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Effect of Polyamine and Salicylic Acid on Growth and Yield of Chili Pepper *Capsicum annuum* L. Plant under Salt Stress

Fatima Haider Subhi*, *Mushtak F. Karomi Kisko*

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

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Abstract

Salt stress has adverse impacts on chili pepper (*Capsicum annuum* L.) growth, development and production. Plant tolerance must be increased to be able to adapt to salinity stress conditions. A factorial experiment with three factors according to Randomized Complete Blocks Design (RCBD) was conducted with three replicates. Polyamine P0, P1, P2 (0, 2, 3 mg L^{-1} Polyamine), salicylic acid A0, A1, A2 (0, 75, 150 mg L^{-1}), sodium chloride S0, S1, S2 (0, 2000, 4000 ppm) with their interactions were investigated. Salinity had a significant negative effect on the phenotypic characteristics and yield. Polyamine P2, salicylic acid A2 and their interaction (P2A2) were superior, as the highest value was recorded in many of the studied characteristics in comparison to the control. Interaction P2A2S0 gave the highest value compared to other parameters for all processing methods. In summary, this result indicates that the application of polyamine and salicylic acid can effectively reduce the harmful effect of salt stress in chili pepper.

Keywords: *Capsicum annuum* L., Chilli pepper, Polyamine, Salicylic acid, salt stress.

Introduction

The chili pepper has been used in Mexico for more than 7000 years, and its origin is South America, as a natural with various types, and moved to tropical regions worldwide [1]. It is used as fresh and cooked vegetables, spices, dried forms, food coloring, ornamental plant breeding, and the manufacturing of extracts for the pharmaceutical and cosmetics industries. Minerals like P, K, Mg, Ca, Fe, Zn, vitamins C, A, E, K, B3, B6, amino acids, and carotenoids found in chili peppers are abundant and crucial for human growth and health [2]. Capsanthin, which gives peppers their red color, is a powerful antioxidant and may help to prevent cancer. Capsaicin is found mostly in the central spongy tissue of fruit, seeds, and ovary. It has powerful medicinal properties and physiological potential that make it a viable candidate for use in a variety of pharmaceutical preparations and ointments for colds, sore throats, asthma, chest congestion, headaches and arthritis[3].

Soil salinity is a measure of the concentration of all the soluble salts in soil and water, usually expressed as electrical conductivity (EC), the salts being mostly NaCl, Na_2SO_4, $CaCO_3$ and $MgCO_3$ [4]. Sodium chloride (NaCl) is particularly problematic for soil salinity, causing slow growth, leaf senescence, reduce plant branching and decrease yields. Sodium ion (Na^+) in high concentrations causes significant damage to the cytosol in leaf cells because it interferes with many metabolic processes such as photosynthesis. Salt stress leads to the formation of cytotoxic activated oxygen, which causes oxidative damage to lipids, proteins, and nucleic acids. In addition, salinity can be involved in the formation of reactive oxygen species (ROS) such as hydroxyl radicals (OH), and superoxide radicals (O^{2-}) [5].

Polyamines (PAs) are ubiquitous low-molecular-weight, aliphatic compounds with wide as well as complex application in fundamental areas of plant growth and development [6], they play a major role in tolerating abiotic stress in plants [7], they are widely distributed in plants and regulate various cellular functions that are important for cell growth. Plants accumulate a large amount of polyamines against various elements important for cell growth and various abiotic stresses [8], polyamines play a key role in plant tolerance to various abiotic stresses, and positive effects of PAs on growth, ion homeostasis, water maintenance, photosynthesis, reactive oxygen species (ROS) accumulation, and antioxidant systems have been revealed in many plant species under abiotic stress [9]. Accumulation of PAs is essential in plant response to salt stress, and thus PAs have crucial importance in salinity tolerance [10]. PAs play a key role in alleviating plant salinity stress by activating biochemical, physiological, and molecular defense systems including photosynthetic pigment defense, antioxidant systems, hormonal interaction, and ionic homeostasis, ultimately alleviating the negative effects of suboptimal conditions on plants [11, 12].

Salicylic acid (SA) is an essential phytohormone that regulates plant growth, development, and defense during stress conditions by stimulating several physiological and metabolic processes[13]. SA, in low amounts, is involved in the coordination of physiological processes such as stomatal closure, nutrient absorption, chlorophyll pigments synthesis, protein synthesis, phytohormones balance, transpiration and photosynthesis in plants. It also plays the role of an important signaling molecule in the local and systemic resistance response of plants [14]. During stress, regulatory molecules, including plant hormones, play key roles in controlling developmental processes and signaling networks, and these molecules have been recognized as having the potential to be used to develop stress-tolerant plants [15]. Plants synthesize the phenolic compound salicylic acid (2-hydroxybenzoic acid), which has subsequently been shown to play a role in some key metabolic activities, including lignin and pigments biosynthesis, allelopathy, and controlling responses to biotic and abiotic stress mediators by increasing plant resistance to System Acquired Resistance (SAR) by stimulating or altering internal signals of the inner leaf anatomy to withstand a large number of stresses [16, 17], it is a powerful phenolic signaling biomolecule and a multifaceted plant growth regulator involved in a wide range of growth, metabolism and defense systems. Exogenous application of SA facilitates seed germination, growth, and flowering, up-regulates photosynthesis and increases enzymatic and non-enzymatic antioxidant activity [18]. According to numerous studies, SA promotes plant growth and development in challenging environments through tolerance of abiotic stress by reducing DNA damage and upregulating antioxidants to reduce oxidative stress [19].

The aim of the field study is to mitigate salinity in the pot by adding polyamine and salicylic acid, as well as to study the morphological and physiological characteristics of chili pepper plants.

