



## Evaluation of Morpho-Physiological Traits and Phytochemical Composition of Selected Microgreens

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### ARTICLE INFO

#### Article history

Received: 16 Oct 2023

Accepted: 18 Dec 2023

Published online: 31 Mar 2024

#### Keywords

Microgreen,  
Morpho-physiological traits,  
Phytochemical composition,  
Nutrition

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### ABSTRACT

People are becoming more conscious about nutritional food because of the significant increase in diseases. Again, agricultural land is decreasing which creates a scarcity of nutritional foods. In this context, microgreens can be an excellent option as they can be grown in a controlled environment using various vertical farming. Microgreens are leafy green vegetables whose edible parts are harvested at the seedling stage. For successful further incorporation into the global food system and evaluation of their nutritional impacts, it is essential to determine microgreens morpho-physiological and nutritional properties. There were two phases of this experiment- investigating morpho-physiological parameters and evaluating biochemical parameters of the selected microgreens under laboratory conditions. The whole experiment was carried out under CRD with four replications. Eight vegetables are treated as mustard (*Brassica juncea* L.), radish (*Raphanus sativus*), chia (*Salvia hispanica*), red amaranth (*Amaranthus tricolor* Linn), coriander leaf (*Coriandrum sativum* L.), garden cress (*Lepidium sativum*), sesame (*Sesamum indicum* L.), and cabbage (*Brassica oleracea* var.). Among these microgreens, the best performed four vegetables (mustard, radish, chia and red amaranth) were selected for evaluating biochemical parameters. This study found that radish microgreens provided better performance concerning morphological characteristics among the eight microgreens and red amaranth microgreens showed the highest bio-active compounds, protein, minerals and anti-oxidant activity among the four microgreens tested.

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## 1. Introduction

Microgreens are young and tender cotyledonary leafy greens found in a palette of different colour, textures and flavours (Xiao et al. 2012; Pinto et al. 2015; Turner et al. 2020). They are specific types of vegetables that are harvested and consumed in an immature stage (Xiao et al. 2014). It is soft in texture, different in colour and adds various quality attributes, enhancing the sensory properties of main dishes. Generally, it is harvested just above the roots after the first true leaves emerge (Kou et al. 2013). This microgreen served to the customers with the attached stem, cotyledons and first true leaves, which have gained popularity as a new culinary trend over the past few years. These 10 to 14 days of seedling emergence are considered novel greens available in the market and are typically distinguished categorically by their size and age (Lee et al. 2004; Pinto et al. 2015; Partap et al. 2023). But when it is harvested in between

20-40 days, it is known as baby greens, which is different from microgreens (Partap et al. 2023). Microgreen is not only popular for its distinguished texture and colour, but also its nutritional value. It contains higher concentration of functional components such as antioxidants, phenolics, vitamins and minerals than mature greens and seeds (Sharma et al. 2022; Polash et al. 2019). This functional food contains health-promoting and/or disease-preventing properties that are additional to their normal nutritional values (Xiao et al. 2015; Polash et al. 2018).

Recently, unauthorized chemicals and frequent pesticides application during the growing period and post-harvest level make the marketable vegetables more dangerous for keeping good health. That's why, fresh and nutritional food demand is on the rise, and microgreens can be a great option in this (Ebert 2022). Again, agricultural land is decreasing day by day due to rapid growth of population and urbanization. For this, scientists are trying to find alternative for vertical farming and microgreens can be an

### Cite This Article

Afrin, S., Shorna, S.I., Polash, M.A.S., Hossain, M.A. 2024. Evaluation of Morpho-Physiological Traits and Phytochemical Composition of Selected Microgreens. *Fundamental and Applied Agriculture*, 9(1): 22–28. <https://doi.org/10.5455/faa.172764>

opportunity that can help in increasing food production and expand agricultural operations. Accordingly, it is in the best interest of specialty crop growers, extension specialists and researchers to tap upcoming trends and opportunities for niche products. i.e., microgreens. Several researches focused on an individual microgreen variety and limited research on comprehensive studies on the morpho-physiological and nutrition properties of various microgreens varieties.

