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Research Article

The effect of aspirin on the Muskmelon plant, *Cucumis melo* under different levels of salt stress

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Abstract: This study aimed to examine the role of aspirin (ASA, acetylsalicylic acid) in mediating the response of *Cucumis melo* (muskmelon) to variable levels of salinity stress. The study examined the impact of aspirin on the growth parameters, photosynthetic pigments, and total sugars of muskmelon plants subjected to salt stress. Five saline concentrations (NaCl solutions) of 0, 50, 100, 150, and 200 mmol were used as the stress stimulant, and five saline concentrations were combined with aspirin (10ppm) as an alleviation treatment. The findings showed that aspirin increased the activity of photosynthetic pigments and growth parameters, and lowering the overall sugar content, hence positively influencing salt tolerance. The results also showed an increase in the negative effect of salinity as the level of salinity increased. In the first group, adding aspirin to the second group and comparing it with non-aspirin treatment, we found that aspirin reduced salinity's negative effect. The results give insightful data and new methods using aspirin solution as a salinity treatment for future cultivation resistant to salt and yields of muskmelon.

Keywords: Muskmelon, Acetyl Salicylic Acid, Salt stress, Photosynthesis.

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Introduction

Muskmelon, *Cucumis melo* L. is a significant crop in the Cucurbitaceae family. Muskmelon is one of the most significant vegetables and significant commercial crops. Abiotic and biotic environmental factors have an impact on crop productivity and growth. One of the most significant environmental factors affecting plant productivity is salinity stress, particularly in arid and semiarid areas. Using biofertilizers for alfalfa improves the dry weight of the roots and shoots and the concentration of nitrogen (Monforte et al. 2014; Nastari-Nasrabadi & Saberali, 2020; Benouis et al. 2022). Popularly grown for its sweet flavor and excellent nutritional content, muskmelon is an economic crop (Pitrat 2008; Alrashedi et al. 2021). The 1.19 million hectares area produced an estimated 29.49 million tonnes of muskmelons worldwide (FAO 2014; Thakur et al. 2019).

A significant barrier to sustainable agricultural development is soil salinity, which reduces agricultural productivity, jeopardizes agricultural profits, and threatens the world's food security. The effects of soil salinity are felt negatively on about 20% of arable land worldwide (Hazell & Wood 2008; Qadir et al. 2014; Yan et al. 2023; Fadhil et al. 2024). Saline soils with high sodium ($Na+$) ion concentrations cause ion toxicity and osmotic stress, which impede photosynthesis, oxidative stress, and plant metabolism (Hamann 2015; Manishankar et al. 2018; Mohammed & Nulit 2019 a, b; Jia et al. 2019; Mohammed and Nulit 2020 a, b; Van Zelm et al. 2020). Moreover, it can even cause plant death because it severely reduces biomass, lateral root growth, and seed germination (Zhang et al. 2019; Fadhil et al. 2024). Consequently, increasing the salt resistance of crops has attained international interest.

Salinity stress causes a decrease in photosynthetic pigments and an increase in soluble sugar and proline levels. By regulating atmospheric nitrogen, improving nutrient accessibility, and obstructing the synthesis of plant hormones like auxin, cytokinin, and gibberellins, growth-promoting bacteria can benefit plants under stressful circumstances (Sarabi et al. 2017). Germination is the first and most crucial stage of plant morphogenesis, growth, and development; it mostly determines the quality of seedling growth (Uniyal & Nautiyal 1998; Yan et al. 2023). Salt stress and other unsavory environmental factors can lead to decreased seedling growth and reduced yields (Liu et al. 2020). For this reason, improving yields requires an understanding of how muskmelon seeds germinate when exposed to salt stress.