Materials and Methods

A field experiment was conducted in pots in the Botanical Garden in the Department of Biology, College of Science for Women, University of Baghdad, during the period from November 2022 to June 2023. A factorial experiment with three factors according to a randomized complete blocks design was conducted with three replicates. Polyamine P0, P1, P2 (0, 2, 3 mg L^{-1} Polyamine), salicylic acid A0, A1, A2 (0, 75, 150 mg L^{-1}), sodium chloride S0, S1, S2 (0, 2000, 4000 ppm) with their interactions. Each pot contained 7 plants. Leaves were sprayed with a solution of distilled water containing 0.1% Tween 20 (polysorbate 20, a polysorbate-type nonionic surfactant consisting of sorbitan ethoxyl) as a surfactant.

Soil samples were taken from the pots at a depth of 0-15 cm randomly and mixed well. The sample was analyzed in the postgraduate studies laboratory of Department of Biology, College of Science, University of Baghdad to conduct some chemical and physical analysis of the soil, as shown in Table 1.

During the plant's growth period, the seedlings were irrigated regularly with non-salted water, and from the first growth stage until they reached the four true leaf stages, they were irrigated with three different treatments (without salt, 2000, 4000 ppm) of NaCl. Salinity was added several times, the first time after the formation of the four leaves, and then whenever irrigation was needed. The seedlings were watered with unsalted water after each irrigation with salt water throughout the plant's life cycle. Weeds were controlled manually. Seeds of chili pepper (salt sensitive) *Capsicum annuum* L. Var. Anaheim TMR23 (from Biostim Protection, Bakker Brothers, China) cultivars, were sterilized and sown in special germination trays on Oct. 1st 2022. Once, seven plants were randomly selected for transplanting, and the plants' leaves were sprayed with Polyamine and salicylic acid.

Growth and yield characteristics were recorded for randomly selected plants: plant height (cm), leaf area (dcm^2 $plant^{-1}$), shoot dry weight (g), capsaicin content (µg ml^{-1}), number of fruit (fruit $plant^{-1}$), fruit weight (g), leave content of total chlorophyll (mg 100 g $F.W.^{-1}$), catalase enzyme (mmol min mg^{-1}) and yield per plant (g $plant^{-1}$).

Leaf area (dcm^2 $plant^{-1}$): The leaf area was calculated by calculating the maximum length and width of three leaves of 27 Pots for each replicate (i.e. a total of 81 experimental units (upper, lower and middle) according to the equation. LA= 0.498LW+0.05 [20]

Leaves content of total chlorophyll (mg 100 g $F.W.^{-1}$): The chlorophyll content of leaves in plants was calculated for 81 experimental units using a chlorophyll meter according to the equation [21]

$Ch= 7.953+1.026*CCI -0.0045*CCI^2$

Capsaicin content in chili pepper fruit: Capsaicin determination: Capsaicins were extracted by mashing 5 g of fresh samples with 25 ml of acetone and leaving the mixture for one hour at 50 °C, then 1 ml of the filtrate was taken and transferred to a test tube and completely dried. 5 ml of hexane was added to each tube and the tubes were left at 25 °C for 10 minutes to dissolve the precipitate in the tubes. Then 4 ml of hexane was withdrawn from each tube and transferred to a new tube without any transfer of the precipitate. Then 10 ml (0.005N) NaOH was added to each tube, the contents of the tubes were mixed using Vortex, then the top layer (hexane) was removed, 1 ml of NaOH was taken and transferred to a new tube, and 50 µl of (1N) of HCl were added to each tube, and 50 µl of dye (2,6-Dichloroquinone-4-chloroimide) and 50 µl of (2.5%) of ammonia. The tubes were left at 25 °C for 10 minutes to complete the color reaction. The optical absorption intensity of the tubes was measured at 600 nm wavelength. Capsaicin was estimated using the capsaicin standard curve as reported by [22] as in Fig. 1.

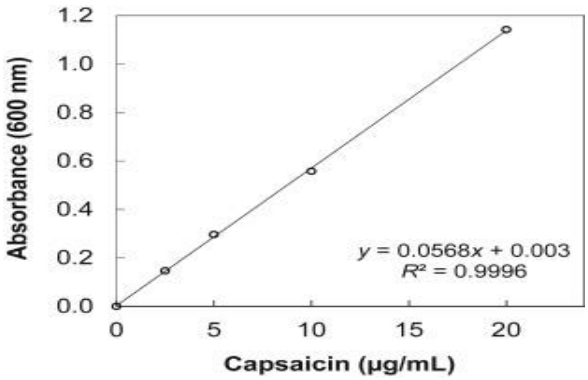

Figure. 1 capsaicin standard curve

Catalase enzyme activity: 2 grams of fresh leaves were mashed with potassium phosphate buffer solution (0.1M) pH = 7.8 at a ratio of (1:2 w/v). The extract was filtered by gauze and centrifuged at 10000g for 30 minutes. The activity of the catalase enzyme was estimated in the leaves, where 0.2 ml of the extract was taken and incubated with 1 ml of a mixture containing H_2O_2 (65 mM) with a phosphate buffer (60 mM) pH = 7.4 at 25 °C for 4 minutes. Then the enzyme action was stopped by adding 1 ml of ammonium molybdate (32.4 mM). Readings are taken to estimate the activity of the enzyme at wavelength 405 nm. The effectiveness is estimated according to the following equation: [23]

$$\text{Catalase activity} = \frac{(\text{Sample} - \text{Blank 1})}{(\text{Blank 2} - \text{Blank 3})} \text{x } 271$$

Whereas:

Blank 1: contains 1 ml of the base material (the H_2O_2 with the buffer solution), 1 ml of molybdate and 0.2 ml of the sample.
Blank 2: contains 1 ml of the base material (the H_2O_2 with the buffer solution), 1 ml of molybdate, and 0.2 ml of the buffer solution.
Blank 3: contains 1 ml of buffer, 1 ml of molybdate, and 0.2 ml of buffer.

