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RESEARCH PAPER

Impact of biostimulants to improve the growth of local fennel (*Foeniculum vulgare)* under salinity stress

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Key Message: This study reveals that the foliar spray of three biostimulants namely seaweed extract, humic acid and vegetable extract significantly alleviated the negative effects of salinity, and hence improved the growth of local fennel (*Foeniculum vulgare*).

ABSTRACT: Three biostimulants (seaweed extract, humic acid and vegetable extract) with varying concentrations were examined to evaluate their effect on the growth of local fennel. Seaweed extract with 1.5 g, 3.0 g and 4.5 g L^{-1} concentrations and humic acid with 1 g, 2 g and 3 g concentrations were applied as foliar spray. While vegetable extract was applied as foliar spray with 0.3%, 0.5% and 0.7% concentrations. Irrigation of saline water having EC = 5.0 dS m^{-1} was done thrice a week. The experiment was laid out using CRD. The main findings show that humic acid at 3 g L^{-1} significantly alleviated the negative effects of salinity and stimulated fennel growth. The maximum number of branches (7.667 branches per plant) were recorded in plants treated with 0.7% vegetable extract. Plants treated with 3 g humic acid were peaked (4.9667 g) in dry weight followed by 0.7% vegetable extract (4.7667 g dry weight). Early flowering was observed in plants treated with 4.5 g seaweed extract (109 days) followed by 3.0 g humic acid (113 days), while it was delayed in control (123 days). Maximum fresh weight was gained in plants treated with 3.0 g humic acid (33.83 g) followed by 0.7% vegetable extract (29.467 g fresh weight). Fruit/seed weight was higher in plants treated with 3.0 g humic acid followed by 0.7% vegetable extract. This study signifies that the mode of action of these stimulants should be understood, and their responses may be examined at physiological, biochemical, and molecular levels.

Keywords: Brackish and canal water, Leaching fraction, Sorghum, Electrical conductivity

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INTRODUCTION

Fennel (*Foeniculum vulgare*) is a local medicinal perennial of Mediterranean region. It is cultivated for its monetary, aromatic and medicinal value (Mona et al., 2008). It belongs to family Apiaceae (Umbelliferae). It is local to Southern European countries and developed widely all over Middle - East, China, India, and Turkey. There is a wide range of wild and adopted fennel varieties varying in seed size, aroma and taste. It is one of the essential garden herbs of Indo-Pak. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as a culinary spice (Shahmokhtar & Armand, 2017). It has antifungal and antiseptic activities against many pathogenic organisms (Kazemi et al., 2012). Fennel seeds comprise of vital oil. Its leaves have diuretic properties. It is also used to cure asthma and kidney problems. Its seeds have anti-spasmodic, antihirsutism and anti-inflammatory activity (Rahimi, & Ardekani, 2013; Choi & Hwang, 2014). It is also reported for its antidemtia, anti-platelet, antithrombatic functions and rich antioxidant properties (Tognolini et al., 2007). Its products have pleasant taste and zesty aroma that might be utilized as a part of soups, sauces, pickles, perfumery, beauty care products, scenting cleansers, pharmaceutical and phytotherapy businesses (Barros et al., 2010).

Soil salinity affects horticultural efficiency globally (Zorb et al., 2004). Salinity affects seed imbibition, germination and root development (Khan & Gulzar, 2003). Plant development and annual production are diminished in salt-affected soil due to the abundance take-up of conceivably harmful ions (Yamaguchi & Blumwald, 2005). Currently, more than one third of the world's arable land is subjected to soil quality and land degradation due to salt stress. Salinity affects the agricultural output badly. It decreases stomatal conductance incredibly and therefore diminishes photosynthetic rate (Bethke & Drew, 1992). In any case, the restraint of photosynthetic rate forced by stomatal inference may advance clumsiness between photochemical movement at photosynthesis framework II (PSII) and electron prerequisite for photosynthesis, prompting abundance excitation and ensuing photosynthesis inhibitory harm of PSII response. The impact of salt stress has been concluded on numerous horticultural products (Maas & Hoffman, 1977) but there are very few data available on the effect of salt stress on fennel growth. Salinity can be mitigated using different methods and substances. Biostimulants are emerging method to combat salinity by providing essential nutrients. Plant biostimulants are different substances and microorganisms used for upgrade plant development. It can enhance growth capability, mitigate abiotic stress or potentially improve quality attributes and enhance nutrition (Du Jardin, 2015).

Various seaweed extracts are accounted for to have plant-development impact and revealed one of their more comprehensive roles in agribusiness and agriculture as natural excrements and manures (Craigie, 2011). Seaweed extracts have now increased significantly more extensive response as "plant biostimulants". Uses of various concentrations of these seaweed extracts have been accounted for to upgrade plants' resilience to an extensive variety of abiotic stresses i.e. saltiness, dry spell and temperature extremes (Bulgari et al., 2019). Seaweed extracts are additionally utilized as foliar sprays on vegetables, flowers and tree to enhance production and quality (Haider et al., 2012). Humic acid (HA) is dark colored organic substance and a natural resource used as an alternative for fertilizer to increase crop production. Humic substance has been accounted to improve nutrient absorption, plant growth, physiology and metabolism (Ahmad et al., 2016). Humic substances have fundamental composition in organic matter due to plant rot, microbial residue, yet furthermore from the metabolic activity by soil organism using humic acid. It is used for enhancing plant development and harvest yield (Eyheraguibel et al., 2008). Its application increases nutrient take-up (Linehan, 1978), cell permeability and improves processes associated with the plant development (Lee & Bartlett, 1976). Vegetable extract is organic extract of different vegetables prepared locally by the help of sugar. It gave plant essential nutrients, hormones and sugars as well. It helps the plants to improve quality and productivity (Abbasi et al., 2019). This study was aimed to search the efficiency of various available commercial biostimulants products on the development of fennel under saline conditions and to sort out the best biostimulant product and its concentration to enhance fennel development under saline condition.

