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# Influence of Cycocel foliar spray on the growth of oleander plants under salt stress

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## ABSTRACT

The study examined the effects of foliar growth regulator (Cycocel) at three concentrations (0, 500, and 1000 mg L<sup>-1</sup>) and three levels of irrigation water salinity (tap water, 6 dS m<sup>-1</sup>, and 9 dS m<sup>-1</sup>) on the vegetative growth of two oleander cultivars (white and pink). The findings indicated that the white cultivar outperformed the pink in most vegetative traits. In addition, applying the highest concentration of Cycocel (1000 mg L<sup>-1</sup>) significantly boosted vegetative parameters. Conversely, irrigation with water at 9 dS m<sup>-1</sup> led to a reduction in all measured vegetative traits, such as plant height, leaf count, leaf area, stem diameter and branch number. Notably, the combination of the pink cultivar with 1000 mg L<sup>-1</sup> Cycocel improved leaf number and stem diameter, while the treatment pairing 1000 mg L<sup>-1</sup> Cycocel with tap water produced the highest branch numbers. Overall, the treatment integrating the pink cultivar, 1000 mg L<sup>-1</sup> Cycocel, and tap water achieved the best results with 123.90 leaves per plant, a stem diameter of 15.35 mm, and 7.40 branches per plant compared to the control. In summary, although the white variety excelled in most vegetative traits, the pink cultivar demonstrated enhanced characteristics, and foliar application of Cycocel was effective in boosting all measured parameters.

**KEYWORDS:** Cycocel application, Oleander cultivars, Growth, Salt stress

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## INTRODUCTION

*Nerium oleander* L., commonly known as oleander, is a member of the Apocynaceae family. Originally from the Mediterranean region, this ornamental shrub is now cultivated worldwide, especially in tropical and subtropical areas such as Australia, South Africa, and the western and southern United States. Typically, oleander grows between 8-20 feet, featuring an upright, multi-branched structure with gracefully arched limbs (Al-Ghitani, 1985). Medicinal plants have long been central to treatment practices among researchers, healthcare professionals, and practitioners. Their diverse types and broad utility have spurred a growing global demand, particularly as concerns rise over the adverse effects of synthetic drugs (Patrício *et al.*, 2022). Oleander itself is valued in traditional medicine because its leaves and bark are rich in cardiac glycosides, including oleandrin, nirin, and adilnerin as well as coumarin alkaloids, which help strengthen heart muscles and regulate heart rhythms. Moreover, the plant is employed in managing skin conditions like itching and leprosy and in relieving joint and sciatic pain. However, its leaves and roots also contain potent toxins such as folinerin, nirianthin, and nerine compounds that once earned it the nickname “donkey poison,”

yet continue to be recognized for their medical significance (Sabatino *et al.*, 2019). Different cultivars of oleander vary in growth habits, responses to environmental factors, and levels of secondary metabolites due to their genetic makeup. These genetic differences not only shape overall development and stress tolerance but also enhance essential processes like photosynthesis, carbohydrate production, and protein synthesis, ultimately boosting vegetative growth and yield (Brito *et al.*, 2020). Salt stress represents a major environmental challenge, reducing the global area of cultivable land, affecting roughly 10% of all soil types and 50% of irrigated lands (Guo *et al.*, 2018; Alkarawi *et al.*, 2022). High salinity alters the soil's osmotic potential, hindering water uptake by roots and often leading to plant death (Pasha, 2022). Furthermore, excessive salt interferes with the availability of key nutrients, such as nitrogen, phosphorus, and potassium, which in turn diminishes plant growth and productivity. These effects are particularly pronounced in dry and semi-arid regions, where salt stress slows photosynthesis and degrades both yield and quality (El Sabagh *et al.*, 2020). The objective of this study is to evaluate the potential of Cycocel foliar sprays to mitigate the adverse effects of salt stress to enhance vegetative growth in oleander plants.

