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Evaluation of Hydroponic Growth Media on Phytochemical Performances of Two Basil Genotypes

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ABSTRACT

The global demand for medicinal plants is rapidly increasing, prompting the exploration of innovative cultivation techniques. Hydroponics, in particular, offers a promising method for producing high-quality medicinal herbs. This study was carried out as a factorial experiment using a completely randomized design with three replications, conducted in a research greenhouse in Zanjan, Iran. The experiment involved two basil genotypes (green and purple) and ten different growth media: cocopeat, perlite, sand, pumice, cocopeat + perlite (1:1), cocopeat + pumice (1:1), cocopeat + sand (1:1), pumice + sand (1:1), pumice + perlite (1:1), and sand + perlite (1:1), all under a hydroponic system. The results revealed that chlorophyll and carotenoid contents in both basil genotypes were significantly higher in the cocopeat + perlite and cocopeat + pumice media. In contrast, plants grown in sand and pumice substrates exhibited lower concentrations of photosynthetic pigments. Additionally, phytochemical parameters, including total phenols, flavonoid and anthocyanin content, antioxidant activity (measured via DPPH and FRAP assays), total protein, and PAL enzyme activity, were notably enhanced in the combined growth media, especially in the cocopeat + perlite medium. Conversely, the lowest values for these phytochemical traits were observed in plants grown in inorganic substrates, such as sand and pumice, either alone or in combination. Overall, the cocopeat + perlite medium was the most effective at improving both physiological and phytochemical parameters. In conclusion, optimizing growth media in hydroponic systems can significantly enhance the production of high-quality vegetables and medicinal herbs with superior nutritional value.

Introduction

Sweet basil (*Ocimum basilicum* L.), a widely used vegetable and medicinal herb, belongs to the Lamiaceae family. Its notable antioxidant activity stems from a rich array of secondary metabolites, particularly phenolic compounds and polyphenols such as flavonoids and anthocyanins (Taie et al., 2010). These secondary metabolites are bioactive compounds involved in plant defense mechanisms and help plants adapt to environmental conditions. As a result, the synthesis and accumulation of these bioactive molecules in plant tissues are significantly influenced by both nutritional and environmental factors during growth and development (Maggini et al., 2021).

The rising global demand for agricultural

products has led to the adoption of soilless cultivation methods, with hydroponic systems offering an alternative to traditional soil-based farming in sustainable agriculture (Yang et al., 2024). Hydroponic systems, in particular, have gained attention for the cultivation of medicinal plants, providing a controlled environment that minimizes the influence of external factors like temperature, pests, and diseases. Since the production of phytochemicals is highly sensitive to environmental conditions, hydroponic cultivation ensures better nutrient availability, ultimately enhancing the quality of medicinal plants (Saxhaug et al., 2022).

Previous studies have demonstrated that hydroponic systems improve the quality, bioactive compound content, and biomass of medicinal plants (Nomnqophiso et al., 2024). For example, Kumar et al. (2024) found that the phytochemical quality of basil and mint was superior in hydroponic systems compared to soil cultivation. Similarly, the bioactive compounds of *Agastache rugosa* increased under hydroponic conditions (Lam et al., 2020), and Bafort et al. (2022) reported similar findings with *Euphoria* plants. Sweet basil, one of the most widely produced aromatic herbs, is now cultivated hydroponically on a global scale (Maggini et al., 2022), and its demand continues to grow (Rodgers et al., 2022).

Hydroponic cultivation systems enable precise management of water and nutrient solutions, leading to increased crop yield and quality while reducing labor requirements (Lim et al., 2020). However, successful plant growth in hydroponic systems depends on various factors, with the choice of growth medium being one of the most critical. Growth media, or substrates, play a key role in promoting plant growth by providing an ideal environment for root development, ensuring adequate air and water retention, and fostering optimal plant health (Shylla et al., 2018). Soilless cultivation systems offer additional benefits, such as reducing soil-borne diseases and preventing the spread of viral infections and nematodes. A variety of organic and inorganic materials, or combinations of both, are used as substrates in these systems. Research has shown that combined growth media often enhance plant growth by improving the physical and chemical properties of the substrate (El-Hanafy et al., 2017). Common substrates used in hydroponics include perlite, cocopeat, peat moss, pumice, and sand (Mathowa et al., 2016; Lim et al., 2020). In some cases, these media can also partially meet the nutritional needs of the plants (Sharifi and Nadei, 2019).

Despite the growing use of hydroponic systems, there is limited knowledge about the effects of different organic and inorganic growth media on the phytochemical characteristics and antioxidant potential of basil cultivars. Therefore, this study aims to investigate the impact of various growth media, both alone (100% v) and in combination (50:50 volume ratio), on the phytochemical and antioxidant properties of two basil genotypes (green and purple) grown under hydroponic conditions.

Materials and Methods

This research was conducted as a factorial experiment using a completely randomized design with three replications, carried out in a research greenhouse located in Zanjan, Iran (36° 40' 0" N, 48° 28' 60" E, 1640 m a.s.l). The experiment involved two factors: basil genotypes at two levels (green and purple), and 10 different growth media. The growth media included cocopeat, perlite, sand, pumice, and the following combinations in 50:50 ratios, i.e., cocopeat + perlite, cocopeat + pumice, cocopeat + sand, pumice + sand, pumice + perlite, and sand + perlite (Table 1), all under a hydroponic system. Seeds were sown in sterilized two-liter plastic pots and watered daily with tap water. Following germination, 10 plants were retained in each pot, and any excess seedlings were removed to ensure uniform plant density.

Establishment of the hydroponic system

In the hydroponic system, plants were irrigated three times a day with 300 mL of nutrient solution. The complete hydroponic fertilizer "Hader," consisting of two separate formulations (A and B), was used to supply all essential macro- and micronutrients. Deionized water was used to prepare the nutrient solution, which contained the following mineral concentrations: 8.7% nitrogen (N), 2.62% phosphorus (P), 19.32% potassium (K), 5.54% sulfur (S), 4.13% magnesium (Mg), 0.24% iron (Fe), 0.045% boron (B), 0.053% manganese (Mn), 0.004% zinc (Zn), 0.002% copper (Cu), and 0.001% molybdenum (Mo). Water and greenhouse temperatures were monitored daily, with the greenhouse temperature maintained at 26°C. The pH and electrical conductivity (EC) of the nutrient solution were adjusted to 6.5 and 2 mS cm^{-1}, respectively.

Chlorophyll and carotenoid content

To measure chlorophyll a, chlorophyll b, and carotenoids, one gram of basil leaves was crushed and ground to form a homogeneous mixture with 10 mL of 80% acetone. One mL of this mixture

was then combined with 9 mL of 80% acetone and centrifuged at 8000 rpm for 15 min. The supernatant was collected for the measurement of chlorophyll and total carotenoids. Chlorophyll a, chlorophyll b, and total carotenoid content were determined using a spectrophotometric method with a VIS/UV spectrophotometer. The absorbance of the supernatant was recorded at wavelengths of 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 480 nm for total carotenoids. 80% acetone was used as the blank. The concentrations of chlorophyll a (Equation 1), chlorophyll b (Equation 2), total chlorophyll (Equation 3), and the carotenoid ratio (Equation 4) were calculated according to Arnon (1949).