## 2. Materials and Methods

### 2.1. Morpho-physiological investigation

The planting materials were mustard, coriander leaf, spinach, red amaranth, radish, chia, bok-choy, water spinach, cabbage, cauliflower, sesame and garden cress seeds. According to Xiao et al. (2015), these were collected for sensory attribute tests. The sensory attribute scales for the test were, Nil = 1; Poor = 2; Fair = 3; Good = 4; and Strong = 5. From the above planting materials eight microgreen species were selected for treatment and used for research. The experiment was carried out with a Completely Randomized Design (CRD) and four replications, resulting in pots 32. Cleaned and dried pots were taken and filled up with soil and cowdung (1:1 ratio) and set the experiment on the pot house. The 50 seeds of eight microgreens were sown in each pot, and for the first three days the pots were kept in an artificially darkening place to ensure etiolated growth. On the fourth day, all the seedlings were given under light requiring spraying water twice a day up to harvest. The microgreens were harvested after ten days from sowing.

#### 2.1.1. Data collection

Germination percentage (GP) and vigor index were taken from the following calculation –

$$GP = \frac{\text{No. of seed germinated}}{\text{No of seed sown}} \times 100$$

Vigor index = Germination percentage × average seedling length (cm)

Shoot length, hypocotyls length and seedling height were measured after ten days old seedlings. It was cut above the soil surface and measured and expressed in cm. Fresh weight and dry weight was recorded after harvesting. The seedlings were cleaned thoroughly by washing with water. Then after wiping well with tissue paper and drying the fresh weight of the seedlings was recorded. Then, the seedlings were oven-dried at 80°C for 72 hours. After drying the dried seedlings were weighed and the dry matter content of the seedlings was recorded.

### 2.2. Bio-chemical Investigation

After investigating morpho-physiological traits, the best performing four microgreen species were selected for bio-chemical study. Four microgreens are – mustard, radish, chia, and red amaranth. Again, the experiment was carried out with CRD with four replications in a pot with previously described media. The seeds of four microgreens were grown in that pot in the pot house. Germination was done according to the previously described method.

#### 2.2.1. Data collection

**Proximate analysis:** Proximate composition of the leaf was determined by adopting procedures of association of analytical chemist (AOAC 1990). In this way ash percentage, carbohydrates, lipid, protein and fiber content were determined.

**Mineral contents:** Dried microgreens (MG) (2 g per experimental replicate) were manually ground into a fine powder using a clean mortar and pestle and placed into clean scintillation vials. Each sample was subjected to standard acid digestion procedures to determine the dry mass content (Weber 2016) of the following elements: P, K, Ca, Mg, S, Na, Fe and Zn.

**Bio-active compounds content:** The leaves of plants (1 g) were extracted in 10 ml of chilled acetone solution in the dark. After centrifugation at 4000 rpm for 10 minutes the absorbance of supernatants was taken at 453, 505, 645 and 663 nm wave lengths. Contents were calculated according to the equation depicted in Barros et al. (2010).

**Vitamin C:** Ascorbic acid was determined following a previously described procedure by Xaio et al. (2012) with 2,6-dichloro indophenol and measured the content by the titrimetric method. The results were expressed as mg of ascorbic acid per 100 g of fresh weight.

**DPPH radical scavenging activity:** 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out with some modifications (Sanja et al. 2009). Different concentrations of methanolic extracts & BHA were taken in different test tubes. The volume was adjusted to 100 µl by adding methanol 3 ml of a 0.1 mM methanolic solution of a DPPH was added to these tubes & shaken vigorously. The tubes were allowed to stand in the dark at room temperature for 30 min. The control was reported as above without any extract. DPPH radical scavenging activity was measured by a reduction in the intensity of purple colour and quantified by the decrease in absorbance at wavelength 517 nm. Radical scavenging activity (RSA) was calculated using the following formula:

$$RSA (\%) = \frac{\text{Control Optical Density} - \text{Sample Optical Density}}{\text{Control Optical Density}} \times 100$$

### 2.3. Statistical analysis

The collected data were statistically analyzed using Minitab 17 statistical Computer Package Programmer in accordance with the principles of Completely Randomized Design. Duncan's Multiple Range Test (DMRT) was used to compare variations among the treatments.

