BILINGUAL PUBLISHING GROUP

Journal of Environmental & Earth Sciences

https://journals.bilpubgroup.com/index.php/jees

ARTICLE

Impact of Bentonite and Humic Acid on the Growth and Flowering of *Catharanthus roseus* L. in Sandy Soil

Raad Farhan Shahad [1*], ***Mohammed Malik Hamid*** [2]

[1] *Department of Soil and Water Resources Sciences, University of Al-Qadisiyah, Al-Diwaniyah 58000, Iraq*

[2] *Department of Soil and Water Technologies, Technical collage of Al-Awsat Technical University, Kufa 54001, Iraq*

ABSTRACT

Bentonite is a very useful material for improving soil properties, which enhances the ability of plants to grow and produce in different conditions. The experiment was carried out in an agricultural nursery in one of the areas of the City of Diwaniyah, in a house covered with green netting, with a shade rate of 25%, to study the effect of bentonite and humic acid on the growth and flowering of a *Catharanthus roseus* L. plant in sandy soil. The experiment included two factors: the first factor was bentonite clay, and the second factor was humic acid. Using a randomized complete block design (R.C.B.D) with three replications, data were analyzed using the analysis of variance (ANOVA) method, and comparison was made according to the least significant difference (L.S.D) test at a probability level of 0.05. The experiment consisted of adding bentonite clay at 0, 2, 6, and 8 g L^{-1}, humic acid at 0, 0.5, 1, and 10 g L^{-1}. The results showed that adding bentonite clay and humic acid to sandy soil can have a significant positive effect on the growth and flowering of the Catharanthus roseus plant grown in poor sandy soil conditions. Bentonite, clay and humic acid were added at concentrations of 8 and 10 g L^{-1}, which led to an increase in plant height and number of leaves and leaf area. They reached 30.07, 23.84 cm^2, 76.62, 63.42 cm^2 for $leaf^{-1}$ and 24.73, 20.22 cm^2 for $leaf^{-1}$, respectively. The results also showed an increase in the content of nitrogen (N), phosphorus (P), and potassium (K) in leaves by 2.27, 1.92, 1.99% and 1.51, 1.22, 1.77%. This also led to an increase in chlorophyll pigment and anthocyanin at the highest concentration and gave the highest value. Therefore, adding bentonite and humic acid together gave the highest values in vegetative and chemical characteristics, compared to treatments without addition.

*CORRESPONDING AUTHOR:

Raad Farhan Shahad, Department of Soil and Water Resources Sciences, University of Al-Qadisiyah, Al-Diwaniyah 58000, Iraq; Email: raad.farhan@qu.edu.iq

ARTICLE INFO

Received: 27 Septembert 2024 | Revised: 10 October 2024 | Accepted: 12 October 2024 | Published Online: 20 November 2024

DOI: https://doi.org/10.30564/jees.v7i1.7368

CITATION

Shahad , R.F., Hamid, M.M., 2024. Impact of Bentonite and Humic Acid on the Growth and Flowering of *Catharanthus roseus* L. in Sandy Soil. Journal of Environmental & Earth Sciences. 7(1): 157–166. DOI: https://doi.org/10.30564/jees.v7i1.7368

Keywords: Bentonite; Humic Acid; Sandy Soil; *Catharanthus roseus* L.

