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Influence of Soil and Light Condition on the Growth and Antioxidants Content of *Amaranthus Cruentus* (Red Amaranth) Microgreen

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Abstract. Light and soil condition has significant effect on the quality of plants cultivated. However, there are limited studies conducted on this research area, specifically on the *Amaranthus Cruentus* (red amaranth) microgreens. Therefore, this research project aims to investigate the influence of light and soil condition on red amaranth microgreens growth as well as its antioxidants contents. Microgreens were grown under different soil condition with the same light condition, and vice versa. The stem length, root length, fresh weight and dry weight of microgreens were measured. The antioxidants and mineral content were also analysed. This paper concludes that neutral pH and loose soil is the best soil condition for cultivating microgreens. Black soil produced microgreens with 1.89 and 2.60-fold higher in terms of stem length, and 1.50 and 2.13-fold higher in terms of fresh weight than those cultivated in orange and tallow soil respectively. Compost can be added to soil to improve its quality. In terms of lighting condition, sunlight provided the best light source for yielding high quality microgreens, reducing the growth time of microgreens from 11 days to 8 days. Red+blue and white artificial light can also be used to substitute sunlight as they yielded similar quality.

1. Introduction

Antioxidants are compounds that protect cell organelles against oxidative damage from free radicals which are generated during cellular respiration and is highly damaging to cell organelles. In human body, this potentially leads to many forms of cancer and neurological diseases. The importance of antioxidants has gained significant traction with the public in recent years, leading to many forms of antioxidants supplement. However, synthetic antioxidants may be harmful to the human body [1]. This increase the public interest and subsequent seeking after natural source of antioxidants in various fruits and vegetables. Therefore, it is desirable to increase the level of antioxidants in commercially available vegetables, which can increase its nutrition and subsequently the commercial value. Many parameters can affect plant growth and subsequently, its nutritional value. These include soil and light condition. Soil is the most commonly used medium for plant cultivation due to its availability. Different type of soil has different characteristics which affect their suitability for plant growth. This is dependent on the plant species as different plants have different adaptability to the soil type. The texture, pH, density, compactness and mineral content of soil can all affect plant growth. In addition, light is fundamental to plant growth as it serves as energy source for plant through the process of photosynthesis, in which light energy is used to produce glucose. The glucose then fuels the cellular activities of the plant. Light also affect regulatory process in plants. This include the biosynthesis and accumulation of various organic compounds, including antioxidants [2]. Light intensity and spectral composition have been shown to affect plant growth and its antioxidants content. However, the ideal value is also dependent on the plant species. The studies of cultivation method for microgreens are severely lacking in literature, especially on varying the soil condition as the cultivating medium. It was unclear on the impact of soil condition on the cultivation of microgreens. Furthermore, the effect of light quality on the nutritional content of red amaranth microgreen is also unclear, in particular the mineral content of the microgreens. Therefore, this paper aims to evaluate the effect of soil and light condition on the growth, mineral and antioxidants content of *Amaranthus Cruentus*.

2. Materials and Method

2.1. Materials

Red amaranth seed, black soil, orange soil and yellow soil and coconut compost were purchased from a local nursery. Nitric acid (ACS grade 70%), perchloric acid (ACS grade 60%) and hydrogen peroxide (99%), which were used as solvent for feedstock digestion, were purchased from Sigma-Aldrich. The solvents for soil mineral extraction, sulphuric acid (99.9%) and hydrochloric acid (ACS reagent 37%), as well as the solvent for carotenoids extraction, acetone (80%), were also purchased from Sigma-Aldrich. Finally, the K, Mg, Fe, Na, Zn, Ca stock solutions for atomic absorption spectroscopy (AAS) analysis were also purchased from Sigma-Aldrich.

2.2. Method

The experiments were divided into two sections. The first evaluated the effect of soil condition whereas the second evaluated the effect of light condition. For the first part, three types of soil were prepared: black soil; yellow soil; and orange soil. Red amaranth was then grown under sunlight. In addition, coconut compost was also added to improve the soil condition. Three concentration of coconut compost was added: 0%, 25% and 50%. The density and pH of soil was measured.