The statistical analysis system SAS (2018) was used for data analysis to study the effect of salinity, polyamine and salicylic acid, and their interactions based on the examined traits according to a factor experiment (3 x 3 x 3) applied by randomized complete blocks design with three replications, and the significant differences between averages were compared with the least significant difference test (LSD). [24]

Table 1. Chemical and physical properties of experiment soil

Physical		**Chemical**	
Property	**Value**	**Property**	**Value**
Soil texture	Sandy	**pH**	7.2
Sand	92%	**EC**	0.7 $dsm.m^{-1}$
Silt	5%	**N**	40.0 mg kg^{-1}
Clay	3%	**P**	16.0 mg kg^{-1}
Bulk density	1.2	**K**	35.0 mg kg^{-1}

Results and Discussion

Plant height (cm)

Table 2 showed the results of increasing salinity levels that led to a decrease in plant height, as S1 and S2 had the highest rates (38.34) cm, (38.29) cm respectively, compared to the control (no salt) S0 (41.20) cm, while the effect of polyamine was obvious, as the highest average was present at P2 (41.07) cm, there was significant difference in the treatment of salicylic acid, as the highest height was reached at A2 (40.66) cm, while at the interaction between polyamine and salicylic acid, there were significant differences, with the highest value reached in P2A2 (42.33) cm. The results of the interaction between polyamine and salt showed clear significant differences, as the highest value at P2S0 (43.29) cm. Furthermore, when the interaction between salicylic acid and salinity factors increased, significant differences were observed, where the highest value at A2S0 (42.77) cm compared to the lowest plant height at A0S1 and A0S2 (37.03) (37.10), respectively. The interaction between P, A, and S exerts highly significant differences, where the highest plant was at P2A2S0 (44.78) cm.

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Table 2. Effect of polyamine and salicylic acid on salt stress, and their interactions on plant height (cm)

Average P	**P*A**	**S2**	**S1**	**S0**	**A**	**P**
37.46 C	36.14 E	34.47 Q	35.19 P	38.76 JKL	A0	**P0**
	37.43 DE	36.83 O	36.45 O	39.01 IJ	A1	
	38.81 CD	38.41 KLM	38.07 MN	39.96 FG	A2	
39.32 B	37.98 CD	38.26 LM	36.85 O	38.81 JK	A0	**P1**
	39.14 C	38.93 J	37.62 N	40.85 E	A1	
	40.84 AB	39.85 HG	39.13 IJ	43.56 C	A2	
41.07 A	39.49 BC	38.36 KLM	39.26 IJ	40.84 E	A0	**P2**
	41.38 A	39.46 HI	40.42 EF	44.25 B	A1	
	42.33 A	40.07 FG	42.12 D	44.78 A	A2	
LSD: P=0.083 *	LSD: P*A=0.281 *	LSD: P*A*S =0.505 *			**Value LSD0.05**	
---					**S x P**	
LSD: P*S =0.281 *		36.57 E	36.57 E	39.25 C	**P0**	
		39.01 CD	37.87 D	41.08 B	**P1**	
		39.30 C	40.60 B	43.29 A	**P2**	
Average A					**S x A**	
37.87 C		37.03 D	37.10 D	39.47 C	**A0**	
39.31 B		38.41 CD	38.16 CD	41.37 AB	**A1**	
40.66 A		39.45 C	39.77 BC	42.77 A	**A2**	
LSD: A =0.083*		LSD: A*S =0.281 *			**Value LSD0.05**	
---		38.29 B	38.34 B	41.20 A	---	**Average S**
		LSD: S = 0.083 *			**Value LSD0.05**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Leaf Area (dcm^2 $plant^{-1}$)

The results in Table 3 showed increasing levels of salinity hurt the leaf area. There were significant differences between treatments, as the highest value was at S0 (4.243) dcm^2 without salt, and the lowest value was at S2 (3.086) dcm^2, while treatment with polyamines had a positive effect on the leaf area the highest rate was recorded at P2 (3.845) dcm^2. Treatment with salicylic acid, showed significant differences between different concentrations, as the highest rate was found at A2 (3.714) dcm^2. Significant differences were observed in the interaction between polyamine and salicylic acid, where the highest rate was at P2A2 (3.930) dcm^2. The interaction between Polyamine and salt, showed significant differences in the leaf area, as the highest value was at P2S0 (4.37) dcm^2. Significant differences were also observed in the interaction of the salicylic acid and salinity, as the highest concentration rate was at A2S0 (4.301) cm^2. The interaction between P, A, and S factors, showed significant differences in the leaf area, as the highest rate was found at P2A2S0 (4.417) dcm^2, followed by P2A1S0 (4.413) dcm^2, with no significant differences between them, and the lowest rate was found at the concentration P0A0S2 (2.825) dcm^2.