MATERIALS AND METHODS

The present study was conducted to evaluate growth of fennel by the application of biostimulants under salinity stress soil environment. The experiment was conducted at Horticulture Research Area, PMAS, Arid Agriculture University, Rawalpindi, Pakistan. Seed of fennel were taken from the Gene Bank of PGRI-NARC Islamabad, Pakistan. Sowing was done directly in plastic pots during November. Three biostimulants i.e. seaweed extract (1.5, 3.0 and 4.5 gmL^{-1}), humic acid (1, 2 and 3 gmL^{-1}) and vegetable extract (0.3, 0.5 and 0.7%) as foliar application were applied. Treatments were applied four times after 15 days interval except the control one. These treatments were started after forty-five days of sowing. Saline water of 5 $dS\ m^{-1}$ EC was used for irrigation purpose thrice a week. The experiment was laid out in CRD with three replications and data analysis was done by two factor factorial method in all experiments through Fisher's examination of variance method using Duncan's Multiple Range Test (DMRT) at 5% probability level for mean comparison.

Morphological parameters

Plant height (cm) of randomly selected fennel plants was recorded with measuring tape from base to tip of plant at maturity stage. Number of branches of fennel plants in all treatments was counted at maturity. Fresh weight (g) of vegetative growth i.e. stems and leaves of fennel plant were taken individually with weighing machine in grams. After getting fresh weight plants were air dried, and then dried in oven at 80°C till constant weight. Oven dry weight (g) was measured on digital balance. Fresh weight (g) of root was also calculated in grams with weighing machine.

Reproductive parameters

The flowering date was calculated from sowing date to first flower set in pot. No. of umbels per plant of randomly selected plants from each treatment was noted at maturity and then total number of umbels per plant were calculated. Fruit/seed weight (g) of fennel plant was taken individually with weighing machine in grams.

Biochemical parameters

Proline contents were assessed with the well-developed protocol (Bates et al., 1973). The frozen sample was regimented in three percent sulphosalicylic acid (0.02g mL^{-1}). Residue from this mixture was eliminated after 10 minutes centrifugation process at 3,000 rpm. One mL of each homogenized tissue reacts, acid-ninhdrin and glacial acetic acid were taken in test tube and subjected to 100°C for an hour ending by placing it in water bath. Toluene (2 mL) mixed in this mixture and let this for half an hour at room temperature. Two separate segments were achieved. The top segment having chromophore and toluene (1 mL) was subjected to measure optical density at 520 nm using toluene as standard. The proline concentration was determined from a standard curve using D-Proline. Sodium and potassium were determined using flame photometry method (Humphries, 1956). A photoelectric flame photometer was used for this chemical analysis. Plant samples were subjected to the flame. Filters selected the colours which are detected by photometer and exclude the influence of other ions. The device was calibrated with a series of standard solutions of the ion to be tested.

Soil analysis

The soil of experimental field was sandy clay loam and soil characteristics after analysis are given in Table 1.

Statistical analysis

The data was analyzed by using CRD two factor factorial methods in all experiments through Fisher's analysis of variance technique using Duncan's Multiple Range Test at 0.05 significance level for comparison of means.

RESULTS

In present study, fennel was grown in pots and salinity stress was given by applying saline water having EC 5 dSm^{-1} thrice a week. Data regarding vegetative, reproductive and biochemical parameters of fennel (*Foeniculum vulgare*) as affected by different biostimulants were statistically analyzed and discussed in the following headings.

Vegetative parameters

Plant height

Plant height is important parameter of any crop. Data regarding plant height is given in Fig. 1. Biostimulants significantly increased the plant height in fennel plant under salinity stress conditions. Maximum plant height in plants treated by 3g humic acid was observed (91.767 cm) followed by 2g humic acid (88.967 cm), while minimum plant height in control was recorded (74.767 cm).

Number of branches per plant

Data regarding number of branches is shown in Fig. 2. Biostimulants significantly increased the number of branches in fennel plant under salinity stress conditions. Maximum number of branches was observed in plants treated with 0.7 % vegetable extract followed by 3 g seaweed having no. of branches 7.667 and 7.333 respectively while minimum in control with 5.333 numbers.

Fresh weight of vegetative growth

Data regarding fresh weight is evident from Fig. 3. Biostimulants significantly increased the fresh weight in fennel plant under salinity stress conditions as compared to control. Maximum fresh weight was observed in plants treated with 3 g humic acid followed by 0.7% vegetable extract having fresh weight 33.833 g and 29.467 g respectively while minimum in control was represented as 18.300 g.

