EFFECT OF SPRAYING WITH BENZYLADENINE AND PHOSPHORUS ON THE GROWTH, FLOWERING AND CONTENT OF ACTIVE COMPOUNDS OF *CALENDULA OFFICINALIS*

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ABSTRACT

This study aimed to investigate the effect of spraying benzyladenine and phosphorus on growth, flowering, and medicinal active compound content of *Calendula officinalis* cultivar "Bon Bon" under wooden shade conditions. The experiment was conducted using a factorial experiment within Randomized Complete Block Design with three replicates, examining two factors: benzyladenine at three concentrations (0, 50, and 100 mg L^{-1}) and phosphorus at three concentrations (0, 2.5, and 5 mg L^{-1}). The results indicated that benzyladenine (BA2) and phosphorus (P2) treatments significantly enhanced vegetative and floral growth, including plant spread, height, leaf number, branch number, and both fresh and dry weights. Additionally, these treatments improved floral characteristics such as flowering duration, number of flowers, flower diameter, peduncle length, peduncle diameter, fresh and dry flower weights, and flower longevity on the plant.

Keywords: growth regulators, herbaceous ornamentals, essential nutrients.

تاثير الرش بالبنزل ادينين والفسفور في نمو وازهار *Calendula officinals* ومحتواها من المواد الفعالة الطبية

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المستخلص

اجري البحث بهدف دراسة تأثير الرش بالبنزل ادينين والفسفور في نمو وازهار ومحتواها من المواد الفعالة الطبية لنبات الاقحوان *Calendula officinals* صنف Bon Bon ، في ظروف الظلة الخشبية اثناء الموسم الخريفي. طبق البحث ضمن تجربة عاملية وفق تصميم تصميم القطاعات الكاملة العشوائية , وبثلاثة مكررات وتضمن البحث عاملين : العامل الأول. منظم النمو البنزل ادينين بثلاث تراكيز (0، 50 ،100) مغم لتر$^{-1}$) العامل الثاني الفسفور وبثلاث تراكيز أيضا (0، 2.5 ،5) ملغم .لتر$^{-1}$. واظهرت النتائج ان تأثير البنزل ادينين كان معنويا في اغلب صفات النمو الخضري والزهري حيث تفوقت المعاملة البنزل ادنين (BA2) ومعاملة الفسفور P2 معنويا في زيادة افتراش النبات وارتفاعه، وعدد الأوراق وعدد الافرع، والوزنين الطري والجاف للنبات . وتفوقت نفس المعاملتين معنويا لصفات النمو الزهري االصفات الزهرية المتمثلة في مدة التزهبر وعدد الازهار , وقطرها وطول الحامل الزهري وقطرهوالوزنين الطري والجاف ومدة بقاء الازهار على النبات

الكلمات المفتاحية: منظمات النمو، نباتات الزينة العشبية، العناصر الغذائية الاساسية.

