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The Role of Humic Acid Application in Reducing Detrimental Effects of Salt in Cauliflower (*Brassica Oleraceae* L. Var. Botrytis)

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ABSTRACT
This study was conducted to determine effects of Humic Acid (HA) on yield and some quality characteristics of cauliflower under saline conditions. Control (0.3), 2, 4, 6and 8 dS·m^{-1} NaCl and 0, 1 and 2 g·kg^{-1} HA combinations were applied to the growth medium. Salt application generally negatively affected yield and curd growth, and negative effects were mostly due to salt concentrations >2 dS·m^{-1}. Increasing salt concentration positively affected quality characteristics of curds, and the highest total acid contents of dry matter and total soluble solids were detected in salt concentrations of 6-8 dS·m^{-1}. The HA application for decreasing harmful effects of salt brought to a successful conclusion in terms of total yield notably, and curd diameter and length, and the total amount of dry matter. While positive effects occurred mostly at salt concentrations ≥4 dS·m^{-1}; effects of HA application did not occur at NaCl concentration of 6 or 8 dS·m^{-1}. Two g·kg^{-1} HA produced better results for yield and curd weight, but application of 1 or 2 g·kg^{-1} HA produced similar effects for other parameters.

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Karnabaharda (*Brassica oleraceae* L. var. botrytis) Tuz Stresinin Azaltılmasında Humik Asit Uygulamalarının Rolü

ÖZET
Bu araştırmanın amacı; tuzlu koşullarda karnabaharın verim ve bazı kalite özellikleri üzerine Humik Asit (HA) uygulamalarının etkilerini ortaya koymaktır. Yetiştirme ortamına kontrol (0.3), 2, 4, 6 ve 8 dS·m^{-1} NaCl ve 0, 1 ve 2 g·kg^{-1} HA kombinasyonları uygulanmıştır. Araştırma sonuçlarına göre, tuz uygulamaları verim ve taç büyüme parametrelerini genel olarak önemli düzeylerde olumsuz etkilemiştir, bu olumsuz etkiler daha çok 2 dS·m^{-1} üzerinde artan tuz konsantrasyonlarında bulunmuştur. Buna karşın, artan tuz konsantrasyonları bitki taç kalite özelliklerini olumlu etkilemiş ve en yüksek kuru madde, suda çözünür kuru madde ve toplam asit içerikleri yüksek tuz konsantrasyonlarında (6 ve 8 dS·m^{-1}) tespit edilmiştir. Tuzun zararlı etkilerinin azaltılmasına yönelik yapılan HA uygulamalarından toplam verim başta olmak üzere taç çap ve boyu, toplam kuru madde miktarından olumlu sonuç alınmıştır. Bu olumlu etkiler daha çok düşük ve orta tuz konsantrasyonlarında (≥4 dS·m^{-1}) gözlemlenirken, yüksek konsantrasyonlarda (6 ve 8 dS·m^{-1}) HA uygulamalarının herhangi bir etkisi gözlemlenememiştir. Verim ve taç ağırlığında 2g·kg^{-1} HA uygulaması daha iyi sonuçlar verdiği ancak, diğer parametrelerde her iki uygulamanın (1 ve 2 g·kg^{-1}HA) benzer etkiler gösterdiği anlaşılmıştır.

Araştırma Makalesi

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INTRODUCTION
Salinity is one of the most important problems affecting production of vegetables with 20% of agricultural lands and 33% of irrigated areas worldwide affected. Soil salinization may occur

because of climate change, overuse of groundwater (in the regions near the seas), using low quality water for irrigation, and over-irrigation (Machado and Serralheiro, 2017). As a result of the impact of increasing temperature and decreasing rainfall in arid lands, salt cannot be washed away resulting accumulations in the topsoil. This situation limits growth of plant roots and cause abiotic challenges in plants. Excessive salt level causes serious physiological functional disorders, limiting vegetative and reproductive growth of vegetables and causes fertilization disorders decreases in marketing values, and plant death (Dolarslan and Gul, 2012). Salinity threshold levels that affect yield are between 1-2.5 dS·m^{-1} (Machado and Serralheiro, 2017). Cauliflower is a moderate salt-sensitive vegetable, and the salinity threshold level of irrigation water yield of cauliflower starts to decline between 1.9 and 2.7 dS·m^{-1} (Snapp et al., 1991; Kotuby et al., 1997).

