Effect of spraying benzyl adenine and chitosan on some chemical traits and Oil quantity in the leaves of Duranta erecta L.

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Abstract

The experiment was conducted in lathhouse of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Basra during the two agricultural seasons (2022 and 2023) on Duranta plants, for the purpose of knowing the effect of spraying with benzyl adenine at three concentrations of 0, 75, and 150 mg L-1 and chitosan at three concentrations of 0, 100, and 200. mg L-1 on some chemical traits of Duranta leaves. The research was conducted following a completely randomized design for factorial experiments, using two agents: benzyl adenine and chitosan, with three concentrations of each and three repetitions of the treatment, so that the number of experimental units reached 27 experimental units. The least significant difference (L.S.D.) test was used. At a level of significance (0.05) to compare the means, the results showed that the plants sprayed with benzyl adenine at a concentration of 150 mg L-1 were significantly superior in increasing the leaves' total content of chlorophyll pigment, reaching (131.757 and 146.044) mg 100 g-1, and also increasing the leaves' content of carbohydrates. The total solutes amounted to (164,421 and 217,067) mg L-1, and the total carotenoids content of the leaves amounted to (14,850 and 20,263) mg L-1 and the amount of oil in the leaves amounted to (0.1197, 0.1252) g, and its percentage was (1.1967, 1.2524)%. respectively, for both seasons of the study. Also, chitosan at a concentration of 200 mg L-1 gave the highest total chlorophyll content in the leaves. The total soluble carbohydrate content was (179,677 and 212,413) mg 100 g-1, and the total carotenoids content of the leaves was (15,008 and 20,827) mg 100 g-1, respectively, for both seasons.

Keywords: benzyl adenine, chitosan, durnata, chlorophyll, total soluble carbohydrates, carotenoids, Oil quantity

Introduction

Duranta erecta L. is one of the evergreen shrubs belonging to the Verbenaceae family. It is native to the Americas. It is widely grown as an ornamental plant and also used as a hedge plant. The Duranta plant is of great importance due to the diversity of its traits and uses, as it is considered an important tree. It has economic and medicinal value, as it is used as an antiseptic, anti-inflammatory, antimicrobial, and pain reliever. The Duranta plant is also characterized by containing many vital compounds such as alkaloids, flavonoids, and glycosides, which contribute to its therapeutic benefits (8). Therefore, this plant is considered dual-purpose. On the one hand, it It is considered an ornamental plant, and on

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the other hand, it is considered a medicinal plant. The main goal of growing medicinal crops is to obtain a high content of biologically active substances known as secondary compounds (23). The production of these active substances is affected by many factors, including the addition of fertilizers. Biotechnology and spraying with biostimulants and growth regulators (5,21), and the success of cultivating these plants depends on providing these stimulants to obtain good vegetative growth, which is reflected in increasing and improving the production of active substances within the plant. One of the most important of these stimulants is benzyl adenine, which is one of the derivatives of cytokines and has a major role in many plants, especially ornamental and medicinal plants, as it stimulates cell division and increases the efficiency of photosynthesis by increasing the total number of leaves and their surface area (18), as well as Its role in plant growth and development, such as delaying senescence, controlling the balance of branches/roots, and stimulating the growth of lateral branches by breaking the apical dominance. It also works to encourage the formation of chlorophyll and prevent it from being destroyed (6), and works to activate RNA and enzymes necessary for the reactions. Vitality and increases the synthesis of proteins, which increases the transfer of nutrients to active tissues and their assimilation (1, 15). Many studies have confirmed that spraying benzyl adenine on ornamental plants and its interaction with other growth regulators within the plant contributed to improving vegetative and flowering growth and medically active substances within the plant. (11) found that when spraying Abu Khanjar plants with four concentrations of benzyl adenine (0, 200, 250, 300) mg L-1, the concentration exceeded 250 mg L-1 and gave the highest total chlorophyll content in the leaves, reaching (4.83 and 5.13) mg g-1, and the content of carotenoids in the leaves amounted to (14.09 and 14.01)% for both growing seasons (2021 and 2022), respectively, while control treatment gave the lowest total chlorophyll content in the leaves, amounting to (4.63 and 4.95) mg g-1 The content of carotenoids in the leaves reached (12.81 and 12.91)% for both growing seasons, respectively.