Guzmán-Murillo et al. (2013) and Luo et al. (2021) found a negative association between seed species' ROS levels and germination rates. ROS metabolism requires catalase, peroxidase, and superoxide dismutase. Enzyme activity increases ROS elimination (Khan et al. 2021). Lipoxygenase (LOX) is involved in seed development, stress tolerance, and other biochemical and physiological activities. Plants' LOX metabolic pathways generate oxygen radicals and hydroperoxides, which are crucial for their ability to withstand environmental stress (Taşgın et al. 2006; Yan et al. 2023).

Plants rely on the signaling chemical acetylsalicylic acid, or aspirin, to react to biotic and abiotic stressors (Senaratna et al. 2000; Bharti & Garg 2019). Two physiological ways SA reduces salt stress are by inducing an antioxidant defense mechanism and lowering membrane lipid peroxidation (Garg & Bharti 2018). SA also reduces ion toxicity (Liu et al. 2019) and controls the interaction between hormones and signaling substances (Wang et al. 2014).

Some research, however, has revealed that SA mediates the molecular reaction to salt stress in muskmelon seed germination. The study of plant adversity has entered the transcriptomics age thanks to the quick advancement of molecular biology and high-throughput sequencing technology. Researchers can comprehensively and dynamically detect changes in plant gene expression throughout various developmental stages and circumstances by utilizing transcriptome sequencing (RNA-seq) technologies (Yan et al. 2023). However, a major issue in the production of muskmelon is the effects of soil salinity and other obstacles on seed germination and growth period. This study aimed to determine whether phenotypic traits, photosynthetic pigments, and sugars are negatively affected by salinity levels; therefore, we used aspirin to improve or reduce this negative effect on plant quality.

Materials and Methods

Experiments were conducted in a plastic house at the College of Science, University of Misan, Iraq, on June 15, 2023, for 58 days, to evaluate aspirin's impact on muskmelon plants under salinity stress.

Plant materials and treatment: Muskmelon seeds (*Cucumis melo* L. var. *cantalupensis*) were provided by a seed sales outlet at the Ministry of Agriculture in Iraq. Muskmelon seeds were planted in boxes (7.5x7.5x9cm) containing peat moss (Table 1) only weighing 300g, irrigated with salt-free distilled water as needed, for a whole month, and then divided into two groups (Table 2). The first group was treated with 5 saline concentrations (NaCl solutions) of 0, 50, 100, 150, 200 mmol, and 3 repeaters for each treatment (without addition of aspirin). The second group was also treated with five saline concentrations with the addition of aspirin (Acetyl Salicylic Acid (ASA)) at a concentration of 10 ppm each as follows: (A0:0 mmol NaCl + 10 ppm ASA; A1:50 mmol NaCl + 10 ppm ASA; A2:100 mmol NaCl + 10 ppm ASA; A3:150 mmol NaCl + 10 ppm ASA; A4:200 mmol NaCl + 10 ppm ASA).

Growth parameters: After 58 days of planting, the parameters of the growth process and productivity were studied. These growth parameters were seedling length, fresh and dry weight, leaf number, and leaf area.

Table 1. Specifications of peat-moss used in experiments.

Description	Rate
Natural Soil	Free
Organic Matter, %	≥85 %
PH (in soil extract 5: 1)	≤7
EC (in soil extract 5: 1)	≤0.6
Sodium Chloride, %	≤1%
Carbon / Nitrogen Ratio, %	≤50: 1
Moisture	≥30%
Palm Waste / Sewage Waste	Free
Volume Bag, Liter	5
Address	Latvia

Table 2. Arrange of the experimental treatments.

Aspirin (ppm), ASA		Salinity level (milli-Molar), NaCl				
		A_0	A_1	A_2	A_3	A_4
No Aspirin	0 ppm	0 NaCl + 0 ASA	50 NaCl + 0 ASA	100 NaCl + 0 ASA	150 NaCl + 0 ASA	200 NaCl + 0 ASA
Addition of Aspirin	10 ppm	0 NaCl + 10 ASA	50 NaCl + 10 ASA	100 NaCl + 10 ASA	150 NaCl + 10 ASA	200 NaCl + 10 ASA

Ten plants from all treatments were considered to measure their dry weight (DW) and fresh weight (root and aboveground portions). A digital weighing balance was used to record the fresh and dry weights of the seedlings. Samples of shoots and roots were oven-dried for 48 hours at 70°C to determine the dry weight. Thus, the fresh and dry weights of the plant components were used to calculate the dry matter content. The root mass fractions were calculated using the dry weights of the plant components based on Poorter et al. (2012). For each treatment, three Muskmelon plants were chosen so that the leaf area on all of the leaves could be measured.

