

## Morphological and physiological performance of petunia (*Petunia hybrida*) cultivated in a growth substrate derived from Tehran pine (*Pinus eldarica*) cone peat

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### ABSTRACT

Petunia is a widely cultivated ornamental plant whose growth and flowering performance are strongly affected by the physical and chemical properties of the growing substrate. Increasing costs and limited availability of cocopeat have encouraged the use of low-cost, locally available alternatives. This study investigated the morphological and physiological responses of petunia grown in Tehran pine cone-based peat (*Pinus eldarica*) compared with cocopeat. The experiment was conducted as a completely randomized design with two substrate treatments, perlite-cocopeat and perlite-pine cone peat, with three replications. Plant height, flower number and diameter, flowering time, shoot and root biomass, leaf chlorophyll and carotenoid contents, total soluble sugars, and petal anthocyanin concentration were evaluated. The results showed that the pine cone peat-perlite mixture increased flower number by 85.7% and significantly enhanced leaf chlorophyll and carotenoid contents by 45.4% and 25.5%, respectively, while cocopeat promoted higher accumulation of soluble sugars. Overall, composted *P. eldarica* cone peat appears to be a sustainable and economically viable alternative to cocopeat for petunia production.

**Keywords:** growth substrate; pine cone peat; cocopeat; ornamental quality; chlorophyll.

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### Introduction

Petunia (*Petunia hybrida*) is an annual or perennial ornamental species in the family Solanaceae, native to Argentina. It is extensively cultivated across the world and is considered a key element in landscaping for gardens, parks, and urban green spaces (Chehregani et al., 2016). Petunias were among the earliest ornamental

plants to be commercially propagated in the ornamental plant market, starting from the 1950s (Hoda et al., 2014). The flowers of petunia display a wide array of colors, including white, red, purple, pink, yellow, blue, and variegated forms, and this broad color variation has made them one of the most favored bedding plants globally (Hoda et al., 2014).

Nowadays, the use of appropriate growing media is recognized as a critical factor in the production and cultivation of ornamental plants (Tzortzakis et al., 2020). The choice of substrate significantly influences the fulfillment of plant requirements, promotes vegetative growth, and improves overall plant performance and productivity (El-Kazzaz et al., 2017; Prasad et al., 2019). An ideal growing medium should ensure efficient oxygen exchange between roots and the surrounding atmosphere, while providing adequate water retention, nutrient availability, and mechanical support for plant anchorage (Al-Far et al., 2019). In greenhouse production, soilless substrates are widely employed because they are lighter, disease-free, readily available, and more suitable for containerized plants compared with mineral soils (Nelson, 1991).

Beyond physical, chemical, and biological suitability, an optimal growing medium should be cost-effective, sustainable, and lightweight to facilitate handling and transportation, while remaining economically practical (Davidson et al., 2000). Various soilless substrates are commonly used for cultivating flowering plants, including peat, peat moss, perlite, vermiculite, coco peat, zeolite, rockwool, sand, and composts derived from diverse organic sources (Fussy et al., 2022). However, limited availability and high import costs of cocopeat, peat moss, and other foreign substrates have created a need for locally available, cost-effective alternatives that can replace expensive imports and reduce production costs (Carpenter et al., 2011; Fox, 2011; Gruda, 2019). In this context, several studies have suggested that materials such as wood chips (Durand et al., 2021), tree barks, including palm peat (Ahmad et al., 2023), and plant fibers (Vandecasteele et al., 2018) may serve as effective substitutes for imported substrates and can be successfully applied in soilless cultivation systems.

According to Akbarzade et al., (2023), incorporating wood waste compost into the growing medium effectively enhanced flower diameter, plant height, leaf number, and chlorophyll content in plants. Similarly, Woznicki et al., (2024) reported that strawberry plants cultivated in a medium containing spruce wood fibers exhibited higher

sugar accumulation along with improved quality and overall performance. Other studies have shown that adding tea waste to the substrate of cut *Anthurium* flowers not only lowered the costs of greenhouse soilless cultivation but also positively influenced growth parameters and quality indices (Asghari, 2017). Furthermore, Česonienė et al., (2023) indicated that the inclusion of pine and spruce fibers in blueberry cutting media can reduce the reliance on peat.

