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ALLEVIATING ADVERSE EFFECTS OF SOIL SALINITY STRESS ON GROWTH, BIOCHEMICAL COMPOSITION AND YIELD OF CUCUMBER BY USING HUMIC ACID AND CHITOSAN AS FOLIAR APPLICATION

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ABSTRACT: The alleviating effect of foliar application of both humic acid and chitosan on cucumber plants grown in saline soil were elucidated. A pot experiment was conducted at the greenhouse of Plant Physiology Division, Faculty of Agriculture, Cairo University. Giza. Egypt during the two successive seasons of 2020/2021 and 2021/2022. Two factors were studied and designed in Randomized Complete Block Design (RCBD) in each season. Cucumber seeds were sown on 20 Sept. at both seasons, in four levels saline soil (Control, 2000; 4000, 6000 ppm), and treated foliar with five treatments; 1: control (sprayed by tap water), 2, 3: humic acid at two levels (10 ml/L and 20 ml/L) and 4, 5: chitosan at two levels (0.25g and 0.5 g per liter), all of them applied twice. Tween 20 was used as a wetting agent. The plant growth parameters and biochemical components were recorded in two samples of different ages, at the vegetative and flowering growth stages, as well as fruit quality traits and total yield / plant. Results obtained that increasing salinity level affected the seeds germination in which the 6000 ppm had not recorded any seed germination. In addition, increasing salinity level led to reducing plant growth parameters, on the other side chlorophyll a and b increased, and proline accumulation, sugars, amino acids and phenols. A significant reduction was observed in average fruit weight and yield. In addition, chitosan and humic acid application enhanced the growth and biochemical components of cucumber. These inducers were found to have dose dependent effect. Moreover, chitosan showed superiority, mainly in concentration of 0.25 g. L^{-1} as compared to all studied treatments in respect of growth characters, dry matter and biochemical response of cucumber as well as yield and fruit length.

Key words: Cucumber (*Cucumis sativus*), soil salinity stress, humic acid, chitosan, growth, yield.

INTRODUCTION

Salinity is one of the major abiotic stresses which threatens agricultural expansion and cause reduction in growth and productivity of the crop. Salinity leads to negative effects on plant growth, increasing sodium and chloride ions uptake that cause cytotoxicity and nutritional imbalance (**Isayenkov and Maathuis, 2019**). Salt-affected land around the world is about 1125 million hectares, includes about 76 million hectares induced salinization by human. About 50% of suitable lands for cultivation will loss if salinization of lands continues in increasing by 2050. Egypt is one of the significant salts affected soil countries (**Hossain, 2019**). Salt-affected soils occur within the sovereign borders of at least 75 countries and occupy more than 20% of the global irrigated area. Salinity impairs the crops and plants growth and productivity. Both water and soil are subjected to be affected by salination either environmentally or human-caused practice.

Egypt is one of the countries that are suffering from salinity where 35% of the cultivated lands are salt affected soils (**Kotb *et al.,* 2000**). Metabolic changes in plant tissues occur due to

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high concentration of salts in soils which causes osmotic effect inducing plant wilting like water defect stress (**Machado and Serralheiro, 2017**). Increasing salts concentration in the plant root zone cause osmotic stress which negatively effect on cell ion balance and lead to reduce nutrients uptake i.e. K^+ and Ca^{2+} and increase Na^+ and Cl^- **(Paranychianakis and Chartzoulakis, 2005**). Accumulation of harmful ions i.e. Na^+ and Cl^- in plant tissues induce ion toxicity that negatively affect the biological processes such as photosynthesis and damage chloroplasts (**Taiz and Zeiger, 2002**). Cucumber plant (*Cucumis sativus* L.) is among the most desirable cucurbitaceous species, which classified as salinity sensitive plant (**Abdel-Wahab *et al.,* 202**4). Cucumber negatively affected by salinity in all growth stages, such as delaying in seeds germination and emergence, impairing root formation and elongation which inhibit nutrients and water uptake, in addition to reduction in plant vigor, biomass, leaf area and dry matter. Photosynthesis process inhibited with high concentration of salts due to decreasing in chlorophyll pigments content and finally yield reduction (**Khan *et al.,* 2013; Abdel-Farid *et al.,* 2020; Al-Momany and Abu-Romman, 2023**).

Expanding the crop productivity either vertically and horizontally against adverse environmental obstacles becomes the principle aim for physiologists and molecular biologists. Salinity is one of the main environmental obstacles that severely limit crop productivity in Egypt and all over the world. Salinity problems and water scarcely are global issues, which attracts scientist's attention for seeking on alternatives and production programs that would alleviate salinity adverse effect **(Zeid *et al.,* 2018a; Zeid *et al.,* 2018b**) as well as enhancing plant stress tolerance.

Those mechanism was mentioned through depressing plant growth and interfering with photosynthesis, protein synthesis, and energy metabolism (**López-Carrión *et al.,* 2008**). The problem was increased seriously along time due conversion of non-saline soil to saline soil. Besides reclamation of new land has obstacles.

The study suggested that using inducers and agents would improve plant acclimatization and adaptation towards the hazard effect of salinity and enhance its response towards these unfavorable situations. The inducers suggested in this study was chitosan and humic acid. Chitosan is a natural polymer in which deacetylation of chitin results in chitosan production. Crustaceans and fungal are the main sources of them. The biocompatibility, biodegradability, cationic nature, and nontoxicity are their main intrinsic properties (**Román-Doval *et al.,* 2023**). Chitosan plays a role in inducing callose formation, acting as a proteinase inhibitor, helping in phytoalexin biosynthesis, enhancing stomatal conductance, increases abscisic acid concentration, and reduces perspiration in plants without altering their height, leaf area, root height, or biomass. It increases proline and sugar concentrations, thereby changing the seed plasma membrane permeability. Chitosan improves the activity of the peroxidase, phenylalanine ammonia-lyase, tyrosine ammonia-lyase, and catalase as mentioned in Figure 1. Chitosan is defined as an economical, secure, and natural biopolymer derived from chitin, which is mostly found in the exoskeletons of arthropods and fungi cell (**Malerba and Cerana, 2018**), chitosan is a multifunction product in agriculture sector. It is utilized as plant bio-stimulant in virous plants *i.e.,* tomato (**Reyes-Pérez *et al.,* 2020; Attia *et al.,* 2021**), okra (**Mondal *et al.,* 2012**), pepper (**Chookhongkha *et al.,* 2012**), and strawberry (**Rahman *et al.,* 2018**). Improvement in plant vigor and fruit quality attributes of cucumber were induced by a foliar application of chitosan (**Sofian *et al.,* 2022**). Chitosan application alleviated several types of abiotic stresses in cucumber such as salinity (**Dong *et al.,* 2009**), cold stress (**Tan *et al.,* 2023**), and high temperature (**Chen *et al.,* 2019**). Induced resistances to numerous pathogens on vegetable crops by chitosan application such as powdery mildew on cucumber plants (**Moret *et al.,* 2009**), cucumber mosaic virus on tomato plants (**Rendina *et al.,* 2019**), root-knot nematode on tomato plants (**Khalil *et al.,* 2022**), *Fusarium sulphureum* on potato plants (**Sun *et al.,* 2008**), and *Alternaria tenuissima* on potato tubers (**Liu *et al.,* 2019**). In addition to utilizing chitosan as edible coating to decrease nutrients losses and prolong shelf life of some vegetables *i.e.* strawberries, carrot, and cantaloupe (**Tamer and Çopur, 2009**). Concerning salinity stress tolerance,

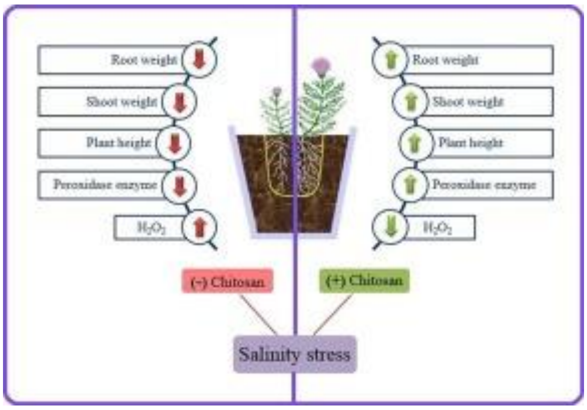


Fig. 1. A diagram of a plant with various levels of stress. (Safikhan *et al.,* 2018)

little literature is available about chitosan role in alleviation salt stress on cucumber.

Humic acid is recognized as complex organic compound that are naturally formed in soil (**Zavarzina *et al.,* 2021**). Humic acid stimulated growth, branching, photosynthetic pigments, dry content of leaves and enhanced the physical characters of fruits which led to significant increment in the productivity of cucumber (**Qassem *et al.,* 2022**). Humic acid played a pivotal role in ameliorating a biotic stresses tolerance of cucumber plants, such as drought stress (**Najafi *et al.,* 2022**), low nitrogen (**Gu *et al.,* 2018**), both low and high temperatures (**El-Remaly *et al.,* 2023**). Regarding salt stress tolerance, **Zarate *et al.* (2023)** found that humic acid clearly enhanced absorption macro elements *i.e.,* N, P, K and Ca, in addition to productivity and quality attributes of cucumber under salt stress. This study investigates the effects of foliar spraying of both chitosan and humic acid on growth, productivity, and the biochemical response of cucumber, which was grown under various levels of salinity stress.

MATERIAL AND METHODS

Experimental Layout, Plant Material and Growth Conditions

A pot experiment was conducted and repeated during the two successive seasons of 2020/2021 and 2021/2022 at the greenhouse of Plant Physiology Division, Faculty of Agriculture, Cairo University. Giza. Egypt. The experiment was designed as a factorial experiment with two factors in a layout of RCBD with three replicates. Cucumber seeds (*Cucumis sativus*), cv. Hisham F1 was used. Seeds were sown on 20 Sept. at both seasons, in pots of 30 cm diameter which were filled with 6 kg soil mixture of pre- washed sand and clay (2:1 v/v). This experiment included 20 treatments which were the combinations between 4 soil salinity levels (3 concentrations and control) and 5 foliar spray treatments (2 concentrations of humic acid (Ha) and 2 concentrations of chitosan (Ch) as well as spraying with water). The four levels of the first factor, salinity, were control, 2000, 4000 and 6000 ppm, and the second factor, foliar application with five treatments, were 1- Control. which sprayed by water, 2,3- foliar spray of humic acid applied twice, in two concentrations; 10 ml (Ha1) and 20 ml (Ha2) per liter and 4,5- foliar spray of chitosan applied in two concentrations; 0.25g (Ch1) and 0.5 g (Ch2) per liter. Tween 20 was added as wetting agent. Saline water was prepared by dissolving Mediterranean seawater salts in water. The salts were prepared by El Nasr Salines Co., by evaporating Mediterranean seawater which was withdrawn from the Mediterranean Sea at depth of 25 km, in El-Hammam village in the North Coast of Egypt (the analysis was illustrated in Table 2). All agricultural practices took place according to recommendations of the Ministry of Agriculture and Land Reclamation (MALR), Egypt. Ammonium sulphate (20.5% N), potassium sulphate (48% K) and calcium superphosphate (15.5% P_2O_5) were added by weight in each pot.

These doses were applied every 10 days with supplement with foliar spray of micronutrients obtained from Shoura Company, Egypt. There was a one plant per pot. Each pot was irrigated until it reached its field capacity to avoid mineral and salt leaching. Then, two samples were collected, 10 days after treatments applications, at vegetative and flowering growth stages (45 and 55 days after planting, respectively).

The soil mixture was randomly taken before cultivation and were subjected for physical and chemical analysis according to **Jackson (1967)**. The mechanical and chemical analysis of the soil mixture was illustrated in Table 1.

Measurements

Morphology and plant growth

In the two successive seasons, the samples were collected randomly after 45 and 55 days of planting, the samples were separated to shoots and roots. The length (cm), fresh weight (g) of roots and shoots were recorded. Also, the number of leaves and shoots to roots ratio were measured. Leaves area was estimated by standardized discs area (cm^2) taken from each leaf against a relation between disc leaf weight and disc area to estimate the total area of whole leaf according to **Pandey and Singh (2011)**. Then both shoots and roots were dried at 70°C/48 hours to measure the dry weight. Each sample was about three replicates. Some fruit quality parameters were measured such as fruit length and average fruit weight, in addition to total yield /plant which measured by calculate the weight for all harvested fruit per plant.

Biochemical components

Photosynthetic Pigments (chl. a, chl. b, total chlorophyll, and total carotenoids) were extracted from early full-grown top leaves using dimethylformamide and expressed as (mg/g fresh weight) according to **Moran (1982)**.

