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Research Article

Effect of Fulvic Acid in Increasing the Resistance of Wheat Plants *Triticum Aestivum* L. To Saline Stress

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Abstract | This study was conducted in the agricultural season of the year 2021-2022 in order to assess the effect of fulvic acid in increasing the ability of the wheat plants to withstand salt stress. The fulvic acid factor included the use of six concentrations (0, 2, 4, 6, 8 and 10) g. L^{-1}, while the salts naturally present in the soil and irrigation water were treated with the equivalent of (50-100) mmol. A number of growth characteristics of the crop suh as (relative water content of leaves (RWC), biomass. $plant^{-1}$ and the rate of total chlorophyll and carotene in the leaves) in addition of K:Na ratio and antioxidants (proline (Pro), ascorbate peroxidase (APX), peroxidase (POX), guaiacol peroxidase (GPX), glutathione reductase (GR). The results showed that sodium chloride led to a decrease in the rates of the growth characteristics, as well as an increase in root: shoot ratio, proline, APX, and GR, while by increasing the concentration of fulvic acid, especially at the level (8 g. L^{-1}), an improvement in the rate of the growth characteristics and a decrease in the rate of antioxidant activity, indicating that the fulvic acid might be enhanced the growth of the wheat plant and protect plant cells from the effects of sodium chloride effect.

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Introduction

The wheat plant, *Triticum aestivum* belongs to the grassy family, and it comes first for cereal crops. Wheat contains small percentages of calcium in addition to being a source of vitamins B6, B1 and vitamin E, and an excellent source of carbohydrates and dietary fibers that are easily digestible. Wheat mainly consists of carbohydrates, most of which are starch or long chains of glucose. Wheat grains also consist of a large amount of fiber, which in turn helps slow digestion and absorption, thus reducing the rise in blood sugar to some extent, there is evidence of the effects of salt in delaying seed germination, seedling growth in general, vegetative growth of the whole plant, leaf swelling pressure, flowering time and number of flowers directly or indirectly inhibiting the process of cell division, reducing the growth of the vegetative system, reducing the size of leaves, increasing the root ratio: shoot, reducing the total dry mass rate, and consequently the decrease in grain production (Moshawih *et al*., 2022). In addition, the salts affect the photosynthetic enzymes, chlorophyll and carotenoids, the ability to photosynthesis, changes in the water potential, where cumulative effects attributed to salt stress were recorded, thus reducing the economic yield and poor quality of grain due to the toxic effect of sodium ions and the occurrence of water stress as a result of osmotic stress And the removal of water from the cytoplasm, as well as the interaction between salt and nutrients, and thus a nutritional imbalance occurs (Ghassemi *et al*., 1995). Fulvic acid is one of the natural organic polymers, and it is extracted from humus found in soil or some marine sediments and aquatic environments, and its composition is C135H182O95N5S2. Fulvic acid is one of the most important organic acids in the world of agriculture. It was found that fulvic acid encourages the germination process by treating the seeds with this organic acid and strengthens the resulting seedlings (Calvo *et al*., 2014). Fulvic corrosive could be a characteristic chelating substance that helps to chelate and encourage the supplements within the soil such as press, zinc, manganese and copper as well as calcium, phosphorous and magnesium. Fulvic acid works to treat soil salinity, as it chelates the calcium element in the soil, then calcium becomes free, active and soft in the soil. With puncture water, which reduces its harmful effect on plants (Kumar, 2019). Fulvic acid improves the physical properties of the soil. The use of fulvic acid makes the soil dark in color, and this helps to absorb sunlight significantly, which leads to raising the temperature of the soil, and this helps to warm the roots on the one hand, as well as stimulate the growth rate in them and increase their branches on the one hand. else. Fulvic acid activates enzymes and energy compounds within plants, which causes activation of endogenous cytokinin and increases the rate of plant cell division, thus increasing the rate of growth and development in plants (Pettit, 2004). Fulvic acid activates and strengthens the roots, as well as increasing the plant's performance efficiency by releasing carbon dioxide gas from the soil. Fulvic acid is a clean and environmentally safe compound that is odorless and non-toxic as it reduces pollution Groundwater and reduces the spread of fungal and bacterial diseases due to the absence of heavy elements (Staggs, 2021). It is based on a lot of research recommended the use of fulvic acid as an alternative source in the case of hydroponic farming systems, that is the culture of nutrient solutions.

