

The effect of salt stress and spraying with proline and humic acid on the vegetative traits and chemical content of wormwood plants. (*Artemisia Herba Alba*)

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Abstract

The study was conducted in the wooden greenhouse of the Department of Horticulture and Landscape Design- College of Agriculture - Tikrit University on the plant *Artemisia herba alba*. During the 2023-2024 and 2024-2025 seasons, the study aimed to investigate the effects of three factors: proline spraying, humic acid addition, and salt stress. The salt stress was applied at three levels (10, 5, 0 g L⁻¹), denoted as S0, S1, and S2, respectively. The second factor is the spraying with proline, which was at three levels (0, 100, 200 mg L⁻¹), denoted as B0, B1, B2 respectively. The third factor, the addition of humic acid, was at two levels (0, 3 g L⁻¹), denoted as H0 and H1, respectively. The experiment was conducted using a split plot design within a completely randomized block design. (R.C.B.D). The results showed the superiority of treatment B2, which provided the highest values in the traits: plant height, chlorophyll content, nitrogen, and proline at 38.391 cm, 57.879%, 2.339%, and 8.094%, respectively. Proline had no significant effect on the other traits. S3 excelled in chlorophyll traits with an average of 4.924%, and salinity stress had no significant effect on the other traits. The H1 treatment significantly excelled in traits such as increased plant height, chlorophyll content, nitrogen, and proline, with rates reaching 36.505 cm, 449%, respectively. 2.280%, 6.189%, respectively, and humic had no significant effect on the other traits. The dual interaction between (B2H1) showed a significant effect on nitrogen, chlorophyll content, and proline traits, with rates of 2.433%, 61.486%, and 8.942% respectively. As for the interaction of the studied factors, the results showed significant differences for the interaction treatment (B2H1) in the traits: nitrogen and proline percentages, with rates of 2.965% and 9.213%, respectively. The interaction between proline and stress did not have an effect on the vegetative traits. The two-way interaction between (S1H1) on the traits: proline percentage and chlorophyll content, with averages of 6.740% and 53.473% respectively, did not have an effect on the vegetative traits. As for the effect of the three-way interaction of the studied factors, the triple combination (B2S2H1) excelled in the traits: nitrogen percentage, proline, and chlorophyll content at 3.413%, 9.870%, and 65.930%, respectively, and the three-way interaction had no effect on the other traits .

Keywords: Wormwood plant, proline, salt stress, humic

Introduction

The Albaherb *Artemisia*, belongs to the Asteraceae family and consists of evergreen, aromatic shrubs with an upright growth habit, ranging in height from 30 to 150 cm. They are multi-branched, densely hairy, and end with flower heads that are yellowish-green or greenish-white in color, containing 2 to 4 flowers [1] [8]. The Albaherb plant is used in folk medicine to treat fever, stimulate the gastric gland, and act as a disinfectant, as well as to aid in wound healing [3] [6]. Many research studies have pointed to the importance and role of the chemical compounds found in the wormwood plant, such as alkaloids, glycosides, types of amino acids, and trace minerals like calcium and sodium [5].

Amino acids play a significant role in the process of nitrogen transfer between roots, leaves, fruits, and others, which are precursors for the synthesis of chlorophyll and other nitrogen-containing compounds in their structure, such as biotin from aspartic acid [4]. Proline is one of the soluble amino acids that is often associated with the severity of environmental stresses, as it accumulates in plants through negative environmental constraints. In addition to its important role in increasing the ability of plants to withstand environmental stresses, proline is considered a crucial factor for the stability of proteins and molecular structures [2]. Proline plays an important role in protecting the plant from moisture stress as it acts as an antioxidant, regulator, and osmotic agent to protect the plant from stress conditions by maintaining membranes and enzymes [9]. Many studies have indicated the positive role of proline in improving the growth and yield characteristics of plants. A study [7] showed that foliar

spraying with proline depends on the type and variety of the plant, the timing of application, and the appropriate concentration, all of which play a role in enhancing growth and yield characteristics.