Ethanol extracts were used for extract of sugars, total free amino acids and total soluble phenols in the fresh cucumber shoots and roots.

Total sugars were estimated using the phenol-sulphuric method according to **Dubois *et al.* (1956)**. The absorbance of developed yellow orange color was measured at 490 nm using spectrophotometer (UNICO UV-2000). Sugars were expressed as mg/g fresh weight of shoot and root of cucumber against glucose.

Total soluble phenols were determined using the folin-Ciocalteau colorimetric method (**Swain and Hillis, 1959**). The absorbance was read at wavelength (725 nm) using the spectrophotometer (UNICO UV -2000). Total soluble phenols were expressed as mg/g fresh weight of shoot and root of cucumber against pyrogallol.

The total free amino acids were determined using Ninhydrin reagent according to **Moore and Stein (1954)** and the absorbance of the developed color was read at 570 nm using spectrophotometer (UNICO UV-2000). A standard curve was measured for total free amino acids were expressed as mg/g fresh weight of shoot and root of cucumber against tryptophan.

Proline was extracted in sulfosalicylic acid 3%, determined using ninhydrin reagent according to **Bates *et al.* (1973)**. A standard curve was measured for free proline was expressed as mg/g fresh weight of shoot and root of cucumber against proline.

Statistics Analysis

Data were processed to homogeneity test and proper statistical analysis of variance of two factorial combined factor design according to the procedures outlined by Snedecor and Cochran, 1980. Dunkin and least significant difference, at 5% level of significance was used to compare between means of treatments. All statistical analysis was performed by using analysis of variance technique of **MStat (1986)** Computer software package. The hierarchy cluster dendrogram for studied parameters was analyzed using IPM SPSS software package, version 25.

RESULTS

A cucumber plant grown in saline soil, with concentrations of 0, 2000, 4000 and 6000 ppm. Cucumber morphology and biochemical study in this article revealed that cucumber cannot germinate, and even germinated seeds cannot withstand growing under 6000 ppm.

Table 1. Mechanical and chemical analysis of the soil experimental site (sands and clay, 2:1, v/v)

<table>
<tr><th colspan="7">Particle size distribution</th></tr>
<tr><th colspan="2">Sand %</th><th colspan="2">Silt %</th><th colspan="2">Clay %</th><th>Texture class</th></tr>
<tr><td colspan="2">72.80</td><td colspan="2">20.00</td><td colspan="2">7.20</td><td>Loamy sand</td></tr>
<tr><th colspan="7">Chemical analysis</th></tr>
<tr><th colspan="2">E.C dS/m</th><td colspan="2">0.33</td><th colspan="2">pH</th><th>7.40</th></tr>
<tr><th colspan="3">Soluble anions (meq/l)</th><th colspan="4">Soluble cations (meq/l)</th></tr>
<tr><td>HCO^-</td><th>Cl^-</th><th>SO_4^{-2}</th><th>Na^+</th><th>K^+</th><th>Ca^{+2}</th><th>Mg^{+2}</th></tr>
<tr><td>1.0</td><td>1.5</td><td>9.6</td><td>1.1</td><td>0.26</td><td>1.14</td><td>0.7</td></tr>
</table>

Table 2. Mediterranean seawater salts analysis performed by El-Nasr Salines Co

M.	INS.	HCO_3^-	CO_3^-	Cl^-	SO_4^{-2}	Ca^{+2}	Mg^{+2}	Na^{+1}
6.3	0.26	0.078	0	55.028	2.15	0.206	0.52	35.461

M: Moisture - **INS.:** Insoluble solids

Effect of Salinity Soil Stress and Foliar Spray of Chitosan and Humic Acid on Cucumber Morphology

The shoot length and shoot fresh and dry weights as well as number of leaves of cucumber grown under salinity stress and foliar sprayed by different doses of humic acid and chitosan were demonstrated in Table 3. Whereas, the root length and root fresh and dry weights, shoot to root ratio of cucumber were demonstrated in Table 4. As well as the leaf area of cucumber at both vegetative and flowering stages, was demonstrated in Table 5. The effect of foliar treatments on plant growth parameters were demonstrated in figures 2 and 3.

In general, results indicated that salinity had significantly affected plant growth and dry matter accumulation, whereas chitosan enhanced plant growth under adverse salinity conditions

Regarding the effect of salinity stress on morphological growth parameters of cucumber plants, results presented in Tables 3 and 4 and Figs 2 and 3 indicated that salinity stress adversely affected cucumber growth significantly, meanwhile with increasing salinity, all growth parameters; shoot length, fresh and dry weights of both roots and shoots, number of leaves and shoot: root ratio were significantly reduced with increasing salinity stress. Root length had a different trend as compared to other studied plant growth parameters, which recorded the highest significant value in cucumber grown in 4000 ppm soil at the vegetative and flowering stages and reduced with reducing salinity stress. These results confirmed an inversely relation between salinity and shoot length, fresh and dry weights, number of leaves as well as shoot to root ratio. On the other hand, root length had a direct proportional with increasing salinity stress.

Concerning the effect of foliar spray treatments on growth parameters of cucumber plants; the same tables indicated that the foliar application of chitosan at concentration of 0.25g l^{-1} (Ch1) enhanced significantly the shoot growth of cucumber plants that was indicated in recording the highest significant value in shoot length, shoot fresh weight, shoot dry weight and number of leaves at the vegetative growth stage. However, foliar application of humic acid on leaves with concentration of 20 cm l^{-1} (Ha2) recorded significantly superior effect on shoot growth parameters at the flowering stage. These results mainly indicated in highest significant record of shoot length, shoot fresh and dry weights as well as number of leaves of cucumber plants.

Table 3. Effect of foliar spray with chitosan and humic acid on shoot length shoot fresh and dry weight and no. leaves of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	Treatments	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Number of leaves	
		Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
	Control	101.29	204.29	54.34	107.12	4.07	12.51	17.42	19.36
	Chitosan (0.25g L^{-1})	145.21	223.83	70.45	62.48	7.32	8.55	17.33	19.49
Control	Chitosan (0. 5g L^{-1})	97.75	170.94	45.80	87.78	4.52	10.44	17.71	19.53
	Humic acid (10ml L^{-1})	114.75	177.56	44.91	80.45	4.21	12.01	16.29	19.15
	Humic acid (20 ml L^{-1})	61.63	236.11	29.35	137.15	2.49	13.94	14.88	17.50
Mean		**104.13**	**202.55**	**48.97**	**94.99**	**4.52**	**11.49**	**16.73**	**19.01**
	Control	70.13	156.31	38.47	66.35	2.69	8.45	14.88	25.03
	Chitosan (0.25g L^{-1})	84.29	164.81	37.57	87.37	3.40	9.52	15.29	15.36
2000 ppm	Chitosan (0. 5g L^{-1})	68.00	193.14	35.66	82.86	2.98	11.42	15.58	15.97
	Humic acid (10ml L^{-1})	80.04	113.81	36.14	48.87	3.07	7.26	13.46	18.11
	Humic acid (20 ml L^{-1})	129.50	182.75	37.75	76.89	6.55	8.83	19.55	19.83
Mean		**86.39**	**162.16**	**37.12**	**72.47**	**3.74**	**9.10**	**15.75**	**18.86**
	Control	46.04	58.49	21.54	17.28	1.57	7.23	12.04	26.52
	Chitosan (0.25g L^{-1})	54.54	90.67	28.43	44.19	2.23	6.62	14.88	17.50
4000 ppm	Chitosan (0. 5g L^{-1})	14.88	67.06	5.76	27.08	0.46	4.05	6.38	15.72
	Humic acid (10ml L^{-1})	46.04	118.06	22.14	44.94	2.62	7.55	13.46	16.28
	Humic acid (20 ml L^{-1})	10.63	156.31	35.71	64.09	5.69	9.02	3.54	24.22
Mean		**34.43**	**99.96**	**22.72**	**40.55**	**2.51**	**6.88**	**10.06**	**19.75**
	Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6000 ppm	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mean		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L.S.D	**Salinity**	7.792	11.60	0.8850	0.8190	0.05794	0.8600	1.045	2.94
	Treatments	10.06	14.98	1.143	1.057	0.07480	1.110	1.349	3.800
	Salinity*Treatments	17.42	25.94	1.979	1.831	0.1296	1.923	2.336	6.582

Table 4. Effect of foliar spray with chitosan and humic acid on root fresh and dry weight, root length and shoot: root ratio of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	Treatments	Root length (cm)		Root fresh weight (g)		Root dry weight (g)		Shoot: Root ratio	
		Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
	Control	20.54	22.85	3.17	7.18	0.38	0.99	10.84	16.06
	Chitosan (0.25g L^{-1})	25.82	19.94	4.49	4.77	1.02	1.07	7.34	8.38
Control	Chitosan (0. 5g L^{-1})	15.58	17.50	2.54	3.47	0.31	1.09	17.14	11.97
	Humic acid (10ml L^{-1})	18.42	14.44	2.20	9.15	0.31	1.06	15.09	18.25
	Humic acid (20 ml L^{-1})	12.04	21.17	1.71	4.99	0.21	0.52	11.97	28.31
Mean		**18.48**	**19.18**	**2.82**	**5.91**	**0.45**	**0.94**	**12.48**	**16.59**
	Control	26.21	22.67	2.81	5.58	0.32	1.33	9.00	7.50
	Chitosan (0.25g L^{-1})	13.46	17.19	2.03	4.20	0.22	0.77	15.63	19.97
2000 ppm	Chitosan (0. 5g L^{-1})	19.13	15.67	2.47	4.33	0.35	1.45	8.11	14.13
	Humic acid (10ml L^{-1})	21.25	14.44	2.35	3.15	0.28	0.57	10.47	16.19
	Humic acid (20 ml L^{-1})	12.75	19.03	2.19	4.21	0.50	0.41	13.71	22.11
Mean		**18.56**	**17.80**	**2.37**	**4.29**	**0.33**	**0.91**	**11.38**	**15.98**
	Control	21.25	19.54	2.48	2.15	0.17	0.22	9.17	33.02
	Chitosan (0.25g L^{-1})	31.88	21.17	3.36	2.37	0.53	0.39	6.01	22.68
4000 ppm	Chitosan (0. 5g L^{-1})	16.29	10.78	1.36	1.28	0.12	0.17	3.62	46.29
	Humic acid (10ml L^{-1})	17.71	17.19	1.67	3.35	0.34	0.12	6.73	70.73
	Humic acid (20 ml L^{-1})	13.21	19.64	2.08	3.12	0.59	0.50	9.65	20.72
Mean		**20.07**	**17.58**	**2.19**	**2.47**	**0.35**	**0.28**	**7.04**	**38.95**
	Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6000 ppm	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mean		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L.S.D	**Salinity**	1.417	2.935	0.04097	0.0748	0.03345	0.041	0.3901	4.535
	Treatments	1.829	3.790	0.053	0.097	0.04318	0.053	0.5036	5.855
	Salinity*Treatments	3.168	6.564	0.09161	0.1673	0.07480	0.092	0.8723	10.14

Table 5. Effect of foliar spray with chitosan and humic acid on leaf area of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	Treatments	Area of leaves (cm^2)	
		Vegetative	Flowering
Control	Control	56.08	40.59
	Chitosan (0.25g L^{-1})	54.56	43.54
	Chitosan (0. 5g L^{-1})	31.96	48.51
	Humic acid (10ml L^{-1})	39.20	44.89
	Humic acid (20 ml L^{-1})	43.14	60.82
	Mean	**44.99**	**47.67**
2000 ppm	Control	44.80	38.32
	Chitosan (0.25g L^{-1})	42.46	36.35
	Chitosan (0. 5g L^{-1})	14.66	41.44
	Humic acid (10ml L^{-1})	37.40	32.63
	Humic acid (20 ml L^{-1})	23.58	37.95
	Mean	**32.58**	**37.34**
4000 ppm	Control	28.32	22.95
	Chitosan (0.25g L^{-1})	34.54	37.20
	Chitosan (0. 5g L^{-1})	16.39	23.90
	Humic acid (10ml L^{-1})	26.32	26.45
	Humic acid (20 ml L^{-1})	12.06	25.20
	Mean	**23.53**	**27.33**
6000 ppm	Control	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A
	Mean	N/A	N/A
L.S.D	**Salinity**	0.1338	0.6035
	Treatments	0.1727	0.7791
	Salinity*Treatments	0.2992	1.349

Ch1 and Ha2 had contradictory effects on growth, means that the enhancing effect of one of them at certain growth stage, was proposed with reducing effect exerted by other treatment at the same growth stage either vegetative or flowering stages.