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**Table 1: Soil analysis laying out its physical and chemical characteristics**

Properties	EC	pH	N	P	K	Na	Organic matter	Sand	Silt	Clay	Soil texture
	2.7	7.6	15.3	3.1	11	22	2.4	750g	50g	200g	Sandy Clay Loam

## MATERIALS AND METHODS

A factorial experiment was conducted under a randomized block design (Al-Rawi, 2000) at Al-Mussaib Technical College, northern Babylon province, in the autumn and spring seasons 2023-2024. The research was carried out in the Lath house of the Department of Plant Production Technologies. The experiment tested the influence of salt stress with three levels of irrigation water salinity (tap water, 6 dS m<sup>-1</sup>, and 9 dS m<sup>-1</sup>) on growth and composition in white and pink oleander cultivars under treatment with Cycocel foliar sprays (0, 500, and 1000 mg L<sup>-1</sup>). Seedlings of both types (*Nerium oleander* L.) were obtained from a private nursery in Babylon province and screened for uniformity at 9 months of age. They were later planted into wooden canopies and allocated into the experimental treatments. Random samples were collected before conducting the experiment to determine the physical and chemical properties of the soil (Table 1). Routine care, which consisted of watering, hoeing, and weeding, was done on a need basis. Various vegetative traits were recorded during the course of the experiment.

## RESULTS

### Plant Height (cm)

The results indicated significant variations in the plant height among oleander cultivars. In particular, the white cultivar produced a mean height of 64.17 cm, which was higher than that of the pink cultivar, 60.34 cm (Table 2).

Yet, when Cycocel was applied at 1000 mg L<sup>-1</sup>, the average plant height was lower at 57.03 cm, while that of the control treatment was taller at 67.33 cm. Irrigation water salinity also had a significant effect on plant height. Plants that were irrigated with 9 dS m<sup>-1</sup> saline solution had an average of 60.17 cm, compared to 64.89 cm for plants irrigated with tap water. The white cultivar with distilled water gave the tallest plants with an average of 68.47 cm. The shortest average height was recorded for the pink cultivar treated with 1000 mg L<sup>-1</sup> Cycocel at 54.83 cm. Tap water irrigated white oleanders achieved an average height of 65.68 cm, while pink oleanders treated with 9 dS m<sup>-1</sup> salinity had a lower average height of 57.64 cm. Cycocel treated plants with 1000 mg L<sup>-1</sup> irrigated with 9 dS m<sup>-1</sup> saline water had the lowest mean height of 54.05 cm. Conversely, those sprayed with tap water had the highest mean height of 70.70 cm. In the interaction between cultivar, Cycocel concentration, and irrigation salinity in a three-way interaction, the white cultivar that was sprayed with distilled water and irrigated with tap water recorded the highest average value of 70.07 cm. Conversely, the pink cultivar that was treated with 1000 mg L<sup>-1</sup> Cycocel and subjected to 9 dS m<sup>-1</sup> salinity had the lowest average value at 57.77 cm. These results highlight the intricacy of the interaction between gene influence, growth regulator treatment,

**Table 2: Effect of Cycocel spray on the height of oleander plants under the salt stress (cm)**

Cultivars	CCC mg L <sup>-1</sup>	Salt stress			Cultivars*CCC
		0 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	9 dS m <sup>-1</sup>	
White oleander	0	70.07	67.00	68.33	68.47
	500	64.43	65.43	64.43	64.77
	1000	62.53	59.79	55.33	59.28
Pink oleander	0	71.33	65.87	61.40	66.20
	500	63.33	57.88	58.77	59.99
	1000	57.63	54.10	52.77	54.83
LSD 0.05					2.988
Average salt stress		64.89	61.71	60.17	
LSD 0.05			2.113		
Cultivar*salt stress					Average cultivars
White oleander		65.68	64.13	62.70	64.17
Pink oleander		64.10	59.28	57.64	60.34
Lsd 0.05			2.988		1.725
CCC*salt stress					Average CCC
0		70.70	66.43	64.87	67.33
500		63.88	61.65	61.60	62.38
1000		60.08	57.03	54.05	57.03
LSD 0.05					2.113

and environmental stress agents in controlling oleander plant growth.