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INTRODUCTION

Pot Marigold "*Calendula officinalis*" commonly known as *Calendula* or, is a well-known ornamental plant valued for its beauty. Ornamental plants are among the most famous and widely used plants in the world, commonly utilized for beautifying gardens, homes, and landscapes. These plants are known for providing an aesthetically pleasing and calming appearance, while also contributing to improving air quality, reducing pollution, and regulating environmental temperatures (3). It is an annual herbaceous winter plant belonging to the *Asteraceae* family and is also referred to as "pot marigold." The name *Calendula* is derived from the Latin word "Calendae," meaning "the first day of the month." Its native habitat is Southern Europe, and it prefers sunny conditions and well-drained, rich soils (20, 27). In addition to its ornamental value, *Calendula* holds significant medicinal uses due to its various active compounds, including tocopherol, volatile oils, and plant pigments such as carotenoids. Historically, its flowers were used by ancient herbalists to treat joint pain, sciatica, heart disease, detoxify the body, and alleviate headaches (5). Moreover, the alcoholic extract of its inflorescences is used in the food industry as a coloring agent for cheese (9). Pot Marigold is one of the plants used in the food and cosmetic industries (15). It has many pharmaceutical, medical and food uses and is used in many industries (7). *Calendula* flower extracts exhibit pharmacological effects, acting as antiseptic, stimulant, diaphoretic, antispasmodic, and antipyretic agents. They also show antiviral properties, including activity against HIV (24), and demonstrate anticancer and antitumor effects. Additionally, the plant has been used internally to treat gastritis and duodenal inflammation (5, 22). In Europe, China, and other countries, *Calendula* flowers are used to color rice as a substitute for the more expensive saffron. It is also used as a tea to treat gastric ulcers (9, 16, 21). One of the important elements for the plant, which significantly influences root formation and flowering, is plant growth regulators (PGRs). These are complex organic substances, either natural or synthetic, that regulates or control one or more physiological processes to stimulate growth and increase flowering (9, 18). Benzyladenine (BA) is a growth-promoting regulator that belongs to the cytokinins group, and it is used as a potential resource in the ornamental plant production system, helping to enhance various economically valuable and marketable traits (19). Rupali *et al.*, (17) found that spraying *Calendula* plants with benzyladenine at a concentration of 25 or 50 mg L^{-1} improved flower production. Alwan and Sadiq (4) also reported an increase in the number of flowers per plant, earlier flowering, longer flowering duration, increased tuberous root production, fresh and dry root weight, flower diameter, plant height, and extended flower longevity on the plant when benzyladenine was applied to *Ranunculus asiaticus*. Phosphorus is one of the essential nutrients for plant growth and production, playing a key role in vital processes such as photosynthesis and nutrient metabolism. It also promotes root development and is responsible for absorbing nutrients from the soil, enhancing both the root and floral systems (10, 23). Mohammad et al. (14) in a study on onion (*Allium cepa*) reported that spraying phosphorus to the plant, had a significant influence in increasing floral and reproductive traits. Mandhare et al. (13) demonstrated the response of *Calendula* to phosphorus application, which included four phosphorus levels (0, 25, 50, and 75 kg ha^{-1}). The results revealed that phosphorus levels significantly influenced vegetative growth, including plant height, stem diameter, number of leaves per plant, leaf area, number of branches per plant, days to the first flower bud, days to the first flower opening, and overall flowering duration. This research aimed to investigate the effect of spraying benzyladenine and phosphorus on the growth, flowering, and medicinal active compound content of *Calendula officinalis* cultivar Bon Bon.

MATERIALS AND METHODS

This study was conducted at the lath house of the Horticulture Department, College of Agricultural Engineering Sciences, University of Baghdad, during the fall season. The objective was to determine the role of benzyladenine and phosphorus on the growth,

flowering, and some medicinal active compounds of *Calendula officinalis* cultivar Bon Bon. Seeds used in the research were obtained from an agricultural office in the Kurdistan region, which had imported them from Turkey. The Bon Bon cultivar seeds were sown in seed trays, and after achieving 100% germination and the seedlings reached the appropriate size, they were transplanted into permanent pots. The pots, 30 cm in diameter, were filled with a growing medium consisting of a 1:3 ratio of sandy loam soil to peat moss. After a month of planting, regular care practices such as irrigation and weeding were carried out. The experiment included two factors: the first was the growth regulator benzyladenine, applied at three concentrations (0, 50, and 100 mg L^{-1}), and the second was phosphorus, also applied at three concentrations (0, 2.5, and 5 mg L^{-1}). The plants were sprayed twice with both treatments. The first application occurred on December 15, 30 days after transplanting the seedlings to their permanent location, and the second application followed 21 days later. The plants were sprayed to the point of runoff using a 3-liter hand sprayer, while control plants were sprayed with distilled water. A 48-hour interval was maintained between spraying the two elements in both applications. Data were recorded after full flowering at the end of April 2020. Standard care practices such as weeding, pest control, and irrigation were conducted as needed. All plants were fertilized every 15 days with *Terra-Sorb Complex* foliar fertilizer at a rate of 1.5 ml L^{-1}, containing 5% organic nitrogen, 5.5% total nitrogen, 20% amino acids, 35% organic matter, 1.5% boron, 0.8% magnesium oxide, 1% iron, and 0.1% molybdenum. This study was conducted as a factorial experiment within a Randomized Complete Block Design (R.C.B.D) with three replicates. Each replicate included nine treatments, with ten plants per treatment, resulting in five plants per treatment being used for data collection. The experimental design was a 3 x 3 factorial, resulting in a total of 270 plants in the experiment. The studied traits included both vegetative and floral characteristics, as well as the medicinal active compound content. Vegetative traits measured were plant height, plant spread, and number of leaves per plant, number of branches per plant, plant fresh weight, and dry weight. Floral traits included the flowering date, flowering duration, number of flowers per plant, flower diameter, number of petals per flower, and flower longevity on the plant. The medicinal active compounds focused on were the carotenoid content in the flowers, measured in mg per 100 g of fresh weight according to Balázs *et al.* (6), and the percentage of saponins, extracted using the method of Szakiel *et al.* (26).