## 3. Results and Discussion

### 3.1. Morpho-physiological Investigation

#### 3.1.1. Planting material for species selection

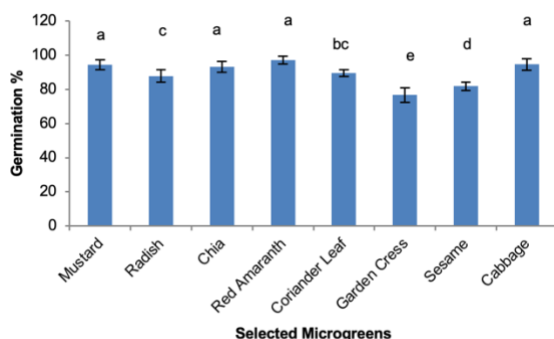
Table 1 shows the sensory attribute test of different microgreens. According to the acceptability of overall eating quality mustard, radish, chia, red amaranth, cabbage, garden cress, sesame and coriander leaves were selected for future research.

**Table 1.** Sensory attribute tests of different microgreens at harvest

Sensory attributes	Intensity of aroma	Intensity of astringency (texture, mouth feel)	Intensity of bitterness	Intensity of grassy	Acceptability of appearance	Acceptability of overall eating quality
Mustard	Nil	Good	Nil	Fairly	Strong	Strong
Coriander leaf	Strong	Fairly	Fairly	Nil	Good	Good
Spinach	Poor	Poor	Poor	Fairly	Fairly	poor
Red Amaranth	Fairly	Strong	Nil	Nil	Strong	Strong
Radish	Good	Good	Poor	Poor	Strong	Strong
Chia	Nil	Good	Fairly	Fairly	Strong	Strong
Bok-choy	Nil	Good	Nil	Nil	Strong	Strong
Water spinach	Nil	Poor	Fairly	Good	Fairly	Poor
Cabbage	Poor	Fairly	Good	Good	Good	Good
Cauliflower	Poor	Poor	Fairly	Good	Fairly	Fairly
Sesame	Nil	Good	Poor	Poor	Good	Strong
Garden cress	Fairly	Good	Poor	Fairly	Good	Good

### 3.1.2. Percent germination

Fig. 1 shows the germination percentage of some selected microgreens. Percent germination were 94.42, 87.88, 93.21, 97.15, 89.56, 76.65, 81.87 and 94.61 for mustard, radish, chia, red amaranth, coriander leaf, garden cress, sesame and cabbage respectively. The lowest germination percentage (76.65) was found in garden cress while it was the highest (97.15) in red amaranth. Among factors that are attributed to poor germination are unfavourable temperature, quality and quantity of light, lack of moisture and inherent factors (Baskin and Baskin, 1998). The difficulties of growing garden cress seeds were mentioned by Lee et al. (2004) who recommended seed priming and application of plant growth regulators to break dormancy.

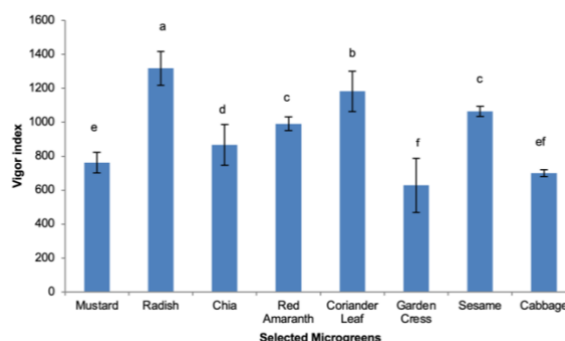


**Figure 1.** Germination percentages of eight selected microgreens. The vertical bars represent the mean  $\pm$ SE (n=8). Dissimilar letter(s) on the top of the bar indicate a significant difference at  $P \leq 0.05$ .

### 3.1.3. Vigor Index

The vigor index was evaluated and found to be significantly different among the eight selected microgreens (Fig. 2). Radish showed the highest (1318) vigor index and the lowest (628) vigor index was seen in garden cress. The vigor index was found to be 762, 867, 991, 1182, 1064 and 700 in mustard, chia, red amaranth, coriander leaf, sesame and cabbage respectively. A similar result was also reported by Maftai et al. (2018) who

reported that radish microgreens had the highest value in terms of vigor index.



**Figure 2.** Vigor index of eight selected microgreens. The vertical bars represent the mean  $\pm$ SE (n=8). Dissimilar letter(s) on the top of the bar indicate a significant difference at  $P \leq 0.05$ .