Despite the efficiency of chemical fertilizers in increasing production and improving its quality, they have harmful effects on the environment and human health. This necessitates reducing the use of chemical fertilizers and using non-harmful organic compounds that enhance the plants' ability to withstand harsh environmental conditions [15]. Many recent studies and research have recommended the use of humic organic fertilizers, particularly humic substances, which enhance the availability of nutrients and plant growth in the soil, improve the physical, chemical, and biological properties of the soil, increase the soil's water retention capacity, and stimulate the growth of biological communities [11]. [10] indicated that decomposed humic substances affect soil properties by improving soil texture and cation exchange capacity, as well as influencing enzymatic reactions and microbial activity, which positively reflected on plant growth.

Salinity stress is considered one of the most significant challenges facing agricultural production and leads to a decrease in the productivity of plant species [14]. Salinity limits the possibility of agricultural expansion in most countries of the world, especially in irrigated farming areas [12]. Salinity stress in the Mediterranean Basin poses a problem for many important staple crops. Due to the presence of significant concentrations of salts in groundwater, especially in desert and semi-desert areas, and the lack of proper drainage systems, the high costs of reclaiming saline lands, the high evaporation rates, and the

unregulated use of fertilizers, the phenomenon of salinity has developed rapidly. [13] Observed this. When conducting two experiments to study the effect of salinity and drought on the growth of chamomile and its essential oil content, it was found that increased salinity caused a decrease in the number of branches and stem length, while drought resulted in a significant reduction in plant height and the number of branches. According to what was mentioned above, this study came for studying the the extent of the impact of stress and proline spraying on some vegetative and chemical properties of wormwood plant leaves and the effect of adding humic acid on vegetative traits and nutrient availability.

Materials and Methods

This study was conducted at the Agricultural Research, Experimentation Station affiliated with the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Tikrit, during the 2023-2024, and 2024-2025 seasons, the seedlings were brought from one of the nurseries affiliated with the Baghdad Governorate on 28/2/2023, which were one year and two months old. These seedlings were transferred to plastic pots (containers) with a diameter of 22 cm, containing soil and peat moss in a 1:2 ratio. Humic acid was added 15 days after transferring the seedlings to the pots, and sodium chloride was added 15 days after adding the humic acid. Proline was sprayed in two doses: the first dose was after adding sodium chloride, 14 days later, and the second dose was 14 days after the first spray, The experiment included three levels of proline, denoted as (B) (0, 100, 200) mg L⁻¹, three levels of salt stress, denoted as (S) (0, 5, 10) g L⁻¹, and two levels of humic acid,

denoted as (H) (0, 3) g L⁻¹. The experiment was designed using a Split plot design within a Randomized Complete Block Design (RCBD), where the addition of proline was placed in the main plots and the salt stress and addition of humic acid were placed in the subplots. The number of plants in each experimental unit was five, with three replications. Results were taken from three plants per experimental unit to measure vegetative traits (plant height and leaf chlorophyll content) and chemical content (percentage of nitrogen, chlorophyll, and proline.)

Plant height (cm plant -1 (

The table (1) shows a significant difference in plant height when sprayed with proline. We observe that treatment B2 gave the highest plant height, reaching (57.104), compared to no spraying (B0), which gave the lowest height of (47.808). Regarding the effect of humic acid, treatment H1 showed superiority by providing the highest height of (55.259) compared to the lowest height of treatment H0 (no spraying), which was (49.606). As for the effect of salt stress, treatment S0 showed superiority by providing the highest height of (57.663) compared to treatment S2, which gave the lowest height of (49.513 .(

The interaction between proline and salt stress shows that treatment B2S0 provided the highest height, reaching (62.161), compared to treatment B0S2, which gave the lowest height of (44.753 .(

As for the interaction between proline and humic acid, we observe the superiority of the H1B2 treatment, which provided the highest concentration at (57.991) compared to the B0H0 treatment, which gave the lowest concentration at (41.847 .(

As for the interaction between salt stress and humic acid, the treatment S0H1 showed superiority by achieving the highest height of

(62.637) compared to the treatment S2H0, which gave the lowest height. (47.677). And in the three-way interaction, we observe significant differences, with the B2S0H1

treatment showing the highest height at (64.006) compared to the lowest height in the B0S0H0 treatment.(40.016.)