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Table 3. Effect of polyamine, salicylic acid on salt stress, and their interactions on leaf area (dcm^2 $plant^{-1}$)

Average P	P*A	S2	S1	S0	A	P
	3.433 B	2.825 H	3.422 F	4.054 C	A0	
3.505 C	3.513 AB	2.843 H	3.537 EF	4.160 BC	A1	**P0**
	3.568 AB	2.933 GH	3.555 EF	4.218 ABC	A2	
	3.526 AB	2.969 GH	3.481 F	4.129 BC	A0	
3.583 B	3.582 AB	2.978 GH	3.527 EF	4.248 ABC	A1	**P1**
	3.642 AB	3.100 G	3.559 EF	4.268 AB	A2	
	3.702 AB	3.108 G	3.715 DE	4.282 AB	A0	
3.845 A	3.903 AB	3.479 F	3.817 D	4.413 A	A1	**P2**
	3.930 A	3.543 EF	3.831 D	4.417 A	A2	
LSD: P=0.033 *	LSD:P*A= 0.437*		LSD: P*A*S = 0.508*		**Value LSD0.05**	
---					**S x P**	
		2.86 G	3.50 DE	4.14 B	**P0**	
LSD: P*S =0.437*		3.01 F	3.52 D	4.21 B	**P1**	
		3.37 E	3.78 C	4.37 A	**P2**	
Average A					**S x A**	
3.554 C		2.967 D	3.539 B	4.155 A	**A0**	
3.666 B		3.100 CD	3.625 B	4.273 A	**A1**	
3.714 A		3.192 C	3.648 B	4.301 A	**A2**	
LSD: A =0.033*			LSD: A*S =0.437*		**Value LSD0.05**	
---		3.086 C	3.604 B	4.243 A	---	**Average S**
			LSD: S =0.033 *		**Value LSD0.05**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Dry weight of the shoot (g)

The results shown in Table 4 indicated that there was a significant effect of treatments on the dry weight of the plant shoot, where the highest rate was for the amino acid P2 (2.769) g. A significant difference was also found for the salicylic treatment, as the highest rate was recorded at the second concentration A2 (2.729) g, followed by the first concentration A1 (2.719) g with no significant differences between the A1 and A2. Whereas, the effect of salt on the shoot dry weight was significant, as the highest rate with S0 (2.894) g, and the lowest rate with S2 (2.725). The interaction between polyamine and salicylic acid was significant, as the highest value was recorded at P2A2 (2.806) g, A significant difference was also noted in the interaction between polyamine and salt, as the highest rate was found at P2S0 (3.073) g. The highest rate was recorded at A2S0 (2.919) g, and the lowest rate was at A0S1 (2.406) g followed by A1S2 (2.535) g in the interaction between salicylic acid and salt. The interaction between the three factors gave significant differences, with the highest value at the P2A0S0 (3.148) g.

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Table 4. Effect of polyamine, salicylic acid on salt stress, and their interactions on dry weight of the shoot

Average P	P*A	S2	S1	S0	A	P
2.564 C	2.457 B	2.422 LMN	2.316 O	2.634 GH	A0	**P0**
	2.596 AB	2.605 HI	2.433 LMN	2.749 DEF	A1	
	2.640 AB	2.833 D	2.422 LMN	2.665 FGH	A2	
2.732 B	2.680 A	2.698 EFG	2.394 NO	2.947 C	A0	**P1**
	2.774 A	2.946 C	2.578 HIJ	2.799 D	A1	
	2.741 A	2.775 DE	2.417 MN	3.033 BC	A2	
2.769 A	2.714 A	2.485 KLM	2.508 KLJ	3.148 A	A0	**P2**
	2.788 A	2.815 D	2.536 IJK	3.012 BC	A1	
	2.806 A	2.946 C	2.411 MN	3.061 AB	A2	
LSD: P= 0.017	LSD:P*A= 0.132		LSD: P*A*S = 0.187		**Value $LSD_{0.05}$**	
---					**S x P**	
	LSD: P*S = 0.132	2.620 G	2.390 E	2.682 BC	**P0**	
		2.806 G	2.463 D	2.926 B	**P1**	
		2.749 F	2.485 C	3.073 A	**P2**	
	Average A				**S x A**	
	2.617 B	2.535 B	2.406 B	2.910 A	**A0**	
	2.719 A	2.417 A	2.515 B	2.853 A	**A1**	
	2.729 A	2.851 A	2.789 B	2.919 A	**A2**	
	LSD: A =0.017		LSD: A*S =0.132		**Value $LSD_{0.05}$**	
	---	2.725 C	2.446 B	2.894 A	---	**Average S**
			LSD: S =0.017		**Value $LSD_{0.05}$**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Number of fruits

The results presented in Table 5 show that there are significant differences in the number of fruits per plant, as the highest value was recorded at the second concentration P2 (8.040) in the treatment of polyamine,. while clear significant differences were observed in the salicylic treatment, as the highest value for the number of fruits was at A2 (7.741), while salinity had a negative effect, as the highest value was at S0 (9.025) compare to the high concentration S2 which gave the lowest value (6.145), in the interaction between polyamine and salicylate, significant differences were found shown in the table, where the highest value was recorded at P2A2 (8.251), when interacting between the P and S factors, a clear significant difference, as the highest value was at the P2S0 concentration (9.823), while the interaction between A and S, there are significant differences in salt concentrations, where the highest value was A2S0 (9.317), regarding the interaction among factors P, A and S, significant differences were observed, as it gave the highest value at P2A2S0 (10.01).