Photosynthetic pigments: The photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were determined using the methods given by Lichtenthaler & Wellburn (1983). Leaf samples (0.1g) were extracted on a heating plate for 20 minutes at 70°C in 1ml of 80% ethanol. After collecting the extracted solutions, the leaves underwent another extraction method before they turned white. All of the chlorophyll contents were measured. The chlorophyll content was measured using the methods given by Witham et al. (1986). Extracts (3 ml of extracts) were measured spectrophotometrically at 665, 649, and 470nm, and 80% ethanol was used as a blank solution. Chlorophyll content was estimated using the following formula:

Chlorophyll a (C_a) = 13.95 (A_{665}) - 6.88 (A_{649})

Chlorophyll b (C_b) = 24.96 (A_{649}) - 7.32 (A_{665})

Carotenoid = (1000A_{470} - 2.05 C_a - 114.8 C_b) / 245)

Total Chlorophyll = 20.2 A_{649} +8.02 A_{665}

Estimation of the total sugar content: Total sugars were estimated using the method of Dubois et al. (1956) with the following steps: In the first stage, 100mg of plant material was taken, and 3 ml of 80% ethanol was added and left in the dark for 48 hours. After this period had elapsed, to evaporate the alcohol, the tubes were submerged in water at 85°C for 10 minutes.

In the second stage, each tube was filled with 20ml of distilled water. In other glass tubes, we took 2 ml of this solution, then 1 ml of liquid phenol at a concentration of 5% was added. Afterward, it was

Table 3. Growth Parameters of Muskmelon plants under different levels of salt stress and the addition of aspirin.

Parameters	Aspirin (ppm)	Salinity level (milli-Molar)				
		A_0	A_1	A_2	A_3	A_4
Seedling Length (cm)	**No aspirin**	54.63±0.88^{ab}	50.67±1.76^{b}	35.10±1.95^{d}	28.07±0.52^{ef}	25.13±0.09^{f}
	Add. Aspirin	59.77±2.72^{a}	53.60±0.75^{b}	40.97±2.97^{c}	34.00±1.27^{d}	32.47±2.05^{de}
Fresh-Weight (g)	**No aspirin**	3.18±0.17^{b}	2.49±.11^{c}	1.96±0.16^{de}	1.69±0.06^{ef}	1.20±0.12^{g}
	Add. Aspirin	3.84±0.29^{a}	2.78±0.10^{bc}	2.35±0.16^{cd}	1.94±0.11^{de}	1.46±0.08^{fg}
Dry-Weight (g)	**No aspirin**	0.55±0.03^{a}	0.44±0.07^{bc}	0.29±0.014^{de}	0.25±0.02^{e}	0.13±0.01^{f}
	Add. Aspirin	0.58±0.03^{a}	0.53±0.03^{ab}	0.49±.04^{ab}	0.37±0.03^{cd}	0.24±0.02^{e}
Leaves Number	**No aspirin**	7.0±0.5^{a}	6.3±0.3^{ab}	5.7±0.3^{bc}	4.7±0.3^{cd}	4.3±0.3^{d}
	Add. Aspirin	7.3±33333^{a}	6.7±0.3^{ab}	5.7±0.3^{bc}	5.0±0.0^{cd}	4.7±0.3^{cd}
Leaf Area (cm^2)	**No aspirin**	20.2±1.0^{b}	20.5±0.9^{b}	15.5±0.6^{cd}	11.7±0.7^{e}	7.9±0.5^{f}
	Add. Aspirin	23.2±0.3^{a}	22.3±0.7^{ab}	17.3±0.1^{c}	14.6±1.0^{d}	10.1±1.0^{ef}

