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Article

Effect of spraying with silicon, humic acid, and proline on the safflower tolerance (*Carthamus tinctorius* L.) to salt stress

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ABSTRACT

The experiment was carried out in the winter of season 2021-2022 in the research field of the College of Agriculture - University of Basra (latitude 30.57° North and longitude 47.80°) to study the effect of foliar spraying of silicon, humic acid, and proline on the endurance of safflower (*Carthamus tinctorius* L.) to salt stress. The experiment was carried out by distributing eight treatments of foliar spraying. Silicon (Si) was sprayed at a concentration of 800 ppm, humic acid (H) at a concentration of 80 ppm, and proline (P) at a concentration of 100 ppm. The studied treatments included control (C), Si, H, P, SiH, SiP, HP, and SiHP) on two soils with salinity (7.63 and 14.24 ds m-1) randomized complete block design with three replicates. The seeds of safflower (Cv. Gila) were planted in the two soils. The results showed significant superiority of the triple treatment (SiHP) in all studied traits, as it recorded the highest mean of height (156.3 cm), number of bells in the plant 59.11, number of seeds in the bell (59.17), weight of 500 seeds (24.30 g), seeds yield (10549 kg seeds ha-1) and biological yield (27582 kg ha-1) with a significant increase over the control treatment, which amounted to 64.2 cm, 26.08 bell plant-1, 24.90 seed bell-1, 14.03 g, 1725 kg seeds ha-1 and 6594 kg.ha-1 respectively—superiority of the proline treatment in all studied traits. Salinity decreased the number of seeds in the plant, the weight of 500 seeds, and seed yield. The foliar spray with the above treatments has proven its effectiveness and efficiency in growth and yield and increased salinity tolerance, in addition to the fact that these materials are environmentally friendly.

Keywords: Foliar Spraying; Salinity; Silicon; Humic acid; proline.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a multi-use crop; it can grow in arid and semi-arid environments because of its tolerance to drought stress, salinity, and lower and higher temperatures [1]. Salinity is considered one of the significant abiotic stresses affecting crop production. Approximately 50% of farmlands and 20% of the world's agricultural land are salt-affected [2]. Salinity inhibits crop growth and productivity via limiting photosynthesis and energy conservation imbalance [3]. Salinity causes an increase in the osmotic pressure of the soil solution, toxicity to some ions, especially sodium and chloride, or an imbalance of ions inside the plant.

The attempt to increase the yield of the safflower crop and its tolerance to salt stress can be achieved through the use of nutrients that are not harmful to the environment, such as the use of silicon, which[4] indicated its role in reducing the harmful effects of salinity and drought and in improving the efficiency of photosynthesis. Several studies have suggested positive growth effects of silicon nutrition, increased biomass, yield, and pollination [5]. Humic acid, which is a biostimulant, can also be used as it increases the plant's ability to grow and photosynthesize by enhancing the absorption of water and nutrients from the soil [6, 7] It was also found that spraying proline on plants enhanced growth with increased biomass and improved photosynthesis, which occurred under saline stress conditions, due to the positive role that proline plays by reducing the absorption of sodium and chlorine ions and enhancing potassium ion absorption [8]. Foliar spraying is preferred because soil salinity affects the absorption of water and nutrients from the soil [9]. Since the soils of central and southern Iraq have high concentrations of salts that make them unsuitable for the cultivation of many field crops, and for the lack of studies in the southern region on the effect of spraying with silicon, humic acid, and proline on the growth and yield of safflower under those conditions, the aim or target.

MATERIALS AND METHODS

The experiment was carried out in the research field of the College of Agriculture - University of Basrah / Karma Ali site (latitude 30.57° N and longitude 80. 47 °) in the winter of the 2021-2022 study. The first factor included spray solutions with silicon (800 ppm), humic, acid (80 ppm), and proline (100 ppm), and the second factor included two soils that differ in their salinity on the tolerance of safflower (Cv. Gila) to saline stress. Eight single and combination treatments were used as foliar applications, including control (C), silicon (Si), humic acid (H), proline (P), silicon +, humic acid (SiH), silicon + proline (SiP), humic acid + proline (HP) and silicon + humic acid + proline (SiHP) and two soils the first with salinity 7.63 ds m-1 (S1) and the second with salinity14.24 ds m-1 (S2) the experiment was applied using a randomized complete block design (RCBD) with three replicates (blocks), each block includes eight spray treatments with 24 experimental units for the first soil and 24 experimental units for the second soil. After the soil was prepared by plowing, smoothing and modifying, random samples were taken from different field areas to estimate some physical and chemical properties (as shown in Table 1).