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Table 5. Effect of polyamine, salicylic acid on salt stress, and their interactions on number of fruits

Average P	P*A	S2	S1	S0	A	P
6.848 C	6.425 C	5.288 P	6.613 L	7.374 HI	A0	**P0**
	7.005 BC	5.855 O	7.232 IJ	7.928 F	A1	
	7.114 ABC	6.040 NO	6.995 H	8.305 E	A2	
7.636 B	7.431 ABC	5.855 O	7.366 HIJ	9.072 D	A0	**P1**
	7.617 ABC	6.233 MN	7.176 JK	9.444 C	A1	
	7.859 AB	6.392 M	7.552 GH	9.632 BC	A2	
8.040 A	7.807 AB	6.040 NO	7.745 FG	9.638 B	A0	**P2**
	8.061 AB	6.611 L	7.756 F	9.817 B	A1	
	8.251 A	6.995 K	7.745 FG	10.01 A	A2	
LSD: P=0.036	LSD:P*A= 0.513		LSD: P*A*S = 0.193		**Value $LSD_{0.05}$**	
---					**S x P**	
		5.728 H	6.946 E	7.869 C	**P0**	
LSD: P*S = 0.513		6.160 G	7.365 D	9.383 B	**P1**	
		6.549 F	7.749 C	9.823 A	**P2**	
Average A					**S x A**	
7.221 C		5.727 E	7.241 C	8.695 B	**A0**	
7.561 B		6.233 DE	7.388 C	9.063 AB	**A1**	
7.741 A		6.476 D	7.431 C	9.317 A	**A2**	
LSD: A =0.036			LSD: A*S =0.513		**Value $LSD_{0.05}$**	
---		6.145 C	7.353 B	9.025 A	---	**Average S**
			LSD: S =0.036		**Value $LSD_{0.05}$**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Fruits weight (g)

The results of Table 6 showed the effect of studied factors on fruit weight. It was found that when salinity increases, the weight of the fruit is decreased, when the fruit was treated with a polyamine, the highest rate of the fruit weight was at P2 (10.15) g, in the salicylic acid treatment, the highest value was at the second concentration, A2 (9.919) g, however, salinity exert harmful effect on fruit weight, as the highest value was at S0 (10.48) g,. The interaction between polyamine and salicylic acid, showed a clear significant difference, as the highest value was found at P2A2 (10.38) g. Similarly, in the interaction between salicylic and salinity, a significant difference was found, as the highest rate was recorded at the concentration A2S0 (10.72) g. The treatment of fruit with combination of polyamine and salt, gave the highest value at P2S0 (10.83) g. In addition, triple interaction of three factors has increased the weight of treated fruits, where the highest value was at P2A2S0 (10.06) g.

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Table 6. Effect of polyamine, salicylic acid on salt stress, and their interactions on fruits weight (g)

Average P	P*A	S2	S1	S0	A	P
9.262 C	8.957 D	8.019 N	8.952 K	9.899 DE	A0	**P0**
	9.287 DC	8.407 M	9.153 J	10.30 C	A1	
	9.541 DC	8.650 L	9.433 HI	10.54 B	A2	
9.603 B	9.282 DC	8.588 L	9.302 IJ	9.956 DE	A0	**P1**
	9.692 BC	8.970 K	9.541 GH	10.56 B	A1	
	9.834 ABC	9.385 HI	9.550 GH	10.56 B	A2	
10.15 A	9.854 ABC	9.452 GHI	9.626 FG	10.48 B	A0	**P2**
	10.21 AB	9.801 EF	9.914 DE	10.93 A	A1	
	10.38 A	10.06 D	10.01 D	11.07 A	A2	
LSD: P=0.028	LSD:P*A= 0.043		LSD: P*A*S = 0.179		**Value LSD0.05**	
---					**S x P**	
		8.359 F	9.179 E	10.24 B	**P0**	
LSD: P*S = 0.043		8.981 E	9.464 D	10.36 B	**P1**	
		9.771 C	9.850 C	10.83 A	**P2**	
Average A					**S x A**	
9.364 C		8.686 E	9.294 CD	10.11 B	**A0**	
9.732 B		9.059 DE	9.536 C	10.60 A	**A1**	
9.919 A		9.365 CD	9.665 C	10.72 A	**A2**	
LSD: A =0.028			LSD: A*S =0.043		**Value LSD0.05**	
---		9.037 C	9.498 B	10.48 A	---	**Average S**
			LSD: S =	0.028	**Value LSD0.05**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Total chlorophyll content of leaves (mg 100 g F.W.$^{-1}$)

The results in Table 7 indicate that there were significant differences in the chlorophyll content of leaves, compared to untreated plants. The harmful effect of salt on chlorophyll pigments was obvious at S2 concentration with the lowest value (40.26), and highest value at S1 (42.17) and S0 (42.02) respectively, while, the treatment of polyamine gave a positive effect, where the highest value for chlorophyll was recorded at the second concentration of polyamine P2 (43.18). Nevertheless, salicylic acid treatment increased the content of chlorophyll, compared to the control, although there were no significant differences between the two concentrations, where the highest value for chlorophyll was recorded at A1 (42.37) followed by A2 (42.12). Significantly, an increase in the content of chlorophyll was noticed when the plant was treated with a combination of polyamine and salicylic acid. The chlorophyll content value was high at P2A2 (43.46). Likely, the interaction between salicylic and salinity showed a significant difference, as the highest value was at A2S0 (43.19), and P2S1 (45.55). The interaction between the three factors was also significant, where the highest value at P2A2S0 (45.09).

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Table 7. Effect of polyamine, salicylic acid on salt stress, and their interactions on total chlorophyll content of leaves

