Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/08nd6t74

Enhancing the Accumulation of Certain Active Compounds in *Aloe Vera* Gel Under the Effect of Vermicompost and Foliar Spray with Spirulina Algae Extract

Asmahan Khalid Allawi *[1], Ziena Mohammed Abdul-Qader* [2]
*[1] Department of Horticulture and Landscape Engineering, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

ABSTRACT

The experiment was conducted at the Agricultural Research Station A, College of Agricultural Engineering Sciences, University of Baghdad – Al-Jadriya, on aloe vera plants during the spring season of 2023, starting from April 19, 2023, to October 19, 2023. The objective was to investigate the individual and interactive effects of soil application of vermicompost and foliar spraying with *Spirulina* algae extract on the content of certain elements, carbohydrates, protein, and some bioactive compounds within the *Aloe* plant. The experiment was implemented according to a Randomized Complete Block Design (RCBD) with a Split-plot arrangement and three replicates. The results indicated the superiority of the two-way interaction treatment involving the addition of 15 tons ha^{-1} of vermicompost to the soil in conjunction with foliar spraying of *Spirulina* extract at a concentration of 8 g L^{-1} in the content of the dried leaves for each of the following: nitrogen percentage (0.463 %), phosphorus (0.133 %), potassium (2.79 %), calcium (215.7 ppm), total carbohydrates (5.65 %), protein (2.89 %), and both aloin compound (271 mg plant^{-1}) and aloinmodin compound (172.57 mg plant^{-1}), as well as phenylalanin and cinnamic acids (39.73 mg 100 g^{-1} and 54.47 parts per million, respectively), total phenols (136.90 mg 100 g^{-1} of dried gel), and total flavonoids (106.5 mg 100 g^{-1} of dried gel).

Key words: aloe plant, biofertilizer , organic fertilizer, secondary compounds.

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Received: 27/4/2025, Accepted: 9/7/2025, Published: 31/7/2026

INTRODUCTION

Aloe (*Aloe vera* L.) is belongs to the family of Asphodelaceae. It is a plant that reproduces by offshoots or seeds and is one of the plants that are typical of arid and semi-arid regions. The African continent and its mountain sides of north and coastal west is considered as the natural habitat of African incense tree (Al-Naimi,2010; Hussain et al.,2020). *Aloe vera*, which is also rich in nearly twenty secondary metabolite, anthraquinone glycosides (mainly aloin and aloe emodin) and many amino acids, is extensively used in the food, pharmaceutical, cosmetic, and health industries. Aloe gel is applied as a soothing balm for burns, cuts, insect bites and other skin conditions, and can also be used to treat genital herpes and psoriasis. It is also used to preserve freshness of skin and is applied for an antifungal agent, anti-dandruff agent, and an anti-inflammatory. In addition, the gel is also used to enhance the immune system for disease resistance including AIDS, leukemia, and diabetes (Kim et al,2021). Vermicompost, it is among the most environmentally acceptable organic fertilizers so far used in agriculture because it provides a good source of macro elements viz N (2.0-3.0%), P (1.55-2.25%) and K (1.55-2.25%). It is also rich in micronutrients, vitamins, enzymes and plant growth hormones that encourage growth and hasten establishment of plants. It is also high in soluble nitrates, phosphates, and calcium (Salman and Abdul-Qader, 2022). Vermicompost improves microbial activity and biomass and the phenolics as well as the availability of macro and micronutrients, growth regulators and humic substances in

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soil, all have been shown to have noticeable positive effects on vegetative growth attributes of plants (Roy,et al.,2010;Al Dayel Aand El Sherif,2022) Plant extracts and marine algae, particularly Spirulina, are considered among the modern agricultural practices used to enhance the yield of horticultural and field crops. They also serve as a means of protecting and treating plants from diseases due to their low or non-existent poisoning for both animals and humans (López-Padrón et al.,2020). *Spirulina platensis*, which belongs to the family Phormidiaceae, is known as a green -blue microalga that can improve the soil's fertility, increase the vegetation growth in plants and contribute to increasing yield. Interest in use has increased in recent years, as it has been included in the production of fertilizers and biostimulants used on plants. This is due to its high protein content of 60-70% and the fact it is a complete protein albeit very rich in also essential amino acids, essential fatty acids and antioxidant pigments like carotene, beta -carotene and phycocyanin. It consisting of calcium, chromium, iron, phosphorus, sodium, zinc, potassium, magnesium, manganese and vitamins A, E, E, B1, B2, B3, B3, B6, B6, B12, B 3, (Biotic acids) (El Sherif et al.,2020;Godlewska et al.,2019) In the context of sustainable environment and clean friendly agriculture, this work was carried out to investigate the individual and combined impacts of soil application by vermicompost and foliar spraying with different levels of Spirulina algae extract on enhancing the concentration of specified primary and secondary metabolites in *Aloe vera*, besides its content of some elements.

MATERIALS AND METHODS

Studied traits: The experiment was conducted at the Agricultural Research Station A, College of Agricultural Engineering Sciences, Baghdad University – Al-Jadriyah, on *Aloe vera* L. plants during the spring season of 2023, starting from April 19, 2023, and continuing until October 19, 2023. The objective was to study the effect of vermicompost growth and gel yield in *Aloe vera* L. plants. Nine random soil samples were collected from different locations within the field at a depth of 0–30 cm. The samples were thoroughly mixed to achieve homogeneity, and then laboratory analyses were carried out. Table 1 presents the results of the laboratory analysis, which was conducted at the Central Laboratory of the College of Agricultural Engineering Sciences, Baghdad University, in order to determine the physical and chemical properties of the soil.

Table 1. Physical and chemical properties of the field soil

Measured parameter	Value	Unit
Electrical conductivity EC $_{1:1}$	2.40	dS m^{-1}
pH $_{1:1}$	7.40	----
Available nitrogen (N)	20	mg kg^{-1} soil
Available phosphorus (P)	6	mg kg^{-1} soil
Available potassium (K)	190	mg kg^{-1} soil
Soil organic matter	7.3	g kg^{-1}
Soil texture class	Loam	------
– Sand	360	g kg^{-1} soil
– Clay	169	g kg^{-1} soil
– Silt	471	g kg^{-1} soil

The field was then plowed in a perpendicularly and divided into three raised beds, each with a width of 0.75 meters and a length of 10 meters. A separation distance of 2 meters was maintained between beds within the same replicate, while a 1-meter isolation distance was kept between sub-subplot treatments. The planting distance between individual plants was set at 40 cm. additionally, a spacing of 2 meters was maintained between replicates to ensure proper isolation. The field was equipped with a drip irrigation system using T-tape lines. *Aloe vera* plants were obtained from Research Station B, from the field specifically allocated to aloe cultivation and affiliated with the Medicinal and Aromatic Plants Research Unit. The plants used were approximately one year old.