### Number of Leaves Plant<sup>-1</sup>

The data presented (Table 3) showed considerable leaf differences in the oleander cultivars under treatment. The white oleander had a greater mean leaf number (107.66 leaves per plant) than the pink cultivar, which had a mean of 103.63 leaves per plant. Cycocel application at 1000 mg L<sup>-1</sup> resulted in an average of 119.37 leaves per plant that was superior to the control group with an average of 97.86 leaves per plant.

Tap water irrigation gave an average of 108.68 leaves per plant, while saline water at 9 dS m<sup>-1</sup> had a decreased average leaf number to 104.21 leaves per plant. The white cultivar and tap water irrigation had the highest mean leaf number of 112.36 leaves per plant. On the other hand, the pink cultivar under 6 dS m<sup>-1</sup> saline water irrigation had the lowest mean of 102.90 leaves per plant. Distilled water sprayed with 9 dS m<sup>-1</sup> saline water irrigated plants had the lowest number of leaves, with an average of 93.67 leaves per plant. In contrast, plants treated with 1000 mg L<sup>-1</sup> Cycocel and irrigated with tap water had the highest mean of 121.97 leaves per plant. The pink cultivar treated with 1000 mg L<sup>-1</sup> Cycocel and watered with tap water had the highest number of leaves at 123.90 leaves per plant. In contrast, the white cultivar sprayed with distilled water and receiving 9 dS m<sup>-1</sup> saline water recorded the lowest at 92.80 leaves per plant. These results highlighted the intricate interaction between genetic traits, application of growth regulators, and environmental conditions in shaping the foliar development of oleander plants.

**Table 3: Effect of Cycocel spray on the number of leaves plant<sup>-1</sup> under salt stress**

Cultivars	CCC mg L <sup>-1</sup>	Salt stress			Cultivars*CCC
		0 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	9 dS m <sup>-1</sup>	
White oleander	0	102.73	97.20	92.80	97.58
	500	114.30	103.53	102.33	102.33
	1000	120.0	114.80	121.17	118.67
Pink oleander	0	102.90	96.97	94.53	98.13
	500	88.20	96.60	93.27	92.69
	1000	123.90	115.13	121.17	120.90
LSD 0.05					5.479
Average salt stress		108.68	104.04	104.21	
LSD 0.05			3.874		
Cultivars*salt stress		Average cultivars			
White oleander		112.36	105.18	105.43	107.66
Pink oleander		105.00	102.90	102.99	103.63
LSD 0.05			5.479		3.163
CCC*salt stress		Average CCC			
0		102.82	97.08	93.67	97.86
500		101.25	100.07	97.80	99.71
1000		121.97	114.97	121.17	119.37
LSD 0.05			6.711		3.874

### Leaf Area (cm<sup>2</sup>)

The results showed a significant impact on leaf area based on different treatments. The white cultivar achieved an average leaf area of 14.49 cm<sup>2</sup>, which was notably larger than the 13.69 cm<sup>2</sup> recorded for the pink cultivar.

Additionally, applying Cycocel at 500 mg L<sup>-1</sup> resulted in the highest leaf area, averaging 15.51cm<sup>2</sup> while the control treatment without any growth regulator yielded the lowest average at 13.22 cm<sup>2</sup> (Table 4). Irrigation water salinity also played a key role; plants irrigated with tap water reached an average of leaf area of 18.12 cm<sup>2</sup>, considerably higher than the 11.97 cm<sup>2</sup> observed at a salinity level of 9 dS m<sup>-1</sup>. The combination of the pink cultivar with 500 mg L<sup>-1</sup> Cycocel and irrigated with tap water reached an average leaf area of 22.66 cm<sup>2</sup>, compared to 11.76 cm<sup>2</sup> when the same concentration was paired with irrigation at 6 dS m<sup>-1</sup>. The interaction among cultivar, Cycocel concentration, and irrigation salinity was also significant. The pink cultivar treated with 500 mg L<sup>-1</sup> Cycocel and irrigated with water at 9 dSm<sup>-1</sup> achieved the highest leaf area of 25.90 cm<sup>2</sup>, whereas the white cultivar sprayed with distilled and irrigated with water at 9 dS m<sup>-1</sup> recorded the smallest leaf area at 9.08 cm<sup>2</sup>.