Recently, in Iran, a cellulose-based substrate derived from the cones of *Pinus eldarica* (Tehran pine) has been developed and patented. Considering its promising properties, evaluating its potential as a growing medium is essential. *P. eldarica*, a pine species native to the Eldar region of Georgia, has been cultivated in Iran for several centuries and is commonly referred to as Iranian pine, Tehran pine, or formal pine (Soltanian et al., 2016). Babarabie et al., (2025) reported that a cellulose-based cone peat substrate from *P. eldarica* increased germination rates of *Adenium*, ornamental sunflower, and *Osteospermum* compared to cocopeat. Another study demonstrated that Tehran pine cone peat could replace cocopeat as a propagation medium for *Zamiifolia*, enhancing root and rhizome length and reducing leaf cutting germination time (Babarabie et al., 2025). Given the significance of ornamental plants, particularly petunia, identifying a suitable growing medium for this species is of considerable importance. Accordingly, the present study aimed to compare the effects of *P. eldarica* cone peat and imported cocopeat on the quantitative and qualitative traits of petunia, and to evaluate their potential as substitutes for cocopeat.

## Materials and Methods

The present study was conducted in December 2024 at the Laboratory of Hormozgan University, using a completely randomized design with two treatments and three replications, with each replication consisting of three plants. The first treatment consisted of a 1:1 (v/v) mixture of perlite and cocopeat, while the second treatment consisted of a 1:1 (v/v) mixture of perlite and *Pinus eldarica* cone compost. The properties of these

growing media are presented in Table 1, including measurements of some essential macro- and micronutrients, pH, electrical conductivity, and cation

exchange capacity, which differed between the two substrates.

Table 1 - Characteristics of the used growth media in the experiment

Substrate	pH	EC	CEC	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mn (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)
Cocopeat	6.57	0.53	207	3.14	382	396	1380	10	597	362	16
Pine Cone Peat	6.43	0.61	241	2.85	413	419	1438	58	1770	800	309

Forty-five-day-old petunia seedlings ('Burgundy' F1) with six leaves were obtained from Shokoofeh Isfahan Company. Seedlings were transplanted into pots with a diameter of 14 cm and a height of 12 cm and grown in a research greenhouse with an average light intensity of 11,000 lux,  $70 \pm 5\%$  relative humidity, and temperatures of  $26 \pm 2^\circ\text{C}$  during the day and  $18 \pm 2^\circ\text{C}$  at night. Upon transplanting, humic acid was applied during the first irrigation to reduce transplant shock. Plants were monitored daily, and each pot was irrigated with 100 mL of water whenever the growing medium began to dry.

One week after transplanting and continuing until the end of the growth and flowering period (95 days), all plants were fertilized weekly with Hoagland's nutrient solution. At the end of the experiment, the following traits were measured: plant height, number of flowers, flower diameter, fresh and dry weight of roots, fresh and dry weight of shoots, total soluble sugars, total chlorophyll and leaf carotenoid contents, and petal anthocyanin content. Flowering onset was also recorded daily throughout the experimental period.

#### Plant height and Flower diameter

Plant height and flower diameter were recorded using a digital caliper. Fresh biomass of shoots and roots was determined with a digital balance. For dry weight measurements, shoot and root samples were separately placed in paper bags and oven-dried at  $72^\circ\text{C}$  for 48 hours,

after which the dry weights were measured using a digital balance.

#### Chlorophyll and Carotenoids

Chlorophyll extraction from leaf tissue was carried out using 80% acetone. Fresh leaf samples (0.1 g) were homogenized with 5 mL of 80% acetone to obtain a uniform solution. The homogenate was centrifuged at 4,800 rpm for 20 minutes, and the supernatant volume was then adjusted to 10 mL with 80% acetone. Absorbance was measured using a spectrophotometer at 645 and 663 nm for chlorophyll and at 480 and 510 nm for carotenoids. Total chlorophyll and carotenoid concentrations were calculated in  $\text{mg g}^{-1}$  fresh weight according to the formulas described by Arnon (1949).