Table (1) Effect of proline, salt stress, humic acid, and their interaction on plant height (cm.plant -1) of Artemisia herba alba leaves Plant -1) for the leaves of the wormwood plant Artemisia herba alba

Proline B) (Humic)H () Salt stressS (Average BXH
		S ₀	S ₁	S ₂	
B ₀	H ₀	40.016q	45.423n	40.103p	41.847f
	H ₁	63.900b	48.003m	49.403k	53.768d
B ₁	H ₀	57.536e	44.513o	50.210j	50.753e
	H ₁	60.006d	52.816h	49.230l	54.017c
B ₂	H ₀	60.516c	55.416f	52.720i	56.217
	H ₁	64.006a	54.550g	55.416f	57.991a
					AverageB
Average overlap BXS	B ₀	51.958e	46.713g	44.753i	47.808c
	B ₁	58.771b	48.665g	49.720f	52.385b
	B ₂	62.261a	54.983c	54.068d	57.104a
					AverageH
Average overlap SXH	H ₀	52.690b	48.451e	47.677f	49.606b
	H ₁	62.637a	51.790c	51.350d	55.259a
AverageS		57.663a	50.120b	49.513c	
: B0 without spraying (distilled water) B ₁ Spraying at a : (¹⁻ mg/L 100) concentration of B2 Spraying at a : (¹⁻ concentration of (200 mg/L			S ₀ Without addition : (distilled water) S ₁ Add at a : (¹⁻ concentration of (5 g/L S2 Add at a : ¹⁻ concentration of (10 g/L (H0 Without addition : : (distilled water) H1 Add at a : (¹⁻ concentration of (3 g/L
Numbers with similar letters mean that there are no significant differences between the averages of the coefficients according to Duncan's multinomial test with a probability level of 5%.					

Chlorophyll b,a content in the leaves (mg.g-1 fresh weight) the total chlorophyll

The table (2) shows a significant difference in chlorophyll percentage when sprayed with

proline. We observe that treatment B2 gave the highest percentage, reaching (47.507), compared to no spray B0, which gave the lowest percentage of (39.113). Regarding the effect of humic acid, treatment H1 showed

superiority by providing the highest percentage of (50.451) compared to the lowest percentage of treatment without spray H₀, which was (38.650). As for the effect of salt stress, treatment S₀ showed superiority by providing the highest percentage of (52.771) compared to treatment S₂, which gave the lowest percentage. (40.217) .(

The interaction between proline and salt stress shows that the B₂S₀ treatment

outperformed with the highest percentage of (54.788) compared to the B₀S₂ treatment, which gave the lowest percentage. (30.756) .(As for the interaction between proline and humic acid, we observe that the treatment H₁ B₂ outperformed with the highest percentage of (54.000) compared to the non-spraying treatment B₀H₀, which gave the lowest percentage of (31.956) .(

Table (2) Effect of proline, salt stress, humic acid, and their interaction on the total chlorophyll content of *Artemisia herba alba* leaves

