J. of Plant Production, Mansoura Univ., Vol. 14 (11): 551 - 558, 2023

Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Potential Effect of Cobalt and Selenium on Croton Plants Grown under Saline Conditions

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ABSTRACT

The search for a viable alternative to freshwater such as the use of wastewater to irrigate ornamental plants has become particularly important due to Egypt's water scarcity problems. Researchers in this field should increase their efforts to introduce innovative approaches that allow for the efficient use of wastewater while maintaining the integrity of the agricultural system. Therefore, this research was conducted to evaluate the effect of irrigation *with* water of different salinity levels [EC=475, 1000 and 2000 ppm] on Croton plant. In addition, the effects of spraying cobalt (Co) and selenium (Se) at different rates (0.0, 2.5, 5.0 mgL^{-1}) were studied. The study evaluated various parameters of the Croton plant, including the plant height, leaf area, nitrogen, phosphorus, potassium, chlorophyll a & b, anthocyanin and antioxidants. It is noteworthy that when Croton plants were irrigated with fresh water (EC=475ppm), they showed the highest values for all the parameters studied except for antioxidants, which showed the opposite trend. Also, as the salinity of the irrigation water increased to 1000 ppm and then to 2000 ppm, the values of all the parameters gradually decreased except for antioxidants which gradually increased with increasing salinity. On the other hand, both cobalt and selenium applications resulted in improvement of the Croton plant performance, especially when the plants were irrigated with saline water (EC=1000 and 2000 ppm). In terms of effectiveness, the order from most effective to least effective treatments was as follows;Se (5.0 mgL^{-1})> Co (5.0 mgL^{-1})> Se (2.5 mgL^{-1})> Co (2.5 mgL^{-1})> control group.

Keywords: Wastewater, Co, Se, ornamental plants

INTRODUCTION

Croton (*Codiaeum variegatum*), commonly known as Croton petra, belongs to the Euphorbiaceae family. It is an ornamental plant of great importance in horticulture, landscaping, and various other fields (Esmail, 2008). Croton is a valuable and visually striking ornamental plant with a number of practical uses, including its contribution to landscaping, air purification, and the horticultural trade. Its vibrant foliage and adaptability make it a popular choice for adding color and visual interest to different environments (Bakheet *et al.* 2018).

The scarcity of freshwater resources in Egypt has prompted a critical search for alternative water sources to sustain agriculture (El-Nashar and Elyamany, 2023). One promising avenue is the use of wastewater to irrigate ornamental plants, which has gained increasing importance. Researchers in this field are challenged to develop innovative strategies that not only maximize the efficient use of wastewater, but also ensure that the integrity of the agricultural system is maintained (Selim *et al.* 2023). This urgency is due to the stress induced by irrigation of ornamental plants with saline water, which often leads to the generation of harmful free radicals or as named ROS in plant tissues. These free radicals pose a significant threat to the health and vitality of ornamental plants (Goyal *et al.* 2020; Dotaniya *et al.* 2023; Elnashar *et al.* 2023).

Furthermore, recent discoveries have highlighted the newfound importance of cobalt in enhancing plant resilience to saline conditions. It is now considered not only a beneficial element, but also a criticalcomponent in mitigating the detrimental effects of salinity stress on plants (Gad and El–Metwally, 2015). The effectiveness of Cobalt in enhancing plant tolerance to salinity stress is attributed to a number of mechanisms. It plays a role in maintaining ionic balance in plant cells (Baddour *et al.* 2021; Brengi *et al.* 2022).

Selenium, a trace element, is important in several biological functions, with one of its key roles being that of an antioxidant (Jiang *et al.* 2017; Abdalla *et al.* 2023; Elsherpiny, 2023).

The specific objectives of this research work are to provide a comprehensive understanding of how salinity, cobalt, and selenium influence the growth, physiology, and stress tolerance of the Croton plant, which is valuable both for ornamental plant cultivation and for sustainable water management strategies in regions facing water scarcity.

MATERIALS AND METHODS

This study was carried out to evaluate the effects of irrigation with water of different salinity levels on Croton plant. Additionally, the effects of spraying cobalt (Co) and selenium (Se) at different rates were studied.

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DOI: 10.21608/jpp.2023.249018.1289

1. Experimental location and experimental period

This research was conducted at the Al-Mansoura Agricultural Research Station, which is affiliated to the Agricultural Research Center in Egypt and is located at approximately 31.0500°N latitude and 31.3833°E longitude in terms of geographical coordinates. The study took place and extended from February 15th to October 15th, covering the 2022 and 2023 seasons, with each season lasting for a duration of 8 months.

2. Plant material

In this study, the Croton cultivar (*Codiaeum variegatum* L.) used was petra c.v., which was obtained from a private nursery in Alqanater Alkhayrih, Egypt.The Petra cultivar is characterized by its wide leaves decorated with red veins and edges, as described by Bakheet *et al.* (2018).

3. Preparation of synthetic saline water

Synthetic saline water was carefully prepared by dissolving El-Rashidi natural salt in tap water to achieve the desired salinity level (1000 and 2000 ppm). This choice to use natural salt, as opposed to pure sodium chloride (NaCl), was motivated by the intention to create a saline water source that closely mimics the diverse ionic composition found in nature, thus avoiding the potential singular effects of sodium or chloride ions.