#### Total Sugars

For the measurement of total sugars, 100 mg of fresh leaf sample was hydrolyzed in 5 mL of 2.5 N hydrochloric acid in a boiling water bath for 3 hours and then neutralized with sodium carbonate. The volume was adjusted to 100 mL, and the solution was centrifuged at 5,000 rpm for 10 minutes. One milliliter of the supernatant was taken for analysis. Four milliliters of anthrone reagent were added, and the mixture was incubated in a water bath at  $70^\circ\text{C}$  for one minute. The sample was then rapidly cooled, during which the color changed from green to gray, and absorbance was

measured at 630 nm. Total sugar content was calculated in mg g<sup>-1</sup> dry weight (Thimmaiah, 1999).

### Anthocyanin

For anthocyanin measurement, 0.1 g of fresh petal tissue was ground with 10 mL of acidic methanol (a 1:99 v/v mixture of pure methanol and concentrated hydrochloric acid) using a porcelain mortar. The extract was transferred to test tubes and incubated in the dark at 25 °C for 48 hours. After incubation, the tubes were centrifuged at 4,000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 550 nm using a spectrophotometer. Anthocyanin concentration was calculated in mg g<sup>-1</sup> fresh weight using a molar absorption coefficient of 33,000 cm<sup>-1</sup> M (Wagner, 1979).

### Data Analysis

The experiment was designed as a completely randomized design (CRD). Statistical analyses were

carried out using SAS software (version 9.4). Treatment means were compared using the LSD multiple range test at a 5% significance level ( $P \leq 0.05$ ), and statistically significant differences were denoted by different letters. Graphs were generated using Microsoft Excel.

### Results

The analysis of variance (ANOVA) indicated that the type of growing medium did not have a significant effect on flower diameter, plant height, fresh and dry weights of roots and shoots, flowering onset, or petal anthocyanin content. In contrast, the treatments significantly influenced the number of flowers at the 5% level. Moreover, total chlorophyll content, leaf carotenoid content, and total soluble sugar concentration were significantly affected by the substrate at the 1% level (Tables 2 and 3).

Table 2 - Analysis of variance of the effect of growing media on morphological traits of petunia.

Source of Variation	df	Flower Diameter	Plant Height	Number of Flowers	Fresh Root Weight	Fresh Shoot Weight	Dry Root Weight	Dry Shoot Weight
Treatment	1	0.054 <sup>ns</sup>	0.881 <sup>ns</sup>	96 <sup>*</sup>	0.074 <sup>ns</sup>	6.468 <sup>ns</sup>	0.005 <sup>ns</sup>	0.564 <sup>ns</sup>
Error	2	0.056	0.611	1.50	0.027	1.981	0.002	0.265
CV%	-	9.399	2.945	9.185	9.868	3.093	8.686	10.701

\* and <sup>ns</sup> indicate significance at the 5% probability level and non-significance, respectively.

Table 3 – Analysis of variance of the effect of growing media on physiological and biochemical traits of petunia.

Source of Variation	df	Petal anthocyanin content	Total sugar	Total Chlorophyll	Leaf carotenoid	Flowering time
Treatment	1	0.001 <sup>ns</sup>	0.014 <sup>**</sup>	0.087 <sup>**</sup>	0.052 <sup>**</sup>	0.166 <sup>ns</sup>
Error	2	0.0001	0.00003	0.0004	0.00006	0.067
CV%	-	2.056	1.872	3.140	1.977	1.108

\* and <sup>ns</sup> indicate significance at the 5% probability level and non-significance, respectively.

### Number of Flowers

The mean comparison results (Table 4) revealed that petunia plants grown in the 50:50 (v/v) mixture of *Pinus eldarica* cone peat and perlite produced the highest number of flowers. This increase in flower production supports the study's goal of identifying a locally available,

nutrient-rich substrate that can match or surpass the performance of imported cocopeat. These results suggest that this medium not only enhances the reproductive performance of petunia but also offers a potential strategy to reduce reliance on foreign substrates.