Equation 1: $(Chl\ a) = \frac{(12.7\ A_{663}) - (2.69\ A_{645}) \times V}{W1000}$

Equation 2: $(Chl\ b) = (\frac{21}{21\ A_{645}}) - (\frac{5}{1\ A_{663}})$

Equation 3: $(Chl\ T) = (Chl\ a) + (Chl\ b)$

Equation 4: $\frac{(1000A_{470}) - (\frac{1}{8\ Chl\ a}) - (\frac{85}{02\ Chl\ b})}{198}$

Table 1. Physical and chemical characteristics of the studied substrate compounds.

substrate	pH	EC	Water holding capacity (%)	Air retention capacity (%)	Porosity (%)	Bulk Density (g cm^{-3})
Cocopeat (100%)	5.3	2.85	77	38	75	0.17
Perlite (100%)	7.2	1.84	63	62	85	0.31
Pumice (100%)	6.7	0.68	58	26	70	0.33
Sand (100%)	6.8	1	43.3	23.2	66.5	1.55
Cocopeat + perlite (v/v = 50%)	7.3	1.9	73	55	78	0.49
Cocopeat + pumice (v/v = 50%)	6.4	1.5	45	25	65	0.24
Cocopeat + sand (v/v = 50%)	6.2	1.6	63	14	77	0.85
Pumice + perlite (v/v = 50%)	6.9	1.15	61	33	75	0.26
Sand + perlite (v/v = 50%)	7.1	1.35	54	42	75	0.95
Pumice + sand (v/v = 50%)	6.9	0.85	53	25	67	0.95

Total protein content

The total protein content was determined using the Bradford method (Bradford, 1976). The absorbance of the samples was measured using a spectrophotometer, and the total protein concentration was calculated by comparing the absorbance values to a standard curve prepared using known protein concentrations.

Determination of total phenolic compounds (TPC)

The total phenolic compounds were determined using the Folin–Ciocalteau method (Singleton et al., 1974). To 0.5 mL of the basil extract, 0.5 mL of deionized water and 2 mL of Folin–Ciocalteau reagent were added. After a 3-min incubation, 10 mL of 20% (w/v) sodium carbonate was introduced, and the mixture was then incubated in the dark at room temperature for 30 min. The absorbance of the samples was measured at 725 nm using a spectrophotometer. Results were expressed as mg of gallic acid equivalents per g of dry weight of basil leaves.

Total flavonoid content (TFC)

The total flavonoid content was measured using the aluminum chloride colorimetric method, as described by Zhishen et al. (1999). In this procedure, 0.5 mL of the methanolic extract was mixed with 150 µL of 15% sodium hydroxide solution. After 6 min, 150 µL of 10% aluminum chloride ($AlCl_3$) was added, followed by the addition of 2 mL of 4% sodium hydroxide (NaOH) and 2 mL of distilled water to achieve a final volume of 5 mL. After 1 h, the absorbance of the samples was measured at 510 nm using a

spectrophotometer. Results were expressed as mg of quercetin equivalents g^{-1} dry weight of basil leaves.

Total anthocyanin

The total anthocyanin content was determined using the method described by Wagner (1979). For this analysis, 0.1 g of fresh leaves was homogenized in 10 mL of acidified methanol (methanol: HCl, 99:1 v/v) and incubated at 25 °C for 24 h in the dark. The extracts were then centrifuged at 4000 g for 10 min at room temperature. The absorbance of each supernatant was measured at 550 nm using a UV-visible spectrophotometer. Total anthocyanin content was expressed as μmol g^{-1} of fresh weight.

Phenylalanine ammonia-lyase (PAL) activity assay

To measure phenylalanine ammonia-lyase (PAL) enzyme activity, an enzyme extract was first prepared. Three-hundred mg of fresh leaf tissue were ground in an ice-cold mortar with 6.5 mL of 50 mM Tris-HCl buffer (pH 8.8) containing 15 mM β-mercaptoethanol for 5 min, followed by an additional 30 min. The mixture was then centrifuged at 50,000 g for 1 min, and the supernatant was collected for PAL enzyme measurement.

PAL enzyme activity was assessed based on the rate of cinnamic acid production, following the method of Wang et al. (2006). The reaction mixture consisted of 1 mmol of extraction buffer, 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of double-distilled water, and 0.1 mL of the enzyme extract. This mixture was incubated at 37 °C for 1 h, after which the reaction was terminated by adding 0.5 mL of 6 M hydrochloric acid. To extract the resulting product, 15 mL of ethyl acetate was added, and the solvent was evaporated.

The remaining solids were suspended in 3 mL of 0.5 mM NaOH, and the concentration of cinnamic acid was measured at an absorbance of 280 nm. One unit of enzyme activity was defined as the amount of PAL produced that results in the formation of 1 μmol of cinnamic acid min^{-1}, expressed as μmol of cinnamic acid mg^{-1} of protein min^{-1}.

Determination of free radical scavenging activity

The free radical scavenging activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, following the method described by Brand-Williams et al. (1995). To prepare the methanolic total extract, 100 g of dried plant powder was obtained from the aerial parts of the plant and extracted using a soaking method in methanol for 48 h.

For the DPPH assay, 80 μL of the methanolic extract was mixed with 1.92 mL of a DPPH solution in methanol. The absorbance of the samples was measured at 515 nm after a 2.5-min incubation period. The ability of the test material to quench DPPH free radicals was calculated using the following equation:

$$Scavenging\% = \frac{100 \times (A0 - As)}{A0}$$

In this equation, A0 = absorbance of control at 0 min, As = absorbance of the sample.

Statistical analysis

Data analysis was performed using the ANOVA procedure in SAS 9.2 software. A comparison of mean values was carried out using the LSD method ($P \leq 0.01$). Graphs were drawn in Microsoft Excel.

Results

As shown in Table 2, both the cultivar and growth medium treatments, as well as their interaction, had significant effects on chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (total Chl), carotenoid content, total phenolic compounds, PAL enzyme activity, and anthocyanin levels. In contrast, for total flavonoids, total protein, and antioxidant activity as measured by the DPPH and FRAP assays, the simple effects of the treatments were significant; however, their interaction did not exhibit any significant effects on these parameters.