### Stem Diameter (mm)

The results indicated large differences in stem diameter among the treatments (Table 5). The pink cultivar had a greater average stem diameter of 13.91 mm compared to 12.62 mm for the white cultivar. Plants treated with 1000 mg L<sup>-1</sup> Cycocel also had thicker stems, averaging 13.76 mm, whereas, those treated with 500 mg L<sup>-1</sup> had only 12.97 mm. Irrigation with saline water 6 dS m<sup>-1</sup> increased stem diameter to 13.84 mm more than irrigation using 9 dS m<sup>-1</sup>, with an average value of 12.93 mm.

**Table 4: Effect of Cycocel spray on leaf area of oleander plants under salt stress**

Cultivars	CCC mg L <sup>-1</sup>	Salt stress			Cultivars*CCC
		0 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	9 dS m <sup>-1</sup>	
White oleander	0	17.87	12.48	9.08	13.14
	500	19.41	11.66	12.80	14.62
	1000	20.29	12.39	14.45	15.71
Pink oleander	0	14.50	12.53	12.88	13.30
	500	25.90	11.86	11.40	16.39
	1000	10.76	12.17	11.22	11.38
LSD 0.05			3.854		2.225
Average salt stress		18.12	12.18	11.97	
LSD 0.05			1.573		
Cultivars salt stress		Average cultivars			
White oleander		19.19	12.18	12.11	14.49
Pink oleander		17.05	12.19	11.84	13.69
LSD 0.05			2.225		12.85
CCC*salt stress		Average CCC			
0		16.18	12.51	10.98	13.22
500		22.66	11.76	12.10	15.51
1000		15.52	12.28	12.84	13.55
LSD 0.05			2.725		1.573

**Table 5: Effect of Cycocel spray on stem diameter of oleander plants under salt stress**

Cultivars	CCC mg L <sup>-1</sup>	Salt stress			Cultivars*CCC
		0 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	9 dS m <sup>-1</sup>	
White oleander	0	12.05	13.39	12.97	12.80
	500	11.91	12.50	12.14	12.18
	1000	12.40	13.40	12.85	12.88
Pink oleander	0	13.25	13.44	13.32	13.34
	500	13.25	15.26	12.67	13.76
	1000	15.35	15.03	13.51	14.63
LSD 0.05			1.554		0.897
Average salt stress		13.04	13.84	12.93	
LSD 0.05			1.573		
Cultivars*salt stress		Average cultivars			
White oleander		12.12	13.10	12.67	12.62
Pink oleander		13.95	14.57	13.20	13.91
LSD 0.05			0.897		0.518
CCC*salt stress		Average CCC			
0		12.67	13.41	13.15	13.07
500		12.58	13.88	12.45	12.97
1000		13.88	14.22	13.18	13.76
LSD 0.05			1.099		0.634

The integration of the pink cultivar with 1000 mg L<sup>-1</sup> Cycocel gave the longest stem at 14.63 mm, while the combination of the white cultivar with 500 mg L<sup>-1</sup> gave the shortest, at 12.18 mm. Likewise, the pink cultivar watered with 6 dSm<sup>-1</sup> water had a greater average diameter (14.57 mm) than the white cultivar watered with tap water (12.12 mm). In regard to the interaction of Cycocel concentration and salinity, a combination of 1000 mg L<sup>-1</sup> Cycocel with tap water gave a mean stem diameter of 14.22 mm, as opposed to 12.45 mm for the treatment with 500 mg L<sup>-1</sup> Cycocel using 9 dS m<sup>-1</sup> saline water. In addition, the three-way interaction between cultivar, Cycocel concentration, and salinity of irrigation was significant. The treatment that involved the pink cultivar, 1000 mg L<sup>-1</sup> Cycocel, and tap water

yielded the thickest stems at 15.35 mm, while the white cultivar with 500 mg L<sup>-1</sup> Cycocel and irrigated with tap water had the thinnest stems, measuring 11.91 mm on average.

