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Effect of Humic Acid and Ascorbic Acid on Seed Germination and Growth of Cucumber (*Cucumis sativus* L.) under Salinity Stress

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Abstract: The aim of this study was to investigate the effects of Humic Acid (HA) and Ascorbic Acid (AsA) on cucumber growth under different levels of soil salinity. The experiment was designed as a factorial, using a completely randomized design in the laboratory of Samangan University in Afghanistan. The levels of salinity stress were set at (0, 50, and 100mM sodium chloride) while AsA and HA were set at (0, 30, and 60mM and 0, 1 and 2. L⁻¹) respectively. The results of the analysis showed that the effect of all factors were significant on all studied traits. The highest mean daily germination (19%), root length (56.22mm), shoot length (39,06mm), fresh shoot weight (183.7mg), and seed vigor index were obtained from the (0mM salinity+60mM AsA+ 2g.L⁻¹ HA) treatment. Also the most germination percentage (95%), fresh root (45.88mg) and dry root (13.76mg), observed in (50mM salinity+0mM AsA+ 2g.L⁻¹ HA) treatment. However, the combined amounts of (60mM AsA+ 2g.L⁻¹ HA) were more effective for reducing different levels of salinity and increasing the growth characteristics of cucumber. Also, 2g L⁻¹ HA and 60mM AsA alone, were more effective. Treatments without AsA and HA showed the lowest growth in most traits. These findings suggest that the application of HA and AsA can help to improve cucumber growth under salt stress conditions.

Keywords: Abiotic stress, Dry shoot, Fresh shoot, Germination and Root length.

Introduction

Crops are affected by a variety of biotic and abiotic stresses. Among abiotic stresses, salinity is one of the factors that reduces agricultural production, which is a major problem in arid and semi-arid regions (Jaraghili *et al.*, 2016). Soil salinity is a significant abiotic stress that reduces crop yields worldwide because it reduces the germination rate and initial seedling growth (Sen & Mandal, 2016). Sodium ion accumulation disrupts many cellular processes such as water conduction, photosynthesis, respiration, and plasma membrane function. It also increases electrical conductivity (EC) and causes food imbalance and toxicity in cells (Volkov & Beilby, 2017). Salinity delays the plant's ability to absorb water due to reduced water potential in the root zone. As a result, salinity stress affects the growth and development of plants, leading to reduced yield (Pourmeidani *et al.*, 2011). Seed germination and seedling growth are the most

sensitive stages to salinity. Salinity stress causes adverse physiological and biochemical changes in germinated seeds, affecting seed germination and root establishment through osmotic stress, ion-specific effects, and oxidative stress (Ibrahim, 2016; Allela & Al-Hamdani, 2019). Approximately 35% of the regions around the world that are exploited to produce crops in arid and semi-arid regions suffer from salinity, leading to reduced yield of cucumber and tomato plants due to reduced germination, seedling growth, poor plant establishment, low weight, and marketable fruits (Alsaeedi et al., 2018). Salinity has negative effects on the yield of cucumber and tomato plants by reducing germination, seedling growth, plant establishment, fruit weight, and marketability (Sato et al., 2006).

Humic acid (HA) is one of the main components of humic matter, which is the main organic compound in soil (Humus). It is produced by the biodegradation of dead organic matter. Although it is not an acid, humic acid is a complex mixture of different acids containing carboxyl and phenol groups. This mixture functions as a biphasic or sometimes as a triphasic acid (Akinci et al., 2009). Commercial humic acid products typically contain 44-58% C, 42-46% O, 6-8% H, and 0.5-4% N, as well as many other elements (Larcher, 2003). Humic acid increased Mn, Fe and Zn elements of cucumber under salinity stress (Demir, et al., 1997). The mechanism of action of humic acid in boosting plant growth is not fully understood, but various explanations have been proposed. These include increased cell membrane permeability, oxygen uptake, respiration, and photosynthesis, as well as phosphate uptake and increased root length (Türkmen et al., 2004). Previous studies and research have confirmed the beneficial effect of humic substances on plant physiology,

including direct effects on cell membrane permeability, respiration, nucleic acid biosynthesis, ion absorption, hormone and enzyme activity (Chen & Schnitzer, 1978). In cucumber seeds, soaking with 5000 mg. L⁻¹ of HA for 48 hours, 10000 mg. L⁻¹ of HA for 12 and 48 hours, and 15000 mg. L⁻¹ of HA for 12 and 48 hours significantly increased fresh shoot and root weight, as well as germination percentage, compared to the control group (Hartwigsen & Evans, 2000). Cucumber plants treated with 5 mgL⁻¹ and 100 mg L⁻¹ concentrations of HA exhibited increased shoot dry weight (Mora et al., 2010). In a study on radish cultivars, humic acid was reported to increase shoot length, seedling fresh weight, and germination rate (Rohani et al., 2016).

Elevating the concentration of humic acid via drenching or spraying applications soil enhances the growth, fruit yield, and quality of cucumbers. (Karakurt, et al., 2015). The use of 100 mg L⁻¹ of humic acid increased plant height by about 10.50%, while the use of 300 mg. L^{-1} of humic acid showed the highest value of dry matter, 10.6% more than the control group (Al-Madhagi, 2019). Humic acid also had a significant impact on reducing the negative effects of salt stress, as the highest germination percentage, germination rate, pedicle length, radicle length, fresh and dry weight, and seed vigor index were measured in the treatments of 0.009 mgL^{-1} HA+6dSm⁻¹ and 0.009 mgL^{-1} HA+12 dS m⁻¹ in Medicago sativa (Sofi et al., 2018). Application 50 mg.L⁻¹, of HA can affect the physiological processes and morphological properties of the Hypericum perforatum under salinity stress (Rasouli & Noroozisharaf, 2022).