In respect to the effect of foliar spray treatments on the root growth, results indicated that plants treated with Ch1 recorded the highest significant root length, root fresh and dry weights at the vegetative stage. Whereas, at the flowering stage, root length was superior in Ha2 treated plants, followed by Ch1 treated plants. In addition, the highest value of root fresh weight was recorded in plants treated by foliar spray of humic acid in concentration of Ha1, however Ch1 obtained the lowest significant value in root fresh weight. Additionally, root dry weight was the highest significant rank in chitosan treated plants with concentration of Ch2, however humic acid reduced the dry weight of roots at both concentrations.

The previous results were proved by the trend of shoot to root dry weight ratio. Ch1 and Ch2 recorded the lowest value significantly at both vegetative and flowering stages. These results indicated that chitosan has no effect on dry matter partitioning in shoot versus roots, which indicated increasing in both organs. However, Ha2 obtained significantly the highest value at the vegetative stage, whereas Ha1 was superior at the flowering stage. Humic acid indicated to play a role in dry matter portioning in shoot versus root, at both vegetative and flowering stages. In addition, dry matter partitioning depends on the humic acid concentration. Ha1 participates in partitioning dry matters towards roots at the vegetative stage, however, shoots accumulate dry matter in the flowering stage. A vice versa was indicated in plants treated by Ha2, which accumulates dry matters in shoot and roots at vegetative and flowering stages, respectively.

Meanwhile, the main effect of interaction between salinity stress and foliar spray of studied treatments on the cucumber morphology which is shown in Figure 2 and Figure 3. Results indicated that the foliar application of humic acid in both concentrations has affected the shoot growth parameters in the vegetative and flowering stages, however, chitosan in both concentrations mainly affected the growth of the root parameters at both growth stages. The treated plants with Ch1 at 4000 ppm soil recorded the highest significant root length values at the vegetative stage. This treatment was superior as compared to control at grown either in non-saline or 2000 ppm. In the flowering stage, the values of root length showed narrow significant differences among foliar spray of studied treatments under different soil salinity. While the treatment with Ch1 which grown in 4000 ppm soil showed no significant difference at both control cucumbers grown under either non-saline soil or 2000 ppm soil salinity. These results ensured that the chitosan effect on root length was superior as compared with other studied treatments. Similar trend was observed in root fresh weight at Ch1 treated cucumber under 4000 ppm soil salinity at the vegetative stage. It also was superior as compared to control plants in non-saline and 2000 ppm soil salinity. Moreover, Ch1 treated plants recorded the highest significant root fresh weight grown in non-saline soil salinity at the same growth stage. At the flowering stage, the treated plants by Ch2 under 2000 ppm soil salinity recorded the highest significant value of root dry weight. However, at the flowering stage, root fresh weight was increased by humic treatment; in which Ha1 obtained the highest significant root fresh weight value in cucumber grown in non-saline soil salinity.

Furthermore, shoot length was recorded the highest significant value in Ch1 treated plants at the vegetative growth under non-saline soil. However, humic acid treated cucumbers grown in 2000 ppm soil salinity recorded significantly the highest value in saline conditions. Moreover, Ch1 treated cucumbers in 4000 ppm soil salinity increased root length as well as root fresh and dry weight as compared to other treatments in the same salinity level, at the vegetative stage. Furthermore, at the flowering stage, humic acid treated cucumbers showed a significantly increase of shoot length as well as shoot/ root fresh and dry weight when compared to other treatments. These results confirmed that the cucumbers grown in 2000 ppm and 4000 ppm, which recorded a significant increase in cucumbers treated by Ha2 as compared with other treatments in the same salinity level. On the same manner, shoot fresh weight showed significant increase with Ha2 treated cucumber, which recorded the highest significant value in non-saline soil, in addition to in 4000 ppm soil salinity, at the flowering stage. Moreover, Ha2 treated cucumbers grown in 2000 ppm soil salinity increased significantly shoot dry weight. Similarly, Ha2 treated cucumbers in 4000 ppm obtained the highest root dry weight when compared to other treatments, at the vegetative stage.

Moreover, results indicated that cucumbers which were not treated with foliar application showed an increase in root dry matter on the expenses of shoot dry accumulation at the vegetative and flowering stages in non-saline and 2000 ppm as well as 4000 ppm soil salinity except flowering stage of 4000 ppm. The plants grown in 4000 ppm at the flowering stage showed the shoot dry matter accumulation rather than root accumulation, which had a beneficial effect on yield. Concerning the effect of the studied treatments, it was found that Ch1 in non-saline soil increased root dry matter accumulation significantly more than shoot accumulation, at both vegetative and flowering growth stages. These results referred to enhancing root growth by chitosan rather than enhancing shoot accumulation. Whereas, cucumber grown in 2000 ppm soil salinity and treated by Ch1, showed an increase in shoot accumulation rather than root accumulation significantly, mainly at the vegetative growth, while at the flowering stages, shoot accumulation was increased. However, Ch1 treated cucumber in 4000 ppm

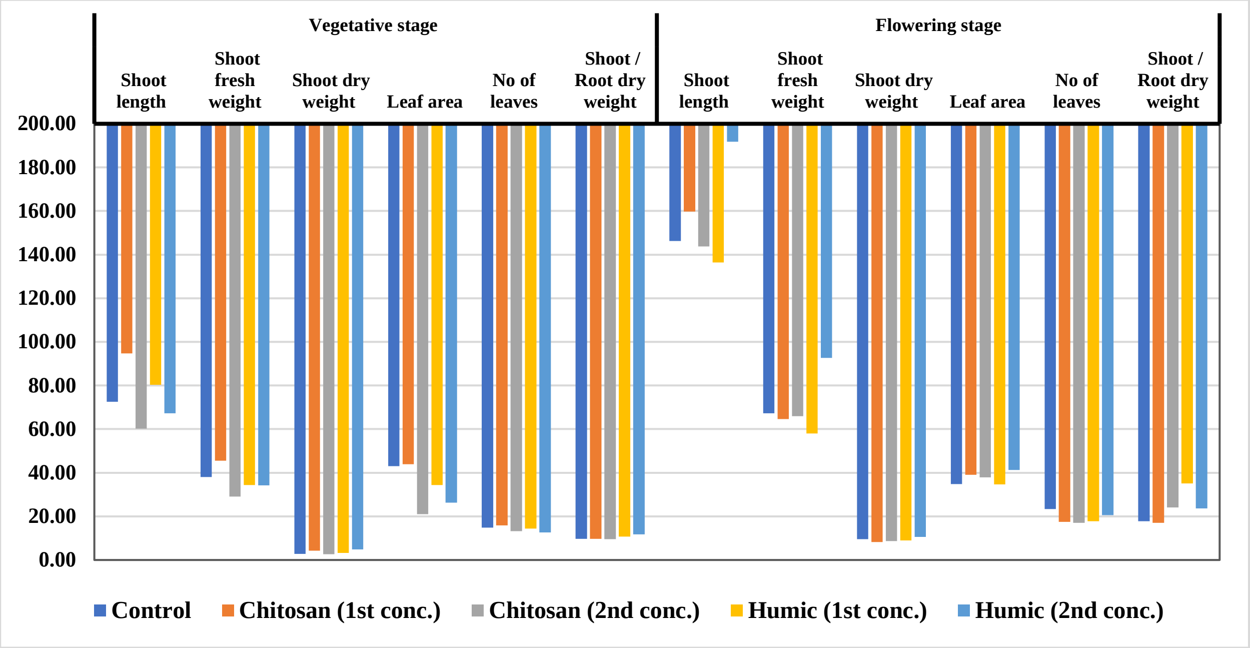


Fig. 2. Effect of foliar spray with chitosan and humic acid on shoot fresh and dry weight, shoot length, no. of leaves, leaf area and shoot: root ratio of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

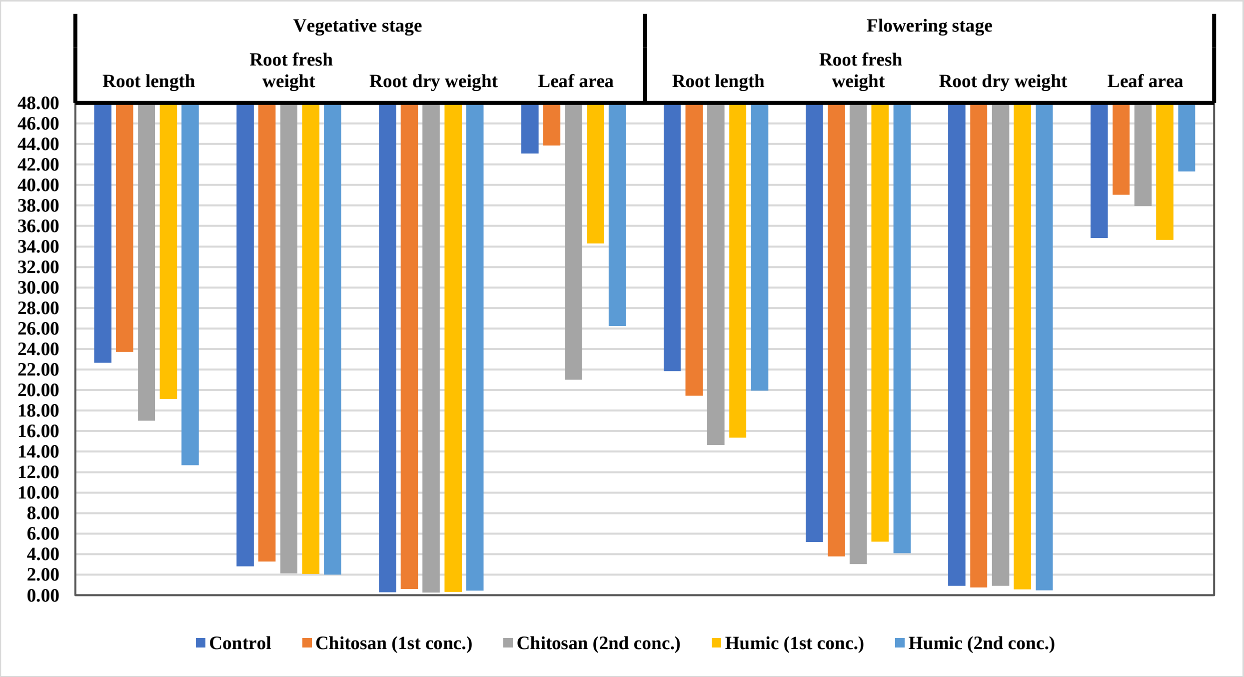


Fig. 3. Effect of foliar spray with chitosan and humic acid on root fresh and dry weight, root length and leaves area of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

soil showed increase in root accumulation significantly at the vegetative stage, otherwise shoot dry matter accumulated significantly at the expense of root, in the flowering stage. These results were considered as adaptation to salinity stress at the vegetative stage as well as enhancing yield accumulation in the flowering stage.

Furthermore, Ch2 and Ha1 treated cucumber showed the higher significant record in shoot dry matter accumulation rather than root dry matter accumulation at the vegetative stage, respectively. On the other hand, Ha1 and Ch2 showed the highest significant shoot dry matter accumulation at the flowering stage, respectively.

Concerning the salinity effect on the area of cucumber leaves which indicated in Table 5, salinity stress showed a significant adversely effect on leaves area of cucumber plants. The grown plants under 4000 ppm soil recorded, significantly the lowest leaves area, and followed by 2000 ppm and then control, at the vegetative and flowering stages (Table 5). Regarding the effect of treatments on leaves area, Also Ch1 recorded the highest significant value at the vegetative stage, whereas Ha2 was significantly superior at the flowering stage. Furthermore, the main effect of the interaction between both salinity stress as well as foliar spray of chitosan or humic acid on leaves area, showed that non-treated plants (control) showed the lowest significant leaves area under different salinity levels; non-saline, 2000 ppm and 4000 ppm soil salinity as compared to other treatments, at the flowering stage. Humic acid treated plants revealed a significant increase in area of leaves, confirming the enhancing effect of humic acid on shoot growth parameters. On the other hand, chitosan treated plants showed a reduction in leaves area when compared with other studied treatments, at the flowering stage. Moreover, Ha2 treated cucumbers in the non-saline soil showed the highest significant leaves area, however, Ch1 showed a significant reduction in leaves area. While, increased salinity level to 4000 ppm showed different pattern for the studied treatments, in which chitosan showed increase in the leaves area, which showed enhancing effect under salinity stress.

Effect of Salinity Soil Stress and Foliar Spray of Chitosan and Humic Acid on Cucumber Biochemical Components Concentration

Salinity stress has affected significantly the biochemical components concentration in cucumber; however, chitosan and humic acid were enhanced plant metabolites which enhanced their beneficial use.

