Physiological and Phytochemical Responses of *Aloe vera* L. to Organic Nutrients and Biofertilizers

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Abstract

This Experiment was conducted in Thi-Qar Governorate - Nasiriyah City - in the plant canopy of the Horticulture and Landscape Engineering Department for the agricultural season (2024-2025) to study the effect of soil application of organic nutrients and biofertilizers on some growth characteristics and active components of *Aloe vera* L. A randomized complete block design (RCBD) was employed for the experiment, which included three concentrations of organic nutrients and biofertilizers: (0), (2), and (4) ml/L. The addition of organic nutrients led to a significant increase in all vegetative and chemical characteristics when treated with the concentration of (4 ml L^{-1}) (plant height 34.82 cm, leaf width 26.26 mm, leaf length 31.52 cm, leaf thickness 11.267 mm), and also significantly affected all chemical characteristics (Aloin content 158.59 µg g^{-1}, Aloe-emodin 106.67 µg g^{-1}, salicylic acid 13.167 µg g^{-1}, cinnamic acid 13.544 µg g^{-1}). The results also showed that treatment with biofertilizers at a concentration of (6 ml L^{-1}) caused significant differences in all vegetative and chemical growth characteristics (plant height 32.04 cm, leaf width 22.17 mm, leaf length 29.39 cm, leaf thickness 9.567 mm, Aloin 147.49 µg g^{-1}, Aloe-emodin 100.00 µg g^{-1}, salicylic acid 11.133 µg g^{-1}, and cinnamic acid 12.678 µg g^{-1}).

Keywords: Biofertilizers, Organic Nutrients, Cinnamic acid, Salicylic acid

Introduction

Aloe vera L. belongs to the Asphodelaceae family and is considered a plant of arid and semi-arid regions, growing in tropical and wilderness areas. Its original habitat is North Africa, and it later spread to eastern and western India, Europe, and most other tropical regions [1]. *Aloe vera* is one of the ancient medicinal plants used for medical purposes; historical manuscripts indicate that it was used more than 6,000 years ago to treat many diseases, as recorded on clay tablets in the Mesopotamian civilization. It is also considered one of the important medicinal plants used in many pharmaceutical industries to treat diseases, and it is included in creams, juices, and many cosmetics.

The leaves of the *Aloe vera* plant are the part used medically, from which the gel is extracted; the gel contains anthraquinone compounds, which include glycosides with medical benefits [12], including Aloin and Aloe-emodin. The gel contains 96-99.5% water, and the remainder is a solid substance containing 75 or more different compounds, including anthraquinones, sugars, vitamins, phenolics, saponins, minerals, sterols, and salicylic acid.

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Algae extract is considered one of the most important organic sources used in agriculture, and is considered a complement to chemical fertilizers rather than a substitute for them [11]. Organic farming has received widespread attention from farmers and consumers due to its important health and environmental properties. However, due to the scarcity of nutrients such as phosphorus, nitrogen, and other elements that are limiting factors in organic farming, slow growth calls for the use of methods to accelerate seedling growth and bring them to the appropriate size. One such method is the addition of biostimulants to improve and accelerate plant growth. Algae extracts are derived from natural organic materials that have the ability to decompose, making them an organic source for improving plant nutrition and growth and leaving no negative effects on the plant or soil [13].

One of the most important biotechnology techniques is the addition of bio-based substances known as biofertilizers. Biofertilizers are preparations that contain microorganisms capable of supplying plants with essential nutrients from natural sources, thereby reducing reliance on various chemical fertilizers. These fertilizers have the ability to continuously release nutrients, making them sufficient to meet the needs of the treated plants. The term "biofertilizers" is broad and is used for products containing organisms and algae, either alone or in combination, which, when applied, help fix atmospheric nitrogen or dissolve soil nutrients. Biofertilizers include many microorganisms that vary depending on the purpose for which the fertilizer is used, and are produced by isolating, purifying, and characterizing different microorganisms and multiplying them in suitable cultures until they are used as inoculants. They can be mixed with seeds before planting, used to soak seedling roots, or added directly in the field near the roots of growing plants. Biofertilizers are used to reduce the addition of chemical fertilizers by at least 25%, and they help mitigate environmental pollution problems and improve microbial pollination of seeds, benefiting the preservation of good soil [9]. Biofertilizers play an important role in compensating for deficiencies in nutrient productivity and are effectively used to support and sustain agriculture [11].

