Effect of cultivars And spraying with Cycocel on the vegetative and chemical traits of oleander plants under salt stress

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Abstract

A Randomized Complete Blocks Design (RCBD) experiment was conducted in lathhouse of the Department of Plant Production Technologies, Al-Musaib Technical College, north of Babylon province, for autumn 2023 and spring 2024 seasons to study the effect of two cultivars of oleander (White oleander and Pink oleander) and three concentrations of (the growth regulator Cycocel 0-500-1000 mg L^{-1}, with four sprays, and three levels of irrigation water salinity (tap water, 6 DS. m^{-1} and 9 DS. m^{-1}) on the growth and active substances of the oleander plant. After the end of the experiment on May 1, 2024, the results were analyzed statistically. The results were as follows-:The results showed that the white cultivar was significantly excelled on A1 for most of the studied traits, except for (carbohydrates, chlorophyll, flavonoids, phenols, saponins), in which the pink cultivar A2 excelled.The growth regulator Cycocel (1000 mg L^{-1}) also showed a significant effect on all the traits studied .Salinity (9 DS.m^{-1}) showed an increase in the leaves' content of active substances (glycosides 1.19%, phenols 106.99 mg g^{-1}, flavonoids 71.67 mg, saponins 0.478%), compared to 0.72, 82.03 mg g-1, 49.42 mg g^{-1} and 0.288%, respectively.

Key words: oleander, Cycocel, Cultivars, saponins, flavonoids

Introduction

The oleander (Nerium oleander L.) is a plant belonging to the oleander family, Apocynaceae. It is considered an ornamental shrub native to the Mediterranean Sea. It is currently grown in all parts of the world, where it is grown as an ornamental plant in tropical and subtropical regions, Australia, South Africa, and the western and southern United States of America. It can reach a height of It reaches a height of 8-20 feet, and has an upright, many-branched shape with a beautiful shape and its branches are arched [1] Medicinal plants were and still are an important and successful means of treatment for scientists, doctors and specialists, as they play an important role in human life due to their abundance, multiplicity of types and wide use. Commercial demand for medicinal plants has increased in various parts of the world with the increase in scientific research aimed at them due to the side effects of chemical medicines [2]. The oleander plant is considered a medicinal plant, as its leaves and peel contain the cardiac glycosides (Oleandrin, Nirin, Adilnerin, and the Coumarine alkaloid), which are used in the treatment of heart diseases (strengthening its muscles and regulating its beats). It is also used in the treatment of some skin diseases (itching and leprosy) and relieves joint pain. Sciatica, and the leaves and roots contain a large number of toxic substances, including Folineriin, Nirianthin, and Nerine, which are found in all parts of the plant. This is why it was called

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"donkey poison," and this substance has become of great medical importance [13] . Study aims to study the effect of two cultivars of oleander (White oleander and Pink oleander) and three concentrations of the growth regulator with four sprays, and three levels of irrigation water salinity on the growth and active substances of the oleander plant.

Materials and methods

A factorial experiment was conducted in a randomized block design, [4] at Al-Musaib Technical College, north of Babylon province, for autumn and spring seasons 2023-2024 in lathhouse of the Department of Plant Production Technologies for the purpose of studying the effect of the white oleander and pink oleander cultivars and spraying with cyclosil on the effect of salt stress on the growth and content of materials. Effective for the warbler Nerium oleander L. Seedlings of the warbler, white oleander and pink oleander, were obtained from a private nursery in Babylon province. Seedlings were as homogeneous as possible at 9 months of age. Seedlings were transferred to lathhouse and seedlings were distributed among treatments. The physical and chemical properties of the anvil soil were taken randomly before starting the experiment. Table (1).