4. Studied elements

Cobalt sulfate ($CoSO_4$, containing 36% cobalt) and sodium selenite (Na_2SeO_3, containing 45.56% selenium) were purchased from Sigma Company (Sigma-Aldrich, St. Louis, MO, USA). To prepare standard solutions for experimental purposes, cobalt and selenium were carefully dissolved in a suitable solvent at precise concentrations. These prepared standard solutions were then used to prepare the cobalt (Co) and selenium (Se) concentrations required for the study.

5. Experimental design and treatments

The experiment included fifteen treatments, covering all possible combinations of three irrigation treatments and five different foliar applications. These treatments were arranged in a split-plot design, with the irrigation treatments serving as the main factor, and the foliar applications of cobalt and selenium as the sub main factors. Each treatment was replicated ten times, resulting in a grand total of 135 pots used for each season of the study. The different treatments denoted by the following symbols: [I_1: Fresh water (EC=475ppm); I_2: Saline water (EC=1000ppm); I_3: Saline water (EC=2000ppm); F_1: Without foliar applications; F_2: Cobalt at rate of 2.5 mg L^{-1}; F_3: Cobalt at rate of 5.0 mg L^{-1}; F_4: Selenium at rate of 2.5 mg L^{-1}; F_5: Selenium at rate of 5.0 mg L^{-1}]. Water with an EC value of 475 represented irrigation with fresh water, while water with EC values of 1000 and 2000 ppm represented irrigation with waste water (synthetic saline water).

6. Experimental medium in final pots

The final pots were filled with a mixture of sand, clay, and peat moss in equal proportions, with a volume-to-volume ratio of 1:1:1. with the addition of individual calcium super phosphate(6.7%P) at a rate of 5 g pot^{-1}. Several characteristics of the experimental medium are detailed in Table 1, with the analysis carried out according to the methodology outlined by Dewis and Freitas (1970).

Table 1. Several attributes of the experimental medium

Traits and units	Values
Availability of nutrients , mg kg^{-1}	
Available N	38.9
Available P	10.25
Available K	200.3
Particles size distribution, g kg^{-1}	
Sand	490
Silt	100
Clay	410
Texture class	Sandy clay
Others	
O.M,%	1.13
EC, dSm^{-1}	3.40
pH	8.05
CEC, cmol kg^{-1}	35.3

7. Experimental set up

Plants were transplanted into their designated plastic pots (25 cm* 18 cm), after receiving the plants from the nursery on the first of February. All seedlings were similar in growth and 20cm in length. The NPK fertilizer solution was prepared by dissolving 5.0 g of a commercial compound called Kristalon (19-19-19) in one liter of tap water. The NPK fertilizer was applied as a soil drench in five equal portions of200 ml each. The first dose was applied 10 days after transplanting and the other doses were applied at monthly intervals. The foliar application of both cobalt and selenium was carried out ten times from 27th April to 15th September with a 15-day interval between each application, using a plastic sprayer, with an application volume of 1.5 liters per pot for each treatment studied. Irrigation was carried out weekly according to the prescribed irrigation treatments. It's worth noting that the application treatments were started from the last week of April, as the application prior to that did not meet the prescribed application protocols under study.

8. Measurements

Physical characteristics of the whole plant

Plant height (cm), fresh and dry leaf weights (g $plant^{-1}$), No. of leaves $plant^{-1}$, No. of branches $plant^{-1}$, leaf area (cm^2 $plant^{-1}$), root length (cm), No. of roots $plant^{-1}$ and fresh and dry root weights (g $plant^{-1}$), were recorded on the 15th of October of each season.

Leaf chemical constituents

On the 15th of October of each season, the percentages of nitrogen (N), phosphorus (P), potassium (K), and carbohydrates (%) in the samples were measured. The assessment of N, P, and K was performed using the Kjeldahl, spectrophotometric, and flame photometer methods, respectively, following the procedures described by Walinga *et al.* (2013). Prior to NPK analysis, samples underwent a preparation step in which they were dried and digested with a 1:1 mixture of H_2SO_4 and $HClO_4$, according to the method described by Peterburgski (1968). The total carbohydrate content of the dried leaves was determined according to the method described by Herbert *et al.* (1971).

Plant pigments

On the 15th of October in each season, chlorophyll a & b (mg g^{-1} F.W), carotene (mg g^{-1} F.W) and anthocyanin (mg $100g^{-1}$) pigments were measured using established standard methods as described by Schoefs (2005).

Antioxidants and indicators of oxidation

On the 15th of October of each season, we measured for proline, malondialdehyde (MDA, a biomarker of lipid peroxidation), peroxidase (POX), and catalase (CAT). Proline levels were determined according to the method described by Ábrahám *et al.* (2010). MDA concentrations were determined according to Davey *et al.* (2005). POX and CAT activity measurements were performed according to Güneş *et al.* (2019).

9. Statistical analysis

Statistical analysis of the data was performed using CoStat (Version 6.303, CoHort, USA, 1986). The least significant difference (LSD) test was used to compare means at a significance level of 0.05, following the methodology as described by Gomez and Gomez (1984). Duncan's test, as described by Duncan (1955), was used to further assess the means between treatments

RESULTS AND DISCUSSION

Results

1. Growth performance

Irrigation with water of different salinities and the application of cobalt and selenium at different rates significantly affected all growth parameters of the Croton plant, including plant height (cm), fresh and dry leaf weights (g plant^{-1}), No. of leaves plant^{-1}, No. of branches plant^{-1}, leaf area (cm^2 plant^{-1}) as shown in Table 2, root length (cm), No. of roots plant^{-1} and fresh and dry root weights (g plant^{-1}) as shown in Table 3.

