

Response of Petunia plant *Petunia hybrida* to brassinolide spraying and (BiO-NPK) Application on terms of vegetative and floral growth indicators.

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Abstract

Due to importance of cultivating ornamental herbaceous flowering ornamental plants the experiment was conducted at the research station of the College of Agriculture, University of Kufa, during the 2025-2026 to demonstrate the role of brassinolide spraying and (BiO-NPK) application in the growth and flowering traits of the Petunia plant” planted in plastic pots. The experimentation comprised two factors; the first one was brassinolide with three concentrations (0, 1 and 2) mg.L⁻¹, and the second factor was (BiO-NPK) with three concentrations (0, 20 and 40) ml.L⁻¹. A randomized complete block design was adopted for this two-factor experiment with three replications. the experiment was implemented using a complete randomized block design (R.C.B.D)3×3 = 9. were analyzed using analysis of variance (ANOVA) and comparisons were made. According to the Least Significant Difference test (L.S.D) at a probability level (0.05). spraying brassinolide at a concentration of 10 mg.L⁻¹ with (BiO-NPK) at a concentration of 40 mg.L⁻¹ as an interaction effect realized a significant increase in plant height, number of leaves, number of flowers, flower diameter, leaf content of total chlorophyll and leaf content of total carbohydrates, which reached 52.01 cm. 59.03 leaf.plant⁻¹, 60.12 flowers.Plant⁻¹, 6.880 cm, 24.09 mg.100 g⁻¹, and 22.94 mg.g- mg. 100g-1, respectively, received from the combination treatment of (brassinolide 2 mg.L-1 with (BiO-NPK)40 ml.L⁻¹). In contrast, the interaction effect of the control treatment gave the lowest mean for the same traits studied 22.01 cm, 20.66 leaf.plant⁻¹, flowers.Plant⁻¹, 5.197 cm, , 12.62 mg.100 g⁻¹, and 19.02 mg.g⁻¹, respectively.

Keywords: *Petunia spp*, spraying, Brassinolide, BiO-NPK.

(It is not necessary that the keywords are taken from the title)

Introduction

Petunia (*Petunia* spp.) is an annual or perennial plant belonging to the Solanaceae family. As an ornamental plant, it is cultivated for its beautiful flowers in flowerbeds, borders, pots, hanging baskets, window boxes, and containers. In warm climates, petunias are considered perennials, but they are grown as annuals in temperate regions. Their flowers exhibit a stunning diversity of shapes, colors, and sizes and are widely used in various landscape designs. Common colors include pink, salmon, red, white, bicolors, and multicolored varieties. Since the beginnings of horticulture, petunia has been considered a primary ornamental plant hybrid petunia is mainly cultivated through seeds [11]. Petunia is originated from South America. The name is derived from French, word 'petun', meaning "tobacco". Garden Petunia (*Petunia* hybrid Vilm.) has been bred since the 1830s. Jussieu established the genus *Petunia* (Sensu jussieu) in 1803. There are over 360 cultivars of petunia available in the market today. Garden petunias are small soft plants having a height of 25-35 cm with straggling or decumbent habit, pubescent and more or less sticky, with large showy flowers. The leaves are alternate, simple and entire, and are viscid pubescent. Flowers are beautiful, funnel shaped, small or large up to 6-12 cm across and produced on about 4-5 cm [18]. In order to obtain high-quality *Petunia* flowers in terms of flower density, inflorescence size, and, the plant was

treated by spraying with brassinolide, which is a class of plant hormones and is the sixth group of plant hormones in addition to other plant hormones, which are similar to steroid hormones in animals [7]. Researchers at the United States Department of Agriculture (USDA) were able to identify a compound that is effective in stimulating plant growth within this group and has been called Brassinolide [17 and 19]. Brassinosteroids were extracted for the first time from the pollen of the *Brassica napus* plant [13]. They are considered plant hormones produced in different parts of the plant and have been proven to play an important role in every stage of plant growth and development through their contribution to the division and cellular elongation, biosynthesis of the cell wall compositions, growth of branches, formation of adventitious roots, and flowering [1]. Biofertilizers are organic sources that stimulate plant growth by providing readily available nutrients and producing growth regulators that promote lateral roots and increase the surface area of the root system, thereby improving its structure. They also help to mitigate the concentration of heavy metals and toxic compounds in the rhizosphere [2]. Given the importance of petunia among flowering ornamental plants, this study aimed to investigate the effect of brassinolide spray and biofertilizer application, as well as their interaction, on the growth and flowering of petunia plants.