In order to increase productivity in plant production in saline soils, soil characteristics need to be improved by adding organic matter (Asık et al., 2009). Organic based fertilizer application increases soil fertility, and improves physical, chemical, and biological features of soil, and resulting increases yield per unit area (Dolarslan and Gul, 2012). Humic substances (humic acid and fulvic acid), generally termed humas, are basic components of soil organic matter (Chen and Aviad, 1990). It was reported that humic substances are effective in decreasing salinity levels of soils and reducing toxic effects of heavy metals (Gumuzzio et al. 1985; Gerzabek and Ulah, 1990).

The effect of water shortage and salt level, use of humic substances for removing negative effects of elements in toxic quantities, and effects on plant growth were studied (Asık et al., 2009). Humic acid application decreases adverse effects of salt.

This study was conducted to determine the salt limit of irrigation water, used in cauliflower cultivation that would affect yield and some quality parameters, alone and in conjunction with humic acid.

MATERIAL and METHODS

This study was conducted in a plastic covered experimental greenhouse (a latitude of 40°01′N, and a longitude of 28°22′ E with an altitude of 22 m above sea level) at the research fields of Mustafakemal PasaVocational College, Uludag University in Bursa, Turkey. To prevent rainwater from reaching plants, the roof of protected culture structure was covered with polyethylene and the remaining parts were left open. In the study, commercial cauliflower (*Brassica oleraceae* L. *var. botrytis cv. Kartopu*) variety was used.

The experiment was a split plot arranged in a complete factorial with 4 repetitions. The main plot was salt concentration and the split was comprised of humic acid levels. Each repetition was comprised of a single plant in a metal barrel with 0.85 m in height and 0.58 m in diameter. 225 kg of air-dry sandy-loam soil (81%sand, 11% clay, 8.1% silt, 1.4% organic matter, 20.56% lime, with P_2O_5=19.6 ppm, K_2O=221.20 ppm, total N=0.56%, pH=7.8, EC=0.29 dS·m^{-1}, bulk density of 1.23 g·cm^{-3}, field capacity=25) was placed into barrels after being passed through a 2 mm sieve. Then, 5 cm sand-gravel mixture was placed at the base of the barrels for possible water drainage. Eighty kg·ha^{-1} of N (as ammonium nitrate), 60 kg of P_2O_5 (as super phosphate), and 5 kg of K_2O (as potassium sulphate), were applied to the barrels (Abd El-All and Mohammed, 2014). All the P and K fertilizers and half of N fertilizer were applied before planting and the other half of the N were applied4 weeks after planting.

Seedlings were obtained from a seedling company. Seedlings with 3-4 true leaves were planted into barrels at the beginning of August and were irrigated daily with tap water for 10 days after planting, and later when needed. No diseases occurred in plants, weeding, and the other necessary procedures were conducted regularly throughout the trial. Average temperature and humidity in the growth medium over the growing period were 24.2°C and 63%, respectively.

Treatments included the NaCl concentrations [0.3 (control), 2, 4, 6 or 8 dS·m^{-1}] as the main factor, and humic acid (HA) 0, 1 or 2 g·kg^{-1} as the split plot. The NaCl (Merck), which has a high solubility in water, was used to prepare saline irrigation water, and the salt was added into the tap water. Application was based on 30-40% drainage. Salt dose was increased gradually to protect plants from osmotic shock and full concentration reached in a 4-day period. Delta Humat, the source of humic acid, was applied by mixing into the soil before planting. The humic acid had characteristics of 12%, pH: 12.86, EC: 32.8 mS·cm^{-1} and was leonardite based.

Plants were harvested t 90 days after transplanting with curds cut at the soil surface.

Number of leaves per plant (NL); in the harvest period, all leaves were counted separately in each plant.

Plant height (PH, cm); plant height was determined by measuring the distance from the soil to the terminal point where the curd ended.

Curd diameter (CD, cm); the diameter of the curds were measured with a ruler and their average was determined as (cm).

Curd height (CH, cm) was measured by ruler

Curd weight per plant (CW); harvested cruds were weighed to determine the average crown weight (g).

Marketable yield (kg da^{-1}) was calculated considering that the plants could be planted at 50x70 cm (Vural et al., 2000) intervals to 1000 m^2 trial area.