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Experimental Design: The experiment was carried out according to a Randomized Complete Block Design (RCBD) within a split-plot arrangement, with three replicates for each of the vegetative growth traits and gel yield of the plant under study.

The experiment included two factors:

1. The first factor was the application of vermicompost to the soil, denoted by the symbol **V.** This factor was randomly distributed among the main plots at three levels, based on the findings of Nematian *et al.* (2012), as follows:

V1 = No soil application of vermicompost (control treatment)

V2 = Soil application of vermicompost at a rate of 7.5 tons ha^{-1}

V3 = Soil application of vermicompost at a rate of 15 tons ha^{-1}

Table 2 presents the composition of the vermicompost:

Table 2. Composition of vermicompost

Measured parameter and unit	Value
pH	6.8
EC (dS/m)	16.51
CEC (Cmol/kg)	200.8
Organic matter (g kg^{-1})	600.3
Available nitrogen (mg kg^{-1})	20000
Available phosphorus (mg kg^{-1})	5000
Available potassium (mg kg^{-1})	25000
Calcium (mmol L^{-1})	35
Magnesium (mmol L^{-1})	25
Sodium (mmol L^{-1})	30
Potassium (mmol L^{-1})	10

2. The second factor was the different concentrations of natural Spirulina algae extract, which was applied to the sub-plots and denoted by the symbol S. It included five concentration levels based on the findings of Al-Dayel and El Sherf (2020), as follows:

S1 = Spraying with distilled water only (control treatment)

S2 = Spraying with Spirulina extract at a concentration of 1 g L^{-1}

S3 = Spraying with Spirulina extract at a concentration of 2 g L^{-1}

S4 = Spraying with Spirulina extract at a concentration of 4 g L^{-1}

S5 = Spraying with Spirulina extract at a concentration of 8 g L^{-1}

Table 3 shows the composition of the Spirulina algae extract as indicated on the label of the commercial product.

Table 3. Composition of Spirulina algae extract

Component	Percentage (%)
Protein	76
Fat	1.73
Calcium	1.20
Phosphorus	1.41
Moisture	3.7
Ash	3

Aloe vera plants were sprayed with *Spirulina* algae extract to the point of full foliar wetting on three occasions. The interval between each spraying was 20 days, as stated by Al-Dayel and El Sherf (2022). Spraying with the various concentrations of *Spirulina* extract was performed at sunset, after mixing the extract with a wetting agent solution (Al-Zahi), since *Aloe vera* is a CAM plant that opens its stomata at night, unlike most other plants. Spraying began one month after the saplings were transplanted into the field where the experiment was conducted. The treatments were distributed randomly in the field, resulting in 45 experimental units (3 × 5 × 3). Each unit contained six plants, leading to a

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total of 270 plants in the field. The measured data were collected and analyzed statistically according to the aforementioned experimental design using GenStat v.12.1 (2017). Mean comparisons were performed using the Least Significant Difference (LSD) test at a 5% probability level (Al-Rawi and Abdel Aziz,2000). Before planting, the field soil was moistened using the drip irrigation system, and irrigation was applied directly after transplanting. The pre-prepared plants were planted on both sides of each bed, with six plants per treatment, and a distance of 40 cm between plants. All agricultural maintenance operations—including weeding, hoeing, and fertilization—were uniformly applied across all treatments. A balanced NPK chemical fertilizer (20:20:20) was applied to the soil at a rate of 2.5 g per plant, In addition, an agricultural shade net (saran) was installed over all treatments in the open field to protect the plants from sunlight during the growing season.

Studied Traits: The study included the following:

Vegetative growth traits:
These included the average increase in:
leaves number per plant (leaves plant^{-1})
Leaf width (mm)
Leaf thickness (mm)
Average fresh weight per plant (g plant^{-1})
Average dry weight per plant (g plant^{-1})
Total chlorophyll concetration in leaves (mg 100 g^{-1} of F.w), the method described by Salman and Abdul-Qader (2022)
Average gel yield from fresh leaves per plant (g plant^{-1})
Average gel yield from dried leaves per plant (g plant^{-1})
Average fresh gel yield per hectare (kg ha^{-1})
Average number of offshoots per plant (offshoots plant^{-1})

Element estimation: This involved determining the following in the leaves:
Nitrogen content (%)
Phosphorus content (%)
Potassium content (%)
Calcium (ppm)
The above elements were analyzed using the methods described by Salman and Abdul-Qader (2022).

Estimation of some primary metabolites:
Percentage of carbohydrates in the leaves (%): were estimated using the method of Joslyn (1970).

Percentage of protein in the leaves (%): Protein content was calculated after determining nitrogen percentage, then multiplying the result by 6.25, as recommended by A.O.A.C. (1980).

Estimation of some bioactive compounds in dried *Aloe vera* Gel: Determination of Total Phenolic Content in Dried *Aloe vera* Gel: the method described by Laonini and Quahrani (2017) **Determination of total flavonoids in dried *Aloe vera* leaf gel:**
The total flavonoid content of the crude extract was determined by the colorimetric method of aluminum chloride, according to the method described by Habibatni et al. (2017).

Estimation of some active compounds in *Aloe vera* gel using a high-performance liquid chromatography (HPLC) device:
Standard active compounds were prepared: 0.01% of the high-purity standard compounds phenylalanin acid, aloin, aloe-emodin, and cinnamic acid were taken and dissolved in 100 ml of high-purity methanol, each separately, resulting in a standard concentration of 100 µg ml-1. Using the dilution law (C1V1=C2V2), the concentrations of the standard compounds mentioned above were prepared and injected into the HPLC device (Chiang et al ,2012), as shown in Figures 3, 4, 5, and 6, respectively.

