

The Effects of Salicylic acid and Silicon on Seed Yield, Oil Content, and Fatty Acids Composition in Safflower under Salinity Stress

Bahareh Jamshidi Jam

University of Zanjan

Farid Shekari (✉ shekari@znu.ac.ir)

University of Zanjan

Babak Andalibi

University of Zanjan

Reza Fotovat

University of Zanjan

Vahab Jafarian

University of Zanjan

Aria Dolatabadiyan

University of Manitoba

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Abstract

Soil and water salinization has global impact on crop production and food security. Application of phytohormones and nutrients management is major approaches to reduce salinity risks. The effects of salicylic acid (0, 600, 1200, and 1800 μM) and silicon (0, 1.5, and 2.5 mM) foliar application on safflower seed yield and quality was investigated under salt stress conditions (1.7, 7.5, and 15 dS m^{-1}). Salinity decreased capitulum number, seed number per capitulum, 100-seed weight, seed yield, oil percentage, oil yield, linoleic acid content, palmitic and linoleic acids yield, and seed potassium content. Application of salicylic acid (SA) and silicon (Si) increased biological yield, seed yield, oil content, oil yield, linoleic acid content, palmitic and linoleic acid yield but decreased stearic and oleic acid content and oleic acid yield. Harvest index was decreased with increasing salinity level, which indicates a stronger effect of salinity on seed yield rather than biomass production. In contrast, SA and Si, whether alone or together increased HI. The appropriate concentration of Si was different in salinity levels. Under non-stress and moderate stress conditions 2.5 mM Si showed better performance, while at severe salinity level, 1.5 mM Si showed a suitable state. Oil content and quality improved by increasing linoleic acid and reducing stearic and palmitic acids by application of SA and Si. Results suggest that the application of 1200 μM SA along with either levels of Si was more effective in improving quantitative and qualitative yield, especially under salinity stress conditions.

1 Introduction

Global agricultural productivity is seriously threatened by rising soil and water salinity. The issue is particularly prevalent in arid and semi-arid regions, such as Iran. [1, 2]. Increased salinity risk has raised concerns about food security and the destruction of natural resources [3]. There are numerous reports of detrimental effects of salinity on plant development, morphological, physiological, and biochemical processes, as well as quantitative and qualitative yield [4, 5]. In the short term, salinity causes osmotic stress, while in the long term it leads to ionic toxicity and induces oxidative stress at the cellular level [4]. Sodium ions damage plant cells by inhibiting photosynthesis, impairing ionic homeostasis, and membrane lipid peroxidation, thereby adversely affecting plant growth and yield [1, 6, 7]. For example, in mustard, salinity caused detrimental effects on photosynthesis by reducing leaf area and chlorophyll content, bursting oxidative damage, and decreased seed yield by reducing photosynthesis, number of pods, seeds, and 100-seed weight [8]. In cotton [9], and safflower [2] salinity decreased leaf area, leaf water potential, and K, Ca, Mg, and N content while increased Na content. On the other hand, seed yield improvement has been reported on account of proper application of nutrients and plant growth regulators in the presence of saline and unconventional water sources and other adverse environmental conditions [8, 10].

Plants need to be supplied by optimal amount of micro and macronutrients to overcome environmental stresses [10]. In this regard, one of the most important strategies to reduce the effects of stress and promote plant adaptation is the external supply of these elements to the plants [11]. Silicon (Si) is a quasi-essential element for plants and exists in the soil in the form of silicate or silicon oxide. Silicon application has shown significant effects on plants growth and development, either under stressful or optimal conditions [10, 12]. Silicon affects micro and macronutrients uptake and distribution in plants [12]. There are many studies reporting Si beneficial effects on plant development and yield as well as its ability to mitigate the detrimental impacts of environmental stress [2, 5, 10, 11]. Silicon has an impact on plant growth through elevating osmolyte accumulation, nutrient absorption, photosynthesis rate, antioxidant activity, phenolic compounds and adjusting water status, and hormones regulation [11]. In a study, Si application increased safflower seed yield compared to control plants under stress conditions [13]. Also, Si treatment raised the growth of sunflower and sorghum grown under salinity stress [14].

Auxin, gibberellin, and salicylic acid (SA), among other PGRs, are crucial for signaling network, plant growth, and tolerance to environmental stress [7, 8]. Salicylic acid is a phenolic molecule that acts as a phytohormone and has major impacts on a variety of physiological and biochemical processes, plant development, and yield, as well as plant resilience to environmental stresses such as salinity [7, 15]. However, higher concentrations may cause plant toxicity and reduce yield [16,

17]. It has been reported that SA application under both non-stress and stress conditions reduced saturated fatty acids and increased linoleic acid and oleic acid content in *Cucurbita pepo* [18].

Silicon and SA can boost plant dry weight and yield stability by enhancing silicon and other nutrients absorption [17]. In a study, the combined application of SA and Si reduced the inhibitory effects of excess boron in chickpeas and helped increase plant tolerance to boron toxicity by preventing oxidative damage to membranes [15]. With the simultaneous application of Si and SA in soybean and bean, it was found that SA foliar application increased leaf Si content which was accompanied by increased CO₂ uptake and stomatal opening [16]. Also, SA increased Si uptake and dry weight in peanut, especially when combined with the Si foliar application [17]. It has been reported that the application of PGRs, such as SA and foliar application of elements, like Si improve the seed yield, oil and fatty acids in oilseed plants [13, 18, 19].

Increasing world population beside to climate change and soil degradations lead to use arable land with lower quality. Soil salinization is rapidly increasing in large scales, especially in countries located in arid and semi-arid regions. Therefore, it is essential to understand mechanisms involved in salt tolerance and take measures to improve salt tolerance. Safflower (*Carthamus tinctorius* L.) is an annual oilseed crop with high economic value of edible oil production. Safflower has a strong root system and remarkable tolerance to salinity and drought [13]. Due to its tolerance to environmental stresses and its production in dryland systems, this plant has a potential to be considered as a promising future crop for being cultivated in arid lands. Although safflower is a salt tolerant crop, its yield and oil quality reduce due to salt stress. Application of PGRs and nutrients may increase crops performance under stressful conditions. Although there is information about the separate effects of SA and Si on safflower, reports about the combined application of these substances on the performance and quality of safflower oil under salinity are limited. This study was conducted to investigate the combined effect of SA and Si foliar application on seed yield and its components, oil yield, and changes in oil fatty acid profiles of safflower grown under salinity stress.

2 Materials And Methods

2.1 Experimental Design, Plant Materials and Treatments

This research was conducted in a research greenhouse at the University of Zanjan, Zanjan, Iran. The experiment was performed as a factorial arrangement based on a randomized complete block design with three factors and three replications in plastic pots with 30 cm top diameter and 35 cm height filled with sifted soil, sand, and manure with a ratio of six: three: one. The soil was a clay loam type and contained pH 7.6, EC 1.74 dS m⁻¹, available P 16.8 mg kg⁻¹, available K 170 mg kg⁻¹, Na 18.86 mg kg⁻¹, Ca 14.4 mg kg⁻¹, total N 0.075%. Safflower seeds, cv. Goldasht were obtained from the Seed and Plant Improvement Institute, Oilseed Crops Research Department, Karaj, Iran. Salinity treatment consisted of three levels (1.7, 7.5, and 15 dS m⁻¹). The NaCl required for each pot was calculated and added to the pots after dissolving in water. To keep constant the salinity concentration in the pot, after irrigation, the collected water in the saucer through the drainage was returned to the pot. Salicylic acid treatment included four levels of foliar application (0, 600, 1200, and 1800 µM). Silicon treatment had three levels of foliar application (0, 1.5, and 2.5 mM) from potassium silicate. The pH of the potassium silicate solution was adjusted to 7 using HCl (1 M) and NaOH (1 M).

2.2 Seed Sowing, and Greenhouse Conditions

Twenty safflower seeds, disinfected with carboxin thiram fungicide, were planted at the depth of 3 cm in each pot. The first irrigation was done immediately after seeding and the next irrigation rounds were performed at two-three days intervals. After emergence and thinning at the 3–4 leaf stage, five healthy plants were preserved in each pot. Abamectin was sprayed twice against two-spot mites, Benomyl was used once against powdery mildew, and Imidacloprid was applied once against aphids. The greenhouse light was provided by sunlight and artificial light. Lighting: darkness duration was 16: 8 h. Radiation intensity was 900–1000 µM m⁻² s⁻¹. The average relative humidity was 63% and the average maximum and minimum temperatures were 32/15°C in day/night.