Note: Different lowercase letters indicate significant differences at the 0.05 probability level (P<0.05) according to Duncan's multiple range tests.

added 5 ml of concentrated sulfuric acid, avoiding putting the acid on the wall of the tube, and placed the tubes in a 30°C water bath for 15-20 minutes. The optical density was read on a spectrophotometer (Do_{490}), and the concentration of sugars is estimated in micrograms/100 g of substance Vegetarian the equation of Total sugars (mg/g $_{\text{Fresh-Plant}}$) = 1.24 + (97.440 Do_{490})

Statistics analysis: A completely randomized design (CRD) was adopted with 10 treatments ((five salinity levels), and (one aspirin concentration × five salinity levels)) and three replicates (10 plants per treatment). Data are presented as the mean±SD from at least three measurements. The data were evaluated using Analysis of Variance (ANOVA) and the General Linear Model (GLM) technique as described by Snedecor & Cochran (1980). Duncan's test was used to differentiate the means at a level of significance (P≤0.05).

Results and Discussion

Effects of aspirin on growth parameters of muskmelon plants under different levels of salt stress: The results indicated significant differences in the plant height by increasing in salt concentration, where the salt concentration of 0 mmol was the highest (54.63cm), and those in the highest salt level (200 mmol) treatment had the lowest plant height (25.13cm) (Table 3). The interaction between the salt concentration and aspirin had a notable impact on the plant height. The treatment with the highest average (59.77cm) was obtained by adding 10 ppm of aspirin to 0 mmol of sodium chloride. The addition of 200 mmol of sodium chloride in the absence of aspirin produced the therapy with the lowest average of 25.13cm.

The results revealed significant differences in the fresh weight due to the increase in salt concentration, where the salt concentration of 0 mmol had the highest rate (3.18 g), and the highest salt level (200 mmol) had the lowest fresh weight of 1.20g (Table 3). The interaction of the factors of salt concentration and adding of aspirin had a significant effect on the fresh weight, as the treatment of 0 x mmol sodium chloride 10 ppm of aspirin) had the highest average weight (3.84g) and the treatment of 200 x mmol sodium chloride 0 ppm aspirin, showed the lowest fresh weight (1.20g).

The results also showed significant differences in the dry weight between treatments, and the salt concentration of 0 mmol had the highest rate (0.55g) and the highest salt level (200 mmol) showed the lowest dry weight of 0.13 g (Table 3). The interaction of the factors of salt concentration and adding of aspirin had a significant effect on the rate of dry weight, as the treatment (0 x mmol sodium chloride 10

Table 4. Photosynthetic Pigments of Muskmelon plants under different levels of salt stress and the addition of aspirin.

Parameters	Aspirin (ppm)	Salinity level (milli-Molar)				
		A_0	A_1	A_2	A_3	A_4
Chlorophyll a(mg/g)	No aspirin	14.01±0.387^{b}	12.25±0.57^{bc}	8.22±0.55^{d}	6.69±0.63^{d}	6.16±0.73^{d}
	Add. Aspirin	15.27±0.33^{ab}	17.91±.33^{a}	12.3±0.45^{bc}	9.36±.30439^{cd}	8.37±0.23^{d}
Chlorophyll b (mg/g)	No aspirin	18.53±0.68^{d}	23.13±0.3b^{c}	24.72±.053^{b}	7.01±1.22^{e}	7.10±0.8^{e}
	Add. Aspirin	18.82±0.48^{d}	35.84±1.38^{a}	22.08±0.91^{c}	8.72±0.77^{e}	8.21±.55^{e}
Carotene (mg/g)	No aspirin	8.54±0.25^{a}	8.83±0.36^{a}	8.15±1.12^{ab}	7.38±0.09^{abc}	6.82±0.01^{bc}
	Add. Aspirin	7.23±.049^{abc}	6.11±0.60^{cd}	4.68±0.85^{d}	6.17±0.13^{cd}	6.38±0.05^{c}
Total Chlorophyll (mg/g)	No aspirin	38.48±0.79^{b}	40.49±0.56^{b}	36.67±0.42^{b}	18.22±1.38^{cd}	15.90±0.92^{d}
	Add. Aspirin	39.14±0.94^{b}	61.49±1.38^{a}	39.36±2.45^{b}	20.80±1.22^{c}	19.09±0.34^{cd}