Properties	**pH**	**ECe**	**Ca++**	**Mg++**	**Na+**	**HCO_3-**	**Cl-**	**SO_4=**	**N**	**P**	**K**
1st soil	7.44	7.63	20.3	17.8	46.5	4.2	49.3	28.1	53.3	5.2	122.3
2nd soil	7.58	14.24	32.4	33.8.	85.7	6.3	104.3	37.5	55.4	5.4	143.3
			Ca++	Mg++	Na+	HCO_3-	Cl-	SO_4=	N	P	K
			20.3	17.8	46.5	4.2	49.3	28.1	53.3	5.2	122.3
Unit		ds m^{-1}	Meq l^{-1}								

Table 1. Some Chemical and Physical Properties of Soil.

Each experimental unit had dimensions of 3 * 2.4 meters, containing four lines with a length of 3 meters; the distance between one line and another was 0.6 m. and 0.25 m within. The seeds were planted on 5/11/2021, and one plant was left in each hole. Fertilizers were added equivalent to 240 kg N ha-1 (in two batches) with 60 kg P ha-1 and 120 kg K ha-1. The eight treatments used were sprayed in three stages; after 40, 70, and 100 days of planting, 10 plants were selected from each experiment to study the different traits. The data were analyzed using the SPSS program ver. 23, and the least significant difference (LSD P<0.05) to compare the means.

RESULTS

Plant height

The analysis of variance (Table 8) and the results of Table (2) showed that all spraying treatments, whether single, double, or triple, had a positive and significant effect and led to an increase in plant height compared to the control. The highest height for the safflower plants (156.3 cm) was recorded using the SiHP treatment with an increase of 143.5 % compared to the control, which recorded the lowest value (64.2 cm.). The data in Table 2 and analysis of variance (Table 8) indicate no significant effect of salinity on plant height, perhaps because the plant height values were calculated as an average of all effects resulting from adding the studied treatments that weakened and reduced the effect of salinity. The interaction between spraying treatments and salinity treatments significantly affected plant height. SiHP treatment in non-saline and saline soil recorded the highest height, reaching 154.7 and 157.9 cm. respectively.

Treatments	C	Si	H	P	SiH	SiP	HP	SiHP	Mean
S1	75.4	99.8	100.6	111.0	116.4	129.0	137.1	154.7	115.5
S2	52.9	86.9	91.3	118.3	124.6	146.4	148.0	157.9	115.8
Mean	64.2	93.4	96.0	114.7	120.5	137.7	142.6	156.3	
L.S.D ($P<0.05$)	Treatments 8.5			Salinity NS.			Interaction 12.03		

Table 2. Effect of spraying solutions (silicon, humic acid and proline) and salinity and their interaction on the plant height (cm.)

Number of bells

The results of the first factor in Table 3 indicate the significant effect of spraying combination SiHP, as it recorded the highest average number of bells per plant, reaching 59.11 bell plant -1, with an increase of 126.64% compared to the lowest average recorded in the control treatment, which gave 26.08 bell plant-1. As for the binary treatments SiH, SiP and HP, it is noticeable that they significantly outperformed the control treatment with an increase of 92%, 106.86% and 112.54%, respectively. No significant difference was observed between the SiP and HP treatments, but they were significantly superior to the SiH treatment. The results of the first factor showed a significant superiority of the single treatment average P compared with the averages of silicon and humic acid, with an increase of 12.26% and 31%, respectively. Table 3 shows a significant difference between treatment S1 and treatment S2, with an increase of 11.50%.

The interaction between spraying the three substances and salinity significantly affected the number of bells per plant, and the treatment SiHP in S1 soil gave the most significant number of bells in the plant, reaching 62.30 bell plant-1.

Treatments	C	Si	H	P	SiH	SiP	HP	SiHP	Mean
S1	31.57	47.43	44.53	53.53	51.00	55.03	56.47	62.30	50.23
S2	20.60	40.97	35.27	51.00	49.33	52.87	54.40	55.93	45.05
Mean	26.08	44.20	39.90	52.27	50.17	53.95	55.43	59.11	——-
L.S.D (P<0.05)	Treatments 2.74			Salinity 1.11			Interaction 3.88		——

Table 3. Effect of spraying solutions (silicon, humic acid and proline) and salinity and their interaction on several bells in the plant.

Number of seeds in bell

The results of Table 4 indicate the significant effect of spraying silicon, humic acid, and proline or their interaction on the number of seeds in the plant, which led to an increase compared to the control. The number of seeds in the bell recorded using the SiHP treatment reached 59.17 seed bell-1, with a rise of 137.63% compared to the control, which gave the lowest value of 24.90 seed bell-1. The increase in the rest of the treatments ranged from 59.84% by spraying humic acid to 105.46% by using humic acid + proline.