2. Material and Methods

The study was conducted at the research station of the College of Agriculture, University of Kufa, during 2025-2026 growing season to demonstrate the role of brassinolide and (BiO-NPK) in the growth and flowering of Petunia. Seeds of the Pinto Grandiflora variety, produced by the Swiss company Syngenta, were sown on February 15, 2025, in plastic growing trays containing peat moss. After

germination and the seedling reaching a height of 4-5 cm (i.e., the appearance of true leaves), they were transplanted into 25 cm plastic pots in diameter and 24 cm high in a growing medium consisting of river soil and peat moss in a 1:3 ratio, weighing 5 kg, at a rate of one plant per pot. The soil was analyzed according [21] (Table 1)

Table 1. Some chemical and physical properties of soil

pH	EC ds m ⁻¹	OM %	CaCO ₃ %	N mg Kg ⁻¹	P mg Kg ⁻¹
7.4	3.1	1.3	10.9	12.4	12.9
K mg Kg ⁻¹		Sand gm Kg ⁻¹	Silt gm Kg ⁻¹	Clay gm Kg ⁻¹	Soil texture
14.3		390	500	110	Silty loam

Table 2. Some chemical properties of peat moss.

pH	EC ds m ⁻¹	P ₂ O ₅ mg L ⁻¹	N mg L ⁻¹	K ₂ O mg L ⁻¹	Mg mg L ⁻¹	S mg L ⁻¹
6.17	2.66	160	140	180	100	120

Three sprays of brassinolide at three levels (0, 5 and 10) mg.L⁻¹ and (BiO-NPK) at three concentrations (0, 20 and 40 ml/L⁻¹) were applied alone or in combination, the first spray after the seedlings were transplanted into pots and new leaves appeared on the plants when they had 4-5 pairs of true leaves; the second spray 21 days after the first; and the third spray 21 days after the second. Liquid bleach was added at a rate of two drops per liter of spray solution as a spreading agent. A layer of **At the end of experiments the following indicators were recorded:**

Vegetative Growth Indicators

1-Plant Height (cm)

Plant height was measured using a measuring tape from soil to the highest point at the flower

agricultural nylon (polyethylene) was laid on the floor of the greenhouse under the pots to prevent weed growth in the soil and to avoid salt leaching into the pots. Place thin plastic dishes under each pot to keep the potting soil moist. The recommended fertilizer was Di ammonium phosphate (DAP) at a rate of 0.5 g /pot⁻¹, three times every 30 days for all treatments, and irrigation and weeding were carried out as needed.

bud stage for each experimental unit, and the average was calculated for each treatment.

2-Total Number of Leaves (leaf plant⁻¹)

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

Second: Flower growth indicator

1-Number of flowers (flower.plant⁻¹)The number of plant flowers for each experimental unit was calculated, and the average number of flowers for each treatment was extracted .

2-Flower diameter (mm).Flower diameter was measured using a (vernier caliper) for each experimental unit..

Chemical parameters

1-Total leaf chlorophyll

Leaf chlorophyll content (mg 100g⁻¹ FW)was estimated according to the method.[12]

2-Total leaf soluble carbohydrate (mg g⁻¹)

Leaf carbohydrate content was estimated according to the [10] method.

The experiment was carried out using a Randomized Complete Block Design (RCBD), and the data were analyzed using one way ANOVA, and comparisons were made according to the Least Significant Difference (LSD) test at a probability level of (0.05).

3. Results and Discussions**1- Plant height (cm)**

Table (3) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹ . increased Plant height to 35.09 cm Compared to the control treatment that reached of 28.04 cm

The same table also shows that fertilization with (BiO-NPK) at a concentration of 40 ml.L⁻¹ increased Plant height by 37.03

cm over the distilled water spray treatment, which was 29.04 cm

Table (3) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹ , along with (BiO-NPK) at a concentration of 40 ml.L⁻¹ with increased Plant height to 52.01 cm Compared to the measurement treatment that reached of 22.01 cm

Table 3. Effect of Spraying with Brassinolide and fertilization with (BiO-NPK), and Their Interaction on Plant height (cm).

Treatment		BIO-NPK ml.L ⁻¹			Average effect of Brassinolid
		0	20	40	
Brassinolid mg.L ⁻¹	0	22.01	25.05	37.07	28.04
	20	20.01	22.01	42.07	28.03
	40	23.06	32.0	52.01	35.09
Average effect of BIO-NPK		29.04	25.08	37.03	
(0.05) L.S.D		Brassinolid		8.73	
		BIO-NPK		8.73	
		Interference		15.12	

2- - number of leaves (leave. Plant⁻¹)

Table (4) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹ . increased number of leaves to 55.03 leaves. Plant⁻¹. Compared to the measurement treatment that reached of 46.05 leave. Plant⁻¹.