Chitosan is a substance extracted from chitin, which is a series of cyclic carbohydrates. Commercial production of chitin and chitosan began in Australia, India, the United States of America, Japan, Poland, Thailand, and Norway (9). It is found naturally in some microorganisms and yeast. Chitosan is It is the most abundant sugar after cellulose in terms of use and spread (3). It is used in sustainable agriculture instead of pesticides because it controls viral, bacterial, and fungal pathogens in soil and plants (24). It works to increase the absorption of Nutrients, increasing the process of photosynthesis and building phenols, lignin, and flavonoids (20), (19) noted that spraying chitosan at a concentration of 300 mg L-1 on Duranta plants gave the highest chlorophyll content in the leaves, reaching 1.319 and 1.383. mg. Using four concentrations of chitosan (0, 100, 200, 400) ppm as a spray on the shoots of lavender plants (Lavandula officinalis, Chaix.), the concentration exceeded 400 ppm and gave the highest chlorophyll content in the leaves, reaching 52.72 and 52.06 SPAD for both growing seasons. (2018 and 2019), respectively, while control treatment gave the lowest chlorophyll content in the leaves, amounting to 46.89 and 46.36 SPAD for both growing seasons, respectively. It also gave the highest total

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carbohydrates in the leaves, amounting to 16.91 and 17.44% for both seasons, respectively, while the values were the lowest. When treating the comparison, it was 14.78 and 15.30% for both seasons, respectively.The research aims to determine the extent of the effect of different concentrations of benzyl adenine and chitosan on the success of growing the Duranta plant and improving its chemical traits under the environmental conditions of southern Iraq.

Materials and methods:

The experiment was conducted in the canopy of the Department of Horticulture and Landscape Engineering, College of Agriculture / University of Basra during the two seasons (2022 and 2023) on Duranta plants, for the purpose of knowing the effect of spraying different levels of benzyl adenine (0, 75, 150) mg L-1 and chitosan at levels (0, 100, 200) mg L-1 in growth traits. Some chemical traits of the leaves of the Duranta plant. Seedlings were brought from one of the private nurseries in Baghdad at one year old and transferred to plastic anvils with a diameter of 25 cm, filled with a medium consisting of 2 mol + 1 peat moss, and several random samples were taken. of the soil before planting, and some chemical and physical traits of the potting soil were estimated in the central laboratory of the College of Agriculture / University of Basra. Table (1.(

Table 1. Some primary traits of the soil used in the study

Particle size					**EC**	**O.M**	**N**	**P**	**K**
Clay (%)	Silt (%)	Sand (%)	**Texture**	**PH**	dsm^{-1}	%	$mg\ l^{-1}$		
10.0	8.0	82.0	Loamy Sand	7.34	1.0	26	53	26.3	66.4

Studied traits:

Total chlorophyll content of leaves (mg 100 g-1 fresh weight(

The total chlorophyll pigment in the leaves was estimated according to the method (13) by taking 0.5 g, adding 10 ml of 80% acetone, crushing the tissue with a ceramic mortar until the tissue was white, then filtering it using filter paper. The optical absorption of the chlorophyll pigment was measured using a spectrophotometer at two wavelengths: (645 and 663) nanometers, and the following equation was applied to calculate the amount of total chlorophyll in the leaves (mg 100 g-1(

Total chlorophyll = 20.2 x D(645) + 8.02 x D(663) (v/w x 1000(

D (663) = Optical absorption reading at a wavelength of 663 nm

D(645) = Optical absorption reading at a wavelength of 645 nm

V = final volume of extract. W = weight of soft tissue in grams

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Leaves content of total soluble carbohydrates (mg 100g-1 dry weight(

It was estimated using the Modification of Phenol- Sulfuric Acid Colorimetric Method described by (10) by taking the weight of dried leaves after grinding them using an electric grinder. By taking 0.5 g of dry leaf sample for each experimental unit and placing it in a 100 ml volumetric flask. Adding 70 ml of distilled water to it and placing it in a water bath at 70°C for an hour and then cooling it at room temperature. The solution was filtered with filter paper, and a volume of 5 ml was taken from the filtrate and 25 ml of distilled water was added to it. Then 1 ml of it was taken and 1 ml of phenol (5%) and 5 ml of concentrated sulfuric acid were added to it and cooled at room temperature. The absorbance was measured at a wavelength of 490 nm with a Spectrophotometer device .

