Biostimulant effects of *Moringa oleifera* on growth and antioxidant activity in red amaranth (*Amaranthus tricolor* L.) microgreens

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Abstract. The development of newfound functional foods is a key driver of innovation in the food industry, aiming to meet the growing demand and expectations of consumers for healthier options. Microgreens are gaining popularity as functional foods due to their high nutrient density and rich content of bioactive compounds or secondary metabolites. To optimize its growth and antioxidant content, natural additives such as *Moringa oleifera* leaf extract can be used. This study aimed to evaluate the antioxidant activity of red amaranth (*Amaranthus tricolor* L) microgreens after the application of MLE as a biostimulant. The red amaranth seeds were planted on rockwool where biostimulants can be easily integrated into plant growth. Furthermore, the relationship between used concentration and biostimulant potential of extracts was observed. To determine the antioxidant properties of all tested extracts, four different concentrations of MLE 0% w/v, 10% w/v, 15% w/v, and 20% w/v were used. The highest ability to scavenge DPPH radical was shown by the extract at the highest tested concentration of MLE 20% w/v. In the highest concentration, the ability to scavenge DPPH radical was on 47,78 ppm, which is categorized as a very strong antioxidant. These results indicate that red amaranth microgreen given the addition of MLE has a high inhibitory value of free radicals. Furthermore, the 20% w/v MLE concentration significantly enhanced plant growth, resulting in nearly a 50% increase in microgreen height, as well as in fresh and dry weight, compared to the control. Considering these findings, suggest that MLE is an effective natural additive for improving the nutritional value and growth performance of red amaranth microgreens.

1 Introduction

In this new century, the global population needs new food sources that are high in nutrients yet easy to consume. In this regard, microgreen can be seen as one of the solutions of the innovation to be carried out [1]. Microgreens are plants that are harvested very young, they are harvested just after the cotyledons appear but before the actual leaves develop. They are harvested from 7 to 21 days after germination, depending on the species [2]. Microgreens are

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researched to be a better substitute for sprouts as they are rich in nutrient content. They contain more phytochemicals, minerals, and vitamins than mature plants [3].

Amaranth seeds are a rich source of iron, which can be particularly beneficial in the treatment of anemia [4]. Recent research by Hsiao et al. [5] indicates that the aqueous extract from steamed red amaranth leaves could be an effective nutritional supplement for preventing diabetic retinopathy. According to Karamac et al. [6], amaranth plants exhibit higher antioxidant activity during the vegetative and early flowering stages, suggesting that these phases are particularly valuable as sources of antioxidants, in addition to containing a high concentration of hydroxycinnamic acid, as one of phenolic compound. Other research by Pasko et al. [7] showed that extracts from the plant during its growth stages and early flowering are valuable sources of antioxidants. These extracts can be used to develop nutraceuticals or serve as functional food ingredients, such as microgreens.

Microgreens contain phenolic compounds, which possess antioxidant properties that can help scavenge free radicals in the body when consumed. Free radicals are highly reactive molecules that can cause oxidative damage to cells and contribute to various diseases. Antioxidants play a crucial role in maintaining overall health and reducing the risk of chronic diseases [8]. In addition to its nutritional value, microgreen not only contains valuable nutrients but also aligns with consumer preferences for novelty and taste. Furthermore, it is highly appealing to producers due to its simple cultivation method, minimal production requirements, and the ability to achieve maximum consumption in a relatively short time.

To optimize the antioxidant content in microgreens, biostimulants are applied to the read amaranth seedling. Biostimulants are substances added to plants to enhance their quality to increase antioxidant activity and boost the content of phenols, flavonols, and osmoprotectants, helping plants mitigate abiotic stresses such as salinity and drought. Biostimulants can be derived from plant extracts, such as Moringa leaves [9]. In some studies, moringa leaf extract can help increase plant growth.

2 Material and Method

2.1 Preparation

Preparation was carried out by organizing the tools and materials to be used. The tools included a blender, sieve, Erlenmeyer flask, measuring cup, plastic container, rockwool media, labels, water sprayer, scale, meter, filter paper, test tubes, spectrophotometer, and cuvette. The materials required were dried Moringa leaves, distilled water, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution, ethanol and red red amaranth seeds.