2.3 Foliar Application of Salicylic Acid and Silicon

After establishing the seedlings and at the three to the four-leaf stage, SA and Si foliar application was performed. Salicylic acid was sprayed first at 7 am, and then Si was sprayed at 6 pm the next afternoon. The plants were harvested after maturity and the following traits were measured by selecting five plants from each pot.

2.4 Phenotypic, Yield, Yield Components and Qualitative Trait Measurements

2.4.1 Plant Height and Biological Yield

At harvest, plant height was measured from the soil surface to the tip of the plants. The plants were then harvested from near the soil surface dried in an oven at 70 °C and then weighted. The average weight of a plant was reported as biological yield in g per plant.

2.4.2 Seed Yield, Yield Components, and Harvest Index

The capitula were separated and counted to report capitulum number per plant. Then seeds were separated manually and counted to record seed number per capitulum. 100 seeds were counted using a seed counter (Pfeuffer, Germany) and weighted by a digital scale (0.001 g accuracy). To calculate seed yield (g plant^{-1}) all capitula obtained from each pot were pounded by hand and all seeds were weighed. The harvest index was calculated as the ratio of seed yield to biological yield and was expressed as a percentage.

2.4.3 Oil Percentage and Oil Yield

Three g of seeds was grounded and packed in filter paper and then its initial weight was obtained. The samples were placed in a Soxhlet device (BUCHI extraction system B-811, Germany) for 11 h to extract their oil with n-hexane solvent. The filter papers containing the sample were then placed in an oven at 50°C for 2 h to remove the excess solvent. After 2 h, the samples were taken out of the oven and transferred to a desiccator to prevent moisture absorption. Then, by taking out each sample from the desiccator, its secondary weight was immediately obtained. Finally, the oil content was calculated as a percentage with the following formula [20]:

$$\text{Oil percentage} = [(\text{Initial weight} - \text{Secondary weight}) / \text{Initial weight}] \times 100$$

Oil yield (g plant^{-1}) was obtained by multiplying the percentage of oil by seed yield per plant.

2.4.4 Fatty Acids Composition

Fatty acids were identified using gas chromatography method. The gas chromatograph-mass spectrometer (GC-MS) device included a gas chromatography model 7890B and a mass spectrometer (model 5977A made by Agilent Company, USA). The GC/MS had a split/splitless injection system, an electron bombardment ionization model, and mass libraries related to the NIST and WILEY. The HP5-MS column with a 60 m length, a 0.25 mm inner diameter, and a 0.25 μm thickness was used. The injection site temperature, interface temperature, and ionization site temperature were set at 290, 300, and 250°C, respectively. The column temperature program was started with an initial temperature of 70°C and was maintained at this temperature for 5 min. Then the column temperature was brought to 150°C with a slope of 15°C min^{-1} and remained constant at this temperature for 2 min. Finally, it was transmitted to 290°C with a slope of 20°C min and remained constant at this temperature for 10 min. The split ratio was set to 1:20. The volume of injection was 1 μL . For the extraction and derivatization process (methyl esterification) of the extracted fatty acids, first, 2 mL of n-hexane solvent and 300 μL of methanolized KOH solution were added to the extracted sample (0.5 g). Then the resulting mixture was stirred for 5 min. Thereafter the samples were centrifuged at 4500 \times g for 5 min. The supernatant phase of the sample was transferred to GC/MS for analyzing fatty acids.

2.4.5 Yield of Fatty Acids and Percentage of Seed Protein

The fatty acid yield (mg plant^{-1}) was calculated by multiplying the percentage of each fatty acid by oil yield per plant. To calculate seed protein percentage, total seed nitrogen percentage measured using the Kjeldahl method. The data was multiplied by a constant coefficient of 6.25 [21].

2.4.6 Seed Potassium and Sodium Contents

The wet digestion method was used to measure seeds potassium and sodium content. A dry sample (0.3 g) was digested with mixed acid (6 g of salicylic acid, 100 ml of 98% sulfuric acid, and 18 ml of distilled water) according to the method of Walinga et al. [22]. Then the extract was used to measure the content of sodium and potassium by the method of flame measurement (flame photometry) using a flame photometer (Jenway, model PFP7/C, UK). Sodium and potassium contents were reported as a percentage.

2.5 Statistical Analysis

The normality of the data was confirmed by the normality test using SAS statistical software (SAS, Institute Inc. 2009). A three-way factorial ($3 \times 4 \times 3$) arranged in a randomized complete block design with three replications was used for data analysis. For the analysis of variance, SAS statistical software (SAS, Institute Inc. 2009) was used. When the effects were significant ($P \leq 0.05$), differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

3 Results

The analysis of variance showed that the main effects of "salinity", "salicylic acid" and "silicon" were significant on all the traits. Only 100 seed weight was affected by salinity and Si and SA had not significant effect on this trait. The interaction effect of "salinity \times salicylic acid \times silicon" was significant ($P \leq 0.001$) on all the traits except for number of seed per capitulum, 100 seed weight, harvest index, Palmitic acid content and Seed sodium.

3.1 Plant Height

Salinity reduced plant height so that the tallest plants were found under non-stress condition and sprayed with $1200 \mu\text{M}$ SA and 2.5 mM Si whilst the shortest plants were belong to the severe salinity level and non-treated plants. The separate and co-application of SA and Si increased plant height under salinity and non-salinity conditions. Under non-stress condition and 7.5 dS m^{-1} salinity, the application of $1200 \mu\text{M}$ SA along with 2.5 mM Si increased plant height by 47% and 38%, respectively, compared to the non-application of these two compounds under the same conditions. At the 15 dS m^{-1} salinity, the application of $1200 \mu\text{M}$ SA along with 1.5 mM Si elevated plant height by 39% compared to the non-application of these two compounds (Table 1).

Table 1

Interaction of salicylic acid and silicon foliar application on height, biomass, number of capitulum per plant, number of seeds per capitulum, seed yield, harvest index, oil percentage, and oil yield at different salinity levels.

Salt stress (dS m ⁻¹)	Salicylic acid (μM)	Silicon (mM)	Height (cm)	Biomass (g plant ⁻¹)	Number of capitulum per plant	Number of seed per capitulum	Seed yield (g plant ⁻¹)	Harvest index (%)	Oil percentage (%)	Oil yield (g plant ⁻¹)
1.7 (control)	0	0	56.92 ± 1.39	7.23 ± 0.59	2.22 ± 0.12	17.72 ± 2.27	1.63 ± 0.114	22.6 ± 0.641	23.40 ± 1.44	0.385 ± 0.049
		1.5	67.80 ± 1.48	9.67 ± 0.2	2.41 ± 0.097	18.96 ± 1.31	2.26 ± 0.112	23.35 ± 0.679	25.03 ± 1.88	0.570 ± 0.069
		2.5	69.45 ± 0.91	10.42 ± 0.28	2.56 ± 0.13	19.74 ± 0.96	2.56 ± 0.093	24.54 ± 0.318	26.12 ± 1.82	0.671 ± 0.069
	600	0	71.91 ± 1.3	11.21 ± 0.34	2.58 ± 0.081	19.80 ± 0.99	2.78 ± 0.096	24.78 ± 0.121	26.29 ± 1.82	0.734 ± 0.076
		1.5	71.97 ± 0.61	11.71 ± 0.33	2.90 ± 0.052	20.01 ± 0.15	2.92 ± 0.126	24.96 ± 0.429	27.98 ± 1.48	0.822 ± 0.079
		2.5	74.03 ± 1.87	12.57 ± 0.42	2.91 ± 0.047	20.89 ± 0.43	3.18 ± 0.121	25.28 ± 0.451	28.60 ± 1.53	0.912 ± 0.083
	1200	0	76.31 ± 1.95	12.68 ± 0.18	3.13 ± 0.067	21.02 ± 0.35	3.28 ± 0.077	25.87 ± 0.295	29.24 ± 2.02	0.961 ± 0.083
		1.5	78.67 ± 3.4	13.74 ± 0.29	3.2 ± 0.12	21.27 ± 1.54	3.61 ± 0.112	26.25 ± 0.279	29.67 ± 2.17	1.075 ± 0.108
		2.5	83.90 ± 0.98	14.02 ± 0.44	3.57 ± 0.033	21.56 ± 1.09	3.73 ± 0.103	26.62 ± 0.108	32.30 ± 1.99	1.209 ± 0.106
	1800	0	69.06 ± 1.06	10.75 ± 0.31	2.69 ± 0.059	20.54 ± 0.67	2.48 ± 0.070	23.09 ± 0.147	26.04 ± 1.74	0.648 ± 0.061
		1.5	70.35 ± 0.92	11.13 ± 0.4	2.72 ± 0.053	20.62 ± 0.87	2.69 ± 0.122	24.18 ± 0.582	26.95 ± 1.36	0.729 ± 0.069
		2.5	68.24 ± 0.70	10.33 ± 0.33	2.44 ± 0.07	19.28 ± 0.18	2.32 ± 0.088	22.45 ± 0.477	25.41 ± 1.83	0.592 ± 0.065
7.5	0	0	50.73 ± 1.83	6.93 ± 0.51	1.83 ± 0.115	16.84 ± 1.32	1.31 ± 0.103	18.85 ± 0.49	21.92 ± 1.60	0.289 ± 0.043
		1.5	56 ± 1.07	7.67 ± 0.13	2.03 ± 0.102	16.96 ± 1.12	1.58 ± 0.011	20.57 ± 0.205	22.72 ± 1.86	0.359 ± 0.032

