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# The Effect of Selenium Biofortification on The Growth and Biochemical Responses of Two Microgreen Species

**Nosa Finalia*, Karno, Rosyida**

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## ABSTRACT

**Microgreens are a type of vegetable crop that may be grown at a young age (7−14 days) and have a high nutritional value. Microgreens from the Brassicaceae family, such as red radish and broccoli, contain a variety of antioxidants. Selenium biofortification improves microgreen quality and selenium content. The purpose of this study was to determine the influence of selenium levels and the differences between microgreen species. This study was carried out in November−December 2023 at a housing development on Kudan Street in Semarang City, Central Java. Chlorophyll and carotenoid analyses were performed at the Plant Physiology and Breeding Laboratory, Faculty of Animal and Agricultural Sciences, the Waste Treatment Laboratory at the Faculty of Engineering conducted the phenol analysis, while the Cendekia Nanotech Hutama Chemical and Biological Analysis Laboratory in Semarang City performed the antioxidant analysis. This study utilized a randomized complete block design with a factorial pattern of 5×2 and four replications. The first factor was selenium concentration, which had five levels: 0, 2, 4, 6, and 8 mg/L. The second factor was microgreen species, which consisted of two components: red radish and broccoli microgreens. The study found that treating broccoli microgreen with selenium at a concentration of 4 mg/L increased total chlorophyll content and antioxidant capability. Broccoli outperformed red radish microgreens on all metrics.**

**Keywords: antioxidant, biofortification, microgreens, weight, selenium**

## INTRODUCTION

Plant biofortification is a way of improving the nutritional value of agricultural products to suit human mineral requirements. Biofortification is a sustainable agriculture practice that effectively fulfills human nutritional needs (Roriz *et al.* 2020). Efforts to biofortify selenium must be intensified because insufficiency is linked to an increased risk of many illnesses. Low selenium intake has been associated to numerous disorders, including degenerative diseases, such as Keshan disease (Wang *et al.* 2023). One biofortification strategy for microgreens is the use of selenium, which helps to correct mineral deficiencies. Selenium biofortification is the method of raising selenium concentrations in plants to boost antioxidants using hydroponics (Kusumaningrum *et al.* 2016). Selenium biofortification in agriculture is an effective way to increase selenium levels in plants. The most efficient way for increasing selenium concentration in crops is biofortification (Hossain *et al.* 2021).

Microgreens, which are collected at an early stage, can benefit from selenium biofortification. Various plant species have the potential to be employed in biofortification, but microgreens stand out due to their short growth cycle, high nutritional content, and low levels of antinutritional chemicals (Poudel *et al.* 2023). These vegetables are becoming in demand, a popular food plant that is harvested 7 to 14 days after sowing, when the first genuine leaves appear (Turner *et al.* 2020). The Brassicaceae family is one of the most popular microgreens, frequently farmed due to their high phytochemical content and promise as useful foods (Alloggia *et al.* 2023). Microgreens come in a wide range of edible forms that can be processed into various food products. Many vegetable seeds have the potential to be grown as microgreens, although the most widely commercialized are from herb or vegetable crops in the Brassicaceae family, such as broccoli and radish (Xiao *et al.* 2019). Broccoli is highly liked because it contains bioactive chemicals such as phenolics, ascorbic acid, mineral nutrients, and glucosinolates (Gao *et al.* 2021). Vegetables containing glucosinolates provide significant health benefits for humans. The glucosinolate content of young vegetables detoxifies toxic chemicals in the body and promotes overall health (Baldelli *et al.* 2025). Radish, like other Brassicaceae plants, has stems that vary in color depending on the type. In addition to broccoli, radish is an adaptable crop that is abundant in nutrients and acts as an antioxidant (Gofar *et al.* 2022). Radish microgreens include a variety of nutrients, including 52.31 mg/100 g of ascorbic acid, 135.74 mg/100 g of phenolics, and 39.83 mg/100 g of flavonoids (Yadav *et al.* 2018).

Diponegoro University, Semarang 50275, Indonesia
* Corresponding Author:
Email: nosafinalia@students.undip.ac.id

This studys look at how varied selenium (Se) biofortification concentrations affect the growth and metabolic responses of two microgreen species. This study also investigated the impact of crop species on microgreen growth and biochemical responses, as well as the interacting effects of different selenium biofortification concentrations and microgreen species on growth and biochemical properties.

## METHODS

This study was carried out from November to December 2023 at a housing development on Kudan Street in Semarang City, Central Java. The Plant Physiology and Breeding Laboratory, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, conducted chlorophyll and carotenoid analyses. The Waste Treatment Laboratory at the Faculty of Engineering did the phenol analysis, while the Chemical and Biological Analysis Laboratory at CV Cendekia Nanotech Hutama in Semarang City used the DPPH method to measure the antioxidant analysis.