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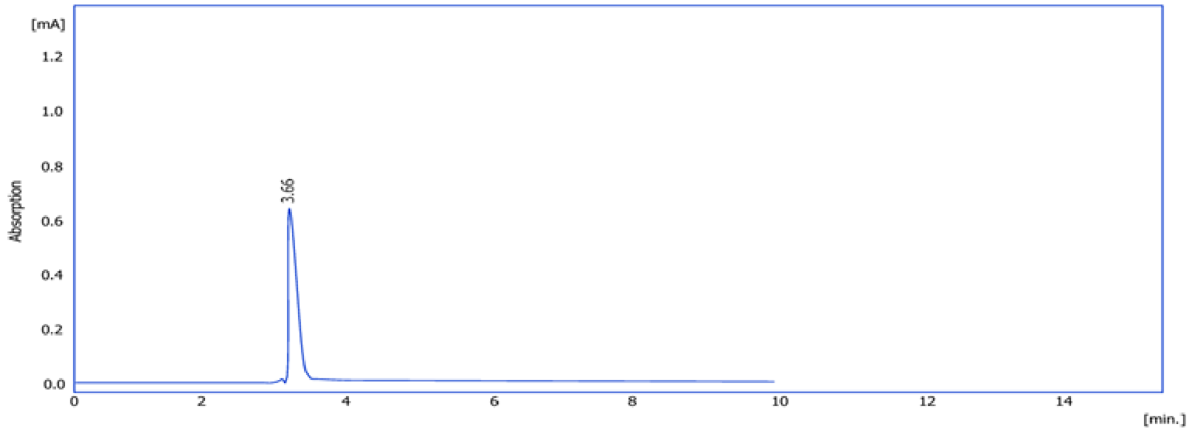
25/03/2024 01:40 م	Chromatogram F:\ phenyl alanine 10 ppm .PRM	Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:
Sample ID : phenyl alanine 10 ppm
Sample : phenyl alanine 10 ppm
Inj. Volume [mL] : 0.1
Amount 0
ISTD Amount 0
Dilution 1

Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change



Result chromatography Table (Uncal - F:\ phenyl alanine 10 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	3.66	2015.80	681.32	100.00	100.00	0.25	
	Total	2015.80	681.32	100.00	100.00		

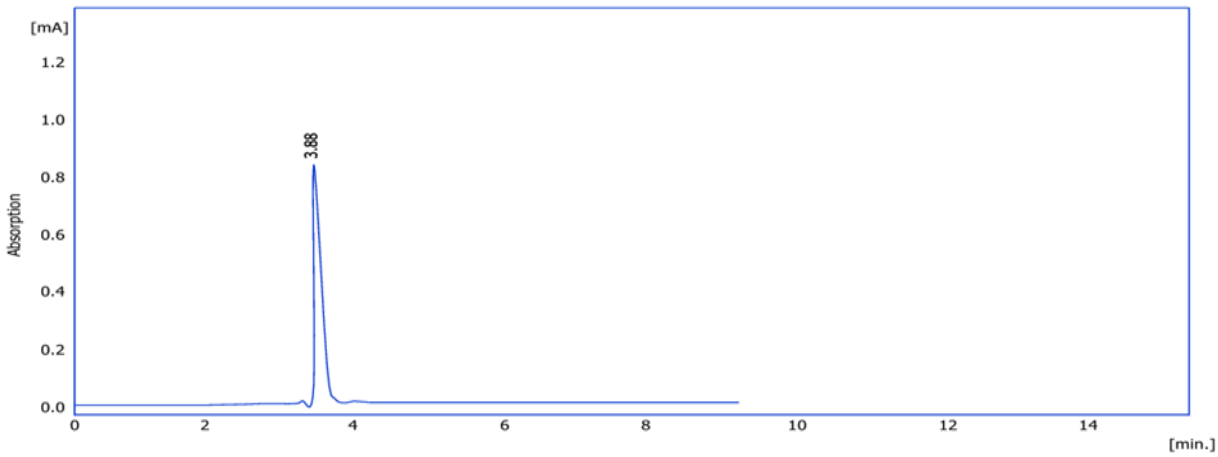
Figure 1. Standard compound of the amino acid phenylalanine

20/03/2024 01:40 م	Chromatogram F:\ Aloin 10 ppm .PRM	Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:
Sample ID : Aloin 10 ppm
Sample : Aloin 10 ppm
Inj. Volume [mL] : 0.1
Amount 0
ISTD Amount 0
Dilution 1

Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change



Result chromatography Table (Uncal - F:\ Aloin 10 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.88	3214.80	820.66	100.00	100.00	0.25	
	Total	3214.80	820.66	100.00	100.00		

Figure 2. Standard compound of Aloin

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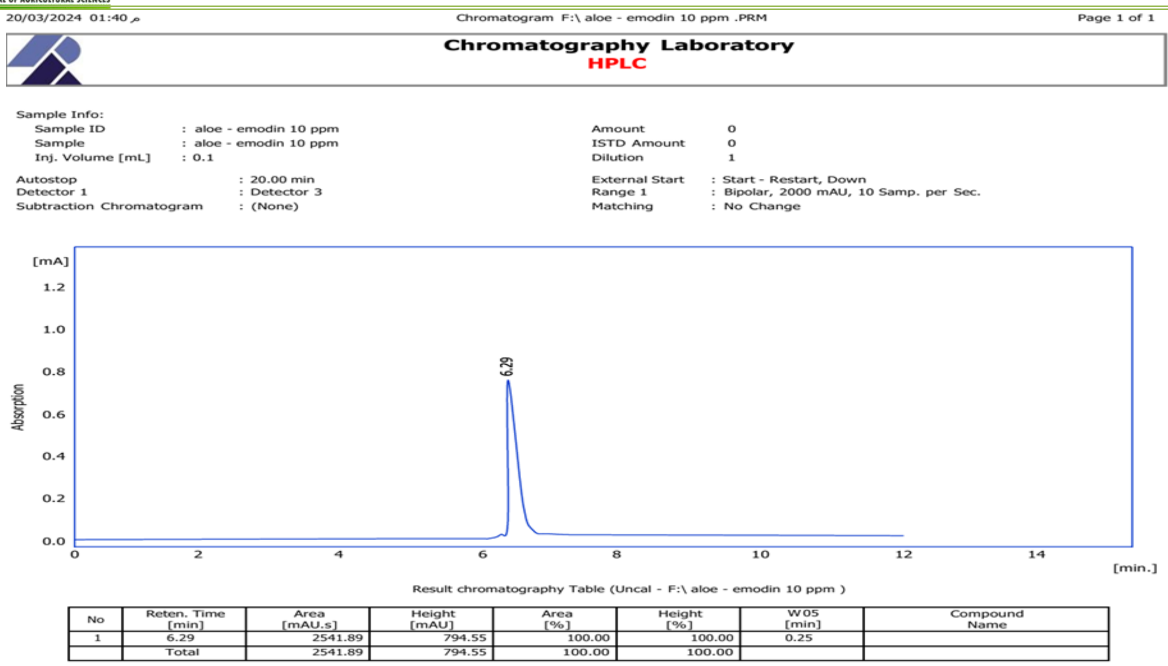