RESULTS AND DISCUSSION

Vegetative Growth Traits: Effect of spraying benzyladenine and phosphorus on the vegetative growth traits of *Calendula officinalis* cultivar *Bon Bon:* Table (1) shows that spraying plants with benzyladenine had a positive effect on the vegetative growth traits of *Calendula officinalis*. The treatment with benzyladenine (BA2) led to an increase in plant spread, plant height, number of leaves, number of branches, and fresh and dry weight, reaching 53.56 cm, 39.97 cm, 115.00 leaves $plant^{-1}$, 21.00 branches $plant^{-1}$, 40.59 g fresh weight, and 8.53 g dry weight, respectively. These values were not significantly different from the BA1 treatment for plant height, but they were superior compared to untreated plants, which recorded 50.56 cm. The concentration of 100 mg L^{-1} (P2 treatment) Table (2) had the most significant impact on plant spread, plant height, number of leaves, number of branches, and fresh and dry weight, recording values of 53.44 cm, 38.79 cm, 112.33 leaves $plant^{-1}$, 21.00 branches $plant^{-1}$, 38.05 g fresh weight, and 7.73 g dry weight, respectively, compared to untreated plants and other treatments, which showed lower results for most traits. The interaction between the studied factors had a significant effect compared to the plants treated individually. The combination treatment BA2P2 excelled in most of the studied traits and did not significantly differ from the BA2P1 treatment in some of the traits, as shown in Table (3). This indicates that the combined application of benzyladenine at 100 mg L^{-1} and phosphorus at 5 mg L^{-1} (BA2P2) produced superior results in terms of vegetative growth, further enhancing plant performance compared to individual treatments.

Table 1. Effect of foliar application with benzyl adenine on vegetative traits of *Calendula officinals*

benzyl adenine level	Plant spread cm	Plant height cm	Leaf number (Leaf Plant^{-1})	Branches Number (Branche Plant^{-1})	Fresh weight g	Dry weight g
BA_0	50.56	35.67	107.00	19,33	34.56	6.64
BA_1	53.00	40.28	111.67	20.44	38.28	7.51
BA_2	53.56	39.97	115.00	21.00	40.59	8.53
LSD0.05	1.853	2.487	2.802	0.925	2.262	0.844

Table 2. Effect of foliar application with Phosphorus on vegetative traits of *Calendula officinals*

Phosphorus level	Plant spread cm	Plant height cm	Leaf number (Leaf Plant^{-1})	Branches Number (Branche Plant^{-1})	Fresh weight g	Dry weight g
P_0	51.67	38.20	109.67	19.56	37.31	7.59
P_1	52.00	38.30	111.67	20.44	38.03	7.35
P_2	53.44	38.79	112.33	21.00	38.05	7.73
$LSD_{0.05}$	1.853	2.478	2.802	0.925	2.262	0.844

Table 3. Effect of foliar application with benzyl adenine and Phosphorus on vegetative traits of *Calendula officinals*

benzyl adenine & Phosphorus level		Plant spread cm	Plant height cm	Leaf number (Leaf Plant^{-1})	Branches Number (Branche Plant^{-1})	Fresh weight g	Dry weight g
BA_0	P_0	50.67	53.42	106.33	19.00	36.53	7.30
	P_1	49.33	34.17	106.67	19.67	34.50	6.47
	P_2	51.67	37.39	108.00	19.33	32.64	6.14
BA_1	P_0	52.00	40.39	112.33	20.00	35.96	7.25
	P_1	52.67	39.99	111.67	20.33	39.14	7.14
	P_2	53.33	40.45	111.33	21.00	39.64	8.13
BA_2	P_0	51.33	38.78	110.33	19.67	39.44	8.21
	P_1	54.00	42.22	117.00	20.67	40.45	8.45
	P_2	55.33	38.92	117.67	22.67	41.88	8.92
$LSD_{0.05}$		3.210	2.032	4.852	1.602	3.919	1.461