Materials and Methods

Research Location

The research was conducted in the shade house belonging to the Department of Horticulture and Landscape Engineering at the College of Agriculture, University of Thi-Qar, during the growing season (2024-2025). The study was conducted on *Aloe vera* L. plants brought on 18/12/2024 from Al-Narjis nursery in Thi-Qar Governorate. The plants were grown in plastic pots with a diameter of 15 cm, then repotted into larger pots with a diameter of 25 cm after sterilization on 22/12/2024, where the growing medium was prepared consisting of peat moss at a ratio of (1:2). The study included two factors: the first factor was organic nutrients at three levels (0, 2, 4 ml L^{-1}), and the second factor was biofertilizer at three levels (0, 2, 4 ml L^{-1}).

Studied Traits

All measurements were taken on March 1, 2025, using three plants per experimental unit with three replicates, and the average was then calculated.

Plant Height (Cm .plant-1

Plant height was measured from the point of contact with the soil to the tip of the leaf for all plants in each experimental unit using a measuring ruler, and the average was then calculated.

Number of Leaves (Leaf.plant-1**)**

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The number of leaves on all plants was calculated, then the number of leaves per plant within each treatment was determined.

Leaf Length (Cm leaf

The lengths of three leaves from the second row of leaves were measured using a measuring tape for each plant in the experimental unit [9].

Leaf Width (mm)

Measured from the lower third of the leaf using vernier calipers.

Leaf Thickness (mm)

Measured from the lower third of the leaf of each plant in each experimental unit using vernier calipers.

Leaf Fresh Weight (g)

Three leaves from the second row were cut at the point of attachment to the plant, weighed, and the weight of each leaf was extracted and multiplied by the number of leaves per plant.

High-Performance Liquid Chromatography (HPLC) Analysis of Active Compounds

Samples were analyzed using a German-made SYKAM high-performance liquid chromatography (HPLC) system with a pump, a UV spectrophotometer (model 3240 S) as detector, and an automatic sampling model (S 5200). The column used for analysis was ODS-C18 (25 cm × 4.6 mm). The mobile phase consisted of water (78%) and acetonitrile; the detection wavelength was 220 nm and the flow rate was 1.0 ml min^{-1}. The injection volume was 50 µL, and the column temperature was maintained at ambient conditions.

After analyzing the standard compounds and samples, the retention time was used to identify the active compounds, and the sample area was used to calculate the concentration of the active compounds according to the following equation, expressed in µg ml^{-1} [10]: Sample concentration = Dilution factor × (sample peak area × standard concentration) / standard peak area.

Results and Discussion

Plant Height (cm)

The results in Table (1) show significant differences in plant height as a result of adding organic nutrients and biofertilizers. The organic nutrients treatment at a concentration of 4 ml L^{-1} produced the highest plant height (34.82 cm), a significant difference from the control treatment, which recorded the lowest value (24.38 cm). The biofertilizer treatment at a concentration of 6 ml L^{-1} produced the highest plant height (32.04 cm) compared to the control treatment, which recorded the lowest height (27.52 cm). The two-way interaction between the 4 ml L^{-1} organic nutrients concentration and the 6 ml L^{-1} biofertilizer concentration recorded the highest average (37.67 cm), while the control treatment recorded the lowest value (21.33 cm).

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Table 1. Effect of soil addition of organic nutrients and biofertilizers on plant height (cm).

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
24.38	26.97	24.83	21.33	0
29.81	31.50	29.50	28.43	2
34.82	37.67	34.00	32.80	4
0.958			1.659	LSD (0.05)
	32.04	29.44	27.52	Biofertilizers average
			0.958	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Leaf Width (mm)

The results in Table (2) show a significant difference in leaf width resulting from the addition of organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the largest leaf width (26.26 mm), a significant difference from the control treatment (14.38 mm). The biofertilizer treatment produced the largest leaf width (22.17 mm) compared to the control treatment (18.73 mm). The two-way interaction between the 4 ml L^{-1} organic nutrients concentration and the 6 ml L^{-1} biofertilizer concentration recorded the highest average (27.50 mm), while the control treatment recorded the lowest value (12.67 mm).

Table 2. Effect of soil addition of organic nutrients and biofertilizers on leaf width (mm).