Free proline concentration

Results presented in Table 6 showed the effect of saline affected soil and foliar spray by different doses of humic acid and chitosan on free proline concentration in leaves and roots of cucumber, at vegetative and flowering stages. Concerning the effect of salinity stress, results revealed that salinity had no significant effect on proline concentration in cucumber roots at both vegetative and flowering growth stages. However, proline concentrations were significantly affected in both vegetative and flowering stages in plant leaves. It was observed that proline concentration increased in cucumber leaves which grown in control and 2000 ppm soil, at the vegetative stage which represented the highest significant value, whereas control was the highest record. Furthermore, proline concentration ranked the first significant value in 2000 ppm soil salinity at the flowering stage.

Concerning the main effect of the foliar spray of chitosan and humic acid on free proline concentration in cucumber plants, data in Table 6 indicated that ch1 recorded significantly highest value of free proline in leaves at the vegetative stage. However, the proline was reduced at the flowering stage in the same treatment. In addition, Ha1 and Ch1abstained significantly the same values of proline in leaves, at the vegetative and flowering stages. Meanwhile, Ch1 and Ha1 recorded the lowest proline accumulation in leaves at the flowering stage. Furthermore, Ha2 showed significantly the highest value in leaves at the flowering stage. Regarding the accumulation of proline in roots, the non-treated plants (control) recorded the highest significant free proline accumulation, followed by Ha1. These treatments were followed by Ch1 in roots at the flowering stage. The lowest significant treatment was Ha1 in roots at the flowering stage.

In the respect of main effect of the interaction between both salinity stress and foliar sprayed treatments, the results indicated that non treated cucumbers showed a significant reduction in proline accumulation in leaves at the vegetative and flowering stages, as well as the same trend was observed in roots at the vegetative stage. On the other hand, a significant increase in proline accumulation was observed in flowering stage in roots. The increment in soil salinity to 2000 ppm resulted the increase in proline accumulation significantly at the flowering stage, however, a significant reduction was observed at the vegetative stage, in leaves and roots. Furthermore, a reduction was observed in leaves at both growth stages except in roots at the flowering stage when salinity level increased to 4000 ppm. The proline recorded the highest significant level in cucumber sprayed with water in roots grown in 4000 ppm, at the flowering stage. The plants grown in 2000 ppm and non-saline soil were significantly increased proline accumulation, subsequently to those grown in 4000 ppm.

Concerning the main effect of the chitosan and humic acid, the grown plants in non-saline soil and treated by Ch1 showed a significant increase in proline accumulation in leaves at the vegetative stage and in roots at the flowering stage. On the other hand, it was reduced significantly in leaves at the flowering stage and in roots at the vegetative stage. Increasing chitosan concentration to Ch2 has reduced proline accumulation in leaves at both vegetative and flowering stages. While treating cucumbers with humic acid Hu1, has cumulated proline significantly in leaves at both vegetative and flowering stages as well as in roots at the vegetative stage. On the other edge, proline was reduced in roots at the flowering stage in the same treatment. Furthermore, Ha2 resulted in similar result of Ch2 effect on these cucumbers, which reduced significantly free proline accumulation in leaves at the vegetative and flowering stages. While an increase has been recorded in roots at the vegetative stage and followed by a significant reduction in roots.

Furthermore, increasing soil salinity to 2000 ppm, the studied treatments had different trend observed in proline accumulation in cucumbers than those grown in non-saline conditions. Ch1 has significantly recorded the highest proline accumulation in leaves and roots as well, at the vegetative stage. On the other hand, a significant reduction has been observed in leaves and roots, at the flowering stage. In addition, Ch2 had recorded the highest significant proline accumulation in leaves and roots, at both vegetative and flowering stages, when compared with other studied treatments under different salinity levels. In respect of the humic acid effect on proline accumulation, results indicated that a significant reduction has been observed in proline in leaves and roots, at the vegetative and flowering stages, in cucumbers treated by Ha1. Whereas Ha2 treating cucumbers resulted in significant increase in proline in leaves at the vegetative and flowering stages, however, a reduction was observed in roots at the vegetative stage. Moreover, a significant increase was detected in roots at the flowering stage.

Consequently, increasing salinity level of soil to 4000 ppm, had resulted in reduction in proline accumulation in leaves and roots, at both vegetative and flowering stages, of cucumbers treated by Ch1. Whereas Ch2 treating cucumbers resulted in a reduction in proline accumulation in leaves and roots at the vegetative and flowering stages, respectively. In addition, results indicated that treating plants by humic acid resulted in significant increase in proline accumulation at the vegetative stage in cucumbers treated by Ha1. On the other hand, Ha1 resulted in a significant reduction in proline in leaves, at the flowering stage as well as in roots at both vegetative and flowering stages. Ha2 treated cucumbers resulted in significant high record in proline accumulation in leaves, at both stages and roots at the vegetative stage, however, a reduction was observed in roots at the flowering stage.

Total free amino acids concentration

The total free amino acids concentration found in leaves and roots of cucumber, at vegetative and flowering stages, grown in saline soil and sprayed by different doses of humic acid and chitosan was demonstrated in Table 7. Concerning the effect of salinity stress, salinity significantly increased total free amino acids concentration in cucumber leaves grown in 4000-ppm soil at both vegetative and flowering growth stages. Whereas 2000 ppm recorded significantly the lowest value of free amino acids. Furthermore,

Table 6. Effect of foliar spray with chitosan and humic acid on free proline concentration in roots and leaves of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	Treatments	Roots (mg/g fresh weight)		Leaves (mg/g fresh weight)	
		Vegetative	Flowering	Vegetative	Flowering
Control	Control	0.260	2.086	0.242	1.086
	Chitosan (0.25g L^{-1})	0.084	1.395	2.872	0.429
	Chitosan (0. 5g L^{-1})	0.184	1.050	1.680	0.713
	Humic acid (10ml L^{-1})	0.669	0.079	3.249	1.638
	Humic acid (20 ml L^{-1})	0.532	0.523	0.191	0.999
Mean		**0.346**	**1.027**	**1.647**	**0.973**
2000 ppm	Control	0.148	2.255	0.099	1.719
	Chitosan (0.25g L^{-1})	0.576	0.401	2.768	0.395
	Chitosan (0. 5g L^{-1})	0.485	1.831	1.997	1.935
	Humic acid (10ml L^{-1})	0.211	0.151	1.288	0.187
	Humic acid (20 ml L^{-1})	0.153	1.542	1.946	2.451
Mean		**0.314**	**1.236**	**1.620**	**1.337**
4000 ppm	Control	0.419	2.768	0.097	0.830
	Chitosan (0.25g L^{-1})	0.070	0.217	1.126	0.425
	Chitosan (0. 5g L^{-1})	1.270	0.163	0.506	1.285
	Humic acid (10ml L^{-1})	0.253	0.353	2.080	0.218
	Humic acid (20 ml L^{-1})	1.035	0.918	1.985	1.541
Mean		**0.609**	**0.884**	**1.159**	**0.860**
6000 ppm	Control	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A
Mean		N/A	N/A	N/A	N/A
Main effect of foliar spray treatments					
Control		0.276	2.370	0.146	1.211
Chitosan (0.25g L^{-1})		0.243	0.671	2.255	0.416
Chitosan (0. 5g L^{-1})		0.646	1.015	1.394	1.311
Humic acid (10ml L^{-1})		0.377	0.194	2.206	0.681
Humic acid (20 ml L^{-1})		0.573	0.995	1.374	1.664
L.S.D	**Salinity**	ns	ns	0.4100	0.2967
	Treatments	ns	0.6005	0.5294	0.3831
	Salinity*Treatments	0.5032	1.040	0.9169	0.6635

Table 7. Effect of foliar spray with chitosan and humic acid on total free amino acid concentration in roots and leaves of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022).

Salinity	Treatments	Roots (mg/g fresh weight)		Leaves (mg/g fresh weight)	
		Vegetative	Flowering	Vegetative	Flowering
Control	Control	1.071	7.770	3.730	7.770
	Chitosan (0.25g L^{-1})	11.483	8.996	4.800	10.032
	Chitosan (0. 5g L^{-1})	1.261	6.389	2.538	2.935
	Humic acid (10ml L^{-1})	3.315	6.665	9.393	12.122
	Humic acid (20 ml L^{-1})	4.921	10.706	1.520	9.929
	Mean	**4.410**	**8.105**	**4.396**	**8.558**
2000 ppm	Control	0.449	9.652	1.779	2.659
	Chitosan (0.25g L^{-1})	6.821	9.411	5.992	8.547
	Chitosan (0. 5g L^{-1})	4.386	13.054	2.417	5.456
	Humic acid (10ml L^{-1})	2.935	9.739	1.485	11.155
	Humic acid (20 ml L^{-1})	3.264	14.591	2.107	8.858
	Mean	**3.571**	**11.289**	**2.756**	**7.335**
4000 ppm	Control	6.441	5.905	10.153	2.901
	Chitosan (0.25g L^{-1})	1.830	5.750	3.384	4.697
	Chitosan (0. 5g L^{-1})	3.333	11.655	4.438	14.194
	Humic acid (10ml L^{-1})	2.780	15.558	3.453	12.553
	Humic acid (20 ml L^{-1})	1.330	9.739	9.791	13.002
	Mean	**3.143**	**9.721**	**6.244**	**9.469**
6000 ppm	Control	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A
	Mean	N/A	N/A	N/A	N/A
Main effect of foliar spray treatments					
Control		2.653	7.776	5.220	4.443
Chitosan (0.25g L^{-1})		6.711	8.052	4.725	7.759
Chitosan (0. 5g L^{-1})		2.993	10.366	3.131	7.529
Humic acid (10ml L^{-1})		3.010	10.654	4.777	11.943
Humic acid (20 ml L^{-1})		3.171	11.678	4.472	10.596
L.S.D	**Salinity**	ns	0.5056	0.7498	ns
	Treatments	2.224	0.6527	0.9680	3.022
	Salinity*Treatments	3.853	1.131	1.677	5.234

free amino acids were recorded with the highest significant concentration in roots of cucumber grown in control and 2000-ppm soils at the vegetative and flowering stage, respectively.

Concerning the main effect of studied treatments on the accumulation of free amino acids in leaves and roots of cucumber plants grown in saline soil; results indicated that treated cucumber either foliar sprayed by chitosan or humic acid were increased amino acids accumulation as compared to the non-treated cucumbers (control). It was observed that chitosan and humic acid recorded the highest significant value of amino acids in leaves at the vegetative stage. However, the values were reduced by chitosan at the flowering stage, while the values were the highest significant in leaves at the flowering stage with humic acid. Concerning the main effect of studied treatments on roots, the results showed that Ch1 recorded the highest significant amino acids in plants which was followed by humic acid at the vegetative stage. On the contradictory, at the flowering stage, Ha2 obtained the highest amino acids accumulated in plants followed by chitosan.

Regarding the main effect of interaction between the studied treatments as well as salinity stress, results indicated that the control plants obtained a significant reduction in free amino acids accumulation in roots and leaves at both vegetative and flowering stages which grown in non-saline and 2000 ppm soil salinity. On the other hand, a significant increase was observed in the same treatment by increasing salinity level to 4000 ppm.

Concerning the effect of the studied treatments, it was observed that free amino acids were increased in leaves at the vegetative and flowering stages, whereas the accumulation was increased in roots at the vegetative stage and reduced at the flowering stageCh1 recorded a significant increase in free amino acids accumulation in leaves and roots at the vegetative stage. Furthermore, these plants showed significant amino acid accumulation in leaves, however, a significant reduction in roots, at the flowering stage which grown in different studied salinity levels as compared to other treatments. In respect to humic acid, Ha1 showed a significant reduction in free amino acids accumulation in leaves at the vegetative stage and a significant increase at the flowering stage, whereas in roots at the vegetative and flowering stages, an increase and reduction was observed significantly, respectively in non-saline, 2000 ppm and 4000 ppm, soil salinity. On the other side, Ha2 recorded a different trend, free amino acids accumulation was significantly increased in leaves at the flowering stage and reduced in the vegetative stage in non-saline and 2000 ppm soil salinity. On the other hand, it was recorded an increase in 4000 ppm. Ha2 showed an increase significantly in 2000 ppm and a reduction significantly in 4000 ppm soil salinity in root at the vegetative and flowering stages.

Ch2 obtained a significant increase in free amino acids in leaves at the vegetative and flowering stages. While, a reduction showed significantly in roots at both vegetative and flowering stages in 4000 ppm soil salinity. In which this treatment showed the highest significant record in leaves at the flowering stage.