Proline B) (Humic)H () Salt stressS (Average BXH
		S ₀	S ₁	S ₂	
B ₀	H ₀	39.000m	30.450q	26.420r	31.956f
	H ₁	58.630c	45.090i	35.093p	46.271c
B ₁	H ₀	50.240e	41.420k	37.180n	42.980d
	H ₁	59.083b	46.100g	48.070f	51.084b
B ₂	H ₀	45.120h	37.000o	40.923l	41.014e
	H ₁	64.456a	43.923j	53.620d	54.000a
AverageB					
Average overlap BXS	B ₀	48.815c	37.770h	30.756i	39.113c
	B ₁	54.711b	43.760e	42.625f	47.032b
	B ₂	54.788a	40.461g	47.271d	47.507a
AverageH					
Average overlap SXH	H ₀	44.820d	36.290e	34.841f	38.650b
	H ₁	60.723a	45.037c	45.594b	50.451a
middleS		52.771a	40.663b	40.217c	
Numbers with similar letters mean that there are no significant differences between the averages of the coefficients according to Duncan's multinomial test with a probability level of 5%.					

The percentage of nitrogen in the leaves (%)

The table (3) shows a significant difference in nitrogen percentage when spraying with proline. We observe that treatment B₂ gave the highest concentration of nitrogen (N) for the plant, reaching (2.442), compared to no spraying (B₀), which gave the lowest

concentration (1.417). Regarding the effect of humic acid, treatment H₁ showed superiority by providing the highest concentration (2.153) compared to the lowest concentration of the no-spray treatment (H₀), which reached (1.651). As for the effect of salt stress, treatment S₀ showed superiority by providing the highest concentration (2.446) compared to

treatment S2, which gave the lowest concentration. (1.583.(

The interaction between proline and salt stress shows that treatment B2S0 has the highest rate, reaching (3.128), compared to treatment B0S2, which has the lowest concentration at (1.138 .(

As for the interaction between proline and humic acid, we observe the superiority of the H1B2 treatment, which provided the highest concentration of (2.647) compared to the B0H0 treatment, which gave the lowest concentration of (1.212 .(

As for the interaction between salt stress and humic, the treatment S0H1 showed superiority by providing the highest concentration, which reached (2.908), compared to the treatment S2H0, which gave the lowest concentration. (1.421 .(

As for the three-way interaction, we observe significant differences, with the B2S0H1 treatment showing the highest concentration at (3.416) compared to the lowest concentration in the B0S2H0 treatment.(1.063.(

Table (3) Effect of proline, salt stress, humic acid, and their interaction on the nitrogen percentage (%) in the leaves of the wormwood plant *Artemisia herba alba*

Proline B) (Humic)H () Salt stressS (Average BXH
		S ₀	S ₁	S ₂	
B ₀	H ₀	1.396m	1.176p	1.063q	1.212f
	H ₁	2.253e	1.403m	1.213o	1.623d
B ₁	H ₀	1.713j	1.463l	1.343n	1.506e
	H ₁	3.056b	1.600k	1.910h	2.188c
B ₂	H ₀	2.840c	2.013g	1.856i	2.236b
	H ₁	3.416a	2.413d	2.113f	2.647a
					AverageB
Average overlap BXS	B ₀	1.825e	1.290h	1.138i	1.417c
	B ₁	2.380b	1.531g	1.626f	1.847b
	B ₂	3.128a	2.213c	1.985d	2.442a
					AverageH
Average overlap SXH	H ₀	1.983b	1.551e	1.421f	1.651b
	H ₁	2.908a	1.805c	1.745d	2.153a
AverageS		2.446a	1.678b	1.583c	
: B ₀ without spraying (distilled water) B ₁ Spraying at a : ~ concentration of (100 mg/L (¹ B ₂ Spraying at a : ~ concentration of (200 mg/L (¹			S ₀ Without addition : (distilled water) S ₁ Add at a concentration : (¹ of (5 g/L S ₂ Add at a concentration : (¹ of (10 g/L		H ₀ Without addition : : (distilled water) H ₁ Add at a : (¹ g/L concentration of (3
Numbers with similar letters mean that there are no significant differences between the averages of the coefficients according to Duncan's multinomial test with a probability level of 5%.					

