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Can Moringa Leaf Spray Treatment Increase the Nutraceutical Properties of Radish Baby Leaf?

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Abstract: Among the ready-to-use products, baby leaf salads (both raw and cooked), especially those belonging to the Brassicaceae family, represent a very interesting food typology, with nutraceutical properties. Recently, to obtain products with lower levels of synthetic chemicals and to improve nutritional quality, attention has been paid to the use of natural biostimulants such as *Moringa oleifera* Lam. In this study, the aim was to investigate the effect of applying this natural biostimulant, at 15, 30, and 45 days from sowing, by spraying seedlings of radish (*Raphanus sativus* L.) each morning with *Moringa oleifera* leaf extract (MLE) at doses of 1:40 and 1:30 L^{-1} until dripping. Different morphological, physiological, and chemical parameters were determined. At harvesting time, the fresh biomass, total leaf area, and unit leaf area showed progressive increases as the dose of MLE was increased, while there were no significant difference in the dry biomass among the treatments. The quantum yield of PSII showed a significant increase in response to MLE treatments. The contents of chlorophylls and carotenoids were higher in both MLE treatments as compared with those of the control plants. The antioxidant capacity (DPPH) was not influenced by MLE treatment, while the influence was significant for total phenolic content (TPC). No significant differences were observed for the total sugar content, while the highest concentration of ascorbic acid was found with both MLE treatments; the MLE treatments did not modify the nitrate content. Therefore, MLE treatment showed a positive influence, although further studies are necessary to individuate the better doses and treatment modalities to improve the characteristics of radish baby leaf.

Keywords: novel foods; *Moringa oleifera* leaf extract; polyphenol content; chlorophylls; total sugars; nitrate

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1. Introduction

Vegetables, if consumed according to the recommendations, contain almost all the essential nutrients for human metabolism, and therefore they are actively involved in the promotion of human health [1]. Among superfoods, baby leaf salads, as ready-to-use products, represent a novel product that is gaining more and more popularity among consumers [2,3]. These products are prepared with young leaves harvested in a very early growth phase (4–8 true leaves), when they are metabolically very active [4]. A different species from different botanical families has been harvested for baby leaf salads, both raw and cooked [5]. The species mostly used as raw baby leaves belong to different botanical families, including Amaranthaceae, Asteraceae, Brassicaceae, and others [5].

In particular, among the species, radish (*Raphanus sativus* L.) is a root vegetable from the Brassicaceae family and is an important crop that is popular throughout the world [6]. Radish has been reported to be a helpful natural additional treatment in the case of degenerative and coronary disease because of its nutraceutical metabolites [7]. The whole plant is edible; generally, the taproot is consumed, although the aerial part can be used as a leaf vegetable [8]. The leaves of this plant are, in fact, an underutilized leaf vegetable; therefore, this plant species has been poorly studied and practically no

information is available on the chemical profile of the leaves themselves. Previous studies on the antioxidant activities of *R. sativus* have principally focused on sprouts; from these investigations, it has emerged that these products contain synapic acid esters and flavonoids as the main phenolic components [9]. Recently, there has been renewed interest in *R. sativus* as an ingredient in the production of functional foods as it contains glucosinolates and several other components with nutritional proprieties [8].

In agriculture, the goal is high-quality production, and fertilizers are always necessary; however, the optimal quantity of fertilizer must be used because excessive use of fertilizer has negative consequences on both the environment and the products, and it can result in uneconomical and unprofitable crop yield [10]. With the aim to reduce the consumption of synthetic chemicals, in recent years, attention has been shifting more and more to the use of natural biostimulants. There is, in fact, a continuous need to look for natural alternatives that can become safe sources of nutrients for plants. Among these products, the extract of *Moringa oleifera* Lam. has gained attention. The *Moringa oleifera* leaf extract (MLE) acts as a hormone for plant growth since it can improve seed germination, plant growth, and crop yield [11]. It is used mainly in developing countries, where yields are very low, and therefore it has been proposed to farmers as a possible complement and/or substitute for inorganic fertilizers [12]. Minerals, proteins, vitamins, β-carotene, amino acids, and phenols are contained in different parts of the *Moringa oleifera* plant. In addition, it provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid, and kaempferol [13–16]. Thus, MLE may represent a good natural source of antioxidant compounds for plants [15–19]. Some research has been carried out on the effects of MLE to evaluate their hormonal effects and changes in the secondary metabolites of plants and their impact on the quality of products.