Total sugar concentration

The total sugar concentration found in leaves and roots of cucumber, at vegetative and flowering stages, grown in saline affected soil and foliar sprayed by different doses of humic acid and chitosan were demonstrated in Table 8. Results concerning the effect of salinity stress, revealed that salinity has significantly increased total sugar concentration in cucumber leaves grown in control soil, at vegetative growth stage, in addition to it recorded the lowest significant value in cucumber grown in 2000-ppm soil. At the flowering stage, leaves of cucumber grown in 2000-ppm soil recorded the highest significant value when compared with other studied treatments. Furthermore, total sugars were recorded as the highest significant concentration in roots of cucumber grown in control and 2000-ppm soils at the flowering stage.

Concerning the effect of the studied treatment on the sugar accumulation in roots and leaves of cucumber plants, results revealed that humic acid was superior to chitosan in enhancing sugar accumulation in roots and leaves at the vegetative and flowering stages. Additionally, control was found to accumulate sugars in roots and leaves at vegetative and flowering stages, which was higher than chitosan and humic acids expect Ch2 in leaves, at the vegetative stage.

Table 8. Effect of foliar spray with chitosan and humic acid on total sugars concentration in roots and leaves of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	Treatments	Roots (mg/g fresh weight)		Leaves (mg/g fresh weight)	
		Vegetative	**Flowering**	**Vegetative**	**Flowering**
Control	Control	0.599	1.241	0.998	0.813
	Chitosan (0.25g L^{-1})	0.399	0.727	0.528	1.069
	Chitosan (0. 5g L^{-1})	0.385	0.542	2.253	1.084
	Humic acid (10ml L^{-1})	0.143	0.955	0.784	1.141
	Humic acid (20 ml L^{-1})	0.086	0.827	0.585	0.970
Mean		0.322	0.858	0.858	1.030
2000 ppm	Control	0.342	2.296	0.599	0.913
	Chitosan (0.25g L^{-1})	0.086	1.155	0.756	2.552
	Chitosan (0. 5g L^{-1})	0.299	0.585	1.126	1.640
	Humic acid (10ml L^{-1})	0.143	1.141	0.585	1.597
	Humic acid (20 ml L^{-1})	0.057	0.998	0.870	1.255
Mean		0.185	1.235	1.235	0.787
4000 ppm	Control	1.069	0.371	1.155	2.538
	Chitosan (0.25g L^{-1})	0.057	0.741	0.442	0.599
	Chitosan (0. 5g L^{-1})	0.314	0.913	1.155	1.184
	Humic acid (10ml L^{-1})	0.114	0.856	0.784	1.084
	Humic acid (20 ml L^{-1})	0.699	1.226	0.770	0.884
Mean		0.451	0.821	0.861	0.861
6000 ppm	Control	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A
Main effect of foliar spray treatments					
Control		0.670	1.302	0.917	1.421
Chitosan (0.25g L^{-1})		0.181	0.875	0.575	1.407
Chitosan (0. 5g L^{-1})		0.333	0.680	1.511	1.302
Humic acid (10ml L^{-1})		0.133	0.984	0.718	1.274
Humic acid (20 ml L^{-1})		0.280	1.017	0.741	1.036
L.S.D	**Salinity**	ns	0.1968	0.1055	0.3051
	Treatments	ns	0.2540	0.1362	0.1362
	Salinity*Treatments	ns	0.4400	0.2360	0.6822

Increasing sugar accumulation in leaves and roots referred to a mechanism of salinity stress, non-treated cucumber (control) had observed to accumulate sugars excessively in roots and shoots when compared with the other treatments. Whereas treating cucumber by chitosan and humic acid were enhanced plant tolerant by other ways to accumulate sugars, which used in other beneficial metabolism pathways. Furthermore, chitosan and humic acid showed different trend in sugar accumulation. Results indicated that humic acid enhanced sugar accumulation rather than chitosan in leaves and roots. At the vegetative stage, sugars were accumulated in leaves in plants treated by Ch2 significantly, followed by both concentrations of humic acids. On the other hand, Ch1 was the lowest sugar accumulation in leaves at the vegetative stage. On the same manner, at the flowering stage, sugars were accumulated in humic acid treated cucumber which recorded significant higher rank when compared with chitosan treated plants.

Regarding the mean effect of the combination between the salinity stress as well as the studied treatments on the accumulation of sugars in roots and leaves of cucumber plant, it was deduced that plants tended to take a defense mechanism in response to salinity stress, which indicated in accumulating sugars in roots was counteracted by sugar concentration reduction in leaves, at the flowering and vegetative stages. These results were indicated in our study, on the other hand, the reduction of sugar in roots was counteracted by increasing of sugars in leaves. It was indicated that cucumbers sprayed by water showed an increase in sugar accumulation significantly in cucumber leaves at the vegetative stage, in which all treated cucumbers reduced sugar accumulation except Ch2, the highest significant record. However, these plants which sprayed by water showed a significant reduction in sugars when grown in 2000 ppm soil salinity, in leaves at the vegetative stage. Moreover, these plants showed significant increase when grown in 4000 ppm soil salinity in leaves at the vegetative stage. Furthermore, these plants showed significant increase in sugar accumulation in leaves and significant reduction in roots, at the flowering stage, when grown in non-saline soil (control) and 2000 ppm soil salinity. On the other hand, at the same stage, sugars recorded the highest significant record in water sprayed (control) leaves of cucumbers grown in 4000 ppm soil salinity among all studied treatments, however, they recorded the lowest significant concentration when compared with other treatments in roots.

These studied treatments showed different trends, in which Ch2 showed a significant highest record in leaves grown in non-saline conditions, at the vegetative stage. Furthermore, they recorded a significant increase in sugar accumulation in 2000 ppm and 4000 ppm soil salinity. On the other hand, it showed a significant reduction in roots at the flowering stage.

Furthermore, Ch1 showed a unique trend in sugar accumulation when compared to other studied treatments. At the vegetative stage, it showed a significant reduction in sugar accumulation in roots and leaves. Whereas it increased sugars accumulation in roots, at the flowering stage, mainly with increasing salinity, in which the sugars were highly significantly recorded in 2000 ppm soil salinity.

Total phenols concentration

The total soluble phenols concentration found in leaves and roots of cucumber, at vegetative and flowering stages, grown in saline affected soil and foliar sprayed by different doses of humic acid and chitosan were demonstrated in Table 9. Results concerning the effect of salinity stress, revealed that salinity has significantly recorded the highest concentration of total soluble phenols in cucumber leaves and roots, which are grown in 2000-ppm soil, at both vegetative and flowering growth stages. Furthermore, it was noticed significant increase in soluble phenols concentration at flowering stage more than vegetative stage in both leaves and roots.

Regarding the mean effect of studied treatments on free phenols accumulation in leaves and roots of cucumber plants grown in saline soil; results indicated that phenols were accumulated by humic acid which was significantly higher than other treatments; control, the non-treated cucumbers and chitosan, in both leaves and roots, at the vegetative and flowering stages. On the contract, chitosan showed a reduction in accumulating free phenols in leaves and roots, at vegetative and

Table 9. Effect of foliar spray with chitosan and humic acid on total phenols concentration in roots and leaves of cucumber at the vegetative and flowering stages under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	**Treatments**	**Roots (mg/g fresh weight)**		**Leaves (mg/g fresh weight)**	
		Vegetative	**Flowering**	**Vegetative**	**Flowering**
Control	Control	0.007	0.033	0.040	0.024
	Chitosan (0.25g L^{-1})	0.006	0.039	0.022	0.044
	Chitosan (0. 5g L^{-1})	0.006	0.030	0.010	0.021
	Humic acid (10ml L^{-1})	0.003	0.035	0.039	0.040
	Humic acid (20 ml L^{-1})	0.005	0.035	0.042	0.025
Mean		**0.005**	**0.035**	**0.031**	**0.030**
2000 ppm	Control	0.007	0.044	0.035	0.016
	Chitosan (0.25g L^{-1})	0.005	0.039	0.043	0.014
	Chitosan (0. 5g L^{-1})	0.008	0.037	0.043	0.075
	Humic acid (10ml L^{-1})	0.001	0.030	0.025	0.042
	Humic acid (20 ml L^{-1})	0.010	0.041	0.039	0.035
Mean		0.006	0.038	0.037	0.036
4000 ppm	Control	0.006	0.032	0.038	0.015
	Chitosan (0.25g L^{-1})	0.006	0.033	0.027	0.027
	Chitosan (0. 5g L^{-1})	0.004	0.040	0.024	0.035
	Humic acid (10ml L^{-1})	0.001	0.032	0.039	0.023
	Humic acid (20 ml L^{-1})	0.006	0.036	0.035	0.036
Mean		0.005	0.035	**0.033**	**0.027**
6000 ppm	Control	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A
Mean		N/A	N/A	N/A	N/A
	Main effect of foliar spray treatments				
Control		0.006	0.037	0.038	0.018
Chitosan (0.25g L^{-1})		0.006	0.037	0.031	0.028
Chitosan (0. 5g L^{-1})		0.006	0.036	0.026	0.043
Humic acid (10ml L^{-1})		0.002	0.032	0.035	0.035
Humic acid (20 ml L^{-1})		0.007	0.037	0.039	0.032
L.S.D	Salinity	ns	0.001628	0.001628	0.001628
	Treatments	0.0006648	0.0006648	0.0006648	0.0006648
	Salinity*Treatments	0.001151	0.001151	0.001151	0.001151

flowering stages, except in roots at the flowering stage. Ha2 recorded the highest significant accumulation in phenols in roots, at vegetative and flowering stages and in leaves at the vegetative stage. However, a reduction in phenols accumulation was observed by Ha2 in leaves at the flowering stage. On the contradictory, Ch1 significantly recorded the lowest free phenols accumulation in roots and leaves at both vegetative and flowering stage except in roots at flowering stage which was observed higher accumulation.

Concerning the mean effect of the combination of studied treatments and salinity stress, it was found the cucumbers which sprayed by water showed a significant reduction in phenols accumulation in leaves, at the vegetative stage and roots at both vegetative and flowering stages that grown in non-saline conditions. whereas, increasing salinity level to 2000 ppm showed a significant increase in roots at flowering stage only, however, leaves in both stages and roots at vegetative stage, did not show any significant increase. Furthermore, these plants showed a significant reduction in leaves and roots at both vegetative and flowering stages, which indicates these plants cannot withstand this level of salination.

In respect of the effect of studied treatments on the phenol accumulation, it was indicated that the studied treatments had different trends among different studied salinity levels. In which chitosan showed highest significance phenols accumulation in 2000 ppm at higher concentration (Ch2) in leaves at both vegetative and flowering stages.

However, Ch2 increased and reduced phenols accumulation in roots at vegetative and flowering stages, respectively which grown in the same salinity level. In addition, a significant reduction has been observed by Ch2 in roots and leaves at both growth stages except in roots at flowering stage, when salinity level has been increased to 4000 ppm. Furthermore, Ch1 showed a reduction in leaves and subsequently increased, at the vegetative and flowering stages, respectively in non-saline soil. Whereas a significant reduction observed in same treatment in roots at both growth stages. Increasing salinity level resulted in increase in phenols in leaves and roots at the vegetative and flowering stages, respectively. Furthermore, increasing soil salinity to 4000 ppm reduced phenols accumulation. Finally, Ch2 showed an increase in phenol accumulation somehow, however, Ch1 did not enhance phenols accumulation.

In the respect of effect of humic acid under different salinity conditions, results indicated that Ha2 significantly increased phenols accumulation in leaves and roots in cucumbers grown in 2000 ppm, at vegetative and flowering stages except its accumulation in leaves at the flowering stage. On the other hand, Ha2 has reduced phenols accumulation in leaves and roots at both growth stages with increasing salinity to 4000 ppm.

The trend of cucumbers under different treatments showed enhancing under 2000 ppm salinity level, whereas increasing salinity showed a reducing in phenols accumulation which could be referred to obstacles in synthesis process.

Photosynthetic Plant Pigments

The chlorophyll a, chlorophyll b, total chlorophyll, carotenoids concentration and total chlorophyll: carotenoids as well as chlorophyll a: chlorophyll b ratio in leaves of cucumber, at the vegetative growth stage were demonstrated in Table 10. Cucumber has grown in saline affected soil and foliar sprayed by different doses of humic acid and chitosan. Results concerning the effect of salinity stress on chlorophyll parameters, revealed that cucumber grown in 2000-ppm soil recorded the highest concentration of total chlorophyll which resulted from the significant increase in both chlorophyll a & chl. b concentrations. Furthermore, carotenoids concentration showed the same trend. Moreover, the ratio between total chlorophyll and carotenoids were recorded as the lowest value showing increasing chlorophyll concentration versus carotenoids concentration. Besides, the same trend was observed with ratio of both chl. a & chl. b.