Curds were cleaned by tap water, grounded with a blender, and dried in a drying oven at 70°C for 2 days, then weighed and dry matter determined (Noshadi et al., 2013). Total soluble solids were determined by amount of juice extracted from curds via a refractometer (Abbe-type refractometer, model 60/Direct Reading, Bellington and Stanley Inc., Kent, UK) (Tigchelaar, 1986). Total acidity was determined with titration of the same fruit juice using 0.1 N NaOH (Anonymous 1968).

Yield and quality parameter data from samples were subjected to the analysis of variance in IBM SPSS Statistics for Windows (ver. 20.0, IBM Corp., Armonk, NY). If the interaction was significant it was used to explain results. If the interaction was not significant means were separated using LSD test ($p<0.05$).

RESULTS and DISCUSSION

Increasing salt concentrations had a significant effect on the number of leaves of cauliflower plants. However, the results of the variance analysis indicated that the number of leaves in a plant was not affected by 4 $dS{\cdot}m^{-1}$ salt concentrations, but increasing salt concentrations from this point and up decreased the number of leaves significantly. Similar with our findings, Miceli et al. (2003) emphasized that the high soil salinity may reduce the number of leaves per plant. On the other hand, increasing HA doses have not affected the number of leaves per plant significantly. Indeed, the effect of NaCl x HA interaction on the number of leaves per plant was found insignificant. This result shows that HA applications treated are not effective in reducing the adverse effects of salt on the number of leaves (Table 1).

As seen in Table 1, plant heights decreased with the increasing NaCl doses, and stunting effect was emerged on the plants.

This effect was observed mainly upon the increasing of salt dose to the level of 4 $dS{\cdot}m^{-1}$. HA applications have impacted plant height positively contrary to the NaCl applications, and it has been detected that 1$g{\cdot}kg^{-1}$and 2 $g{\cdot}kg^{-1}$ applications have had similar impacts on the plant height.

Table 1. Effects of different NaCl and humic acid applications on cauliflower yield, some curd development parameters and quality properties

Treatments	NL	PH	CH	CD	CW	MY	DM	TSS	TA
		(cm)			(g)	(kg da^{-1})	(%)	(^{0}Briks)	(g l^{-1})
T_0 H_0	13.25	40.90 b	13.75 c	16.90 b	1363.75 b	3886.68 b	7.73 h	6.28	1.23
T_0 H_1	13.00	44.73 a	15.58 ab	18.20 a	1425.75 a	4063.38 a	7.98 gh	6.64	1.21
T_0 H_2	13.00	44.90 a	15.30 ab	18.48 a	1459.50 a	4159.58 a	8.13 fg	6.69	1.23
T_2 H_0	13.00	41.24 b	15.13 b	18.10 a	1348.50 b	3843.23 b	8.46 f	6.83	1.32
T_2 H_1	13.00	43.89 a	15.68 ab	18.25 a	1448.00 a	4126.80 a	8.39 f	6.80	1.31
T_2 H_2	13.00	43.98 a	16.53 a	18.55 a	1440.25 a	4104.71 a	8.44 f	6.81	1.31
T_4 H_0	12.75	38.90 b	11.90 d	15.08 c	1151.00 d	3280.35 d	9.87 cd	7.70	1.49
T_4 H_1	13.25	39.03 b	12.50 d	15.75 bc	1179.00 cd	3360.15 cd	9.95 c	7.73	1.48
T_4 H_2	12.50	39.20 b	12.85 cd	16.09 bc	1200.75 c	3422.14 c	10.20 c	7.74	1.51
T_6 H_0	9.00	27.40 cd	9.48 e	11.25 d	971.00 e	2767.35 e	10.59 b	8.75	1.93
T_6 H_1	9.50	29.08 c	9.03 e	11.75 d	960.75 e	2738.14 e	10.99 a	8.75	1.96
T_6 H_2	9.25	29.38 c	9.35 e	11.53 d	976.00 e	2781.60 e	11.26 a	8.72	1.94
T_8 H_0	7.75	23.30 e	7.03 f	7.50 e	821.50 f	2341.28 f	9.24 e	7.10	2.01
T_8 H_1	7.75	23.44 e	6.70 f	8.25 e	811.25 f	2312.06 f	9.51 de	7.06	2.02
T_8 H_2	8.00	25.55 e	6.78 f	8.00 e	834.50 f	2378.33 f	9.43 e	7.15	2.01
NaCl (dS m^{-1})									
0.3 (Control)	13.08 a	43.51 a	14.88 b	17.86 a	1416.33 a	4036.55 a	7.95 e	6.53 e	1.22 e
2	13.00 a	43.03 a	15.78 a	18.30 a	1412.25 a	4024.91 a	8.42 d	6.81 d	1.31 d
4	12.83 a	39.04 b	12.42 c	15.63 b	1176.92 b	3354.21 b	10.00 b	7.72 b	1.50 c
6	9.25 b	28.64 c	9.28 d	11.51 c	969.25 c	2762.36 c	10. 95 a	8.74 a	1.94 b
8	7.83 c	24.10 d	6.83 e	7.92 d	822.47 d	2343.89 d	9.40 c	7.11 c	2.01 a
Humicacid (HA, g kg^{-1})									
0	11.15	34.36 b	11.46 b	13.77 b	1131.15 c	3223.78 c	9.18 b	7.33	1.58
1	11.30	36.03 a	11.9 ab	14.44 a	1164.95 b	3320.11 b	9.36 a	7.40	1.60
2	11.15	36.60 a	12.16 a	14.53 a	1182.20 a	3369.27 a	9.49 a	7.42	1.60
NaCl	**	**	**	**	**	**	**	**	**
HA	ns	**	**	**	**	**	**	ns	ns
NaCl × HA	ns	**	**	**	**	**	**	ns	ns

