomata level, depending on the salinity and exposition time, species, and age of the plant Zahra *et al*. (2022). The humates has mitigated the inhibitory effect of salinity on the photosynthesis Alamer *et al*. (2022), while the respiratory process could also be favored, leading to a better flow of energy in plants treated with humates Sani *et al*. (2022).

photosynthesis since decreased as the concentration of NaCl increased; this is because NaCl toxicity reduces the photosynthesis Irakoze *et al*. (2020).

5. Effect of treatment with humic and salt stress on chlorophyll content in leaves (µg/g):

The data in Figure (5) indicate that there are significant differences at the level ($P<0.01$) between the studied treatments in terms of the chlorophyll content in leaves of sweet basil plants.

Salt stress led to a decrease in chlorophyll content in leaves, and this decrease increased with increasing concentration of added salt.

While treatment with humic acid at two concentrations (1 and 3 g/l) increased chlorophyll content in leaves compared to the control

Treatment with humic acid at a concentration of 1 g/l and salinity of 6 dS/m also outperformed all other parameters and the control.

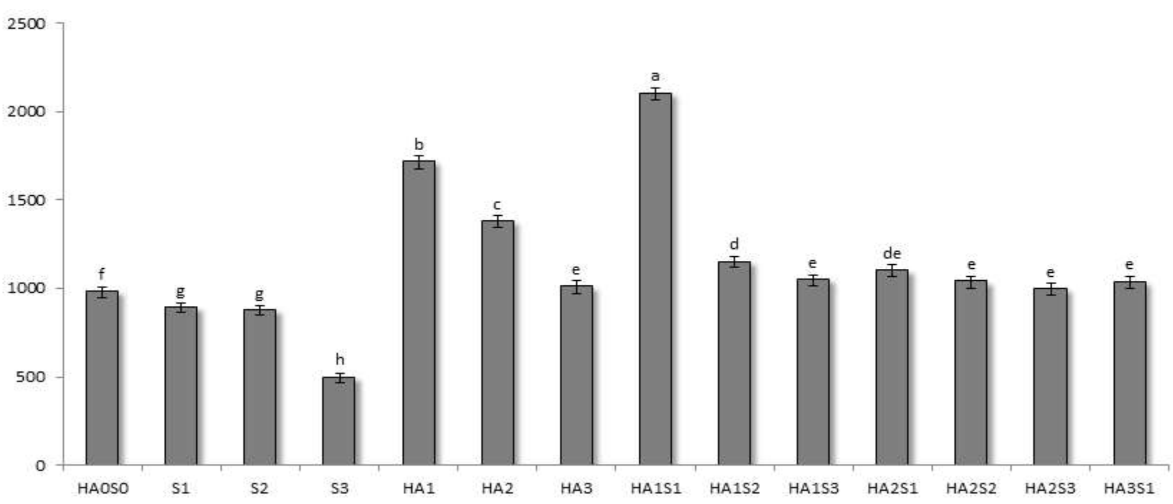

Figure (5): Effect of humic on chlorophyll content in leaves under salt stress.

This noticeable decrease in the content of photosynthetic pigments (chlorophyll and carotenoids) in basil leaves is explained by the fact that high concentrations of sodium chlorine salts lead to increased decomposition of chlorophyll molecules, the destruction of chloroplasts, and a decrease in their physiological activity in the plant Taleisnik-Gertel *et al.* (1983).

Concerning the effect of HA on photosynthetic pigment concentration, the results were similar to that obtained by Aydin *et al.* (2012) on bean (*Phaseolus vulgaris* L.) plant grown under salt stress. In this respect, The results obtained were in agreement with those reported by Cavalcante *et al.* (2011). Karakurt *et al.* (2009) reported that the foliar sprays of these substances (HAS) also promote growth, and increases yield and quality in a number of plant species.

6. Effect of treatment with humic and salt stress on proline content in leaves (µmol/g):

The data in Figure (6) indicate that there are significant differences at the level (P<0.01) between the studied treatments in terms of proline content in the leaves of sweet basil plants.

Salt stress increased the proline content, and the proline content increased with increasing concentration of added salt.

While treatment with humic acid at two concentrations (1 and 3 g/L) led to a decrease in the proline content in plants compared to the high concentration and the control.

Treatment with humic acid at a concentration of 1 g/L and a salinity of 6 dS/m also gave the lowest proline content compared to the rest of the treatments.

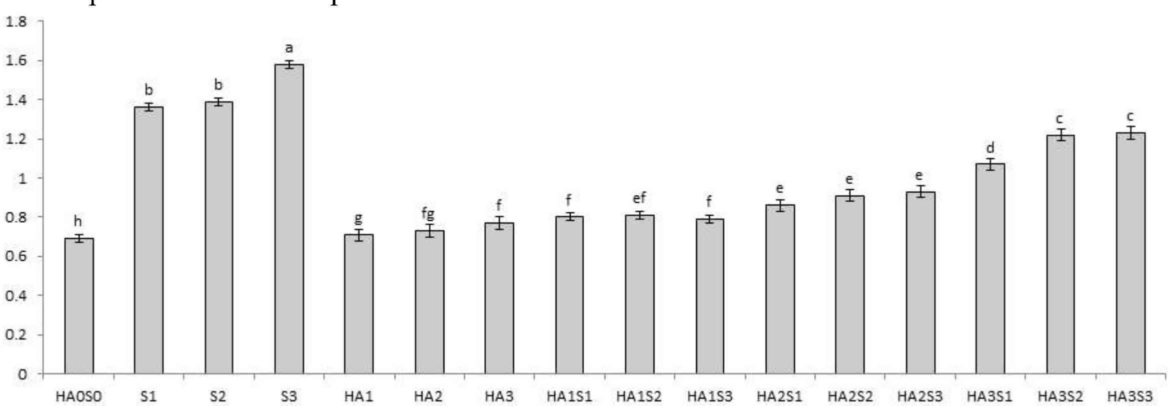

Figure (6): Effect of humic on proline content under salt stress.

Said-Al Ahl and Hussein (2010) reported that on oregano (Origanum vulgare L.) plants were irrigated with tap water (0.40 dsm1), and with NaCl solution (4 dsm-1) with two levels of potassium humate (0.0 and 1.5 g pot-1) was applied to the soil with water irrigation application at three equal portions before each cut. That the foliar application of HA caused a significantly positive trend in increasing herb fresh yield (g.plant-1) in the all cuts.

The increase in proline content as NaCl concentrations increased is a typical response of this species under NaClstress conditions, coinciding with the results reported by Jadczak *et al.* (2022) and Safwat *et al.* (2022).

Conclusions:

Increasing NaCl concentrations negatively affected the basil traits *viz*. Plant Height, Number of branches, Plant leaf area, chlorophyll content in leaves, and Net Photosynthesis Rate, however humic acid reduced the negative effects of NaCl. 1 g/L concetration of humic acid usually select to minimize the negative effect of NaCl. According to the study it is necessary to conduct more research on developing salt-tolerant plants by utilizing humic acid in laboratory and field conditions.

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