2.2 Moringa leaf extraxt

Fresh and healthy Moringa leaves were harvested, preferably in the morning when nutrient levels were most concentrated. The leaves were thoroughly rinsed in clean water to remove any dirt, then dried in an oven at 80°C for 1.5 hours. Once dried, the leaves were ground into a fine powder using a blender. Subsequently, the extraction method was using boiling technique. The powder of moringa leaves were added with distilled water in a beaker flask at a a variety ratio of concentrations 10% w/v, 15% w/v, and 20% w/v, and then heated in a water bath for 30 minutes at a temperature of 90 ° C. Subsequently, the solution paste was strained with filter paper [10]. The liquid extract was used as a natural biostimulant.

2.3 Microgreen planting and MLE application

Four plastic containers were prepared for seeding, and red amaranth seeds were sown on the surface of evenly moistened rockwool. The seeds were then sprayed with MLE until fully wet. The containers were placed in a location away from direct sunlight for 7-15 days.

2.4 Physiological test

Physiological tests of microgreen were carried out by observing the growth of microgreen that had been planted on rockwool media with the addition of MLE concentrations of 10% w/v, 15% w/v, and 20% w/v. Physiological observations in the form of wet weight and dry weight were carried out on 5, 10, and 15 days after sowing (DAS). Meanwhile, plant height was carried out on 15 DAS or at harvest time, then tested using the ANOVA statistical test.

2.5 Extraction of red amaranth microgreen

The harvested microgreen was washed, then dried in an oven at 80°C for 1.5 hours. Furthermore, microgreen was grinded using mortar until it becomes a fine powder. The powder was then mixed with 95% ethanol in a beaker flask at a 1:1 ratio. Subsequently, the extract ethanol was left for 72 hours to allow the ethanol to evaporate naturally then strained with filter paper [10].

2.6 DPPH test

For the stock solution, 19.7 mg of DPPH was dissolved with absolute ethanol in a 100 mL volumetric flask, then wrapped in aluminum foil. Furthermore, from each parent solution a concentration series of 20 ppm, 40 ppm, 60 ppm and 80 ppm were made. For measurement by spectrophotometry, 1 ml of each concentration series, sample and DPPH were taken into the cuvette, then allowed to stand for 20 minutes. Then the absorbance was measured by spectrophotometry at a wavelength of 517 nm. A blank solution was also made as a negative sample by entering 3 ml of DPPH solution that had been mixed with ethanol into the cuvette. As a comparison, 25 mg of vitamin c was mixed with 100 ml of ethanol. Then a concentration series of 20 ppm, 40 ppm, 60 ppm, and 80 ppm were made. For spectrophotometric measurements, 2 ml each was taken, and 1 ml DPPH was added. Measured with a wavelength of 517 nm on spectrophotometry.

3 Result and Discussion

3.1 Physiological test

The addition of external nutrients and biostimulants can help microgreen seedlings grow optimally. Moringa leaf extract contains cytokinin hormones, especially zeatin, which can increase plant growth, yield, and regeneration response, and increase resistance to environmental stress [11].

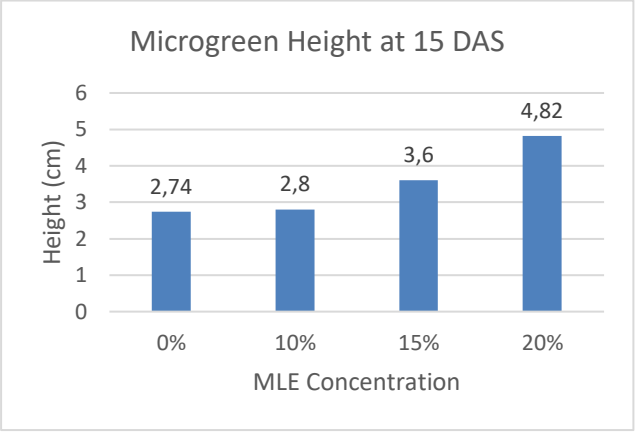

Fig. 1. Microgreen height data at 15 DAS.