Table (1) Physical and chemical traits of the soil before the start of the experiment

	traits
7.6	pH
2.7	Electrical conductivity ($ds.m^{-1}$)
15.3	Available nitrogen ($mg\ kg^{-1}$ soil)
3.1	Available phosphorus ($mg\ kg^{-1}$ soil)
11	Available potassium ($mg\ kg^{-1}$ soil)
22	Sodium ppm
2.4	Organic matter ($g.kg^{-1}$ soil)
Soil components	
750 g	Sand (g. kg-1 soil)
50 g	silt (g. kg-1 soil)
200 g	Clay (g. kg-1 soil)
Silty clay sand	texture

Service operations of irrigation, hoeing and weeding were conducted whenever necessary.The content of the leaves of active substances was estimated at the Ministry of Science and Technology in Baghdad provainc, where the samples were dried at a temperature of 70 degrees Celsius, and the leaves were placed in perforated conditions for 48 hours in the oven.

1- Glycoside content in leaves:

The sample dried leaf powder (10 g) was soaked with 80% methanol at room temperature and the solvent was replaced every 48 hours (3 × 8 L). The total extract was concentrated under vacuum. To determine the glycosides, 10% extract, after replacing the solvent each time, and the total leaf extract were mixed with 10 ml of freshly prepared

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Baljet reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). After an hour of mixing, the mixture was diluted with 20 ml. ml of distilled water, and the absorbance was measured at 495 nm using a Shimadzu UV/VIS spectrophotometer, model 1600A (Kyoto, Japan), and after calibration with the standard curve, the standard curve was prepared by taking 10 ml of different concentrations (12.5-100 mg L^{-1} From Securidaside. Securidaside was isolated from the plant sample extract. The glycoside content of the three replicates was expressed as mg of Securidaside per gram dry weight of dried extracts [11].

2- Total Saponins Content:

Total saponins were estimated according to the method presented by (Chinelo et al., 2014) by a double gravity method using (50 ml) of 20% ethanol, then both extracts were poured together and the combined extract was reduced to about (40 ml) at 90 °C, and transferred to Add a separating funnel (40 ml) of diethyl ether and shake it vigorously. The extraction was repeated by sectioning repeatedly until the aqueous layer became clear in color. Saponins were extracted with (60 ml) of regular butanol. The collected extracts were washed with a 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporating dish. They were dried at a temperature of 60 °C in an oven and re-calibrated after cooling in a desiccator, then the process was repeated twice to obtain the average. The saponin content was determined by difference and calculated as a percentage of the original sample according to the equation:

Saponin ratio = (W2 - W1 / weight of sample) x 100/1

W1 = Weight of evaporating plate

W2 = weight of evaporating dish + sample

3- Total flavonoid content:-

The total flavonoid content in the crude extract was determined by the aluminum chloride colorimetric method. Briefly, 50 µL of crude extract (1 mg/mL ethanol) was prepared from 1 mL of methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% NaNO2 solution with 0.3 mL of 10% AlCl3 solution after 5 Minutes of incubation, then leave for 6 minutes. After that, 2 ml of 1 mol/L NaOH solution was added, the solution was stirred well and distilled water was added to the mixture until the final mixture reached 10 ml. The mixture was then left for 5 minutes, after which the optical absorbance was read by a spectrophotometer at a wavelength of 510 nm. Total flavonoid content was calculated from the standard curve, as mg rutin equivalent per gram dry weight [11].

4- Determination of total phenolic compounds. Total Phenolic Content

The leaf sample was dried in the shade at room temperature for (24 hours), then ground into a fine powder in an electric blender. (5 g) were taken from it, placed in a Soxhlet device, and extracted with (300 ml) ethanol at (50-55 degrees Celsius) for a period of time. After 3-4 hours, the extract was filtered through Whatman filter paper No. 1, and the extract was concentrated using a rotary evaporator under low pressure at 40 degrees Celsius. The weight of the extract after the concentration process was (2.6 g) and it was stored at 4 degrees Celsius in a storage vial until analysis was performed. The content of total phenols was estimated according to the method presented by [14] using gallic acid and Folin-Ciocalteu reagent, which stipulates taking (150 microlitres) of the alcoholic extract with (500 microlitres) of Folin's reagent. Add (1.5 ml) of (20% sodium carbonate), mix well, and bring the final volume to (10 ml). After two hours of reaction, the absorbance value is

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recorded at a wavelength of 765 nm. The concentration of total phenols was calculated according to the calibration curve for gallic acid and in units of (mg/g dry weight-1).

