



THE IMPACT OF NANO FERTILIZATION AND SALICYLIC ACID ON GROWTH, YIELD AND ANTI-OXIDANT CONTENTS IN ROCKET PLANT UNDER SALT STRESS

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ABSTRACT. This investigation aimed to study the effect of organic fertilizers, nano-fertilizers and salicylic acid on the growth and yield of rocket (*Eruca sativa* L.) and the content of active compounds and antioxidants when the plants were exposed to salt stress. The experiment was conducted using a randomized complete block design (RCBD) according to the split-plot system. The main factor was water quality (1.2 dS m⁻¹ and 8 dS m⁻¹). While the combination treatments of Nano fertilizer, Salicylic acid and poultry manure were distributed in sub-plots and each treatment included three replicates. The treatments irrigated with saline water showed a reduction of glucosinolate and ascorbate contents (58 µg g⁻¹ and 105.71 µg g⁻¹, respectively). Salinity led to an increase in glutathione and proline in the leaves (1146 and 2.2 µg g⁻¹, respectively), while the fertilization treatments (poultry manure + nano-NPK; poultry manure + salicylic acid + nano-NPK) resulted in an increase in the glucosinolate content of the leaves under salt stress (85.6 and 89.2 µg g⁻¹, respectively). The nano-NPK treatment achieved a high value of the leaves' ascorbic acid content under the unstressed conditions (166.73 µg g⁻¹), while the salicylic acid + nano-NPK treatment achieved the highest value of ascorbic acid under salt stress (137.4 µg g⁻¹). The combination of salicylic acid + poultry manure + nano-NPK obtained the highest value of glutathione content in the leaves (1950 µg g⁻¹) under the stress conditions. There is a positive correlation between salt stress and glutathione + proline, while the salt stress condition had a negative effect on glucosinolate, ascorbate and yield.

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Introduction

Rocket (*Eruca sativa* L.) is a useful commercial crop with beneficial nutritional properties. Rocket plant leaves are rich in vitamins, biotin, thioglycosides, ascorbic acid, glutathione and retinol. Thioglycoside is considered one of the most important sulfur compounds responsible for the spicy taste in plants. Salinity is the most influential environmental factor limiting the productivity of crops (Chakrabarti, Ahmad, 2009).

Many plants depend on salt stress resistance, by producing effective antioxidants because of oxidative stress in cells due to the accumulation of free radicals, which cause the oxidation of the internal structures of the cell, especially the cell membranes (Kharusi *et al.*, 2019).

Water scarcity is one of the constraining factors for crop production; it plays a primary role in reducing crop

production more than the reduction resulting from all other environmental factors (Bader *et al.*, 2020; Gar *et al.*, 2021).

Most strategically, crops are sensitive to salinity and crops drop between 20 and 50% of productivity under salt stress conditions, these reductions due to drought and soil salinity have two reasons. The first is the scarcity of irrigation water and the second is the high concentration of dissolved salts in the waters of the Tigris and Euphrates rivers, which are associated with low levels of water (Pengfei *et al.*, 2017; Al-Taey *et al.*, 2019). Because most crops are glycophytes and sensitive to salt stress, a high level of salinity is expected to cause a significant reduction in crop growth and production (Liu, Suarez, 2021). Salinity has plagued soil fertility and drastically affected the growth and survival of glycophytes in irrigated regions of the world



since the beginning of recorded history. It is particularly common in arid and semi-arid areas where evapotranspiration exceeds annual precipitation and where irrigation is, therefore, necessary to meet crops' water needs (Manchanda, Garg, 2008).

Nanotechnology is a new multidisciplinary solution, especially in the agricultural and food sciences, which has led to new methods of solving many agricultural problems. Nanoparticles have other potential applications in agricultural systems, especially in foliar or ground fertilization operations (Ghorbani *et al.*, 2011). Furthermore, foliar fertilization should be supported with an organic fertilizer system to provide the needs of nutritional elements, especially under conditions of salt stress. Al-Juthery *et al.* (2020) noted that foliar application of nano-NPK 20-20-20 fertilizer optimized the growth, yield and concentration of nutrients in grains of wheat.

Salicylic acid is one of the plant hormones produced in plants and it plays an important role in regulating most of the biological activities of plants, such as plant growth, photosynthesis and flower regulation. Salicylic acid has received the attention of scientists and researchers due to its link with the defence systems inside the plant against the stresses to which the plant is exposed, particularly under conditions of salt stress, by reducing the production of oxidative systems and reducing the damage caused by these free oxygen radicals (Lateef *et al.*, 2021).