The percentage of proline (%)

The table (4) shows a significant difference in the proline percentage when sprayed with proline. We observe that treatment B₂ gave the highest concentration, reaching (7.218), compared to the non-spray treatment B₀, which gave the lowest concentration of (4.137). Regarding the effect of humic acid, treatment H₁ showed superiority by providing the highest concentration of (5.661) compared

to the lowest concentration of the non-spray treatment H₀, which was (5.306). As for the effect of salt stress, treatment S₂ excelled by providing the highest concentration of (6.361) compared to the non-spray treatment S₀, which gave the lowest concentration of (4.137). (4.861 .(

The interaction between proline and salt stress shows that treatment B₂S₂ outperformed with the highest rate of (8.265)

compared to the control treatment B0S0, which had the lowest concentration of (3.501).

As for the interaction between proline and humic acid, we observe the superiority of the treatment H1 B2, which provided the highest concentration of (7.475) compared to the non-spray treatment B0H0, which gave the lowest concentration of (4.055).

As for the dual interaction between salt stress and humic, the treatment S2H1 showed superiority by providing the highest

concentration, which reached (6.546), compared to the non-spray treatment S0H0, which gave the lowest concentration. (3.785).

As for the triple interaction, we observe significant differences, and the treatment B2S0H1 was distinguished by giving the highest concentration, which reached (8.720) compared to the lowest proline concentration in the treatment B0S0H0, which reached (2.756).

Table (4) The effect of proline, salt stress, humic acid, and their interaction on the proline percentage (%) in the leaves of the plant *Artemisia herba alba*

ProlineB) (Humic)H () Salt stressS (Average BXH
		S ₀	S ₁	S ₂	
B ₀	H ₀	2.756q	4.660l	4.750k	4.055f
	H ₁	4.246n	4.130o	4.280m	4.218e
B ₁	H ₀	3.180p	5.800f	5.726g	4.902d
	H ₁	4.843j	4.143o	6.883e	5.290c
B ₂	H ₀	5.420h	7.410d	8.053c	6.961b
	H ₁	8.720a	5.230i	8.476b	7.475a
					AverageB
Average overlap BXS	B ₀	3.501i	4.395g	4.515f	4.137c
	B ₁	4.011h	4.971e	6.305d	5.096b
	B ₂	7.070b	6.320c	8.265a	7.218a
					AverageH
Average overlap SXH	H ₀	3.785f	5.956c	6.176b	5.306b
	H ₁	5.936d	4.501e	6.546a	5.661a
middle S		4.861c	5.228b	6.361a	
: B0 without spraying (distilled water) B1 Spraying at a concentration : (1 ⁻ of (100 mg/L B2 Spraying at a concentration : (1 ⁻ of (200 mg/L			S ₀ Without addition : (distilled water) S ₁ Add at a : (1 ⁻ concentration of (5 g/L S ₂ Add at a : (1 ⁻ concentration of (10 g/L		H0 Without addition : : (distilled water) H1 Add at a : : concentration of (3 g/L (1 ⁻
Numbers with similar letters mean that there are no significant differences between the averages of the coefficients according to Duncan's multinomial test with a probability level of 5%.					

The percentage of chlorine (%)

The table (5) shows a significant difference in the percentage of chlorophyll when spraying with proline. We observe that the non-spray treatment B0 gave the highest concentration of chlorophyll in the plant, reaching (4.181), compared to treatment B1, which gave the lowest concentration of (3.105). Regarding the effect of humic acid, the non-spray treatment H0 showed superiority by providing the highest concentration of (4.357) compared to the lowest concentration of treatment H1, which reached (3.037). As for the effect of salt stress, treatment S2 showed superiority by providing the highest concentration of (5.250) compared to the non-spray treatment S0, which gave the lowest concentration. (1.421). (The interaction between proline and salt stress shows that the B0S2 treatment outperformed by providing the highest rate of (6.180) compared to the non-spray B0S0 treatment,

which gave the lowest concentration of (1.116). (

As for the dual interaction between proline and humic acid, we observe the superiority of the non-spray treatment H0 B0, which provided the highest concentration of (5.042) compared to the treatment B1H1, which gave the lowest concentration of (2.407). (