Results

Estimation of glycoside content of leaves (%)

The results of Table (2) indicate that there are no significant differences between the cultivars in the glycoside content of the leaves, while Cycosil (1000 mg L^{-1}) gave the highest rate of 1.32% compared to the treatment (spraying with distilled water), which gave the lowest rate of 0.58%. The salinity of irrigation water also had a significant effect. The treatment (9 $DS.m^{-1}$) recorded the highest rate of glycosides, which gave 1.19%, compared to the salinity level (tap water), which recorded the lowest rate, which amounted to 0.72%.

The bi- interactions between the study factors also had a significant effect on the glycoside content of the leaves. The treatment (white cultivar + Cycosil 1000 mg L^{-1}) recorded the highest rate of 1.34% compared to the treatment (white cultivar + tap water), which recorded the lowest rate of 0.57%. As for the bilateral interaction between cultivars and salinity levels, the treatment (white cultivar + 9 $DS.m^{-1}$) excelled and gave the highest rate of 1.20% compared to the treatment (white cultivar + tap water), which gave the lowest average of 0.71%. As for the interaction between cycosil and salt stress, where The treatment (Cycosil 1000 mg L^{-1} + 9 dSm^{-1}) recorded the highest rate of 1.48% compared to the treatment (spraying with distilled water + tap water), which gave the lowest rate of 0.36%.As for the triple interaction between the study factors, the treatment (white cultivar + Cycosil 1000 mg L^{-1} + 9 DS.m-1) excelled and gave the highest rate of 1.50% compared to the treatment (white cultivar + spraying with distilled water + tap water), which gave the lowest rate. For glycosides, it reached 0.34%.

Table (2): Effect of cultivar and spraying with Cycosil on the glycoside content of leaves as a result of salt stress (%)

Cultivars	CCC mg L^{-1}	Salt stress			Cultivars*CCC
		0 dsm^{-1}	6dsm^{-1}	9 dsm^{-1}	
White oleander	0	0.34	0.54	0.84	0.57
	500	0.66	1.01	1.25	0.98
	1000	1.13	1.38	1.50	1.34
Pink oleander	0	0.37	0.56	0.86	0.59
	500	0.71	0.96	1.26	0.97
	1000	1.12	1.35	1.46	1.31
L.S.D 0.05		0.05			0.03
Average salt stress		0.72	0.97	1.19	L.S.D 0.05
L.S.D 0.05		0.02			
Cultivars* salt stress					Average Cultivars

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White oleander	**0.71**	**0.98**	**1.20**	**0.96**
Pink oleander	**0.73**	**0.96**	**1.19**	**0.96**
L.S.D 0.05		**0.03**		**0.01**
CCC * salt stress				**Average CCC**
0	**0.36**	**0.55**	**0.84**	**0.58**
500	**0.68**	**0.99**	**1.25**	**0.97**
1000	**1.13**	**1.36**	**1.48**	**1.32**
L.S.D 0.05	**0.04**			**0.02**