The experiment followed a factorial Randomized Complete Block Design (RCBD). The first factor was the concentration of selenium dioxide ($SeO_2$) added to the nutritional solution at five levels: 0 (S0), 2 (S1), 4 (S2), 6 (S3), and 8 mg/L (S4). The second element was microgreen species, which included two species: red radish (V1) and broccoli microgreens (V2). Treatments were placed in a 5 × 2 factorial arrangement with four replications, totaling 40 experimental units.

Plant material preparation and grow chamber setup were among the study procedures used. The growing substrate consisted of 30 × 20 × 2.5 cm of rockwool. The grow room was outfitted with a multi-level microgreen rack, and T5 LED strip lights (4000 K, 9 W) were added on each shelf. The nutritional solution was made using the half-strength Hoagland approach, which involved correctly weighing, premixing, and dissolving the essential components in water. Sowing was done by placing the rockwool in a microgreen tray with the desired thickness and soaking it in water. Microgreen seeds were placed on the rockwool, watered with a sprayer, and covered for four days in darkness.

The selenium treatment was administered at the specified concentration. Selenium was added to a half-strength Hoagland nutrition solution on the fourth day after germination. The solution was applied by watering the rockwool every two days until the harvest. Each microgreens tray received the exact same volume of nutritional solution. The microgreen seedlings were grown in a floating hydroponic system under LED illumination with a photoperiod of 16 hours light/8 hours dark, fertilized with a standard of half-strength Hoagland nutrient solution at pH 6.5 and electrical conductivity (EC) at 650 ppm. Red radish and broccoli microgreens were grown for 14 days after sowing and harvested by cutting the stems with scissors at 1 cm above the rockwool surface.

This study's parameters were shoot fresh weight evaluated using a digital scale and chlorophyll; carotenoid, phenol, and antioxidant capacity determined with a UV-Vis spectrophotometer. The acquired data was examined to detect treatment effects. If significant differences were found, the results were further analyzed using Duncan's Multiple Range Test at a significance threshold of α = 5%.

## RESULTS AND DISCUSSION

The shoot fresh weight of microgreens is shown in Table 1. According to the results of Duncan's test, each species treatment produced a substantial variation in shoot weight. This heterogeneity was most likely caused by the varying seed densities of each microgreen species, which influenced the density of the growing substrate. This finding is consistent with the discovery of Portales *et al.* (2024), that selenium biofortification has no effect on microgreen biomass, but that differences in biomass between species may be due to physiological factors such as seed density. Similar studies have found that seed density influences plant shoot weight. These are congruent with Thuong and Minh's (2020) findings, which showed that radish seed density of 8 seeds/cell, or around 109 g, resulted in the maximum fresh weight, while 6 seeds/cell yielded the lowest.

Selenium treatment at all doses had no significant effect on shoot fresh weight. This could be because

Table 1 Fresh weight of microgreen shoots treated with selenium

| Selenium Treatments (mg/L) | Species | | Average |
|---|---|---|---|
| | Red radish | Broccoli | |
| | g | | |
| 0 | 99.45 | 82.85 | 91.15 |
| 2 | 104.75 | 82.93 | 93.84 |
| 4 | 100.60 | 82.43 | 91.51 |
| 6 | 103.50 | 83.73 | 93.61 |
| 8 | 103.35 | 83.18 | 93.26 |
| Average | 102.33$^{a}$ | 83.02$^{b}$ | |
| Coefficient of variation (%) | 4.56 | | |

Remarks: Numbers followed by different superscripts in the average row denote significant differences derived from Duncan's test ($p < 0.05$).

selenium is more crucial for metabolic activity, which may not have a direct impact on shoot biomass in microgreens. This is consistent with the results of Islam *et al.* (2020), that selenium biofortification did not raise the fresh weight of wheat microgreens due to competition between selenium and important ion transporters in the growth medium. At some levels, selenium concentration may have a deleterious impact on shoot biomass. According to Naseem *et al.* (2021), selenium treatment may reduce pigment activity and so impede plant growth. This is consistent with the results of Puccinelli *et al.* (2019), that selenium concentrations of 4 and 8 mg/L had no significant effect on the fresh weight of microgreens.