Regarding baby leaves, there is limited research on the use of *Moringa oleifera* as a biostimulant (i.e., there is limited research on the effect of a concentrated aqueous extract of this plant). Although *Moringa oleifera* is a tree native to eastern India, this plant adapts very well to the climatic conditions of Sicily [20], where for some years it has been cultivated and displayed as an ornamental plant, for the aesthetic features of the whole plant, including its foliage, flowers, and fruits [21]. Thus, investing in the cultivation of *Moringa oleifera* could represent a possible new source of fresh material for obtaining MLE.

In this context, our experiment aimed at investigating the exogenous application of aqueous MLE to understand how it could influence both yield and the nutraceutical properties of radish leaves, which represent an aspect of particular interest for this kind of product.

2. Materials and Methods

2.1. Plants Materials

Raphanus sativus L. (radish) seeds were purchased from CN Seeds, Ltd., Pymoor, Ely, Cambridgeshire, UK and were utilized in the trial. Seeds were sown in cellular trays using Vigorplant Italia SRL (Fombio (LO), Italy) as the substrate (pH 5.5–6.5, EC 0.15–0.25 dS/m), and the seedlings were irrigated every day. The containers were placed in a cold greenhouse located in Catania (southern Italy, 37°31′010″ N 15°04′018″ E, 105 m above sea level (m.a.s.l.)) with natural light (from 5.9 to 11.6 $MJm^{-2}d^{-1}$) and temperature (18.4 ± 6.8 °C) conditions, from February to March 2021.

The *Moringa oeifera* leaf extract (MLE) was sprayed in three applications: 15 and 30 days after planting and 2 days before harvesting (45 days after planting), following the information reported by Alì et al. [22]. The seedlings were sprayed in the morning (between 9:00 and 10:00 a.m.) until dripping, with different doses (1:40, 1:30 L^{-1}, or distilled water), according to Zulfiqar et al. [23].

2.2. Moringa oleifera Leaf Extract

The *M. oleifera* leaf extract (MLE) was prepared according to Toscano et al. [20]. The leaves of *M. oleifera* were shade-dried and then finely ground with a mill. The powder was

mixed in distilled water (50 g in 200 mL). The mixture was maintained for 48 h at 25 °C, and then the solution was filtered through filter paper Whatman No 1 and diluted in water (1:40 and 1:30, *v*/*v*). Tween 20 (0.05%) was added to the spray solutions as a wetting agent.

The MLE extract was analyzed, and its chemical constituents have been reported by Toscano et al. [20].

2.3. Measurement of Growth Parameters

Twelve randomly collected seedlings were used for each replication to detect growing parameters such as fresh and dry biomass, the longitudinal (Wx) and transversal (Wy) lengths of the leaves, and total and unit leaf area. The leaf characteristics and the total leaf area were acquired using an area meter (Delta-T Devices Ltd., Cambridge, UK). The dry biomass was obtained by placing weighted samples in an electric ventilated oven set at 70 °C until a constant weight was attained. Color indices were measured using a Minolta portable colorimeter (CR-400 Konica Minolta, Inc., Osaka, Japan) to detect the CIEL*a*b* color parameters, L* (lightness), a* (green to red), and b* (yellow to blue).

2.4. Chlorophyll a Fluorescence

The quantification of quantum yield of PSII (Fv/Fm ratio) was measured using a modulated chlorophyll fluorimeter OS1-FL fluorimeter (Opti-Sciences Corporation, Tyngsboro, MA, USA). Twelve plant leaves for each replication were dark-adapted by cuvette clips (Opti-Sciences Corporation, Tyngsboro, MA, USA) for 15 min. The Fv/Fm ratio was obtained from the Fv and Fm and represented the potential maximal PSII quantum yield. The variable fluorescence (Fv) was calculated using the difference between F0 and Fm; F0 represented the minimum fluorescence, and Fm represented the maximal fluorescence of the dark-adapted state.

2.5. Quantification of Chlorophyll and Carotenoids

The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid were quantified by spectrophotometer. For each sample, 100 mg of leaf sample was ground with liquid nitrogen and thereafter incubated in a dark room (4 °C for 24 h) in 99% methanol (Sigma-Aldrich, Milan, Italy). The samples were read at 665.2 nm, 652.4 nm, and 470 nm (7315 Spectrophotometer, Jenway, Staffordshire, UK). The chlorophyll content was determined following the procedure described by Lichtenthaler et al. [24]. Three biological replicates were used for the analysis (n = 3).