Increasing soil salinity to 4000-ppm affected total chlorophyll concentration in cucumber leaves significantly, which noticed to be recorded the lowest value when compared with other studied salinity concentrations. Furthermore, the ratio between chl. a & chl. b concentration

Table 10. Effect of foliar spray with chitosan and humic acid on chlorophyll a, chlorophyll b, Total chlorophyll, carotenoids concentrations and ratio of chlorophyll a: b as well as total chlorophyll: carotenoids in leaves of cucumber under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

Salinity	Treatments	Leaves (mg/g fresh weight)					
		Chlorophyll a	**Chlorophyll b**	**Total chlorophyll**	**Carotenoids**	**Chlorophyll a: b**	**Total chlorophyll: carotenoids**
Control	Control	6.906	3.216	10.122	3.890	2.149	2.602
	Chitosan (0.25g L^{-1})	7.783	4.473	12.257	5.091	1.961	2.473
	Chitosan (0. 5g L^{-1})	9.070	5.849	14.920	6.989	1.572	2.140
	Humic acid (10ml L^{-1})	9.631	7.928	17.558	8.416	1.221	2.088
	Humic acid (20 ml L^{-1})	6.143	2.628	8.770	3.148	2.341	2.790
Mean		**7.907**	**4.819**	**12.725**	**5.507**	**1.849**	**2.419**
2000 ppm	Control	6.531	3.153	9.684	3.698	2.108	2.616
	Chitosan (0.25g L^{-1})	8.724	4.495	13.218	5.189	1.943	2.552
	Chitosan (0. 5g L^{-1})	9.494	7.764	17.259	8.244	1.297	2.121
	Humic acid (10ml L^{-1})	8.698	5.554	14.252	6.260	1.737	2.333
	Humic acid (20 ml L^{-1})	7.307	4.768	12.075	4.961	1.646	2.502
Mean		**8.151**	**5.147**	**13.298**	**5.671**	**1.746**	**2.425**
4000 ppm	Control	4.821	2.400	7.222	2.819	2.078	2.569
	Chitosan (0.25g L^{-1})	6.680	3.325	10.005	3.655	2.060	2.749
	Chitosan (0. 5g L^{-1})	5.981	3.478	9.459	4.407	2.139	2.090
	Humic acid (10ml L^{-1})	6.786	4.115	10.900	4.248	1.767	2.582
	Humic acid (20 ml L^{-1})	7.632	3.962	11.595	4.524	1.946	2.582
Mean		**6.380**	**3.456**	**9.836**	**3.931**	**1.998**	**2.515**
6000 ppm	Control	N/A	N/A	N/A	N/A	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A	N/A	N/A	N/A	N/A
Mean		N/A	N/A	N/A	N/A	N/A	N/A
		Main effect of foliar spray treatments					
	Control	6.086	2.923	9.009	3.469	2.111	2.596
	Chitosan (0.25g L^{-1})	7.729	4.098	11.827	4.645	1.988	2.591
	Chitosan (0. 5g L^{-1})	8.182	5.697	13.879	6.547	1.669	2.117
	Humic acid (10ml L^{-1})	8.372	5.866	14.237	6.308	1.575	2.334
	Humic acid (20 ml L^{-1})	7.027	3.786	10.813	4.211	1.978	2.625
L.S.D	Salinity	0.9703	0.9938	1.919	0.9583	ns	0.024
	Treatments	1.253	1.283	2.478	1.237	0.2154	0.037
	Salinity*Treatments	ns	2.222	ns	2.143	0.3731	0.53

was increased as well as same trend observed in ratio between total chl. and carotenoids concentration which indicating the adverse effect exerted by salinity stress on chlorophyll parameters.

Regarding the mean effect of studied treatments on chlorophyll parameters, results indicated that total chlorophyll has significantly increased in cucumbers treated by Ch1, Ch2 and Ha1 when compared with other treatments. Moreover, Ch2 and Ha1 were recorded as the highest significant total chlorophyll concentrations when compared with other studied treatments. The observed increase in the total chlorophyll was deduced from an increase in the chlorophyll a and chlorophyll b concentrations in these treatments. In addition, a significant increase was observed in carotenoids concentration. On the other hand, these treatments indicated a reduction in the ratio between chlorophyll a and chlorophyll b, which confirmed that chlorophyll b was increased on the expenses of chlorophyll a concentration reduction. Furthermore, control plants, the non-treated cucumbers were the lowest total chlorophyll concentration among all the studied treatments. This reduction was due to the reduction in chlorophyll a and b concentrations, besides carotenoids concentration was observed to be reduced. Moreover, the ratio between chlorophyll a and chlorophyll b was significantly recorded as the highest record indicating the ratio between chlorophyll a and b was not also affected concentration of each chlorophyll was reduced. Regarding the chitosan effect on chlorophyll concentration, Ch1 was significantly recorded as the highest chlorophyll a concentration among all other studied treatments. In addition to a reduced chlorophyll b and carotenoids concentration when compared with other studied treatments. Furthermore, the ratio between chlorophyll a and b was high referring to the normal ratio between both chlorophylls. As a result, Ch1 was the superior treatment in respect of chlorophyll parameters. Consequently, Ha2 was followed Ch1, which increased chlorophyll a, as well as chlorophyll b and carotenoids were reduced, subsequently the ratio between Chl. a, and Chl. b was reduced. However, both Ch1 and Ha2 had a similar trend, but Ch1 was the superior which ranked the higher values in chlorophyll concentration and suitable ratios between chlorophylls concentration.

The mean effect of the combination of studied treatments and salinity stress demonstrated in the Table 10, which indicated that in non-saline soil conditions, cucumber sprayed by water showed a significant increase in chlorophyll and significant reduction in chlorophyll b and total carotenoids. Besides, the same results were observed with the same treatment under different soil salinity levels; both 2000 and 4000 ppm. In the respect of chitosan and humic acid treating effect, results indicated that Ch1 has increased chlorophyll a significantly, whereas an increase observed significantly in chlorophyll b and carotenoids, which considered reduction when compared to Chl. a. these results were found in cucumbers grown in non-saline soil, and 2000 ppm salinity level as well as in 4000 ppm soil salinity conditions and treated by Ch1. Which indicated Ch1 enhanced concentration of Chl. a under different salinity levels, besides an increase in Chl. b and carotenoids. On the same manner, Ha2 showed similar trend in cucumbers grown in non-saline soil salinity as well as those grown under 4000 ppm soil salinity, in which Chl. a recorded the highest significant value in non-saline soil, as well as its concentration had been increased under 4000 salinity level with non-significantly difference with those grown under non-saline conditions. In addition, Chl. b recorded an increase and carotenoids recorded a reduction, while both was reduced when compared with Chl. a, in the same plants. On the contradictory, Ch2 as well as Ha1 increased Chl. b concentration significantly that recorded highest recorded when compared with the other treatments under 2000 ppm soil salinity and non-saline soil, respectively. In addition, carotenoids were increased significantly by these treatments under both non-saline conditions and 2000 ppm soil salinity. Whereas these treatments were increased Chl. a along with increasing Chl. b, however, a reduction in carotenoids under 4000 ppm salinity level. These results were confirmed by the ratio between chlorophyll a and b concentrations, which indicated that Ch1 and Ha2 had recorded the highest ratio when compared with other treatments, under different non-saline conditions as well as under other salinity levels.

Effect of Salinity Soil Stress and Foliar Spray of Chitosan and Humic Acid on Yield and Quality of Cucumber Plants

Salinity stress has affected significantly the yield and quality parameters; however, chitosan and humic acid were enhanced plant metabolites which enhanced their beneficial use.

The effect of salinity stress on fruit length has demonstrated in Figure 4. Results indicated that the highest salinity level to 6000 ppm had affected the plant germination, in which there were no cucumber seeds germinated in this soil. Whereas results showed a significant reduction in fruit length at 4000 ppm. Fruit length was affected significantly by highest salinity soil, 4000-ppm, however, no significant difference was observed between non-saline soil and 2000-ppm on fruit length.

Furthermore, the mean effect of studied treatments; foliar spray of chitosan (0.25g l^{-1} and 0.5g l^{-1}) and humic acid (10 cm l^{-1} and 20 cml^{-1}) on fruit length were showed in Figure 4. Results showed the highest significant record of fruits length were recorded by foliar spray of first concentration of chitosan (Ch1) on cucumber leaves. However, humic acid recorded a significant reduction when compared to the other studied treatments on fruit length.

Data presented in Table 11, where average fruit weight and yield per plant of cucumber were illustrated in which results showed they significantly due to effect of salinity stress, whereas, foliar spray of Ch or Hu treatments and their interactions. Regarding the effect of salinity stress on average fruit weight and yield per plant of cucumber, data indicated that salinity stress adversely affected average fruit weight and yield significantly, meanwhile with increasing salinity, both of average fruit weight and yield per cucumber plant were significantly reduced trend.

Concerning the effect of foliar spray treatments on average fruit weight and yield of cucumber plants; it was indicated that the foliar application of chitosan on leaves with concentration of 0.25g L^{-1} (Ch1) only has significantly enhancing effect on yield per plant compared to control, whereas all foliar spray treatments showed a significant increment of average fruit weight compared to control. Generally, the highest values of both average fruit weight and yield per plant of cucumber were recorded with the treatment of Ch1.

Cluster analysis

The dendrogram of cluster analysis of combination effect of both salinity and foliar spray of chitosan (0.25 g l^{-1} and 0.5 g l^{-1}) and humic acid (10 cm l^{-1} and 20 cm l^{-1}) on cucumber growth characters, biochemical components, at vegetative and flowering stages and yield and fruit quality were demonstrated in Figure 5.

The clusters were classified the combination effect of the studied treatments under various soil salinity levels into three main categories; group A, B and C. there was far distance between group A and C, in which Group C composed of six treatments which are all studied treatments grown in 4000-ppm soil salinity plus 1st conc. of humic acid applied in 2000-ppm soil salinity. This indicated that these treatments showed similar effects on plants in studied traits, besides recording the lowest values among studied traits. Moreover, group C showed similarity which recorded increased values, as well as showing the superiority of their treatments in which non-sprayed cucumbers under non-saline conditions which suggested the favorable condition for growing cucumbers was in similarity with chitosan (1st conc) in 2000-ppm soil salinity, Chitosan (2nd conc) in non-saline and 2000-ppm soil, non-sprayed cucumbers in 2000-ppm, humic acid (1st and 2nd conc) in non-saline and 2000-ppm, respectively. This proved that these treatments were categorized in the same groups in affecting on studied traits.

DISCUSSION

Salinity is a dramatic global problem which threatens the agricultural expansion and population growth (**Atta *et al.*, 2023a**). Finding solutions to overcome these environmental challenges will diminish the adverse effects of these obstacles. Crop improvement is a way to overcome these problems, which would be treating crops with inducers which enhance their tolerance towards stresses. In this study, salinity stress dramatically affected the cucumber growth which observed in shoot and root growth as mentioned above cucumber seed couldn't germinate in soil affected

Table 11. Effect of foliar spray with chitosan and humic acid on average fruit weight and yield per plant of cucumber under different levels of soil salinity (combined seasons of 2020/ 2021 and 2021/2022)

Salinity	Treatments	Average fruit weight (g)	Yield/ plant (kg)
Control	Control	149.20	1.882
	Chitosan (0.25g L^{-1})	168.70	2.598
	Chitosan (0. 5g L^{-1})	155.00	2.233
	Humic acid (10ml L^{-1})	148.00	2.195
	Humic acid (20 ml L^{-1})	161.80	2.442
	Mean	**156.50**	**2.270**
2000 ppm	Control	115.10	1.890
	Chitosan (0.25g L^{-1})	150.40	2.142
	Chitosan (0. 5g L^{-1})	110.20	1.803
	Humic acid (10ml L^{-1})	130.60	1.607
	Humic acid (20 ml L^{-1})	150.20	1.555
	Mean	**131.30**	**1.799**
4000 ppm	Control	124.00	1.540
	Chitosan (0.25g L^{-1})	135.70	1.708
	Chitosan (0. 5g L^{-1})	131.20	1.460
	Humic acid (10ml L^{-1})	132.00	1.500
	Humic acid (20 ml L^{-1})	123.00	1.437
	Mean	**129.20**	**1.529**
6000 ppm	Control	N/A	N/A
	Chitosan (0.25g L^{-1})	N/A	N/A
	Chitosan (0. 5g L^{-1})	N/A	N/A
	Humic acid (10ml L^{-1})	N/A	N/A
	Humic acid (20 ml L^{-1})	N/A	N/A
	Mean	N/A	N/A
Main effect of foliar spray treatments			
Control		97.08	1.328
Chitosan (0.25g L^{-1})		113.70	1.612
Chitosan (0. 5g L^{-1})		99.12	1.374
Humic acid (10ml L^{-1})		102.70	1.326
Humic acid (20 ml L^{-1})		108.80	1.359
L.S.D	**Salinity**	1.37	0.035
	Treatments	1.67	0.054
	Salinity*Treatments	3.33	0.109