The same table also shows that fertilization with (BiO-NPK) at a concentration of 40 ml.L⁻¹ increased number of leaves by 54.08 leaves. Plant⁻¹. over the distilled water spray treatment, which was 64.09 leaves. Plant⁻¹.

Table (4) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹, along with (BiO-NPK) at a concentration of 40 ml.L⁻¹ with increased number of leaves

to 59.03 leaves. Plant⁻¹. Compared to the measurement treatment that reached of 35.07 leaves. Plant⁻¹.

Table 4. Effect of Spraying with Brassinolide and fertilization with (BiO-NPK), and Their Interaction on number of leaves (leaves. Plant⁻¹).

Treatment		BIO-NPK ml.L ⁻¹			Average effect of Brassinolid
		0	20	40	
Brassinolid mg.L ⁻¹	0	35.07	48.0	56.04	46.05
	20	43.02	60.03	62.02	52.07
	40	44.07	54.01	59.03	55.03
Average effect of BIO-NPK		46.09	53.0	54.08	
(0.05) L.S.D		Brassinolid		6.87	
		BIO-NPK		6.87	
		Interference		11.90	

3- leaf content of total chlorophyll (mg.100 g⁻¹)

Table (5) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹. increased leaf content of total chlorophyll to 20.34 mg.100 g⁻¹. Compared to the measurement treatment that reached of 17.41 mg.100 g⁻¹.

The same table also shows that fertilization with (BiO-NPK) at a concentration of 40 ml.L⁻¹ increased leaf content of total chlorophyll by 19.99 mg.100 g⁻¹. Compared

to distilled water spray treatment was 16.67 mg.100 g⁻¹.

Table (5) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹, along with (BiO-NPK) at a concentration of 40 ml.L⁻¹ with increased leaf content of total chlorophyll to 24.09 mg.100 g⁻¹. Compared to the measurement treatment that reached of 12.62 mg.100 g⁻¹.

Table 5. Effect of Spraying with Brassinolide and fertilization with (BiO-NPK), and Their Interaction on chlorophyll (mg.100 g⁻¹)

Treatment		BIO-NPK ml.L ⁻¹			Average effect of Brassinolid
		0	20	40	
Brassinolid mg L ⁻¹	0	12.62	18.33	21.27	17.41
	20	21.10	21.42	14.62	19.04
	40	16.67	20.26	24.09	20.34
Average effect of BIO-NPK		16.80	20.01	19.99	
(0.05) L.S.D		Brassinolid		2.189	
		BIO-NPK		2.189	
		Interference		3.791	

4- leaf content of total soluble carbohydrates(mg.g⁻¹)

Table (6) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹ increased leaf content of total carbohydrates to 24.31 mg.g⁻¹. Compared to the measurement treatment that reached of 20.99 mg g⁻¹.

The same table also shows that fertilization with (BiO-NPK) at a concentration of 40 ml.L⁻¹ increased leaf content of total carbohydrates by 20.64 mg.g⁻¹. Compared

to distilled water spray treatment was 22.94 mg.g⁻¹.

Table (6) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹, along with (BiO-NPK) at a concentration of 40 ml.L⁻¹ with increased leaf content of total carbohydrates to 26.50 mg.g⁻¹. Compared to the measurement treatment that reached of 19.02 mg.g⁻¹

Table 6. Effect of Spraying with Brassinolide and fertilization with (BiO-NPK), and Their Interaction on carbohydrates (mg.g⁻¹).

Treatment		BIO-NPK ml L ⁻¹			Average effect of Brassinolid
		0	20	40	
Brassinolid mg L ⁻¹	0	19.02	21.55	22.40	20.99
	20	20.04	21.13	22.87	21.35
	40	22.86	23.56	26.50	24.31
Average effect of BIO-NPK		20.64	23.06	22.94	
(0.05) L.S.D		Brassinolid		2.983	
		BIO-NPK		2.983	
		Interference		5.166	

5- number of flowers (flowers.Plant⁻¹).

Table (7) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹ increased number of flowers to 55.03 flowers. Plant⁻¹. Compared to the measurement treatment that reached of 46.05 flowers. Plant⁻¹.

The same table also shows that fertilization with (BiO-NPK) at a concentration of 40 ml.L⁻¹ increased number of flowers by 54.08 flowers. Plant⁻¹. over the distilled

water spray treatment, which was 64.09 flowers. Plant⁻¹.