Ascorbic acid, also known as vitamin C, plays a regulatory role in increasing the productivity of many plants by acting as a factor for several enzymes and regulating phytohormone-mediated signaling processes

(Barth et al., 2006). Seed treatment with ascorbic acid before sowing is widely used to improve performance and stability under various external factors such as high salinity. Studies have shown that seed treatments with ascorbic acid and salicylic acid significantly increased the germination percentage, but the mean germination time (MGT) was not affected by all initial treatments. However, the use of 50 ppm ascorbic acid significantly increased the root and stem length, as well as the fresh and dry weight of seedlings in wheat (Afzal et al., 2005). It has various roles in plant growth, including cell division function, cell wall expansion, and other growth processes (Behairy et al., 2012). The ascorbic acid can regulate antioxidative metabolism in many plants (Anjum et al., 2014) and ascorbic acid can be improved by exogenous by foliar spray and its application through rooting medium (Athar et al., 2008). As a bio-regulator, ascorbic acid plays an essential role in salinity tolerance and has been shown to enhance osmolytes accumulation by effective at low concentrations in cucumber under salt stress (Maach et al., 2021).

Cucumber is the most important greenhouse plant in semi-arid regions with saline groundwater. Therefore, more research is needed to investigate the effects of salinity on germination, growth, and chemical composition of this plant (Allela & Al-Hamdani, 2019). Due to the increasing salinity of soil and water and its limiting effects on growth and crop production of many plants, the aim of this study is to determine the direct effects of humic acid, ascorbic acid, and the interaction between humic acid and ascorbic acid on the germination and initial growth of cucumber seedlings under different levels of salinity.

For studying the effect of HA and AsA with salinity stress on cucumber germination, seeds were prepared in Samangan Agriculture Research Institute. The study was done under condition (25+3°C, 57-60% controlled humidity and 13h light/11h dark) for a period of seven days in Botany Laboratory located in Samangan University in June 2021. For antiseptic of seeds, 5% sodium hypochlorite was put them in this solution for 1 minute and washed in distilled water (Zu et al., 2017). This experiment was conducted as factorial based on a completely randomized design with 27 treatments and three replications. The experiment included three levels of salinity (0mM, 50mM, and 100mM sodium chloride), three levels of ascorbic acid (0, 30, and 60mM), and three levels of humic acid (0, 1 and 2g.L⁻¹) and 20 seeds were placed in each petri dish containing filter paper. Six milliliters of the desired solutions were added to each petri dish. The first counting of germinated seeds took place after 48 hours. After seven days, having finished the growth period. Root and shoot length were measured by software (J Micro Vision 1.2.7), after that fresh shoot and root were dried in an oven with a temperature of 65°C to measure dry biomass. Fresh and dry weights of roots and shoots were measured by a digital scale with an accuracy of 0.001g. The germination percentage (GP), coefficient velocity of germination (CVG), main daily germination (MDG) and seed vigor index (SVI) were measured based to equations. 1-4):

1- Germination Percentage = $\frac{\text{No.of seeds germinated}}{\text{Total No.of seeds planted}} \times 100$ (Abdul-Baki & Anderson, 1973).

2-
$$CVG = (\sum_{i=1}^{K} \int i / \sum_{i=1}^{K} \int i x_i) 100$$

(Ranal & Santan, 2006).

Materials & Methods

Where *fi* is number of seeds germinating every day *i*; *xi*: number of days from sowing, and *k*: last day of germination.

3- MDG =
$$\frac{GP}{\text{length of test period}}$$
 (Scott *et al.*, 1984).

4 SVI = mean of initial stem length + the mean of initial root length) × final germination percentage (Ajithkumar*et al.*, 1998).

Data factorial analysis of variance based on one way analysis of variance (ANOVA) was performed using the SAS software (version 9.1) and analysis of comparison of means were done by the least significant difference (LSD) method at $p \le 0.05$ level and Excel program was used to draw the tables.

Results & Discussion

ANOVA result showed a significant difference between salinity, humic acid and ascorbic acid about in all traits. The LSD test was run only on the following interactions considering the significant of the two interactions.

 Table (1): ANOVA of Main Daily Germination (MDG), Germination Percentage (GP), Seed

 Vigor Index (SVI), and Coefficient Velocity of cucumber under different treatments.

Mean Squares								
Source of Variation	DF	MDG	GP	SVI	Coefficient velocity	Root length		
Salinity	2	100.50**	2511.4**	39731593.0**	0.0*	271.0**		
Ascorbic Acid (AsA)	2	3.7**	92.9*	7407850.8**	0.0*	77.5**		
Humic Acid (HA)	2	2.6**	67.0**	4376158.3**	0.0*	32.3**		
Salinity*AsA	4	1.9*	48.9*	475920.5ns	$0.0^{\rm ns}$	49.2**		
Salinity *HA	4	0.9**	23.0**	79314.9*	0.0*	1.1**		
AsA* HA	4	0.2**	7.3*	48304.1*	0.0*	0.5*		
Salinity *AsA* HA	8	0.1*	3.5 ^{ns}	42441.3*	0.0*	0.4*		
Error		0	1.9	14613.3	0	0.2		
CV		1.5	1.6	1.8	6.9	0.9		
		0.01.0						