For the second part, red amaranth was grown with black soil under different light condition. It was grown under red, blue and red+blue light at 20 $\mu mol/(m^2 \cdot s)$. A control set was grown under sunlight for comparison purposes. The red amaranth was harvested after reaching microgreen stage and freeze dried. The steam length, root length, fresh weight and dry weight were measured, and the antioxidants content evaluated. The antioxidants tested were carotenoids and ascorbic acid. DCPIP titrimetric method was used to analyse the ascorbic acid content. The formula used to calculate the ascorbic acid content is shown in (1).

$$\text{Ascorbic acid} = \text{Conc. DCPIP} \times \text{Titrant volume} \times 176.12 \tag{1}$$

The carotenoids analysis was carried out with the absorption method proposed by Chen et al. (2016) [3]. The concentration of carotenoids was calculated with (2), (3) and (4).

$$\text{Chl a} = 1/1000W\ (12.72 \times OD_{663} - 2.59 \times OD_{645})V \tag{2}$$

$$\text{Chl b} = 1/1000W\ (22.88 \times OD_{645} - 4.67 \times OD_{663})V \tag{3}$$

$$\text{Car} = 1/1000W(1000 \times OD_{470} - 3.27 \times \text{Chl a} - 104 \times \text{Chl b}/229)V \tag{4}$$

where W is sample weight and V is volume of extract.

The mineral content of the soil and red amaranth were analysed with AAS method, using Perkin-Elmer AAnalyst 400. Zinc (Zn), Magnesium (Mg), Iron (Fe), Calcium (Ca), Potassium (K) and Sodium (Na) were analysed. Different concentration standards were prepared for different mineral analysis to obtain the calibration curve. The concentration standards were taken from recommendation by the manufacturer. K, Fe, Na and Zn, were prepared with concentration of 0.1 ppm, 0.5 ppm and 1.0 ppm, whereas those of Mg and Ca were prepared with concentration of 1.0 ppm, 2.0 ppm, 3.0 ppm and 4.0 ppm.

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3. Results and Discussion

3.1. Red Amaranth Grown in Different Soil Condition

Table 1. Effect of different compost concentration in different soil on the fresh and dry weight of microgreens.

Compost concentration	Fresh weight (g)			Dry weight (g)		
	Orange	*Yellow*	*Black*	*Orange*	*Yellow*	*Black*
0%	2.82±0.21	2.04±0.31	5.34±0.28	0.34±0.05	0.24±0.02	0.51±0.08
25%	4.76±0.58	4.17±0.62	5.98±0.44	0.44±0.01	0.33±0.03	0.53±0.04
50%	5.74±0.23	5.21±0.12	6.14±0.51	0.49±0.01	0.48±0.08	0.56±0.10

Table 2. Effect of different compost concentration in different soil on the stem and root length of microgreens.

Compost concentration	Stem length (cm)			Root length (cm)		
	Orange	*Yellow*	*Orange*	*Yellow*	*Orange*	*Yellow*
0%	2.8±0.3	2.3±0.3	2.8±0.3	2.3±0.3	2.8±0.3	2.3±0.3
25%	4.2±0.3	4.0±0.3	4.2±0.3	4.0±0.3	4.2±0.3	4.0±0.3
50%	5.2±0.4	5.2±0.4	5.2±0.4	5.2±0.4	5.2±0.4	5.2±0.4

Table 3. Effect of different compost concentration on the bulk density and pH of the soil.

Compost concentration	Bulk density (g/cm^3)			Soil pH		
	Orange	*Yellow*	*Black*	*Orange*	*Yellow*	*Black*
0%	1.13±0.05	1.16±0.13	1.11±0.12	3.94±0.32	4.01±0.21	6.67±0.52
25%	0.95±0.08	0.93±0.08	0.91±0.05	4.38±0.38	4.44±0.33	6.93±0.37
50%	0.83±0.09	0.77±0.07	0.79±0.03	4.38±0.23	4.81±0.49	6.97±0.36

Table 4. Mineral content of microgreens grown under different soil condition[a]

El.	Black soil			Yellow soil			Orange soil		
	0%	*25%*	*50%*	*0%*	*25%*	*50%*	*0%*	*25%*	*50%*
Zn	0.92±0.06	0.77±0.05	0.79±0.05	0.88±0.05	0.73±0.04	0.64±0.04	0.74±0.05	0.93±0.06	1.03±0.06
Mg	92.3±1.99	79.4±1.71	83.92±1.81	96.83±2.09	61.25±1.32	48.19±1.04	71.24±1.53	135.4±2.92	77.6±1.67
Fe	1.15±0.02	1.65±0.04	1.66±0.04	4.25±0.09	2.23±0.05	1.17±0.03	7.73±0.17	4.07±0.09	2.64±0.06
Ca	65.7±4.01	56.2±3.44	61.44±3.73	58.11±3.55	62.12±3.77	54.27±3.31	55.04±3.35	84.8±5.18	72.55±4.42
K	118±7.19	119±7.30	120.0±7.32	87.06±5.3	127.6±7.77	111.2±6.77	108.5±6.61	173.2±10.5	145.5±8.86
Na	3.03±0.07	3.55±0.08	4.56±0.10	3.33±0.07	5.06±0.11	3.73±0.08	5.09±0.11	7.97±0.17	5.55±0.12