Estimation of the leaves' content of flavonoids, mg g^{-1}

The results of Table (3) indicate that there are significant differences between the cultivars in the content of flavonoids in the leaves, as the pink cultivar was significantly excelled and gave the highest rate of 60.12 mg g^{-1} compared to the white cultivar, which recorded 59.92 mg g^{-1}, while Cycosil gave a concentration of (1000 mg. L^{-1}) The highest rate of flavonoids content was 76.87 mg g^{-1} compared to the treatment (spraying with distilled water), which gave the lowest rate, amounting to 43.71 mg g^{-1}. The salinity of the irrigation water also had a significant effect, as the treatment recorded (9 DS.m-1) The highest rate of flavonoids content was 71.67 mg g^{-1}, compared to the salinity level (tap water), which recorded the lowest rate of 49.42 mg g^{-1} .The bi- interactions between the study factors also had a significant effect on the content of flavonoids in the leaves. The treatment (pink cultivar + Cycosil 1000 mg L^{-1}) recorded the highest rate, reaching 77.74 mg L^{-1}, compared to the treatment (Pink cultivar + spraying with distilled water), which recorded the lowest rate. 43.06 mg L^{-1}. As for the bilateral interaction between the cultivars and salinity levels, the treatment (pink cultivar + 9 $DS.m^{-1}$) excelled and gave the highest average of 72.18 mg L^{-1} compared to the treatment (pink cultivar + tap water), which gave the lowest average of 49.26. mg L-1. As for the interaction between Cycocel and salt stress, the treatment (Cycocel 1000 mg L^{-1} + 9 $DS.m^{-1}$) recorded the highest rate of 87.12 mg L^{-1} compared to the treatment (spraying with distilled water + tap water) which gave the lowest rate of 87.12 mg L^{-1}. 35.85 mg L^{-1}.As for the triple interaction between the study factors, the treatment (pink cultivar + Cycosil 1000 mg L^{-1} + 9 $DS.m^{-1}$) excelled and gave the highest rate of 88.63 mg L^{-1} compared to the treatment (pink cultivar + spraying with distilled water + tap water), which gave The lowest rate was 34.77 mg L^{-1}.

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Table (3): Estimation of the leaves' content of flavonoids, mg g^{-1}

Cultivars	CCC mg L^{-1}	Salt stress			Cultivars*CCC
		0 dsm^{-1}	6dsm^{-1}	9 dsm^{-1}	
White oleander	0	36.93	41.10	55.07	44.37
	500	46.57	58.83	72.80	59.40
	1000	65.27	77.13	85.60	76.00
Pink oleander	0	34.77	40.03	54.37	43.06
	500	46.87	58.27	73.53	59.56
	1000	66.13	78.47	88.63	77.74
L.S.D 0.05		1.860			1.074
Average salt stress		49.42	58.97	71.67	L.S.D 0.05
L.S.D 0.05		0.759			
Cultivars* salt stress					Average Cultivars
White oleander		49.59	59.02	71.16	59.92
Pink oleander		49.26	58.92	72.18	60.12
L.S.D 0.05		1.074			0.620
CCC * salt stress					Average CCC
0		35.85	40.57	54.72	43.71
500		46.72	58.55	73.17	59.48
1000		65.70	77.80	87.12	76.87
L.S.D 0.05		1.315			0.759

Phenols content of leaves (mg g^{-1})

The results of Table (4) indicate that there are significant differences between the cultivars in the content of phenols in the leaves, as the pink cultivar was significantly excelled and gave the highest rate of 96.09 mg g-1 compared to the white cultivar, which recorded 92.50 mg g^{-1}, while Cycosil gave a concentration of (1000 mg g^{-1}) The highest rate was 114.87 mg g^{-1} compared to the treatment (spraying with distilled water), which gave the lowest rate of 76.07 mg g^{-1}. The salinity of the irrigation water also had a significant effect. The treatment (9 $DS.m^{-1}$) recorded the highest rate of phenols, which It gave 106.99 mg g^{-1} compared to the salinity level (tap water), which recorded the lowest rate of 82.03 mg g^{-1} .The bi- interactions between the study factors also had a significant effect on the content of phenols in the leaves. The treatment (pink cultivar + Cycosil 1000 mg L^{-1}) recorded the highest rate, reaching 116.39 mg L^{-1}, compared to the treatment (white cultivar + spraying with distilled water), which recorded the lowest rate. 74.37 mg g^{-1}. As for the bilateral interaction between the cultivars and salinity levels, the treatment (pink cultivar + 9 DS.m-1) excelled and gave the highest average of 108.48 mg g^{-1} compared to the treatment (white cultivar + tap water), which gave the