Table 2 shows chlorophyll content, with Duncan's test results suggesting that selenium concentration interacts with microgreen species. Selenium concentrations of 2, 4, 6, and 8 mg/L in broccoli differed significantly from the control. This suggests that selenium is efficient at increasing chlorophyll content in broccoli microgreens. These findings are comparable with Vicas *et al.* (2019), that selenium treatments increased chlorophyll content in broccoli microgreens while maintaining a steady ratio of chlorophyll-a to -b at each treatment level. Puccinelli *et al.* (2021) support this finding, stating that selenium improves photosynthetic pigment production by shielding chloroplasts from abiotic stress and ROS damage. Selenium can minimize ROS damage using a variety of biocatalysts. According to Liu *et al.* (2022), selenium supplementation boosts antioxidant enzyme activity, hence reducing ROS effects and preventing oxidative damage in plants. Danso *et al.* (2023) support this, reporting that selenium is vital in a variety of physiological activities such as plant peroxidation prevention, antioxidant enzyme activity change, and chloroplast repair and regeneration. This is reinforced by Portales (2024), that selenium biofortification did not increase selenium content in kale, radish, cabbage, or wheat microgreens due to a variety of factors influencing phytochemical synthesis at varying selenium levels. The varied responses of broccoli and red radish to selenium treatment suggest that each species has distinct physiological pathways for selenium accumulation. Wu *et al.* (2022) stated that broccoli is a known accumulator of Se and S, which have comparable chemical properties; hence, selenium supplementation impacts sulfur uptake and glucosinolate metabolism, increasing both in broccoli roots. In contrast, red radish exhibited a different response than broccoli. Tenesaca *et al.* (2024) found that selenium treatment affects radish microgreens differently depending on their tolerance level. Radish microgreens respond positively to moderate selenium concentrations because they support the homeostasis process, whereas higher concentrations have a negative effect on their physiology and can cause toxicity.

Table 3 displays the carotenoid content of the plants and the results of Duncan's test, showing that the treatment of different microgreen species was significantly different, with broccoli having a higher carotenoid content (1.87 mg/g) than red radish microgreens. This could be because the broccoli

Table 2 Chlorophyll content of microgreen species treated with selenium

| Selenium Treatments (mg/L) | Species | | Average |
|---|---|---|---|
| | Red radish | Broccoli | |
| | mg/g | | |
| 0 | 5.16$^{b}$ | 5.36$^{b}$ | 5.26 |
| 2 | 6.82$^{b}$ | 14.50$^{a}$ | 10.66 |
| 4 | 5.12$^{b}$ | 15.00$^{a}$ | 10.06 |
| 6 | 6.42$^{b}$ | 13.00$^{a}$ | 9.71 |
| 8 | 5.03$^{b}$ | 14.25$^{a}$ | 9.64 |
| Average | 5.71 | 12.42 | |
| Coefficient of variation (%) | 19.26 | | |

Remarks: Numbers followed by different superscripts in the column or average row denote significant differences derived from Duncan's test ($p < 0.05$).

Table 3 Carotenoid content of microgreen species treated with selenium

| Selenium Treatments (mg/L) | Species | | Average |
|---|---|---|---|
| | Red radish | Broccoli | |
| | mg/L | | |
| 0 | 1.19.. | 1.49.. | 1.38 |
| 2 | 1.58.. | 1.90.. | 1.74 |
| 4 | 1.24.. | 2.02.. | 1.63 |
| 6 | 1.49.. | 2.02.. | 1.75 |
| 8 | 1.28.. | 1.95.. | 1.62 |
| Average | 1.35$^{b}$ | 1.87$^{a}$ | |
| Coefficient of variation (%) | 19.16 | | |

Remarks: Numbers followed by different superscripts in the average row denote significant differences derived from Duncan's test ($p < 0.05$)

control group had higher carotenoid levels than red radish, indicating a larger potential for carotenoid production. These findings are in line with Pannico *et al.* (2020), that each plant genotype has a different response to plant biosynthetic activity and secondary metabolites because selenium absorbed by the plant affects phytoene synthase. The phytoene synthase enzyme (PSY) is a key catalyst for plant carotenoid production. This is consistent with Zhou *et al.* (2022), that PSY is the initial catalyst in the carotenoid biosynthesis pathway and acts as a carotegenic enzyme. Selenium concentration treatment at all levels had no significant influence on carotenoid content in plants. This is congruent with Tenesaca *et al.* (2024) report that selenium treatment had no effect on carotenoid content. This is thought to be because carotenoids, which act as non-enzymatic antioxidants to battle ROS in chloroplasts and peroxisomes, are unaffected by selenium. The same study produced results that are similar to the findings of this investigation. This is reinforced by Germ *et al.* (2019), that specific selenium compounds, such as SeO42- or SeO32-, cannot boost carotenoid concentration in microgreens, whereas iodine compounds may.

The phenol content of the plants is shown in Table 4, that the results of Duncan's test, indicating that treatment with different microgreen species showed significant differences, with broccoli having a higher phenol content (1823.93 mg GAE/100 g) than red radish microgreens (739.04 mg). This is most likely because red radish microgreens react differently than broccoli in terms of phenol production. According to Puccinelli *et al.* (2021), species differences considerably affect phenol production, with sorrel (Rumex acetosa) producing the greatest phenol content of 2.70% at a selenium level of 1.5 mg/L, although selenium concentration had no significant effect on total phenol content. Plant species have a substantial influence on the phenol content of microgreens. Fuente *et al.* (2019) found that the phenol content of four varieties of microgreens varied greatly, with broccoli carrying 2037.38 mg GAE/100 g and radish holding 2111.19 mg GAE/100 g.