1. Introduction

Catharanthus roseus L. is one of Apocynaceae plants, a perennial, evergreen plant. It is a semi-shrub plant. Stems are single, cylindrical, erect, with many branches at top. Leaves are simple, opposite each other, oval, oblong, smooth, with full edges. Inflorescences are determined with a terminal or axillary growth. Flowers are layered in shape. They are arranged in groups without a stand, their numbers ranging from 2–7 The plant is of high economic importance, and demand for it increases greatly annually because it is one of the plant groups with aesthetic values. It is considered one of the plants in pots and flower beds, and it is used in several ways, such as exhibitions and decorating balconies and windows due to the beauty of its flowers [1]. It has many colors and lasts a long time, in addition to its rapid growth [2]. It does not require large service operations, and it is considered an important medicinal plant because it contains alkaloids that are used in many industries. Its cultivation succeeds in sunny and shaded places. It resists diseases and insects, and its cultivation succeeds in sandy and poor lands [3]. Sandy soil is defined as soil that contains 85% sand, while the percentage of silt and clay does not exceed 15% [4]. Sandy soils are characterized by their good aeration because their large pores and quick-draining properties. Therefore, it is also considered a soil with a poor ability to store water and retain nutrients. Potting soil (sandy soil) suffers from many problems despite its use in greenhouses and most nurseries for the production of various plants due to low Cation Exchange Capacity (CEC), as its cation exchange capacity reaches (6–10 C_{+}mmol kg^{-1}) because of the lack of clay minerals in it. It is considered a poor soil in the availability of nutrients due to the lack of organic matter [5, 6]. Sustainable agriculture aims to use the best methods for sandy soils, through the use of soil improvers such as bentonite and humic acid [7]. Among these enhancers are clay deposits represented by bentonite clay, which is a type of natural clay consisting of mineral smectite and often montmorillonite. It is added "to soil to improve its properties and increase the availability of nutrients" as well as to increase the moisture content and water conductivity. It works to increase specific surface area of soil and its ability to exchange cations [8]. Bentonite clay is characterized by a cation exchange capacity of up to 150 C_{+}mol kg^{-1} [9]. The addition of bentonite to sandy or gypsum soil improves many physical and chemical properties (water conductivity, moisture content at field capacity and bulk density) and readiness for nutritional absorption by plants [10]. Bentonite improver is characterized by its ability to expand, which allows it to absorb water and double its volume, and thus one can benefit from its colloidal properties. It is composed of mineral montmorillonite, which is characterized by its ability to swell and contract, absorb amounts of water, and increase the volume of ion exchange because of the increase in negative charge on the surfaces of colloidal particles [11]. It is an effective mineral enhancer of the root system and increases biological activity in the soil of the root system [12]. Bentonite improves the chemical properties of soil such as electrical conductivity (EC), pH, cation exchange capacity (CEC), and organic matter (OM) content. Higher levels of bentonite (P2) were especially effective in enhancing these properties [13]. Bentonite enhances soil structure by filling the spaces between sand particles, improving water retention and reducing erosion rates. Even small amounts (0.2% to 1%) can significantly enhance soil physical properties and stability [14].

Humic acid is an organic compound resulting from the decomposition of organic matter in soil. Its composition is $C_{75}H_{33}O_{17}N_3(COOH)_3(CO)_2$. They are brown and dark-colored solutions that do not have a fixed and specific structural structure. Among the natural features of humic acids is the diversity of the size of their particles and the heterogeneity of their structural composition. Because of these features and characteristics, its molecular weight has become very complex, and commercial material is prepared from mineral Leonardite, which contains 60% humic, fulvic, and humic acids. Humic acid is the most important part of humus because it contains a high exchange capacity for positive ions [15]. The use of amino acid (humic acid) is necessary to supply the growing medium with nutrients, as adding humic acids to soil leads to plant absorption of nutrients [16]. The mixture of bentonite clay and humic acid increases the concentration of organic carbon in the soil, microbial biomass, enzyme activities, and germination quality in sandy soil. Increasing organic carbon in the soil leads to enhanced soil fertility and enhances plant growth [17]. Studies have shown that the addition significantly enhances the growth and flowering of Catharanthus roseus L. in sandy soil by improving soil structure, nutrient retention, and microbial activity, as well as increasing flower growth and most plant characteristics [18, 19]. Treatment with bentonite clay and humic acid increases biomass and soil microbes. Increasing the number of microbes is beneficial because it contributes to nutrient cycling and soil health in general [20]. Increasing enzymatic activities improves the synthesis and breakdown of organic materials and facilitates the availability of nutrients to plants. This indicates an improvement in the biological activity of the soil [21]. The quality of germination in potting soil is greatly affected by the type of grain, and the germination rate often does not reach 100%. The use of bentonite and humic improvers increases the germination rate of seeds in sandy soil, leading to reduced losses and increased profits [21].The research aims to study the role of Al-bentonite clay and Al-humic acid improvers in the growth and flowering of *Catharanthus roseus* L. plants in sandy soil.

2. Material and Methods

The study was conducted during one agricultural season in one of the nurseries of the City Diwaniyah, located in the center of the Governorate, in a house covered with green saran The cultivation was done in a house covered with green sorghum, and the shade percentage was 25%. A sandy soil type (river soil) with a sandy texture was chosen. The physical and chemical properties of the soil were estimated as in **Table 1** according to Richards [22] or Page, Miller and Kenney [23]. Local seeds were planted in plastic agricultural dishes with dimensions of 55*28 cm containing river soil and peat moss. **Tables 2** and **3** show some properties of peat moss and bentonite. After a period, the seeds germinated and the seedlings reached a height of 4–5 cm and the appearance of true leaves. They were carefully separated, and transferred at a rate of one plant per plastic container containing a growing medium prepared from peat moss and soil, as in **Figure 1**.