Figure 3. Standard compound of Aloe-emodin

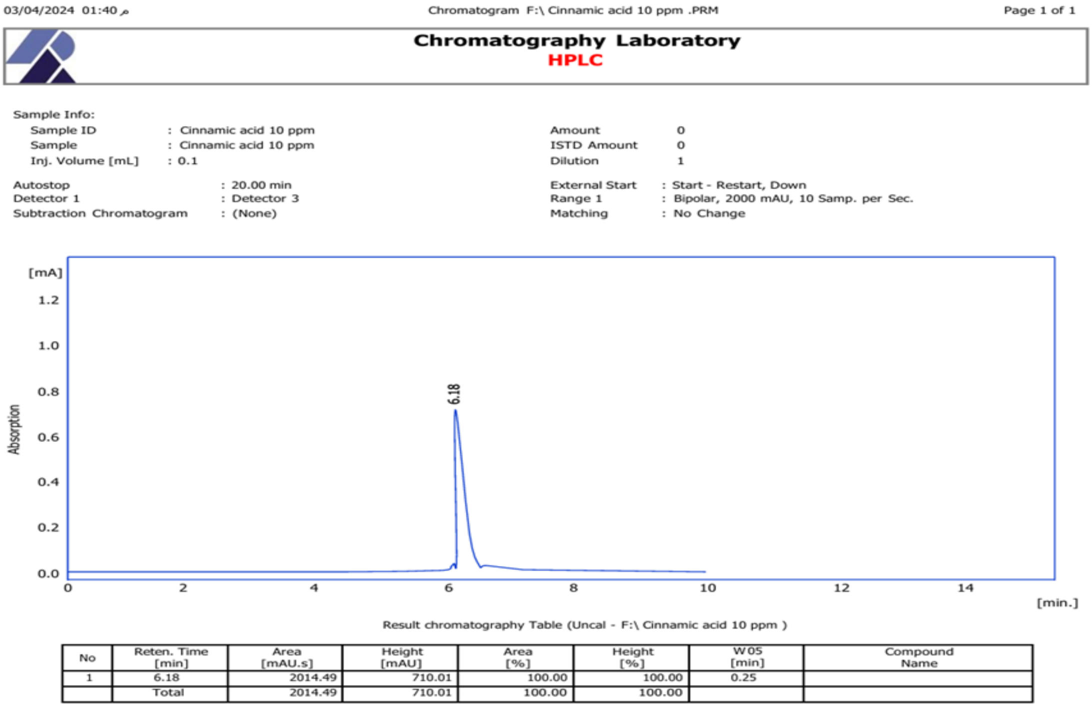

Figure 4. Standard compound of the amino acid Cinnamic acid

Preparation of Crude Extract from Dried *Aloe vera* Gel: The crude extract was prepared from dried *Aloe vera* gel and analyzed for its content of aloin and aloe-emodin using an HPLC device, according to the method described by Logaranjan et al. (2013). The concentrations of the active ingredients (aloin and aloe-emodin) were determined quantitatively by comparing the standard compound with the model compound under the same conditions using the following formula:

Concentration of Sample $=$ $\frac{\textbf{Area of Sample Compound Peak}}{\textbf{Area of Standard Compound Peak}}$ x **Conc. of Standard Solution × Number of dilutions**

Estimation of the amino acids phenylalanine and cinnamic acid: Phenylalanine and cinnamic acid were extracted following the procedure of Rasmus et al. (2018). Analyses A 3 g sample was

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introduced into a 25 mL volumetric flask with 25 mL of 1 M hydrochloric acid. The resulting mixture was heated at 55°C for 3 h. After heating, the sample was dried under a rotary evaporator. 5 ml of sodium citrate buffer (pH 2.2) was then added. The filtrate was filtered by 0.45 µm plastic filter, and the filtrate was prepared for the HPLC analysis.

Preparation of the extracted sample for injection into an HPLC device: One ml of the extracted sample was taken, and 200 microliters of 5% orthophthalein aldehyde (OPA) were added. The sample was shaken for two minutes, after which 100 microliters of the final mixture were taken and injected into a high-performance liquid chromatography (HPLC) device.

Estimation of the Amino Acids Phenylalanine and Cinnamic Acid in Dried *Aloe* Gel Using High-Performance Liquid Chromatography (HPLC): The analysis was conducted in the laboratories of the Ministry of Science and Technology / Department of Environment and Water using a high-performance liquid chromatography (HPLC) [1] system (German-made, model Sykam). The Scriver et al. (31) with a mobile phase of (methanol: acetonitrile: 5% formic acid) at 20:60:20v/v (flow rate: 1 mL min^{-1}). A C18-NH2 separation column (250 mm X 4.6 mm) separation of phenylalanine and cinnamic acid after that the phenylalanine was detected with the fluorescence detector (Ex =445nm and Em =465nm) and (the software that you were used to analyze the amino acids which included both phenylalanine and cinnamic acid. The concentration of the two amino acids was calculated according to the following equation:

$$\textbf{Concentration of Sample} = \frac{\text{Area of Sample Compound Peak}}{\text{Area of Standard Compound Peak}} \times \textbf{Conc. of Standard Solution} \times \textbf{Number of dilutions}$$

RESULTS AND DISCUSSION

The results in Table 4 indicate a significant effect of adding vermicompost fertilizer on the vegetative traits studied in the plant. The treatment of adding vermicompost fertilizer to the soil by 15 tons ha^{-1} (V2) outperformed by recording the highest rate of increase in the number of leaves per plant in each of the traits of the rate of increase in leaf width, leaf thickness, average fresh weight of the plant, leaf content of total chlorophyll, average fresh weight of the gel, average leaf yield of dry gel, average yield of fresh gel per hectare, and number of offspring per plant, which reached 8.80 leaves per $plant^{-1}$, 28.45 mm, 10.09 mm, 1819 g, 23.50 mg 100 g^{-1}, 1251.1 g $plant^{-1}$, 9.18 g $plant^{-1}$, 13787.4 kg ha^{-1}, 7.04 offshoots $plant^{-1}$, respectively, compared to the control treatment (V0), which recorded the lowest average in the same traits above, which reached 7.37 leaves per $plant^{-1}$, 18.82 mm , 8.40 mm, 1027 g, 21.69 mg 100 g^{-1}, 771.3 g .$plant^{-1}$, 7.66 g $plant^{-1}$. 8499.3 kg ha^{-1} 3.78 offshoots $plant^{-1}$, respectively. The spraying treatment with 8 g L^{-1} Spirulina extract exhibited higher increase in the rate of the number of leaves of plant to which it reached, 9.04 leaves $plant^{-1}$, in comparison with the spraying treatment with 4g L^{-1} Spirulina extract, 2 g L^{-1} Spirulina extract, 1 g L^{-1} Spirulina extract and the control treatment (spraying of distilled water only). The rate of increase in $plant^{-1}$ leaves of each treatment was reduced to 8.26, 8.11, 8.08, and 7.89 leaves $plant^{-1}$, respectively. The spraying treatment with 8 g L^{-1} Spirulina extract and the spraying treatment with 4 g L^{-1} Spirulina extract were superior in the rate of increase in leaf width, reaching 25.22 and 22.70 mm, respectively, compared to the standard treatment of spraying with distilled water alone, which reached 20.90 mm. The 8 g L^{-1} Spirulina extract spray treatment (S4) also outperformed by recording the highest average leaf thickness increase rate of 10.66 mm, while the control treatment (S0) sprayed with distilled water (S0) recorded the lowest average of 7.70 mm. The 8 g L^{-1} Spirulina extract spray treatment (S4), the 4 g L^{-1} Spirulina extract spray treatment (S3) and the 2 g L^{-1} Spirulina extract spray treatment (S2) also outperformed in the same trait, as plant weights reached 1717, 1622 and 1591 g, respectively, compared to the 1 g L^{-1} spray treatment (S1) and the control treatment (S0), which recorded 1439 and 1310 g, respectively. Foliar spray of Spirulina extract on the foliage in 8 g L^{-1} (S4) increased the vegetative growth of aloe plant. This treatment also gave the maximum values of total chlorophyll in the