Note: Different lowercase letters indicate significant differences at the 0.05 probability level (P<0.05) according to Duncan's multiple range tests.

ppm of aspirin) had the highest average dry weight (0.58g), and the treatment of 200 x mmol sodium chloride 0 ppm aspirin, revealed the lowest at 0.13g.

Based on the findings, greater salt concentrations cause significant leaf number changes. In particular, the rate was 7.0 at 0 mmol salt and 4.3 at 200 mmol salt. A substantial association between salt concentration and aspirin presence affected the rate of leaf growth. The therapy with 0 × mmol sodium chloride and 10 ppm aspirin had the highest average value of 7.3, whereas the treatment with 200 × mmol sodium chloride and 0 ppm aspirin had the lowest average value of 4.3 (Table 3).

Table 3 shows that the rate of leaf area increased with salt concentration, with 0 mmol having the highest rate of 20.2 cm^2 and 200 mmol having the lowest rate of 7.9 cm^2. The interaction of the factors of salt concentration and the addition of aspirin had a significant effect on the rate of leaf area, as the treatment (0 x mmol sodium chloride 10 ppm of aspirin) had the highest averages, reaching 23.2 cm^2, while the treatment (200 x mmol sodium chloride 0 ppm aspirin) the lowest was 7.9cm^2. The addition of aspirin had a significant effect on the rate of growth parameters (seedling length, fresh weight, dry weight, leaf number, and leaf area) and improved plant resistance under salt stress. Thus, it reduces the negative effects of salinity on growth parameters, especially at high salinity levels.

Effects of aspirin on the Photosynthetic Pigments of Muskmelon plants under different levels of salt stress: The results indicated significant differences in the rate of chlorophyll a, because of the increase in salt concentration, where the salt concentration of 0 mmol had the highest rate of 14.01 mg/g, while the highest salt level of 200 mmol had the lowest rate of chlorophyll a (6.16 mg/g) (Table 4). The interaction of the factors of salt concentration and the addition of aspirin had a significant effect on the rate of chlorophyll a, as the treatment (0 × mmol sodium chloride 10 ppm of aspirin) had the highest averages, reaching 15.27 mg/g, while the treatment (200 mmol sodium chloride 0 ppm aspirin) was the lowest 6.16mg/g.

The results showed significant differences in the rate of chlorophyll b due to the increase in salt concentration, where the salt concentration of 0 mmol had the highest rate of 18.53mg/g, while the highest salt level of 200 mmol had the lowest rate of chlorophyll b (7.10mg/g) (Table 4). The chlorophyll b rate was notably influenced by the combined effect of salt concentration and aspirin. The treatment that yielded the highest average concentration (18.82mg/g) contained 0 × mmol sodium chloride and 10ppm of aspirin. Conversely, the treatment with the lowest average concentration (7.10mg/g) consisted of 200 mmol sodium chloride and 0ppm of aspirin.

Based on the results, the carotenoid rate exhibits a substantial change as the salt content increases. At 0 mmol, the rate was 8.54mg/g, while at 200 mmol, the rate was 6.82mg/g, i.e. the lowest level of salt (Table

Table 5. Total sugars of Muskmelon plants under different levels of salt stress and the addition of aspirin.