Average P	P*A	S2	S1	S0	A	P
40.78 B	39.28 C	38.43 D	39.28 CD	40.13 BCD	A0	**P0**
	41.24 BC	40.72 BCD	41.59 BCD	41.41 BCD	A1	
	41.81 BC	40.97 BCD	41.43 BCD	43.03 BCD	A2	
40.50 B	40.10 C	39.43 CD	39.54 CD	41.34 BCD	A0	**P1**
	40.32 BC	40.36 BCD	40.07 BCD	40.5 BCD	A1	
	41.09 BC	40.81 BCD	41.00 BCD	41.45 BCD	A2	
43.18 A	40.52 BC	39.93 BCD	40.34 BCD	41.29 BCD	A0	**P2**
	45.55 A	40.12 BCD	52.57 A	43.95 BC	A1	
	43.46 AB	41.56 BCD	43.74 BCD	45.09 B	A2	
LSD: P=1.821	LSD:P*A= 2.556		LSD: P*A*S = 5.497		**Value $LSD_{0.05}$**	
---					**S x P**	
LSD: P*S = 2.556		40.04 C	40.77 BC	41.52 BC	**P0**	
		40.20 BC	40.20 BC	41.11 BC	**P1**	
		40.54 BC	45.55 A	43.44 AB	**P2**	
Average A					**S x A**	
39.97 B		39.26 C	39.72 C	40.92 BC	**A0**	
42.37 A		40.40 BC	44.74 A	41.96 ABC	**A1**	
42.12 A		41.11 BC	42.06 ABC	43.19 AB	**A2**	
LSD: A =1.821			LSD: A*S =2.556		**Value $LSD_{0.05}$**	
---		40.26 B	42.17 A	42.02 AB	---	**Average S**
			LSD: S =1.821		**Value $LSD_{0.05}$**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Capsaicin content in chili pepper fruit (μg ml^{-1})

The effect of treatments on the nature of capsaicin in hot pepper fruit is shown in Table 8, the results indicate that increasing the level of salinity led to an increase in the concentration of capsaicin. In S2 treatment, the highest value was (3.028), and the lowest value was (2.891) in the control S0. In the presence of polyamine, the highest value at P2 (3.180), while, in treatment with salicylic acid, the highest value was at A2 (2.986). Moreover, clear significant differences were noticed in the interaction of polyamine and salicylic acid, where the highest rate was recorded at P2A2 (3.214). When the growth regulator interacted with salt, a significant difference was found as shown in the table, where the highest value was at P2S2 (3.221), significant differences were also found between salicylic acid and salinity, where the highest value was at A2S2 (3.077). At the triple interaction, the significant differences were very clear, and the highest value of capsaicin concentration was at P2A2S2 (3.246).

Table 8. Effect of polyamine, salicylic acid on salt stress, and their interactions on capsaicin content in chili pepper fruit

Average P	P*A	S2	S1	S0	A	P
2.769 C	2.746 E	2.866 IJ	2.726 M	2.646 O	A0	**P0**
	2.776 E	2.913 GH	2.740 M	2.676 NO	A1	
	2.785 E	2.943 G	2.706 MN	2.706 MN	A2	
2.887 B	2.817 DE	2.880 HI	2.790 KL	2.783 L	A0	**P1**
	2.885 CD	2.946 G	2.826 JK	2.883 HI	A1	
	2.958 C	3.043 F	2.880 HI	2.953 G	A2	
3.180 A	3.127 B	3.193 CD	3.146 E	3.043 F	A0	**P2**
	3.197 AB	3.223 ABC	3.200 BCD	3.170 DE	A1	
	3.214 A	3.246 A	3.236 AB	3.160 DE	A2	
LSD: P=0.005	LSD:P*A= 0.048		LSD: P*A*S = 0.040		**Value $LSD_{0.05}$**	
---					**S x P**	
		2.907 D	2.724 F	2.676 F	**P0**	
LSD: P*S = 0.048		2.956 C	2.832 E	2.873 DE	**P1**	
		3.221 A	3.194 A	3.124 B	**P2**	
Average A					**S x A**	
2.897 C		2.980 ABC	2.887 BC	2.824 C	**A0**	
2.953 B		3.027 AB	2.922 ABC	2.910 ABC	**A1**	
2.986 A		3.077 A	2.941 ABC	2.940 ABC	**A2**	
LSD: A =0.005			LSD: A*S =0.048		**Value $LSD_{0.05}$**	
---		3.028 A	2.917 B	2.891 C	---	**Average S**
			LSD: S =0.005		**Value $LSD_{0.05}$**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$.

Catalase enzyme activity

The results shown in Table 9 indicate that the potential activity of catalase enzyme in plant leaves is salt concentration dependent. In S2 treatment, the highest rate was (141.5), compared to S0, which exerted the lowest rate (127.7), and the highest rate was reached at P0 (141.6). A significant difference was also noticed when salicylic acid is used, where the highest value was at A0 (138.8). The interaction between polyamine with salicylic acid also had a significant effect, reaching the highest value at the control P0A0 (145.5), and the lowest value at the second concentration P2A2 (127.3). In salicylic acid and salinity interaction, significant differences were recorded as shown in this table, as it gave the highest value at A0S2 (144.1), and the second interaction of polyamine and salinity also showed a significant difference, with the highest value reaching at P0S2 (144.4),. Similarly, significant differences were found when the combination of polyamine, salicylic and salt was used, the highest value for enzyme activity was found at P0A0S2 (148.7).

Table 9. Effect of polyamine, salicylic acid on salt stress, and their interactions on catalase enzyme

Average P	P*A	S2	S1	S0	A	P
141.6 A	145.5 A	148.7 A	145.4 B	142.6 EF	A0	**P0**
	141.9 AB	144.2 CD	141.4 G	140.0 H	A1	
	137.4 BCD	140.3 H	137.5 J	134.6 L	A2	
134.7 B	138.8 BC	143.4 DE	144.3 C	128.7 P	A0	**P1**
	134.9 CDE	142.3 F	140.4 H	122.1 S	A1	
	130.4 EF	138.4 I	133.2 M	119.7 T	A2	
129.8 C	132.1 DEF	140.2 H	131.7 N	124.4 R	A0	**P2**
	130.1 EF	139.7 H	130.5 O	120.1 T	A1	
	127.3 F	136.4 K	127.7 Q	117.7 U	A2	
LSD: P=0.130	LSD:P*A= 3.122		LSD: P*A*S = 0.887		**Value $LSD_{0.05}$**	
---					**S x P**	
LSD: P*S = 3.122		144.4 A	141.4 AB	139.0 B	**P0**	
		141.3 AB	139.3 B	123.5 D	**P1**	
		138.8 B	129.9 C	120.7 D	**P2**	
Average A					**S x A**	
138.8 A		144.1 A	140.4 AB	131.9 DE	**A0**	
135.6 B		142.1 AB	137.4 BCD	127.4 EF	**A1**	
131.7 C		138.4 ABC	132.8 CDE	124.0 F	**A2**	
LSD: A =0.130			LSD: A*S =3.122		**Value $LSD_{0.05}$**	
---		141.5 A	136.9 B	127.7 C	---	**Average S**
			LSD: S =0.130		**Value $LSD_{0.05}$**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$