**, * and ns indicate statistical significance at 0.01, 0.05 levels, and non-significance, respectively.

Main Daily Germination (MDG)

The results of analysis variance showed that the main effect of HA, AsA, and salt stress and the interactions of Salinity+HA and AsA+HA had a significant at P< 0.01 and interaction of Salinity+AsA and Salinity+AsA+HA had a significant at P< 0.05 for mean daily germination (Table 1). The highest percentage of MDG (19.00%) was observed in the control treatments, 30mM AsA, 19.00%, and 60mM AsA, 19.00%. Additionally, the treatments 50mM salinity+30mM AsA+2g.L⁻¹ HA, 18.00%; 50mM salinity+60mM AsA+2g.L⁻¹ HA, 18.00%, and 50mM salinity+60mM AsA+1g L⁻¹ HA, 18.00 had the highest MDG, which were significantly different from the salinity. treatment without The lowest percentage was observed in 100mM salinity+0mM AsA+0g.L⁻¹ HA; 100mM salinity+0mM AsA +1g.L⁻¹ HA, and 100mM salinity+0mM AsA +2g L⁻¹ HA treatments respectively (Table 2). As seen in the 100mM AsA+0g.L⁻¹ 14.00% salinity+0mM HA, treatments, salt reduced stress seed germination, while in the treatment 50mM salinity+60mM AsA+2g.L⁻¹ HA, 18.00%, it was seen that the combination of ascorbic acid and humic acid reduced salinity stress and increased germination, as Sofi et al. (2018) has stated that humic acid also had a significant impact on reducing the negative effects of salt stress and also (Maach *et al.*, 2021) pretended ascorbic acid plays an essential role in salinity tolerance and has been shown to enhance osmolytes accumulation.

Germination Percentage (GP)

The results of the ANOVA showed significant effects of HA, salt stress, and the interaction between salinity and HA at P< 0.01 on the germination percentage of cucumber, while the main effect of AsA and the interaction between salinity and AsA and AsA with HA had a significant effect at P < 0.05, (Table 1). The highest germination percentages obtained from the 50mM salinity+0mM AsA+2g.L⁻¹ HA, 95%; 50mM salinity+0mM AsA+1g.L⁻¹ HA, 95%; 100mM salinity+30mM AsA+1g L⁻¹HA, 95% and 100mM salinity+30mM AsA+2g.L⁻¹ HA, 95%, which, except of the treatment 100mM salinity+30mM AsA+0g.L⁻¹ HA, 92.22% had a significant difference with the other treatments. The lowest germination percentage was obtained in the treatment of 0mM salinity+30mM AsA+0g.L⁻¹HA, 70.22% (Table 2). As Behairy et al. (2012) expressed, the ascorbic acid has various roles in plant growth, including cell division function, cell wall expansion. The part that was observed in the above treatments, the combined treatments of HA and AsA reduced salinity stress and led to more seed germination and this result was similar to those reported with Hartwigsen & Evans (2000) and Sofi et al. (2018).

Seed Vigor Index (SVI)

The results of the ANOVA indicated that the main effects of HA, AsA, and salt stress were significant at (P< 0.01), and the interaction effects of salinity with HA; AsA with HA and salinity with AsA and HA were significant at (P< 0.05) on the SVI. However, the interaction effect of salinity with AsA was not significant on SVI, (Table 1). The treatment with 0mM Salinity+60mM AsA+2g L⁻¹ HA, 9052 showed

the highest SVI, which was significantly different from the other treatments, and the lowest SVI was obtained from the 100mM Salinity+0mM AsA+0g L⁻¹ HA, 4746), which had a significant difference from the other treatments (Table 2). As it was observed, the combination of humic acid and ascorbic acid increased the vigor index compared to the control, but it seems that they do not have much effect in reducing the salinity stress. This result was similar by founding of (Awad & Ahmed, 2020), that they reported, when cucumber seed's soaked in 03% HA that increased SVI up to 846.56. The positive effects of humic acid on germination and plant growth of seedlings can be for the reason to better water absorption and transporting of the stored materials to the root and shoot in addition Positive effect of HA on plant growth is, may be due to hormone activity of HA on membrane permeant ability of root cells, cellular respiration, various enzymatic reactions, photosynthesis and protein synthesis (Canellas et al., 2002).

Coefficient Velocity

ANOVA result showed a significant effect (P< 0.05) of all treatments on the coefficient velocity, except for the salinity+AsA treatment (Table 1). The highest coefficient velocity was obtained from the 50mM Salinity + 60mM AsA + 0g.L⁻¹HA and 100mM Salinity + 30mM AsA + $1g.L^{-1}$ HA (0.304 and 0.3) treatments, respectively. The lowest coefficient velocity was observed in the 0mM salinity + 60mM AsA+ 0g L⁻¹ HA, 0.205; 50mM salinity + 60 mM AsA + 2g.L⁻¹ HA, 0.211 and 0mM salinity + 60mM AsA+ 1g L⁻¹ HA, 0.212 treatments (Table 2). As the results showed that increasing salinity with decreasing HA caused increasing coefficient velocity. As can be seen in the table, ascorbic acid decreased the salinity level and increased the coefficient velocity, but humic acid did not show much effect on the coefficient velocity. According to the results of this study (Weerasekara et al., 2021) indicated that in contrast to humic acid, which had no significant impact on the coefficient of velocity, ascorbic acid was able to reduce salinity levels and enhance the coefficient of velocity. Furthermore (Kadhim, and Hamza, 2021) illustrated that Ascorbic acid was effective in reducing salinity and increasing coefficient velocity, whereas humic acid had no substantial impact on coefficient velocity