2.1. Study Factors

He studied two factors in experiment (first refers to the addition of Bentonite improver at 4 concentrations (0, 2, 4, 6 g L^{-1}); the second was adding four concentrations of humic acid, which were (0, 0.5, 5, 10) g L^{-1}. The first addition was made twenty days after transferring plants from the plastic dishes to the pots (separating plants), and the second was made 21 days after the first addition. The treatments were applied in a factorial experiment using a "randomized complete block design" with two factors and three replicates. Each replicate contains 16 treatments (4*4*3 = 48 treatments). The experimental unit includes 3 pots of plants. The total number of pots for the experiment was 144. Analysis of variance was conducted, and means were compared according to the Least Significant Difference test at the 0.05 level using program VSN [24].

Table 1. Chemical and physical properties of soil.

Property	Value	Unit
pH	7.01	
EC	3.25	ds m^{-1}
N P K	18.20 12.45 10.50	mg kg^{-1}
$CaCO_3$ OM	180 17	gm kg^{-1}
CEC	9.22	$Cmol^{+}kg^{-1}$
Texture	Sandy loamy	
Particle Size Analysis		
Clay Slit Sand	105 155 740	gm kg^{-1}

Figure 1. The stages of plant germination.

Table 2. properties of peat mousse.

Property	Value	Unit
pH	7.82	-
Ec	2.63	ds m^{-1}
N	140	
P_2O_5	160	
K_2O	180	mg kg^{-1}
S	120	
Mg	100	

Table 3. Chemical and physical properties of bentonite.

Property	Bentonite Value	Unit
pH	6.85	
EC	2.90	ds m^{-1}
$CaCO_3$	140	gm kg^{-1}
CEC	70.50	$Cmol^{+}kg^{-1}$
Texture	Clay	
Particle Size Analysis		
Clay	920	
Slit	65	gm kg^{-1}
Sand	5	

2.2. Study Indicators

2.2.1. Plant Height (cm)

Plant height was measured with a tape measure from the soil surface of the pot, up to the highest end of the shoot, and for all treatment plants at the end of flowering for each experimental unit, from which the rate was calculated for each treatment.

2.2.2. Number of Leaves per Plant (Leaf $Plant^{-1}$)

The number of leaves for each experimental unit was calculated, and the average values for each treatment were calculated.

2.2.3. Leaf Area (cm^2 $Plant^{-1}$)

Average leaf area was calculated by the method used in Reference[25]. It was photographed using a scanner, and transferred to the Digitizer program loaded on the computer, by taking three full-length leaves from each plant for treatment.

2.2.4. Leaf Content of Total Chlorophyll (mg $100g^{-1}$)

The chlorophyll content of leaves was estimated according to the method of Reference[26], where a sample of fresh leaves weighing 0.5 grams was taken from the fourth leaf of the plant and 10 ml of acetone at a concentration of 80% was added to it. A ceramic mortar was used and the tissue was completely crushed, and then 10 ml of acetone was added to it. If the tissue becomes white, the solution is filtered using Whatman No.1 filter paper, then the volume is increased to 30 ml and the UV-Visible Spectrophotometer is used to measure the optical absorption of dyes at the two wavelengths 645 and 663 nm in the Postgraduate Studies Laboratory, College of Agriculture, University of Al-Qadisiyah. It was calculated by applying the following equation:

Total chlorophyll = 20.2 × D645 + 8.02 × D663 (V/W × 1000) 100

D = optical absorption

V = final volume of extract

W = weight of paper tissue (g)

D663 = optical absorbance reading at wavelength 663 nm

D645 = optical absorbance reading at wavelength 645 nm.

2.3. Leaves Content Elements

2.3.1. Nitrogen (N)

Nitrogen was estimated according to method of Reference[27] using a Kjeldahl device. 10 ml of the digested sample was taken and placed in a reaction flask. 10 ml of sodium hydroxide (NaOH) was added at a concentration of 40%. The ammonia liberated after the distillation process was collected in a glass flask containing 20 ml of acid. The concentration of boric acid was 2%, and two drops of the indicator mixture Methyl Red and Bromocresol Green were added, and then the ammonia collected in the receiving flask was ground with acid HCL, which was measured at (0.01), and the following equation was applied: N%=

Volume of Acid Consumed dilution X Standard of acid X 14 X volume of dilution

---

X 1000

Volume of Sample taken during distillation X Weight of digested sample X1000

2.3.2. Phosphorus (P)

Phosphorus was determined by taking 10 ml of the digested sample and placing it in a 50 ml volumetric flask. Bring the volume to the mark by adding distilled water, then withdraw 10 ml of the previous solution, add 0.1 g of ascorbic acid and 4 ml of ammonium moly date. Heat the flask to boiling, then the color of the solution changed to blue and the contents of the beaker were transferred to another 100 ml volumetric beaker, the volume was completed to the mark with distilled water, and the samples were measured by a UV-Visible Spectrophotometer, at a wavelength of 420 nm[27] in the Graduate Laboratory.