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
14.38	16.30	14.17	12.67	0
20.48	22.70	20.17	18.57	2
26.26	27.50	26.30	24.97	4
0.574			0.995	LSD (0.05)
	22.17	20.21	18.73	Biofertilizers average
			0.574	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Leaf Length (cm)

The results in Table (3) indicate significant differences in leaf length resulting from the addition of organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the highest leaf length (31.52 cm $leaf^{-1}$), a significant difference from the control treatment (22.30 cm $leaf^{-1}$). The biofertilizer

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treatment at 6 ml L^{-1} produced the highest value (29.39 cm $leaf^{-1}$) compared to the control treatment (25.13 cm $leaf^{-1}$). The two-way interaction recorded the highest average (34.67 cm $leaf^{-1}$) at the 4 ml L^{-1} organic nutrients × 6 ml L^{-1} biofertilizer combination, while the control treatment recorded the lowest value (19.50 cm $leaf^{-1}$).

Table 3. Effect of soil addition of organic nutrients and biofertilizers on leaf length (cm).

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
22.30	24.57	22.83	19.50	0
27.20	28.93	28.13	26.63	2
31.52	34.67	30.63	29.27	4
0.764			1.323	LSD (0.05)
	29.39	27.20	25.13	Biofertilizers average
			0.764	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Leaf Thickness (mm)

The results in Table (4) show a significant difference in leaf thickness as a result of adding organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the highest leaf thickness (11.267 mm $leaf^{-1}$), a significant difference from the control treatment (6.433 mm). The biofertilizer treatment at 6 ml L^{-1} produced the highest leaf thickness (9.567 mm $leaf^{-1}$) compared to the control treatment (7.756 mm $leaf^{-1}$). The two-way interaction recorded the highest average (12.433 mm $leaf^{-1}$), while the control treatment recorded the lowest value (5.500 mm $leaf^{-1}$).

Table 4. Effect of soil addition of organic nutrients and biofertilizers on leaf thickness (mm).

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
6.433	7.233	6.567	5.500	0
8.467	9.033	8.567	7.800	2
11.267	12.433	11.400	9.967	4
0.3722			0.6447	LSD (0.05)
	9.567	8.844	7.756	Biofertilizers average
			0.3722	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

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Estimation of Chemically Active Compounds

Aloin Concentration in Leaves (µg g^-1^)

The results in Table (5) show significant differences in Aloin concentration in leaves as a result of treatment with organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the highest Aloin concentration (158.59 µg g^{-1}), a significant difference from the control treatment (123.93 µg g^{-1}). The biofertilizer treatment at 6 ml L^{-1} produced the highest concentration (147.49 µg g^{-1}) compared to the treatment recording the lowest value (135.16 µg g^{-1}). The two-way interaction recorded the highest average (170.00 µg g^{-1}), while the control treatment recorded the lowest value (121.30 µg g^{-1}).

Table 5. Effect of soil addition of organic nutrients and biofertilizers on Aloin concentration (µg g⁻ ¹) in Aloe vera leaves.

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
123.93	126.47	124.03	121.30	0
140.07	146.00	140.07	134.13	2
158.59	170.00	155.73	150.03	4
1.554			2.691	LSD (0.05)
	147.49	139.94	135.16	Biofertilizers average
			1.554	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Aloe-emodin Concentration in Leaves (µg g^-1^)

The results in Table (6) show significant differences in Aloe-emodin concentration as a result of treatment with organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the highest concentration (106.67 µg g^{-1}), a significant difference from the control treatment (78.00 µg g^{-1}). The biofertilizer treatment at 6 ml L^{-1} recorded the highest concentration (100.00 µg g^{-1}) compared to the control treatment (82.33 µg g^{-1}). The two-way interaction recorded the highest average (114.33 µg g^{-1}), while the control treatment recorded the lowest value (62.33 µg g^{-1}).

Table 6. Effect of soil addition of organic nutrients and biofertilizers on Aloe-emodin concentration (µg g⁻ ¹) in Aloe vera leaves.

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
78.00	92.67	79.00	62.33	0
86.44	93.00	80.00	86.33	2

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106.67	114.33	107.33	98.33	4
2.818			4.882	LSD (0.05)
	100.00	88.78	82.33	Biofertilizers average
			2.818	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Salicylic Acid in Leaves (µg g^-1^)

The results in Table (7) show significant differences in salicylic acid concentration as a result of adding organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the highest concentration (13.167 µg g^{-1}), a significant difference from the control treatment (7.422 µg g^{-1}). The biofertilizer treatment at 6 ml L^{-1} produced the highest concentration (11.133 µg g^{-1}) compared to the treatment recording the lowest value (9.133 µg g^{-1}). The two-way interaction recorded the highest value (14.133 µg g^{-1}), while the control treatment recorded the lowest value (6.667 µg g^{-1}).

Table 7. Effect of soil addition of organic nutrients and biofertilizers on salicylic acid concentration (µg g^{-1}) in Aloe vera leaves.