Table 4. Means Comparison for the effect of growing media on physiological and biochemical traits of petunia.

growing media	Number of flowers	Total sugar	Total Chlorophyll	Leaf carotenoid
Pine cone peat + perlite (50 + 50 v/v)	17.333 <sup>a</sup>	0.630 <sup>b</sup>	0.772 <sup>a</sup>	0.926 <sup>a</sup>
Cocopeat + perlite (50 + 50 v/v)	9.333 <sup>b</sup>	0.729 <sup>a</sup>	0.531 <sup>b</sup>	0.738 <sup>b</sup>

Different letters indicate significant differences at the 5% probability level.

### Leaf Chlorophyll and Carotenoid Content

The mean comparison results (Table 4) showed that petunia plants grown in a 50:50 (v/v) mixture of *Pinus eldarica* cone peat and perlite exhibited the highest leaf chlorophyll and carotenoid contents.

Regarding physiological traits, leaf chlorophyll and carotenoid contents were highest in plants grown in the pine cone peat + perlite medium (0.772 and 0.926 mg g<sup>-1</sup> FW, respectively), whereas plants grown in cocopeat + perlite exhibited lower levels (0.531 and 0.738 mg g<sup>-1</sup> FW, respectively).

### Total Sugar Content

The mean comparison results (Table 4) showed that petunia plants grown in the 50:50 (v/v) mixture of cocopeat and perlite exhibited the highest total sugar content. This result highlights the effect of the growing medium and nutrient availability on soluble sugar accumulation. Cocopeat improves plant growth by enhancing water retention, which indirectly supports carbohydrate and sugar synthesis.

### Discussion

The higher flower number in the pine cone peat + perlite treatment demonstrates the potential of this locally produced, nutrient-rich substrate to improve reproductive performance in petunia and reduce

dependence on imported cocopeat. Nutrient availability, especially of potassium, phosphorus, nitrogen, and other macro- and micronutrients (Ca, Mg, Fe, Mn, Cu), is crucial for flower development and yield (Gülser et al., 2019; Hussain et al., 2017; Rahmani et al., 2020). The elevated cation exchange capacity and nutrient content of pine cone peat likely contributed to the observed increase in flower number.

Previous studies support the positive effect of organic substrates on flower production. Rahmani et al., (2020) reported that wood chip compost increased bud numbers in the lily, while Asghari (2017) observed higher flower numbers in Anthurium with tea waste in the medium. In contrast, pine wood fiber was reported to reduce flower production in petunia (Dickson et al., 2022; Harris et al., 2020), possibly due to allelopathic compounds or differences in material composition. In the present study, *P. eldarica* cone peat had a positive impact on flower number, highlighting its suitability compared with pine wood fiber (*P. taeda*).

The higher leaf chlorophyll and carotenoid contents in plants grown in pine cone peat + perlite are likely related to the increased availability of iron, manganese, and magnesium, which are essential for pigment biosynthesis (Marschner, 2012; Mohammadzadeh Toutouchi et al., 2016; Verbruggen et al., 2013). These findings are consistent with previous reports showing enhanced chlorophyll and carotenoid accumulation in

plants grown with organic amendments such as wood ash, green compost, and wood waste compost (Akbarzade et al., 2023; Mohsenzadeh et al., 2023; Nabeela et al., 2015; Sardoei, 2014).

Although pine cone peat provided adequate porosity and water retention, total soluble sugar content was higher in cocopeat + perlite. This effect may result from the superior water-holding capacity of cocopeat, which indirectly supports carbohydrate synthesis and translocation (Tiwari, 2015). Similar trends were reported in strawberry and Stevia plants grown in cocopeat-containing substrates (Asghari, 2017; Sharifi et al., 2019). Overall, the results indicate that pine cone peat is a promising alternative to imported cocopeat, improving flower number, chlorophyll, and carotenoid content, while cocopeat may favor sugar accumulation.

## Conclusion

The findings of this study indicate that the choice of growing medium significantly affects the quantitative and qualitative traits of petunia. Pine cone peat-based media had a more pronounced positive effect on most traits, including flower number, chlorophyll, and carotenoid contents, compared with cocopeat. Considering that pine cone peat is locally produced, reduces dependence on imported substrates, is more cost-effective, and contains higher nutrient levels, it can be recommended as a sustainable and economical alternative to imported cocopeat for petunia seedling production. Although this study was conducted under controlled greenhouse conditions, results may differ under field conditions; therefore, further field-based research is recommended to validate and generalize these findings.

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