2.3.3. Potassium (K)

The potassium content of the leaves was estimated according to the method[27, 28]. 10 ml of the digested sample was taken, diluted in a 50 ml glass beaker, supplemented to the mark with distilled water, and the sample was read in a flame photometer, and a series of concentrations of standard potassium were prepared.

2.4. Flower Content of Anthocyanin (mg 100g^{-1})

Anthocyanin pigment in flower petals was estimated by Reference[29], where 1 g of fresh flower petals was taken and extracted in a solvent consisting of ethyl alcohol and HCl (1.5N) at a ratio of 85:15, and 10 ml of the mixture was added and crushed using a ceramic mortar, then 10 ml of the mixture was added and the solution was filtered using filter paper and the volume was completed to 30 ml and the samples were read using a UV-Visible Spectrophotometer, at a wavelength of 535 nm and calculated using the following equation in the graduate studies laboratory, College of Agriculture, Al-Qadisiyah University (amount of anthocyanin = volume of the solution used × extraction × dilutions × 100).

3. Results and Discussion

3.1. Plant Height

The results in **Table 4** demonstrate that adding clay bentonite 6 gm L^{-1} increased plant height (30.07 cm) compared to control (12.02 cm). Findings additionally demonstrated that the applying humic acid 6 gm L^{-1}, resulted in a considerable increase in plant height (23.84 cm), Compared to control (18.00 cm). While the interaction findings showed that adding bentonite 6 gm L^{-1}, humic acid 10 gm L^{-1}, resulted in a substantial increase in plant height (37.52 cm) compared to control (11.11 cm).

Table 4. Effect of bentonite clay and humic acid on plant height (cm) of *Catharanthus roseus* L.

Treatment		Humic Acid (gm L^{-1}) 0	0.5	1	10	Mean Bentonite
Bentonite (gm L^{-1})	0	11.11	10.69	12.20	14.09	12.02
	2	14.27	16.35	19.47	19.96	17.51
	4	21.48	21.67	22.38	23.81	22.33
	6	25.14	27.52	30.11	37.52	30.07
Mean humic acid		18.00	19.06	21.04	23.84	
L.S.D (0.05)		Bentonite	0.395			
		Humic acid	0.395			
		Bentonite × Humic acid	0.790			

3.2. Number of Leaves Plant

Table 5 shows that adding bentonite clay 6 gm L^{-1}, resulted in an increase of (76.62 leaf plant^{-1}) compared to control (34.82 leaf plant^{-1}). Additionally, adding humic acid 10 gm L^{-1} resulted in a significant increase in plant height,, reaching 63.42 leaf plant^{-1}, compared to control (54.35 leaf plant^{-1}). As a consequence, interaction findings revealed that adding bentonite 6 gm L^{-1} and humic acid 10 gm L^{-1} resulted in a substantial increase in plant height (83.37 leaf plant^{-1}) when compared to the control (30.00 leaf plant^{-1}).

3.3. Leaf Area Plant

Table 6 shows that adding clay bentonite 6 gm L^{-1}, increased the leaf area of plant (24.73 cm^2 plant^{-1}), compared to control (10.23 cm^2 plant^{-1}). Results also revealed that adding humic acid 10 gm L^{-1} resulted in a considerable increase in plant height (20.22 cm^2 plant^{-1}),when compared to the control treatment's (16.16 cm^2 plant^{-1}), As a consequence, interaction findings revealed that mixing bentonite 6 gm L^{-1} and humic acid 10 gm L^{-1} resulted in a substantial increase in plant height (27.05 cm^2 plant^{-1}), as compared to the control (8.22 cm^2 plant^{-1}).

Table 5. Effect of bentonite clay and humic acid on the number of leaves (leaf plant^{-1}) of *Catharanthus roseus* L.

Treatment		Humic Acid (gm L^{-1})				Mean Bentonite
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	30.00	33.44	36.44	39.41	34.82
	2	52.47	55.44	57.23	60.41	56.38
	4	62.56	65.45	68.83	70.49	66.83
	6	72.38	74.03	76.71	83.37	76.62
Mean humic acid		54.35	57.09	59.80	63.42	
L.S.D (0.05)		Bentonite	0.376			
		Humic acid	0.376			
		Bentonite × Humic acid	0.752			

Table 6. Effect of bentonite clay and humic acid on leaves area plant (cm^2 plant^{-1}) of *Catharanthus roseus* L.