Root length

The ANOVA result showed that the main effects of HA, AsA, and salt stress, as well as the interaction effects of salinity+HA and salinity+HA, had significant effects at (P <0.01), while the interaction effect of HA+ AsA and salinity+AsA+HA had significant effects at (P < 0.05) on the root length of cucumber plant (Table 2). The longest root length was obtained from the treatment of 0mM salinity + 60mM AsA+2g.L⁻¹ HA, 56.22mm, which was significantly different from all other treatments. After that the treatments of 0mM salinity + 60mM AsA +1g.L⁻¹ HA and 0mM salinity + 60 mM AsA + 0g.L^{-1} HA had the longest root lengths (54.65 and 52.9 mm)

respectively. The lowest root length was obtained from the treatments of 100mM salinity + 30mM AsA + 0g. L⁻¹ HA, 42.62mm) and 100mM salinity + 0mM AsA+1g.L⁻¹ HA, 43.46mm which were significantly different from the other treatments, (Table 2). Based on the results, that the AsA had the main effect on root length, and its combination with 2g.L⁻¹ of humic acid was the most effective in promoting root length and the part that can be seen in the 100mM salinity + 60mM AsA + 2g L^{-1} HA, 49.15mm; 100mM salinity + 60mM AsA+1g.L⁻¹ HA, 47.82mm and 50mM salinity +30mM AsA+ 2g.L⁻¹ HA, 47.51mm treatments, the combination of ascorbic acid and humic acid has reduced the level of salinity stress to some extent and has caused an increase in the root length. This result similar to (Akladious et al., 2018), they stated, when 50mM, 100mM AsA was applied in turnip, there was significant increase in root length, may be due to increased cell division within the apical meristem of seedling roots due to enhanced indole acetic acid and cytokinin levels in the root tissues.

summy stress at L.S.D (p ^{-0.05}).								
Treatments	Seed vigor index	Coefficien t velocity	MDG%	GP %	Root length (mm)			
0mMSalinity+0mMAsA+0g L ⁻¹ HA	7156 ^f	0.229 ^{efg}	19.00 ^a	86.11 ^{bcde}	45.61 ^h			
0mMSalinity+0mMAsA+1g L ⁻¹ HA	7577 ^e	0.235^{defg}	19.00 ^a	86.3 ^{bcde}	46.89 ^{fg}			
0mMSalinity+0mMAsA+2g L ⁻¹ HA	8122 ^d	0.244^{bcdefg}	19.00 ^a	84.63 ^{de}	48.67 ^d			
0mMSalinity+30mMAsA+0g L ⁻¹ HA	7260^{f}	0.2517^{abcdef}	19.00 ^a	70.22 ⁱ	46.56 ^g			
0mMSalinity+30mMAsA+1g L ⁻¹ HA	7640 ^e	0.235^{defg}	19.00 ^a	86.3 ^{bcde}	47.52 ^{ef}			
0mMSalinity+30mMAsA+2g L ⁻¹ HA	8096 ^d	0.228 ^{efg}	19.00 ^a	75.74^{h}	48.63 ^d			
0mMSalinity+60mMAsA+0g L ⁻¹ HA	8346°	0.205^{fg}	19.00 ^a	71.3 ⁱ	52.9°			
0mMSalinity+60mMAsA+1g L ⁻¹ HA	8670 ^b	0.212^{fg}	19.00 ^a	85.56 ^{bcde}	54.65 ^b			
0mMSalinity+60mMAsA+2g L ⁻¹ HA	9052ª	0.2143^{fg}	19.00 ^a	87.59 ^{bcd}	56.22 ^a			
50mMSalinity+0mMAsA+0g L ⁻¹ HA	5766 ^{mn}	0.239 ^{cdefg}	16.67 ^d	71.22 ⁱ	43.6 ^j			
50mMSalinity+0mMAsA+1g L ⁻¹ HA	6164 ^{jk}	0.236 ^{cdefg}	17.33°	95ª	44.85 ⁱ			
50mMSalinity+0mMAsA+2g L ⁻¹ HA	6427 ⁱ	0.243^{bcdefg}	17.33°	95 ^a	46.51 ^g			
50mMSalinity+30mMAsA+0g L ⁻¹ HA	5799 ^m	0.239 ^{cdefg}	16.00 ^e	85.56 ^{bcde}	45.6 ^h			

Table (2): Main and interaction Effects of HA and AsA on some cucumber traits, under salinity stress at L.S.D (p<0.05).