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lowest average of 80.24. As for the interaction between Cycocel and salt stress, the treatment (Cycocel 1000 mg L^{-1} + 9 $DS.m^{-1}$) recorded the highest rate of 129.47 mg g-1 compared to the treatment (spraying with distilled water + tap water) which gave the lowest rate of 69.92 mg g^{-1} . .As for the triple interaction between the study factors, the treatment (pink cultivar + Cycosil 1000 mg L-1 + 9 $DS.m^{-1}$) excelled and gave the highest rate of 131.30 mg g^{-1} compared to the treatment (white cultivar + spraying with distilled water + tap water) which It gave the lowest rate of phenols, amounting to 68.10 mg g^{-1}.

Table (4) Leaves' content of phenols (mg g^{-1})

Cultivars	CCC mg L^{-1}	Salt stress			Cultivars*CCC
		0 dsm^{-1}	6dsm^{-1}	9 dsm^{-1}	
White oleander	0	68.10	71.83	83.17	74.37
	500	74.60	88.97	105.77	98.78
	1000	98.03	114.37	127.63	113.34
Pink oleander	0	71.73	75.77	85.83	77.78
	500	80.63	93.40	108.23	94.09
	1000	99.07	118.80	131.30	116.39
L.S.D 0.05		2.655			1.533
Average salt stress		82.03	93.86	106.99	L.S.D 0.05
L.S.D 0.05		1.084			
Cultivars* salt stress					Average Cultivars
White oleander		80.24	91.72	105.52	92.50
Pink oleander		83.81	95.99	108.46	96.09
L.S.D 0.05		1.533			
CCC * salt stress					Average CCC
0		69.92	73.80	84.50	76.07
500		77.62	91.18	107.00	91.93
1000		98.55	116.58	129.47	114.87
L.S.D 0.05		1.878			1.084

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Leaves content of saponins %

The results of Table (5) indicate that there are significant differences between the cultivars in the content of saponins in the leaves, where the pink cultivar was significantly excelled and gave the highest rate, reaching 0.387%, compared to the white cultivar, which recorded 0.379%, while Cycosil (1000 mg L^{-1}) gave the highest rate. The saponins content of the leaves reached 0.534% compared to the treatment (spraying with distilled water), which gave the lowest rate of 0.235%. The salinity of the irrigation water also had a significant effect. The treatment (9 $DS.m^{-1}$) recorded the highest rate of saponins content, which gave 0.478% compared to the level of salinity. (Tap water), which recorded the lowest rate of 0.288%. The bi- interactions between the study factors also had a significant effect on the saponins content of the leaves. The treatment (pink cultivar + Cycosil 1000 mg L^{-1}) recorded the highest rate of 0.540% compared to the treatment (white cultivar + spraying with distilled water), which recorded the lowest rate of 0.232%. As for the bilateral interaction between cultivars and salinity levels, the treatment (pink cultivar + 9 DS.m-1) excelled and gave the highest rate of 0.482% compared to the treatment (white cultivar + tap water), which gave the lowest average of 0.286%. As for the interaction between cycosil and salt stress, where The treatment (Cycosil 1000 mg L-1 + 9 DS.m-1) recorded the highest rate of 0.620% compared to the treatment (spraying with distilled water + tap water) which gave the lowest rate of 0.166%.As for the triple interaction between the study factors, the treatment (pink cultivar + Cycosil 1000 mg L-1 + 9 $DS.m^{-1}$) excelled and gave the highest rate of 0.620% compared to the treatment (white cultivar + spraying with distilled water + tap water), which gave the lowest rate. For saponins, it reached 0.166%.