### Number of Branches Plant<sup>-1</sup>

The results indicated significant differences between cultivars regarding the number of branches per plant (Table 6). The white cultivar had a mean of 4.57 branches per plant, significantly greater than the pink cultivar with 4.30 branches per plant. Additionally, spraying with Cycocel at 1000 mg L<sup>-1</sup> gave the highest number of branches at 4.98 per plant, while the lowest mean of 4.15 branches per plant was obtained using the control treatment (spraying with distilled water). Tap water irrigated plants had a mean of 5.37 branches per plant,

**Table 6: Effect of Cycocel spray on the number of branches plant<sup>-1</sup> under salt stress**

Cultivars	CCC mg L <sup>-1</sup>	Salt stress			Cultivars*CCC
		0 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	9 dS m <sup>-1</sup>	
White oleander	0	5.00	4.30	3.83	4.38
	500	4.97	3.87	4.17	4.33
	1000	6.37	4.63	3.97	4.99
Pink oleander	0	5.50	2.97	3.30	3.92
	500	3.00	4.93	4.07	4.00
	1000	7.40	4.83	2.76	4.97
LSD 0.05			1.486		0.858
Average salt stress		5.37	4.26	3.67	
LSD 0.05			0.607		
Cultivar *salt stress		Average cultivars			
White oleander		5.44	4.27	3.99	4.57
Pink oleander		5.30	4.24	3.34	4.30
LSD 0.05			0.858		0.495
CCC*salt stress		Average ccc			
0		5.25	3.63	3.57	4.15
500		3.98	4.40	4.12	4.17
1000		6.88	4.73	3.32	4.98
LSD 0.05			1.050		0.607

which is significantly greater than the 3.67 branches per plant developed at a salinity of 9 dS m<sup>-1</sup>. All these factors interacted and affected the branch numbers. For instance, the union of white cultivar with 1000 mg L<sup>-1</sup> Cycocel resulted in a mean of 4.99 branches per plant, while the pink cultivar that was treated with distilled water resulted in a mean of 3.92 branches per plant. In terms of cultivar and salinity, white oleanders irrigated with tap water reached an average of 5.44 branches per plant, whereas pink oleanders under 9 dS m<sup>-1</sup>, salinity regime averaged 3.34 branches plant<sup>-1</sup>.

Additionally, the interaction between Cycocel concentration and salinity, the highest branch count 6.88 branches plant<sup>-1</sup> was observed in plants receiving 1000 mg L<sup>-1</sup> Cycocel with tap water, while the lowest count 3.32 per plant occurred under the same Cycocel level with 9 dS m<sup>-1</sup> saline water. The three-way interaction among cultivar, Cycocel treatment, and irrigation salinity was significant. The pink cultivar treated with 1000 mg L<sup>-1</sup> Cycocel and tap water irrigation recorded the highest average of 7.40 branches plant<sup>-1</sup>, while the pink cultivar under the same treatment with 9 dS m<sup>-1</sup> irrigation water had the lowest average of 2.67 branches plant<sup>-1</sup>.