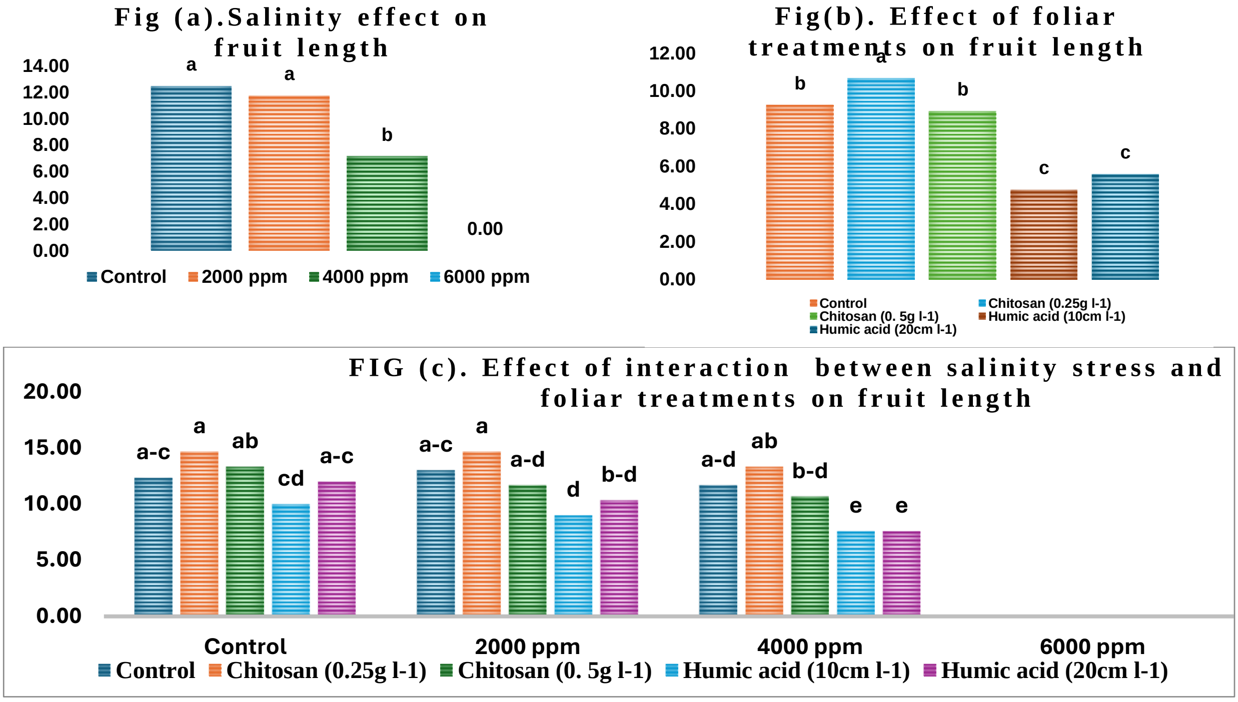
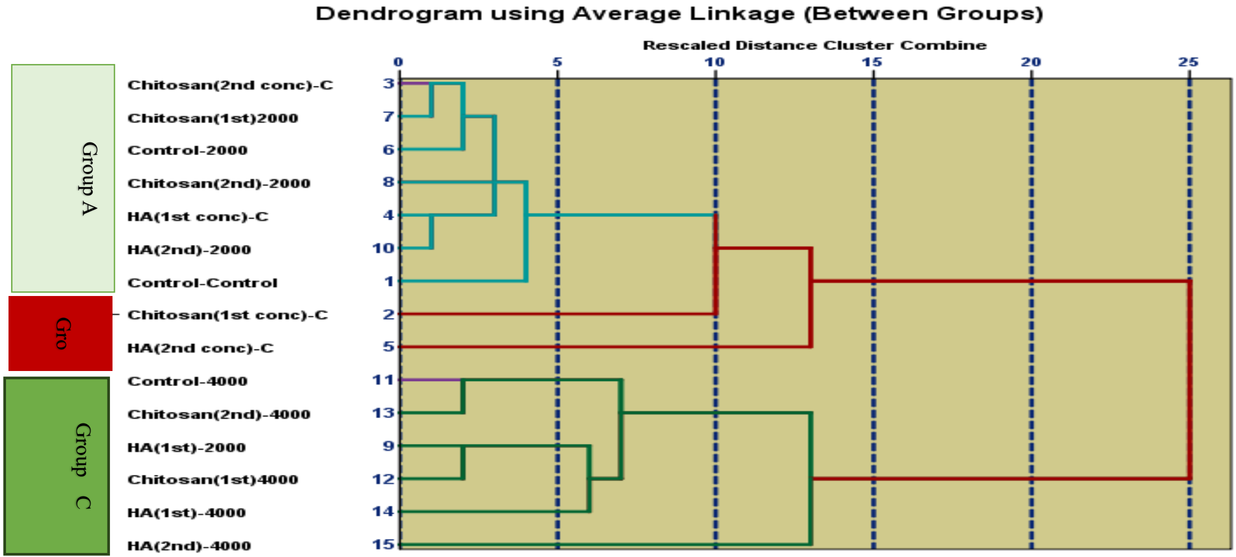


Fig. 4. Effect of different levels of soil salinity (A), foliar treatments (B), and the interaction between salinity stress and foliar treatments (C) on fruit length, (combined seasons of 2020/ 2021 and 2021/2022)



Group A: Control-control: control (non-sprayed cucumber in non-saline soil), Control-2000: non-sprayed cucumber in 2000-ppm soil, Chitosan (1st)-2000: 0.25 g l^{-1} chitosan in 2000-ppm soil, Chitosan (2nd)-C: 0.5 g l^{-1} chitosan in non-saline soil, Chitosan (2nd)-2000: 0.5 g l^{-1} chitosan in 2000-ppm soil, HA(1st conc)-C: 10 cm l^{-1} humic acid in non-saline soil and HA(2nd conc)-2000: 20 cm l^{-1} humic acid in 2000-ppm soil.

Group B: Chitosan (1st conc)-C: 0.25 g l^{-1} chitosan in non-saline soil, HA (2nd conc)-C: 20 cm l^{-1} humic acid in non-saline soil.

Group C: Control-4000: control (non-sprayed cucumber in 4000-ppm soil), Chitosan (2nd)-4000: 0.5 g l^{-1} chitosan in 4000-ppm soil, HA(1st conc)-2000: 10 cm l^{-1} humic acid in 2000-ppm soil, Chitosan (1st)-4000: 0.25 g l^{-1} chitosan in 4000-ppm soil, HA(1st conc)-4000: 10 cm l^{-1} humic acid in 4000-ppm soil, and HA(2nd conc)-4000: 20 cm l^{-1} humic acid in 4000-ppm soil.

Fig. 5.Effect of foliar spray with chitosan and humic acid on dendrogram hierarchical cluster analysis of cucumber under different levels of soil salinity (combined seasons of 2020/2021 and 2021/2022)

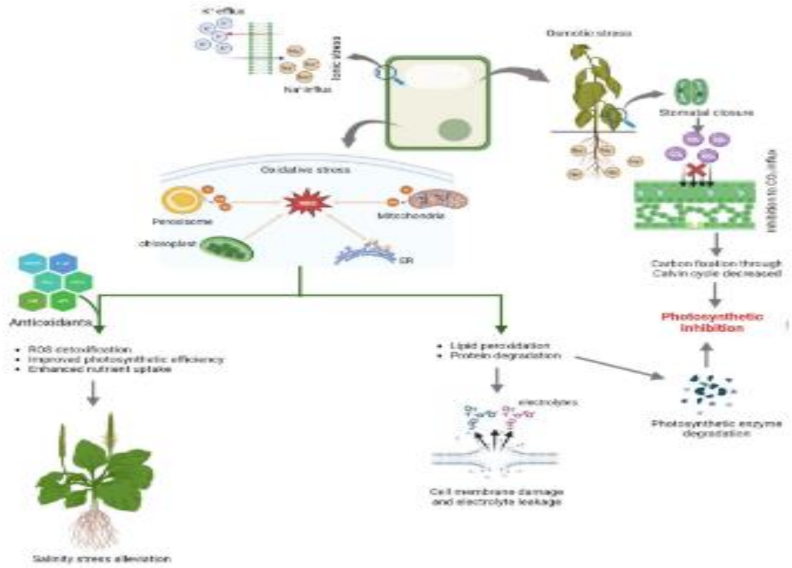


Fig. 6. Different physiological alterations in plants under salinity and role of antioxidants in stress alleviation as illustrated by Atta *et al.* (2023b)

by 6000 ppm (8.5 dS/m), besides decreasing soil salinity to 2000 ppm and 4000 ppm affect cucumber growth and biochemical components. These results were in harmony with other studies, which mentioned that cucumber (*Cucumis sativus* L.) is evaluated for salinity as a moderately sensitive crop. They reported that cucumber tolerated an *Ece*; electrical conductivity, of soil paste extract to 2.5 dS/m. As well as the cucumber fruit yield reduced about 13% directly to each increase in *ECe* unit above the threshold value. These results were reported by **Maas and Hoffman 1977**). In addition to **Sonneveld and Voogt (1978)** who indicated when cucumber irrigated by saline water with ranged from 0.1 to 4.5 dS/m, the yield decreased linearly in response to *ECiw* increased. They reported that the reduction was reported about 17% in correlation with *ECiw* unit increase. **Jones *et al.* (1989)** found that 4.0 dS/m was significantly decreased the cucumber fruit yield. All these studies reported the reduction in yield and growth of cucumber plants in response to the salinity increases either in water irrigation or in soil, which was declared by **Wan *et al.* (2010) and Abdel-Farid *et al.* (2020)**, which were in harmony with our results.

Under salinity conditions, the normal metabolic pathways were disrupted in plants, in which plants possess several metabolic pathways to withstand and response to stress elicitors. Salinity stress mentioned to effect plants by both osmotic stress and ionic stress, and subsequently plants suffer nutritional and oxidative stresses (**He *et al.,* 2018**).

The negatively effects of salinity were mentioned to begin with abiotic stresses mainly osmotic and salinity stresses, which represented in diminishing the plant root's ability to absorb water and then followed by ionic stress, which represented the nutritional imbalance, and subsequent resulted in formation of ROS species, hormonal imbalance, and susceptibility to infection by the pathogen **(Talbi Zribi *et al.,* 2018; Singh *et al.,* 2022**). In the respect of osmotic stress derived from salinity, plant roots senses changes in osmotic pressure of soil solution which in turned by several mechanisms had carried in response to this situation. These plants experience alternations in carbohydrate and oil metabolism which contribute to energy conservation and production of osmo-protectants against the elicited stress. On the same manner, plants tended to enhance its ability to absorb water from surroundings, through increasing its osmotic potential.

Plants pose changes in glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway that leads to alternations in sugars, phenols, amino acids metabolic changes as well, which contribute to osmotic adjustment in the cell. Their accumulation indicated a mechanism posed by plants in a way of osmoregulation and osmotic protection for plant cells which enhance their tolerance to osmotic stress as mentioned by **Nadeem *et al.* (2020)**, which was demonstrated in Fig. 6. Besides the altering in water relations, transpiration, nutritional imbalances, stomatal conductance, and oxidative damage happened due to salinity stress, which contributed to a yield drop, the ionic stress because of competition of the Na^+ and Cl^- with other needed minerals like potassium and nitrogen as well as the toxicity effect of these ions are impairing the plant growth and yield production of plants as mentioned by **Kaveh *et al.* (2011), He *et al.* (2018) and Atta *et al.* (2023b)**. In plants, the pathway from glutamine is the primary route for the synthesis under osmotic stress conditions, and the pathway from ornithine is known to operate under nitrogen limitation, which could occur under salt stress **(Delauney *et al.,* 1992; Suprasanna *et al.,* 2016a)**. Meanwhile, there are studies indicated biochemical and chemical inducers were mentioned to play a role in enhancing plant tolerance in which each had a specific effect on plants which alter the metabolic activities to produce osmo-protectants. In which all these agents are acting in achieving the same aim that is osmoregulation and homeostatic regulation for the cell (**Suprasanna *et al.,* 2016b**). On the other hand, as found in our results, when a reduction in osmo-regulators accumulation, such as sugars, phenols, and amino acids, has been observed in the stressed conditions due to applying protective agents, this could be concluded that these agents were enhanced the plant mediation in a way to withstand the existing stress and directing the metabolites to growth and enhancing beneficial use of metabolites instead of accumulating these osmo-regulators. By this way, plants can use these products in a way to enrich beneficial use of photosynthetic assimilates as a strategy of reduction of primary metabolic costs.