Floral Growth Traits: Effect of spraying benzyladenine and phosphorus on the floral growth traits of *Calendula officinalis* cultivar *Bon Bon* is shown in Table (4). Spraying plants with benzyladenine, especially at the concentration of 100 mg L^{-1} (BA2), significantly improved floral growth traits. This treatment reduced the flowering duration, increased the number of flowers, and enhanced the flower diameter, peduncle length and diameter, as well as fresh and dry weights, and extended the flowering period in terms of days. The values recorded were 120.44 days for flowering duration, 30.11 flowers per plant, 8.59 cm flower diameter, 4.43 mm peduncle diameter, 12.93 petals per flower, 62.78 g fresh weight, 26.07 g dry weight, and 52.44 days for flower longevity. These results were superior compared to the untreated control plants, which showed lower values for all traits. Similarly, phosphorus application, as presented in Table (5), had a significant positive impact on floral growth traits. Spraying with phosphorus (P2) reduced the flowering duration to 119.23 days, increased the number of flowers to 29.09 plant^{-1}, and enhanced both peduncle diameter and length to 4.07 mm and 12.09 cm, respectively. The fresh and dry weights reached 59.74 g and 23.11 g, respectively, while the flowering longevity was extended to 49.33 days. The interaction between benzyladenine and phosphorus showed significant effects on floral growth traits, as outlined in Table (6). The BA2P2 treatment excelled in most of the studied floral traits, with no significant differences in some

traits compared to the other interactions, further highlighting the beneficial combined effect of benzyladenine and phosphorus on *Calendula* flower development.

Table 4. Effect of foliar application with benzyl adenine on floral traits of *Calendula officinals*

benzyl adenine level	flowering time (days)	flowering date (days)	flowers Number (flower Plant^{-1})	Flower diameter (cm)	Flower holder diameter (cm)	Flower stand length (cm)	Fresh weight (g)	Dry weight (g)
BA_0	44.89	123.6	25.44	7.06	4.074	10.44	55.47	18.88
BA1	48.00	121.89	28.78	7.89	3.921	12.25	59.11	21.95
BA2	52.44	120.44	30.11	8.59	4.438	12.93	62.78	26.07
LSD0.05	2.175	1.362	1.384	0.813	0.2258	0.963	1.747	0.612

Table 5. Effect of foliar application with Phosphorus on floral traits of *Calendula officinals*

Phosphorus level	flowering time (days)	flowering date (days)	flowers Number (flower Plant^{-1})	Flower diameter (cm)	Flower holder diameter (cm)	Flower stand length (cm)	Fresh weight (g)	Dry weight (g)
P_0	121.78.	27.44	7.71	4.152	11.57	58.30	22.47	46.89
P1	122.00	28.56	7.74	4.211	1186	59.31	22.32	49.11
P2	119.23	28.33	9.09	4.070	12.09	59.74	23.11	49.33
LSD0.05	1.362	1.384	0.813	0.2258	0.963	1.747	0.612	2.175

Table 6. Effect of foliar application with benzyl adenine and Phosphorus on floral traits of *Calendula officinals*

benzyl adenine & Phosphorus level		flowering time (days)	flowering date (days)	flowers Number (flower Plant^{-1})	Flower diameter (cm)	Flower holder diameter (cm)	Flower stand length (cm)	Fresh weight (g)	Dry weight (g)
BA0	P0	123.00	25.33	10.58	4.250	55.01	10.03	10.26	43.33
	P1	123.33	25.67	10.47	3.983	55.55	18.94	10.47	46.33
	P2	124.33	25.33	10.26	3.990	55.87	19.68	13.68	45.00
BA1	P0	121.67	28.67	12.44	3.867	58.33	21.45	12.44	47.00
	P1	122.33	28.67	11.99	4.280	59.31	21.85	11.99	48.67
	P2	121.67	29.00	12.32	3.617	9.68	22.55	12.32	48.33
BA2	P0	120.67	28.33	11.99	4.340	61.55	24.93	11.99	50.33
	P1	120.33	31.33	13.12	4.370	63.08	26.18	13.12	52.33
	P2	120.33	30.67	13.68	4.603	63.66	27.09	19.56	54.67
LSD		2.358	2.396	4.302	0.391	3.026	1.060	1.669	3.767