Yield per Plant (g)

The results presented in Table (10) indicate that there were noticeable differences in the plant yield traits. Increasing salinity levels led to a decrease in plant yield, with the highest value recorded at S0 (94.83) g and the lowest value at a high concentration of salt S2 (55.82) g. When treated with polyamines, the highest value was found at concentration P2 (82.29) g. Likewise, when salicylate treatment was applied, a significant difference was observed, with the highest rate at A2 (77.61) g, when salicylate reacts with salt, a significant difference was also found, the highest value was at A2S0 (100.0) g, as polyamine reacts with salt, the table displays significant differences, the highest value was at P2S0 (106.4) g. As for the interaction between amino acid P and growth regulator A, the results indicate a significant difference as the highest value was at P2A2 (86.27) g. Regarding the triple interaction of polyamine, salicylate, and salinity, there were notable differences, the concentrations for which the highest average was obtained was P2A2S0 (110.8) g.

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Table 10. Effect of polyamine, salicylic acid on salt stress, and their interactions on yield per plant (g)

Average P	**P*A**	**S2**	**S1**	**S0**	**A**	**P**
64.17	58.21	42.41	59.20	73.01	A0	
C	D	O	L	GH		**P0**
	65.70	49.23	66.20	81.68	A1	
	CD	N	JK	E		
	68.60	52.26	65.98	87.55	A2	
	CDE	N	JK	D		
74.14	69.72	50.28	68.55	90.34	A0	
B	BCD	N	IJ	D		**P1**
	74.72	55.91	68.47	99.78	A1	
	ABC	M	IJ	C		
	77.98	60.00	72.14	101.8	A2	
	ABC	L	GH	C		
82.29	77.58	57.10	74.56	101.0	A0	
A	ABC	LM	FG	C		**P2**
	83.03	64.81	76.90	107.3	A1	
	AB	K	F	B		
	86.27	70.38	77.55	110.8	A2	
	A	HI	F	A		
LSD: P=0.431	LSD:P*A= 4.402		LSD: P*A*S = 3.054		**Value $LSD_{0.05}$**	
---					**S x P**	
		47.96	63.79	80.75	**P0**	
LSD: P*S =		H	F	C		
4.402		55.40	69.72	97.31	**P1**	
		G	E	B		
		64.10	76.34	106.4	**P2**	
		F	D	A		
Average A					**S x A**	
68.50		49.93	67.44	88.14	**A0**	
C		F	CD	B		
74.48		56.65	70.52	96.28	**A1**	
B		EF	C	A		
77.61		60.88	71.89	100.0	**A2**	
A		DE	C	A		
LSD: A =0.431			LSD: A*S =4.402		**Value $LSD_{0.05}$**	
---		55.82	69.95	94.83	---	**Average S**
		C	B	A		
			LSD: S =0.431		**Value $LSD_{0.05}$**	

For each parameter, averages followed by the same letter are not significantly different at $p \leq 0.05$

Discussion

The results of the current study highlighted the effect of spraying the amino acid polyamine, the growth regulator salicylic acid, and their interactions on salt-sensitive plants under salt stress conditions. The response of hot pepper plants to salt stress may differ, as the vegetative growth characteristics were decreased under both salt levels compared to the control, increasing salinity levels led to a significant decrease in the values of growth indicators, such as plant height, leaf area, shoot dry weight, fruit weight, and yield per plant compared to untreated plants (control). The reason for this is due to the disturbance in physiological processes [25,26], also due to an imbalance in ionic imbalance, which leads to the accumulation of harmful ions in the plant cells such as Na^+ and Cl^- [27], and the absorption capacity of macro-nutrients such as N, P, K, Ca, and Mg is reduced, and thus, nutrients are also affected, especially N, which is one of the components involved in the synthesis of the

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chlorophyll molecule [28,29]. It is also harmful to the redox environment of the cell, as ionic toxicity disrupts the non-covalent interaction between amino acids and decreases concentrations of cations such as K and Ca [30,31]. The results are consistent with other studies [25,32]. By increasing salt stress, an inhibition of the photosynthesis process occurs, as a result of partial closure of stomata and a decreased gas exchange, which affects the growth of chloroplasts and an imbalance in the concentration of pigments, including the chlorophyll pigment [33], which is very sensitive to salinity and other stresses, so the chlorophyll content and photosynthesis efficiency decreases with increasing NaCl concentration in the hot pepper plant as shown in table 7, and this is similar to what the researcher found [10,34], while there is an increase in the content of antioxidant enzymes with an increase in the concentration of NaCl, as indicated in table 9, as they are considered indicators of the occurrence of stresses in the plant, such as catalase, this may be due to an increase the ability to scavenge oxygen radicals, and maintain cellular membranes. Likewise, an increase in the enzyme activity can be considered an inhibitor for catalytic secondary metabolism [35]. Tables 2-6 showed that salinity stress hurts plant growth and fruit yield, however, it leads to an increase in the capsaicin content, as the synthesis of capsaicinoids occurs in the placenta of hot pepper fruits as shown in table 8 [36], which agrees with [37].