### 3.1.4. Seedling size and weight

Hypocotyl length at harvest showed a significant difference among the selected microgreens (Table 2). The longest hypocotyl (11.5 cm) was recorded in radish while the shortest (6.37 cm) was in garden cress. The possible reason for this could be the growth of garden cress is inhibited by long day time light because it is known that the vegetative and reproductive growth of garden cress require short day time light (Polash et al. 2020). The hypocotyl length was 7.50, 7.20, 9.50, 10.32, 10.58 and 6.50 cm for mustard, chia, red amaranth, coriander leaf, sesame and cabbage respectively. The longest (12.48 cm) shoot was found in radish while it was the shortest (6.75 cm) in garden cress. The shoot length was 7.80, 7.50, 9.78, 11.25, 11.10 and 7.10 cm for mustard, chia, red amaranth, coriander leaf, sesame and cabbage respectively. Statistical analysis of the data revealed that the average seedling height at harvest was significantly different among the species (Table 2). The longest seedling (15 cm) was recorded in radish while the shortest (7.41 cm) was in garden cress. The seedling height was 8.08, 9.30, 10.20, 13.20, 13.10 and 8.30 cm for mustard, chia, red amaranth, coriander leaf, sesame and cabbage

respectively. Morphological quality indicators for microgreens have been suggested in a number of publications, especially hypocotyl length which has been reported as a feature that could be useful in determining quality (Harakotr et al. 2019).

**Table 2.** Hypocotyl length, shoot length, seedling height, fresh weight and dry weight of eight selected microgreens at harvest

Vegetables	Hypocotyl length (cm)	Shoot Length (cm)	Seedling Height (cm)	Fresh weight (g)	Dry Weight (g)
Mustard	7.50e	7.80d	8.08e	30.31e	1.53e
Radish	11.50a	12.48a	15.00a	34.94a	2.23a
Chia	7.20f	7.50e	9.30d	29.48f	1.45e
Red Amaranth	9.50d	9.78c	10.20c	32.28d	1.65d
Coriander Leaf	10.32c	11.25b	13.20b	33.53b	2.13b
Garden Cress	6.37g	6.75g	7.41f	25.30h	1.13g
Sesame	10.58b	11.10b	13.10b	32.74c	1.85c
Cabbage	6.50g	7.10f	8.30e	27.45g	1.25f

Values marked with the same letter within the columns do not differ significantly @ 5% level of probability.

**Table 3.** Proximate analysis of four selected microgreens at harvest (on dry weight basis g/100g)

Microgreens	Moisture	Ash	Fiber	Carbohydrates	Protein	Fat
Mustard	88.3b	2.0a	0.3c	5.5a	4.2b	0.7a
Radish	91.0a	0.6b	0.5b	5.8a	0.6d	0.09c
Chia	91.3a	1.5ab	0.6a	3.5b	3.6c	0.5b
Red amaranth	92.3a	0.8ab	0.6a	6.3a	4.7a	0.6ab

Values marked with the same letter within the columns do not differ significantly @ 5% level of probability

**Table 4.** Macro minerals content of eight selected microgreens species at harvest (on dry weight basis mg/100g)

Vegetables	Na	K	S	P	Ca	Mg
Mustard	0.21b	0.45b	0.53c	9.30d	0.75d	0.40c
Radish	0.15c	0.28d	0.59b	9.82c	1.13c	0.35d
Chia	0.11d	0.35c	0.34d	10.41b	1.90b	0.81b
Red Amaranth	0.35a	0.66a	0.75a	11.32a	2.10a	0.95a

Values marked with the same letter within the columns do not differ significantly @ 5% level of probability.

**Table 5.** Micro minerals content of four selected microgreens at harvest (on dry weight basis mg/100g)

	Mustard	Radish	Chia	Red amaranth
Fe	291.57b	215.53d	260.73c	330.52a
Zn	649.73b	302.62d	365.27c	750.35a

Values marked with the same letter within the columns do not differ significantly @ 5% level of probability.

**Table 6.** Bio-active compounds (mg/10ml) of four selected microgreens at harvest

Microgreens	Chl a	Chl b	Total Chl	β-carotene	Lycopene
Mustard	0.56b	0.25b	0.81b	0.25b	0.44a
Radish	0.39c	0.22b	0.61c	0.21c	0.43a
Chia	0.35d	0.18d	0.53c	0.16d	0.41a
Red amaranth	0.61a	0.35a	0.96a	0.29a	0.51a

Values marked with the same letter within the columns do not differ significantly @ 5% level of probability

Fresh weight of the plants at harvest showed significant differences (@5% level) among the species. The highest (34.94 g) fresh weight was recorded in radish while it was the lowest (25.3 g) in garden cress. The fresh weight was 30.31, 29.48, 32.28, 33.53, 32.74 and 27.45 gm for mustard, chia, red amaranth, coriander leaf, sesame and cabbage.