**: 0.01 significant, ns: no significant

T_0, T_2, T_4, T_6, T_8 respectively, 0.3 (control), 4, 6, 8 dS m^{-1} NaCl; H_0, H_1, H_2 respectively,0, 1, 2 g kg^{-1} HA

NL: Number of Leaves per plant, PH: Plant height, CH:, Curd Height CD: Curd Diameter, CW: Curd weight per plant, MY: Marketable Yield, DM: Dry Matter, TSS: Total Soluble Solids, TA: Total Acidity

When the NaCl x HA interaction is taken into account, it can be understood that the adverse effect of NaCl (control, 2dS·m^{-1}, and 6 dS·m^{-1}) on plant height could partially be tolerated by HA. Meganid et al. (2015) obtained similar results in a study they conducted on bean. The researchers reported that they found significant increases in the heights of seedlings on which salt stress was applied with humic acid application.

In this study, the relationships between NaCl and HA applications, and curd heights of cauliflower were examined. Also, as seen in Table 1, the impact of NaCl x HA interaction on curd heights was found significant. Cauliflower curd height showed an increase until 2 dS·m^{-1} salt concentrations, and decreased with increasing salt concentrations above this level significantly. The positive impact of HA applications was mostly shown in 2 and 4 dS·m^{-1} salt applications. As compared 1 and 2 g·kg^{-1} HA applications, the impact of 2 g·kg^{-1} HA application on curd height was higher even though the difference was small (Table 1). In spite of that, any impact of HA application could not be found in high salt concentrations (6 and 8 dS·m^{-1} NaCl).

Head diameter of cauliflower showed a significant change depending on the NaCl application and the highest diameter was obtained from the control and 2 dS·m^{-1} doses. As the salt concentrations increased, curd diameter decreased at a statistically significant amount and smaller heads were harvested. When the NaCl x HA interaction is examined, it can be observed that the losses in curd diameters of the plants in the control group and 4 dS m^{-1} salt application group could partially be tolerated with adding HA into the plantation medium, and 1 g·kg^{-1} and 2 g·kg^{-1} HA applications had similar effects. However, the positive effect of HA was not observed in 2, 6 and 8 dS·m^{-1} salt doses (Table 1). The researchers obtained similar results in a previous study on pepper (cv. Demre), and they emphasized that HA application was an effective and cheap method in reducing the negative effects that emerge under moderate saline conditions (Cimrin et al., 2010).