Tables 2 and 3 show that when the Croton plants were irrigated with water with an electrical conductivity (EC) value of 475ppm, they exhibited the highest values of all the above-mentioned parameters. Conversely, as the salinity of the irrigation water increased to 1000 ppm and then 2000 ppm, the values of these parameters decreased. The Croton plants irrigated with water with an EC value of 2000 ppm showed the lowest values for all the parameters studied.

The same Tables also show that both cobalt and selenium applications improved the performance of the Croton plants, expressed in terms of plant height (cm), fresh and dry leaf weights (g plant^{-1}), No. of leaves plant^{-1}, No. of branches plant^{-1}, leaf area (cm^2 plant^{-1}) (Table 2), root length (cm), No. of roots plant^{-1} and fresh and dry root weights (g plant^{-1}) (Table 3), especially when the plants were irrigated with saline water with EC values of 1000 and 2000 ppm. In terms of efficacy, the order from the most effective to the least effective treatments was as follows; Se (5.0 mgL^{-1})> Co (5.0 mgL^{-1})> Se (2.5 mgL^{-1})> Co (2.5 mgL^{-1})> control group (no foliar application).

It's worth noting that for most of the parameters observed, plants that were irrigated with saline water (with an electrical conductivity of either 1000 or 2000 ppm) and simultaneously sprayed with either cobalt or selenium showed values close to, and in some cases exceeding, those of plants irrigated with fresh water (with an electrical conductivity of 475 ppm) without any foliar application, and in some cases, even exceeded them. This trend was particularly evident when using a concentration of 5.0 mgL^{-1} for both cobalt and selenium in plants irrigated with saline water (EC=1000). The same trend was observed for both seasons studied.

Table 2. Effect of irrigation water with different salinity levels under cobalt and selenium spraying at different rates on the vegetative growth performance of Croton plant during two consecutive seasons (2022-2023)

Treatments		Plant height, cm		Fresh weight, g plant^{-1}		Dry weight, g plant^{-1}		No. of leaves plant^{-1}		No. of branches plant^{-1}		Leaf area, cm^2 plant^{-1}	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Main factor : Salinity levels of irrigation water													
I_1		51.28a	52.90a	60.91a	65.00a	15.96a	16.77a	44.60a	45.13a	7.60a	8.00a	147.57a	154.96a
I_2		47.52b	48.87b	57.94b	62.78b	15.12a	15.91b	40.87b	41.53b	6.73b	6.87b	135.49b	142.38b
I_3		40.58c	41.99c	48.46c	50.68c	12.55b	13.16c	34.80c	35.20c	4.67c	5.20c	114.04c	119.60c
LSD 5%		0.98	1.84	2.21	1.89	0.84	0.61	1.38	1.79	0.59	0.92	4.45	1.23
Sum main factor : Cobalt and selenium levels													
F_1		40.92c	42.29c	48.59c	50.85d	12.71c	13.35c	36.22e	36.89e	5.11c	5.56b	120.10d	126.24d
F_2		46.24b	47.74b	54.18b	60.02c	14.07b	14.77b	39.33d	39.89d	6.11b	6.56ab	129.87c	136.27c
F_3		48.37ab	49.86ab	59.01a	61.80b	15.32a	16.09a	41.89b	42.33b	6.78ab	7.11a	136.91b	143.95b
F_4		47.22b	48.70ab	57.68a	59.06c	15.05a	15.80a	40.22c	40.67c	6.44ab	6.67a	133.99b	140.48b
F_5		49.55a	51.02a	59.40a	65.71a	15.56a	16.38a	42.78a	43.33a	7.22a	7.56a	140.96a	147.94a
LSD 5%		2.16	2.38	3.03	1.75	0.59	0.79	0.78	0.70	0.83	1.07	3.83	3.52
Interaction													
I_1	F_1	45.52	47.18	56.52	55.64	14.84	15.68	38.00	38.67	6.33	6.33	129.00	135.93
	F_2	50.80	52.75	60.30	68.25	15.69	16.55	45.33	46.00	7.33	7.67	146.73	154.00
	F_3	53.35	54.79	62.63	68.70	16.55	17.24	46.67	47.00	8.00	8.67	153.63	161.26
	F_4	52.50	53.88	61.53	60.33	16.11	16.86	46.00	46.33	7.67	8.33	150.89	157.87
	F_5	54.24	55.92	63.59	72.08	16.60	17.50	47.00	47.67	8.67	9.00	157.58	165.74
I_2	F_1	44.99	46.23	55.35	62.63	14.58	15.25	37.67	38.33	5.67	5.67	124.68	131.18
	F_2	46.82	47.97	57.00	61.66	15.05	15.71	38.67	39.33	6.67	7.00	132.70	138.78
	F_3	48.94	50.32	59.34	60.81	15.15	16.13	43.33	44.00	7.00	7.00	139.59	147.12
	F_4	46.73	48.21	58.32	62.69	15.19	15.98	39.67	40.33	7.00	7.00	136.78	144.02
	F_5	50.09	51.61	59.71	66.11	15.61	16.47	45.00	45.67	7.33	7.67	143.69	150.78
I_2	F_1	32.23	33.44	33.89	34.27	8.70	9.13	33.00	33.67	3.33	4.67	106.63	111.61
	F_2	41.09	42.50	45.25	50.13	11.46	12.06	34.00	34.33	4.33	5.00	110.17	116.03
	F_3	42.83	44.48	55.05	55.88	14.25	14.90	35.67	36.00	5.33	5.67	117.49	123.48
	F_4	42.42	44.00	53.20	54.17	13.86	14.55	35.00	35.33	4.67	4.67	114.30	119.55
	F_5	44.31	45.52	54.92	58.94	14.49	15.18	36.33	36.67	5.67	6.00	121.60	127.31
LSD 5%		3.75	4.13	5.25	3.04	1.02	1.73	1.38	1.20	1.43	1.85	6.67	6.10

