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The Effect of Humic Acid and Salicylic Acid to Improving Salt Tolerance in Yellow Hot Chili (*Capsicum annuum* L.)

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Abstract

Salt stress adversely affects the physiological processes of plants, causing negative changes in the morphology and anatomy of cells, tissues, and organs. To adapt to the increasingly complex situation of saline intrusion, the application of exogenous plant growth regulators shows many positive results to improve the crop's tolerance. This study was carried out in the greenhouse of Kien Giang University with the aim of determining the most suitable concentrations of humic acid and salicylic acid on yellow hot chili (Capsicum annuum L.) under salt stress.The experiment was arranged in a completely randomized design, including two factors: humic acid (with four concentrations: 0 ppm, 1000 ppm, 1500 ppm, and 2000 ppm) and salicylic acid (with four concentrations: 0 mM, 1 mM, 1.5 mM, and 2 mM) were treated separately or in combination (including 16 treatments with three replicates). The results showed that the yellow hot chili could grow and bear fruit when irrigated with saline water that the plant is 30 days after planting, but the yield was still low. The record indicated that supplying humic acid at 1500 ppm into the soil and spraying with salicylic acid at 2 mM to the leaves gave the most effective results, increasing 82% of the yield compared with the control.

Keywords: humic acid, saline stress, salicylic acid, yellow hot chili.

1. Introduction

Salt stress adversely affects the physiological processes of plants, causing negative changes in the morphology and anatomy of cells, tissues, and organs (Hieu and Ve, 2017). Na^+ and Cl^- of salt inhibit the absorption of nutrients such as NH_4^+, K^+, Ca^{2+}, Mg^{2+} or NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, etc., causing the plant to lose nutritional balance. The most obvious symptom is the loss of chlorophyll in the leaves, then the leaves turn yellow and then die. In addition, the transpiration in the leaves causes the salt concentration in the leaves to rise to a toxic threshold that causes cell death and leaf burn. Leaf lesions severely affect plant growth and yield (Hasanuzzaman *et al*., 2012; Nghia and Trung, 2016).

To adapt to the increasingly complex salt content situation, improving the salt tolerance of crops to saline conditions is one of the best solutions, and the application of exogenous plant growth regulators shows many positive results (Bhanwar *et al*., 2019). Humic acid and salicylic acid are growth regulators used to promote plant growth and yield under various stress conditions, including drought and salinity stress (Uttam *et al*., 2016b). The mechanism of humic acid is to enhance the absorption of nutrients and reduce the absorption of some toxic elements. Besides, humic acid also increases cell membrane permeability, oxygen uptake, respiration, photosynthesis, phosphate uptake, and stem cell elongation, these functions of humic acid help plants to tolerate stress conditions of the environment (Adil *et al*., 2012). Salicylic acid also plays an important role in responding to abiotic stresses such as drought and salinity. At the physiological level, exogenous salicylic acid treatment helps plants maintain the content of photosynthetic pigments (such as Chl a, Chl b) and increase the production of compounds that play the role of osmotic pressure regulation, molecular protection, or antioxidants such as proline, anthocyanin in drought environment (Cao Phi Bang *et al*., 2018).

This study aimed to investigate the effects of humic acid and salicylic acid on the growth and yield of yellow hot chili plants under salinity conditions. Chili (*Capsicum annuum* L.) is a spiced vegetable with high economic value (Hai and Thanh, 2017) and high efficiency in adapting to salinity situations (Selim *et al*., 2017). Moreover, chili belongs to the Solanaceae family, many studies such as Fathima and Denesh (2013), Uttam *et al.* (2016a), Naser *et al.* (2017), Fredy *et al.* (2018), and Muhammad and Muhammad (2019) showed that humic acid and salicylic acid exhibited positive effects on plants of the Solanaceae family, promoting good growth and yield under saline conditions.

2. Materials and Methods

Varieties: Africa TN223 yellow hot chili (*Capsicum annuum* L.) of Trang Nong seed company. Seeds are soaked in water for an hour, then sown in a nursery tray, sowing one seed in each hole, watering just enough moisture. When the seeds germinate, place them in a well-lit place. 5-7 days before planting, reduce water slowly, 2-3 days before planting, stop watering for root development. 2-3 hours before transplanting, water thoroughly. Then transfer the plant to a pot, press the base firmly, and water immediately after planting. Planted seedlings have 5-6 leaves.

Growth regulators used in the study i.e Humic acid of Hoang Lien Son Co., Ltd., purity 95% and salicylic acid of Xilong (China), purity ≥ 99.8%.