Chlorophyll a, b and total

The chlorophyll content in green and purple basil leaves varied significantly across different growth media. As shown in Figure 1, the highest concentration of chlorophyll a (1.77 mg g^{-1} fresh weight) was observed in green basil grown in the cocopeat + pumice substrate, while the lowest concentration (0.87 mg g^{-1} fresh weight) was found in the sand substrate. For purple basil, the highest amount of chlorophyll a (1.44 mg g^{-1} fresh weight) was recorded in the cocopeat + perlite medium, whereas the lowest concentration (0.83 mg g^{-1} fresh weight) was associated with the sand growth medium. As illustrated in Figure 2, the highest concentration of chlorophyll b (0.44 mg g^{-1} fresh weight) in green basil was associated with the cocopeat and perlite mixed substrate, while the lowest concentration (0.18 mg g^{-1} fresh weight) was observed in the sand substrate. Additionally, in purple basil, the highest

chlorophyll b content (0.39 mg g^{-1} fresh weight) was achieved in the cocopeat + perlite medium, whereas the minimum content (0.17 mg g^{-1} fresh weight) was related to the sand growth medium.

Table 2. Analysis of variance of phytochemical and antioxidant activities of two basil genotypes were grown in 10 growth media under hydroponic system.

S.V	df	MS										
		Chla	Chlb	TChl	CC	TPC	TFC	ACN	PAL	DPPH	FRAP	TPrT
Cultivar	1	0.0576**	0.0001**	0.0576**	0.0001**	0.0582**	0.02413**	0.1402**	0.0020**	63.860**	0.6181**	0.0220**
Growth media	9	0.0870**	0.0813**	0.3119**	0.01337**	0.4348**	0.2381**	0.0013**	0.0438**	35.649**	32.375**	0.1035**
Cultivar× Growth media	9	0.0094**	0.00001**	0.0094**	0.0001**	0.0582**	0.0023^{ns}	0.0002**	0.00486**	0.2438^{ns}	0.1115^{ns}	0.0002^{ns}
error	40	0.0194	0.0229	0.0290	0.0028	0.217	0.06905	0.0008	0.0072	128.920	0.3254	0.0119
CV (%)	-	2.02	8.725	1.97	5.88	2.96	5.03	3.90	2.10	3.31	4.02	2.11

Chl a: Chlorophyll a, Chl b: Chlorophyll b, TChl: Total chlorophyll, CC: Carotenoid content, TPC: Total phenolic content, TfC: Total flavonoid content, CAN: Anthocyanin, PAL: Phenyl alanine amonialyase, TPrT; Total protein. S.V: Source of variation, df: Degree of freedom, MS: Mean squares, CV: Coefficient of variation. **; Significant at $P \leq 0.01$, ^{ns}: No Significant.

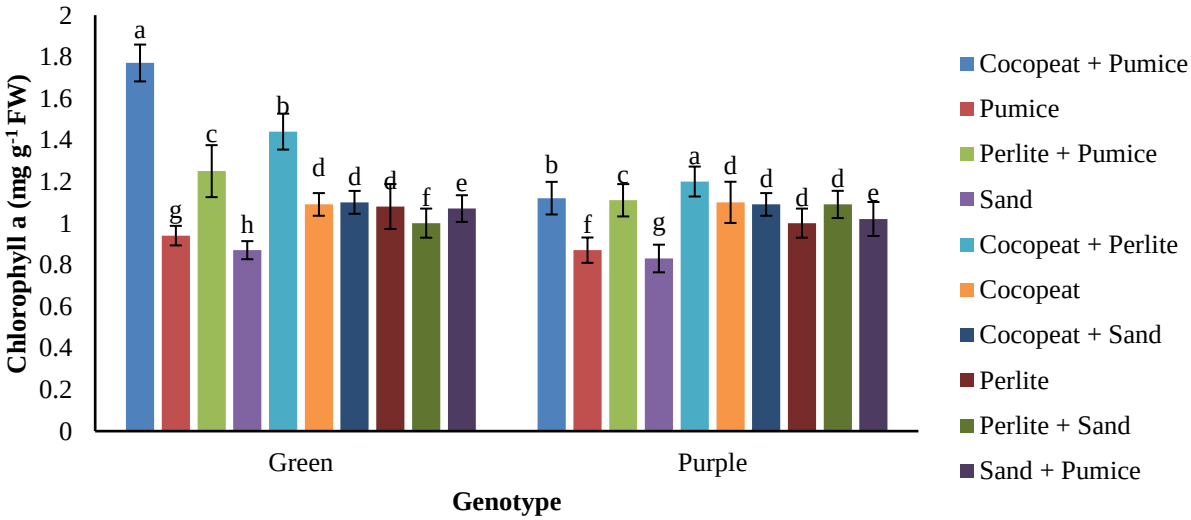

Fig. 1. Effect of growth media in two genotypes of basil (green and purple) on chlorophyll a. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

The highest total chlorophyll (2.2 mg g^{-1} FW) in green basil was recorded in the cocopeat + pumice growth medium, whereas the lowest amount (1.01 mg g^{-1} FW) was obtained in the sand substrate. Furthermore, in the purple cultivar, the highest mean value of total chlorophyll (1.59 mg g-1 FW) and lowest (1 mg g^{-1} FW) were recorded in cocopeat + perlite and sand growth media, respectively (Fig. 3).

Carotenoid

According to the results, the highest concentration of carotenoid (0.23 mg g^{-1} FW) in green basil occurred in cocopeat + pumice growth medium, and the minimum (0.12 mg g^{-1} FW) was recorded in sand. Also, in purple basil, the highest carotenoid content (0.24 mg g^{-1} FW) was observed in cocopeat + pumice growth media, and the lower concentration (0.1 mg g^{-1} FW) was associated with the sand substrate (Fig. 4).

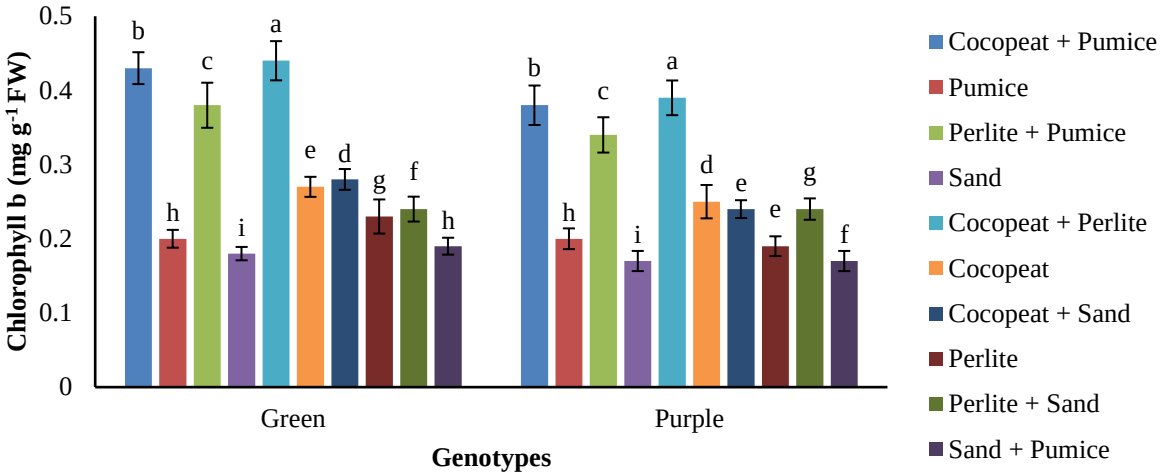

Fig. 2. Effect of the growth media in two genotypes of basil (green and purple) on chlorophyll b. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