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Since, I_1: Fresh water (EC=475ppm); I_2: Saline water (EC=1000ppm); I_3: Saline water (EC=2000ppm); F_1: Without foliar applications; F_2: Cobalt at rate of 2.5 mg L^{-1}; F_3: Cobalt at rate of 5.0 mg L^{-1}; F_4: Selenium at rate of 2.5 mg L^{-1}; F_5: Selenium at rate of 5.0 mg L^{-1}

Table 3. Effect of irrigation water with different salinity levels under cobalt and selenium spraying at different rates on root characteristics of Croton plant during two consecutive seasons (2021/2022-2022/2023)

Treatments		Root length, cm		No. of roots plant^{-1}		Root fresh weight, g plant^{-1}		Root dry weight, g plant^{-1}	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Main factor : Salinity levels of irrigation water									
I_1		26.87a	27.33a	16.47a	16.93a	18.30a	19.22a	6.30a	6.40a
I_2		25.70a	26.31a	15.27b	15.60b	16.95b	17.82b	5.85b	5.94b
I_3		23.28b	23.80b	11.47c	11.80c	15.02c	15.76c	5.17c	5.25c
LSD 5%		1.32	1.32	1.19	1.04	0.43	0.12	0.11	0.06
Sum main factor : Cobalt and selenium levels									
F_1		23.70c	24.19c	11.67c	12.00b	15.39d	16.18c	5.28c	5.37c
F_2		25.06b	25.58b	14.44b	14.89a	16.74c	17.57b	5.76b	5.84b
F_3		25.91ab	26.49ab	15.33ab	15.78a	17.27ab	18.16b	5.96ab	6.05a
F_4		25.55ab	26.14ab	14.89ab	15.33a	16.92bc	17.77a	5.86a	5.95ab
F_5		26.21a	26.66a	15.67a	15.89a	17.45a	18.32a	6.00a	6.09a
LSD 5%		1.15	1.06	0.99	1.13	0.50	0.23	0.14	0.15
Interaction									
I_1	F_1	25.16	25.57	13.67	14.00	16.54	17.43	5.73	5.82
	F_2	26.77	27.22	16.67	17.00	18.62	19.54	6.34	6.43
	F_3	27.53	28.07	17.33	18.00	18.81	19.73	6.43	6.53
	F_4	27.10	27.66	17.00	17.67	18.59	19.52	6.44	6.54
	F_5	27.78	28.13	17.67	18.00	18.93	19.88	6.54	6.67
I_2	F_1	24.54	25.10	13.33	13.67	16.46	17.31	5.62	5.74
	F_2	25.29	25.89	15.33	16.00	16.56	17.36	5.72	5.79
	F_3	26.07	26.82	16.00	16.00	17.39	18.33	6.04	6.11
	F_4	26.10	26.83	15.33	15.67	16.75	17.64	5.82	5.92
	F_5	26.50	26.92	16.33	16.67	17.58	18.48	6.03	6.12
I_2	F_1	21.41	21.89	8.00	8.33	13.18	13.80	4.49	4.56
	F_2	23.10	23.64	11.33	11.67	15.04	15.82	5.22	5.30
	F_3	24.12	24.59	12.67	13.33	15.62	16.41	5.41	5.49
	F_4	23.44	23.92	12.33	12.67	15.43	16.16	5.32	5.39
	F_5	24.34	24.93	13.00	13.00	15.83	16.60	5.42	5.49
LSD 5%		1.98	1.84	1.73	1.96	0.87	0.87	0.24	0.25

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Since, I_1: Fresh water (EC=475ppm); I_2: Saline water (EC=1000ppm); I_3: Saline water (EC=2000ppm); F_1: Without foliar applications; F_2: Cobalt at rate of 2.5 mg L^{-1}; F_3: Cobalt at rate of 5.0 mg L^{-1}; F_4: Selenium at rate of 2.5 mg L^{-1}; F_5: Selenium at rate of 5.0 mg L^{-1}

2. Chemical constituents and plant pigments

Irrigation with water of different salinities and the application of cobalt and selenium at different rates had a significant effect on the chemical composition of the Croton plant. This influence extended to the main chemical constituents, including nitrogen content (%), phosphorus content (%), potassium content (%), and carbohydrate content (%) as shown in Table 4. Furthermore, these treatments also had significant effects on plant pigments, specifically chlorophyll a & b (mg g^{-1} F.W), carotene (mg g^{-1} F.W) and anthocyanin pigment (mg $100g^{-1}$), as detailed in Table 5.