Dry weight of vegetative growth

Data regarding dry weight of vegetative growth is evident from Fig. 4. Biostimulants significantly increased the dry weight in fennel plant. Maximum dry weight was observed in plants treated with 3g Humic acid followed by 0.7%

vegetable extract having dry weight 4.9667 g and 4.7667 g respectively while minimum dry weight in 1 g seaweed was recorded as 2.6333 g.

Reproductive parameters

Root weight

Data regarding root weight is apparent from Fig. 5. Biostimulants significantly increased the root weight in fennel plant under salinity stress conditions. Maximum root weight was observed in plants treated with 4.5 g seaweed followed by 0.3% vegetable extract having root weight 8.80g and 8.43g respectively while minimum root weight was recorded in 1g seaweed.

Flowering date

Flowering date is another important trait. Data regarding flowering date is apparent from Fig. 6. Biostimulants significantly increased the flowering date in fennel plant under salinity stress conditions. Minimum flowering date were observed in plants treated with 4.5 g seaweed extract followed by 3 g humic acid having flowering date 109 and 113 respectively while maximum flowering date was recorded in control having flowering date 123.

Number of umbels per plant

Data regarding no. of umbels as shown in Fig. 7. Biostimulants significantly increased the no. of umbels in fennel plant under salinity stress conditions. Maximum number of umbels was observed in plants treated with 3g Humic acid followed by 2 g Humic acid seaweed having no. of umbels 11.6767 and 11.333 respectively while minimum number of umbels was recorded in 1 g seaweed having 6.333 umbels.

Fruit/seed weight per plant

Seed weight is an important trait as more seed result in more yields. Data regarding fruit/seed weight is evident from Fig. 8. Biostimulants significantly increased the fruit/seed weight in fennel plant under salinity stress conditions. Maximum seed weight was observed in plants treated with 3 g humic acid followed by 0.7% vegetable extract having seed weight 8.9333 g and 8.4000 g respectively while minimum seed weight was recorded 1g seaweed having seed weight 3.5333.

Biochemical parameters

Proline contents

Data regarding proline content is evident from Fig. 9. Biostimulants significantly decreased the fruit weight in fennel plant under salinity stress conditions. Maximum proline was observed in control plants followed by 1.5 g seaweed having proline 3.8 and 3.7333 μmol g^{-1} respectively while minimum proline was recorded 4 g seaweed having proline 1.2666 μmol g^{-1}.

Sodium and potassium concentration

Data regarding sodium concentration is evident from Fig. 10. Maximum Na^+ was observed in control plants followed by 1.5 g seaweed having sodium 1.8666 and 1.7 mg g^{-1} respectively while minimum sodium was recorded in 0.5% vegetable extract having sodium 1.3666 mg g^{-1}. Data regarding potassium concentration is evident from Fig.11. Maximum K^+ was observed in 0.7% vegetable extract followed by 0.5% vegetable extract having K^+ 20 and 19.666 mg g^{-1} respectively while minimum K^+ was recorded in 1.5 g seaweed extract having K^+ 11.666 mg g^{-1}.

Table 1 Some physical and chemical characteristics of the used soil

Components	Values
Sand	56%
Silt	22.8%
Clay	21.2%
Na^{+}	89.7 meq L-1
K^{+}	140 meq L-1
Mg^{++}	35.3 meq L-1
Ca^{++}	50.5 meq L-1
pH	7.87
Electrical conductivity	0.53

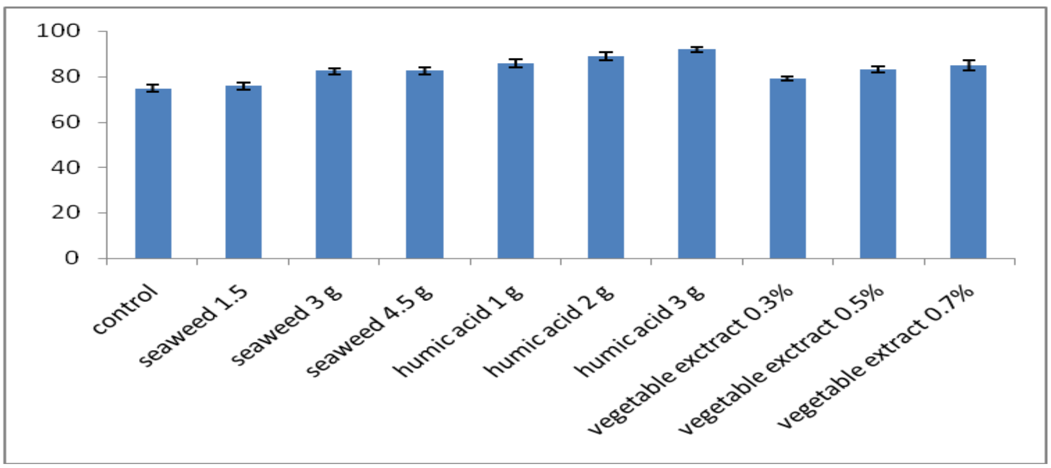

Fig. 1 Effect of different biostimulants on plant height of fennel under salinity stress