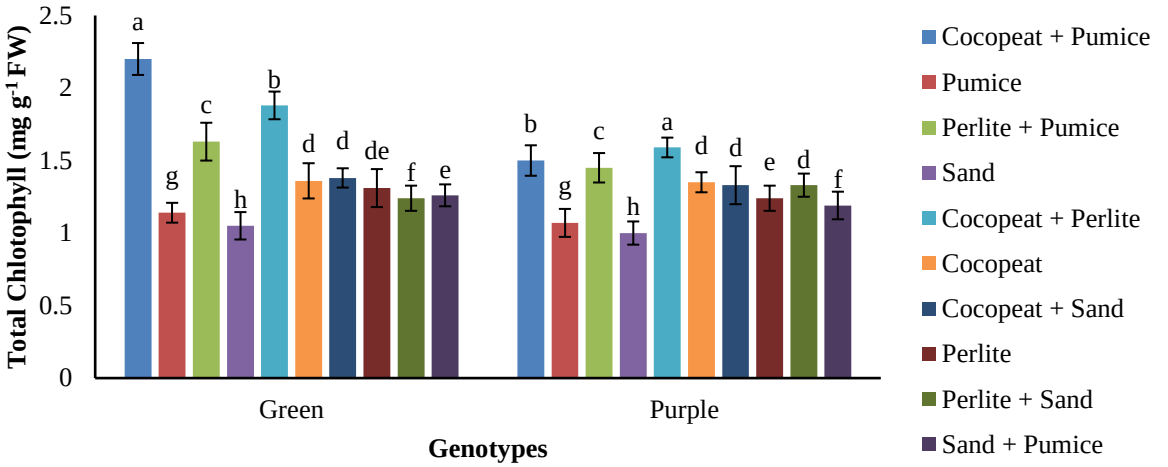

Fig. 3. Effect of the growth media in two genotypes of basil (green and purple) on total chlorophyll. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

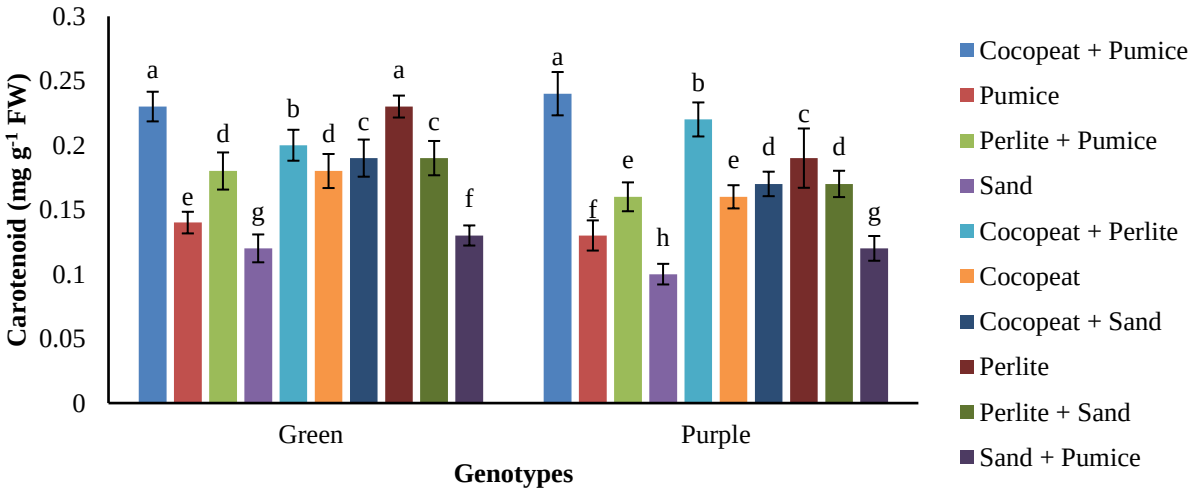

Fig. 4. Effect of the growth media in two genotypes of basil (green and purple) on carotenoid. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

Total protein

The comparison of mean values (Fig. 5) indicated that the highest total protein was obtained in the purple cultivar (8.75%) in comparison with the green basil. Also, the highest value (1.11 mg g^{-1} FW) of protein was recorded in cocopeat + perlite substrate, although no significant difference was observed between this substrate and cocopeat + pumice growth media. Furthermore, the lowest value of protein (0.64 mg g^{-1} FW) occurred in plants of the sand substrate (Fig. 6).

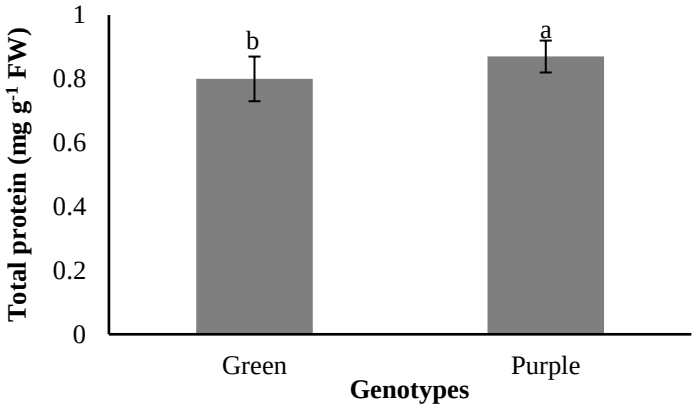

Fig. 5. Simple effect of genotype on total protein. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

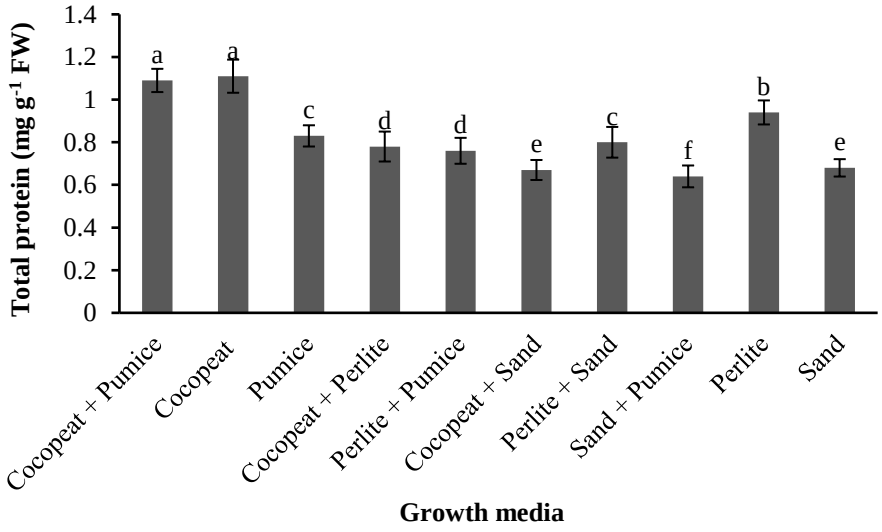

Fig. 6. Simple effects of growth media on total protein in *O. basilicum*. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

Total phenolic compound

Figure 7 illustrates that the highest accumulation of total phenolic (3.6 mg GAE g^{-1} DW and 2.8 mg GAE g^{-1} DW) compounds in both green and purple basil were obtained in cocopeat and perlite growth media respectively, whereas the minimum content in green and purple basil (2.2 mg GAE g^{-1} DW, 2.2 mg GAE g^{-1} DW, respectively) were observed in the sand growth medium.