Table (7) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹, along with (BiO-NPK) at a concentration of 40 ml.L⁻¹ with increased number of flowers to 59.03 flowers. Plant⁻¹. Compared to the measurement treatment that reached of 35.07 flowers. Plant⁻¹.

Table 7. Effect of Spraying with Brassinolide and fertilization with (BiO-NPK), and Their Interaction on number of flowers (leaves. Plant⁻¹).

Treatment		BIO-NPK ml L ⁻¹			Average effect of Brassinolide
		0	20	40	
Brassinolid mg L ⁻¹	0	33.27	38.10	44.40	38.59
	20	58.33	59.31	55.78	54.08
	40	51.84	54.68	60.12	57.77
Average effect of BIO-NPK		47.78	50.70	51.97	
(0.05) L.S.D		Brassinolid		4.015	
		BIO-NPK		4.015	
		Interference		6.955	

6- Flower diameter (cm).

Table (8) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹ increased flower diameter to 6.096 cm Compared to the measurement treatment that reached of 5.656 cm

The same table also shows that fertilization with (BiO-NPK) at a concentration of 40 ml.L⁻¹ increased flower diameter by 6.132

cm over the distilled water spray treatment, which was 5.713 cm

Table (8) shows that spraying with brassinolide at a concentration of 10 mg.L⁻¹, along with (BiO-NPK) at a concentration of 40 ml.L⁻¹ with increased flower diameter to 6.880 cm Compared to the measurement treatment that reached of 5.197 cm

Table 8. Effect of Spraying with Brassinolide and fertilization with (BiO-NPK), and Their Interaction on Flower diameter (cm).

Treatment		BIO-NPK ml.L ⁻¹			Average effect of Brassinolide
		0	20	40	
Brassinolid mg L ⁻¹	0	5.197	5.877	5.893	5.656
	20	6.407	6.210	5.760	5.916
	40	5.713	5.953	6.880	6.096
Average effect of BIO-NPK		5.772	5.881	6.132	
(0.05) L.S.D		Brassinolid		0.440	
		BIO-NPK		0.440	
		Interference		0.763	

This is consistent with a previous study showing that spraying the Roselle (*Hibiscus sabdariffa*) plant with brassinolide significantly increased flower growth indicators, including flower diameter, inflorescence length, and inflorescence number [3]. Spraying brassinolides improved floral growth parameters, indicating that they stimulate cell divisions in the plant through a hormone-like mechanism of action, which positively impacted floral growth.

Brassinolides facilitated the translocation of sugars and proteins produced in the leaves and their accumulation in the flower growth areas [4]. The increased inflorescence number resulting from spraying brassinolides may be due to the improved total protein and carbohydrate content in the leaves,[14] which indirectly leads to the accumulation of these biochemicals in the consumption areas, and their potential to improve floral growth parameters [5]. In the same context, it was

indicated that treatment with brassinosteroids led to a significant increase in the activity of the ATPase enzyme that stimulates the Carboxylase enzyme responsible for increasing the soluble protein, which consequently increases the processes of construction, cell division and elongation, and then increases the growth of flowers [20]. vegetative and flowering characteristics. This can be attributed to the biofertilizer's ability to promote plant growth by producing plant growth regulators such as auxins, gibberellins, and cytokinins, leading to an increase in the number of leaves and branches. Auxins act as growth promoters in apical regions and support the elongation of roots and stems, as well as cell division and elongation. This is consistent with the findings of Bessai et al.

Conclusion

The interaction of brassinolide and (BiO-NPK) had a highly significant effect on improving the nutritional efficiency of the plant. It increased the concentration of Parameters det Plant height, number of leafs, leaf content of total chlorophyll, leaf content of total carbohydrates , number of flowers, and flower diameter The

[15]. Biofertilizers also have a high capacity to colonize plant roots, thereby increasing their growth and productivity and enhancing the absorption of water, nutrients, and plant hormones from the soil [6, 8]. A significant increase in flowering growth indicators, such as the number and diameter of flowers, was also observed with the addition of biofertilizer. This may be due to the increase in leaf chlorophyll content, which enhances the efficiency of carbon assimilation and the synthesis of nutrients, including carbohydrates. This, in turn, positively affects the activity of the floral system, thereby increasing the plant's ability to improve its flowering and increase the number of flowers [16].

integrated treatment resulted in the highest, flower growth indicator brassinolide rate (2 mg.L^{-1}) with foliar application of (BiO-NPK) at 40 ml L^{-1} , which confirms the role of growth plant hormones and Biofertilizers in improving nutrient uptake efficiency.

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