The effects of the NaCl application on curd weight become statistically significant and the highest values were observed in the control and 2 dS·m^{-1} applications. When the salt dose was increased to 4 dS·m^{-1}, a decrease in the curd weight began and the weight decreased at higher rates as the salt doses increased. The impact of the NaCl x HA interaction was found significant. In the control, 2 dS·m^{-1} and 4 dS·m^{-1} NaCl applications, positive effects of the HA added the soil were observed. In other words, heavier cauliflower curds were obtained by the HA that was added in the plantation medium. Especially, the impact of 2 g·kg^{-1} HA application was found higher. However, any impact of the HA applications with high salt doses (6 and 8 dS·m^{-1}) could not be detected (Table 1). Karungi et al. (2010) reported that humic substance application increased growth in cauliflower, cabbage, and tomato plants. Also, Verma et al. (2014) reported that the physical characteristics of cabbage heads improved and yield per head could be increased by adding humic acid.

The yield decreased gradually depending on the increasing salt doses, and the yield reached the lowest level at the highest salt dose. However, cauliflower yield was not affected by low salt doses (control and 2 dS·m^{-1}) and the highest yield was detected at these applications. Ehret and Ho (1986) reported similar findings with our research. The researchers reported that increasing salt concentration of irrigation water decreased the water intake of cauliflowers, and resulting a loss in curd yield. Giuffrida et al. (2017) revealed that 4 dS·m^{-1} salt applications in cauliflower (cv. Conero) decreased the yield conspicuously. On the other hand, many researchers indicated that humic acid may be an effective tool in decreasing soil salinity levels and improving salt tolerance under moderate salt conditions (Machado and Serralheiro 2017). Indeed, it was proved that humic acid applications reduced the impact of salinity in strawberries, peppers, and corns under saline conditions (Masciandaro et al., 2002; Pilanali and Kaplan, 2003; Turkmen et al., 2004). When NaCl x HA interaction were examined, HA applications increased the yield in salt-free conditions, 2 dS·m^{-1}and 4 dS·m^{-1} salt concentrations. When 1 and 2.0 g·kg^{-1} HA applications were compared, higher yield was obtained from 2 g·kg^{-1} HA application. It was observed that these positive effects of HA applications on yield lost at high salt concentrations (6 dS·m^{-1}and 8 dS·m^{-1}) (Table 1).

The impact of NaCl application on the dry matter in cauliflower was significant and in general, increase in salt concentrations also increased the amount of dry matter. Six dS·m^{-1} salt levels have been the application with the highest amount of dry matter. Positive impact of salinity on the amount of dry matter was also stated in the studies conducted on melons, peppers, tomatoes, and cucumbers. However, De Pascale et al. (2015) reported that salinity also increased the amounts of acid and brix in these fruits. When the values of NaCl x HA interaction were taken into account; it was observed that the amount of dry matter at 6 dS·m^{-1} salt dose reached the highest with HA applications (1 g·kg^{-1}and 2 g·kg^{-1}). Similarly, HA applications increased the amounts of dry matter of the plants that salt was not applied, and positive effects of the HA applications occurred at lower rates in the 4 dS·m^{-1} application. In spite of that, it was observed that this positive impact of HA disappeared at the high salt concentration (8 dS·m^{-1}). That the effects of 1 g·kg^{-1}and 2 g·kg^{-1} HA applications on the amount of dry matter are similar can also be seen in Table 1.

Rouphael et al. (2012) reported that salinity had some positive effects on some quality characteristics in vegetables. Giuffrida et al. (2017) detected that increasing salinity enhanced dry matter of curd, total soluble solids and acid values in a study they conducted on cauliflower. In also our study, it was determined that the amounts of total soluble solids increased significantly with increasing salt doses. The highest amount of total soluble solids was obtained from the 6 dS·m^{-1}application in cauliflowers. According to the results of the variance analysis, the impact of the interaction effect on the amount of total soluble solids was not significant (Table 1).

In the research, depending on the increasing salt concentrations, acid contents of cauliflower curds increased significantly and the highest content was found in 8 dS·m^{-1} salt dose. The impact of the NaCl x HA interaction on the acid content was found insignificant. This result shows that HA that is added into the plantation medium does not have any positive or negative impact on the acid contents of curds (Table 1).

CONCLUSIONS

The yield and curd growth was affected negatively by salt (<2 dS·m^{-1}) in cauliflower. It was understood from the research results that these negative effects could partially be reduced with humic acid applications. The salt concentrations that increased in the plantation medium affected curd quality of cauliflower positively. These positive effects increased more after more of humic acid added into the growth medium. However, these positive effects of humic acid were observed at low and moderate salt concentrations (≥4 dS·m^{-1}). Any positive impact of HA application was not observed at higher salt concentrations.

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