Parameters	Aspirin (ppm)	Salinity level (milli-Molar)				
		A_0	A_1	A_2	A_3	A_4
Total sugar (mg/g)	**No aspirin**	62.43 ± 2.16^{g}	84.26 ± 5.17^{ef}	105.66 ± 8.14^{cd}	136 ± 4.24^{ab}	144.21 ± 3.62^{a}
	Add. Aspirin	36.45 ± 2.35^{h}	68.05 ± 3.88^{fg}	71.36 ± 4.86^{fg}	98.71 ± 6.27^{de}	120.31 ± 12.19^{bc}

Note: Different lowercase letters indicate significant differences at the 0.05 probability level ($P<0.05$) according to Duncan's multiple range tests.

4). The interaction of the factors of salt concentration and the addition of aspirin had a significant effect on the rate of carotenoids, as the treatment of 0 × mmol sodium chloride 10 ppm of aspirin had the highest averages, reaching 7.23mg/g, while the treatment of 200 mmol sodium chloride 10ppm aspirin was the lowest at 6.38mg/g. The addition of aspirin had a significant effect on the rate of photosynthetic pigments (Chlorophyll a, Chlorophyll b, and carotenoids) and improved plant resistance under salt stress. Thus, it reduces the negative effects of salinity on photosynthetic pigments, especially at high levels of salinity.

Effects of aspirin on total sugars of Muskmelon plants under different levels of salt stress: The results indicate significant differences in the rate of total sugars by increasing the salt concentration. The salts concentration of 0 mmol treatment had the lowest rate of 62.43mg/g, while the highest salt group of 200 mmol had the highest total sugars of 144.21mg/g (Table 5). The interaction of the factors of salt concentration and adding of aspirin had a significant effect on the total sugars, as the treatment 0 x mmol sodium chloride 10ppm of aspirin had the lowest averages of total sugars of 36.45mg/g, and the treatment of 200 x mmol sodium chloride 0ppm aspirin showed the highest total sugars of 144.21mg/g. Adding aspirin had a significant effect on the total sugars and improved plant resistance under salt stress. Thus, it will reduce the negative effect of salinity on total sugars, especially at high levels of salinity. Because salt stress negatively affects many elements of physiology and biochemistry, including photosynthesis, superoxide ion balance, antioxidant responses, osmolyte buildup, and proline metabolism, and limits plant development and morphology (Misra & Saxena 2009; Roussos et al. 2013). Because SA may control a plant's resistance to a variety of environmental challenges, especially salt stress, it is typically utilized in plant agriculture (Senaratna et al. 2000; Hayat et al. 2010).

Nowadays, one of the problems with cultivatable land is the salinity of the soil. Improving salt tolerance in crops with a wide range of application prospects can be accomplished simply and practically by applying exogenous regulating chemicals. Thus, it is imperative to clarify the mechanism by which SA encourages Muskmelon seed development in salt stress conditions. While SA can cause salt resistance, which has also been observed in several plant species (Dong et al. 2011; Hasan, Mohaddeseh 2011; Liu et al. 2014; Horváth et al. 2015; Arif et al. 2020).

One of the most important variables influencing photosynthetic efficiency and plant growth is the quantity of photosynthetic pigment (Shao et al. 2014). The results showed an increase in the negative effect of salinity as the level of salinity increased, compared to the control one. In the first group, adding aspirin to the second group and comparing it with non-aspirin treatment, we found that treatment with aspirin reduced the negative effect of salinity. According to our findings, muskmelon is a salt-tolerant plant. By activating the photosynthetic process and growth parameters more effectively, an exogenous supply of SA could mitigate the negative effects of moderate salinity on muskmelon growth.

Conclusions

We examined how SA supports Muskmelon plants under salt stress using a combination of growth parameters, photosynthetic pigments, and total sugars. According to our findings, SA increases the activity of photosynthetic pigments and growth parameters while lowering the number of total sugars, which has a proper impact on salt tolerance. This finding provides new data and modern techniques such as applying aspirin solution as a salinity treatment for future salt-resistant farming and high muskmelon yields.

Data availability: The paper and Supplementary Materials provide all of the datasets that were acquired for this investigation.

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