Treatment with selenium concentrations at all levels had no significant influence on plant phenol content. This is thought to be because high levels of selenium can interfere with the plant's phenol production mechanism. The lowest selenium concentration treatment of 2 mg/L increased phenol content in broccoli microgreens by 28.03%, whereas 6 mg/L selenium resulted in a lesser rise of 2.86% compared to the control. This finding is in line with Cheng *et al.* (2023), that selenium treatment can be beneficial to plants at specific doses, but if the dosage is too low or too high, it can hinder plant growth. Selenium treatment at 4 mg/L in red radish microgreens produced the maximum phenol level, while dosages of 6 mg/L and 8 mg/L resulted in a drop in phenol content. According to Pannico *et al.* (2020), selenium can generate abiotic stress comparable to that caused by other metal compounds, potentially interfering with the phenylpropanoid pathway.

The antioxidant capabilities of the plants are shown in Table 5. According to Duncan's test results, selenium concentration treatment at 6 mg/L did not change substantially from 0, 4, and 8 mg/L, but did differ

Table 4 Phenol content of microgreen species treated with selenium

| Selenium Treatments (mg/L) | Species | | Average |
|---|---|---|---|
| | Red radish | Broccoli | |
| | mg GAE/100g | | |
| 0 | 717.10.. | 1619.25.. | 1168.23.. |
| 2 | 804.39.. | 2072.21.. | 1438.30.. |
| 4 | 804.63.. | 1839.78.. | 1322.21.. |
| 6 | 682.40.. | 1664.59.. | 1173.49.. |
| 8 | 686.58.. | 1923.80.. | 1305.19.. |
| Average | 739.04 $^{b}$ | 1823.93 $^{a}$ | |
| Coefficient of variation (%) | 9.31 | | |

Note: Numbers followed by different superscripts in the average row denote significant differences derived from Duncan's test ($p < 0.05$)

Table 5 Antioxidant capacity of microgreen species treated with selenium

| Selenium Treatments (% DPPH) | Species | | Average |
|---|---|---|---|
| | Red radish | Broccoli | |
| | % DPPH inhibition | | |
| 0 | 74.38.. | 80.01.. | 77.19$^{ab}$ |
| 2 | 71.30.. | 77.14.. | 74.22$^{b}$ |
| 4 | 71.95.. | 81.05.. | 76.50$^{ab}$ |
| 6 | 75.26.. | 86.17.. | 80.72$^{a}$ |
| 8 | 75.95.. | 83.08.. | 79.51$^{ab}$ |
| Average | 73.77$^{b}$ | 81.49$^{a}$ | |
| Coefficient of variation (%) | 5.16 | | |

Remarks: Numbers followed by different superscripts in the column or average row denote significant differences derived from Duncan's test ($p < 0.05$)

significantly from the 2 mg/L selenium concentration. Selenium concentrations of 4−8 mg/L can elicit a favorable response, enhancing antioxidant activity. This is consistent with the findings of Puccinelli *et al.* (2019), that administering selenium to sprouting lupin at doses of up to 8 mg/L increased antioxidant content. Selenium content influences microgreens' antioxidant capacity. This is most likely due to selenium's effect on microgreens' antioxidant capacity via activating the plant's antioxidant defense system. According to Gupta and Gupta (2017), selenium in plants acts as a catalytic center for various selenoproteins, which neutralize free radicals and defend against stress.

Plant antioxidant concentration differed significantly when treated with several microgreens. species, including broccoli and red radish. This is thought to be because broccoli contains more phytochemicals, which influence antioxidant activity. Woch and Noawak (2019) found that selenium can boost plant antioxidant capacity, but this varies by species. This finding is reinforced by Giordano *et al.* (2022), that differences in antioxidant activity capacity among Apiaceae microgreens influenced by genotype, extraction process, growth conditions, and fertilization. The method used to determine antioxidant capacity influences the results because each microgreen species reacts differently to the chemicals used. Fuente *et al.* (2019) revealed that antioxidant capacity analysis in broccoli microgreens using the Trolox Equivalent Antioxidant Capacity Assay (TEAC) and Oxygen Radical Absorbance Capacity Assay (ORAC) yielded lower results than other types of microgreens, whereas analysis using the DPPH method yielded the opposite results when compared to six different species.

## CONCLUSION

Based on the research, selenium treatment at 4 mg/L in broccoli microgreens was found to boost total chlorophyll content and antioxidant capacity. Broccoli microgreens outperformed red radish microgreens in all metrics. There was a link between selenium concentration treatment and species differences in plant chlorophyll content.

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