Data represents the average of three replicates (n = 3) ± standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

Salt stress (dS m ⁻¹)	Salicylic acid (μM)	Silicon (mM)	Height (cm)	Biomass (g plant ⁻¹)	Number of capitul per plant	Number of seed per capitul	Seed yield (g plant ⁻¹)	Harvest index (%)	Oil percentage (%)	Oil yield (g plant ⁻¹)	
600	0	2.5	58.86 ± 1.41	8.08 ± 0.24	2.17 ± 0.003	17.27 ± 1.82	1.68 ± 0.037	20.87 ± 0.169	23.55 ± 1.95	0.398 ± 0.042	
		0	60.76 ± 1.62	8.29 ± 0.21	2.20 ± 0.063	17.91 ± 0.31	1.77 ± 0.072	21.27 ± 0.315	23.78 ± 1.89	0.422 ± 0.051	
		1.5	62.75 ± 2.28	9.06 ± 0.13	2.38 ± 0.096	18.67 ± 0.45	2.00 ± 0.101	22.09 ± 0.807	24.26 ± 1.40	0.488 ± 0.051	
	1200	0	2.5	63.8 ± 0.13	9.48 ± 0.04	2.52 ± 0.023	19.33 ± 0.60	2.10 ± 0.055	22.12 ± 0.52	24.32 ± 1.86	0.512 ± 0.052
			1.5	64.31 ± 1.32	9.68 ± 0.32	2.78 ± 0.036	19.79 ± 0.57	2.15 ± 0.096	22.17 ± 0.354	25.36 ± 1.94	0.548 ± 0.066
			2.5	68.02 ± 0.24	10.01 ± 0.55	2.84 ± 0.124	19.92 ± 0.70	2.29 ± 0.103	22.89 ± 0.466	25.41 ± 1.69	0.585 ± 0.064
	1800	0	2.5	70.05 ± 0.81	10.67 ± 0.61	2.95 ± 0.047	20.58 ± 1.02	2.57 ± 0.159	24.09 ± 0.156	27.23 ± 1.95	0.706 ± 0.089
			1.5	61.49 ± 0.7	8.64 ± 0.24	2.10 ± 0.053	19.14 ± 0.9	1.80 ± 0.061	20.87 ± 0.17	23.30 ± 1.86	0.423 ± 0.048
			2.5	62.87 ± 0.44	9.70 ± 0.37	2.32 ± 0.093	19.47 ± 1.09	2.04 ± 0.123	20.98 ± 0.486	24.16 ± 1.27	0.496 ± 0.055
15	0	2.5	58.53 ± 1.86	8.55 ± 0.28	2.05 ± 0.047	18.97 ± 0.24	1.68 ± 0.069	19.66 ± 0.182	23.02 ± 1.41	0.389 ± 0.040	
		0	46.67 ± 1.2	5.83 ± 0.089	1.61 ± 0.057	14.83 ± 0.9	1.04 ± 0.031	17.78 ± 0.27	19.61 ± 1.66	0.204 ± 0.023	
		1.5	53 ± 1.70	6.34 ± 0.36	1.71 ± 0.084	16.10 ± 0.29	1.17 ± 0.058	18.43 ± 0.406	21.29 ± 1.20	0.250 ± 0.026	
	600	0	2.5	53.56 ± 1.44	6.76 ± 0.42	1.86 ± 0.084	16.26 ± 0.50	1.33 ± 0.068	19.62 ± 0.204	21.37 ± 1.64	0.285 ± 0.036
			0	56.16 ± 2.23	7.21 ± 0.35	1.92 ± 0.116	16.84 ± 0.79	1.45 ± 0.091	20.06 ± 0.525	21.48 ± 1.51	0.314 ± 0.041
			1.5								

Data represents the average of three replicates (n = 3) ± standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

Salt stress (dS m ⁻¹)	Salicylic acid (μM)	Silicon (mM)	Height (cm)	Biomass (g plant ⁻¹)	Number of capitulum per plant	Number of seed per capitulum	Seed yield (g plant ⁻¹)	Harvest index (%)	Oil percentage (%)	Oil yield (g plant ⁻¹)
15		1.5	56.67 ± 2.03	7.54 ± 0.56	2.04 ± 0.104	17.62 ± 1.20	1.61 ± 0.098	21.46 ± 0.405	22.15 ± 1.32	0.360 ± 0.043
		2.5	60.83 ± 0.75	8.18 ± 0.84	2.11 ± 0.056	17.79 ± 1.70	1.78 ± 0.160	21.85 ± 0.33	23.05 ± 1.43	0.415 ± 0.061
	1200	0	62.69 ± 1.01	8.29 ± 0.41	2.31 ± 0.077	18.78 ± 0.57	1.86 ± 0.035	22.49 ± 0.72	23.57 ± 2.05	0.440 ± 0.046
		1.5	64.99 ± 0.94	9.24 ± 0.36	2.61 ± 0.15	18.93 ± 0.33	2.15 ± 0.082	23.25 ± 0.027	25.10 ± 2.05	0.543 ± 0.063
		2.5	63.28 ± 0.73	8.95 ± 0.21	2.34 ± 0.032	18.83 ± 0.47	2.05 ± 0.040	22.85 ± 0.298	22.55 ± 1.51	0.46 ± 0.039
	1800	0	53.74 ± 2.6	6.50 ± 0.4	1.88 ± 0.060	16.44 ± 0.14	1.36 ± 0.084	20.88 ± 0.051	21.09 ± 1.35	0.288 ± 0.035
1.5		55.56 ± 1.13	7.15 ± 0.27	2.05 ± 0.090	17.08 ± 0.43	1.54 ± 0.039	21.52 ± 0.56	21.77 ± 1.44	0.335 ± 0.029	
2.5		51.66 ± 1.92	5.96 ± 0.032	1.70 ± 0.075	16.36 ± 0.35	1.20 ± 0.013	20.08 ± 0.137	20.68 ± 1.56	0.248 ± 0.021	
LSD		3.035	0.555	0.158	2.49	0.122	1.13	0.923	0.064	

Data represents the average of three replicates (n = 3) ± standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

3.2 Capitulum per Plant, Number of Seeds per Capitulum and 100-Seed Weight

Increase in NaCl concentration decreased capitulum number per plant and seed number per capitulum. Application of SA and Si increased capitulum number per plant and seed number in capitulum under both stress and non-stress conditions. The highest values of these traits was found in 1200 SA μM and all Si concentrations and lowest values was in the 15 dS m⁻¹ and without application of SA and Si (Table 1). Application of 1200 μM SA along with 2.5 mM Si increased capitulum number by 60.8% and 61.2%, and seed number per capitulum by 22% and 22%, respectively, compared to the non-application of these two compounds under non-stress and 7.5 dS m⁻¹ salinity conditions. While at 15 dS m⁻¹ salinity, 1200 μM SA along with 1.5 mM Si increased capitulum number by 62%, and seed number per capitulum by the 28% compared to the non-application of these two compounds (Table 1).

The 100-seed weight was affected only by salinity ($P \leq 0.001$) and the application of SA or Si had not any significant effect on 100 seed weight. Increase in NaCl concentration reduced 100-seed weight. No significant difference was observed between salinity levels. Under salinity conditions, 100-seed weight showed a 12% reduction compared to the control conditions (Fig. 1).