The experiment was conducted from June to December, 2021 at the greenhouse, Kien Giang University, Vietnam. The soil used has a sandy soil texture and is taken from the topsoil with a depth of 0-20 cm; pH = 8.4; EC = 0.2 Ms/cm. The soil was air-dried and placed in plastic pots (25x20 cm). Plastic pots are placed on plastic tarpaulin and placed in a greenhouse with a distance of 1.0x0.3 m.

This study was arranged in a factorial design based on a Completely Randomized Design (CRD) with three replications, each replication includes one plant/pot. The first factor was humic acid with four levels (0 ppm, 1000 ppm, 1500 ppm, and 2000 ppm) is applied directly to the soil right after transplanting. The second factor was salicylic acid treated with four levels (0 mM, 1 mM, 1.5 mM, and 2 mM) at 30 and 60 DAP, spraying on both leaf surfaces in the morning. The treatment combinations between humic acid and salicylic acid were presented in Table 1.

Irrigation water is artificially saline-treated with NaCl solution with at 4 dS/m. Watering was carried out around the trunk two times per day in the early morning and late afternoon from 30 days after planting (DAP).

Table 1. Symbols of the combination treatments

	0 ppm (H0)	1000 ppm (H1)	1500 ppm (H2)	2000 ppm (H3)
0 mM (S0)	H0S0	H1S0	H2S0	H3S0
1 mM (S1)	H0S1	H1S1	H2S1	H3S1
1.5 mM (S2)	H0S2	H1S2	H2S2	H3S2
2 mM (S3)	H0S3	H1S3	H2S3	H3S3

The amount of fertilizer for each pot includes 1 kg of manure, 14 g of urea, 31 g of superphosphate, and 13.4 g of potassium chloride. Before planting, apply all manure + superphosphate + 1/3 urea + 1/3 potassium chloride. The remaining urea and potassium chloride are evenly divided equally four times to supply.

Growth parameters including plant height (cm), trunk diameter (mm), canopy diameter (cm) were recorded 28, 49, and 70 days after planting (DAP). Yield parameters: fruit weight (g), fruit length (mm), fruit diameter (mm), number of fruits per plant (fruit/plant), fruit weight per plant (g/plant), yield (ton/ha). The data were statistically processed using SPSS 25.0 software. Analysis of variance (ANOVA) evaluated the difference of treatments by further testing Duncan's test at the 5 % significant level.

3. Results and Discussion

A. The effect of humic acid and salicylic acid on the growth of yellow hot chili under saline stress

The results in Table 2 show that treating humic acid and salicylic acid with different levels significantly affects the growth of yellow hot pepper plants. All monitoring parameters such as plant height (cm), trunk diameter (mm), and canopy diameter (cm) showed statistically significant differences (at 5% significant level) at 28, 49, and 70 days after planting.

According to Fathima and Denesh (2013), there was no statistical difference in the heights of chili plants when treated with humic acid in the first year of the experiment. Statistically significant differences in height were recorded at the second year, with the highest plants being sprayed with 6 ml/L of humic acid at 35 and 50 days after planting. Fredy *et al.* (2018) reported that, acid humic affected to roots of chili. Double root lengths were observed in the treatments of 0.1%, and 0.2% humic acid applied to the soil before planting. In this study, the plant heights were not improved when invidually treating humic acid or salicylic acid at 1 mM and 1.5 mM, but the height was improved with 2 mM salicylic acid. At 28 and 49 days after planting, the treatments were treated separately with salicylic acid at the concentration of 2 mM gave the best results in terms of plant height (43.67 cm and 51.83 cm, respectively), followed by that was the treatment treated with humic acid at the concentration of 1000 ppm in combined with salicylic acid at the concentration of 2 mM (39.5 cm at 28 DAP and 47.33 cm at 49 DAP).

Table 2. Growth recording of yellow hot chili over the stages of 28, 49, and 70 days after planting

