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

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**ABSTRACT**

**The experiment was carried out in a wooden shade structure by applying foliar spray of Benzyladenine and phosphorus to observe their effects on growth, flowering, and medicinal active compound content of Calendula officinalis cultivar "Bon Bon". A two-factor experiment was conducted within Randomized Complete Block Design with three replications. The factors were Benzyladenine applied at three different concentrations (0, 50, and 100 mg L-1) and phosphorus at three concentrations (0, 2.5, and 5 mg L-1). Results obtained show that Benzyladenine (BA2) and phosphorus (P2) treatments significantly improved the vegetative and floral growth of the plant in terms of plant spread, height, leaf number, and branch number as well as their fresh and dry weights. These also enhanced some floral characteristics such as flowering duration, number of flowers, flower diameter, peduncle length, and peduncle diameter as well as fresh and dry flower weights and flower longevity on the plant**

**Keywords: plant growth substances, flowering plants, vital nutrients.**



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

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**المستخلص**

**تم تنفيذ التجربة داخل هيكل مظلة خشبية من خلال تطبيق رش ورقي بمادة البنزيل أدينين والفوسفور بهدف دراسة تأثيرهما على النمو والتزهير ومحتوى المركبات الفعالة دوائيًا في نبات Calendula officinalis من الصنف "Bon Bon" .وقد أجريت التجربة بتصميم القطاعات الكاملة العشوائية (RCBD) بعاملين وبثلاث مكررات. تمثل العاملان في البنزيل أدينين بثلاث تراكيز مختلفة (0، 50، و100 ملغم/لتر) والفوسفور بثلاث تراكيز (0، 2.5، و5 ملغم/لتر). أظهرت النتائج أن المعاملتين BA2(بنزيل أدينين) و P2(فوسفور) قد حسّنتا بشكل معنوي النمو الخضري والزهري للنبات من حيث انتشار النبات، ارتفاعه، عدد الأوراق، وعدد الأفرع، بالإضافة إلى الوزن الطري والجاف لها. كما ساهمت هذه المعاملات في تحسين بعض الصفات الزهرية مثل مدة الإزهار، عدد الأزهار، قطر الزهرة، طول السويقة الزهرية وقطرها، وكذلك الوزن الطري والجاف للأزهار وطول عمر الزهرة على النبات.**

**الكلمات المفتاحية :منظمات نمو النبات، نباتات مزهرة، مغذيات حيوية.**

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## INTRODUCTION

Pot Marigold is known as Calendula or "Calendula officinalis," a popular ornamental plant that is grown for the value of its beauty. Ornamental plants are within the most famous and broadly used plants across the globe. They are major means applied to add aesthetics to gardens, homes, and landscapes. These types of species provide a beautiful and serene sight and carry the value of making the air clean and good for further pollution control as well as for temperature regulation (3). It is an herbal winter annual belonging to the family Asteraceae and is sometimes called "pot marigold." Calendula, from the habitat of Southern Europe, requires a sunny position with well-drained, fertile soil (20, 27). Besides the ornamental value, Calendula proves to be very important medicinally due to a series of active compound nutritive elements present in it; for example, tocopherol, oils that paint the flag, and plant pigments/bioflavonoids such as carotenoids. In history, the flowers of this were used by herbalists in ancient times for treating the pain of joints, sciatica, heart diseases, detoxification of the body, and for treating headaches. In addition, the ethanolic extract from its inflorescences finds application in the food industry as a coloring preparation 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) reported the reaction of Calendula to phosphorus application. It included four levels of phosphorus: (0, 25, 50, and 75 kg ha-1). Results obtained that the levels of phosphorus significantly influenced the vegetative growth parameters recorded. Such parameters included plant height, stem diameter, number of leaves per plant, leaf area, number of branches per plant, days to first flower bud, days to first flower opening, and total duration of flowering. The current study was conducted to investigate the effect of foliar application of Benzyladenine and phosphorus on growth, flowering, and the content of active compounds of medicinal value in Calendula officinalis L. variety Bon Bon.

## MATERIALS AND METHODS

This experiment took place in the lath house of the Horticulture Department, College of Agricultural Engineering Sciences, University of Baghdad. It was within the fall season. The first objective was to establish the influence of Benzyladenine and phosphorus on the growth,

flowering, and some medicinal active compounds of Calendula officinalis var. Bon Bon. Seeds used in the experiment were obtained from a farm supply center in the Kurdistan region. These were imported from Turkey. The seeds of Bon Bon were sown in seed trays. After achieving 100% germination and the seedlings attaining a proper size, the seedlings were then transplanted to pots as permanent homes. The pots, 30 cm in diameter, were filled with a growing medium, a 1:3 ratio of sandy loam soil to peat moss. After planting for one-month, cultural practices such as irrigation and weeding were done regularly. The experiment used two factors: the first one is the growth regulator Benzyladenine applied at three concentrations (0, 50, and 100 mg L-1) and the second factor is phosphorus 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. Measured vegetative traits comprised plant height, spread, number of leaves and branches per plant, fresh and dry weight of the plant. The floral traits were the flowering date and flowering duration, number of flowers and their diameter, number of petals, and how long the flower stays on the plant. The active compounds of medicinal importance focused on the content of carotenoids in flowers in mg/100 g of fresh weight Balázs et al., (6) and the % of saponins 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:*** Spraying plants with Benzyladenine positively affected the vegetative growth traits of Calendula officinalis as is evident from the data reported in Table (1). Regarding the BA2 treatment, plant spread, and plant height are significant at 53.56 cm and 39.97 cm, respectively. The number of leaves and branches are 115.00 leaves plant-1 and 21.00 branches plant-1, respectively. These are followed by fresh weight and dry weight which are 40.59 g and 8.53 g, 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 of flowering growth indicators of the Pot Marigold plant, which received benzyl adenine and phosphorus spray, in vital activities reflective of vegetative growth factors, probably produces good indication in the capacity of carbon assimilation of the plant. Therefore, it can be said that there is a direct relationship between earlier flowering, the duration of flowers, the number and diameter of flowers, and the fresh and dry weights of the flowers. The increase in carotenoids in flowers due to the application of benzyl adenine and phosphorus is probably related to their nutritive, vitamin, and acidic contributions besides being growth hormones which enhance 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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