Spraying with salicylic acid has been used to reduce the effect of salt stress damage on plants. Salicylic acid plays a role in increasing the absorption of ions from the soil solution, enhancing the efficiency of the photosynthesis process, and increasing the plant's ability to produce antioxidants, and reducing plant sensitivity [38,39]. These results are consistent with [40]. Exogenous SA application improved photosystem II efficiency and chlorophyll content as shown in table 7 [38]. Many previous studies demonstrated that SA effectively alleviates photosynthetic damage in plants[41]. Since ROS production and induction of oxidative stress by salinity are the main causes of reduction in plant growth and productivity, ROS regulation is an important process to avoid cytotoxicity and oxidative damage, and the exogenous SA treatment enhances the activation of antioxidant enzymes, such as catalase as shown in table 9 [42,43].

It has been shown that the treatment of polyamine with different levels reduces the effect of salinity conditions on different plants, as it reduces the damage through the accumulation of enzymatic antioxidants [12]. PAs treatment under salt stress significantly affected the accumulation of secondary metabolites involved in redox homeostasis [44]. Exogenous polyamine (Putrescine) alleviates the accumulation of Na^+ in salt-sensitive rice cultivars under salt stress. In addition, putrescine treatment leads to an increase in the endogenous PAs content, especially spermine and spermidine, resulting in an increase in fatty acid mobilization which is attributed to the stabilization of photosynthetic machinery [45]. Polyamine applications involve the cell membrane protection, stimulating the expression of osmotic response genes, lowering H_2O_2 levels, increasing antioxidant enzyme activity and reducing the accumulation of Na^+ and Cl^- ions in plant organs [46,47]. Catalase activity was increased with salt stress and polyamine treatments, the type of polyamine Putrescine led to an increase in chlorophyll content, leaf area, and leaf fresh weight in beans under salt stress[48]. It was reported that exogenous polyamine treatments reduced the negative effect of salt stress at different levels [49, 50]. It is believed that this may be effective in reducing the effects of salinity. In addition to what was mentioned previously, PAs are involved in many physiological and metabolic processes, including photosynthetic pigment defense, antioxidant systems, and ionic balance, which ultimately mitigate the negative effects of salt stress conditions on plants [12].

Conclusion

The current study concluded that: polyamine, salicylic acid and their interactions led to a significantly improvement in the growth characteristics and production of moderately salt-sensitive chilli pepper plants under salt stress. Salt had a negative effect on pepper plant in most vegetative growth traits, which led to a decrease in the yield of the chili pepper plant. It can also be concluded that the second concentration of spraying polyamines (3 $mg.l^{-1}$), and salicylic acid (150 $mg.l^{-1}$), was superior in many characteristics (plant height, leaf area, dry weight of shoot, chlorophyll content of leaves, fruits weight and number, capsaicin contain in fruits, and yield per plant) under salt stress. And the interaction between the treatments exceeded the second concentration for both polyamine and salicylic acid (P2A2) in most characteristics. Finally the triple interaction of the factors at a concentration (3 $mg.l^{-1}$) polyamine, (150 $mg.L^{-1}$) salicylic acid and 4000 ppm salt stress showed that (P2A2S2) was superior in most characteristics.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contribution Statement

M.K. designed the study. F.S. performed this work, F.S. and M.K. contribute to writing the manuscript. M.K. read and approved the final manuscript.

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تأثير البولي امين وحامض الساليسيليك في نمو وحاصل نبات الفلفل الحريف *Capsicum annuum* L. تحت الإجهاد الملحي

فاطمة حيدر صبحي، مشتاق فرج كرومي كسكو

قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة

الإجهاد الملحي له اثار سلبية على نمو وانتاجية الفلفل الحريف (.Capsicum annuum L) ، ويجب زيادة تحمل النبات ليتمكن من التكيف في ظروف إجهاد الملوحة. ولهذا الغرض أجريت تجربة عاملية بثلاثة عوامل وفق تصميم القطاعات العشوائية الكاملة(RCBD) وبثلاث مكررات تضمنت التجربة ثلاثة مستويات من البولي امين P0، P1، P2 (0، 2، 3 ملغم.ل$^{-1}$)، وثلاثة مستويات من حامض الساليسيلك A0، A1، A2 (0، 75، 150 ملغم.ل$^{-1}$) ، أما العامل الثالث فيتضمن ثلاثة مستويات من ملح كلوريد الصوديوم S0، S1، S2 (0، 2000، 4000 جزء في المليون). أظهرت نتائج التجربة أن اختلاف مستويات الملوحة كان له تأثير سلبي معنوي على الصفات المظهرية وحاصل الفلفل الحار، ولوحظ ان البولي امين P2 وحامض الساليسيليك A2 كانت متفوقة حيث اعطت أعلى قيمة في العديد من الصفات المدروسة، أما عند التداخل بين البولي امين وحامض الساليسيليك فقد لوحظ أن أعلى قيمة كانت P2A2 لنفس الصفات المدروسة عند مقارنتها بمعاملة السيطرة، بينما أعطى التداخل الثلاثي P2A2S0 أعلى قيمة مقارنة مع باقي الصفات المدروسة لجميع طرق المعالجة، باختصار، تشير هذه النتائج إلى أن اضافة البولي امين وحامض الساليسيليك يمكن أن يقلل بشكل فعال من التأثير الضار للإجهاد الملحي في الفلفل الحار.

الكلمات المفتاحية: *Capsicum annuum* L، فلفل حار، البولي امين، حامض الساليسليك، الاجهاد الملحي.