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50mMSalinity+30mMAsA+1g L ⁻¹ HA	6404 ⁱ	0.243^{bcdefg}	17.00 ^{cd}	85 ^{cde}	46.56 ^g
50mMSalinity+30mMAsA+2g L ⁻¹ HA	7129 ^f	0.2193^{fg}	18.00^{b}	88.33 ^{bc}	47.51 ^{ef}
50mMSalinity+60mMAsA+0g L ⁻¹ HA	6258 ^{ij}	0.304 ^a	17.00 ^{cd}	71.11 ⁱ	46.53 ^g
50mMSalinity+60mMAsA+1g L ⁻¹ HA	6854 ^g	0.237 ^{cdefg}	18.00 ^b	76.67 ^{gh}	47.5 ^{ef}
50mMSalinity+60mMAsA+2g L ⁻¹ HA	7156^{f}	0.198 ^g	18.00^{b}	79.44^{fg}	48.51 ^d
100mMSalinity+0mMAsA+0g L ⁻¹ HA	4746 ^p	0.2803 ^{abcde}	14.00 ^g	72.3 ^j	43.58 ^k
100mMSalinity+0mMAsA+1g L ⁻¹ HA	5004°	0.288 ^{abc}	14.33^{fg}	86.3 ^{bcde}	43.46 ^j
100mMSalinity+0mMAsA+2g L ⁻¹ HA	5199°	0.2867^{abcd}	14.33^{fg}	86.85 ^{bcd}	44.48^{i}
100mMSalinity+30mMAsA+0g L ⁻¹ HA	5068°	0.288 ^{abc}	14.67^{f}	90.22ª	42.62 ^k
100mMSalinity+30mMAsA+1g L ⁻¹ HA	5589 ⁿ	0.3 ^a	15.67 ^e	95ª	43.68 ^j
100mMSalinity+30mMAsA+2g L ⁻¹ HA	5811 ^{lm}	0.294 ^{ab}	15.67 ^e	95 ^a	44.6 ⁱ
100mMSalinity+60mMAsA+0g L ⁻¹ HA	6005 ^{kl}	0.2407^{cdefg}	15.67 ^e	82.78 ^{ef}	46.61 ^g
100mMSalinity+60mMAsA+1g L ⁻¹ HA	6323 ^{ij}	0.2183 ^{fg}	16.00 ^e	87.22 ^{bcd}	47.82 ^e
100mMSalinity+60mMAsA+2g L ⁻¹ HA	6654 ^h	0.211^{fg}	16.00 ^e	88.89 ^b	49.15 ^d

Means followed by the same letter (s) within the column are not significantly different based to LSD test at (P < 0.05)

 Table (3): ANOVA of root length, shoot length, fresh shoot, dry shoot, fresh root and dry root of cucumber under different treatments.

Mean Squares							
Source of Variation	DF	Shoot length	Fresh shoot	Dry shoot	Fresh root	Dry root	
Salinity	2	317.3**	7748.6**	133.9*	280.5**	25.2**	
Ascorbic Acid (AsA)	2	99.5**	2390.3**	435.4**	587.0**	52.8**	
Humic Acid (HA)	2	124.8**	400.5**	85.3**	34.9**	3.1**	
Salinity*AsA	4	13.8**	129.2**	17.5**	4.9**	0.4**	
Salinity *HA	4	5.0**	32.6**	2.2**	7.5**	0.7**	
AsA* HA	4	$0.7^{ m ns}$	37.7**	0.8ns	7.7**	0.7**	
Salinity *AsA* HA	8	1.6**	34.0 ^{ns}	2.2**	3.4 ^{ns}	0.3 ^{ns}	
Error		0.4	4.1	0.4	1.8	0.2	
CV		2	1.3	2	3.5	3.5	

**, * and ns indicate statistical significance at 0.01, 0.05 levels, and non-significance, respectively.

Shoot length

The ANOVA result showed that the main effect of HA, AsA, salt stress, and the interaction effect of salinity+HA, salinity+ AsA and salinity+ HA+AsA, had a significant difference effect on shoot length at (P<0.01), but the HA+AsA had no significant difference effect on shoot length (Table 3). The longest shoot length was obtained from the 0mM salinity+60mM AsA+2g L⁻¹ HA, 39.06mm treatment, which was significantly different from the other treatments. After that, the highest shoot length was observed in the 0mM salinity+60mM AsA+1g.L⁻¹ HA, 0mM salinity+0mM AsA+2g.L-1 HA, and 0mM salinity+30mM AsA+2g.L⁻¹ HA, 36.61, 36.82 and 36.59mm respectively, which was significantly different from the other treatments and control. The lowest shoot length was obtained from the 100mM salinity+0mM AsA+0g.L⁻¹ HA, 25.21mm treatment, which was significantly different from all other treatments, (Table 4). As shown in 100mM salinity+60mM AsA+20g.L⁻¹ HA, 33.9mm treatment, the combination of ascorbic acid and humic acid was effective in reducing salinity and increased the Shoot length compared to the control. It can be concluded that ascorbic acid and humic acid reduced salinity stress and increase shoot length of cucumber. (Abdel Nabi & Obaid, 2019) reported that HA led to increase the level of endogenous substances like gibberellins, auxin, and cytokinin, also (Noreen *et al.*, 2021) reported that the salt stress significantly affected the shoot lengths in barley but foliar spray of AsA+Zn increased (113 and 103%) this parameter. Similar results were also observed by (Rohani *et al.* 2016 and Almadhagi 2019).

Fresh shoot weight

The ANOVA results showed that the main effects of HA, AsA, and salinity, as well as the interaction effects of salinity+HA, salinity+AaA and HA+AaA had a significant effect at (P<0.01) on the fresh shoot weight of Cucumis sativus L. (Table 3). However, the interaction effect of salinity+ HA+AsA was not significant. The highest fresh shoot weight was observed in 0 mM(183.7mg)salinity+60mM AsA+2g L⁻¹ HA treatment, which was significantly different from all other except the treatments for (0 mM)salinity+30mM AsA+2g.L⁻¹ HA, 181.0mg and salinity+60mM AsA+1g.L⁻¹ HA, 0mM 182.0mg treatments (Table 4). The treatments with HA and AsA increased fresh shoot weight compared to the without HA and AsA and as seams in treatment, (100mM salinity+60mM AsA+2g L⁻¹ HA) (158.3mg) and 50mM salinity+60mM AsA+2g.L⁻¹ HA, 168.3mg the combination of these two substances also decreased the salinity stress and increased fresh shoot weight than the control (0mM salinity+00mM AsA+0g L⁻¹ HA, 154.0mg). This increase may be due to the increased absorption of elements in the plants, as reported by Demir et al. (1997) Humic acid increased Mn, Fe and Zn elements of cucumber under salinity stress. As reported by Hartwigsen & Evans (2000) and Sofi et al.