3.3 Biological Yield, Seed Yield and Harvest Index

Foliar application of SA and Si reduced the damage caused by salinity stress and increased the biological and seed yield of safflower. In non-stress and 7.5 dS m^{-1} salinity conditions, $1200 \mu\text{M}$ SA along with 2.5 mM Si increased biological yield by 194% and 154% and seed yield by 129% and 96%, respectively, compared to the non-application of these two compounds at the same conditions. At the highest salinity level, the highest biological and seed yield was related to $1200 \mu\text{M}$ SA along with 1.5 mM Si, which caused a 158% and 107% increase respectively compared to the non-application of these two compounds at the same stress level (Table 1). Our results show that at the highest concentration of SA, biological and seed yield decreased in both stress and non-stress condition regardless of the Si levels. It shows a negative effect of SA at this concentration in safflower. Harvest index decreased under salinity stress (Table 1). This reduction was sharp from non-stress condition to 7.5 dS m^{-1} , but from this level to severe salinity stress level the reduction was slight. These findings suggest that although salinity decreased the seed and biological yield, salinity had more effects on seed yield than biomass production. In contrast, the separate and combined treatments of SA and Si both under stress and non-stress conditions increased HI. In the non-stress and 7.5 dS m^{-1} salinity conditions, $1200 \mu\text{M}$ SA along with 2.5 mM Si caused the highest HI, an increase of 18% and 28% compared to the non-application of these two compounds at the same stress levels, respectively. At 15 dS m^{-1} salinity, the highest HI was related to $1200 \mu\text{M}$ SA along with 1.5 mM Si treatment, which increased HI by 31% compared to the non-application of these two compounds (Table 1). It shows that spraying of SA and Si healing effects also increased with increasing salt levels.

3.4 Oil Percentage and Oil Yield

Salt stress reduced seed oil content and oil yield per plant. Although seed oil content was reduced rapidly under moderate salt stress compared to non-stress condition, this reduction was slight but significant between the salt levels (Table 1). The results showed that SA and Si were able to increase oil percentage and oil yield under stress and non-stress conditions. Under the non-stress condition the highest effect of spraying of SA and Si was found in $1200 \mu\text{M}$ SA along with 2.5 mM Si which enhanced seed oil content from 23.4–32.3%. Like to non-stress condition in 7.5 dS m^{-1} salt level, $1200 \mu\text{M}$ SA and 2.5 mM Si increased oil percentage and oil yield. Treated plants with $1200 \mu\text{M}$ SA and 1.5 mM Si showed the highest oil percentage and oil yield compared to the non-application of these two compounds at the highest level of salinity stress (Table 1).

3.5 Content of Palmitic, Stearic, Linoleic, and Oleic Fatty Acids

The fatty acids profile showed that salinity caused a change in the oil quality. Salinity stress increased the content of palmitic, stearic, and oleic acids and decreased the amount of linoleic acid in safflower. At all salinity levels, the combination of SA and Si reduced the content of palmitic acid, stearic acid and oleic acid and increased the linoleic acid content (Table 2). For instance, in non-stress condition linoleic acid content was increased from 64.8% in non-treated plants to 82.8% in application of $1200 \mu\text{M}$ SA and 2.5 mM Si. Also, the content of palmitic acid decreased in the non-stress and 7.5 dS m^{-1} salinity conditions, by mentioned SA and Si concentrations by 19% and 10%, respectively, compared to the non-treated plants at the same levels. A 13% reduction in the content of palmitic acid was observed with the application of $1200 \mu\text{M}$ SA along with 1.5 mM Si compared to the non-application of these two compounds at the highest level of salinity stress (Table 2). Similarly, this situation was found for stearic and oleic acid.

Table 2

Interaction of salicylic acid and silicon foliar application on palmitic acid, stearic acid, linoleic acid and oleic acid percentage, palmitic acid, stearic acid and linoleic acid at different salinity levels.

Salt stress (dS m ⁻¹)	Salicylic acid (μ M)	Silicon (mM)	Palmitic acid (%)	Stearic acid (%)	Linoleic acid (%)	Oleic acid (%)	Palmitic acid yield (mg plant ⁻¹)	Stearic acid yield (mg plant ⁻¹)	Linoleic acid yield (mg plant ⁻¹)
1.7 (control)	0	0	12.88 \pm 0.88	12.65 \pm 0.35	64.82 \pm 0.85	5.48 \pm 0.94	50.02 \pm 9.05	48.88 \pm 7.28	249.4 \pm 32.86
		1.5	12.43 \pm 0.68	11.56 \pm 0.35	67.73 \pm 0.39	4.26 \pm 0.54	71.40 \pm 11.82	66.16 \pm 9.54	386.05 \pm 47.62
		2.5	12.08 \pm 0.42	10.83 \pm 0.41	70.02 \pm 1.03	0.64 \pm 0.071	81.26 \pm 9.85	72.80 \pm 8.75	469.11 \pm 44.43
	600	0	11.87 \pm 0.42	8.13 \pm 0.38	72.88 \pm 1.48	0.53 \pm 0.043	87.61 \pm 11.68	59.86 \pm 7.73	534.63 \pm 52.89
		1.5	11.83 \pm 0.36	7.88 \pm 0.39	75.31 \pm 1.54	0.46 \pm 0.041	97.76 \pm 12.32	65.00 \pm 8.3	617.9 \pm 53.77
		2.5	11.56 \pm 0.21	7.72 \pm 0.22	77.31 \pm 0.62	0.36 \pm 0.047	105.78 \pm 11.34	70.54 \pm 7.39	704.65 \pm 59.52
	1200	0	11.55 \pm 0.28	4.72 \pm 0.37	79.95 \pm 0.99	0.3 \pm 0.047	111.41 \pm 11.97	45.52 \pm 6.2	767.84 \pm 62.64
		1.5	11.52 \pm 0.31	4.54 \pm 0.31	80.84 \pm 1.2	0.22 \pm 0.050	124.19 \pm 14.77	48.94 \pm 6.82	867.83 \pm 81.88
		2.5	10.4 \pm 0.24	4.29 \pm 0.25	82.81 \pm 1	0.16 \pm 0.012	126.12 \pm 13.12	52.11 \pm 6.62	1000.43 \pm 81.2
1800	0	12.24 \pm 0.1	8.88 \pm 0.48	71.66 \pm 0.98	0.9 \pm 0.22	79.45 \pm 8.07	57.83 \pm 7.69	463.43 \pm 40.29	
	1.5	12.07 \pm 0.24	8.64 \pm 0.98	73.72 \pm 1.25	0.78 \pm 0.131	88.10 \pm 9.46	63.91 \pm 12.79	535.61 \pm 42.3	
	2.5	12.39 \pm 0.27	11.24 \pm 0.33	69.42 \pm 1.09	1 \pm 0.076	73.66 \pm 9.47	66.94 \pm 9.19	410.16 \pm 39.76	
7.5	0	0	13.12 \pm 0.25	14.68 \pm 0.24	61.39 \pm 0.42	9.3 \pm 0.12	38.11 \pm 6.36	42.61 \pm 6.99	177.25 \pm 25.57
		1.5	12.92 \pm 1.02	13.53 \pm 0.53	63.76 \pm 1.14	6.74 \pm 0.13	46.77 \pm 7.3	48.7 \pm 5.52	228.43 \pm 18.11
		2.5	12.82 \pm 0.24	12.69 \pm 0.87	64.18 \pm 128	6.51 \pm 0.14	51.22 \pm 6.25	51.13 \pm 8.63	254.86 \pm 23.4
	600	0	12.67 \pm 0.28	11.03 \pm 0.24	67.56 \pm 0.72	6.14 \pm 0.99	53.60 \pm 6.92	46.67 \pm 6.07	284.89 \pm 31.6

Data represents the average of three replicates (n = 3) \pm standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