Materials and Methods

The study was conducted at the open field of Karbala research station with coordinates (32.61603N, 44.02488E) on October 15, 2018, the experiment was conducted with a randomized complete block design (RCBD) according to the split-plot system. The irrigation water quality (1.2 and 8.0 dS m⁻¹) were symbolized (W1 and W2) distributed to the main plot and the combination treatments (Table 1) were distributed in sub-plots. Each treatment included three replicates and soil analysis was conducted (Table 2). Irrigation was determined according to the plants' needs by placing sensors (Firstrate FST100–2006C, China) in each experiment unit. Irrigation was conducted based on depleting 50% of the field capacity.

Combination treatment (Table 1) poultry manure and traditional NPK fertilizer were applied directly to the soil, while salicylic acid and nano-NPK fertilizer were added as foliar spray applications three times every 14 days.

Table 1. The combination treatments

Treatment	Study dosages and concentrations
T1 Control	–
T2 NPK	1 g m ⁻¹
T3 NPK ⁰	0.5 g m ⁻¹
T4 Poultry manure	300 g m ⁻¹
T5 Salicylic acid	50 mg L ⁻¹
T6 SA + poultry manure (PO)	50 mg L ⁻¹ +300 g m ⁻¹
T7 SA + NPK ⁰	50 mg L ⁻¹ +1 g m ⁻¹
T8 PO + NPK ⁰	300 g m ⁻¹ + 1 g m ⁻¹
T9 PO + SA + NPK ⁰	300 g m ⁻¹ + 50 mg L ⁻¹ +1 g m ⁻¹

Table 2. Chemical and physical soil properties

Parameter	Value	Units
Clay	89	
Sand	141	Sandy loam, g kg ⁻¹
Silt	770	
pH	7.23	
EC	3.4	dS m ⁻¹
NaCl%	8.5	%
CO ³⁻	Nil	
HCO ³⁻	13×10 ⁻²	
Ca ⁺²	92×10 ⁻³	
Mg ⁺²	18×10 ⁻³	
K ⁺	86×10 ⁻⁴	mol·L ⁻¹
Na ⁺	15×10 ⁻³	
SO ⁴⁻²	12×10 ⁻³	
Organic matter	1.6	%

Glucosinolate determination (mg g⁻¹ dry weight)

The iron cyanide reduction method was used to determine the leaf content of glucosinolate sulfur glycosides (GLS), as described by Jezek *et al.* (1999). This depended on the reduction of iron cyanide by glucosinolate; then, the product was broken down in an alkaloid medium, liberating a yellow-coloured compound 1-thioglucose and its colour intensity was measured using a spectrophotometer (PG Instruments T60, UK) with a wavelength of 420 nm.

Glutathione determination (mg g⁻¹ dry weight)

The glutathione content in the leaves was determined by Alscher (1989) using a dye (DTNB) that combines with the thiol group SH, in which the glutathione molecule is an alkaloid medium (pH = 8.9) to form the mixed disulphide, which releases the ion complex (yellow thiols). Colour intensity was measured using a spectrophotometer (PG Instruments T60, UK) with a wavelength of 412 nm.

Ascorbic acid contents in leaves (mg 100 g⁻¹ fresh weight)

The vitamin C in leaves was estimated using the direct colour method (Horwitz *et al.*, 1970), which depended on the extent of 2,6-Dichlorophenolindophenol colour reduction by ascorbic acid in the sample based on the standard ascorbic acid.

Proline contents in leaves (mcg g⁻¹ dry weight)

Proline colourimetric determination preceded, based on proline's reaction with ninhydrin, the ratio of 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100 °C for 1 hour. The reaction was arrested in an iced bath and the chromophore was extracted with 1 ml toluene, the absorbance was determined at 520 nm, 0.1 gm of the shoot and root tissues were suspended with 1 ml of 3% sulfosalicylic acid and after centrifugation (10 min at 12 000 rpm). The reaction and determination of proline were carried out similarly, to those described above, the concentration of proline in tissues was determined depending on a standard curve of pure proline (Bates *et al.*, 1973).

Data analysis

Data were recorded and entered into MS Excel 2010. Data were analyzed using the GenStat 12 program analyze data. two-way ANOVA has been used and the means were compared with the LSD test (P ≤0.05; Table 5).

Results and Discussion

Tables 3 and 5 showed significant differences when irrigating with different qualities of water, W1 (1.2 dS m⁻¹) achieved the best values of the vegetative and dry yield, leaves no., the leaf content of chlorophyll, glucosinolate and ascorbic acid, while the best values of glutathione and proline contents in leaves were achieved at W2 (8 dS m⁻¹) irrigation.

A significant difference among the combination treatments (Table 4), all treatments achieved a significant difference compared to control, the treatment T9 (poultry manure + SA + nano-NPK) achieved the highest values in vegetative and dry yield, leaves no., chlorophyll, glucosinolate, glutathione and proline content.