[a] Mineral content is expressed in mg/kg soil

Table 5. Mineral content of microgreens grown under different soil condition[a]

El.	Black soil			Yellow soil			Orange soil		
	0%	*25%*	*50%*	*0%*	*25%*	*50%*	*0%*	*25%*	*50%*
Zn	0.87±0.07	2.63±0.21	2.61±0.21	3.46±0.28	0.81±0.06	1.08±0.09	0.83±0.07	1.72±0.14	1.27±0.10
Mg	6.46±0.14	33.66±0.72	39.82±0.86	24.78±0.67	25.23±0.41	32.29±0.68	19.62±0.42	31.91±0.69	37.09±0.80
Fe	67.2±1.45	61.4±1.32	42.71±0.92	66.35±1.43	50.82±1.09	46.49±1.00	27.68±0.60	18.44±0.4	17.63±0.38
Ca	1.67±0.10	52.56±3.20	78.38±4.77	31.18±1.90	15.05±0.92	59.77±3.64	9.93±0.61	45.28±2.76	57.61±3.51
K	13.1±0.79	63.89±3.89	67.24±4.10	61.99±3.78	37.70±2.29	56.79±3.46	39.31±2.39	52.40±3.20	57.83±3.52
Na	8.08±0.17	9.85±0.21	11.82±0.25	11.82±0.25	9.46±0.20	11.82±0.25	37.73±0.81	39.64±0.85	39.54±0.85

[a] Mineral content is expressed in mg/kg soil

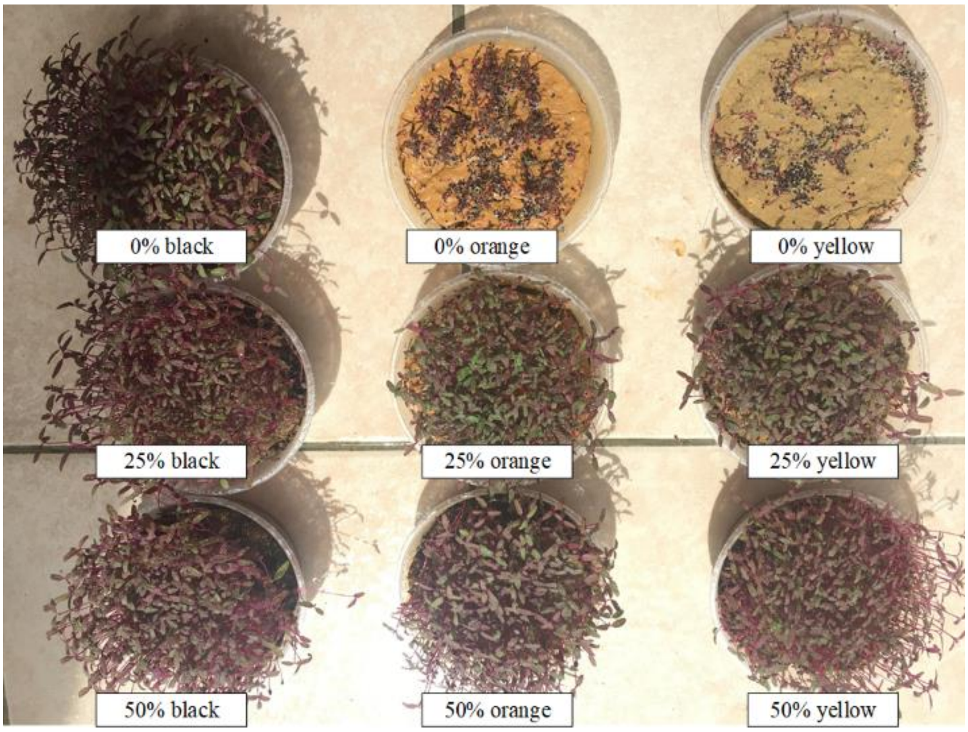

Figure 1. Red amaranth microgreens grown under black, yellow and orange soil.