Organic Nutrientsaverage	**Biofertilizers 4**	**Biofertilizers 2**	**Biofertilizers 0**	**Organic Nutrients(ml L^{-1})**
7.422	8.133	7.467	6.667	0
9.811	11.133	9.833	8.467	2
13.167	14.133	13.100	12.267	4
0.3026			0.5241	LSD (0.05)
	11.133	10.133	9.133	Biofertilizers average
			0.3026	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Cinnamic Acid in Leaves (µg g^-1^)

The results in Table (8) show significant differences in cinnamic acid concentration as a result of adding organic nutrients and biofertilizers. The organic nutrients treatment at 4 ml L^{-1} produced the highest concentration (13.544 µg g^{-1}), a significant difference from the control treatment (9.356 µg g^{-1}). The biofertilizer treatment at 6 ml L^{-1} produced the highest concentration (12.678 µg g^{-1}) compared to the control treatment (10.378 µg g^{-1}). The two-way interaction recorded the highest average (15.067 µg g^{-1}), while the control treatment recorded the lowest value (8.400 µg g^{-1}).

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Table 8. Effect of soil addition of organic nutrients and biofertilizers on cinnamic acid concentration (µg g^{-1}) in Aloe vera leaves.

Organic Nutrientsaverage	Biofertilizers 4	Biofertilizers 2	Biofertilizers 0	Organic Nutrients(ml L^{-1})
9.356	10.367	9.300	8.400	0
11.844	12.600	11.833	11.100	2
13.544	15.067	13.933	11.633	4
0.1701			0.2947	LSD (0.05)
	12.678	11.689	10.378	Biofertilizers average
			0.1701	LSD (0.05)

Means followed with the same letters are not significantly different according to Duncan's multiple range test at 5% significance level.

Discussion

From the above, it is noted from Tables 1 to 8 that there is significant superiority in vegetative and chemical traits, especially when feeding the plant with organic nutrients at a concentration of 4 ml L^{-1}. This superiority may be attributed to the organic nutrients containing auxins, which play an important role in increasing cell division and elongation, leading to increased vegetative growth, increased leaf branching, increased plant height, increased leaf area, and increased dry weight [7]. The results obtained were similar to those achieved by Cheena and Sreenivas [4] when spraying seedlings with organic extract, which produced a significant increase in most vegetative indicators, and were similar to the response of bitter orange seedlings to organic nutrients reported by [5], where a significant increase occurred in most vegetative characteristics.

This may be attributed to the fact that these extracts, in addition to auxins and cytokinins, contain salicylic acid and humic acid, which reduce the stress to which the plant is exposed, improving the ability of the roots to absorb nutrients, grow, stimulate vegetative growth, and resist drought. The increase in growth may also be attributed to gibberellins in the organic nutrient extracts, which help in cell division in the meristematic cells of the apical meristem and increase their length; in addition, they play an important role in the activity of the intercalary meristem, increasing cell division and elongation at the internodes, leading to an increase in their length [2].

The effect of biofertilizer addition on vegetative growth traits and chemical content may be explained by the fact that the additive gives the seedlings direct and rapid access to nutrients through the leaf surface area, raising the osmotic activity of the cells and increasing their effectiveness, thereby increasing the absorption of nutrients and water present in the soil, which has a positive role in the formation of proteins and the regulation of hormones controlling cell division and elongation, thus increasing vegetative growth. These increases may also be attributed to the biofertilizer containing essential nutrients such as nitrogen, phosphorus, and potassium, which have a broad impact on the vital activity of the plant and their role in cell division and elongation, stimulating carbon metabolism, which is positively

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reflected in vegetative growth [3]. This significant superiority may also be due to the role of the fertilizer used in improving the nutritional status of the plant as a result of increased photosynthetic efficiency and carbohydrate accumulation in the leaves, which is positively reflected in stimulating bud growth, in addition to its role in secreting plant hormones that promote cell division, rapid growth, and photosynthetic efficiency through vitamins and activated enzymes [8].

Conclusions

The response of *Aloe vera* L. plants to fertilization with organic nutrients at a concentration of 4 ml L^{-1} was evident in all vegetative growth characteristics and the production of medicinally active substances. Fertilizing the soil with 6 ml L^{-1} of biofertilizer had a positive effect on all vegetative growth traits and most of the qualitative characteristics of *Aloe vera* L. The interaction between organic nutrients and biofertilizers had a significant effect on increasing vegetative growth traits and producing medicinally active compounds such as Aloin, Aloe-emodin, cinnamic acid, and salicylic acid.

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