Salt stress (dS m ⁻¹)	Salicylic acid (μ M)	Silicon (mM)	Palmitic acid (%)	Stearic acid (%)	Linoleic acid (%)	Oleic acid (%)	Palmitic acid yield (mg plant ⁻¹)	Stearic acid yield (mg plant ⁻¹)	Linoleic acid yield (mg plant ⁻¹)	
1200		1.5	12.48 \pm 0.47	10.51 \pm 0.51	67.92 \pm 0.41	3.64 \pm 0.68	61.08 \pm 7.5	51.43 \pm 6.54	331.5 \pm 33.11	
		2.5	12.42 \pm 0.34	9.98 \pm 0.47	68.39 \pm 0.49	3.02 \pm 0.69	63.77 \pm 7.53	51.34 \pm 6.84	350.11 \pm 34.43	
	1200	0	12.11 \pm 0.28	8.48 \pm 0.32	69.45 \pm 0.34	2.68 \pm 0.64	66.67 \pm 9.32	46.67 \pm 6.7	380.59 \pm 44.56	
		1.5	11.98 \pm 0.25	7.95 \pm 0.25	71.11 \pm 0.58	2.21 \pm 0.35	70.30 \pm 9.07	46.6 \pm 5.98	415.06 \pm 42.47	
	1200	2.5	11.86 \pm 0.31	5.51 \pm 0.25	73.87 \pm 0.57	2.09 \pm 0.41	83.95 \pm 11.95	38.91 \pm 5.54	520.2 \pm 62.07	
		1800	0	12.58 \pm 0.65	10.98 \pm 0.37	64.16 \pm 0.86	6.24 \pm 0.157	53.56 \pm 8.41	46.56 \pm 6.33	270.38 \pm 27.38
	1800	1.5	12 \pm 0.31	9.34 \pm 0.33	65.69 \pm 0.57	6.06 \pm 0.137	59.69 \pm 7.66	46.42 \pm 5.96	325.4 \pm 34.35	
		2.5	12.11 \pm 0.48	9.89 \pm 0.28	65.54 \pm 0.99	6.72 \pm 0.55	47.42 \pm 6.47	38.61 \pm 4.66	254.52 \pm 22.79	
	15	0	0	14.36 \pm 0.46	16.7 \pm 0.36	55.26 \pm 1.26	12.16 \pm 0.33	29.5 \pm 4.13	34.17 \pm 4.19	112.58 \pm 11.11
			1.5	13.9 \pm 0.28	15.76 \pm 1.04	57.67 \pm 1.21	11.68 \pm 0.06	34.82 \pm 4.28	39.61 \pm 6.12	143.64 \pm 13.47
			2.5	13.87 \pm 0.44	14.71 \pm 0.53	60.23 \pm 1.04	9.84 \pm 0.34	39.75 \pm 5.89	42.07 \pm 6.03	171.5 \pm 20.13
		600	0	12.98 \pm 0.2	14.33 \pm 0.66	62.24 \pm 0.68	8.17 \pm 0.23	40.82 \pm 5.82	45.15 \pm 7.18	194.84 \pm 24.01
1.5			12.78 \pm 0.93	13.92 \pm 0.61	62.94 \pm 1.23	6.62 \pm 0.38	46.5 \pm 8.31	50.35 \pm 7.41	226.12 \pm 24.46	
2.5			12.59 \pm 0.5	13.69 \pm 0.25	64.15 \pm 0.88	6.23 \pm 0.27	52.69 \pm 9.29	56.92 \pm 8.7	265.79 \pm 36.55	
1200		0	12.46 \pm 0.23	12.93 \pm 0.22	66.24 \pm 0.32	6.17 \pm 0.18	54.93 \pm 6.55	56.92 \pm 6.45	290.94 \pm 29.05	
		1.5	12.5 \pm 0.55	11.94 \pm 0.81	67.08 \pm 1.59	6.14 \pm 0.25	68.25 \pm 10.3	65.17 \pm 10.55	263.04 \pm 38.61	
		2.5	12.9 \pm 0.47	10.3 \pm 0.28	70.17 \pm 0.91	4.11 \pm 0.70	59.86 \pm 6.74	47.79 \pm 5.17	323.88 \pm 24.36	
1800		0	13.01 \pm 0.55	14.78 \pm 0.31	60.79 \pm 1.37	9.55 \pm 0.27	37.73 \pm 5.82	42.65 \pm 5.59	174.69 \pm 19.14	
		1.5	12.82 \pm 0.46	12.71 \pm 0.32	62.6 \pm 1.11	8.61 \pm 0.42	43.17 \pm 4.96	42.74 \pm 4.49	209.69 \pm 16.73	

Data represents the average of three replicates (n = 3) \pm standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

Salt stress (dS m ⁻¹)	Salicylic acid (μ M)	Silicon (mM)	Palmitic acid (%)	Stearic acid (%)	Linoleic acid (%)	Oleic acid (%)	Palmitic acid yield (mg plant ⁻¹)	Stearic acid yield (mg plant ⁻¹)	Linoleic acid yield (mg plant ⁻¹)
		2.5	13.87 \pm 0.81	16.47 \pm 0.23	57.76 \pm 1.19	10.18 \pm 0.49	34.54 \pm 4.39	40.86 \pm 3.75	142.91 \pm 10.6
LSD			0.660	0.641	1.537	0.972	8.191	5.82	52.44

Data represents the average of three replicates ($n = 3$) \pm standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

3.6 Yield of Palmitic, Stearic, Linoleic, and Oleic Fatty Acids

Like to the seed oil content, salinity stress decreased the yield of palmitic, stearic and linoleic acids and increased the yield of oleic acid in safflower. At all salinity levels, the application of SA and Si increased palmitic acid and linoleic acid yield (Table 2). Under non-stress condition, oleic acid yield was decreased due to application of SA and Si, but surprisingly, it was increased under moderate and severe stress conditions (Table 3). In the salted treatments spraying with 1800 μ M SA and all Si concentrations showed the highest amounts oleic acid yield (Table 3). In the non-stress and 7.5 dS m⁻¹ salinity conditions, the application of 1200 μ M SA with 2.5 mM Si showed the greatest increase in the yield of palmitic and linoleic acids (Table 2). At the highest level of salinity stress, the treatment of 1200 μ M SA and 1.5 mM Si caused an increase in the yield of palmitic acid and linoleic acids compared to the non-application of these two compounds (Table 2).

Table 3

Interaction of salicylic acid and silicon foliar application on oleic acid yield, seed protein and seed potassium at different salinity levels.

Salt stress (dS m ⁻¹)	Salicylic acid (μM)	Silicon (mM)	Oleic acid yield (mg plant ⁻¹)	Seed protein (%)	Seed potassium (%)
1.7 (control)	0	0	20.6 ± 3.45	13.04 ± 3.2	0.201 ± 0.0075
		1.5	24.49 ± 4.49	15.06 ± 4.06	0.2047 ± 0.0043
		2.5	4.38 ± 0.90	16 ± 1.62	0.2093 ± 0.0096
	600	0	3.85 ± 0.42	17.29 ± 3.07	0.2183 ± 0.0022
		1.5	3.84 ± 0.66	17.75 ± 2.53	0.2242 ± 0.0062
		2.5	3.31 ± 0.63	17.81 ± 2.35	0.2286 ± 0.0038
	1200	0	2.93 ± 0.67	18.63 ± 3.5	0.2307 ± 0.0039
		1.5	2.44 ± 0.78	19.23 ± 2.01	0.2342 ± 0.0038
		2.5	1.941 ± 0.25	20.69 ± 2.53	0.2436 ± 0.0058
	1800	0	5.98 ± 1.89	15.61 ± 4.55	0.2156 ± 0.0015
		1.5	5.85 ± 1.55	16.31 ± 1.44	0.2201 ± 0.0061
		2.5	5.93 ± 0.761	14.94 ± 1.44	0.2119 ± 0.0032
7.5	0	0	26.99 ± 4.41	16.27 ± 2.49	0.1845 ± 0.0015
		1.5	24.13 ± 1.93	18.77 ± 5.48	0.189 ± 0.0034
		2.5	26.04 ± 3.30	21.63 ± 1.62	0.1906 ± 0.0036
	600	0	26.54 ± 7	21.81 ± 1.08	0.1984 ± 0.0034
		1.5	18.15 ± 4.83	23.38 ± 2.17	0.2114 ± 0.0037
		2.5	15.90 ± 5.00	24.98 ± 1.77	0.2159 ± 0.0021
	1200	0	15.3 ± 5.32	25.56 ± 2.53	0.2167 ± 0.0037
		1.5	13.22 ± 3.40	26.38 ± 3.43	0.2239 ± 0.0031
		2.5	15.08 ± 4.45	28.81 ± 2.46	0.2280 ± 0.0058
	1800	0	26.45 ± 3.34	22.88 ± 1.98	0.1997 ± 0.0028
		1.5	30.21 ± 3.95	24.63 ± 3.14	0.2155 ± 0.0063
		2.5	26.48 ± 4.69	19.94 ± 2.78	0.1925 ± 0.005
15	0	0	24.95 ± 3.37	17.88 ± 2.44	0.1421 ± 0.015
		1.5	29.2 ± 3.23	21.83 ± 2.26	0.1713 ± 0.0068
		2.5	28.23 ± 4.28	24.79 ± 1.14	0.1835 ± 0.0016
	600	0	25.75 ± 3.95	25.88 ± 1.26	0.1836 ± 0.0044
		1.5	24.09 ± 4.09	27.37 ± 0.902	0.1901 ± 0.007

Data represents the average of three replicates (n = 3) ± standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

Salt stress (dS m ⁻¹)	Salicylic acid (μM)	Silicon (mM)	Oleic acid yield (mg plant ⁻¹)	Seed protein (%)	Seed potassium (%)
1200		2.5	26.06 ± 4.63	27.44 ± 2.53	0.1967 ± 0.0019
		0	27.25 ± 3.52	29.94 ± 5.23	0.1988 ± 0.0021
		1.5	33.52 ± 4.98	31.92 ± 2.44	0.2097 ± 0.0077
1800		2.5	19.34 ± 4.69	27.69 ± 4.7	0.2004 ± 0.0004
		0	27.57 ± 3.7	24.75 ± 5.77	0.1826 ± 0.0024
		1.5	29.01 ± 3.58	25.82 ± 1.59	0.1859 ± 0.001
		2.5	25.37 ± 3.16	20.37 ± 3.9	0.1756 ± 0.0027
LSD			6.08	3.57	0.0136

Data represents the average of three replicates (n = 3) ± standard error. Differences between means were evaluated by using the least significant difference (LSD) test ($P \leq 0.05$).