(2018), humic acid has been significantly influencing the N%, protein%, and TSS% content in cucumber. Additionally, it may also be used as carrier for trace growth regulators and elements. Thus, increasing in nutrient uptake improve the vegetative growth of the plants, increasing cell division and stimulates plant growth hormones (Atiyeh *et al.*, 2002).

Dry shoot weight

The ANOVA result showed that the main effect of HA and AsA and the interaction effect of salinity+ HA, salinity+ AsA, and (salinity+ HA+AsA) had a significant effect at (P<0.01) on the dry shoot weight of Cucumis sativus L. (Table 3). However, the interaction effect of HA+AsA did not have a significant effect on the dry shoot weight. The treatment of 0mM salinity+60mM AsA+2g.L⁻¹ HA, 37.67mg had the highest dry shoot weight, which had a significant difference with all other treatments, then the highest dry shoot weight was observed in the 0mM salinity+60mM AsA+1g L⁻¹ HA, 50mM salinity+60mM AsA+2g L⁻¹ HA, and 100mM salinity+60mM AsA+2g L⁻¹ HA, 35.67, 35.67 and 35.33mg respectively, which had a significant difference with all other treatments except for the 0mM salinity+60mM AsA+2g L⁻¹ HA treatment. The lowest dry weight was obtained from the 100mM salinity+0mM AsA+0g L⁻¹ HA treatment, which had a significant difference with all other treatments except for the 100mM salinity+0mM AsA+1g.L⁻¹ HA and 100mM salinity+0mM AsA+2g.L⁻¹ HA treatments (Table 4). Although Interaction between ascorbic acid and humic acid, reducing salinity stress and had increased the dry shoot weight but, humic acid and ascorbic acid alone did not play a significant role in reducing salinity stress and increasing dry shoot weight, this increase in weight may be due to the absorption of more water and nutrients and the better performance of the plant for growth. As reported by a number of researchers, the ascorbic acid can regulate antioxidative metabolism in many plants (Anjum et al., 2014), ascorbic acid significantly increased the fresh and dry weight of seedlings in wheat (Afzal et al., 2005), ascorbic acid can improved by exogenous by foliar spray and it application through rooting medium (Athar et al., 2008) and elevating the concentration of humic acid via soil drenching or spraying applications enhances the growth, fruit yield, and quality of cucumbers. (Karakurt, et al., 2015). Also, humic acid increased plant height by about 10.50%, while the use of 300 mg L^{-1} of humic acid showed the highest value of dry matter 10.6% more than the control (Almadhagi, 2019) and cucumber plants treated with 5 mg L⁻¹ and 100 mg L⁻¹ of HA exhibited increased shoot dry weight (Mora et al., 2010).

Fresh root weight

The result of analysis of variance showed that the main effect of HA, AsA, and salinity, and the interaction effect of (salinity+HA+AsA) and HA+AsA had a significant effect at (P<0.01), but salinity+HA+AsA had no significant effect on the cucumber fresh root weight, (Table 3). The highest fresh root weight (45.88mg) was obtained from the (50mM salinity+0mM AsA+2g L⁻¹ HA) treatment, which had a significant difference with all other treatments except for the 100mM salinity+30mM AsA+2g.L⁻¹ HA; 50mM salinity+0mM AsA+1g.L⁻¹ HA, and 0mM salinity+60mM AsA+2g.L⁻¹ HA, 43.64, 43.16 and 42.71mg treatments, respectively. The lowest fresh root weight was obtained from the 100mM salinity+0mM AsA+0g.L⁻¹ HA, salinity+30mM and 100mM 27.31mg AsA+0g.L⁻¹ HA, 27.32mg, which had a significant difference with control (0mM salinity+0mM AsA+0g L⁻¹ HA) 38.09mg (Table 4). The comparison of the average treatments showed that the combination of HA

and AsA was more effective than using it alone to reduce salinity stress, as seen in the treatment of 100 mM salinity culture medium, where these two substances were not used, the root weight decreased. According of the results Mora et al. (2010) showed that HA is a phytonutrient and plant growth regulator that has been shown to significantly promote cucumber shoot growth through nitrate-related changes and modification of root-to-shoot distribution of polyamines, cytokinin and mineral nutrients, this increase in weight may be due to the absorption of more nutrients and the better performance of the plant for growth. As reported by a number of researchers, the ascorbic acid can regulate antioxidative metabolism in many plants (Anjum et al., 2014), cucumber seeds, soaking in HA, with significantly increased fresh shoot and root weight, compared to the control (Hartwigsen & Evans, 2000). Studies have shown, AsA as a bio-regulator, plays an essential role in salinity tolerance and has been shown to enhance osmolytes accumulation by effective at low concentrations in cucumber under salt stress (Maach et al., 2021). However, the use of AsA significantly increased the fresh and dry weight of seedlings in wheat (Afzal et al., 2005). Also, AsA has various roles in plant growth, including cell division function, cell wall expansion, and other growth processes (Behairy et al., 2012).