Table (5) % saponins content of leaves

Cultivars	**CCC mg L^{-1}**	**Salt stress**			**Cultivars*CCC**
		0 dsm^{-1}	**6dsm^{-1}**	**9 dsm^{-1}**	
White oleander	**0**	**0.163**	**0.206**	**0.326**	**0.232**
	500	**0.266**	**0.386**	**0.476**	**0.376**
	1000	**0.430**	**0.536**	**0.620**	**0.528**
Pink oleander	**0**	**0.170**	**0.200**	**0.346**	**0.238**
	500	**0.256**	**0.410**	**0.480**	**0.382**
	1000	**0.443**	**0.556**	**0.620**	**0.540**
L.S.D 0.05					**0.016**
Average salt stress		**0.288**	**0.382**	**0.478**	**L.S.D 0.05**
L.S.D 0.05		**0.011**			
Cultivars* salt stress					**Average Cultivars**

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White oleander	**0.286**	**0.376**	**0.474**	**0.379**
Pink oleander	**0.290**	**0388**	**0.482**	**0.387**
L.S.D 0.05		**0.016**		**0.009**
CCC * salt stress				**Average CCC**
0	**0.166**	**0.203**	**0.336**	**0.235**
500	**0.261**	**0.398**	**0478**	**0.379**
1000	**0.436**	**0.546**	**0.620**	**0.534**
L.S.D 0.05	**0.020**			**0.011**

Discussion

The results of tables (15-16-18) showed significant differences between the cultivars, as the pink cultivar was significantly excelled in most traits if it gave the highest average content of active substances, or this may be due to differences in the genetic makeup between the cultivars (Al-Sahhaf and Al-Marsoumi, 2003.(

It is also noted from Tables (18,17,16,15) that the growth regulator Cycosil is available at a concentration of 1000 mg L^{-1}. It had a significant effect on the content of active substances in plant tissues and gave the highest results (glycosides, flavonoids, phenols, saponins), compared to the comparison treatment (spraying with distilled water), which gave the lowest results. The reason may be due to the increased content of active compounds in the leaves when treated with inhibitors. Growth, such as Cycosil, which leads to an increase in the numbers and sizes of chloroplasts, which has a positive effect on increasing the production of active substances [6]. This is due to the fact that spraying with Cycosil causes an obstruction in plant growth, and therefore the sugary and starchy substances that are generated inside the plant are not consumed in the process. Growth is therefore stored within the plant [3] On the other hand, there was an increase in the accumulation of active substances. These results are consistent with the findings of [7]. Spraying tomato plants with Cycosil significantly affected the content of intrinsic solids in the fruits.We notice from Tables (18, 17, 16, 15) that the salinity of irrigation water has significantly affected the content of active substances in the leaves, as high levels of salinity (9 dSm-1) showed a significant accumulation of active substances in the plant leaves, and this is consistent with what was found. During his study [9] he noticed an increase in the total content of phenolic compounds in red pepper using moderate levels of salinity. The increase in the yield of active substances due to the influence of NaCl can be explained by the fact that plants growing under the influence of stress may resort to supporting their growth by increasing the concentration of secondary metabolic compounds. This is one of the means that the plant may resort to for the purpose of reducing the impact of the salt stress imposed on the plant. [10]. [12] also showed that sodium ions, Na, work to increase the activity of the enzymes responsible for the production of alkaloids in the plant, and this was confirmed by [8] and that high levels of active compounds are a necessary factor for the plant to remain on the non-enzymatic defense system against the danger of oxidative processes. For the plasma membrane, enzymes and nucleic acids, they work to protect the plant from damage that may occur as a result of oxidative stress due to salinity, as salinity increases the production of (ROS) that are

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harmful to the plant and reduces their damage as the plant produces alkaloids that play an effective role in protecting the plant from the danger of stresses applied to the plant. on her.

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