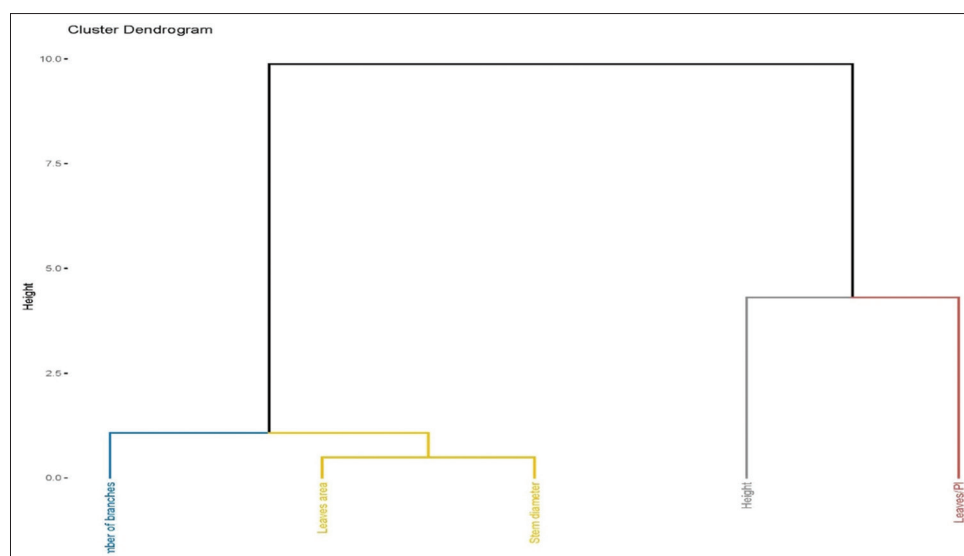
### Dendrogram, Heat Map, Principal Component Analysis

#### Dendrogram

The first small cluster with the number of branches, leaf area and stem diameter showed a similar trend under the experimental conditions. The traits height and leaf size exhibited a close association.

#### Heat map

The heatmap showed height and leaf area with a mix of red and yellow being more sensitive to treatments. Stem diameter and number of branches followed similar patterns. While the traits



**Figure 1: Dendrogram showing effect of Cycocel spray on vegetative traits of oleander plants under salt stress**

with greenish blue color resulted in positive growth responses with balanced Cycocel concentration and salinity levels.

Principal component analysis

The Dim1 46.8% represented the primary variation in data. The y-axis with Dim2 showed 26% variation. One group of traits with leaves plant<sup>-1</sup>, number of branches and stem diameter respond similarly to treatments. Another group (height and leaf area)

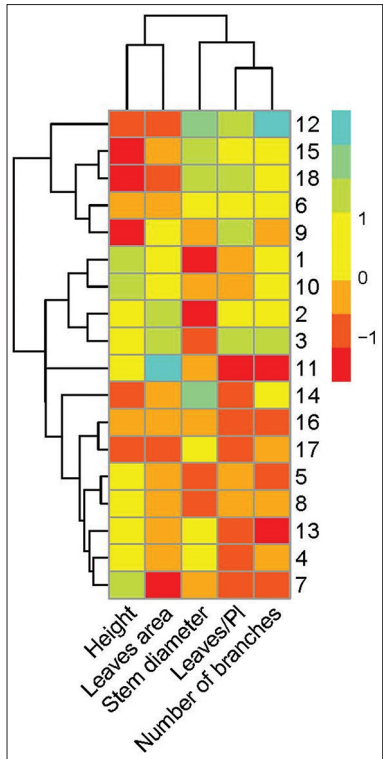


Figure 2: Heatmap showing effect of Cycocel spray on vegetative traits of oleander plants under salt stress

behaved differently, meaning they may be influenced more by certain salinity levels or Cycocel concentrations.

DISCUSSION

It is evident (Tables 2-6) that the white cultivar performed better than the pink in all vegetative traits, such as plant height, number of leaves, leaf area and number of branches. Nevertheless, the pink cultivar exhibited a wider stem diameter (Table 5). These variations are probably due to genetic reasons and the way each cultivars genetic profile responds to environmental conditions, thus affecting the carbon-based compound uptake (Cox, 2018; Al-Maamory & Al-Shammari, 2022).