Total flavonoid

The results indicated that the greatest values of total flavonoids (5.85 mg quercetin equivalent g^{-1} dry weight) were observed in green basil, representing a 5% increase compared to purple basil. Additionally, the highest concentration of total flavonoid compounds (1.08 mg quercetin equivalent g^{-1} dry weight) was recorded in the perlite + cocopeat substrate, with no significant difference compared to the cocopeat + pumice substrate. Conversely, the lowest amount of flavonoids (0.38 mg quercetin equivalent g^{-1} dry weight) was associated with the sand growth medium (Figs. 8 and 9)

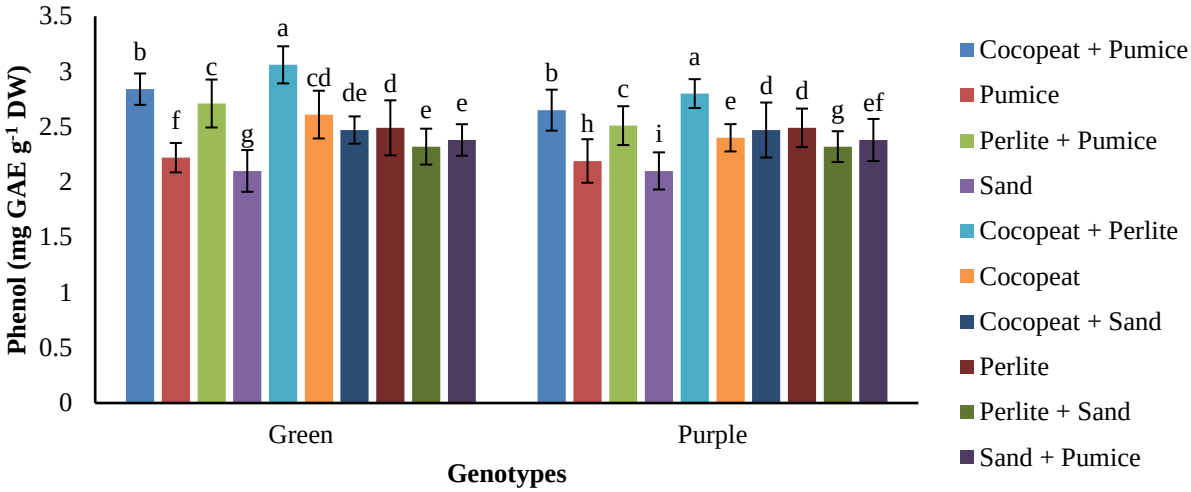

Fig. 7. Effect of the growth media in two genotypes of basil (green and purple) on total phenolic compounds. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

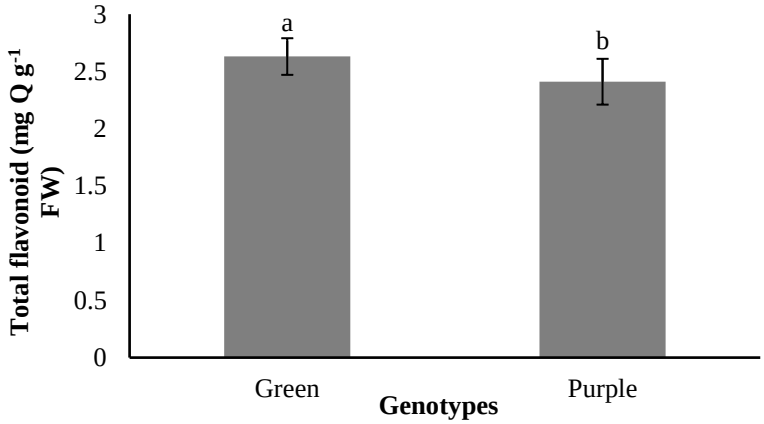

Fig. 8. Simple effects of genotype on total flavonoids in *O. basilicum*. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

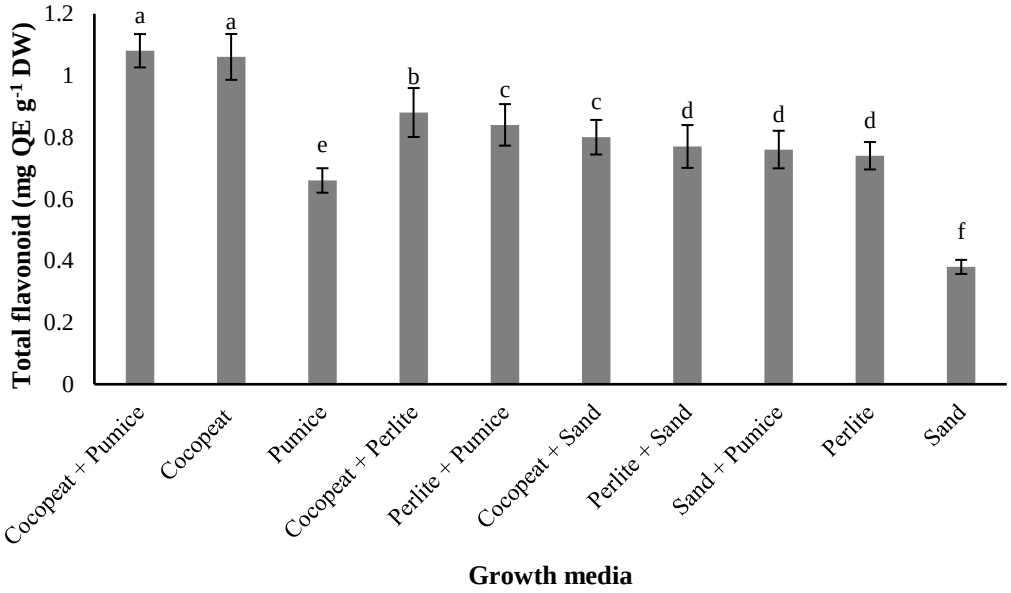

Fig. 9. Simple effects of growth media on total flavonoids in *O. basilicum*. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

Anthocyanin

The maximum anthocyanin content (0.19 µmol g^{-1} FW) in purple basil was observed in plants cultivated in perlite + cocopeat and cocopeat + pumice, whereas the minimum (0.13 µmol g^{-1} FW) was recorded in plants cultivated in the sand growth media. Furthermore, in green basil, the highest mean value of anthocyanin (0.08 µmol g^{-1} FW) belonged to the perlite + cocopeat substrate and the lowest (0.051 µmol g^{-1} FW) was observed in the sand substrate (Fig. 10).