Table 4. Effect of irrigation water with different salinity levels under cobalt and selenium spraying at different rates on leaf chemical constituents of Croton plant during two consecutive seasons (2022-2023)

Treatments		Nitrogen,%		Phosphorus,%		Potassium,%		Carbohydrates %	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Main factor : Salinity levels of irrigation water									
I_1		2.96a	3.11a	0.405a	0.422a	2.69a	2.82a	15.89a	16.19a
I_2		2.81b	2.96b	0.387b	0.403b	2.53b	2.66b	15.12b	15.42b
I_3		2.53c	2.65c	0.350c	0.364c	2.22c	2.33c	13.80c	14.08c
LSD 5%		0.09	0.02	0.002	0.003	0.06	0.02	0.09	0.53
Sum main factor : Cobalt and selenium levels									
F_1		2.60d	2.73d	0.361d	0.376d	2.30d	2.43e	14.15c	14.43d
F_2		2.74c	2.88c	0.377c	0.393c	2.45c	2.57d	14.81b	15.11c
F_3		2.84ab	2.99a	0.389a	0.405a	2.55ab	2.68b	15.27a	15.57ab
F_4		2.79bc	2.92b	0.383b	0.399b	2.50bc	2.63c	15.01b	15.29bc
F_5		2.88a	3.02a	0.393a	0.409a	2.59a	2.72a	15.46a	15.75a
LSD 5%		2.60d	2.73d	0.361d	0.376d	2.30d	2.43e	14.15c	14.43d
Interaction									
I_1	F_1	2.72	2.86	0.375	0.389	2.42	2.54	14.68	14.95
	F_2	2.95	3.10	0.405	0.423	2.68	2.81	15.80	16.09
	F_3	3.05	3.21	0.416	0.434	2.78	2.93	16.39	16.71
	F_4	3.01	3.15	0.410	0.427	2.74	2.88	16.02	16.33
	F_5	3.10	3.26	0.420	0.437	2.81	2.95	16.58	16.84
I_2	F_1	2.67	2.81	0.371	0.388	2.38	2.51	14.43	14.72
	F_2	2.78	2.91	0.383	0.399	2.50	2.63	15.00	15.32
	F_3	2.87	3.03	0.395	0.410	2.59	2.72	15.45	15.74
	F_4	2.82	2.97	0.390	0.405	2.56	2.68	15.16	15.45
	F_5	2.91	3.06	0.398	0.413	2.64	2.78	15.58	15.88
I_2	F_1	2.42	2.53	0.338	0.351	2.12	2.23	13.35	13.61
	F_2	2.49	2.62	0.344	0.358	2.17	2.27	13.62	13.92
	F_3	2.60	2.73	0.357	0.371	2.28	2.39	13.97	14.27
	F_4	2.53	2.65	0.350	0.364	2.21	2.32	13.84	14.10
	F_5	2.63	2.75	0.362	0.378	2.32	2.44	14.24	14.52
LSD 5%		0.14	0.06	0.009	0.009	0.10	0.06	0.35	0.56

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Since, I_1: Fresh water (EC=475ppm); I_2: Saline water (EC=1000ppm); I_3: Saline water (EC=2000ppm); F_1: Without foliar applications; F_2: Cobalt at rate of 2.5 mg L^{-1}; F_3: Cobalt at rate of 5.0 mg L^{-1}; F_4: Selenium at rate of 2.5 mg L^{-1}; F_5: Selenium at rate of 5.0 mg L^{-1}

Table 5. Effect of irrigation water with different salinity levels under cobalt and selenium spraying at different rates on the leaf pigments of Croton plant during two consecutive seasons (2022-2023)

Treatments		Chlorophyll a, mg.g^{-1} F.W		Chlorophyll b, mg.g^{-1} F.W		Carotene, mg.g^{-1} F.W		Anthocyanin mg.100g^{-1}	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Main factor : Salinity levels of irrigation water									
I_1		0.467a	0.480a	0.270a	0.277a	0.547a	0.563a	4.88a	5.13a
I_2		0.450b	0.463b	0.261b	0.269b	0.524b	0.539b	4.17b	4.39b
I_3		0.414c	0.426c	0.244c	0.251c	0.469c	0.483c	3.10c	3.26c
LSD 5%		0.003	0.008	0.002	0.003	0.005	0.008	0.08	0.04
Sum main factor : Cobalt and selenium levels									
F_1		0.423d	0.435d	0.242e	0.249e	0.489s	0.504d	3.36e	3.54e
F_2		0.440c	0.453c	0.256d	0.264d	0.504c	0.518c	3.94d	4.14d
F_3		0.449b	0.462b	0.265b	0.272b	0.523b	0.539b	4.34b	4.56b
F_4		0.446b	0.459b	0.261c	0.268c	0.518b	0.533b	4.09c	4.30c
F_5		0.458a	0.472a	0.269a	0.277a	0.534a	0.549a	4.52a	4.75a
LSD 5%		0.005	0.005	0.003	0.003	0.005	0.005	0.10	0.05
Interaction									
I_1	F_1	0.437	0.449	0.246	0.253	0.515	0.531	3.79	3.98
	F_2	0.466	0.480	0.270	0.279	0.545	0.559	4.87	5.09
	F_3	0.472	0.486	0.276	0.285	0.553	0.568	5.28	5.55
	F_4	0.468	0.483	0.274	0.281	0.548	0.566	5.05	5.31
	F_5	0.490	0.503	0.282	0.290	0.574	0.591	5.44	5.70
I_2	F_1	0.433	0.445	0.244	0.252	0.501	0.515	3.64	3.84
	F_2	0.442	0.457	0.262	0.270	0.508	0.524	3.99	4.21
	F_3	0.458	0.470	0.266	0.273	0.537	0.554	4.43	4.65
	F_4	0.451	0.463	0.265	0.273	0.530	0.544	4.15	4.34
	F_5	0.465	0.480	0.268	0.276	0.545	0.560	4.66	4.90
I_2	F_1	0.400	0.412	0.235	0.241	0.451	0.465	2.67	2.80
	F_2	0.413	0.424	0.236	0.243	0.458	0.471	2.97	3.11
	F_3	0.418	0.430	0.251	0.259	0.480	0.494	3.32	3.48
	F_4	0.418	0.432	0.243	0.250	0.476	0.490	3.08	3.24
	F_5	0.420	0.432	0.257	0.264	0.483	0.497	3.47	3.65
LSD 5%		0.009	0.009	0.008	0.005	0.006	0.010	0.18	0.08