As for the interaction between salt stress and humic acid, the treatment S2H0 showed superiority by providing the highest concentration, which was (5.934), compared to the treatment S1H0, which gave the lowest concentration. (5.294). (

As for the three-way interaction, we observe significant differences, with the treatment B0S2H0 showing the highest concentration at (7.356) compared to the lowest chlorine concentration in the plant treatment B2S0H1.(1.003). (

Table (5) Effect of proline, salt stress, humic acid, and their interaction on the chlorophyll percentage (%) of *Artemisia herba alba* leaves

Proline B) (Humic)H () Salt stressS (Average BXH
		S ₀	S ₁	S ₂	
B ₀	H ₀	1.366o	6.403b	7.356a	5.042a
	H ₁	0.866r	4.093j	5.003e	3.321e
B ₁	H ₀	2.183m	4.396h	4.830g	3.803c
	H ₁	1.126p	2.293l	3.803k	2.407f
B ₂	H ₀	1.983n	5.083d	5.616c	4.227b
	H ₁	1.003q	4.256i	4.890f	3.383d
AverageB					
Average overlap BXS	B ₀	1.116h	5.248b	6.180a	4.181a
	B ₁	1.655f	3.345e	4.316d	3.105c
	B ₂	1.493g	4.670c	5.253b	3.805b
AverageH					
Average overlap SXH	H ₀	1.844e	5.294b	5.934a	4.357a
	H ₁	0.998f	3.547d	4.565c	3.037b
AverageS		1.421c	4.421b	5.250a	

: B0 without spraying (distilled water)	S 0 Without addition : (distilled water)	H0 Without addition : : (distilled water)
B 1 Spraying at a : (1⁻ concentration of (100 mg/L	S 1 Add at a concentration : (1⁻ of (5 g/L	H1 Add at a : (1⁻ concentration of (3 g/L
B2 Spraying at a : (1⁻ concentration of (200 mg/L	S2 Add at a concentration : (1⁻ of (10 g/L	
Numbers with similar letters mean that there are no significant differences between the averages of the coefficients according to Duncan's multinomial test with a probability level of 5%.		

Conclusion

We observe from the above table that the plant *Artemisia* showed resistance to salt stress at the concentrations used in the study. We note the three-way interaction (proline spraying, addition of humic acid, and salt stress) at concentrations of (200 mg L⁻¹, 3 g L⁻¹, 10 g L⁻¹) respectively .

The plant has shown its ability to adapt and grow under these salt concentrations, with resistance varying according to plant species. Plant resistance occurs due to several mechanisms that allow the plant to complete its metabolic activities without being affected by the highly stressful external environment. One of these mechanisms is osmotic adjustment. The term "osmotic adjustment" was first introduced by the scientist Bernstein in 1961 to describe the changes in osmotic potential in leaves due to changes in soil osmotic potential caused by salinity. This term has since been widely used in salt or water stress research. It is the increase in osmotic pressure of the cellular content due to the

accumulation of salts and soluble substances for the purpose of the resistance mechanism [6]. We observe that spraying with proline along with the addition of humic acid has reduced salt stress in the triple interaction treatments, which positively reflected in the increase of the concentrations of (nitrogen, proline, chlorophyll) in the triple interaction treatments of the table.(5.4.3). This is attributed to the fact that proline is a soluble substance, and its accumulation is often associated with the severity of environmental stresses, as it accumulates in plants during negative environmental constraints. It plays an important role in stress tolerance and is considered a factor for the stability of proteins and molecular structures. It is noted that proline, an amino acid, regulates osmotic pressure and increases the plant's efficiency in water absorption under saline conditions in which it grows. This capability makes the plant more resistant to increasing salt concentrations in the soil.

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