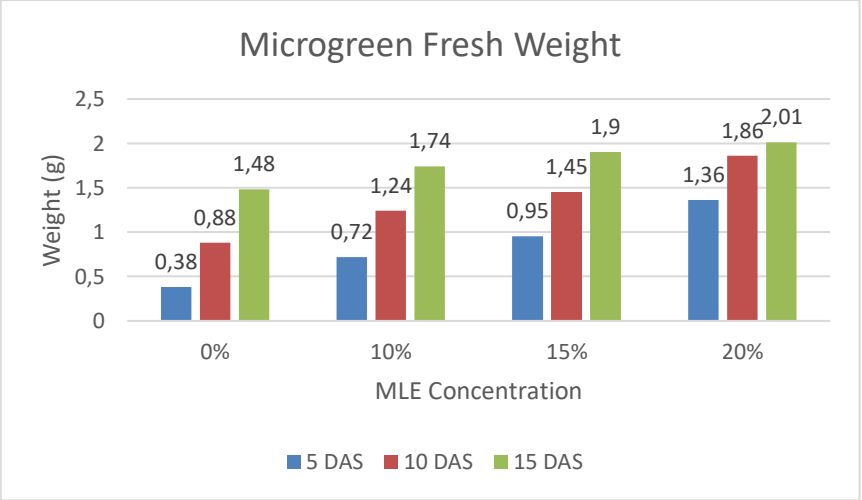

Fig. 2. Red amaranth microgreen fresh weight data of four different Moringa leaf biostimulant concentration on 5, 10, and 15 DAS.

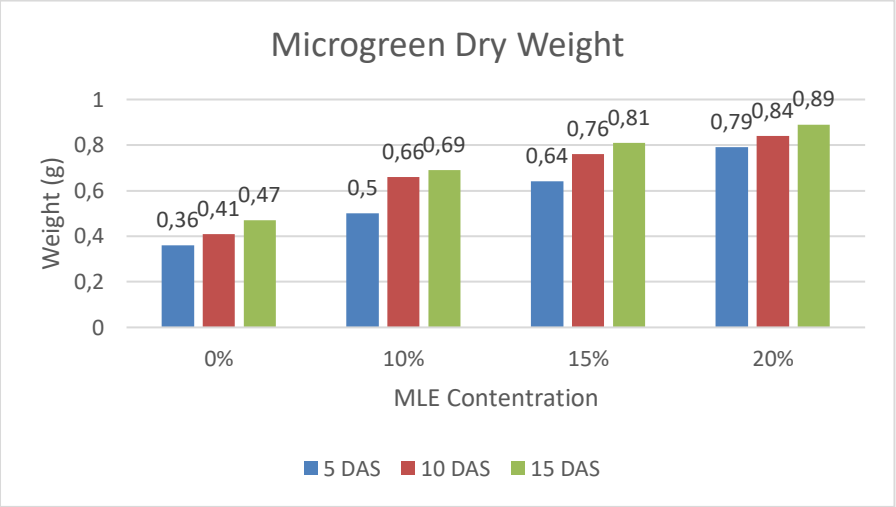

Fig. 3. Red amaranth microgreen dry weight data of four different Moringa leaf biostimulant concentration on 5, 10, and 15 DAS.

In diagram 1, diagram 2, and diagram 3 show the difference given by the variation of Moringa leaf concentration on the growth of red amaranth microgreen. From the concentration variations given, the 20% w/v concentration gives better results than other concentrations, where at a concentration of 0% microgreen has a height of 4.8 cm. Then it is also shown that the highest wet weight and dry weight are at 20% concentration (2.01 g and 0.89 g). This shows that the right concentration to optimize nearly by a half the growth of microgreen is 20% concentration. This outcome could have significant economic implications, as the use of MLE biostimulants in microgreen production results in increased biomass, according in the literature [12].

3.2 Extraction of red amaranth microgreen

Extraction of red amaranth microgreen was carried out by maceration method using 95% ethanol solvent to obtain 10 ml of extract. Ethanol was used as a solvent because ethanol has been reported to provide extracts with better pharmacological quality than water extracts [13]. Ethanol can dissolve polyphenol and flavonoid compounds that can bind free radicals. Ethanol can easily penetrate cell membranes so that it can dissolve polar, semipolar, and non-polar compounds.