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Since, I_1: Fresh water (EC=475ppm); I_2: Saline water (EC=1000ppm); I_3: Saline water (EC=2000ppm); F_1: Without foliar applications; F_2: Cobalt at rate of 2.5 mg L^{-1}; F_3: Cobalt at rate of 5.0 mg L^{-1}; F_4: Selenium at rate of 2.5 mg L^{-1}; F_5: Selenium at rate of 5.0 mg L^{-1}

Among the Croton plants subjected to different irrigation treatments, those irrigated with water with an electrical conductivity (EC) value of 475 ppm showed the highest values for all the parameters mentioned. They were followed by the plants irrigated with water with an EC value of 1000 ppm, and finally, by the plants irrigated with water with an EC value of 2000 ppm. This trend suggests that lower salinity levels in irrigation water correlates positively with improved growth and chemical composition of Croton plants, as indicated by the measured parameters.

Across all irrigation treatments, Croton plants that were sprayed with selenium (Se) at a rate of 5.0 mgL^{-1} showed the highest values for all the traits mentioned. They were followed by the plants sprayed with cobalt at a rate of 5.0 mgL^{-1}. Next, the group treated with selenium (Se) at a rate of 2.5 mgL^{-1} had slightly lower values, and the group treated with cobalt at a rate of 2.5 mgL^{-1} had lower values. Finally, the control group, which received no foliar application, showed the lowest values for the measured traits. This suggests that foliar application of selenium, especially at the higher rate of 5.0 mgL^{-1}, had the most significant positive effect on the traits measured in Croton plants.

It can also be noted that both cobalt and selenium applications enhanced the chemical constituents and plant pigments of Croton plant, especially when the plants were irrigated with saline water with EC values of 1000 and 2000 ppm. The same pattern or trend was observed in both seasons studied.

3. Proline, malondialdehyde (MDA) and antioxidant enzymes (POX, CAT)

The data in Table 6 illustrate the effect of the treatments studied on proline (µg.g^{-1} F.W), malondialdehyde (MDA, µmol.g^{-1} F.W) and antioxidant enzymes *i.e.*, peroxidase (POX, unit mg^{-1} protein^{-1}) and catalase (CAT, unit mg^{-1} protein^{-1}). The data show that the highest values of proline (µg.g^{-1} F.W), MDA (µmol.g^{-1} F.W), POX (unit mg^{-1} protein^{-1}) and CAT (unit mg^{-1} protein^{-1}) were obtained when Croton plants were irrigated with water with an EC value of 2000 ppm followed by 1000 ppm. While the Croton plants irrigated with water with an EC value of 475 ppm (fresh water) had the lowest values of proline, MDA, POX and CAT.

The data in the same table also show that both cobalt and selenium applications significantly increased the Croton plant's own production of proline, MDA, POX and CAT. Since the spraying of Se at a rate of 5.0 mgL^{-1} led to obtaining the maximum values under irrigation *with* both fresh water and saline water, followed by the spraying of Co at rate of 5.0 mgL^{-1} then Se (2.5 mgL^{-1}), then Co (2.5 mgL^{-1}) and finally the control group (without foliar application).

Regardless of the specific foliar treatment applied, there is a noticeable trend that Croton plants irrigated with water with an EC value of 2000 ppm had higher levels of endogenous proline, MDA, POX and CAT compared to the plants irrigated with water with an EC of 1000 ppm and 475 ppm, respectively. This trend was consistent across all the different foliar treatments studied. In essence, this suggests that higher salinities in the irrigation water (EC = 2000 ppm) induced a more pronounced response in terms of these biochemical parameters in the Croton plants, indicating an increased stress response and antioxidant activity when compared to lower salinities (EC = 1000 ppm and 475 ppm). The same pattern was observed for both seasons studied.

Table 6. Effect of irrigation water with different salinity levels under cobalt and selenium spraying at different rates on leaf proline and antioxidant enzymes of Croton plant during two consecutive seasons (2022-2023)