Medicinal Active Compounds

The results of table 7 show that treatment B2 was superior in terms of Content of carotene pigment in flower petals and Percentage of saponins, as it recorded 20.26 mg.100g-1 and 5.022% respectively, while the phosphorus treatment table **8** did not differ significantly from the other treatments. As for the interference treatment B2P2 table **9** was superior to the other treatments, as it recorded 20.31 petal content and 5.083 Percentage of saponins.

Table 7. Effect of foliar application with benzyl adenine on medicinal active substances of *Calendula officinals*

benzyl adenine level	carotene content of flower petals mg. 100 gm fresh weight	Percentage of saponins
BA0	16.44	4.348
BA1	18.70	4.860
BA2	20.26	5.022
LSD	1.821	0.2276

Table 8. Effect of foliar application with Phosphorus on medicinal active substances of *Calendula officinals*

Phosphorus level	carotene content of flower petals mg. 100 gm fresh weight	Percentage of saponins
P0	18.23	4.870
P1	18.59	4.672
P2	18.58	4.688
LSD	1.821	0.2276

Table 9. Effect of foliar application with benzyl adenine and Phosphorus on medicinal active substances of *Calendula officinals*

benzyl adenine & Phosphorus level		carotene content of flower petals mg. 100 g fresh weight	Percentage of saponins
	P0	16.41	4.610
BA0	P1	16.55	4.290
	P2	16.35	4.143
	P0	18.13	4.927
BA1	P1	18.92	4.817
	P2	10.06	4.837
	P0	20.16	5.073
BA2	P1	20.31	4.910
	P2	20.31	5.083
LSD		3.153	0.3942

The improvement in vegetative growth indicators when spraying with benzyl adenine may be attributed to its role in increasing cell division and elongation, which enhances plant height. This positively impacts the number of leaves, leading to an increase in the outputs of biochemical reactions and photosynthesis, ultimately promoting plant growth and branching. As a result, the fresh and dry weight of the chrysanthemum plant increases, reflecting the physiological processes within it (19,1,25). The increase in vegetative growth indicators from spraying with phosphorus may be attributed to the plant's need and response due to the lack of phosphorus in Iraqi soil. This is also related to the important role phosphorus plays in plant growth and productivity, as it is one of the essential nutrients required for plants to complete their growth (2, 11, 12, 28). The improvement in flowering growth indicators of the Pot Marigold plant when sprayed with benzyl adenine and phosphorus may be attributed to their roles in many vital activities necessary for plant growth, which is reflected in vegetative growth indicators. This, in turn, enhances the plant's capacity for carbon assimilation, leading to improved flowering indicators such as earlier flowering, the duration of flower, the number and diameter of flowers, and an increase in both the fresh and dry weight of the flowers (3). The increase in the carotenoid content of flowers when sprayed with benzyl adenine and phosphorus may be attributed to their nutrient, vitamin, and acid content, in addition to growth hormones and their role in improving vegetative growth indicators. This positively impacts carbon assimilation products; when these sugars are broken down, they produce Acetyl CoA, which is the primary material for synthesizing carotenoids (8). Meanwhile, the increase in saponin content in the flowers may be linked to the improvement in vegetative and flowering indicators due to the study factors, resulting in increased carbon assimilation products that produce secondary metabolites, which in turn enhance the saponin levels in the flowers (26). In could be concluded that plants sprayed with the growth regulator benzyladenine significantly outperformed in most vegetative, floral, and qualitative traits. Plants treated with phosphorus showed significant improvements in all vegetative and floral traits. All interactions between the study factors resulted in clear and significant improvements in all the studied floral traits.

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