Dry root weight

The ANOVA result showed that the main effect of HA, AsA, and salinity and the interaction effect of salinity+HA; salinity+AsA and HA+AsA had a significant effect at (P<0.01), but salinity+ HA+ AsA did not have a significant effect on the dry root weight of cucumber (Table 3). The highest dry root weight (13.76, 13.09 and 12.95mg) respectively, was observed in the 50mM salinity+0mM AsA+2g.L⁻¹ HA; 100mM salinity+30mM AsA+2g.L⁻¹ HA and 50mM salinity+0mM AsA+1g.L⁻¹ HA treatments, which had a significant difference with control and all other treatments. The lowest dry root weight (7.21, 7.21, 7.32, 8.14 and 8.32mg) respectively, was obtained from the 50mM AsA+0g L⁻¹ HA; salinity+0mM 0mM salinity+60mM AsA+0g L⁻¹ HA; 100mM salinity+0mM AsA+0g L⁻¹ HA; 0mM salinity+30mM AsA+0g L⁻¹ HA and 100mM salinity+30mM AsA+0g L⁻¹ HA, respectively and had a significant difference with the other treatments (Table 4). The composition of HA with AsA reduced salinity stress and have a greater contribution to increasing dry root weight and also it seems that HA alone was more effective than AsA to reduce salinity stress and increase root dry weight of cucumber. Humic acid had a significant impact on reducing the salt stress, as the highest fresh and dry weight were measured in the treatments of 0.009 mgL⁻¹ HA+6dSm⁻¹ and

 $0.009 \text{ mg } \text{L}^{-1} \text{ HA+12 } \text{dS } \text{m}^{-1} \text{ in Medicago}$ sativa L. (Sofi et al., 2018). This increase in the dry weight of the root may be due to the increase in the absorption of elements as reported by some researchers, cucumber plants treated with HA exhibited increased shoot dry weight (Mora et al., 2010), humic acid increased Mn, Fe and Zn elements of cucumber under salinity stress (Demir et al., 1997) and HA increased cell membrane permeability, oxygen uptake, respiration, and photosynthesis, as well as phosphate uptake and increased root growth (Türkmen et al., 2004). As a bio-regulator, AsA plays an essential role in salinity tolerance and has been shown to enhance osmolytes accumulation by effective at low concentrations in cucumber under salt stress (Maach et al., 2021). review that Literature showed little information is available in effectives of the interaction of both substances.

Table (4): Main and interaction effects of HA and AsA on some cucumber traits, under salinity stress at L.S.D (p<0.05).

v	<u>u</u>	/		
U		•		Dry root
· /	(mg)			(mg)
29.72 ^{gh}		27.33 ^{ij}	38.09 ^{ef}	11.43 ^{ef}
32.87 ^d		31.33 ^{fg}	38.4 ^{ef}	11.52 ^{ef}
36.82 ^b	177.0 ^{cd}	34°	38.68 ^e	11.6 ^e
29.86 ^g	174.7 ^{de}	30.33 ^{gh}	29.21 ⁱ	8.14 ⁱ
32.90 ^d		31.67 ^{ef}	37.93 ^{ef}	11.38 ^{ef}
36.59 ^b	181.0^{ab}	34°	35.43^{fg}	10.63 ^{fg}
34.95°	179.3 ^{bc}	34°	28.3 ⁱ	7.21 ⁱ
36.61 ^b	182.0 ^{ab}	35.67 ^b	39.01°	11.7°
39.06ª	183.7 ^a	37.67ª	42.71 ^{abc}	12.81 ^{abc}
25.60 ^{op}	136.3 ^{nop}	24.33 ^{mn}	29.3 ⁱ	7.21 ⁱ
26.54 ^{no}	143.0 ¹	25.33 ^{lm}	43.16 ^{ab}	12.95 ^{ab}
27.65 ^{lm}	144.3 ¹	26.67^{jk}	45.88ª	13.76 ^a
26.89 ^{mn}	154.0 ^{jk}	26.67^{jk}	32.68 ^{gh}	$9.8 \mathrm{g}^{\mathrm{h}}$
28.78^{hij}	156.0 ^{ij}	29.67^{h}	38.68 ^e	11.6°
31.71 ^e	157.0^{hij}	31.33^{fg}	42.42 ^{bcd}	12.73 ^{bcd}
27.09 ^{lmn}	$160.0^{\rm h}$	33 ^{cd}	31.23 ^h	9.37^{h}
28.66 ^{ijk}	163.7 ^g	34°	35.21 ^{fg}	10.58 ^{fg}
31.00^{ef}	168.3 ^f	35.67 ^b	39.83 ^{cde}	11.95 ^{cde}
25.21 ^p	133.0 ^p	23°	27.31 ⁱ	7.32 ⁱ
26.24 ^{no}	135.0 ^{op}	23.67 ^{no}	38.74 ^e	11.62 ^e
	Shoot length (mm) 29.72gh 32.87d 36.82b 29.86g 32.90d 36.59b 34.95c 36.61b 39.06a 25.60°p 26.54n° 27.65lm 26.89mn 28.78hij 31.71e 27.09lmn 28.66 ^{ijk} 31.00 ^{ef} 25.21 ^p	r r	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