PAL enzyme activity

The highest PAL enzyme activity (0.71 mmol catechol equivalent mg^{-1} min^{-1}) was observed in green basil cultivated in the cocopeat + perlite substrate, while the minimum activity (0.51 mmol catechol equivalent mg^{-1} min^{-1}) was associated with the sand substrate. However, no significant difference was found between the sand and pumice growth media. In the purple basil cultivar, the maximum PAL enzyme activity (0.74 mmol catechol equivalent mg^{-1} min^{-1}) was recorded in the cocopeat + perlite growth medium, whereas the lowest activity (0.54 mmol catechol equivalent mg^{-1} min^{-1}) was obtained in the sand substrate, with no significant differences noted compared to the pumice and sand + pumice treatments (Fig. 11).

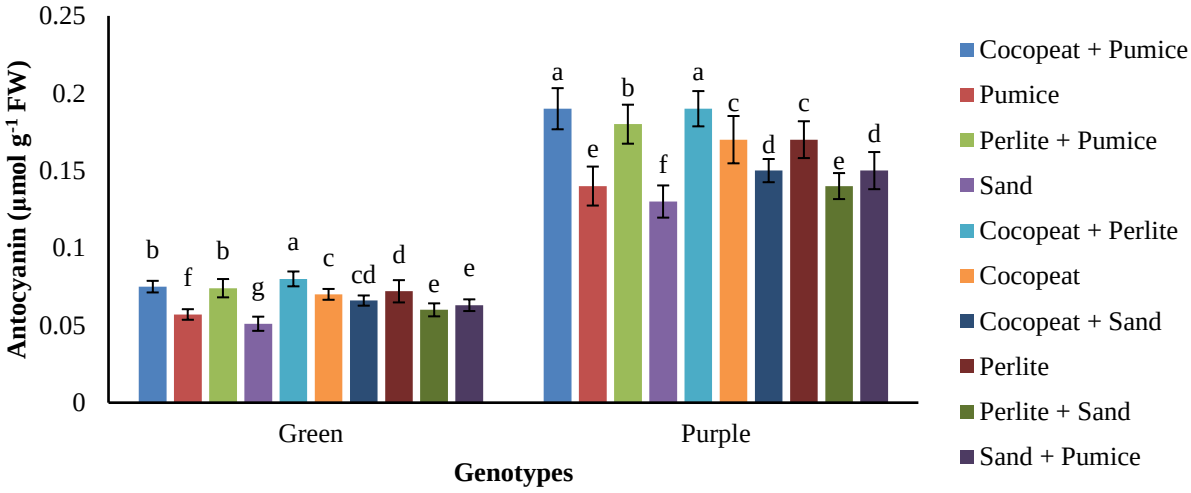

Fig. 10. Effect of the growth media in two genotypes of basil (green and purple) on total anthocyanin. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

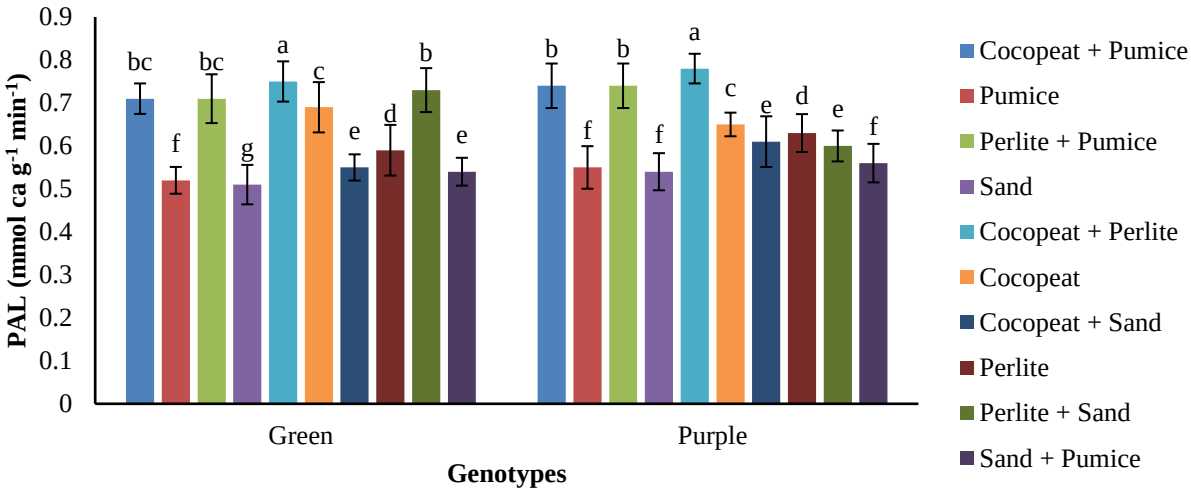

Fig. 11. Effect of growth media on two genotypes of basil (green and purple) on PAL enzyme activity. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

Antioxidant activities (DPPH, FRAP)

The maximum antioxidant activity measured by the DPPH method (57.23%) was associated with purple basil, representing a 5.94% increase compared to green basil (Fig. 12). Furthermore, the highest antioxidant activity was obtained in the cocopeat + perlite growth medium, while the lowest activity (53.09%) was observed in the sand substrate (Fig. 13).

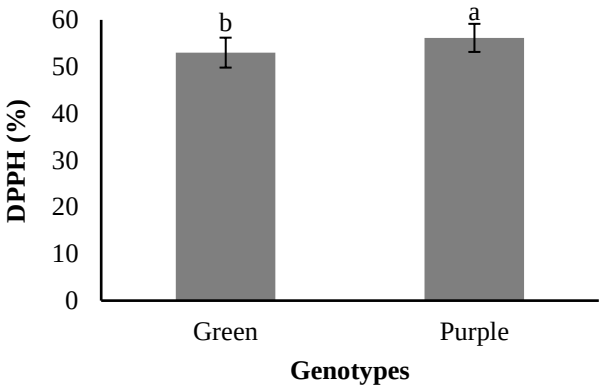

Fig. 12. Simple effects of genotype on DPPH antioxidant activity in *O. basilicum*. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