Based on the data from **Table 1**, **Table 2** and **Table 3**, and as shown in **Figure 1**, red amaranth grown in black soil displayed the best growth, followed by orange and then yellow soil. The fresh weight increased by 38.24% when grown in orange soil, and 161.76% when grown in black soil in comparison with yellow soil. The poor growth of red amaranth in yellow and orange soil are believed to be caused by the compactness and pH value of the soil. The bulk density of soil primarily affects root growth. High density correlates to high compactness which increases the resistance for root penetration and subsequently, the development of rooting system and the overall plant growth. In addition, soil strength also affects the nutrient availability for roots to adsorb. A more compact soil hinders root penetration, which affect the nutrient absorption of the plants. This is supported by the results of adding compost into the soils, whereby the soil bulk density reduces. The compost is believed to have reduced the soil strength as the red amaranth grown has longer root length and displayed higher fresh weight.

Besides, soil pH also affects the red amaranth growth. Orange and yellow soil, which has lower pH than black soil, produced red amaranth with poorer growth. With the addition of compost, the soil pH had increased and was able to produce red amaranth with significantly better growth. It is deduced that the pH value of soil has an effect on the nutrient content inside the soil. Soil with neutral pH value has more nutrient available for plants to extract. This result is also agreed by Singh and Whitehead (1993) where similar results were found [4].

Based on the results above in **Table 4** higher mineral content in soil does not correlate to higher mineral contents in the red amaranth grown. However, for certain elements such as sodium and potassium, a positive correlation was observed. Different plant species requires different mineral content to increase its mineral uptake. This was observed on the plant species, *Curcuma Longa* and *Amaranthus Tricolor* by Akamine et al. (2007) and Sarkar et al. (2010) [5, 6]. Moreover, it was also observed that the addition of compost into the soil had improved its mineral content as shown in **Table 5**. Magnesium, calcium, potassium and sodium content were improved whereas other minerals declined slightly. This result was also observed by Evanylo et al. (2016) where compost improve the soil mineral content [7]. Thus, adding coconut compost is a feasible and economical method to improve soil with poor nutrient content.

Table 6. Ascorbic acid and carotenoids content of red amaranth grown in different soil conditions[a]

Cultivating system		Ascorbic Acid	Carotenoids
Black soil	0%	82.2±3.3	47.31±1.39
	25%	82.0±0.8	48.16±1.42
	50%	90.3±1.2	49.96±0.87
Yellow soil	0%	69.6±2.4	40.50±1.16
	25%	69.6±2.4	44.65±1.25
	50%	78.6±1.4	48.43±0.90
Orange soil	0%	73.8±2.0	45.26±1.25
	25%	73.8±3.3	46.17±1.24
	50%	77.6±3.7	47.77±1.32

[a] Antioxidants content are expressed in mg/100g FW

In addition to improving growth, soil with higher compost addition produced red amaranth with better antioxidants content as shown in **Table 5**. The highest improvement was observed in 50% compost yellow soil where the ascorbic acid content increased by 13%. It is believed that this is affected by the soil mineral content. This is supported by Abanto-Rodriguez et al. (2016) on *Myrciria dubia*, where ascorbic acid has a positive correlation with the concentration of magnesium in soil [8]. As shown evidently in **Table 4**, higher compost composition in soil improved its magnesium content, and therefore, higher antioxidants content was found in the red amaranth grown.

3.2. Red Amaranth Grown Under Different Lighting Condition

The results from **Table 7** showed that red amaranth grown under natural sunlight produced the highest fresh weight, dry weight and the least amount of days to grow. This is due to the higher intensity of light provided by the Sun in comparison to other lighting condition. Higher light intensity induces higher photosynthetic rate in plant. This is supported by study conducted by Mortensen and Grimstad (1990) where they discovered that under 120 μmol/(m^2·s), *F. benhamina*, *F. elastica*, *N. exaltata*, *R. sinica* and *S. podophyllum* displayed higher dry weight than 60 μmol/(m^2·s), which is the result of higher photosynthesis rate [9].

Table 7. Effect of different lighting condition on the growth of red amaranth microgreens.

Lighting condition	Red	Red+Blue	White	Natural sunlight
Fresh weight (g)	4.22±0.21	4.53±0.23	4.73±0.72	5.21±.38
Dry weight (g)	0.36±0.05	0.53±0.04	0.52±0.05	0.56±0.08
Stem height (cm)	5.31±0.55	4.70±0.23	4.80±0.36	5.10±0.22
Harvest (Days)	11	11	12	8
Light intensity (μmol/(m^2·s))	20.0	20.0	20.0	200.7