Total soluble carbohydrates were estimated using the glucose standard curve.

Total carotenoids content of leaves (mg 100 g-1 fresh weight(

The total carotene pigment in the leaves of the Duranta plant was estimated by taking 0.5 gm and adding 10 ml of 80% acetone to it. It was crushed with a ceramic mortar until the texture turned white, then filtered using filter paper. The optical density of the pigment was measured using a spectrophotometer at a wavelength of 480 nm. The total amount of carotenoids was calculated by applying the equation

X=EYe 100 x 1000

Since:

X: number of milligrams of carotene. Y: volume of final solution after dilution with acetone. e: carotene constant, equal to 2500. The results were then converted to units (mg 100 g-1.(

Oil extraction:

Samples (leaves) were taken from the field, washed from dust, the leaves were dried at room temperature, ground, and a weight of 10 grams was taken and the sample was weighed using a sensitive balance. 10 grams of the sample was taken and placed in the extraction thimble using the Soxhlet extractor device at a temperature of 50 degrees Celsius, and 150 grams were added to it. ml of petroleum solvent, then the device was operated for 3 hours and concentrated using a rotary evaporator.

The amount of fixed oil in the leaves and its percentage:

Quantity of oil: (g): Calculated by subtracting the weight of the container with oil from the weight of the empty container

The percentage of oil was estimated according to what was stated (2) according to the equation:

Percentage of fixed oil = amount of oil / weight of leaf sample x 100

statistical analysis:

The experiment was carried out according to a Randomized Complete Block Design (R.C.B.D) and in a factorial experiment with two factors: the first represents the spraying of benzyl adenine at three concentrations (0, 70, 150) mg L-1 and the second factor represents the spraying of chitosan at three concentrations (0, 100, 200).) mg L-1, the number of experimental treatments was (9) factorial treatments, the number of replicates for each treatment was 3 replicates, and the total number of experimental units was (27) experimental units. The statistical analysis of the experiment data was done using the Genestat statistical program, and the Least Significant Differences (L.S.D) test was used at the probability level of 0.05 to compare the averages of the coefficients.

Results and Discussion:

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Chemical properties of Duranta leaves:

The results in Table (2) show that the effect of spraying with benzyl adenine was significant on the leaf content of total chlorophyll, total soluble carbohydrates, and total carotenoids. When comparing the averages of the treatments, we note that there are significant differences between them (Table 2), as the 150 mg L-1 treatment recorded the highest The chlorophyll content in the leaves reached (131,757 and 146,044) mg 100 g-1, respectively, compared to the control plants, whose average chlorophyll content reached (103,929 and 118,640) mg 100 g-1, respectively, for both seasons of the study. It also gave the highest content of total soluble carbohydrates in their leaves. For both seasons of the study, which reached (164,421 and 217,067) mg 100 g-1, respectively, compared to the control plants, whose average carbohydrate content reached (129,744 and 135,223) mg 100 g-1, respectively, for both seasons of the study, and it gave the highest content of carotene pigment in the leaves for both seasons. The study, which reached (14,850 and 20,263) mg 100 g-1, respectively, compared with the comparison plants, whose carotene pigment average reached (11,166 and 16,652) mg 100 g-1, respectively, for both seasons of the study.As for the effect of spraying with chitosan, the results of Table (2) showed excelled for the plants sprayed with chitosan at a concentration of 200 mg L-1 in the studied traits, as the highest content of total chlorophyll in the leaves reached (126,546 and 143,189) mg 100 gm-1, respectively, for both seasons of the study, compared to with control plants, the content of total chlorophyll reached (111,593 and 122,367) mg 100 g-1, respectively, for both seasons of the study. It also gave the highest content of total soluble carbohydrates, amounting to (179,677 and 212,413) mg 100 g-1, respectively, for both seasons of the study, compared to with control plants, the content of total carbohydrates in the leaves was (111,770 and 138,438) mg 100 g-1, respectively, for both seasons of the study, and the highest content of carotene pigment was (15,008 and 20,827) mg 100 g-1, respectively, for both seasons of the study, compared with plants. The comparison in which the carotene content reached (11,700 and 16,179) mg 100 g-1, respectively, for both seasons of the study.