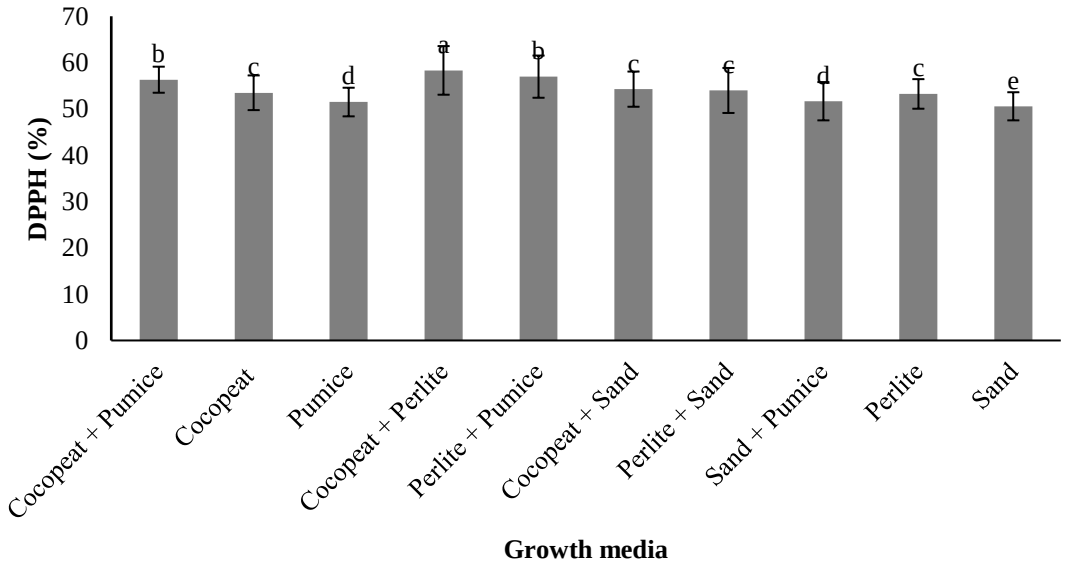

Fig. 13. Simple effects of growth media on DPPH antioxidant activity in *O. basilicum*. Different letter(s) on the bars showed significant difference (P≤0.01). Error bars represent standard deviation.

The antioxidant activity in the FRAP method was higher in purple basil by 9.34%, compared to green basil (Fig. 14). In addition, the maximum antioxidant activity (3.38 mmol Fe^{+} g^{-1}) was recorded in plants cultivated on cocopeat + perlite growth media, although no significant difference was found between this substrate and cocopeat + pumice. Also, the minimum (1.16 mmol Fe^{+} g^{-1}) antioxidant activity in the FRAP method was observed in plants cultivated on sand (Fig. 15).

Discussion

The present study compared the phytochemical properties and antioxidant activities of two cultivated basil genotypes (green and purple) grown in ten different growth media under a hydroponic system. The findings revealed significant variations in chlorophyll and carotenoid contents, phytochemical parameters, and antioxidant activities across the different substrates. The combined substrate of perlite and cocopeat (50:50) yielded the best results in terms

of phytochemical properties, whereas all investigated traits considerably decreased in sand and pumice substrates.

The combination of perlite and cocopeat as growth media improved the physical and chemical properties of the root zone in the hydroponic system, resulting in enhanced phytochemical performance of basil compared to using sole perlite, cocopeat, or other substrates in this study. The addition of organic materials to mineral substrates, including sand and perlite, increased pore spaces, improved electrical conductivity, and enhanced water-holding capacity, thereby promoting better plant growth (MirseyedHosseini et al., 2023). Cocopeat, known for its high water-holding capacity and antifungal properties, also provides substantial amounts of phosphorus and potassium for plant growth. In contrast, inorganic substrates like pumice and sand typically have low nutrient levels (Chhetri et al., 2022).

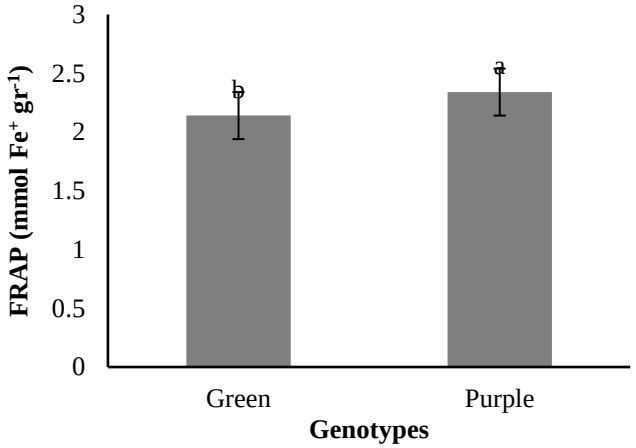

Fig. 14. Simple effects of genotype on FRAP antioxidant activity in *O. basilicum*. Different letter(s) on the bars showed statistically significant differences (P<0.01). Error bars represent standard deviation.

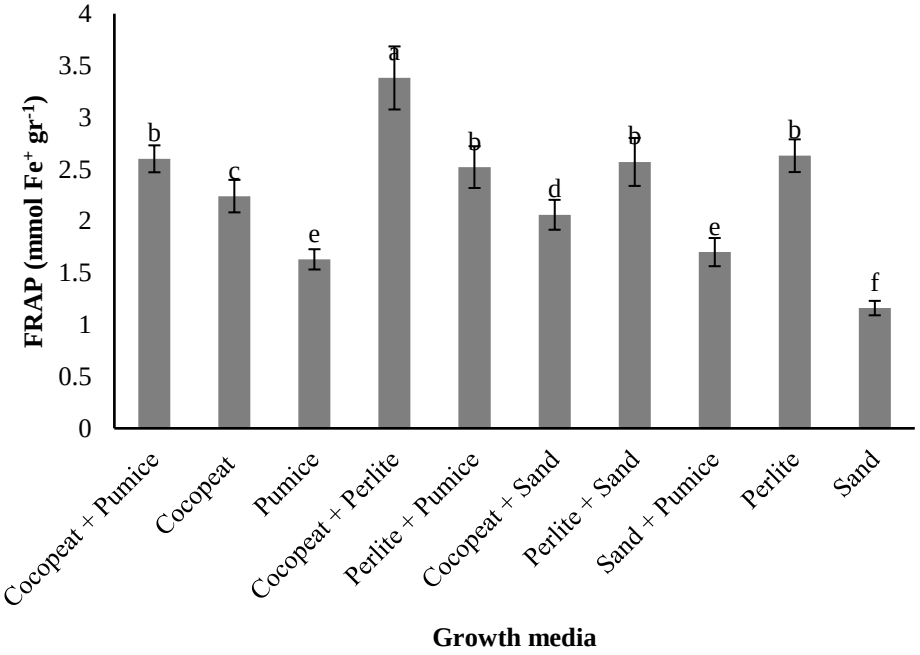

Fig. 15. Simple effects of growth media on FRAP antioxidant activity in *O. basilicum*. Different letter(s) on the bars showed statistically significant differences (P<0.01). Error bars represent standard deviation.

The unique chemical and physical properties of organic substrates contribute to high nutrient levels while reducing the risk of toxicity or nutrient deficiencies (Heidari et al., 2021).