Treatment		Humic Acid (gm L^{-1})				Mean Bentonite
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	8.22	9.71	10.86	12.15	10.23
	2	12.96	14.77	17.40	18.77	15.89
	4	20.88	21.65	23.24	22.93	22.17
	6	22.57	24.19	25.11	27.05	24.73
Mean humic acid		16.16	17.58	19.15	20.22	
L.S.D (0.05)		Bentonite	0.350			
		Humic acid	0.350			
		Bentonite × Humic acid	0.700			

3.4. Leaves Content Elements NPK %

The results in **Tables 7–9** demonstrated that adding bentonite clay 6 g L^{-1} increased the plant's leaf content elements NPK, as it reached (2.27, 1.92, 1.99)%, compared to the comparison treatment's (1.13, 0.18, 0.79)%, Additionally, the results demonstrated that adding humic acid at 10 g L^{-1} led to a significant increase in the plant's leaf content elements NPK, as it reached (1.51, 1.22, 1.77)%, respectively. compared to the comparison (1.07, 0.88, 1.50)%. Consequently, the interaction demonstrated that adding bentonite 6 g mL^{-1}, with humic acid 10 g mL^{-1}, resulted in increase the content of leaves elements NPK (2.10, 1.22, 1.77)% compared to comparison, which amounted to (0.36, 0.36, 1.06)%.

Table 7. Effect of bentonite clay and humic acid on the (N%) of *Catharanthus roseus* L.

Treatment		Humic Acid (gm L^{-1})				Mean Bentonite
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	0.36	0.40	1.30	1.11	0.79
	2	0.84	0.33	1.40	1.10	0.92
	4	1.33	1.16	1.72	1.73	1.48
	6	1.75	2.03	2.08	2.10	1.99
Mean humic acid		1.07	0.98	1.62	1.51	
L.S.D (0.05)		Bentonite	0.270			
		Humic acid	0.270			
		Bentonite × Humic acid	0.540			

Table 8. Effect bentonite clay, humic acid, their (P%) *Catharanthus roseus* L.

Treatment		Humic Acid (gm L^{-1})				Mean Bentonite
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	0.36	0.10	0.12	0.13	0.18
	2	0.43	1.12	1.14	1.18	0.96
	4	1.25	1.27	1.34	1.38	1.31
	6	1.47	1.88	2.13	2.19	1.92
Mean humic acid		0.88	1.09	1.18	1.22	
L.S.D (0.05)		Bentonite	0.155			
		Humic acid	0.155			
		Bentonite × Humic acid	0.311			

Table 9. Effect bentonite clay, humic acid, their (N%) *Catharanthus roseus* L.

Treatment		Humic Acid (gm L^{-1})				Mean Bentonite
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	1.06	1.12	1.14	1.19	1.13
	2	1.24	1.29	1.34	1.39	1.32
	4	1.49	1.71	2.11	2.16	1.86
	6	2.21	2.26	2.29	2.34	2.27
Mean humic acid		1.50	1.59	1.72	1.77	
L.S.D (0.05)		Bentonite	0.050			
		Humic acid	0.050			
		Bentonite × Humic acid	0.100			

3.5. Leaf Content of Total Chlorophyll

Table 10's results showed that adding clay bentonite increased the amount of total chlorophyll in leaves when applied at a rate of 6 gm L^{-1}, or 23.74 mg $100g^{-1}$, in comparison to 12.25 mg $100g^{-1}$ of the control treatment. The results also demonstrated that, in comparison to the control (16.54 mg $100g^{-1}$), adding humic acid at 10 g mL^{-1} , significantly increased plant height, reaching 19.89 mg $100g^{-1}$. Thus, findings of the interaction indicated that adding bentonite at 6 g mL^{-1} along with humic acid at 10 g mL^{-1} resulted in a significantly higher plant height (26.92 mg $100g^{-1}$) than the control (0.82 mg $100g^{-1}$).

3.6. Flower Content of Anthocyanin

The results in **Table 11** showed that adding clay bentonite increased floral content of total anthocyanin (5.49 mg $100g^{-1}$), compared to the control (2.60 mg $100g^{-1}$). The results also revealed that adding humic acid 10 g mL^{-1} resulted in a substantial increase in plant height (4.22 mg $100g^{-1}$), when compared to the control (3.51 mg $100g^{-1}$). As a result, interaction findings revealed that combining bentonite 6 g mL^{-1} and humic acid 10 g mL^{-1} , resulted in a substantial increase in plant height (6.14 mg $100g^{-1}$) when compared to the control (2.15 mg $100g^{-1}$).