Treatment	Plant height (cm)			Trunk diameter (mm)			Canopy diameter (cm)		
	28 DAP	49 DAP	70 DAP	28 DAP	49 DAP	70 DAP	28 DAP	49 DAP	70 DAP
H0S0	35.83 c	47.67 b	49.33 d	8.00 bc	10.33ab	12.33ab	36.67ab	42.67 b	49.00 e
H0S1	31.67 fg	42.00 e	48.67 d	8.33ab	10.67a	12.67a	34.67 cd	41.83 c	47.00 f
H0S2	33.50 de	41.33 ef	55.00 b	7.00 bcd	9.00 bcd	11.00abcd	35.67 bc	40.67 c	50.67 cd
H0S3	43.67a	51.83a	54.67 b	9.67a	11.00a	12.33ab	38.00a	43.33 b	50.33 de
H1S0	31.17 fg	40.33 fg	43.67 f	8.00 bc	9.00 bcd	12.33ab	36.00 bc	42.00 bc	52.00 bc
H1S1	32.33 ef	45.33 d	47.33 e	6.67 cd	8.33 cde	9.33 def	29.67 f	34.67 f	41.00 h
H1S2	26.00 ij	37.33 h	41.67 gh	6.67 bcd	7.67 de	9.00 ef	22.33 h	32.67 g	39.33 i
H1S3	39.50 b	47.33 bc	56.33a	5.33 d	7.00 e	8.67 f	26.67 g	37.00 e	53.00ab
H2S0	34.83 cd	40.00 fg	42.67 fg	8.00 bc	10.00abc	11.33 abc	26.33 g	35.33 ef	47.00 f
H2S1	31.00 fg	39.00 g	42.33 gh	7.00 cd	9.00 bcd	12.00ab	31.00 f	37.67 d	50.00 de
H2S2	32.50 ef	46.00 cd	50.67 c	6.67 bcd	7.67 de	9.33 def	31.33 ef	43.00 b	51.00 cd
H2S3	30.67 g	45.67 d	54.67 b	7.00 bcd	9.67 abc	12.00ab	38.00 a	47.00a	54.33a
H3S0	25.33 j	28.67 j	35.33 i	6.33 cd	7.67 de	10.67 cde	30.67 f	34.67 f	37.67 j
H3S1	30.67 g	40.67 ef	46.67 e	7.67 bc	8.67 bcde	9.67 cdef	30.67 f	36.33 def	40.67 hi
H3S2	28.00 h	37.33 h	43.67 f	7.67 bc	8.67 bcde	10.00 cdef	33.00 de	40.67 c	44.00 g
H2S3	26.83 hi	34.33 i	41.33 h	6.67 bcd	8.33 cde	10.00 cdef	27.00 g	30.83 h	33.00 k

Notes: The average numbers followed by the same small letter in the same column are not significantly different at the 5% probability level according to Duncan's test

At 70 days after planting, the treatment treated with 1000 ppm humic acid + 2 mM salicylic acid recorded a higher growth rate in plant height than the treatment treated with only 2 mM salicylic acid (at 56.33 cm and 54.67 cm respectively), the treatment of 1000 ppm humic acid + 2 mM salicylic acid was also the treatment that gave the highest plant height at this time and was followed by the treatment 2 mM acid salicylic. Treatment treated with 1500 ppm humic acid + 2 mM salicylic acid showed very low plant height at 28 days after planting (only 30.67 cm), but after 2 sprays of salicylic acid, plant height in this treatment had a fast growth rate and reached the height average equal to plants treated with 2 mM salicylic acid (54.67 cm) at 70 days after planting (increase by 24 cm after 6 weeks).

The average growth of the trunk diameter yellow hot chili on the treatments was not similar to the growth in the height plant. At 70 days after planting, the treatment with salicylic acid separately at a concentration of 1 mM resulted in the biggest trunk diameter with an average of 12.67 mm. This was followed by the control (not treated with both acids), the separate 2 mM salicylic acid treatment, the separate 1000 ppm humic acid treatment with means of 12.33 mm and 1500 ppm humic acid combined with 1 mM or 2 mM salicylic acid with 2 mM with means of 12 mm. All of the above-mentioned treatments did not give values that were statistically different from each other. The treatment treated with 1000 ppm humic acid + 2 mM salicylic acid, which gave the most optimal results in plant height, resulted in the lowest trunk diameter with an average of only 8.67 mm at 10 weeks after planting.

Abdullah *et al.* (2019) studied the effect of foliar application of humic and salicylic acids on some sweet pepper kinds *(Capsicum annuum)*. The concentrations were used at 0, 0.5, 1.0, and 1.5 g/L for each acid. The results showed that the higher the acid concentration, the larger the leaf area/plant. In this study, high humic acid treatment at high concentrations (1500 ppm, and 2000 ppm) reduced canopy diameter of yellow hot chili. The best results were obtained with the combined treatment of 1500 ppm humic acid + 2 mM salicylic acid (with means of 38.00 cm at 28 DAP; 47.00 cm at 49 DAP; and 54.33 cm at 70 DAP). Although plants did not reach the highest height in this treatment, the wide foliage were advantages to increase the photosynthetic efficiency, which help plants synthesize and accumulate organic matter for the reproductive stage (Ve and Tai, 2010).