This study aims to identify the harmful effect of salts and the upper limit of the salt concentration that the wheat plants can tolerate and the role of fulvic acid in reducing the harmful effects of salts on wheat plants.

Material and Methods

Preparation of soil and the experimental factors

This study was conducted in the fields of the Faculty of Agriculture and Marshes for the year 2021-2022, using pots with a capacity of 2 kg containing 2 peat moss: 1 sand, the study included two factors, the first one is the use of fulvic acid in six different levels (0, 2, 4, 6, 8 and 10) g. L^{-1}, while the salts naturally present in the soil and irrigation water were treated with the equivalent of (50-100) mmol, with three replicates, after soaking them in normal water for 6 hours and 10 the seeds were cultivated, and watered with the nutrient solution only until the germination completed, and when the seedlings reach the age of two weeks, the plants irrigated with the nutrient solution that contains a fulvic acid solution (according to the plants' need). After 8 weeks from the date of germination, the plants were carefully removed and the different growth characteristics and antioxidant activity were calculated as follows:

Relative water content of leaves (RWC)

The relative water content was based on the procedure described by (Shivakrishna *et al*., 2018).

Total chlorophyll

The total chlorophyll was based on the procedure described by (CI and Indira, 2016).

Carotene

The total carotene in the leaves of the wheat plant was based on the procedure described by (Chanda *et al*., 2018).

K^+ and Na^+ estimation

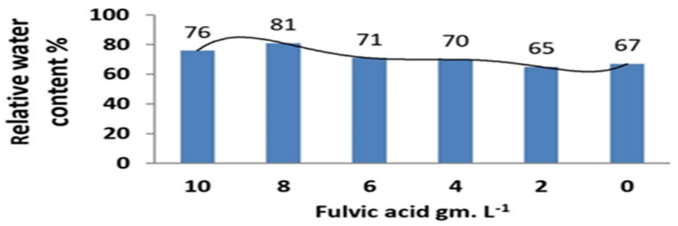
The concentration of K^+ and Na^+ was measured according to the method of (20).

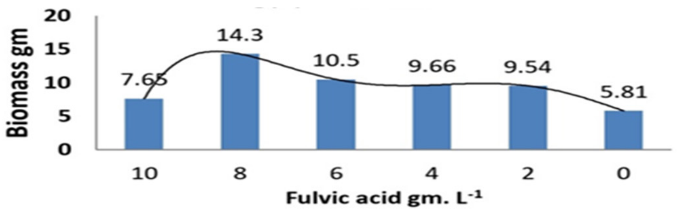
Proline

The total proline in the leaves of the wheat plant was determined according to the method of (Ábrahám *et al*., 2010).

Enzyme extraction and assay
Approximately (0.5g) of the wheat leaf sample was homogenized in a cold 0.1M phosphate buffer (pH = 7.5).the protein content of the samples was determined using a bovine albumin method (Bradford, 1976).

Ascorbate peroxidase (APX)
Ascorbate peroxidase was assayed spectrophotometer at 290 nm in an UV (model M 36, Beckman,CA, USA) equipment.





Peroxidase (POX)
Peroxidase activity was assayed as an increase in optical density due to the formation of tetraguaiacol (Castillo and Greppin 1984).

Guaiacol peroxidase (GPX)
GPX activity was determined according to (Upadhyaya *et al*., 1985).

Glutathione reductase (GR)
Glutathione activity was assayed according to (Sairam, and Srivastava, 2002).

Statistical analysis
The statistical analysis of this experiment was completed using SPSS software version 23, using A one-way ANOVA and significance was identified at $P \leq 0.05$.