4. Discussion

We note from **Tables 4–6** that the addition of bentonite clay and humic acid achieved an increase in the vegetative growth characteristics (plant height, number of leaves, and leaf area) of *Catharanthus roseus* L. This increase is due to the fact that the addition of bentonite improved the physical, chemical and biological properties of the soil, including cation exchange [30], Meanwhile, the addition of humic acid activated physiological processes and caused an increase in vegetative growth, which led to increased plant, height and number of plant leaves. Humic acid is distinguished by its ability to provide an acidic medium that helps bind with positive ions and protects them from precipitation. It also plays an important role in the permeability of the cell membrane and its importance in the transport and readiness

Table 10. Effect of bentonite clay and humic acid on the leaf content of total chlorophyll (mg $100g^{-1}$) of *Catharanthus roseus* L.

Treatment		**Humic Acid (gm L^{-1})**				**Mean Bentonite**
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	10.82	11.87	12.78	13.52	12.25
	2	14.34	16.40	16.82	17.85	16.35
	4	19.22	19.18	20.25	21.29	19.98
	6	21.80	22.47	23.77	26.92	23.74
Mean humic acid		16.54	17.48	18.41	19.89	
L.S.D (0.05)		Bentonite	0.226			
		Humic acid	0.226			
		Bentonite × Humic acid	0.452			

Table 11. Effect of bentonite clay and humic acid on the flower content of total anthocyanin (mg $100g^{-1}$) of *Catharanthus roseus* L.

Treatment		**Humic Acid (gm L^{-1})**				**Mean Bentonite**
		0	**0.5**	**1**	**10**	
Bentonite (gm L^{-1})	0	2.15	2.28	2.85	3.13	2.60
	2	3.25	3.32	3.37	3.41	3.34
	4	3.52	3.88	4.14	4.20	3.93
	6	5.13	5.19	5.52	6.14	5.49
Mean humic acid		3.51	3.67	3.97	4.22	
L.S.D (0.05)		Bentonite	0.108			
		Humic acid	0.108			
		Bentonite × Humic acid	0.217			

of micronutrients and nutrient absorption [31]. It also has a role in modifying the degree of soil interaction and increasing the readiness of nutrients, which have an important role in plant nutrition. It increases the activity of microorganisms and transforms them into forms from which the plant benefits, and thus increases indicators of vegetative growth, including plant height, number of leaves, and increase in leaf area [32]. While **Tables 7–9** show that the significant response to adding bentonite and humic acid in chemical indicators is due to the role of these improvers by increasing the nitrogen and carbon elements and reducing the degree of reaction, which helps in the growth of the root system and thus increases the absorption of nutrients [33]. Bentonite, which is a source of montmorillonite, works to increase the specific surface area, which contributes to improving soil fertility and readiness plant nutrients [34]. The reason for the increase in phosphorus, is due to addition of bentonite clay and humic, which led to improving the enzymatic properties in the soil, including the phosphate enzyme in soil, which releases phosphorus and improves its availability in soil [35].

The reason is the increase in potassium in the plant in **Table 10**, which is due to the ability of humic acid to stimulate nitrogen-fixing isobacteria and increase surface area root hairs, which leads to absorption of potassium and thus improves plant growth through the secretion of chemical compounds that improve plant growth, such as the secretion of cytokinins [19, 36]. **Tables 10** and **11** showan increase in chlorophyll, and anthocyanins. This is due to the increase in nitrogen concentration in leaves, which led to the absorption of elements from humic acid, resulting in higher concentrations in the plant and increased vital activitiest, such as enhanced photosynthesis, which leads to an increase in chlorophyll and thus an increase in plant's need for the elements [37–39].

5. Conclusions

The study indicates the significant effect of bentonite clay and humic acid on the absorption of nutrients in the pink *Catharanthus plant* L., specifically regarding the interference parameters. Adding a mixture of bentonite clay and humic acid led to a significant increase in plant growth (increased height, number of leaves, and surface area of leaves), as well as improving the chemical characteristics of the plant (increased leaves' content of chlorophyll, increased leaves' content of nitrogen, phosphorus, and potassium, and increased flowers and their content of anthocyanin pigment), compared to the non-additive agent. The study indicates that these environmentally friendly natural amendments, such as bentonite clay, can work in a better way. It is effective in improving the absorption of nutrients from the soil, which is reflected in plant growth, and reduces the use of chemical fertilizers and pesticides that cause effects on the environment. The study also showed that these amendaments can serve as a viable alternative to chelators, which cause many side effects.

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision R.F.S.; Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision M.M.H.

Funding

This work received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Al-Batal, N.N., 2010. Outdoor ornamental plants. Damascus University Publications, Faculty of Agriculture, Al-Rawda Press: Damascus, Syria. pp. 20–28.