It is also noted from Table 4 that the increase in soil salinity (S2) led to a decrease in the number of seeds per bell to 41.67 compared to 47.45 seeds per bell at soil S1, with a reduction of 18%.

The interaction between spraying the three substances and salinity significantly affected the number of seeds per bell, and the treatment SiHP in S1 soil gave the most significant number of seeds in the bell, reaching 62.33.

Treatments	C	Si	H	P	SiH	SiP	HP	SiHP	Mean
S1	992.2	2011.0	2072.2	2555.2	2376.6	2770.0	2951.4	3883.4	2383.4
S2	378.2	1461.2	1166.2	2371.8	2402.5	2375.3	2723.6	3132.3	1877.2
Mean	649.4	1725.1	1588.0	2463.5	2390.6	2569.6	2835.8	3497.5	
L.S.D (P<0.05)	Treatments 175.7			Salinity 87.9			Interaction 248.5		

Table 4. Effect of spraying solutions (silicon, humic acid and proline) and salinity and their interaction on seed number in bell.

Weight of 500 seed

The statistical analysis results (Table 8) showed the significant effect of spraying silicon, humic acid, or proline (individually or mixing them) in increasing the weight of 500 seeds. The data in Table (5) showed that all spraying treatments led to an increase in seed weight compared to the control; the SiHP treatment recorded the highest weight of 500 seeds, reaching 24.3 gm with a significant increase of 73 % over the control, which gave 14.03 gm. As for the rest of the treatments, it led to an increase in weight, and the percentage of increase was between 32% when spraying humic acid and 53% when spraying HP.

Other results in Table 5 indicate a significant decrease in seed weight by 5.2% due to the high salinity (S2) level, where the treatment recorded 19.34 g per 500 seeds. At the same time, it was 20.40 g in the S1 interaction.

Treatments	C	Si	H	P	SiH	SiP	HP	SiHP	Mean
S1	14.90	20.33	20.13	21.37	20.23	21.07	20.77	24.40	20.40
S2	13.17	17.60	16.90	20.13	19.93	20.70	22.10	24.20	19.34
Mean	14.03	18.97	18.52	20.75	20.08	20.88	21.43	24.30	
L.S.D (P<0.05)	Treatments 1.28			Salinity 0.64			Interaction NS.		

Table 5. Effect of spraying solutions (silicon, humic acid and proline) and salinity and their interaction on the weight of 500 seeds.

Seeds yield

The results of the analysis of variance (Table 8) explained the significant effect of spraying silicon or humic acid or proline (individually or mixing them) in increasing the seed yield. The results in Table 6 showed the superiority of the SiHP treatment in recording the highest seed yield per unit area, amounting to 10549 kg seeds ha-1 with a significant increase that reached more than five times the yield recorded in the control (which gave 1725 kg seeds ha-1). As for the other treatments, a significant increase was recorded compared to the control.

From the results, the single factor most influential in increasing the yield was the proline spray, which recorded 7254 kg. ha-1, followed by the silicon treatment. In general, the double mixtures gave a higher yield than spraying the agents individually, especially in HP treatments, which recorded a yield of 8213 kg. ha-1. As for the effect of soil salinity, there was a significant decrease in seed yield by 23.8% for saline soil (S2), which gave 5522.3 kg. ha-1 compared to 7248.4 kg. ha-1 for non-saline soil (S1). The interaction between spraying treatments and soil salinity significantly affected the seed yield, and the treatment SiHP planted in non-saline soil recorded the most outstanding seed yield of 13302 kg. ha-1, with a significant difference from all other interactions. The results recorded here are a summation and a reflection of the results recorded for the number of seeds per plant, the weight of 500 seeds, and the different effects produced by the studied treatments.

Treatments	C	Si	H	P	SiH	SiP	HP	SiHP	Mean
S1	2523	5712	5028	7700	7251	7842	8626	13302	7248
S2	927	3776	2951	6808	6899	7217	7801	7796	5522
Mean	1725	4744	3990	7254	7075	7529	8213	10549	
L.S.D (P<0.05)	Treatments 932.7			Salinity 466.4			Interaction 1319.1		

Table 6. Effect of spraying solutions (silicon, humic acid, and proline) and salinity and their interaction of seed yield (kg. ha-1).