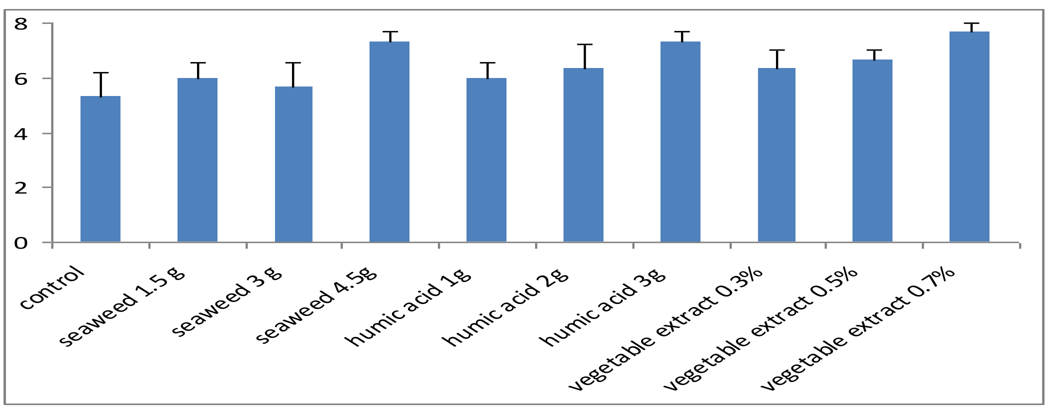

Fig. 2 Effect of different biostimulants on number of branches/plant of fennel under salinity stress

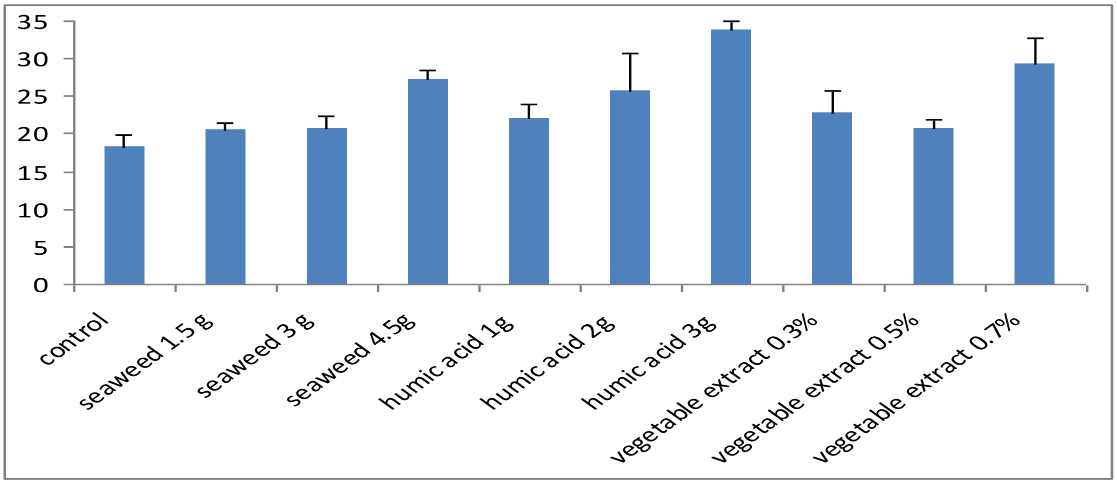

Fig. 3 Effect of different biostimulants on fresh weight of vegetative growth of fennel under salinity stress

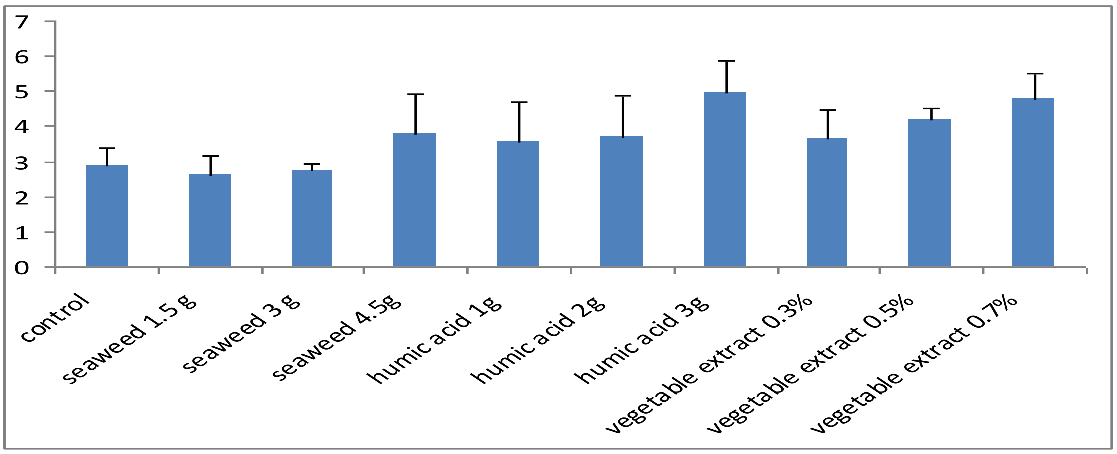

Fig. 4 Effect of different biostimulants on dry weight of vegetative growth of fennel under salinity stress