Table 3. Yield performance recording of yellow hot chili

Treatment	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit number per plant (fruit)	Fruit weight per plant (g/plant)	Yield (ton/ha)
H0S0	9.35 c	90.30 b	16.25 d	35.33 d	330.39 de	8.26 de
H0S1	9.39 c	86.32 d	16.02 def	30.33 f	285.10 fg	7.13 gh
H0S2	9.66 c	91.69 a	16.35 d	36.67 d	354.55 cd	8.86 cd
H0S3	9.56 c	89.94 b	15.60 efg	40.33 c	385.62 c	9.64 c
H1S0	7.39 e	78.90 i	14.65 hi	36.00 d	266.04 gh	6.65 h
H1S1	8.25 d	82.90 f	17.44 c	36.33 d	299.78 ef	7.49 ef
H1S2	7.30 e	80.24 gh	14.69 hi	31.33 ef	228.58 i	5.72 i
H1S3	11.29 a	91.41 a	19.61 a	34.00 de	383.91 c	9.60 c
H2S0	7.21 e	79.65 hi	14.40 i	41.67 c	300.36 ef	7.51 fg
H2S1	6.57 f	76.16 j	15.26 gh	47.33 b	311.03 ef	7.78 ef
H2S2	8.46 d	84.93 e	15.38 fg	50.00 b	423.10 b	10.58 b
H2S3	10.56 b	88.15 c	18.38 b	56.67 a	598.63 a	15.00 a
H3S0	5.97 g	74.65 k	16.18 de	40.67 c	242.94 hi	6.07 hi
H3S1	8.08 d	74.81 k	18.36 b	40.67 c	328.71 de	8.22 de
H3S2	7.34 e	80.67 g	15.55 efg	31.33 ef	230.19 i	5.75 i
H2S3	8.34 d	82.88 f	15.45 fg	33.67 def	281.00 fg	7.02 gh

Notes: The average numbers followed by the same small letter in the same column are not significantly different at the 5% probability level according to Duncan's test.

B. The effect of humic acid and salicylic acid on the yield performance of yellow hot chili under saline stress

According to Table 3, the yield performance of yellow hot chili is affected by humic acid and salicylic acid. When treating 1000 ppm humic acid combined with 2 mM salicylic acid, fruit weight reached the highest with an average of 11.29 g/fruit. In this treatment, the fruit size also gave optimal results, with the average maximum diameter of 19.61 mm and the length of the fruit, though not the longest (91.41 mm on average), with had no difference in fruit length on statistical significance from the treatment with the highest result, treatment of 1.5 mM salicylic acid, at the mean value of 91.69 mm.

Treating salicylic separately with a concentration of 1.5 mM gave the longest fruit length (91.69 mm), but the fruits of this treatment were thin, only 16.35 mm on average, and the fruits were light (9.66 g/fruit). Furthermore, the number of fruits per plant of this treatment was not high (average 36.67 fruits/plant), resulting in low total fruit weight per plant and low yield (with 354.55 g/plant and 8.86 tons/ha in turn).

In the treatment treated with 1000 ppm humic acid + 2 mM salicylic acid, although the fruits were long, big, and heavy, the number of fruits per plant was small (mean of 34 fruits/plant), so the total fruit weight/plant and yield are not high (383.91 g/plant and 9.6 tons/ha, respectively).

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In the treatment treated with 1500 ppm humic acid + 2 mM salicylic acid, although the fruit length was not the longest (only 88.15 mm), the fruit diameter was big (mean of 18.36 mm), and the fruit weight was the second-highest one among all treatments (with an average of 10.56 g/fruit), in particular, the number of fruits per tree was highest (with an average of 56.67 fruits/plant), that resulted in the total weight obtained per plant and the yield were highest (with 598.63 g/plant and 15 tons/ha, respectively).

The data showed that the yellow hot chili was still able to grow and reproduce under saline stress when irrigating with saline water at 4 dS/m, but the yield was not high (8.26 tons/ha). As shown in Figure 1b, treating humic acid separately at levels of 1000, 1500, or 2000 ppm did not improve chili yield compared with the control (without treatment of both acids). Even, that reduced the yield of the crop to 26.5% compared to the control when applied at a concentration of 2000 ppm; to 19.5% when applied 1000 ppm humic acid, and to 9% when applied 1500 ppm humic acid.