Treatments		Proline, µg.g^{-1} F.W		MDA, µmol.g^{-1} F.W		POX, unit mg^{-1} protein^{-1}		CAT, unit mg^{-1} protein^{-1}	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Main factor : Salinity levels of irrigation water									
I_1		7.81c	7.94c	9.14c	9.37c	0.375c	0.381c	0.047c	0.049c
I_2		9.21b	9.38b	10.68b	10.95b	0.436b	0.446b	0.072b	0.075b
I_3		10.54a	10.70a	12.28a	12.56a	0.494a	0.504a	0.098a	0.102a
LSD 5%		0.10	0.41	022	0.31	0.006	0.012	0.003	0.002
Sum main factor : Cobalt and selenium levels									
F_1		8.72e	8.87d	11.21a	11.45a	0.413e	0.421d	0.063e	0.066e
F_2		9.00d	9.13cd	10.95b	11.24ab	0.425d	0.433cd	0.067d	0.070d
F_3		9.44b	9.61ab	10.43d	10.70c	0.445b	0.455ab	0.077b	0.081b
F_4		9.20c	9.34bc	10.68c	10.93bc	0.434c	0.444bc	0.072c	0.075c
F_5		9.60a	9.75a	10.22e	10.49c	0.456a	0.465a	0.082a	0.086a
LSD 5%		0.10	0.40	0.12	0.47	0.004	0.019	0.003	0.003
Interaction									
I_1	F_1	7.26	7.35	9.59	9.83	0.356	0.362	0.038	0.040
	F_2	7.64	7.76	9.40	9.60	0.366	0.373	0.041	0.043
	F_3	8.09	8.23	8.89	9.15	0.383	0.391	0.053	0.056
	F_4	7.85	7.98	9.11	9.31	0.374	0.381	0.046	0.048
	F_5	8.24	8.40	8.68	8.95	0.394	0.400	0.057	0.060
I_2	F_1	8.75	8.99	11.24	11.49	0.411	0.420	0.060	0.063
	F_2	8.95	9.07	10.91	11.20	0.424	0.431	0.067	0.070
	F_3	9.46	9.66	10.40	10.68	0.450	0.460	0.077	0.081
	F_4	9.19	9.37	10.66	10.95	0.435	0.445	0.073	0.076
	F_5	9.70	9.80	10.19	10.44	0.462	0.472	0.083	0.087
I_2	F_1	10.13	10.27	12.79	13.03	0.473	0.482	0.091	0.095
	F_2	10.40	10.58	12.55	12.91	0.486	0.494	0.093	0.097
	F_3	10.77	10.93	12.00	12.26	0.503	0.516	0.102	0.107
	F_4	10.57	10.67	12.28	12.54	0.495	0.505	0.097	0.102
	F_5	10.85	11.05	11.79	12.07	0.512	0.523	0.105	0.111
LSD 5%		0.18	0.69	0.21	0.81	0.008	0.035	0.006	0.006

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Since, I_1: Fresh water (EC=475ppm); I_2: Saline water (EC=1000ppm); I_3: Saline water (EC=2000ppm); F_1: Without foliar applications; F_2: Cobalt at rate of 2.5 mg L^{-1}; F_3: Cobalt at rate of 5.0 mg L^{-1}; F_4: Selenium at rate of 2.5 mg L^{-1}; F_5: Selenium at rate of 5.0 mg L^{-1}

Discussion

The study shows that increasing salinity in irrigation water negatively affects several parameters of Croton plants. This phenomenon can be attributed to salinity stress, which is characterized by the accumulation of salts (*e.g.*, sodium and chloride ions) in the soil and plant tissues. As salinity increases, plants find it increasingly difficult to absorb water and essential nutrients, leading to reduced growth and physiological changes. High salt concentrations disrupt the ionic balance in plant cells. Excess sodium ions can replace essential nutrients such as potassium, causing nutrient deficiencies and interfering with essential cellular processes. This leads to reduced plant height, fewer leaves, and smaller root systems, as observed in the study. Salinity also induces osmotic stress in plants. As soil salinity increases, water becomes less available to plants due to the lower water potential in saline soils. This results in reduced water uptake and can lead to wilting, reduced leaf area, and altered carbohydrate metabolism, which is consistent with the results of the study. Salinity can inhibit photosynthesis by damaging chloroplasts and disrupting ion transport. Chlorophyll a and b levels are reduced, leading to reduced photosynthetic activity, reduced carbohydrate production, and reduced overall plant vigor. When Croton plants are exposed to irrigation water with high salinity levels, they experience salt-induced stress. This stress leads to the production of reactive oxygen species (ROS) in plant tissues. ROS, such as superoxide radicals and hydrogen peroxide, can cause oxidative damage to cell membranes, proteins, and DNA, ultimately compromising plant health and growth.

Proline, an amino acid, plays a key role in plant stress responses, where its accumulation under environmental stressors such as salinity, serves as a protective mechanism, and is an indicator of the plant's stress response. Meanwhile, MDA is a marker for the assessment of lipid peroxidation in plant tissues, reflecting oxidative stress as a result of salinity stress. Peroxidase (POX) and catalase (CAT) are antioxidant enzymes found in plant cells that are involved in the detoxification of reactive oxygen species (ROS) under salinity stress conditions. Therefore, their levels increased in plants grown under stress. This explains the possession of Croton plants irrigated with water at an EC of 2000 ppm the maximum values of these biochemical parameters, indicating an increased stress response and enhanced antioxidant activity in the presence of higher salinity levels, regardless of the foliar treatment applied. These results are consistent with those of Okon (2019); Mushtaq *et al.* (20120); Arif *et al*. (2020).