Perlite, one of the most commonly used inorganic substrates, offers good physical and chemical characteristics (Kennard et al., 2020). In this research, sand growth media demonstrated poor performance compared to other substrates. Although sand is an inexpensive and readily available substrate widely used in the soilless cultivation of various plants, its water-holding and cation exchange capacities are significantly lower than those of other substrates. Additionally, it leads to the loss of nitrate, ammonium, and orthophosphate from the cultivation substrate, negatively impacting growth parameters (Velichkova et al., 2019). Consequently, incorporating other compounds, such as perlite or cocopeat, into sand has been linked to improved phytochemical traits (Agarwal et al., 2021).

Overall, the results from various studies suggest that the growth response of plants to growth media can vary considerably among different species (Graceson et al., 2014). Furthermore, the combination of substrates exhibited synergistic effects on the phytochemical properties of basil cultivars. Each growth medium possesses distinct physicochemical and biological properties, each with its own positive and negative attributes (Agarwal et al., 2021). The substrate combinations used in this experiment provided a more balanced environment for water and air retention, ultimately enhancing nutrient availability.

The results of this study demonstrated that the chlorophyll content of basil genotypes was significantly influenced by the growth media. Basil grown in combined growth media, including cocopeat + pumice and cocopeat + perlite, exhibited higher mean values of chlorophylls and carotenoids. This suggests that optimizing environmental conditions, such as substrate composition, supports better growth and development. A sufficient amount of manganese and nitrogen in substrates promotes chlorophyll pigment synthesis, which directly impacts light absorption and photosynthetic efficiency, ultimately leading to enhanced growth (Shahi et al., 2018). Conversely, a reduction in water storage capacity can hinder the absorption of essential elements, particularly iron, manganese, and phosphorus, resulting in decreased photosynthetic pigments (Roosta et al., 2018). Overall, low porosity in a substrate, coupled with reduced water-holding capacity, leads to inadequate absorption of these essential elements (Pivot et al., 1998; Shinohara et al., 1999).

In this study, the protein content of purple basil was found to be higher than that of green basil. Furthermore, plants grown in cocopeat + perlite and cocopeat + pumice exhibited the maximum protein content compared to other substrates. High protein levels in vegetables enhance their nutritional value, acting as immune system boosters and essential nutraceuticals. Thus, optimizing cultivation techniques, such as selecting suitable growing media, can yield high-value vegetables and medicinal herbs (Tshayingwe et al., 2023).

In related research, Tshayingwe et al. (2023) evaluated the effects of light intensity and four growth media (LECA clay, silica sand, peat, and vermiculite) on the growth and phytochemical content of *Trachyandra divaricata* in a soilless culture system. Their findings indicated that the highest mean values of polyphenols and DPPH activity were achieved in silica sand growth media under 20% shade, while the highest flavonoid content was associated with the LECA clay substrate under both 20% and 40% shade. This indicates that growth media not only influence plant yield but also the synthesis of bioactive compounds such as phenolic compounds, flavonoids, and antioxidant activity (Bulgari et al., 2019).

Some studies have reported a direct correlation between the levels of phenolic compounds and the antioxidant activity of basil (Oonsivilai and Prasongdee, 2014). However, other studies have not observed a direct correlation between antioxidant activities, phenolic compounds, and anthocyanins. This discrepancy may be attributed to the presence of different phenolic and anthocyanin compounds within plant extracts (Prinsi et al., 2019).

In this experiment, the maximum amounts of total phenolic compounds and flavonoids were associated with green basil compared to purple basil. However, the highest antioxidant activities, as measured by the DPPH and FRAP methods, as well as the anthocyanin content, were observed in the purple basil cultivar. Additionally, the highest PAL enzyme activity for both green and purple basil was recorded in the cocopeat + perlite growth medium. Phenylalanine ammonia-lyase (PAL) is a crucial enzyme that regulates the biosynthesis pathway of phenylpropanoid compounds in plants (Ziaei et al., 2012). Rezaei et al. (2020) demonstrated a significant positive correlation between the total phenolic and flavonoid content of basil, DPPH antioxidant activity, and PAL enzyme activity.

In the current study, the higher concentration of anthocyanins in purple basil likely contributed to the increased antioxidant activities of its extracts compared to green basil. This suggests that anthocyanin compounds exert a more potent

antioxidant effect than phenolic and flavonoid compounds. Supporting this notion, Prinsi et al. (2019) investigated anthocyanin levels in different organs of basil across various green and purple genotypes, revealing that the highest anthocyanin content was observed in the leaves of the "Red Rubin" variety (a purple basil cultivar). Similarly, Ferrarezi and Baily (2019) reported higher anthocyanin values in purple cultivars of basil compared to green cultivars.

Contrary to these findings, other studies have indicated a direct relationship between antioxidant activity and the amount of phenolic compounds (Oonsivilai and Prasongdee, 2014). In particular, Walters and Currey (2016) reported that the antioxidant activity, as measured by the FRAP and DPPH methods, was higher in the green basil variety compared to the purple variety. This difference was attributed to the presence of higher percentages of rosmarinic acid and gallic acid in the green variety, which contributed to its antioxidant activity.

A study evaluated the levels of phenolic, flavonoid, and antioxidant compounds in *Acmella oleraceae* under different cultivation conditions, including hydroponic, tissue culture, and conventional soil culture. The results indicated that hydroponic cultivation significantly increased the concentration of secondary metabolites compared to soil and tissue culture. The researchers concluded that using a hydroponic culture system is an effective method for enhancing the medicinal value of this plant (Abeysinghe et al., 2014).

In another study by Kumar et al. (2024), among various growing conditions, the quality parameters of sweet basil, specifically tannin and flavonoid content, were found to be higher in soilless growing media than in other cultivation conditions. Cultivating medicinal plants in controlled environments such as hydroponic systems allows for more precise control of environmental parameters, including temperature, humidity, irrigation, and nutrition (Giurgiu et al., 2014). Furthermore, Surendran et al. (2017) demonstrated that the concentration of organic acids and antioxidant activity in *Mentha spicata* L. were higher in hydroponically grown plants.

Conclusions

The present study aimed to investigate the impact of growth media, a crucial factor in soilless crop production, on the growth and phytochemical responses of two basil genotypes. This valuable medicinal herb is recognized for its significant antioxidant activities, largely attributed to high levels of secondary metabolites, particularly anthocyanins, phenolic compounds, and flavonoids. The findings indicated that the combined use of cocopeat and perlite (50:50) was more effective in enhancing both morphological and phytochemical parameters of the two sweet basil genotypes in a hydroponic system, compared to the individual application of cocopeat, perlite, and other substrates. Additionally, purple basil exhibited greater antioxidant activity, correlating with a higher concentration of anthocyanins compared to green basil. Overall, the phytochemical composition of basil genotypes is significantly influenced by the choice of growth media in hydroponic systems. Thus, the selection of appropriate substrates can enhance the medicinal properties and nutritional value of sweet basil in soilless cultivation systems.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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