There was a significant difference (at 5% level) in the dry weight of the plants at harvest among the species. The highest (2.23 g) dry weight was found in radish while it was the lowest (1.13 g) in garden cress. The dry weight was 1.53, 1.45, 1.65, 2.13, 1.85 and 1.25 gm for mustard, chia, red amaranth, coriander leaf, sesame and cabbage respectively

### 3.2. Bio-chemical Investigation

#### 3.2.1. Proximate analysis

Proximate analysis of four selected microgreens is shown in Table 3. In mustard moisture content was 88.3 g, while it was 91.0 g in radish. Chia contained 91.3 g of moisture. The highest moisture content (92.3 g) was found in red amaranth. The lowest ash content, 0.6 g was found in radish. Ash content was 1.5 g and 0.8 g in chia and red amaranth respectively. The highest ash content was detected in mustard (2 g). Fiber content was 0.3 g and 0.5 g in mustard and radish respectively. Chia and red amaranth showed the highest (0.6 g) fiber content. Carbohydrate content was 5.5, 5.8, 3.5 and 6.3 g for mustard, radish, chia and red amaranth, respectively while protein content was 4.2, 0.6, 3.6 and 4.7 g. Fat content was found the lowest (0.09 g) in radish and 0.5, 0.6 g in chia and red amaranth respectively. Mustard contained the highest (0.7 g) fat. Microgreens contain higher amounts of moisture in contrast to the low amount of carbohydrates and fat. This could protect the human body from weight gain and type-2 diabetes (Seidemann et al. 2018). A diet rich in protein not only maintains and help to lose weight; it also stabilizes blood sugar, boost energy level and supports the absorption of important nutrients (Westerterp-Plantenga et al. 2009). Fiber plays a crucial role in digestive, heart and skin health and can also help in lowering blood cholesterol and glucose levels (Anderson et al. 2009).

#### 3.2.2. Mineral contents:

##### Macro minerals

Table 4 showed significant differences in the macro minerals content among the selected microgreens. Sodium (Na) and potassium (K) content were 0.21, 0.15,

0.11, 0.35 g and 0.45, 0.28, 0.35, 0.66 g for mustard, radish, chia and red amaranth, respectively. The lowest Na content (0.11 g) was found in chia while it was the highest (0.35 g) in red amaranth and the lowest K content (0.28 g) was found in radish while the highest (0.66 g) in red amaranth. Mustard, radish, chia and red amaranth contained 0.53, 0.59, 0.34, 0.75 g sulfur (S) and 9.30, 9.82, 10.41, and 11.32 g phosphorous (P). The lowest (0.34 g) S content was found in chia and it was the highest (0.75 g) in red amaranth. In the case of P, mustard showed the lowest values (9.30 g) while it was the highest (11.32g) in red amaranth. Calcium (Ca) content was the lowest (0.75 g) in mustard but it was relatively higher in radish (1.13 g) and chia (1.90 g). The highest Ca content (2.10 g) was found in red amaranth. Magnesium (Mg) content was 0.40, 0.35, 0.81 and 0.95 g for mustard, radish, chia and red amaranth, respectively. Generally, plant cells tend to accumulate K for essential functions such as cellular metabolism and stomatal opening and exclude Na resulting in a high K/Na ratio in plant tissues (Flyman and Afolayan 2008). A high amount of potassium was also reported in other leafy vegetables such as garden cress (Adera et al. 2022).

##### Micro minerals

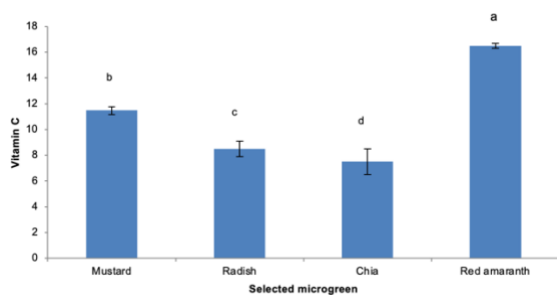
Table 5 divulged the Iron (Fe) content 291.57, 215.53, 260.73 and 330.52 mg for mustard, radish, chia and red amaranth, respectively. Radish showed the lowest Fe (215.53 mg) and the highest in red amaranth (320.52 mg). The lowest Zinc (Zn) content (303 mg) was detected in radish, and it was the highest (750 mg) in red amaranth. Chia and mustard contained 365 and 650 mg Zn, respectively. Polash et al. (2018) also found micro minerals in microgreens.