Cobalt is known to play a role in mitigating the adverse effects of salinity on plants. It can increase plant tolerance to salt stress by improving nutrient uptake, particularly of essential micronutrients such as iron and manganese. Cobalt acts as a cofactor for enzymes involved in nitrogen metabolism and can facilitate nitrogen uptake, which is essential for plant growth. The study confirms that cobalt application (especially at 5.0 mg L^{-1}) has a positive effect on Croton plant performance and mitigates some of the negative effects of salinity. Cobalt is an essential micronutrient that plays an important role in plant physiology. Under saline conditions, the excessive accumulation of sodium ions can interfere with the uptake of essential nutrients such as iron (Fe) and manganese (Mn). This disruption can lead to nutrient deficiencies that adversely affect plant growth. Cobalt acts as a cofactor for enzymes involved in nitrogen metabolism, particularly nitrogenase. Nitrogenase is essential for nitrogen fixation, the process by which atmospheric nitrogen (N_2) is converted to ammonia (NH_3), which plants can use as a nitrogen source. This is important because salinity stress often interferes with nitrogen uptake. By improving nitrogen metabolism and facilitating nutrient uptake, cobalt helps maintain nutrient balance in Croton plants even in the presence of saline irrigation water. This improved nutrient availability supports various plant functions, including the synthesis of amino acids, proteins, and chlorophyll, which are essential for growth and photosynthesis. Cobalt's role in improving nutrient uptake and nitrogen metabolism helps to mitigate the adverse effects of salinity stress on Croton plants. As a result, cobalt- treated plants exhibit better growth parameters, including increased plant height, leaf number, and root development, even when exposed to high salinity levels. These findings are consistent with the results reported by Gad and El–Metwally, (2015); Brengi *et al.* (2022).

Selenium is known for its antioxidant properties. Salinity stress can lead to the production of reactive oxygen species (ROS) in plant tissues, causing oxidative damage. Selenium, as an antioxidant, can scavenge ROS and protectplant cells from oxidative stress. This antioxidant capacity increases the resilience of Croton plants, especially when exposed to saline irrigation. The study shows that selenium application, especially at 5.0 mg L^{-1}, is highly effective in improving Croton plant performance under salinity stress. Selenium acts as an antioxidant by efficiently scavenging ROS. It does this by participating in the formation of selenoproteins, which include antioxidant enzymes such as glutathione peroxidase. These enzymes play a crucial role in neutralizing ROS and preventing oxidative stress-related damage in plant cells. By scavenging ROS, selenium helps to protect vital cellular components, including chloroplasts and mitochondria, from oxidative damage. Chloroplasts are essential for photosynthesis, and their integrity is critical for plant growth and overall health. Mitochondria are responsible for cellular respiration, providing energy for various metabolic processes. Selenium's role in maintaining the functionality of these organelles is essential for plant survival in saline conditions. Selenium supplementation increases the salt tolerance of Croton plants by reducing oxidative stress and maintaining cellular function. This results in improved growth parameters, including increased leaf area, chlorophyll content, and overall plant vigor, even in the presence of saline irrigation water. These results are consistent with those documented by Abdalla *et al.* (2023); Elsherpiny, (2023).

CONCLUSION

It can be concluded that increasing salinity levels in irrigation water has a detrimental effect on Croton plants. This is manifested by reduced growth parameters, reduced nutrient content, altered carbohydrate metabolism, and reduced chlorophyll levels. Both cobalt and selenium applications have been shown to improve Croton plant performance, particularly when exposed to saline irrigation. Cobalt improves nutrient uptake and nitrogen metabolism, while selenium acts as a potent antioxidant, mitigating oxidative stress caused by salinity. Incorporating these recommendations into future research and agricultural practices can contribute to the development of sustainable and efficient strategies for ornamental horticulture and water resource management in regions facing water scarcity and salinity challenges.

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التأثير المحتمل للكوبلت والسيلينيوم علي نبات الكرتون النامي تحت ظروف ملحية

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الملخص

السعي لإيجاد بديل مستدام للمياه العذبة، مثل استخدام المياه العادمة في ري النباتات الزينة، أصبح ذا أهمية بارزة نظرًا لمشكلات ندرة المياه في مصر. وينبغي للباحثين في هذا المجال تعزيز جهودهم لإدخال نهج مبتكرة تسمح بالاستفادة الفعالة من المياه العادمة مع الحفاظ على سلامة النظام الزراعي. ولذا تم تنفيذ هذا البحث لتقييم تأثير الري باستخدام مياه ذات مستويات ملوحة مختلفة [475، 1000 و2000 جزء في المليون] على نبات الكرتون. بالإضافة إلى ذلك، تم فحص تأثير الرش الورقي بالكوبالت والسيلينيوم بمعدلات مختلفة (0.0، 2.5، 5.0 ملجم/لتر). قامت الدراسة بتقييم مدلولات عديدة لنبات الكرتون، مثل طول النبات، المساحة الورقية، محتوي النيتروجين والفوسفور والبوتاسيوم، كلوروفيل أ، وكلوروفيل ب، وصبغة الأنثوسيانين، ومضادات الأكسدة. لوحظ أنه عندما تم ري نباتات الكرتون بالمياه العذبة (475 جزء في المليون)، أظهرت أعلى القيم بالنسبة لجميع المدلولات المدروسة ما عدا مضادات الأكسدة التي اخذت اتجاه معاكس. أيضًا، مع زيادة ملوحة مياه الري إلى 1000 ثم 2000 جزء في المليون، تناقصت قيم جميع المدلولات تدريجيًا ما عدا قيم مضادات الأكسدة التي زادت تدريجيًا مع زيادة مستوى الملوحة. على الجانب الاخر، أسفرت معاملات الكوبالت والسيلينيوم عن تعزيز أداء نبات الكرتون، خاصة عندما تم ري النباتات بمياه ملحية (1000 و2000 جزء في المليون). من حيث الفعالية، كانت الترتيب من الأكثر فعالية إلى الأقل فعالية كالتالي: سيلينيوم (5.0 ملجم/لتر) > سيلينيوم (2.5 ملجم/لتر) > كوبالت (5.0 ملجم/لتر) > كوبالت (2.5 ملجم/لتر) > مجموعة الكنترول.