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Table 2. Effect of spraying with benzyl adenine and chitosan on some chemical traits in the leaves of Duranta plants

Benzyl adenine	Chitosan	Total chlorophyll content in leaves (mg $100g^{-1}$)		Total soluble carbohydrate content in leaves)mg $100g^{-1}$(		Total carotenoid content in leaves) mg $100g^{-1}$(	
		2022	2023	2022	2023	2022	2023
	0	89.613	106.493	96.087	96.343	9.133	14.140
0	**100**	108.160	120.940	128.650	139.690	10.930	16.387
	200	114.013	128.487	164.497	169.637	13.433	19.430
	0	118.487	125.040	111.803	146.687	12.047	16.127
75	**100**	124.160	139.907	145.447	186.983	14.093	19.810
	200	129.473	145.940	177.213	208.747	15.637	20.933
	0	126.680	135.567	127.420	172.283	13.920	18.270
	100	132.440	147.427	168.523	220.060	14.677	20.403
150	**200**	136.150	155.140	197.320	258.857	15.953	22.117
	LSD	**3.2218**	**3.4153**	**N.S.**	**N.S.**	**0.7188**	**0.7157**
	0	103.929	118.640	129.744	135.223	11.166	16.652
Benzyl adenine	**75**	124.040	136.962	144.821	180.806	13.926	18.957
	150	131.757	146.044	164.421	217.067	14.850	20.263
	LSD	**1.8601**	**1.9718**	**6.4317**	**7.1620**	**0.4150**	**0.4132**
	0	111.593	122.367	111.770	138.438	11.700	16.179
	100	121.587	136.091	147.540	182.244	13.233	18.867
Chitosan	**200**	126.546	143.189	179.677	212.413	15.008	20.827
	LSD	**1.8601**	**1.9718**	**6.4317**	**7.1620**	**0.4150**	**0.4132**

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As the results show in Table (2), there are significant differences in the interaction between the two agents benzyl adenine and chitosan, the chlorophyll content and the leaves' content of carotene pigment in the leaves for both seasons of the study, where the interaction treatment of 150 mg L-1 of benzyl adenine with 200 mg L-1 of chitosan was significantly excelled on the rest. The treatments gave the highest content of chlorophyll in the leaves, which reached (136,150 and 155,140) mg 100 g-1, respectively, for both seasons of the study, compared to the control plants, whose average chlorophyll content reached (89,613 and 106,493) mg 100 g-1, respectively, for both seasons of the study. The total carotenoids in the leaves for both seasons of the study amounted to (15,953 and 22,117) mg 100 g-1, respectively, compared to the control plants, whose content reached (9,133 and 14,140) mg 100 g-1, respectively, for both seasons of the study. As for the effect of the interaction in the total carbohydrate content in leaves were non significant.

The Oil quantity in the leaves of Duranta plants:

The results in Table (3) show that there is a significant effect of spraying with benzyl adenine on the amount and percentage of oil in the leaves. When comparing the averages of the treatments, significant differences appeared between all treatments (Table 3), as the 150 mg L-1 treatment recorded the highest Oil quantity in The leaves reached (0.1167 and 0.1200) g, and the highest percentage of oil in the leaves amounted to (1.1670 and 1.2000)% for both seasons of the study, respectively, in comparison with control treatment that gave the lowest Oil quantity in the leaves, amounting to (0.0676 and 0.0726) g, the lowest percentage of oil. In leaves, it reached (0.6757 and 0.7257)% for both study seasons, respectively. As for the effect of spraying with chitosan, the results of Table (3) showed that plants sprayed with chitosan at a concentration of 200 mg L-1 were significantly excelled in these two traits, where the highest Oil quantity in the leaves reached (0.1034 and 0.1075) g, and the highest percentage of oil in the leaves reached (1.0342 and 1. 0752% for both seasons of the study, respectively, in comparison with control treatment, which gave the lowest Oil quantity in the leaves, amounting to (0.0797 and 0.0838) grams, and the lowest percentage of oil in the leaves, amounting to (0.7974 and 0.8384)%, for both seasons of the study, respectively. As the results show in Table (3), there were no significant differences in the interaction between the agents benzyl adenine and chitosan in the Oil quantity and the percentage of oil in the leaves.