100mMSalinity+0mMAsA+2g L ⁻¹ HA	27.90 ^{jkl}	136.0n ^{op}	24.67 ^{mn}	39.31 ^{de}	11.79 ^{de}
100mMSalinity+30mMAsA+0g L ⁻¹ HA	26.46 ^{no}	136.7 ^{no}	26^{kl}	27.32 ⁱ	8.32 ⁱ
100mMSalinity+30mMAsA+1g L ⁻¹ HA	27.66 ^{klm}	138.7 ^{mn}	28.33 ⁱ	42.37 ^{bcd}	12.71 ^{bcd}
100mMSalinity+30mMAsA+2g L ⁻¹ HA	29.56^{ghi}	141.7l ^m	30 ^h	43.64 ^{ab}	13.09 ^{ab}
100mMSalinity+60mMAsA+0g L ⁻¹ HA	30.02^{fg}	151.3 ^k	32.67 ^{de}	37.22 ^{ef}	11.17 ^{ef}
100mMSalinity+60mMAsA+1g L ⁻¹ HA	31.21 ^e	144.3 ^{jk}	34°	38.41 ^{ef}	11.52 ^{ef}
100mMSalinity+60mMAsA+2g L ⁻¹ HA	33.99°	158.3 ^{hi}	35.33 ^b	38.41 ^{ef}	11.44 ^{ef}

Means followed by the same letter (s) within the column are not significantly different based to LSD test at (P < 0.05).

Conclusion

The results of the experiment showed that the combined treatments of 60mM AsA + $2g.L^{-1}$ HA and 60mM AsA + 1g L^{-1} HA were more effective in reducing the impact of different levels of salt stress and promoting cucumber growth about in all attributes. However, the main effect of humic acid was found to be more significant in reducing salinity stress and promoting growth compared to the main effect of ascorbic acid. If we look at tables (2 and 4), we come to the conclusion that the greater amounts of both substances have been more effective in reducing salinity stress and increasing the growth characteristics of cucumber, as 2g.L⁻¹ HA as alone has been more effective for reducing salinity stress and increasing traits of cucumber growth such as seed vigor index, coefficient, shoot length, root length, fresh shoot, dry shoot, fresh root and dry root compared to 1g.L-1 HA and effectiveness of 2g.L⁻¹ HA and 1g.L⁻¹ HA for the mean daily germination and germination percentage was the same. And also, 60mM AsA as alone has been more effective for reducing salinity stress and increasing cucumber traits such as dry shoot, fresh root, seed viguor index, coefficient, shoot length, root length, fresh shoot and dry root compared to 30mM AsA and the level of 30mM AsA was more effective only for the germination percentage trait.

To enhance the effectiveness of their research, researchers should explore higher concentrations of humic acid and ascorbic acid. Additionally, further studies are necessary to elucidate the interactive effects of both substances and determine their physiological impact.

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Contributions of authors

A. M. Y.: Constructed the idea and wrote the manuscript.

B. R.: Did the experiment, collection and analysed the data.

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Conflict of interest

There is no potential conflict of interest for the authors.

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تحت Cucumis sativus L. تاثير حامض المسكوربيك على إنبات البذور ونمو الخيار Cucumis sativus L. تحت ظروف الاجهاد الملحى

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المستخلص: تهدف هذه الدراسة إلى تأثير حامض الهيوميك (HA) وحامض الأسكوربيك (AsA) على نمو نبات الخيار تحت مستويات مختلفة من ملوحة التربة. صممت التجربة كتجربة عاملية باستخدام التصميم العشوائي الكامل في مختبر جامعة سمنگان في أفغانستان. وتم تحديد ثلاث مستويات من الاجهاد الملحي في التربة بالتراكيز 0، 50 و 100 ملى مولار من NaCl، في حين تم تحديد تراكيز حامضي الاسكوربيك والهيومك AsA و HA على 0، 30 و 60 ملى مولار. لتر⁻¹ 0، 1 و 2 جرام. لتر⁻¹ على الترتيب. أظهرت نتائج التحليل الاحصائي أن جميع الصفات المقاسة تأثرت بشكل كبير بالمعاملات المختلفة. اذ تم الحصول على الترتيب. أظهرت نتائج التحليل الاحصائي أن جميع الصفات المقاسة تأثرت بشكل كبير بالمعاملات المختلفة. اذ تم الحصول على أعلى متوسط للانبات وطول الجذر وطول الساق ووزن الساق الطازج ومؤشر قوة البذور في معاملة المقارنة من الملوحة مع 60 ملى مولار. لتر⁻¹ من AsA و 2 جرام. لتر⁻¹ من من المان الطازج ومؤشر قوة البذور في معاملة المقارنة من الملوحة مع 60 ملى مولار. لتر⁻¹ من AsA و 2 جرام. لتر⁻¹ من AsA و مع ذلك تم ملاحظة تقليل تأثير إجهاد الملوحة في المعاملات التي التي والهيومك AsA و (10 من الطازج ومؤشر قوة البذور في معاملة المقارنة من الملوحة مع 60 ملى مولار. لتر⁻¹ من AsA و 2 جرام. لتر⁻¹ من AH و مع ذلك تم ملاحظة تقليل تأثير إجهاد الملوحة في المعاملات التي تحتوي على مولار. لتر⁻¹ من ASA و 2 جرام. لتر⁻¹ من ASA و (00 ملى مولار. لتر⁻¹ من ASA و 5 جرام. لتر⁻¹ من ASA و (00 ملى مولار. لتر⁻¹ من ASA و 3 مكان و (00 ملى مولار. لتر⁻¹ من ASA و 4 AL) و (00 ملى مولار. لتر⁻¹ من ASA و 4 AL) و (00 ملى مولار. لتر⁻¹ معهم و معاملات التي (0.3 معملات التي المعاملات التي التي المالي المالي المعاملات التي المعاملات التي المالي معاملات التي المعاملات التي المالي و المالي و المالي و المالي و المالي معاملات التي و مول الل المالي المالي و و ASA و 4 AL و معاد و مالي المالي و 4 معملات المعاملات المعاملات المالي و 4 AL و 4 AL