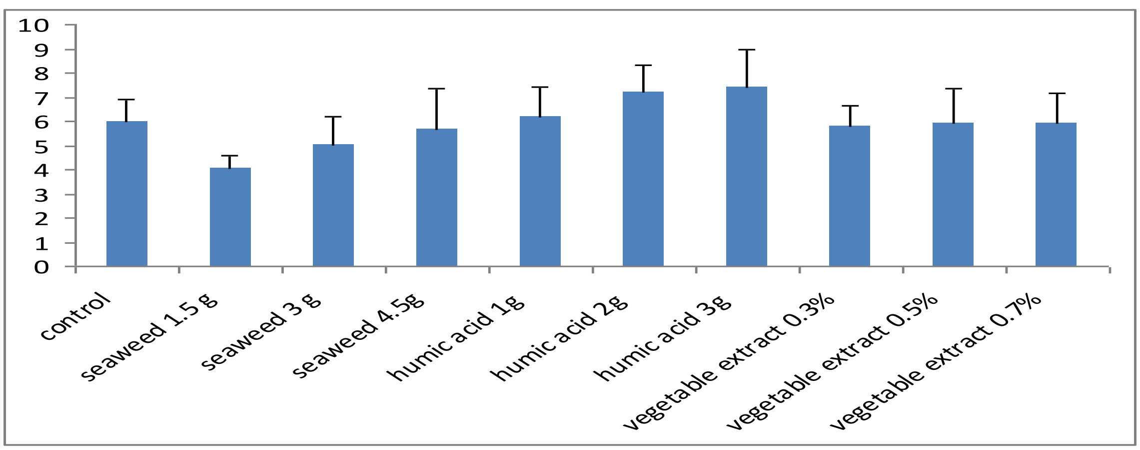

Fig. 5 Effect of different biostimulants on root weight of fennel under salinity stress

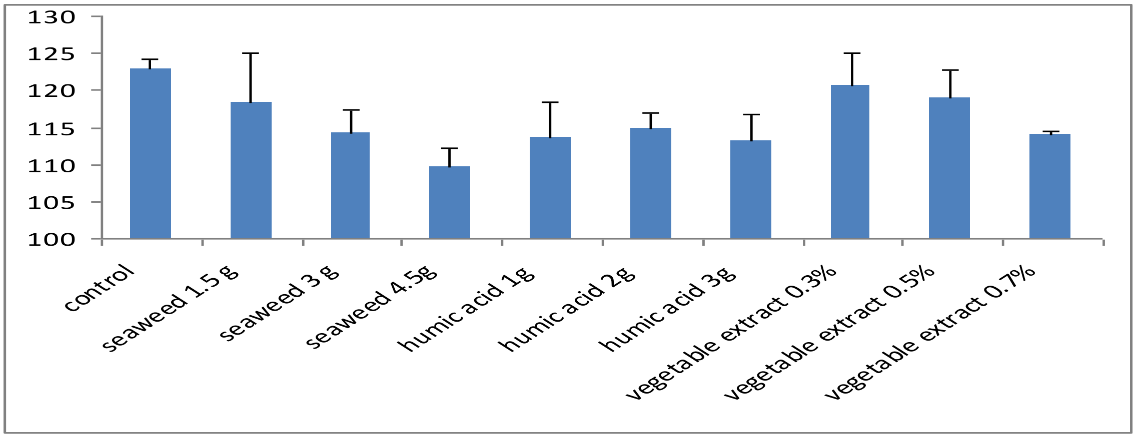

Fig. 6 Effect of different biostimulants on flowering days of fennel under salinity stress

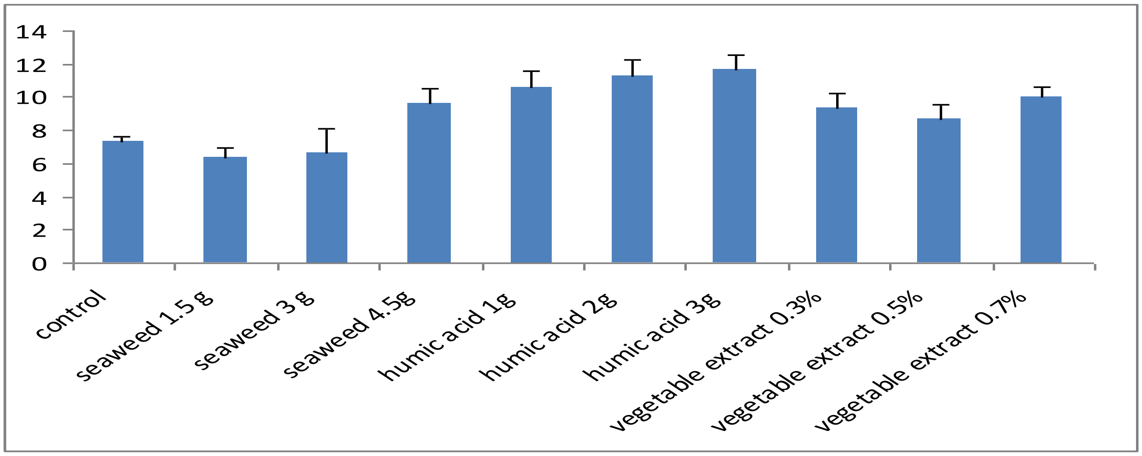

Fig. 7 Effect of different biostimulants on number of umbels/plant of fennel under salinity stress