[2] Al-Shahat, N.A.Z., 2002. Cultivation and production of flower and ornamental plants. Arab House for Publishing and Distribution: Cairo. Arab Republic of Egypt. pp. 15–22.

[3] Al-Khayat, N.K., 2018. Herbaceous Flowers. World of Knowledge for Printing and Publishing, College of Agriculture, University of Baghdad: Baghdad, Republic of Iraq. pp. 13–26.

[4] Soil Survey Staff, 1999. Soil Taxonomy: A basic system of soil classification for making and interpreting soil surveys, 2nd ed. USDA Natural Resources Conservation Service: Washington, DC, USA. pp. 50–66.

[5] Al-Janabi, H.Y., 2016. Applied land reclamation. Al-Qasim Green Univiversity Printing Press, Faculty of Agriculture: Babylon, Iraq. pp. 1–245.

[6] Karbout, N. Mlih, R., Latifa, D., et al., 2021. Farm manure and bentonite clay amendments enhance the date palm morphology and yield. Arabian Journal of Geosciences. 14, 818–825. DOI: https://doi.org/10.1007/s12517-021-07160-w

[7] Lerma, T.A., Chamorro, A.F., Palencia, M., 2024. Effect of soil conditioners based on geomimetic materials on plant growth in degraded soils: Poly (acrylic acid)/bentonite. Journal of Environmental Chemical Engineering. 12(5), 113567.

[8] Al-Mishyikh, S.H., Jaralla,A.K.A., 2023. Effect of biochar and bentonite application in availability and uptake of N, P and K for faba bean in desert soil. Iraqi Journal of Desert Studies. 13(1), 76–84. DOI: https://doi.org/10.36531/ijds.2023.139203.1032

[9] Borah, D., Nath, H., Saikia, H., 2022. Modification of bentonite clay & its applications: A review. Reviews in Inorganic Chemistry. 42(3), 265–282.

[10] Ali, M.A., Mohamed, H.M., Elsayed, S.A., et al., 2023. Effect of integrate water shortage and soil conditioners on water productivity, growth, and yield of Red Globe grapevines grown in sandy soil. Open Agriculture. 8(1), 20220240.

[11] Kumari, N., Mohan, C., 2021. Basics of clay minerals and their characteristic properties. In: Nascimento, G.M.D. (ed.). Clay and Clay Minerals. IntechOpen: London, UK. pp. 1–70. DOI: https://doi.org/10.5772/intechopen.97672

[12] Kamińska, J., Puzio, S., Angrecki, M., et al., 2020. The effect of the addition of bentonite clay to traditional sand mixtures on the surface quality of iron castings. Journal of Ecological Engineering. 21(1), 160–167.

[13] Al shammari, A.H.A., Al-Wotaify, A.S.S., 2024. The Effect of Bentonite, poultry manure and elements on some chemical properties of sandy calcareous soil. IOP Conference Series Earth and Environmental Science. 1371(8), 082049.

[14] Abulimiti, M., Wang, J., Congjuan, L., et al., 2023. Bentonite could be an eco-friendly windbreak and sand-fixing material. Environmental