Plants treated with 100 mg L<sup>-1</sup> Cycocel were significantly shorter than control plants, indicating that raising the concentration of this growth regulator induces stunting of stem elongation. The stunted stature is likely to be caused by Cycocel inhibiting the synthesis of gibberellin hormone in plants responsible for cell division and elongation. The findings are in accordance with earlier results (Vanlalhrui *et al.*, 2021). Aside from height Cycocel at 1000 mg L<sup>-1</sup> enhanced other vegetative traits by enhancing the number of leaves, stem thickness, and more branch formation. This could be due to the fact that growth retardants have a tendency to suppress apical dominance, thus stimulating lateral branching and more leaves, perhaps through the suppression of endogenous auxin (IAA) levels, which tend to promote apical dominance (Ali & Baidya, 2021). Moreover, the significant increase in leaf area (Table 4) might be attributed to Cycocel stimulation of cell division and elongation, as well as its role in boosting cellulose fiber production in the cell wall, resulting in wider leaves due to altered sugar composition (Bhatla & Lal, 2023). Irrigation water salinity also has a pronounced impact on vegetative growth (Tables 2-6). It is evident from the data that increasing salinity caused a reduction in vegetative characters for both white and pink cultivars. This decrease is probably caused by high salt levels hindering water

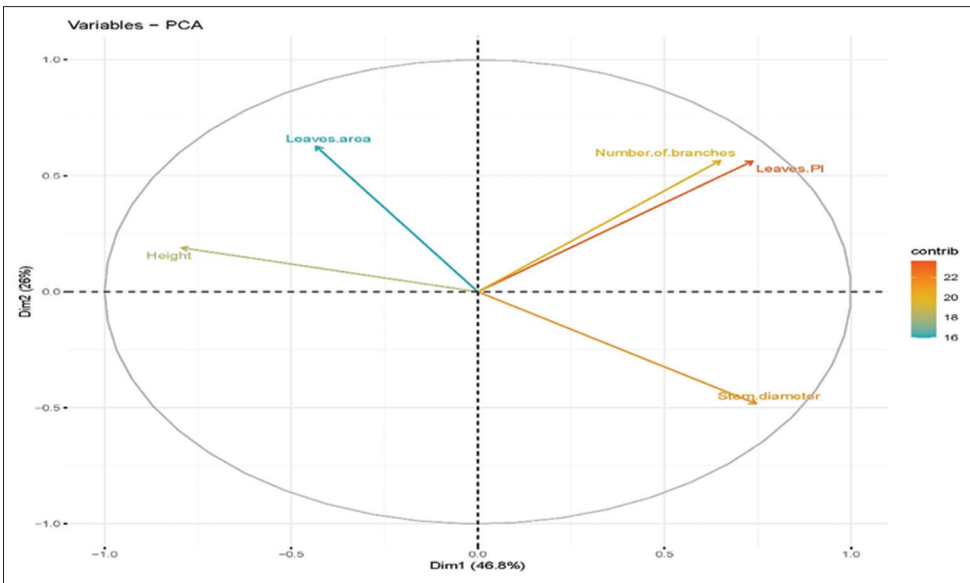


Figure 3: PCA showing effect of Cycocel spray on vegetative traits of oleander plants under salt stress



absorption, cell division, and cell growth. Such salinity stress causes an imbalance in the nutrient and hormonal content in plant cells, influencing cell development and cell size in vascular tissues (Ali *et al.*, 2021; Radhi & Alkarawia, 2021; dos Santos *et al.*, 2022). The application of NaCl in the experiment might also hinder some physiological activities, retarding cell division and restricting cell enlargement at root tips. Thus, increased salinity leads to shorter leaves and diminished photosynthetic performance, reducing overall leaf area (Al-Jubouri & Alwan, 2014). It is also evident (Figures 1-3) that branches, leaf area and stem diameter are strongly linked to each other, whereas height and total leaf count are influenced by different treatment factors. Plants respond differently on specific treatment combinations of Cycocel and salinity. PCA analysis confirmed that leaves per plant, branches and stem diameter are the most important traits in determining the response of plants. The analysis might help in choosing the right Cycocel concentration and irrigation salinity levels to optimize oleander growth while minimizing stress effects.

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Author Query???

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