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leaves (25.58 mg/100 g fresh weight), fresh gel weight/plant (1130.1 g), dry gel yield from the leaves (9.09 g/plant), and number of offshoots/plant (6.18). The S0 treatment (distilled water only) led to the lowest amounts of these traits values, which were equal to 18.67 mg 100 g^{-1}, 983.0 g $plant^{-1}$, 7.98 g $plant^{-1}$, and 5.11 offshoots $plant^{-1}$, respectively. In addition, the effect of 8 gL^{-1} Spirulina spray was not confined to one specific trait, the total gel yield per hectare was also significantly increased to 12,647.7 kg ha^{-1}. This value of yield was significantly greater than that under 1 g L^{-1} treatment (S1) and the control (S0), which were 11,176.7 and 10,617.1 kg ha^{-1}, respectively. In addition to the individual effects, the interaction between soil application of vermicompost and foliar spraying with Spirulina extract showed a significant influence on leaf number per plant. The highest readings were verified in the treatments at 15 t ha^{-1} of vermicompost combined with 4 g L^{-1} of Spirulina extract (V2S3) and 8 g L^{-1} (V2S4), and in the combinations V1S4 (7.5 t ha^{-1} + 2 g L^{-1}), V0S2 (without vermicompost + 2 g L^{-1}) and V2S1 (15 t ha^{-1} + 1 g L^{-1}), which presented 9.56, 9.45, 9.22, 9.22 and 9.11 leaves per plant, respectively. V0S0 (no vermicompost, and no Spirulina) had significantly lowest values, which were followed by V1S2, V0S3 and V1S1, which produced 7.55, 7.45, 7.23 and 7.11 leaves per plant respectively. Moreover, the V2S4 treatment stood out in terms of leaf width, reaching a maximum of 35.85 mm. This increase was considerably greater than that observed in V0S4 (18.34 mm), V0S3 (17.80 mm), and V0S0 (17.23 mm), confirming the positive impact of combining high vermicompost rates with higher concentrations of Spirulina extract on leaf development. The results revealed statistically significant differences among the interaction treatments. The combination of applying 15 tons ha^{-1} of vermicompost with foliar spraying of *Spirulina* extract at 8 g L^{-1} (V_2S_4) showed the highest performance in several growth and biochemical parameters. It recorded the maximum increase in leaf thickness (13.81 mm) and total chlorophyll content in fresh leaves (26.52 mg per 100 g) . In contrast, the control treatment involving no vermicompost and no *Spirulina* application (V_0S_0) gave the lowest values for these traits, at 7.36 mm and 16.54 mg per 100 g , respectively. Additionally, the interaction treatment V_1S_4 (7.5 tons ha^{-1} of vermicompost + *Spirulina* at 8 g L^{-1}) achieved the highest plant fresh weight of 2218 g , significantly surpassing the control treatment V_0S_0, which recorded only 986 g . Regarding fresh gel weight, the V_2S_4 and V_2S_3 treatments outperformed all others, recording 1455.3 g $plant^{-1}$ and 1355.7 g $plant^{-1}$, respectively. These weights were significantly higher than all the interaction treatments weighed: V_0S_0 (control, 805 g $plant^{-1}$), V_0S_2 (no manure + 2 g L^{-1} Spirulina, 797.7 g $plant^{-1}$), V_0S_1 (no manure + 1 g L^{-1} Spirulina, 755.7 g $plant^{-1}$), V_0S_4 (no manure + 8 g L^{-1} Spirulina, 751.7 g $plant^{-1}$), and V_0S_3 (no manure + 4 g L^{-1} Spirulina, 746.3 g $plant^{-1}$) . As to dry gel per plant yield, once more the V_2S_4 treatment was the most effective, with the highest response of 10.62 g $plant^{-1}$ (compared to the lowest of 7.07 g $plant^{-1}$ of the V_0S_3 treatment). Both the combined treatment of 15 tons ha^{-1} vermicompost with Spirulina extract at 8 g L^{-1} (V2S4) and the treatment with the same compost rate paired with 2 g L^{-1} of Spirulina extract (V2S2) exhibited notable superiority, achieving the highest fresh gel yields of 16,037.7 and 14,939.3 kg ha^{-1}, respectively. These values were higher than in all treatments that did not receive vermicompost. More precisely, fresh gel yields were significantly lower in these combined treatments (V0S4, V0S3, V0S2, V0S1, and the control V0S0) (respectively 8,871.0, 8,790.0, 8,327.7, 8,283.3, and 8,224.7 kg ha^{-1}). Moreover, the combination of 7.5 tons ha^{-1} vermicompost and 8 g L^{-1} Spirulina extract (V1S4) resulted in maximum number of offshoots (8.33 offshoots $plant^{-1}$). In contrast, the control treatment (V0S0), which received neither vermicompost nor Spirulina spray, recorded the lowest number of offshoots at only 2.88 per plant.

Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
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Table 4 . Effect of Vermicompost Fertilizer and Spraying with Different Levels of Spirulina Algae Extract on: Leaf Number (leaf plant^{-1}) , Leaf Width (mm^{-1}), Leaf Thickness (mm^{-1}), Fresh Weight (g plant^{-1}) ,Total Chlorophyll Content in Leaves (mg per 100 g of fresh leaf weight) , Fresh Gel Yield (g plant^{-1}) , Dry Gel Yield (g plant^{-1}), Fresh Gel Yield per Hectare (kg ha^{-1}) ,Number of offshoots (offshoot plant^{-1})

treatment **Vermicompost**	**the number of leave (leaf plant^{-1})**	**leaf width (mm^{-1})**	**leaf thickness (mm^{-1})**	**Fresh weight (g plant^{-1})**	**Total chlorophyll**	**Fresh gel yield (g plant^{-1})**	**Leaf Gel Yield (g plant)**	**fresh gel yield per hectare (kg/ha)**	**Number of offshoots (offshoot plant^{-1})**
V0	7.73	18.82	8.40	1027	21.69	771.3	7.66	8499.3	3.78
V1	8.29	22.53	8.62	1761	22.86	1123.3	8.48	12501.7	5.51
V2	8.80	28.45	10.09	1819	23.50	1251.1	9.18	13787.4	7.04
LSD 0.05	0.48	0.37	0.55	127	1.08	50.6	0.99	687.7	0.36
Spirulina Algae Extract									
S0	7.89	20.9	7.70	1310	18.67	983.0	7.98	10617.1	4.59
S1	8.08	23.02	8.26	1439	21.88	1015.6	8.24	11176.7	5.45
S2	8.11	22.51	8.29	1591	23.08	1048.4	8.39	11744.8	5.37
S3	8.26	24.70	10.28	1622	24.22	1065.8	8.50	11779.1	5.48
S4	9.04	25.22	10.66	1717	25.58	1130.1	9.09	12649.7	6.33
LSD 0.05	0.47	0.80	0.74	130	0.97	60.5	0.40	691.9	0.46
V* S									
V0 S0	7.55	.2317	7.36	986	16.54	805.0	8.25	8224.7	2.88
V0 S1	8.00	20.42	7.72	1005	21.45	755.7	7.54	8283.3	3.70
V0 S2	9.22	19.43	8.32	1007	23.42	797.7	7.38	8327.7	3.89
V0 S3	7.23	17.80	9.04	1028	22.74	746.3	7.07	8790.0	4
V0 S4	8.45	18.34	9.57	1108	24.32	751.7	8.05	8871.0	4.44
V1 S0	7.89	22.09	8.27	1407	19.06	1155.3	7.43	12731.7	4.11
V1 S1	7.11	23.03	9.30	1538	22.02	1083.0	8.10	11934.7	5.44
V1 S2	7.45	20.34	8.41	1905	22.49	1099.7	8.14	12070.3	4.77
V1 S3	8.00	24.06	8.52	1739	24.83	1095.3	8.14	12731.7	4.89
V1 S4	9.22	21.46	8.60	2218	25.90	1183.3	8.61	13040.3	8.33
V2 S0	8.22	23.38	7.48	1537	20.41	988.7	8.26	10895.0	6.77
V2 S1	9.11	25.62	7.76	1774	22.18	1208.0	9.07	13312.0	7.22
V2 S2	7.67	27.67	8.13	1862	23.32	1248.0	9.65	14939.3	7.44
V2 S3	9.56	32.24	13.28	2098	25.08	1355.7	8.31	13753.0	7.55
V2 S4	9.45	35.85	13.81	1824	26.52	1455.3	10.62	16037.7	6.22
LSD 0.05	0.81	1.26	1.21	221	n.s	100.2	1.03	1179.4	0.76

V0 = **Treatment without the addition of vermicompost to the soil ,** V1 = **Addition of vermicompost to the soil at a rate of 7.5 tons/ha ,** V2 = **Addition of vermicompost to the soil at a rate of 15 tons/ha** S1 = **Spraying with distilled water only (Control treatment without Spirulina extract) ,** S2 = **Spraying with Spirulina extract at a concentration of 1 g/liter** , S3 = **Spraying with Spirulina extract at a concentration of 2 g/liter** , S4 = **Spraying with Spirulina extract at a concentration of 4 g/liter** S5 = **Spraying with Spirulina extract at a concentration of 8 g/liter**

The results presented in Table 5 indicate a statistically significant effect of earthworm manure application on several biochemical parameters in *Aloe vera* leaves, including nitrogen percentage, phosphorus content, potassium content, calcium content, carbohydrate content, and protein content. The treatment involving the addition of 15 tons ha^{-1} of earthworm manure (V_2) recorded the highest average values: 0.381% nitrogen, 0.099% phosphorus, 2.10% potassium, 188.8 ppm calcium, 4.55% carbohydrates, and 2.38% protein, respectively. These values were significantly higher compared to the control treatment (V_0) , which recorded the lowest averages: 0.305% nitrogen , 0.067% phosphorus , 1.54% potassium , 163.8 ppm calcium , 3.40% carbohydrates , and 1.90% protein , respectively. Additionally, the V_1 treatment (7.5 tons ha^{-1}) also showed a significant effect, recording a protein content of 2.16% . Regarding the effect of foliar spraying with Spirulina extract, the treatments S_4 (8 g L^{-1}) and S_3 (4 g L^{-1}) recorded the highest average values for nitrogen content, at 0.431% and 0.397% , respectively. These two treatments also achieved the highest protein content in leaves , at 2.69% and 2.48% , respectively. In contrast, the control treatment (S_0 , no spray) recorded the lowest average

Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/08nd6t74

values for nitrogen (0.247%) and protein (1.54%) . There were significant reductions in phosphorus that were recorded in these same treatments, with S_4 and S_3 having phosphorus content of 0.117 % and 0.105 %, respectively, against 0.052 % for the control (S_0).Further, foliar application of Spirulina extract at 8 g L^{-1} (S_4) increased significantly potassium (2.50%), calcium (203.4 ppm), carbohydrates (5.20%) and protein (2.69%) levels in leaves in comparison with control (S_0) treatment levels of 1.19% potassium, 151.9 ppm calcium, 2.56% carbohydrates and 1.54% protein, respectively. In terms of the interaction between soil application of earthworm manure (V) and foliar spray with *Spirulina* extract (S) , the results showed significant differences. The interaction treatment V_2S_4 achieved the highest values for all measured elements and compounds in leaves: 0.463% nitrogen, 0.133% phosphorus, 2.79% potassium , 215.7 ppm calcium , 5.65% carbohydrates , and 2.89% protein . On the other hand, the control interaction treatment V_0S_0 recorded the lowest values: 0.217% nitrogen , 0.043% phosphorus, 1.03% potassium , 143.7 ppm calcium , 2.07% carbohydrates, and 1.35% protein , respectively.