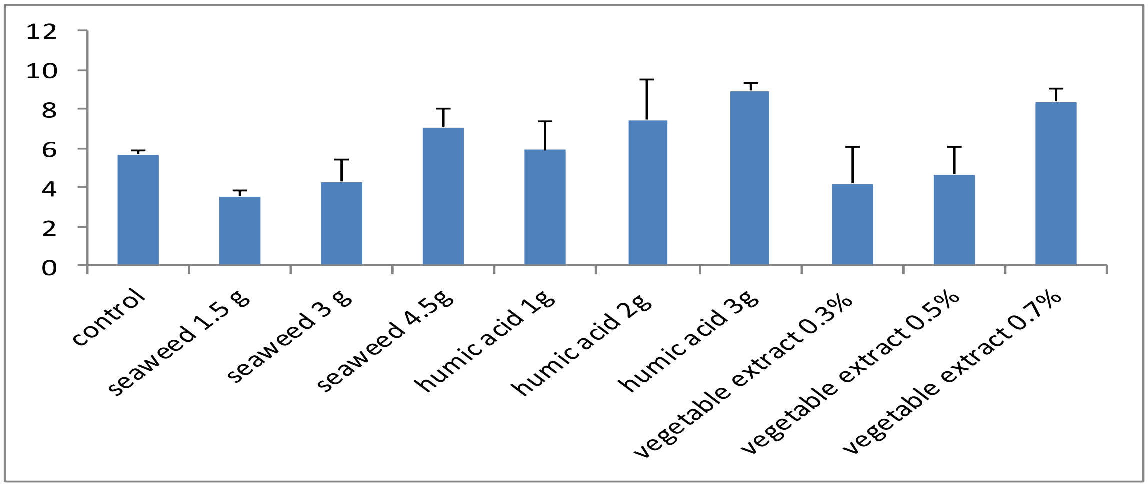

Fig. 8 Effect of different biostimulants on seed weight of fennel under salinity stress

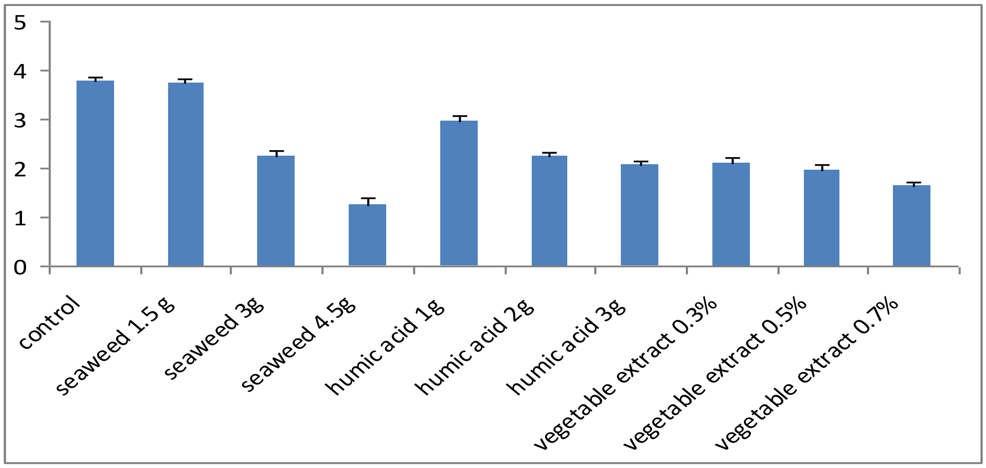

Fig. 9 Effect of different biostimulants on proline contents under salinity stress

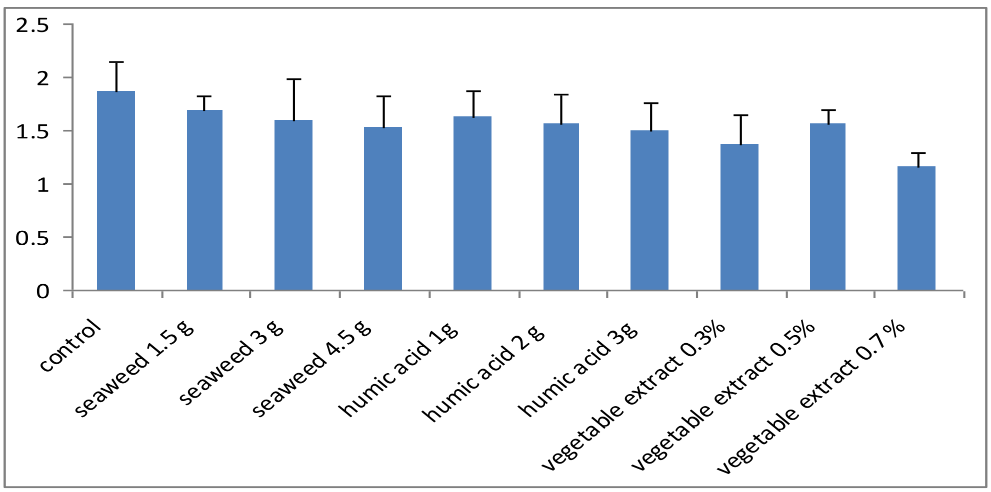

Fig. 10 Effect of different biostimulants on sodium concentration under salinity stress