These results were in harmony with previous evidence had proved by investigators worked on transgenic plants development with overexpressing biosynthetic enzymes for osmo-protectants, such as mannitol, GB, D-ononitol, or sorbitol, which had resulted in accumulation of these compounds in levels too low to give protective benefits solely through osmotic mass action (**Huang *et al.,* 2000**).

On the same manner, our study has showed a variation and changes in osmolytes accumulation under effect of chitosan and humic acid treatment in cucumbers grown in saline soil. Our study noticed that chitosan and humic acid were found to play a role in accumulating sugars in cucumber organs in a way to protect and enhance plant tolerance to stress. In our study, chitosan showed the highest significant treatment which reduced sugar accumulation in cucumber organs, leaves and roots. In the same manner, a similar trend was observed in free proline accumulation also, in which non-treated plants (control) were accumulated free proline in roots, at flowering growth stage, however, the foliar treated cucumber by either chitosan or humic acid was observed a reduction in free proline accumulation. These results referred to role played by these inducers in increasing cucumber meditation to the salinity stress in which these plants had reduced their accumulation to the osmo-regulator, the free proline in roots. Furthermore, roots are the main plant organ that require osmo-regulators accumulating substance which play a role in osmotic adjustment in roots suffered from osmotic and salinity stress. Chitosan recorded the lowest significant free proline accumulation in leaves and roots at the flowering stage. This result indicated the protective role of chitosan to the plants that posed a reduced proline accumulation in leaves at the flowering stage. Whereas humic acid had increased accumulating free proline in roots and leaves at the flowering stage when compared to chitosan. These results in proline accumulation proved the protective role of chitosan and humic acid in plants, whereas chitosan was showed the superior in playing this role when compared to humic acid and control. Furthermore, our results showed an accumulation of amino acids in the vegetative stage in leaves which subsequently increase its osmotic potential against osmatic effect exerted by salinity stress. Furthermore, the osmotic potential in leaves was increased which subsequently maintains the water stream flow feeding to cucumber leaves tissue. Despite of accumulating these osmolytes had a beneficial role in osmotically adjustment, which was observed its excessive accumulation in the vegetative growth however, the contradictory has been observed at the flowering stage, in which the use-efficiency of these product was rewarded towards fruits development. Thus, a reduction in amino acids accumulation at flowering stage has been observed than the vegetative stage, which referred to cucumber mediation to stress along withstanding higher yield production. Thus, chitosan showed superior treatment when compared with humic acid as well as non-treated cucumbers. Because chitosan showed higher adaptation in accumulating amino acids at the vegetative stage and showed higher use-efficiency in transferring these osmolytes to withstand the fruit development requirements. Moreover, humic acid showed higher accumulation in free phenols accumulation in both roots and leaves at both growth stages, the vegetative and flowering. On the other contradictory, accumulating phenols were observed at the flowering stage only in roots in chitosan treated plants, especially Ch1. These results confirmed that both chitosan and humic acids enhanced cucumber adaptation to elicited stressed conditions, which indicated by accumulating osmolytes in roots and leaves, at both vegetative and flowering stages.

Whereas chitosan showed higher use-efficiency of these osmolytes, the biproducts of photosynthesis process, which were observed to enhance its accumulation in leaves at the vegetative stage rather than flowering stage in respecting to fruit development stage requirements. Moreover, chitosan enhanced their accumulation in roots in respect of osmotic adjustment. These evidence was confirmed by several investigators, (**Suprasanna *et al.,* 2016a**) had mentioned that osmo-protectants (proline, glycine betaine, gamma-aminobutyric acid and sugars) accumulation is a response found in different plant systems in response to stresses which encouraged the researchers to devise strategies for improving the plant tolerance through manipulation of the osmolytes accumulation in plants through alternations in the expression of core biosynthetic enzymes or their derivatives and expression of related transporters. They mentioned that abiotic stresses such as drought, salinity, low temperature, and flooding will result in increased sugar.

Additionally, some studies investigated the role played by antioxidants in enhancing the resistance of the transgenic plants in which sugar alcohols could function as scavengers of activated oxygen species.

In the respect of chlorophyll parameters, the increase in chlorophyll concentration referred to enhanced photosynthesis apparatus and adapting the surrounding conditions, and consequently affect the productivity of the plants. As a result, the plant showed higher growth activity and productivity. In addition, the ratio between chlorophyll a and chlorophyll b showed to be changed in which the increase in chlorophyll b concentration on the expenses of chlorophyll a showed plants suffering from stress, which increased the accessory pigments to support Chlorophyll a performance. In the respect of chlorophylls parameters, Ch1 and Ha2 were the highest performing treated cucumbers when compared to the other studied treatments.

Both humic acid and chitosan showed a protective role played in plants against stress, mainly in osmotic adjustment. Furthermore, chitosan was superior in efficient using photosynthetic products to adapt the osmotic adjustment besides withstanding fruit development requirements.

Our results were in harmony with who mentioned that osmotic adjustment in plants suffering stresses, which showed an effective strategy for stress tolerances, as well as accumulation proline, the osmo-protectants, as well as glycine betaine and sugars was defined a defense in different plant systems. In addition, investigators have contributed a progress in increasing plant tolerance to stresses in which metabolic conduit genetically engineered has been conducted for number of compatible solutes accumulation, for instance, glycine betaine, proline, mannitol, sorbitol and trehalose.

Consequently, this led to a successful demonstration for osmolytes role in plant resistance which indicated the transgenic plants had displayed an increase in resistance to drought stress, high salinity and cold stress as reported by **Suprasanna *et al.* (2016)**. Moreover, **Radomiljac *et al.* (2013)** added that the sugars increase in plant organs is well associated with osmotic adaptation and protection in which sugars are also known to play a role in signal transduction mechanisms during abiotic stress environment. In addition, metabolomics has often been crucial in clarifying the unique and shared biochemical changes that occur in plants. Additionally, proline play a role as antioxidant, play a role of ROS scavenging activity besides to its singlet oxygen quenching ability (**Suprasanna *et al.,* 2016a**). **Liang *et al.* (2013)** stated that proline is synthesized from glutamate and ornithine, with ornithine being converted into P5C/GSA via ornithine-δ-aminotransferase. According to **Delauney *et al.* (1992)**, the pathway from glutamate is the primary route for proline synthesis under osmotic stress, while the pathway from ornithine operates under nitrogen limitation. In chloroplasts, an increased rate of proline biosynthesis during stress helps maintain a low NADPH: NADP+ ratio, supports electron flow between photosynthetic excitation centers, stabilizes redox balance, and reduces photoinhibition and damage to the photosynthetic apparatus (**Hare and Cress, 1997**). Upon recovery from stress, proline is converted back to glutamate by P5C dehydrogenase (P5CDH). Additionally, proline accumulation has been reported under conditions of drought, salinity, and freezing (**Verslues *et al.,* 2006**). All these investigations confirmed the increase of osmolytes in plant cell during abiotic stress is a way of defense in such stresses; oxidative, drought and salt stress, however, if these solutes were reduced under stress as well as plant is still conserving its growth, this is indicating the protective role played by chitosan. Chitosan is known to support plant growth and development and protect against microbial threats such as fungi, bacteria, viruses, nematodes, and insects. Additionally, recent updates on chitosan's antimicrobial and insecticidal properties were discussed. These effects are further examined concerning chitosan’s interactions with phytohormones like jasmonic acid (JA), salicylic acid (SA), indole acetic acid (IAA), abscisic acid (ABA), and gibberellic acid (GA). The stress-induced redox shift in cellular organelles may be influenced by chitosan's involvement with reactive oxygen species (ROS) and antioxidant metabolism, including hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Chitosan has been found to enhance plant tolerance to osmotic and salinity stress, as well as to act as an anti-transpirant (**Pandey *et al.,* 2018**). Our findings align with other researchers who reported that chitosan (CTS) promotes plant growth and increases tolerance to abiotic stress. Exogenous CTS application can mitigate the harmful effects of salt stress on lettuce plants, increasing proline and soluble sugar accumulations and enhancing peroxidase and catalase activities, thus reducing oxidative damage to leaves. CTS also reduced sodium accumulation while increasing potassium accumulation in the leaves of NaCl-treated plants. These results may help optimize lettuce production under saline conditions.

However, the mechanism by which CTS alleviates salinity damage is not fully understood. Future research should focus on analyzing Na+/K+ transporter gene expressions and possible signal transduction pathways involved in CTS-regulated increased tolerance to salt stress. The amine and hydroxyl groups in CTS may also prevent Na+ from reaching photosynthetic tissues by chelating it at the root or lower tissue level, depending on the CTS application method (**Zhang *et al.,* 2021**).

Humic acid, the active constituent of organic humus, is not a fertilizer but a soil conditioner or plant bio-stimulant that improves plant growth and productivity through various beneficial effects on soil and plant attributes. Humic acid directly and indirectly affects plant growth and development by stimulating enzymes involved in many biological processes, enhancing plant resistance to biotic stress, improving stomatal conductance, transpiration rate, chlorophyll synthesis, and photosynthesis rate, promoting sugar and amino acid metabolism, and increasing cell wall thickness, thereby prolonging fruit storage periods. Humic acid application also improves nutrient uptake and enhances the vegetative growth, flowering, and fruiting characteristics of papaya. The effects of humic acid are dose-dependent, with high concentrations inhibiting nutrient accumulation. Humic acid has been shown to improve shoot length and diameter, leaf area, fruit weight, dimensions, firmness, anthocyanin content, total soluble solids (TSS), and the TSS: acid ratio, while decreasing fruit drop and acidity in ‘Anna’ apple trees (**Ennab *et al.,* 2023**).

Conclusion

Cucumber was detrimentally affected by salinity stress in respect of plant growth, and the biochemical components as well as yield and fruit quality. However, cucumber response to salinity was withstand to some limit of salinity which was 2000-ppm soil salinity. The increasing salinity of more than 2000-ppm was not acceptable to cucumber and hardening its recovery. The leaves application of humic acid and chitosan enhanced plants under effect of salinity, whereas their effect was dose dependent. Nevertheless, foliar application of chitosan in concentration of 0.25 g L^{-1} was the superior treatment for enhancing cucumber growth characters, biochemical composition and yield as well as fruit quality under 2000 ppm of soil salinity. Thus, it is recommended to apply 0.25 g L^{-1} chitosan on cucumber leaves which reflect plant yield and fruit length.

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التخفيف من التأثيرات الضارة لإجهاد ملوحة التربة على النمو والتركيب الكيميائي الحيوي ومحصول الخيار باستخدام الرش الورقي لحامض الهيوميك والشيتوزان

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التأثير المخفف للرش الورقي بكل من حامض الهيوميك والشيتوزان على نمو الخيار في الاراضي الملحية تم توضيحه. أجريت تجربة أصص خلال موسمين ناجحين2021/2020 و2022/2021 وكان التصميم الإحصائي قطاعات كاملة العشوائية. زرعت بذور الخيار يوم 20 سبتمبر من كل موسم في تربة ملحية من أربع تركيزات (صفر و2000 و4000 و6000 جزء في المليون) مع استخدام خمس معاملات وهي الرش بالماء كمعاملة الكنترول بالإضافة الى تركيزين للرش الورقي من حامض الهيوميك (10 و20 مللي/اللتر) وكذلك الرش بتركيزين من الشيتوزان (0.25 و0.5 جرام/ لتر) وتم استخدام مادة توين 20 كمادة ناشرة. تم أخذ العينات لتحديد قياسات نمو النبات والتركيب الكيميائي الحيوي في كلا من المجموع الخضري والجذري خلال مرحلتي النمو الخضري والتزهير بالإضافة الى صفات الجودة والمحصول الكلي للنبات. أشارت النتائج الى ان زيادة التركيز الملحي في التربة أثر على انبات البذور حيث انه لم يكون هناك انبات للبذور في تركيز 6000 جزء في المليون. أثرت زيادة الملوحة بشكل معنوي على صفات النمو حيث أدت الى انخفاضها. نسبة كلوروفيل أ: ب زادت وكذلك تراكم البرولين والسكريات والأحماض الأمينية والفينولات وعلى الجانب الأخر انخفض متوسط وزن الثمرة والمحصول. استخدام كلا من الهيوميك والشيتوزان حسن من النمو والتركيب الكيميائي الحيوي داخل النبات. وكان للتركيز المستخدم لهذه المواد تأثير مستقل. الشيتوزان بتركيز 0.25 جرام/اللتر تفوق على الهيوميك بالنسبة لصفات النمو وتوزيع المادة الجافة بالإضافة للمحصول وطول الثمرة.

الكلمات الاسترشادية: الخيار (*Cucumis sativus*) والملوحة وحامض الهيوميك والشيتوزان.

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