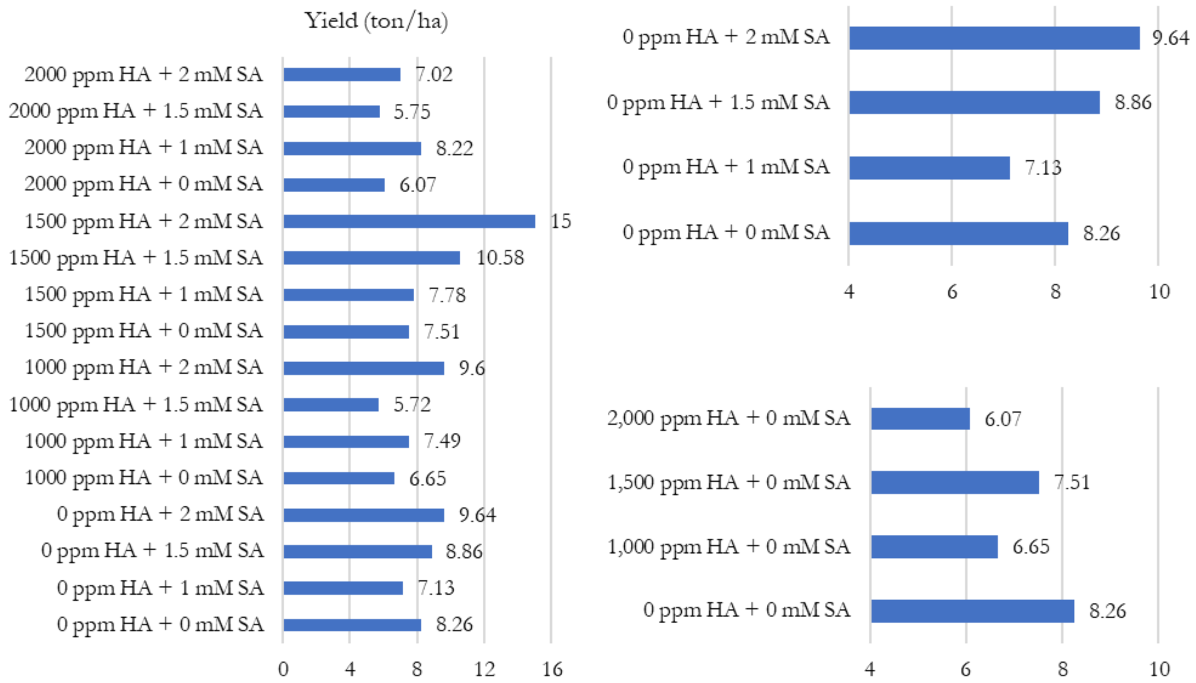

Figure 1. Yield performance of yellow hot chili (ton/ha) under salty stress
a) Yield of yellow hot chili (ton/ha) between treatments without humic acid application
b) Yield of yellow hot chili (ton/ha) between treatments without salicylic acid application

As shown in Figure 1a, when comparing the yield between the treatments supplied separately with salicylic acid and the control, the results showed that after spraying salicylic acid to the chili plants twice at 30 and 60 days after planting with the high concentrations of at 1.5 mM or 2 mM, the yield of the crop was improved, but not high. Treatment with 1.5 mM salicylic acid resulted in an increase of about 7%, and treatment with 2 mM salicylic acid increased yield by about 17% compared with the control under saline irrigation conditions.

Ahmed (2021) reported that supplying 100 ppm salicylic acid as foliar application at 30, 45 and 60 days after transplanting increased about mean of 40% fruit weight per chili plant in 2 cultivation year. According to Figure 1, in the combined treatment of 1500 ppm humic acid and 2 mM salicylic acid, the yield of yellow hot chili increased by 82% in comparison to the treatment without both acids. On the other hand, increasing the concentration of humic acid from 1500 ppm to 2000 ppm in combination with 2 mM for the crop strongly reduced the yield (53% compared with 1500 ppm humic acid). Therefore, it is necessary to combine suitably the concentrations of two acids to supply plants, overuse can lead to counterproductive effects.

4. Conclusions

Under complicated saline intrusion conditions leading to the scarcity of fresh water for irrigation, saline water up to 4 dS/m can be used to irrigate yellow hot chili crops. To adapt well to saline intrusion, a combination of humic acid and salicylic acid should be treated for better adaptation by watering with 1500 ppm humic acid right after planting the seedlings in the soil and spraying 2 mM salicylic acid on the leaves at 30 and 60 days after planting, that can improve the yield of yellow hot chili by 82% compared with not applying these two acids.

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