Technology and Innovation. 29, 102981. DOI: https://doi.org/10.1016/j.eti.2022.102981
[15] Chen, G.Q., Wei, K., Hassanvand, A., et al., 2020. Single and binary ion sorption equilibria of monovalent and divalent ions in commercial ion exchange membranes. Water Research. 175, 115681.
[16] Yongxu, J., Yuan, Y., Liu, Z., et al., 2024. Effect of humic substances on nitrogen cycling in soil-plant ecosystems: Advances, issues, and future perspectives. Journal of Environmental Management. 351, 119738. DOI: https://doi.org/10.1016/j.jenvman.2023.119738
[17] Zhang, Y.L., Zhen, Q., Cui, Y.X., et al., 2020. Use of montmorillonite-enriched siltstone for improving water condition and plant growth in sandy soil. Ecological Engineering. 145, 105740. DOI: http://dx.doi.org/10.1016/j.ecoleng.2020.105740
[18] Vembu, G., Singaravel, R., 2013. Effect of NPK levels on the nutrient content and uptake of periwinkle [*Catharanthus roseus* (L) G. Don] in coastal sandy soil. Plant Archives. 13(2), 969–971.
[19] Al-Taey, D.K., Hussain, A.J., Khadum, H., 2023. Bentonite impact on soil properties and biological activity in the face of drought : A review. IOP Conference Series Earth and Environmental Science. 1262(4), 042058. DOI: https://doi.org/10.1088/1755-1315/1262/4/042058
[20] Daunoras, J., Kačergius, A., Gudiukaitė, R., 2024. Role of soil microbiota enzymes in soil health and activity changes depending on climate change and the type of soil ecosystem. Biology. 13(2), 85. DOI: https://doi.org/10.3390/biology13020085
[21] Ibraheem, F., Abu-Ria, M.E., Abo-Hamed, S.A., et al., 2023. Humic acid improves germination, vegetative growth, and yield in rice (*Oryza sativa* L.) under salinity stress. The Egyptian Society for Environmental Sciences. 27(1), 75–91.
[22] Richards, L.A., 1954. Diagnosis and improvement of saline and alkaline soil. Soil Science Society of America Journal. 18, 348.
[23] Page, A.L., Miller, R.H., Kenney, D.R., 1982. Methods of soil analysis: Chemical and microbiological properties, 2nd ed. American Society of Argonomy: Madison, WI, USA. pp. 1–1159.
[24] Al-Rawi, K.M., Khalaf, A.A., 2000. Design and analysis of agricultural experiments. Dar Al-Kutub for Printing and Publishing, University of Mosul, Ministry of Higher Education and Scientific Research: Mosul, Republic of Iraq. pp. 33–43.
[25] O'Neal, M.E., Landis, D.A., Isaacs, R., 2002. An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. Journal of Economic Entomology. 95(6), 1190–1194.
[26] Goodwin, T.W., 1976. Chemistry and biochemistry of plant pigments, 2nd ed. Academic Press: New York, NY, USA. pp. 1–373.
[27] Al-Sahaf, F.H., 1989. Applied plant nutrition. University of Baghdad, Ministry of Higher Education and Scientific Research: Baghdad, Republic of Iraq. pp. 50–72.
[28] Horneck, D.A., Hanson, D., 1998. Determination of potassium and sodium by flame emission spectrophotometer. In: Karla, Y.P. (ed.). Handbook of Reference Methods for Plant Analysis. CRC Press: Boca Raton, FL, USA. pp. 153–155.
[29] Abbas, M.F., Abbas, M.J., 1992. Practical care and storage of fruits and vegetables. College of Agriculture. University of Basra. Ministry of Higher Education and Scientific Research: Basra, Republic of Iraq.
[30] Minhal, F., Ma'as, A., Hanudi, E., et al., 2020. Improvement of the chemical properties and buffering capacity of coastal sandy soil as affected by clay and organic by-product application. Soil and Water Research. 15, 93–100.
[31] Olk, D.C, Dinnes, D.L., Scoresby, J.R., et al., 2018. Humic products in agriculture: Potential benefits and research challenges a review. Journal of Soils and Sediments. 18(8), 2881–2891.
[32] Bhatla, S.C., Lal, M.A., 2018. Plant physiology, development and metabolism. Springer: New York, NY, USA. pp. 66–80.
[33] A-Sharaa, M.A., 2020. The effect of biofertilizers and humic acid on the growth of *Ranunculus asiaaticus* Chinon L. [Master's Thesis]. Karbala, Republic of Iraq: Al-Qasim Green University. pp. 70–45.
[34] Al-Watifi, A.S.S., Al-Jubouri, R.F.S., 2015. The effect of brucite layer deposition on the transformation of expanded clay minerals into chlorite in sabkha soils of the cities of Diwaniyah and Hillah-Iraq. Al-Qadisiyah Journal of Agricultural Sciences. 5(2), 39–45.
[35] Chen, L., Chen, X.L., Zhou, C.H., et al., 2017. Environmental-friendly montmorillonite-biochar composites: Facile production and tunable adsorption-release of ammonium and phosphate. Journal of Cleaner Production. 156, 648–655. DOI: https://doi.org/10.1016/j.jclepro.2017.04.050
[36] Nofal, E.M.S., Fardous, A.M., Abd El-Hardy, W.M., et al., 2021. Effect of bio-stimulants (humic acid, salicylic acid and chitosan) on rose periwinkle (*Catharanthus roseus* L.). Applied Ecology & Environmental Research. 19(2), 971–980.
[37] Lerma, T.A., Chamorro, A.F., Palencia, M., 2024. Effect of soil conditioners based on geomimetic materials on plant growth in degraded soils: Poly (acrylic acid)/bentonite. Journal of Environmental Chemical Engineering. 12(5), 113567.
[38] Mi, J., 2017. Effect of bentonite amendment on soil hydraulic parameters and millet crop performance in a semi-arid region, Field Crop. Res.
[39] Salih, A.A., Moalla, M., Harba, N., 2020. Heterosis and combining ability for some quantitative Traits in Faba bean (*Vicia faba* L.). Syrian Journal of Agricultural Research. 7(5), 243–255.