3.3 DPPH test

Antioxidant activity of red amaranth extract was tested using DPPH method with UV-Vis spectrophotometry at 517 nm wavelength. The amount of antioxidant activity is indicated by the IC50 value, which is the concentration of sample solution required to inhibit 50% of DPPH free radicals. The test results showed that the microgreen extract treated with the addition of MLE when planting the highest results were shown at a concentration of 100% with a percent inhibition of 53.2% which can be seen in Table 1. A 53.2% inhibition in DPPH measurement indicates that the microgreen added with biostimulant has reduced the DPPH radical's activity by 53.2%.

Table 1. Antioxidant testing of red amaranth microgreen extract

Concentration (%)	%Inhibisi	IC_{50} value
0%	20.2	120.23
10%	28	95.99
15%	38.5	71.19
20%	53.2	47.78
Vitamin C	57.23	38.05

The IC_{50} value was obtained by making a standard calibration curve as follows:

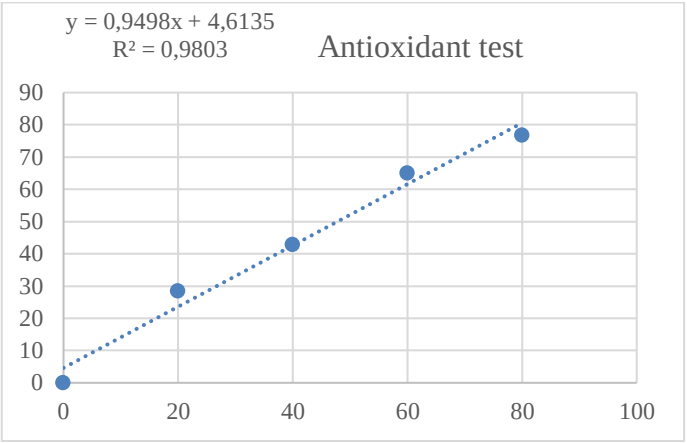

Fig. 4. Antioxidant testing of red amaranth extracts

Based on the calibration curve, the IC_{50} value is calculated by replacing the Y value with the number 50 in the linear regression equation, thus obtained:

$y = 0.9498x + 4.6135$

$IC_{50} = (50 - 4.6135) / 0.9498x$

$= 47.78$

The IC_{50} value obtained from the analysis was 47.78 ppm, indicating a very strong antioxidant activity. Antioxidants are classified as very strong if their IC_{50} value is less than 50 ppm, strong if between 50 and 100 ppm, moderate if between 100 and 150 ppm, weak if between 150 and 200 ppm, and very weak if above 200 ppm [14]. Therefore, the IC_{50} value of 47.78 ppm confirms the antioxidant potency as very strong. An IC_{50} value of 47.78 ppm means that a concentration of 47.78 ppm is required to inhibit 50% of free radical activity in the assay. This result was notably lower compared to other treatments, including the untreated control (Table 1). Among the phytochemicals in the microgreens, Vitamin C (ascorbic acid) was the most abundant [15]. As a result, both Vitamin C and red amaranth microgreens with MLE also demonstrated very strong antioxidant activities, with similarly low IC_{50} values.

3.4 Statistical analysis

ANOVA hypothesis testing criteria at a significant level of 0.05 is if F count > F table then Ho is rejected, while if F count < F table then Ho is accepted. In the results of one-way ANOVA testing in Figure 5, it can be seen that F count> F table (18.96> 3.31) so that there is a significant influence between variations in MLE concentration and increased

growth of red amaranth microgreen.

Fig. 5. One-way ANOVA statistical test

ANOVA						
Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between	4,386261	2	2,19313	18,96386	4,74E-06	3,31583
Within Gr	3,469436	30	0,115648			
Total	7,855697	32				

4 Conclusions

Based on the results of the study, red amaranth supplemented with moringa extract biostimulant has very strong antioxidant activity, which is indicated by the IC_{50} value of 47.78 ppm at 20% w/v extract concentration. Based on physiological tests, it is also seen that 20% w/v moringa leaf extract concentration has an effect on height, wet weight, and dry weight. The results of the one-way ANOVA test also showed that the concentration variation had a significant effect.

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