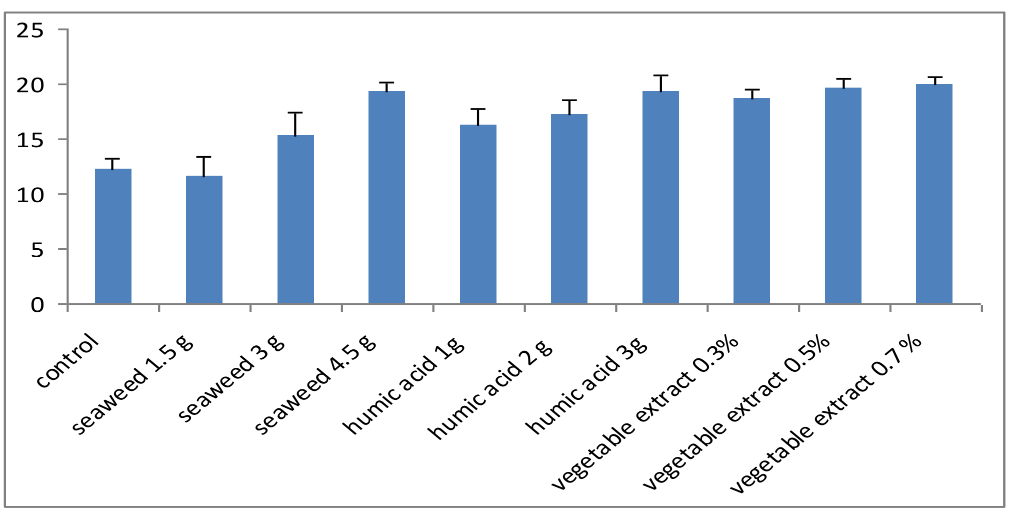

Fig. 11 Effect of different biostimulants on potassium concentration under salinity stress

DISCUSSION

Humic acid beneficially enhance plant height by improving nutrient uptake by plants and hormones effect. The present findings are also similar with the results of the earlier researchers as El-Nemr et al. (2012) reported that spray of 3g L^{-1} humic acid had positive effect on plant height in cucumber plants. Humic substances are essential soil component since these contain a steady portion of carbon and enhance water holding limit, pH buffering and thermal protection. Humic acid also increases cell permeability as a result its use is proposed to increase plant height. Also supported by Fagbenro and Agboola (1993) that plant height was improved by efficient uptake of N, P, K, Mg, Ca, Fe, and Zn. Present results also support the findings of Bohme & Lua (1997) that humic acid enhanced height in pepper as compared with control. Sani (2014) also observed that foliar spray of humic acid had positive effects on all morphological parameters including height, leave numbers, fresh weight and yield in canola plants. Hagag et al., (2011) studied humic acid application results in increment of plant height in olives. Seaweed extract plants observed maximum height at 4.5 g concentration with value of 82.36 cm. Seaweed shows increase in development and the utilization of seaweed as biostimulants in crop yield is improved. Jannin et al. (2013) observed that application of *Ascophyllum nodosum* (seaweed extract) significantly increased brassica plant height and improve plant characteristics. Crouch & Van Staden (1991) observed that seaweed incorporate microelement supplements, amino acids, vitamins, cytokinins, auxins & abscisic acid that affect cell metabolism in treated plants, prompting improved development and product yield. Blunden et al. (1996), found that seaweed extracts contain different betaines, good solute i.e. eases osmotic pressure caused due to saline soil.

Plants treated with vegetable extracts showed maximum height at 0.7 g concentration with the values of 84.86 cm. In vegetable extract calcium is present which gave rise in the height (Bukhari et al., 2014; Sajid et al., 2014). Extracts can also control the phytopathogens and can also be used as an alternative control for plant diseases (Stangarlin et al., 2008). Plants treated with humic acid indicated maximum number of branches at 3 g concentration with the values of 7.33. Brownell et al. (1987) also examined that humic acid application has advantageous impact on nutrient absorption leads to increase plant vegetative and reproductive growth of tomato, cotton and grapes. Present results are similar with the work of these scientists as Stirk et al., (2014) found that seaweed contain auxins, cytokinins and gibberellins, amino acids and mineral supplements, that emphatically affect plant development and division. Battacharyya et al. (2015), studied that seaweed change physical and biological properties of soil and may likewise influence the structure of plant roots subsequently helps in productive take-up of supplements. Nabati et al. (1994) found that seaweed enhanced growth of Kentucky Bluegrass (*Poa pratensis* L. cv. Rich) in saline stress i.e. upgraded both above and below development of the grass at 0.15 S m^{-1} salinity. Vegetable extract contain macronutrients i.e. nitrogen and phosphorus. Nitrogen increase number of branches per plant in rapeseed, while the least number of branches are observed in control (Uddin et al., 1992).

Bakry et al. (2014) detailed that humic acid at 50 kg raised fresh weight of flax plant. Fresh weight expanded by 66.6% than control. El-Nemr et al. (2012) studied that cucumber plants were applied three times after every 15 days with different measure of humic acid and bio-stimulators three weeks after planting. Recorded data showed that each and all morphological characters including number of leaves and stems per plant, new weights of leaves per plant and yield and its parts of cucumber plants demonstrated positive and basic responses with the high 3 g L^{-1} humic acid. Plants treated with seaweed extract observed maximum fresh weight at 4.5 g concentration with the values of 7.33. Seaweed extract was used as the calcium in seaweed extricates helps in protein activation, cell lengthening, and cell stability. This calcium is required by N-fixing bacteria, which fix the nitrogen and made it available to plants.