In general, the amount of stearic acid yield increased with the application of SA and Si both in stress and non-stress conditions. Foliar application of 2.5 mM Si without SA at non-stress conditions had the highest yield of stearic acid (49% increases) compared to the non-application of these two compounds at non-salinity conditions. The lowest yield of stearic acid was achieved in the non-application of these two compounds at 15 dS m⁻¹ salinity level (Table 2).

3.7 Seed Protein Percentage

Salinity stress increased seed protein percentage in the seeds. An increasing trend in the seed protein percentage was observed with the application of SA and Si under saline and non-saline conditions. The highest protein content was found in sever salinity treatment and application of 1200 μM SA and 1.5 mM Si spraying. On the other hand, the lowest seed protein was in the non-treated plants grown under non-stress condition. In the non-stress and 7.5 dS m⁻¹ salinity conditions, the combined treatment of 1200 μM SA with 2.5 mM Si was the superior treatment in terms of increasing the seed protein percentage (Table 3). Separate application of Si and SA raised the seed protein percentage in safflower compared to the control treatment, but the co-application of these two compounds had a greater additive effect on the seed protein percentage.

Seed potassium and sodium contents

The seed sodium content was not affected by salinity, salicylic acid, silicon, and their interactions. In contrast, seed potassium content was affected by salinity and SA and Si application. Salinity reduced seed K content but separate and combined treatments of SA and Si increased it in both salinity stress and without stress. Seed K content was increased up to 47.6% by spraying 1200 μM SA and 1.5 mM Si at 15 dS m⁻¹ salinity compared to the non-application of them at the same salinity level (Table 3).

4 Discussions

High salt levels in soils can poison plants, alter their morphology, and interfere with their physiological, biochemical, and molecular functions [1]. Exogenous application of Si and SA can positively affect the quantitative and qualitative yield of plants by improving growth characteristics, increasing the uptake of essential elements, and decreasing the content of harmful elements in the shoot and root parts under environmental stresses, such as salinity [2, 15, 23].

4.1 Plant Height and Biological Yield

Safflower height and biological yield were decreased by applying salinity. Increasing the accumulation of salt in the soil by reducing the potential of soil solution causes osmotic stress in plants [25]. Continuation of such conditions affects the plant tissues' water status. On the other hand, the production of toxic ions, oxidative damage, and nutritional disorders resulting from salinity stress affect plant water relations and reduce cell division and development, ultimately decreasing plant growth [1, 4, 6, 7]. Also, it is reported that all growth parameters decreased with increasing NaCl concentration in safflower [26, 27].

Exogenous application SA at optimal concentrations has shown beneficial effects on plant growth and development grown under both normal and stressful conditions [7, 28]. Numerous studies have reported improvement in plant growth with SA treatment, especially under stress. For example, height and biomass in cotton [9] and dry weight in peanut [17] and mung bean [29] increased with SA application under salinity. Salicylic acid modulates cell division and expansion by regulating the transcription of key genes, such as cell cycle-related genes and cell wall loosening genes [30]. The positive effect of SA on chlorophyll content is due to the stimulation of mineral assimilation and the inhibition of free radical synthesis [7, 31]. Ethylene affects stomatal closure in plants and SA limits ethylene production by inhibiting the activity of 1-aminocyclopropane-1-carboxylic acid synthase. On the other hand, decreased potassium content stimulates ethylene formation while potassium accumulation due to SA treatment inhibits ethylene formation. It is reported, SA improved the stomatal density and conductance notably under stress [32]. Salicylic acid affects some metabolic factors in carbon fixation including Rubisco enzyme concentration and activity, and/or photosynthetic carbon reduction cycle [33]. Also, SA treatment may affect the SOS (salt over sensitive) pathway, notably SOS4 and SOS5 and regulate the sodium and potassium homeostasis [32]. Also, this hormone enhanced absorption of essential elements and water by improving root growth [28]. Finally, improvement of biological yield with SA treatment can be due to the incremental effects on cell division and development, height, chlorophyll, leaf area, photosynthesis, and the content of K, N, Ca, and Mg as well as its reducing effect on Na content [9, 28].

Silicon can lead to an increment in the chlorophyll content by improving the uptake of essential elements for chlorophyll biosynthesis, such as nitrogen and iron. On the other hand, silicon raises the stomatal conductance by increasing the stomatal density and stomatal aperture size. Finally, by increasing the chlorophyll content and stomatal conductance, silicon leads to an increase in the photosynthetic rate and as a result plant growth [34, 35]. Silicon-treated plants under environmental stress showed increased biomass production and improved tolerance due to the adequate uptake of Si [36, 37]. This increase was associated with an increase in chlorophyll and photosynthesis and a reduction in oxidative damage by increasing gene expression and activity of antioxidant enzymes [5, 34, 38]. As well as an increment in the relative water content and water absorption by improving root hydraulic conductivity [24]. It has been reported that the shoot's dry weight increased with the application of Si in the peanut [17] and safflower [2], and this increase was effective due to the increment of Si accumulation in the shoot. Under salinity stress, Si application improved plant height, leaf area, and consequently plant biomass via increasing photosynthesis and associated traits and RWC [2, 38].

In this study, the synergistic effect of SA and Si was observed on growth, seed yield, and quantity and quality of oil in safflower. Silicon and SA separately have positive effects on the physiological characteristics, photosynthesis, relative water content, soluble sugar content, Mg, Ca, and K uptake, and growth of plants. Also, Si application increases the endogenous SA level, notably under stress. On the other hand, a combination of Si and SA can alleviate environmental stresses in plants by increasing the compatible solutes, raising K uptake, decreasing Na and Cl ionic toxicity, and increasing the antioxidant defense system that this causes to retain the balance of reactive oxygen species and malondialdehyde content. These complex interactions ultimately improve plant growth, development, and yield traits [37]. However, the exact mechanism of the synergistic effect of silicone and salicylic acid has not been clearly defined yet. In some studies, the synergistic effect of SA and Si on plant growth and environmental stress tolerance has been identified too. Combined foliar application of salicylic acid and silicon in mung bean and spinach plants reduced sodium uptake and increased RWC, stomatal conductance, chlorophyll index, leaf area index, potassium uptake, biomass, and seed yield by improving root growth in saline conditions, which indicates that the accumulation of sodium ions in roots somehow helped to decrease the concentration of accumulated sodium ions in shoots [23, 39]. Also, these researchers reported that during salinity stress, an

increment in water uptake by improving root growth due to the combined application of SA and Si increased RWC in leaves, leading to stomatal opening and increasing stomatal conductance and photosynthetic rate finally growth and yield [39]. In wheat, the separate application of Si and SA increased RWC, soluble sugars, soluble protein, the content of K, Ca, and Mg, antioxidant enzyme activity, and biological yield but the co-application of these two compounds had a greater additive effect on the above traits and drought tolerance [24]. In chickpea plants exposed to boron toxicity, the co-application of SA and Si raised the fresh and dry biomass of the shoot by increasing the antioxidant defense system and the content of chlorophyll and carotenoids [15]. The application of SA, especially when combined with the foliar application of Si, increased the dry weight of the peanut plant [17]. Contrary to the above studies, it has been reported that the foliar application of Si and SA or the separate application of them had no significant effect on increasing resistance to nitrogen deficiency stress in rice [36]. Also, the co-application of Si and SA in soybean improved photosynthesis, while in beans it had the opposite effect [16]. Based on this, it seems that the interaction of Si and SA varies depending on the plant species, plant age, and Si and SA concentrations at the time of application.

4.2 Seed Yield, Yield components, Oil Percentage, and Oil Yield

Salinity reduced seed yield, yield components, oil content, and oil yield in safflower, which was consistent with the results of other researchers in this field [19, 40]. It has been reported that the high concentration of salinity in soil and water can reduce the quantitative and qualitative yield of the plant by causing toxicity in the plant [7]. Data showed that both seed yield and oil percent was reduced under salt stress condition, but the oil yield was more related with seed yield than seed oil percent. In other word, a direct relationship was observed between seed yield and oil yield. In soybean, despite the increase in oil content under salinity stress, the oil yield is also reduced with decreasing seed yield [41].