Results and Discussion

The results of this study showed that sodium chloride salinity in the soil and irrigation water caused significantly ($p<0.05$) decreased ranges in the relative water content (Fig. 1), plant biomass (Fig. 2), total chlorophyll (Fig. 3) , carotene (Fig.4) and K: Na ratio (Fig. 5).

By using the increase in the concentration of fulvic acid, a significant and gradual increase was observed in most of the growth characteristics studied, where the concentration 8 gm. L^{-1} recorded the highest rates in relative water cycle RWC, Biomass, Total chlorophyll, Carotene and K: Na ratio (81%, 14.3 gm, 0.46 mg. L^{-1} FW, 18.6 µg. gm^{-1} FW and 1.62%) respectively.

The decrease in growth rates may be due to, since the salts have caused ionic stress and thus an increase in the rate of free radicals as a result of the increase in the accumulation of sodium and chlorine ions, followed by changes in the water relations of the plant, which It results in closing of the stomata and thus a decrease in the rate of the plant's enzymatic activity and a weakness in the formation of the total rate of chlorophyll (Zheng *et al*., 2009), and thus there was a decrease in the rate of cell elongation and a reduction in the rate of plant growth completely, which affected the decrease in the rate of plant height, a decrease in the rate of leaf area and a decrease in the rate of relative water content, those harmful ions (sodium and chlorine) worked to disrupt the balance of hormones and vital activities, the most important of which are the processes of photosynthesis and respiration, in addition to consuming the largest part of energy for osmotic adjustment instead of using it in the processes of growth and development, our results and this is consistent with what was mentioned (Francois *et al*., 1986).

Kumar and Chen, 2008 has demonstrated that the enmity between Potassium and sodium assimilation happens on the floor of the root underneath saltiness push. A moo proportion of K:Na underneath the intemperate concentration of NaCl conceivably demonstrated that Ca, K , and Mg transportes were impeded through Na particle underneath stature mindfulness of NaCl, this might too mediate with plant digestion system and decrease plant development characteristics. In expansion the rebate in standard gas alter and closure of leaf stomata may well be due to noxious Na and Cl particles which minimize the transport of photosynthetic electrons with decreased carbon assimilation and digestion system and oxidative hurt to PSII beneath NaCl push, this might lead to supplement awkwardness, our comes about are steady with (Ashraf, *et al*., 2017; Azizpour, *et al*., 2010).

The low rate of growth characteristics in general and the low rate of chlorophyll formation in particular may be due to the fact that wheat plants are sensitive to salinity and this agrees with what was found by (Ahmad *et al*., 2007; Ashraf and Harris, 2004). The reason for the significant increase in the rates of the studied growth characteristics as a result of the increase in the concentration of fulvic acid may be due to the fact that fulvic acid is considered a regulating medium for plant growth, a significant increase in all the studied growth characteristics as a result of the increase in the concentration of the added fulvic acid, which means an increase in the rate of the photosynthesis process, as fulvic acid contributed to an increase in the rate of water absorption as a source of hydrogen necessary for the carbon dioxide reduction process in the photosynthesis process, our results agree with (Dai *et al*., 2004).

The results of this study showed that the increasing in the concentration of fulvic acid caused decreased gradualy in the activity of (proline (**Fig. 6**) where it reached 3.09 μg. gm^{-1} FW when using 6 gm. L^{-1} fulvic acid, ascorbate peroxidase APX (**Fig. 7**) 7.11 mmol. $min^{-1}.mg^{-1}$ FW when 10 gm. L^{-1} Fulvic acid, guaiacol peroxidase GPX (**Fig. 9**) 9.13 U.mg-1 protein when 6 gm. L^{-1} Fulvic acid and glutathione reductase GR (**Fig. 10**) 8.33 $U.mg^{-1}$ protein when using 8 gm. L^{-1} Fulvic acid.