Biological yield

The results of the statistical analysis in Table 8 indicate that the spraying materials, whether single, double or triple, had a significant and positive effect and led to an increase in the biological yield compared to the control treatment. It is noted from the data in Table 7 that the triple spray (SiHP) recorded the highest biological yield of 27582 kg. ha-1 significantly increased more than three times compared to the control treatment, which gave 6594 kg. ha-1. Regarding the effect of individual factors, proline spraying was the most

effective in increasing the biological yield, which gave 16413 kg. ha-1 with an increase of 149% compared to the control, followed by silicon (12541 kg. ha-1) and then humic acid (10783 kg. ha-1). As for the dual spraying, the treatment HP recorded a biological yield of 20468 kg. ha-1. Soil salinity did not affect the biological yield of safflower, perhaps because it did not affect plant height (Table 2).
The interaction between spraying treatments and soil salinity significantly affected the biological yield, and the therapy SiHP planted in non-saline soil recorded the most significant biological yield of 29702 kg. ha-1, with a significant difference from all other interactions.
The factors that affect growth characteristics, including plant height yield, will impact the biological yield.

Treatments	C	Si	H	P	SiH	SiP	HP	SiHP	Mean
S1	9261	13371	11862	17012	17567	18179	19945	29702	17112
S2	3928	11712	5970	15813	19350	20734	20990	22546	15962
Mean	6594	12541	10783	16413	18459	19456	20468	27582	
L.S.D (P<0.05)	Treatments 2136.9			Salinity NS.			Interaction 3022.0		

Table 7. Effect of spraying solutions (silicon, humic acid, and proline) and salinity and their interaction on biological yield (kg. ha-1).

S.O.V	d.f	Plant height	No. bells plant^{-1}	No. seeds plant^{-1}	Weight of 500 seeds	Seeds yield	Biological yield
Block	2	25.45	1.00	9642.4	3.21	3323351.4	2864071.6
T	7	5510.90**	682.70**	4459360.1**	51.97**	45747158.8**	255080041.4**
S	1	1.08	322.92**	2431305.1**	13.44**	35754090.2**	15882652.5
T*S	7	280.39**	19.58*	146890.1**	3.33	4088217.2**	11884165.8*
Error	30	75.39	7.83	32159.2	1.70	906285.0	4756811.5

Table 8. Analysis of variance represented by mean square for the studied traits.

DISCUSSION

Even in saline soils, the effect of spraying silicon, humic acid, and proline was evident in increasing the plant's tolerance to salt stress, as an increase in plant height was observed with the spraying of these substances. This result is consistent with what [10] observed regarding the effect of silicon on the height of safflower plants. Silicon plays an essential role in many vital processes within the plant, the most important of which is improving photosynthesis efficiency, increasing the roots' effectiveness in absorbing water and nutrients, and increasing its tolerance to biotic and abiotic stress conditions [5, 13]. Also, humic acid, a biostimulant, can increase the plant's ability to grow and photosynthesize by enhancing water absorption and nutrients from the soil [7]. The most significant effect of proline, which works on the osmotic balance during stress, is that it improves the activity of red antioxidants, reduces the absorption of sodium and chlorine ions, and enhances potassium absorption [8]. The number of bells per plant decreased when the salt concentration in the soil increased. This, of course, comes from the negative impact of salinity, whether in reducing the rate of absorption of water and nutrients from the soil or from the nutritional imbalance within the plant, as well as the negative impact of salinity on many physiological processes, that salinity hinders protein synthesis and metabolism and causes oxidative stress (ROS) that leads to oxidation of fats and proteins [11]. The number of seeds in the bell is due to the role of the studied factors in increasing the content of chlorophyll and the rate of photosynthesis, changing the behavior of stomata and reducing the rate of transpiration in leaves, which means an increase in the efficiency of photosynthesis in the different stages of growth, leading to flowering, to the stage of flowering and seed formation, and the transfer of the products of the photosynthesis process to the seeds with greater efficiency.

The most important observation is that spraying the above materials helped increase the seed's weight, even in saline soils, in proportions similar to those in non-saline soils. Proline, an amino acid, plays an essential role in plants. It protects the plants from various stresses and helps them recover more rapidly. Silicon plays a vital role in many biological processes within the plant, the most important of which is improving photosynthesis efficiency, increasing roots' effectiveness in absorbing water and nutrients, and increasing its tolerance to biotic and abiotic stress conditions [12, 14]. Proline, an amino acid, is highly beneficial in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three significant roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule [15, 16].

CONCLUSIONS

Based on what the results of the study gave of the beneficial effect of foliar spraying with silicon, humic acid and proline in promoting growth and seed yield under saline stress conditions, even with a high salt concentration in the soil, it was recommended to use the combination (SiHP) in safflower productivity. More studies can be conducted on other plants to confirm the results obtained.

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