One of the factors reducing the yield of safflower under salinity stress can be a decrease in the lateral branches and capitulum number, which occurred due to a decrease in the number of flowers and the loss of capitula. Hussain and Al-Dakheel [27] declared a similar report on the safflower under salinity stress. The reduction in oil percentage may be due to the participation of some fatty acids, such as linoleic acid in the cellular hardening. In salinity stress, fatty acids raise the production of certain enzymes, such as lipoxygenase to increase salinity tolerance [42]. Reduced oil content and oil yield in safflower due to salinity were in line with the study of Flagella et al. [43] on sunflower.

An increase in safflower seed yield with the use of SA is due to its positive role on flowering, the number of capitula per plant and the number of seeds per capitulum (Table 1). This result was consistent with the results of Lotfi et al. [29] who reported that the number of pods per plant, the number of seeds per plant, and seed yield increased with the application of SA in mung beans. The application of SA in soybean plants, despite reducing the oil percentage, increased oil yield due to increased seed yield [41]. The results of this study about the positive effects of SA on the quantitative and qualitative yield of safflower, especially under saline conditions, agreed with the above results. It has been reported that improving root growth and subsequently improving water and nutrient uptake by the application of SA at salinity stress can lead to improved plant growth and quantitative and qualitative yield [7, 28]. Raised seed yield in chickpea with SA treatment has been attributed to an increase in root length, photosynthetic rate, stomatal conductance, number of pods, and 100-seed weight due to this hormone [44].

In the present study, a positive effect of Si on the growth and quantitative and qualitative yield of safflower was observed under salinity stress (Tables 1, 2 and 3). Previous research has indicated that plants treated with Si under environmental stresses show an increase in seed yield due to adequate Si uptake [36, 37]. Si increased some physiological traits and capitulum number in safflower and in this way led to an increase in safflower seed yield [13]. It has been reported that the application of Si in sesame increased the number of capsules per plant, the number of seeds per capsule, 1000-seed weight, seed yield, and oil content [45]. The results of this study about the increase in seed yield, yield components, and oil content with the application of Si under salinity stress were consistent with the results of the above studies. This increase can be attributed to the positive role of Si in improving chlorophyll, water content, and photosynthesis, which leads to an increment in flowering and seed formation as well as the availability of more photosynthetic assimilates for the developing seeds.

In this study, there was a synergistic effect of SA and Si on seed yield and yield components of safflower. In this regard, it has been reported that in saline conditions, the co-application of SA and Si improved the seed yield of mung beans via reducing Na uptake and increasing chlorophyll index, leaf area index, and K content [39]. Also, the co-application of SA and Si had a greater incremental effect on the number of seeds per spike, 1000-grain weight, and seed yield in wheat [24] and pod formation in peanut [17] compared to the separate application of Si and SA. It seems that the synergistic effect of SA and Si can be attributed to the important roles of these compounds in the morphophysiological, and biochemical processes of plants and the uptake of water and nutrients.

The positive effect of the interaction of Si and SA in safflower under salinity stress could be due to the effect of these two compounds on gene expression. In this regard, it has been reported that in stressful environments, both Si and SA positively regulate key genes involved in rhizosphere acidification, antioxidant defense, SA biosynthesis, and Si uptake in plants and inhibit the expression of genes responsible for the biosynthesis of abscisic acid in the roots and shoots [37]. On the other hand, it has been specified that Si together with SA can reduce the polymerization reactions of Si to help its uptake [17]. This indicates that the co-application of these two compounds is a useful approach to providing silicon for oilseed crops with limited root uptake for Si.

4.3 Harvest Index

Salinity stress reduced the harvest index in safflower plants. Although both seed yield and biological yield decreased under stress conditions, seed yield showed a greater decrease compared to biological yield. Reduction of HI under salinity stress was associated with a greater reduction in seed yield compared to biological yield in salinity conditions and in contrast, the improvement of HI with the application of SA and Si showed an incremental effect of them on the seed yield compared to the biological yield. The results of this study about the reduction of HI in safflower under salinity stress were in line with the results of the study by Hussain and Al-Dakheel [27] who stated that HI in safflower decreased with increasing NaCl concentration. In the mung bean HI also increased with the application of SA [29]. The results of this study about the positive effect of Si on the HI were in accordance with the results of the study by Manaf et al. [45] who declared that the application of Si in sesame increased seed yield and HI. In our study, the observed synergistic effect between SA and Si on the HI was consistent with the results of the study by Maghsoudi et al. [24] in wheat, and Lotfi et al. [29] in mung bean who stated that the co-application of Si and SA raised the HI.

4.4 Fatty Acids content and Yield

Fatty acids content and quality in are quantitative traits that are affected by genetic and environmental factors [46]. Changes in the amount and composition of seed fatty acids due to salinity stress have been reported in several studies. For example, the content of palmitic and oleic acids decreased in safflower under salinity conditions, while the amount of stearic, linoleic, and linolenic acids increased [19]. Salinity in soybean decreased the amount of linoleic acid and the yield of palmitic, stearic, oleic, and linoleic acids and increased the amount of palmitic, stearic, and oleic acids [41], which was in line with the results of our study. Decreased linoleic acid content and increased oleic acid content have been reported in sunflower under salinity stress [43]. The significant change in the percentage of safflower fatty acids under salinity stress indicates that the saturated and unsaturated fatty acids of safflower seeds have been affected by changes in environmental conditions. The water deficit under salinity stress conditions can shorten the lipid accumulation stage, which damages all desaturase enzymes [43, 47]. Increased oleic acid percentage and decreased linoleic acid percentage in salinity conditions can be due to the rapid lipid accumulation and limited activity of all enzymes, such as the 12 Δ desaturase enzyme (responsible for the unsaturation of oleic acid to linoleic acid) because sodium and chloride ions can inactivate these enzymes and this condition can be harmful to lipid metabolism [43]. Oleic acid synthesis occurs by C18: 1 formation in plastids and unsaturation location to C18: 2 and C18: 3 is in the cytosol. Since environmental stresses, such as salinity limit the transfer of oleic acid to the cytosol, thus, the percentage of oleic acid increases, and the percentage of linoleic acid decreases [48]. There was a direct relationship between the yield of fatty acids and seed yield, which indicates that the decrease in the yield of fatty acids at salinity could be due to reduced seed yield and the percentage of oil accumulated in the seed.

Improvement of fatty acid profiles with SA has been reported in several studies. Exogenous application of SA under salinity stress has led to an increase in oleic acid and a decrease in palmitic, stearic, linoleic, and linolenic acids [19]. Under drought stress, spraying plants with SA increased the amount of oleic and linoleic fatty acids and decreased palmitic and stearic acids in *Cucurbita pepo* L. [18]. Under salinity stress, the application of SA in soybean increased the amount of linoleic acid and the yield of palmitic, stearic, oleic, and linoleic acids and decreased the amount of palmitic, stearic, and oleic acids [41], which was in line with the results of this research. Decreased oleic acid percentage and increased linoleic acid percentage with the application of SA can be attributed to the increased fluidity of lipid membranes and the activity of the oleoyl-phosphatidylcholine D12 desaturase enzyme [49]. The decrease in stearic acid percentage can be due to a negative correlation with linoleic acid. It has also been reported that SA can play a role in reducing the detrimental effect of stresses on fatty acid metabolism in safflower so that the expression level of unsaturation genes of fatty acid (*FAD3* and *FAD7*) increased in SA treatments compared to the control group after 72 h [50]. Increased yield of fatty acids due to SA treatment was associated with raised seed yield and oil yield.

Si application has been proven not only to increase the growth and yield of plants, but also to improve their quality, such as fatty acids, proteins, sugars, and vitamins [51]. The results of this study about the positive effect of Si on the quality of fatty acids in the oil were in line with the results of the study by Manaf et al. [45] who stated that the application of Si in sesame increased the percentage of linoleic acid and decreased the percentage of stearic and palmitic acids. Improving the quality of fatty acids by Si treatment can be due to the role of Si in the increased uptake of required elements in their biosynthesis pathway by improving the root system [12] and also the increment of photosynthetic assimilation by improving photosynthesis [38]. Increased yield of fatty acids with Si treatment can be attributed to increased seed yield and oil yield by Si foliar application. Also, the synergistic effect of Si and SA on the quality of fatty acids in safflower under salinity stress can be because of these two compounds on the uptake of elements and gene expression. It has been reported that in stressful environments, both Si and SA positively regulate genes involved in antioxidant defense, nutrient uptake, growth, yield, and tolerance in plants [37].