The comes about of this consider demonstrated a critical increment within the levels of proline and antioxidant chemicals APX, GPX and GR as a result of the increment within the concentration of sodium chloride salt, this may be due to the truth that salt push can actuate a state of oxidative push that enacts the plants defense frameworks to diminish its hurtful impacts because it produces Responsive Oxygen Species (ROS) such as super oxide (O2-), Gracious- and H2O2 (Bakht, *et al*., 2012).

The correlation analysis between Fulvic acid concentrations and growth traits (relative water cycle, biomass, total chlorophyll, carotene and K: Na ratio showed that significant positive correlation ($p<0.05$) (r= 0.711*, r= 0.876*, r= 0.865*, r= 0.998*, r= 0.998, n=15), respectively, while significant negative with the antioxidant traits (Pro, APX, GPX and GR) (r=-0.882*, n=3) r=-0.815*, r= -0.877* and r= -0.922*) respectively.

It was observed that with an increase in the concentration of fulvic acid, a decrease in the activity of proline, ascorbate peroxidase, quaiacol peroxidase and glutathione reductase that's to a certain extent near the concentration between 6-8 gm. L^{-1}, after that, a decrease in the effectiveness of these enzymes was observed, which means that the high concentration of fulvic acid contributed to breaking the bond linking sodium with chlorine, then eliminating sodium with puncture water, thus reducing its danger to the plant, while the peroxidase enzyme POX (**Fig. 8**) was not significantly affected by the Fulvic acid concentrations. A critical increment within the viability of the plant's defense system such as Proline, APX, GPX and GR was observed when the concentration of fulvic acid was increased to a certain extent, stimulate the protective effects of plants by expanding the rate of effectiveness of antioxidant enzymes such as APX, GPX and GR. In addition, fulvic acid works to chelate and facilitate the absorption of nutrients from the soil such as manganese, copper, iron and boron, and because these enzymes need these elements as they are included in their composition, so it is expected to increase their activity with the availability of fulvic acid, which provides greater protection for the plant from the harmful effects of sodium chloride salt (Al-Badri *et al*., 2021). In addition, a decrease in the rate of these enzymes was observed when the concentration of fulvic acid was raised more than 8 g.L^{-1}, It reduces the need for the high activity of antioxidant enzymes, and thus its rate decreases, as well as the rate of absorption of potassium increases, and thus increases the ratio of potassium: sodium, which is an important indicator for the plant of its tolerance to salt stress conditions (Janke, 2022).

Conclusions and Recommendations

The result of this study showed significant negative effects when cultivate the wheat in saline soil and using saline water for irrigation water, an increasing in sodium ion in plant cells caused reduction in growth traits of wheat plant as results of effected on the water relationships of plant tissues, in addition to increasing in the activation in antioxidant enzymes. Fulvic acid contributed significantly to enhancing the resistance of wheat plants to sodium chloride salt, as it improved the rates of the studied growth characteristics and at the same time reduced the rate of activity of antioxidant enzymes, and this is evident from the high potassium: sodium ratio, this percentage is an indication of the resistance of wheat plants.

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Novelty Statement

The novelty of this study is the detailed quantitative analysis of the mechanistic action of fulvic acid in mitigating saline stress in wheat.This research goes beyond general yield assessment by precisely quantifying the role of fulvic acid in modulating key antioxidant enzymes and osmotic adjusters under high salinity. Identifying the optimal fulvic acid dose that maximizes wheat resistance parameters. Developing a comprehensive model that links fulvic acid application to the maintenance of ionic homeostasis thus filling a critical gap regarding the biochemical pathway offulvic acid mediated stress tolerance.

Author's Contribution

Dunya Sabeeh Mohamed: Conceptualisation, Methodology, Investigation and Validation.
Waqeed Mahdi Hadif: Article preparation, Review and editing.
Izyanti Ibrahim: Supervision

Generative AI and AI-assisted technology statement
The artificial intelligence analysis of all sections of this manuscript showed that none of the text plagiarized and was with a score of zero.

Conflict of interest
The authors declare no conflict of interest.

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