The addition of poultry manure, SA and nano-NPK individually achieved a significant increase in compari-

son with the control, in all studied parameters. The overlap of poultry manure, SA and NPK resulted in achieving the highest rates in all studied indicators compared to their use separately especially under irrigation level 8 dS m⁻¹, while the treatment T8 (poultry manure + nano-NPK) achieved the highest value in ascorbic acid contents in leaves.

The reduction of the studied indicators, because of irrigation with water level 8 dS m⁻¹, may be attributed to the osmotic effect caused by the increased salts in the soil and this leads to a decrease in the absorption of water by the plant. In turn, leads to a decrease in the permeability of nutrients, reflected negatively on cellular metabolism and vital activities inside the cell (Al-Khafajy *et al.*, 2020).

Table 3. Effect of water quality and treatment on some study parameters of rockets

Factors	Yield, kg ha ⁻¹	Dry yield, kg ha ⁻¹	Leaves, no. plant ⁻¹	Chlorophyll, mg 100 g ⁻¹	Glucosinolate, µg g ⁻¹	Glutathione, µg g ⁻¹	Ascorbic acid, mg 100 g ⁻¹	Proline, mg g ⁻¹
W1	10 700.8	1 531	20.41	109.96	110.7	754	136.51	1.18
W2	8 412.8	1 211	18.11	100.64	58	1 146	105.71	2.2
LSD _(0.05)	1 457.28	224.2	1.28	2.091	18.98	278.6	1.01	0.63
T1	5 632.0	978	15.83	93.07	49.5	556	93.3	0.91
T2	12 284.8	1 456	24.67*	109.77	90.8	978	140.99	2.22
T3	9 310.4	1 330	18.87	111.95	95.1	822	141.98	2.11
T4	9 979.2	1 151	16.43	99.65	73.3	675	114.64	1.64
T5	6 969.6	1 014	17.40	100.25	80.8	1 157	96.36	1.37
T6	10 612.8	1 466	20.60	110.73	86.9	1 259	109.33	1.54
T7	8 219.2	1 164	18.27	103.15	94.2	881	113.68	1.47
T8	9 152.0	1 276	18.07	101.45	90.1	867	144.35*	2.09
T9	1 3904.0*	2 103*	23.23*	117.69*	98.5*	1 355*	135.36	1.9
LSD _(0.05)	491.843	193.0	1.53	1.931	8.88	241.6	4.1	0.55

W – water qualities, 1.2 dS m⁻¹ W1, 8 dS m⁻¹ W2; Treatments: Control –T1, 1 g m⁻¹ NPK – T2, 0.5 g m⁻¹ nano-NPK – T3, poultry manure (PO) 300 g m⁻¹ – T4, salicylic acid (SA) 50 mg L⁻¹ – T5, SA + PO – T6, SA + nano NPK – T7, PO + NPK⁰ – T8, PO + SA + NPK⁰ – T9

Table 4. The interaction treatments between the study factors

Factors	Yield, kg ha ⁻¹	Dry yield, kg ha ⁻¹	Leaves, no. plant ⁻¹	Chlorophyll, mg 100 g ⁻¹	Glucosinolate, µg g ⁻¹	Glutathione, µg g ⁻¹	Ascorbic acid, mg 100 g ⁻¹	Proline, mg g ⁻¹
W1T1	6 195.2	1 018	16.27	102.43	3.07	0.750	5.52	0.80
W1T2	13 675.2	2 068	26.93	110.67	4.26	1.130	5.96	1.75
W1T3	11 352.0	1 491	19.27	114.23	4.95	1.270	6.16	1.52
W1T4	11 686.4	1 392	17.80	103.73	4.49	1.277	6.32	1.89
W1T5	7 884.8	1 236	18.20	100.53	4.23	1.060	6.11	1.35
W1T6	11 475.2	1 573	21.20	118.54	3.66	1.170	6.43	1.71
W1T7	8 782.4	1 345	19.20	105.43	4.75	1.440	6.21	1.51
W1T8	9 891.2	1 417	19.47	104.30	4.32	1.300	6.38	1.94
W1T9	15 382.4	2 237	25.40	129.75	5.03	1.610	6.33	2.00
W2T1	5 086.4	938	15.40	83.70	2.56	0.250	4.36	1.76
W2T2	10 894.4	1 844	22.40	108.87	3.54	0.450	5.1	3.54
W2T3	7 251.2	970	18.47	109.67	3.92	0.890	5.59	3.18
W2T4	8 272.0	910	15.07	95.57	3.29	0.797	5.46	2.85
W2T5	6 054.4	791	16.60	99.97	4.03	0.960	5.5	2.28
W2T6	9 750.4	1 359	20.00	102.93	2.97	0.630	5.58	2.63
W2T7	7 656.0	983	17.33	100.87	3.32	1.060	5.65	2.46
W2T8	8 395.2	1 135	16.67	98.60	3.83	0.840	5.77	2.72
W2T9	12 425.6	1 968	21.07	105.63	4.04	1.020	5.61	2.22
LSD _(0.05)	4 699.2	279.2	2.04	2.761	0.887	0.483	0.757	0.72