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Table 3. Effect of spraying benzyl adenine and chitosan on some chemical traits in the leaves of Duranta plants

Benzyl adenine	Chitosan	Oil quantity (g)		Oil percentage(%)	
		2022	2023	2022	2023
	0	0.0494	0.0544	0.4943	0.5443
0	**100**	0.0715	0.0765	0.7150	0.7650
	200	0.0818	0.0868	0.8177	0.8677
	0	0.0841	0.0881	0.8410	0.8810
75	**100**	0.0920	0.0960	0.9197	0.9597
	200	0.1045	0.1085	1.0450	1.0850
	0	0.1057	0.1090	1.0570	1.0900
	100	0.1204	0.1237	1.2040	1.2370
150	**200**	0.1240	0.1273	1.2400	1.2730
	LSD	**N.S.**	**N.S.**	**N.S.**	**N.S.**
	0	0.0676	0.0726	0.6757	0.7257
	75	0.0935	0.0975	0.9352	0.9752
Benzyl adenine	**150**	0.1167	0.1200	1.1670	1.2000
	LSD	**0.00911**	**0.00806**	**0.09110**	**0.08064**
	0	0.0797	0.0838	0.7974	0.8384
	100	0.0946	0.0987	0.9462	0.9872
Chitosan	**200**	0.1034	0.1075	1.0342	1.0752
	LSD	**0.00911**	**0.00806**	**0.09110**	**0.08064**

We notice from Table (2) that high concentrations of spraying with benzyl adenine are significantly excelled in the chemical traits of the leaves of Duranta plants. This may be due to the effectiveness of cytokinins in stimulating the formation of

chlorophyll in the leaves because it works to maintain the integrity of the cell wall and prevent the degradation of proteins in the chloroplast (7), as well as cytokines have an important role in the development of chloroplast function and thus work to increase the chlorophyll content within the plant (14). Cytokines are also known to activate enzymes that regulate carbohydrate metabolism and thus increase the accumulation of carbohydrates due to a decrease in their decomposition (4). Thus, increasing these enzymes is reflected in an increase in the efficiency of the metabolic process, which reflects positively on the increase in the leaves' content of total carotenoids. There was also a significant superiority of the high concentrations of chitosan in the studied traits. The reason for improving the chemical traits of the leaves of Duranta plants due to the effect of chitosan may be attributed to its role in encouraging the effectiveness of the endogenous hormones cytokinin and auxin, which have an important role in increasing cell division and elongation, which leads to improving the vegetative growth of the plant, and this is reflected in Positive effect on the chemical traits in the leaves (16), and this was confirmed by (25). In addition, the chitosan molecule varies in action from one cell to another depending on the physiological chemistry, and this causes an increase in the root system, and increasing the effectiveness of the roots means an increase in the absorption of nutrients, which is reflected in the activity of the photosynthesis process and the construction of carbohydrates and sugars that work to increase the accumulation of nutrients. In the shoots of the plant.

As for fixed oils in leaves, the reason for their increase in Dorenta leaves is due to the role of benzyl adenine as a growth regulator that leads to an increase in the process of photosynthesis by stimulating the formation of chlorophyll pigment in chloroplasts (Table 2).and this is the main factor in the process of photosynthesis and the production of carbohydrates. Which encouraged an increase in the production of secondary compounds, especially volatile oils (22)(17.(

Conclusions:

In light of the field experiment, spraying benzyl adenine led to an increase in the leaf content of total chlorophyll, total soluble carbohydrates, total carotenoids, and the amount and percentage of oil in the leaves. Spraying Duranta plants with chitosan also led to positive results in improving chemical traits through a significantly positive relationship and impact on the photosynthesis process. Growth and nutritional content in plants under different treatment conditions, which led to improving the cultivation of Duranta plants and their tolerance to climatic conditions in the southern region of Iraq.

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