Treatment **Vermicompost**	**N %**	**P%**	**K%**	**Ca (ppm)**	**Carbohydrates in leaves (%)**	**protein in leaves (%)**
V0	0.305	0.067	1.54	163.8	3.40	1.90
V1	0.347	0.087	1.87	178.2	3.94	2.16
V2	0.381	0.099	2.10	188.8	4.55	2.38
LSD 0.05	0.0016	0.0017	0.009	0.88	0.06	0.027
Spirulina Algae Extract						
S0	0.247	0.052	1.19	151.9	2.56	1.54
S1	0.285	0.061	1.42	160.4	3.11	1.78
S2	0.362	0.086	1.89	177.9	4.21	2.26
S3	0.397	0.105	2.16	191.2	4.73	2.48
S4	0.431	0.117	2.50	203.4	5.20	2.69
LSD 0.05	0.0023	0.0012	0.009	1.51	0.06	0.025
V* S						
V0 S0	0.217	0.043	1.03	143.7	2.07	1.35
V0 S1	0.252	0.052	1.25	152.3	2.70	1.57
V0 S2	0.315	0.067	1.58	165.4	3.59	1.96
V0 S3	0.355	0.082	1.77	173.6	4.04	2.22
V0 S4	0.387	0.094	2.06	184.0	4.59	2.42
V1 S0	0.231	0.049	1.12	148.1	2.29	1.44
V1 S1	0.276	0.057	1.35	157.1	2.83	1.72
V1 S2	0.374	0.090	1.92	178.1	4.25	2.34
V1 S3	0.412	0.115	2.29	197.5	4.95	2.57
V1 S4	0.442	0.124	2.66	210.4	5.38	2.76
V2 S0	0.292	0.064	1.44	164.0	3.32	1.82
V2 S1	0.327	0.076	1.67	171.7	3.80	2.04
V2 S2	0.397	0.102	2.16	190.3	4.80	2.48
V2 S3	0.424	0.118	2.42	202.4	5.18	2.65
V2 S4	0.463	0.133	2.79	215.7	5.65	2.89
LSD 0.05	0.0038	0.0023	0.016	2.41	0.10	0.040

Table 5. Effect of vermicompost fertilizer and spraying with *Spirulina* extract on nitrogen, phosphorus , potassium, calcium, protein and carbohydrates

V0 = **Treatment without the addition of vermicompost to the soil ,** V1 = **Addition of vermicompost to the soil at a rate of 7.5 tons/ha ,** V2 = **Addition of vermicompost to the soil at a rate of 15 tons/ha** S1 = **Spraying with distilled water only (Control treatment without Spirulina extract) ,** S2 = **Spraying with Spirulina extract at a concentration of 1 g/liter ,** S3 = **Spraying with Spirulina extract at a concentration of 2 g/liter ,** S4 = **Spraying with Spirulina extract at a concentration of 4 g/liter** S5 = **Spraying with Spirulina extract at a concentration of 8 g/liter**

The results reported on Table 6 revealed a significant effect of vermicompost supplementation into the soil on the content of aloin (mg $plant^{-1}$) and aloe-emodin (mg

Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/08nd6t74

plant^{-1}) in the gel, phenylalanine and cinnamic acid in the dried Aloe gel as well as total phenols and total flavonoids in the dried Aloe gel. For the V3 treatment (soil treatment, source NPK and 15 ton ha^{-1} of vermicompost), it was the superior treatment of the study traits with estimates ranging from 221.0 to 89.90 mg 100 g^{-1} dry gel weight for all traits, respectively, followed by V2 (soil treatment, source NPK, 10 tons ha^{-1} of vermicompost) and then V1 (soil treatment, source NPK and without vermicompost) of 171.7-74.80 mg 100 g^{-1} dry gel weight for all traits, respectively. In addition, spray extract of species of Spirulina treatment S4 was significantly showed the highest aloin and aloe-emodin content in the gel, percent of phenylalanine and cinnamic acid in the dried Aloe gel, (b) Total phenols in the dried Aloe gel, and (c) total flavonoid compounds in the dried gel with values of 251 mg plant^{-1}, 150.43 mg plant^{-1}, 35.67 mg 100 g^{-1}, 47.51 ppm, 129.50 mg 100 g^{-1} dry gel weight, and 99.20 mg 100 g^{-1} dry gel weight compared to the control treatment (S1) magnitude of 142.5 mg plant^{-1}, 71.73 mg plant^{-1}, 13.20 mg 100 g^{-1}, 16.46 ppm, 98.20 mg 100 g^{-1} dry gel weight, and 66.50 mg 100 g^{-1} dry gel weight, respectively. The results of the interaction between soil application of vermicompost and spraying with Spirulina species extract showed a significant effect. The interaction treatment between the addition of 15 tons ha^{-1} of vermicompost (V3) and spraying with Spirulina species extract at a concentration of 8 g L^{-1} (S4) excelled by recording the highest estimations for the content of aloin (mg plant^{-1}), aloe-emodin (mg plant^{-1}), phenylalanine and cinnamic acid in the dried *Aloe* gel, total phenols in the dried *Aloe* gel, and total flavonoids in the dried gel, reaching 271.0 mg plant^{-1}, 172.57 mg plant^{-1}, 39.73 mg 100 g^{-1}, 54.47 parts per million, 136.90 mg 100 g^{-1} dry gel weight, and 106.50 mg 100 g^{-1} dry gel weight, respectively, compared to the control treatment V1S1 which recorded 142.5 mg plant^{-1}, 62.13 mg plant^{-1}, 9.41 mg 100 g^{-1}, 12.47 parts per million, 93.20 mg 100 g^{-1} dry gel weight, and 62.50 mg 100 g^{-1} dry gel weight, respectively.

Vermicompost positively influences vegetative growth characteristics and yield, which is reflected positively in the content of medicinally active compounds in medicinal plants (14, 23). This is supported by the results obtained in Tables 4 and 5, as the addition of vermicompost to the soil led to an increase in most vegetative growth characteristics and in the content of elements, carbohydrates, and protein in the leaves (Tables 4-5). This subsequently led to an increase in the content of secondary metabolites in the gel as a result of the increased carbohydrate and protein content in the leaves (Table 5). This increase might be attributed to the fact that the enhanced photosynthesis led to a higher total chlorophyll content in the leaves, which in turn means an increase in the percentage of both carbohydrates and protein, being primary metabolic products. The continuous increase in these primary metabolites then led to an increase in the content of secondary metabolites in the gel (Table 6). The increase in secondary metabolites in the *Aloe* gel resulting from foliar spraying with *Spirulina* species algae extract might be due to the fact that the use of *Arthrospira platensis* extract is beneficial in improving the quantitative and qualitative production of crops because it stimulates growth and the accumulation of primary metabolic compounds in horticultural and medicinal plants (Priyadarshani and Rath ,2012). This is confirmed by the results obtained in Table 5, which is positively reflected in the content and components of the gel in terms of active compounds (Table 6). The results obtained in this study are consistent with the findings of Anastasia et al. (2012), Anitha et al. (2016), Godlewska et al. (2019), El-Sherif et al. (2020), and Yanni et al. (2020).

Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/08nd6t74

Table 6. Effect of Vermicompost Fertilizer and Spraying with Spirulina Extract on , Aloin Compound, Aloeemodin Compound , Phenylalanine Acid , Cinnamic Acid, Total Total Phenols and Flavonoids

treatment Vermicompost	Aloin Compound (mg plant⁻¹)	emodin-Aloe Compound (mg plant⁻¹)	Phenylalanine Acid (mg 100 g⁻¹)	Cinnamic Acid (ppm)	Total Phenols (mg 100 g⁻¹ dry gel weight)	Total Flavonoids (mg 100 g⁻¹ dry gel weight)
V0	171.7	90.44	19.96	24.38	105.40	74.80
V1	201.1	110.83	25.23	32.63	114.70	83.00
V2	221.0	126.36	29.80	38.95	120.30	89.90
LSD 0.05	2.56	1.67	0.19	0.16	1.52	1.09
Spirulina Algae Extract						
S0	142.5	71.73	13.20	16.46	98.20	66.50
S1	163.0	82.87	17.88	21.63	102.90	71.10
S2	203.9	111.14	26.69	32.84	114.40	84.40
S3	229.2	129.87	31.55	41.49	122.50	91.60
S4	251.0	150.43	35.67	47.51	129.50	99.20
LSD 0.05	2.78	1.33	0.18	0.22	0.68	1.07
V* S						
V0 S0	123.3	62.13	9.41	12.47	93.20	62.50
V0 S1	146.8	73.63	13.77	17.71	98.10	65.50
V0 S2	175.6	88.47	21.32	24.60	105.10	74.10
V0 S3	197.6	106.57	25.64	30.49	111.70	82.50
V0 S4	214.9	121.40	29.66	36.64	119.20	89.60
V1 S0	137.7	67.77	11.49	14.35	96.70	63.70
V1 S1	155.1	78.57	16.18	19.49	102.10	68.50
V1 S2	208.5	114.03	27.52	33.57	115.80	86.80
V1 S3	237.2	136.43	33.34	44.32	126.60	94.60
V1 S4	267.1	157.33	37.62	51.42	132.40	101.50
V2 S0	166.4	85.30	18.71	22.57	104.50	73.30
V2 S1	187.2	96.40	23.68	27.68	108.60	79.20
V2 S2	227.7	130.93	31.23	40.37	122.40	92.40
V2 S3	252.8	146.60	35.67	49.66	129.30	97.80
V2 S4	271.0	172.57	39.73	54.47	136.90	106.50
LSD 0.05	4.67	2.40	0.31	0.35	1.63	1.84

V0 = **Treatment without the addition of vermicompost to the soil ,** V1 = **Addition of vermicompost to the soil at a rate of 7.5 tons/ha ,** V2 = **Addition of vermicompost to the soil at a rate of 15 tons/ha** S1 = **Spraying with distilled water only (Control treatment without Spirulina extract) ,** S2 = **Spraying with Spirulina extract at a concentration of 1 g/liter ,** S3 = **Spraying with Spirulina extract at a concentration of 2 g/liter ,** S4 = **Spraying with Spirulina extract at a concentration of 4 g/liter** S5 = **Spraying with Spirulina extract at a concentration of 8 g/liter**

CONCLUSION

The present study indicated that combined application of vermicompost as a soil amendment and foliar spray with Spirulina species extract exhibited improvement in vegetative growth as well as accumulation of important primary and secondary metabolites in *Aloe vera*. The combined application of 15 tons ha^{-1} vermicompost 8 g L^{-1} Spirulina extracts foliar spray showed the most significant improvements in growth parameters, nutrient contents and bioactive compounds namely: aloin, aloe-emodin, total phenols and total flavonoids in the Aloe gel. These results highlight the potential of integrating organic fertilizers and natural bio stimulants to improve the quality and productivity of medicinal plants in sustainable agricultural systems.

ACKNOWLEDGEMENT

The authors extend their sincere thanks and gratitude to the Dep. of Hoti. and Land scape gardening and the University of Baghdad for their research support.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/08nd6t74

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds:

AUTHOR'S CONTRIBUTION STATEMENT

Asmahan Khalid Allawi ,conducted the practical experimental work, gathered relevant references, and wrote the initial draft of the manuscript.

Ziena Mohammed Abdul-Qader, conceived the research idea, oversaw its execution,and conducted the final review of the manuscript

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Iraqi Journal of Agricultural Sciences –2026:57(7):2012-2026
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/08nd6t74

تعزيز المركبات الفعالة في هلام نبات الصبار باستخدام ا سماد دودة الارض والرَّش الورقي بمستخلص الإسبيرولينا

اسمهان خالد علاوي ، زينه محمد عبدالقادر
جامعة بغداد –كلية علوم الهندسة الزراعية

المستخلص

أجريت التجربة في المحطة البحثية الزراعيةA في كلية علوم الهندسة الزراعية / جامعة بغداد– الجادرية على نبات الصبار للموسم الربيعي 2023 بهدف دراسة التأثير المفرد والمتداخل للاضافة الارضية لسماد دودة الارض والرش بمستخلص طحالب السبيرولينا في محتوى نبات الصبار من بعض العناصر والكاربوهيدرات والبروتين والبعض من المركبات الفعاله.إذ نفذت التجربة وفقاً لتصميم القطاعات الكاملة المعشاة (RCBD) بترتيب الالواح المنشقة بثلاثة مكررات . اشارت النتائج الى تفوق معاملة التداخل الثنائي اضافة 15 طن ه$^{-1}$ سماد دودة الارض للتربة مع الرش بمستخلص السبيرولينا بتركيز 8 غم لتر$^{-1}$ في محتوى الهلام المجفف من النسبة المئوية للنيتروجين (0.463 %) و والبروتين (2.89 %) وكل من مركب الالوين (271 ملغم نبات$^{-1}$) ومركب الالوايمودين (172.57 ملغم نبات$^{-1}$) وحامضي الفنيل الانين والسيناميك (39.73 ملغم 100 غم $^{-1}$ ، 54.47 جزء بالمليون بالتتابع)والفينولات الكلية (136.90 ملغم 100 غم$^{-1}$ من الهلام المجفف) والفلافونيدات الكلية (106.5ملغم 100 غم$^{-1}$ من الهلام المجفف) .ومحتوى الاوراق المجففة لكل من الفسفور (0.133 %) والبوتاسيوم (2.79 %) وتركيزالكالسيوم (215.7 ppm) والنسبة المئويةا للكاربوهيدرات الكلية (5.65 %) .

الكلمات المفتاحية: نبات الصبار، سماد عضوي، مركبات ثانوية، سماد حيوي .