Nabati et al. (1994) reported that seaweed extract enhanced both below an above the ground growth of grass at soil salinity. Hayat et al. (2018) watched that concentrate of garlic significantly affected morphological and physiological parameters in tomato plant as contrasted to control treatment. It enhanced plant height, leaf area, stem diameter, plant fresh/dry weight. Crouch and Van Staden (1991) observed that seaweed application significantly increases the root in tomatoes as compared to control. Our results are also in line with findings of Thompson (2004) who observed that seaweed had positive effects on all morphological parameters including root weight in tomato plants by increasing total volume of root system. Nelson and van Staden (1994) also observed that seaweed components had significant effect on root development in wheat. Nabati et al. (2008), found noteworthy reduction of sodium particles accumulation in the plant after utilizing seaweed. Grass treated with seaweed had less sodium in the tissue, when appeared differently in relation to grass that did not get seaweed extract treatment. Yazdani et al., (2014) observed that humic acid at 50 mg L^{-1} improved quality and amount of gerbera through enhancing root design prompting upgraded supplement take-up and influence hormone-like. Maibodi et al. (2015) also found that humic acid at 100 mg L^{-1} improved root length, root surface and root fresh weight of ryegrass. These results suggest humic acid foliar application enhance root development and nutrient uptake.

Mean comparison of this study also depicted the superiority of seaweed treatment at 4.5 g. Crouch and Van Staden (1991) also observed that tomatoes treated with seaweed components had more and early flower set than control plants. Similar results are observed by Arthur et al. (2003) that seaweed extract application induces early flower and fruiting in plants. Ali et al. (2014) found that humic acid at 1.25 ml when applied to *Tulipa gesneriana* revealed that it was most effective on compared with control. All vegetative and reproductive attributes significantly influenced by addition of humic acid. This treatment gave inimitable outcomes concerning early flowering and fresh and dry flower biomass. Early flowering was recorded in plants treated with 1.25ml humic acid followed by other treatments. Same pattern was observed in this study in which least days were observed in plants treated with 3g humic acid and control treatment took more time in flowering.

Results support the findings of Abetz and Young (1983) who reported that seaweed application significantly increase the flowering per umbels in number of crop plants as compared to control. Results also lined with van Staden et al. (1994) as he treated marigold seedlings with seaweed that increased no. of flowers per umbels. Similar results were also found by Nofal et al. (2015) that number of umbels per plant was significantly enhanced in *Calendula officinalis* with application of seaweed extract (vokozim). Nour et al. (2010) also revealed that the application of foliar spray with seaweed extracts showed considerably enhanced number of fruit set on different trusses in tomato plants. Humic acid also enhanced number of umbels per plant. Plants treated with humic acid observed maximum number of umbels per plant at 3 g concentration with the values of 11.67. Smidova (1962) also found his findings in line with this study that humic acid at concentration of 100 mg L^{-1} accelerated the take-up of water by swelling seed in beginning phase of imbibition. The way that seeds take up adequate measure of water sooner make it workable for the initiation of proteins frameworks which guarantee more blossoming.

Study performed by Azarpour et al. (2012) on eggplant by addition of humic acid also revealed similar impact that number of fruits per square meter was considerably enhanced. These results are also lined with Yildirim (2007) that humic acid increased fruit per plant by both foliar and soil treatments in tomato. Same results were reported by Hassan & Mukhtar (2015) that the foliar spray of humic acid showed considerable enhanced number of pods per plant in snap bean plants. Results are also lined with Mohammed (2012) as he observed that positive impact on pods weight per plant and higher total yield in cowpea *Vigna unguiculata* L. plants was as a result of humic acid application. Bakry et al. (2014) studied that highest yield was obtained at 50 kg fed^{-1} humic acid.

Proline as compared to other amino acids increases more rapidly in water stress condition. It considered a source of carbon and nitrogen for quick recovery and growth in stress. It also helps to stabilize membrane and fee radical scavenger (Hayat et al., 2012). Hayat et al. (2012) also reported higher concentration of proline in plants under stress conditions. It reflects the response of plant against osmotic and environmental stress. All treatments reduced proline content significantly as compared to the control. Tammam (2003) observed proline as a sink of energy in plant organ, which are subjected to salt stress. This energy can regulate redox potential, as a hydroxy radical scavenger, a solute that protects macromolecules against denaturation. Na, K, Ca, Mg ions concentration in begonia upper ground parts were affected by biostimulant application, with higher Na^+, K^+, Ca^{2+} concentration in treated plants and lower centralization of Mg^{2+}. El-Nemr et al. (2012) announced expanded aggregate substance of Na, K, Ca, Mg in *Cucumis sativus* leaves while treating them with humic acid. Tuteja (2007) found in his investigations that humic acid mitigate reducive impact of osmotic stress by expanding K ions that represents tissue extensibility by keeping up turgid of plant tissues under stress conditions.

CONCLUSION

A significant difference of effect was found among the three used biostimulants and within their concentrations. Seaweed extract enhance the early flowering and root biomass in the plants. Higher concentration of vegetable extract influenced the number of branches per plant in the experiment. While higher concentration of humic acid leads by boosting plant fresh and dry biomass, plant height and number of umbel per plant. So it is concluded that during this study humic acid (3 g L^{-1}) gave maximum positive effect on fennel plants growth and development under salinity stress condition.

Author Contribution Statement: Iqra Zulfiqar conducted and carried out this research study. Najma Yousaf Zahid planned, designed and supervised the research study. Abdul Ahad Qureshi wrote and edited the manuscript. Ishfaq Ahmed Hafiz contributed in data analysis. All the authors read and approved the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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