Red amaranth cultivated under red light yielded the highest stem height (5.31±0.55 cm) while red+blue light yielded the lowest (4.70±0.23 cm). However, the physical appearance of red amaranth stem grown under natural light, white light and red+blue light was reddish purple in colour and rigid. On the other hand, red amaranth grown under red light displayed elongated and thin stem, with it being colourless. Plants are very susceptible to changes in narrow band of red light spectrum. Leaves are very effective filters of red, blue and green light. Therefore, a far-red light enriched environment represents fierce competition among neighbouring plants for access to sunlight. When exposed to a far-red light enriched environment, for example in shaded environments, plant developed changes in their growth strategies. Under this environment, stem elongation, leaves inclination and elongation are observed [10]. Despite having elongated stem, red amaranth grown under red light displayed the lowest fresh and dry weight. This is supported by other studies where plant under monochromatic red-light condition often display a lower biomass in comparison to white light despite having elongated stem and leaves [3, 11, 12]. This showed that growing under red light yielded red amaranth with poor quality, whereas natural light produced red amaranth with the best quality. In terms of artificial light, red+blue light and white light are able to produce red amaranth with similar quality to natural sunlight. This also agrees with literature where *Ocimum basilicum* (basil) grown under red and blue light have higher dry weight than those under monochromatic lighting [13]. This suggests that red+blue and white artificial light can sufficiently substitute natural sunlight as light source for cultivation.

Table 8. Ascorbic acid and carotenoids of red amaranth microgreens grown under different cultivating system.

Cultivating system	Ascorbic Acid (mg/100g FW)	Carotenoids (mg/100g FW)
Fully grown	49.8±1.2	36.30±0.99
Sunlight	82.2±3.3	47.31±1.39
White light	82.2±2.8	59.06±1.69
Red light	93.6±0.9	53.71±1.59
Red+blue light	90.8±1.3	59.23±1.80

From **Table 8**, red amaranth grown under red light yielded the highest amount of ascorbic acid while white light yielded the lowest. This does not agree with literature where monochromatic lighting should yield plant with the lowest nutritional content. In some cases, the plants even yielded lower amount of biomass. *Triticum aestivum* produced smaller plant sizes and lower amounts of seeds under monochromatic red light [14]. This can be explained by the low light intensity used which can affect the synthesis of antioxidants under white light, where only 20 µmol/(m^2·s) of light intensity was employed. At 40 µmol/(m^2·s), monochromatic red light is favourable for seed germination and early plantlets development [15]. This might further explain the results obtained as the red amaranth were only grown until microgreens stage. In addition, one study showed that at around 25 to 42.25 µmol/(m^2·s), the medicinal herb *Rhodiola imbricata* was shown to have highest growth rate under monochromatic red light while still having higher antioxidants content than white light [16]. This indicates that red light plays a role in activating certain compounds that synthesizes ascorbic acid such as L-galactose [17]. Under low light condition, the red spectrum composition within white light might not be sufficient enough to activate the synthesis of ascorbic acid, and thus lead to poor ascorbic acid content. Under higher light intensity, plants often displayed higher nutritional content. At 600 µmol/(m^2·s), lettuce grown under red+blue light had higher nutritional content in terms of its sugar, ascorbic acid and protein content, which exceeded even that of white light [18].

Under red+blue and white light, red amaranth yielded the highest amounts of carotenoids. This agrees with literature where under certain light condition, the plants yielded might have a greater antioxidants content than that of natural sunlight. Lighting ratio of 95% red and 5% blue light produced lettuce with higher dry mass and larger leaf area than the controlled sample grown under sunlight [19]. While red light yielded the lowest among the artificial lighting, it is still greater than that of natural sunlight. This might be due to the biosynthesis mechanism of carotenoids. According to Czeczuga (1987), higher light intensity has a negative impact on the carotenoids content of various plant species [20].

4. Conclusion

Among the 3 different soil, black soil is best suited for microgreens growth, yielding the best quality. The addition of compost into soil improved the soil quality and caused improvement in microgreen growth rate. This is due to the reduced density of the soil which help root development. The addition of compost also neutralizes the soil pH, thus improving the quality of microgreens cultivated. Compost addition also increase the mineral content of the soil. However, higher soil mineral content does not correlate to higher microgreens mineral content. In addition, microgreen cultivated under sunlight had the fastest growth rate and yielded better quality in terms of height, mass and antioxidants content, than those under artificial light. Red+blue and white artificial light produced microgreens with similar quality to that of sunlight. To produce microgreens with the best quality, soil with neutral pH and low compactness is preferred. Soil quality can be improved with the addition of compost, which can help in producing higher quality microgreen. Sunlight is the best light source for cultivating microgreens. Red+blue and white light can also be used to substitute sunlight.

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