W – water qualities, 1.2 dS m⁻¹ W1, 8 dS m⁻¹ W2; Treatments: Control –T1, 1 g m⁻¹ NPK – T2, 0.5 g m⁻¹ nano-NPK – T3, poultry manure (PO) 300 g m⁻¹ – T4, salicylic acid (SA) 50 mg L⁻¹ – T5, SA + PO – T6, SA + nano NPK – T7, PO + NPK⁰ – T8, PO + SA + NPK⁰ – T9

Table 5. ANOVA responses due to study factors

Source	Yield, kg ha ⁻¹	Dry yield, kg ha ⁻¹	Leaves, no. Plant ⁻¹	Chlorophyll, mg 100 g ⁻¹	Glucosinolate, µg g ⁻¹	Glutathione, µg g ⁻¹	Ascorbic acid, mg 100 g ⁻¹	Proline, mg g ⁻¹
Water qualities (W)	*	*	*	*	*	*	*	*
Treatments (T)	*	*	*	*	*	*	*	*
W × T	*	*	*	*	*	*	*	*

Significant at P < 0.05, ANOVA; since the 2-way interaction was significant, it was used to explain the results

The increase of salts in the soil solution leads to a rise in the osmotic pressure of the soil solution, causing a decrease in the water availability and obstructing the transfer of water through the root system. In addition to the accumulation of sodium and chloride ions to the toxic limit, leading to a decrease in the activity of the meristematic tissues and inhibition of cell division and elongation (Sakr *et al.*, 2007). Furthermore, it affects the vegetative and root growth, causing a decrease in the rate of plant growth, which is ultimately reflected in yield and these results are consistent with what was reached by (Golezani *et al.*, 2011; AL-Taey *et al.*, 2019).

The irrigation water at level 8 dS m⁻¹ decreased the leaf content of glucosinolate, Martínez-Ballesta *et al.*, (2015) indicated that glucosinolate compounds are affected by environmental stress negatively or positively and it has been found that the content of Arabidopsis leaves of total glucosinolate decreased during salt stress. Glucosinolate creation may be one of the acclimatization methods followed by plants. The decrease in total glucosinolate may be the result of the negative effect of salt stress on cellular metabolism processes and the production of glucosinolate has been affected. This may be due to the increase in the formation and activity of the myrosinase, which increases the hydrolysis of the glucosinolate (Radoicic *et al.*, 2008). The saline water led to a significant decrease in the content of ascorbic acid. It may be due to the negative effect of salinity, which disrupted the processes of cellular metabolism and affected the process of photosynthesis, thus reducing the carbohydrate compounds and carbon chains necessary to build other compounds (Deridder, Salvucci, 2007).

Tables 3 and 4 has been shown an increase in the glutathione content in the plant leaves during irrigation with 8 dS m⁻¹ and this result has been indicated by (Koprivova *et al.* 2010; Lee *et al.* 2013). So, glutathione content is affected by environmental stress such as salt stress, leading to alteration of internal levels of glutathione under stress due to stimulation or modification in the gene expression responsible for glutathione production by reducing free oxygen radicals and delaying senescence (Hussain, 2016).

The salt stress led to an increase in the proline content in the leaves, as this is a mechanism followed by the plant in reducing Reactive oxygen species. The increase in salt leads to an increase in Reactive oxygen species inside the cells and this leads to damages inside the cell in association with the lipids that forms the plasma membrane. The plant works to increase the production of proline to reduce against cell ROS damage and regulate of osmotic pressure under stress conditions (Türkan, Demira, 2009).

Conclusion

Salinity led to an activated the non-enzymatic system against oxidative stress by an increase in glutathione and proline in the leaves. It reduced the other study growth parameters and yield while the combination treatment (poultry manure + nano-NPK; poultry manu-

re + salicylic acid + nano-NPK) increased the glucosinolate content of the leaves under salinity.

Nano-NPK fertilizer achieved the highest value of the leaves' ascorbic acid content.

The treatment combination of (salicylic acid + poultry manure + nano-NPK fertilizer) got the highest value of glutathione content in the leaves under the salinity conditions.

Finally, there is a positive correlation between Salinity and glutathione + proline, while the salt stress condition has a negative effect on glucosinolate, ascorbate and yield.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

DKAA-T – designed the experimental setup, analyzed the data and results, wrote the manuscript.

ZJMA-M – performed greenhouse experiments and biochemical analyses.

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