##### Bio-active compounds

Table 6 shows the values of bio-active compounds in four selected microgreens. Chlorophyll a and chlorophyll b content were 0.56, 0.39, 0.35, 0.61 mg and 0.25, 0.22, 0.18, 0.35 mg for mustard, radish, chia and red amaranth, respectively. The lowest total chlorophyll content was 0.53 mg, found in chia while it was the highest (0.96 mg) in red amaranth. The total chlorophyll content in mustard and radish was 0.81 and 0.61 mg respectively. Beta (β)-carotene content was 0.25, 0.21, 0.16 and 0.29 mg in mustard, radish, chia and red amaranth, respectively and in the case of lycopene, it was 0.44, 0.43, 0.41 and 0.51 mg. A recent report demonstrated that microgreens contain higher amounts of phytonutrients such as chlorophyll (Pinto et al. 2015).

## Vitamin 'C' content

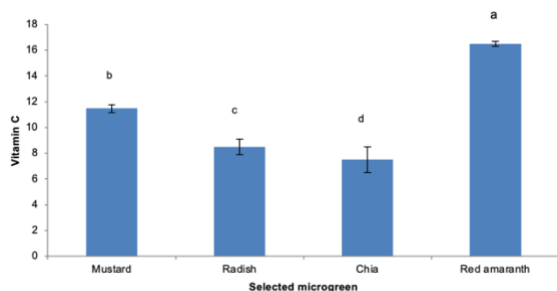
Fig. 3 shows the vitamin C content in four selected microgreens. Chia showed the lowest (7.51 mg) vitamin C content, whereas radish contained a moderate concentration (8.50 mg) of vitamin C per 100 g fresh sample. Mustard showed higher (11.48) vitamin C content than that in radish. Red amaranth showed the highest (16.5 mg) vitamin C content. Recent report demonstrated that microgreens contain higher amounts of phytonutrients such as ascorbic acid (vitamin C) (Pinto et al. 2015; Xiao et al. 2012). A study on microgreens reported that even the microgreen sample with the lowest levels of vitamin C contained a whopping 20 milligrams of vitamin C per 100 grams almost twice the amount of vitamin C found in tomatoes (Xiao et al. 2012).



**Figure 3.** Vitamin C content in four selected microgreens. The vertical bars represent the mean  $\pm$ SE (n=4). Dissimilar letter(s) on the top of the bar indicate a significant difference at  $P \leq 0.05$ .

## DPPH radical scavenging activity

Fig. 5 shows the DPPH radical scavenging activity of four microgreens. Among the species red amaranth exhibited the highest radical scavenging activity with an IC<sub>50</sub> value 0.78  $\mu$ g/ml. Mustard showed high radical scavenging activity with an IC<sub>50</sub> value 1.35  $\mu$ g/ml. Moderate radical scavenging activity was shown by radish whose IC<sub>50</sub> value was 2.70  $\mu$ g/ml. Chia showed the lowest radical scavenging activity with an IC<sub>50</sub> value 3.64  $\mu$ g/ml. The lowest IC<sub>50</sub> value indicated the highest anti-oxidant capacity. The phenomenon is that, the DPPH binds with the free radicals and makes the free radical neutral (Polash et al. 2018; Partap et al. 2023).



**Figure 4.** DPPH radical scavenging activity in four selected microgreens. The vertical bars represent the mean  $\pm$ SE (n=4). Dissimilar letter(s) on the top of the bar indicate a significant difference at  $P \leq 0.05$ .

## 4. Conclusion

Exploring new crops and introducing a wider variety of microgreens will provide consumers with more options and flavors, and expand the market potential for growers. The present study showed that red amaranth microgreen was more suitable for most physiological and biochemical parameters. But the longest hypocotyl, the highest shoot length, seedling height, fresh weight and dry weight were found in radish microgreen. Garden cress showed the lowest performance in the context of most of the morpho-physiological and bio-chemical parameters. Therefore, it may be concluded that red amaranth microgreen might be an excellent source of bioactive compounds and antioxidants.

## 5. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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