4.5 Protein, Potassium, and Sodium Contents of Seeds

Salinity stress increased seed protein percentage and decreased seed potassium content. In this regard, it has been reported that increased salinity stress may activate mechanisms for dealing with oxidative stress to prevent the degradation of structural and functional proteins. Also, the increase in protein content under salinity conditions can be due to increased synthesis of de novo induced proteins from salinity or decreased activity of proteolytic enzymes [52]. Mervat et al. [49] reported that the seed protein percentage in sunflower increased due to irrigation with salt water, which is consistent with the results of our study. The application of SA increased the seed protein content of chickpea and wheat [44, 53]. In this context, it has been reported that phytohormones increase the sink size at the level of seeds and direct the flow of metabolites into the growing seeds, thereby increasing the seed protein content and improving seed yield per plant [54]. This increase in seed protein content may be due to increased nitrate reductase activity with the application of SA [53]. The results of our study about the positive effect of Si on seed protein were in line with the results of the study by Manaf et al. [45] who declared that the application of Si increased the protein percentage in sesame. Si can improve seed protein by increasing the uptake of necessary nutrients in the protein biosynthesis pathway [12] and by raising photosynthesis and photosynthetic assimilates [38]. The synergistic effect of Si and SA on the amount of seed protein in safflower can be considered due to the important roles of these two compounds in the processes of photosynthesis and the uptake of water and nutrients involved in protein production.

In many glycophytes, there is a significant and negative correlation between plant growth and shoot sodium concentration. These plants usually exhibit excluder behavior and limit sodium levels in the shoot in two ways: 1) reduction of loading through the xylem and 2) return of sodium from the shoot to the root through the phloem [55]. Also, one of the mechanisms of plant tolerance to salinity is to increase the absorption of potassium and prevent the entry of sodium into the roots and its transfer to various organs of the plant [4]. It seems that the non-significant differences in seed sodium concentration between salinity and non-stress treatments can be due to the prevention of sodium transfer to shoots and seeds. Decreased

potassium content has been reported in various organs of plants under salinity stress [7, 9]. In wheat, the amount of sodium in leaves, stems, roots, spikes, and seeds increased and the potassium content decreased with increasing salinity. The reason for the decrease in seed potassium content was attributed to the inhibition of potassium uptake and accumulation via the root system by high sodium concentrations [56]. Sodium competes with potassium because of a similar ionic radius, and high levels of sodium in the rhizosphere cause sodium to be absorbed by root cells through potassium transporters, which ultimately inhibits the uptake of potassium in the plant [4]. Increasing the uptake of potassium and calcium and decreasing the uptake of sodium in different organs of plants with the separate application of SA [9] and Si [14] and their combined application [24] has been reported, which was in line with the results of our study. Researchers reported that the total K content in wheat seeds was higher in SA-treated plants under stress conditions [53]. Si application increased the amount of potassium in rice grain and straw [57], which was consistent with the results of present study. It has been specified that Si increases H⁺-ATPase activity in cell membranes, so it can increase cellular potassium uptake [58]. Also, due to the role of SA and Si in regulating the uptake processes of various elements, such as K, N, and Ca [9, 14]; the combined application of SA and Si can have a greater effect on increasing the uptake of essential elements and reducing harmful elements, such as sodium.

5 Conclusions

In conclusion, the results showed that although safflower is a salt resistant plant, a significant reduction observed in this crop's performance under salt stress. Our findings show that the application of SA and Si could increase safflower quantity and quality both under stress and non-stress condition. Salinity reduced plant height, capitulum number, seed number per capitulum, biological yield, seed yield, seed oil percentage, oil yield, and seed potassium content in safflower, while the application of SA and Si increased these traits under the same condition. Salinity increased oleic acid and decreased linoleic acid content. In contrast, foliar application of SA increased seed oil content and oil yield, and improved the oil quality of safflower seeds by increasing linoleic acid. Also, application of Si and SA reduced the amount of palmitic acid compared to the control. The co-application of these two compounds showed a greater effect than separate application. For instance, co-application of SA and Si showed more reduction effect on the amount of palmitic acid. At the highest level of salinity, the combined treatment of 1200 µM SA along with 1.5 mM Si increased the oil percentage, seed potassium content, and seed yield. The higher concentration of SA could not show this positive effect. Our experiment results showed that application of 1200 µM SA along with 1.5 mM Si was the best treatment that can reduce the risk of salinity in safflower. Further research is needed in the field conditions to optimize the combinations of SA and Si for the proper increment in oil quality and seed yield. Also, it is suggested that future works focus on evaluating plant growth and oil quality in terms of genes involved in the interaction pathway of SA with Si.

Declarations

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Accessibility of Data and materials The datasets and materials used and/or analyzed during the current study are available from the authors on reasonable request.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals 'Not applicable'

Ethical Approval 'Not applicable'

Consent to Participate 'Not applicable'

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Authors Contributions BJJ: Greenhouse management, plants husbandry, data collecting in greenhouse and laboratory, statistical analyses of data, original draft preparation. FS: First supervisor, conceptualization, methodology, formal analysis, writing and review of original draft preparation, project administration. BA: Second supervisor, conceptualization, Material preparation, review manuscript. RF: Statistical analyses of data, scientific advisory. VJ: Methodology, scientific advisory. AD: Review and editing of manuscript. All authors listed on the title page have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to online "Silicon" Journal. The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration.

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Figures

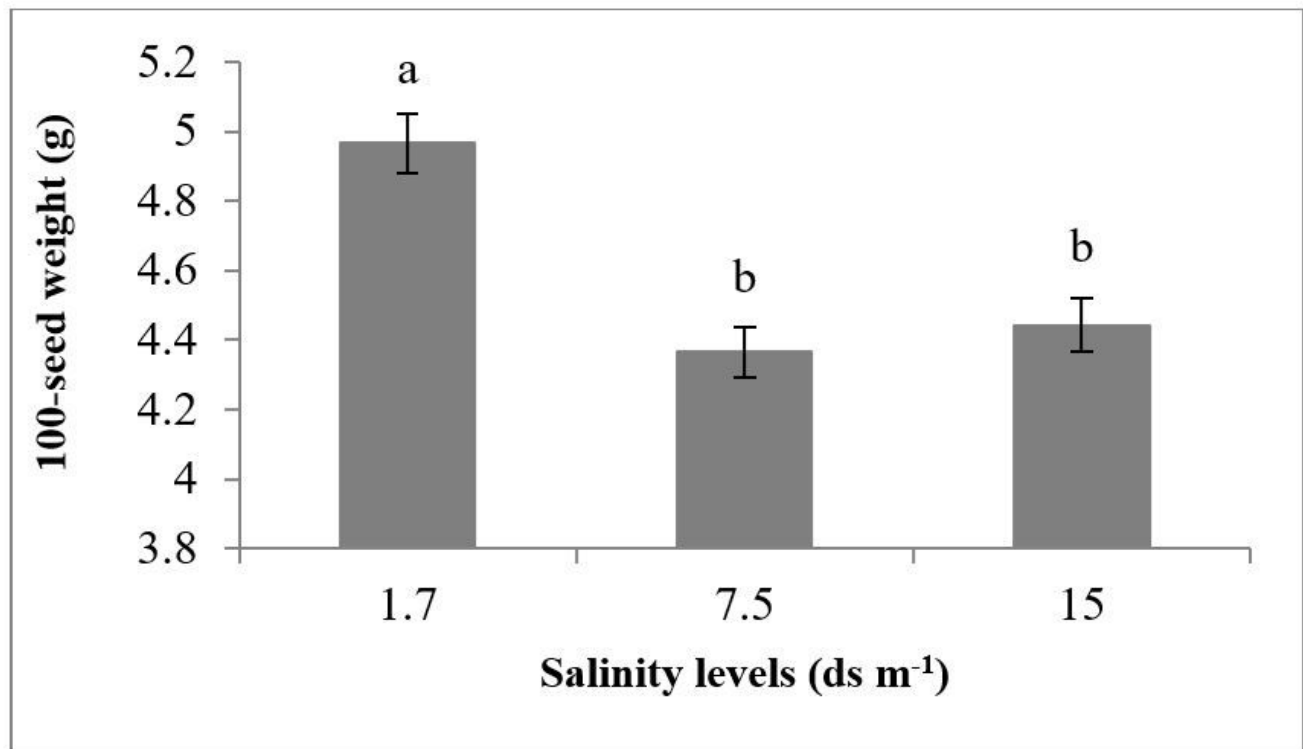


Figure 1

Effect of salinity on the 100-seed weight of safflower.

Columns with the same letters do not differ significantly (LSD test $P \leq 0.05$).