2.6. 2.2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical-Scavenging Activity and Total Phenolic and Flavonoid Contents

The free radical scavenging activity was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH); 100 mg of lyophilized powder was mixed with 1.5 mL of the solvent mixture methanol/water (80%); the mixture was sonicated for 30 min, and then centrifuged at 5000× *g* for 10 min at 5 °C. Thereafter, 0.01 mL of supernatant was added to 1.4 mL of DPPH solution (150 µM) and incubated for 30 min in the dark. The absorbance of the solution was determined to be 517 nm. The results were expressed using a standard curve prepared with Trolox under the same experimental conditions (mg TE g^{-1}) (R^2 = 0.9124). Three biological replicates were used for the analysis (n = 3).

The total phenolic content was determined using the Folin–Ciocalteu method. Samples of 0.2 g of lyophilized leaf powder were homogenized in a solution with 10 mL of the solvent mixture acetone-water (1:1, *v*/*v*) and then left to stand at 20 °C in the dark for 15 h. Next, 100 µL of supernatant was added to 0.5 mL of Folin–Ciocalteu reagent, 6 mL of distilled water, and 1.5 mL of 20 g/100 mL sodium carbonate. Then, the mixture was incubated at 20 °C for 2 h, and thereafter, absorbance was read at 765 nm. The TPC was reported as gallic acid equivalent (mg GAE g^{-1}) (R^2 = 0.9998). Three biological replicates were used for the analysis (n = 3).

The total flavonoid content was determined according to Wang et al. [25], with slight modifications using the aluminum chloride colorimetric method. The results were expressed using rutin as a standard (R^2 = 0.9993). Thereafter, 1 g of lyophilized powder was extracted with 80% methanol (10 mL); 1 mL of the extract was removed and diluted to 10 mL in a volumetric flask (10 mL) using ethanol/water (60:40, *v*/*v*) solvent. One milliliter was mixed with 2% (*w*/*v*) aluminum tri-chloride (2 mL) and CH_3COONa (1 mol/L) solution (3 mL) and then diluted to 25 mL using ethanol. The absorbance was read at 510 nm. The results were expressed as mg of rutin equivalents (RU) per g DW. Three biological replicates were used for the analysis (n = 3).

2.7. Total Sugars

The protocol by Yemm and Willis [26] was used for total sugar determination. One gram of fresh sample was extracted by homogenization in 3 mL of distilled water. The resulting extracts were centrifuged at 3000× *g* for 15 min at room temperature (RT). Thereafter, 1 mL of extract sample was added to 5 mL of anthrone solution (0.2 g in 100 mL of H_2SO_4), cooled in ice for 5 min, and then mixed thoroughly. The samples were kept in a thermostatic water bath (95 °C) for 5 min and then cooled on ice. The absorbance at 620 nm was determined against a blank. The total sugar concentration was expressed using a calibration plot with glucose as a standard (R^2 = 0.9995) [27]. Three biological replicates were used for the analysis (n = 3).

2.8. Ascorbic Acid (Asc) Analysis and Nitrate Concentration

One gram of fresh samples was homogenized in 10 mL of 5% oxalic acid. Then, the samples were centrifuged at 4000× *g* for 5 min. Thereafter, 1 mL of supernatant was mixed with 2 mL of 0.1% methyl viologen and 2 mL sodium hydroxide (2 mol L^{-1}). The absorbance of samples was measured at 600 nm against the radical blank [28]. Three biological replicates were used for the analysis (n = 3).

The nitrate analysis and calibration curve (R^2 = 0.9924) were determined by the method developed by Cataldo [29]. One gram of fresh sample was homogenized in 3 mL of distilled water. The slurry was centrifuged at 3000× *g* for 15 min. After, the supernatant was recovered, and 20 µL was added to 80 µL of 5% (*w*/*v*) salicylic acid in H_2SO_4 and 3 mL of sodium hydroxide (1.5 N). The samples were cooled at RT, and the absorbances were read at 410 nm. The results were expressed using a standard curve prepared with KNO_3 under the same experimental conditions. Three biological replicates were used for the analysis (n = 3).

2.9. Statistical Analysis

The trial was a randomized complete design with three replications. The significance of differences among the treatments was determined by one-way analysis of variance (ANOVA). The Tukey's test was applied and the significant differences established as $p < 0.05$. The statistical analyses were performed using CoStat release 6.311 (CoHort Software, Monterey, CA, USA). The data presented in the figures are the means ± standard error (SE) (Graphpad 7.0, software Inc., San Diego, CA, USA).

3. Results

3.1. Plantlets Characteristics and Photosytem Efficiency

The fresh biomass showed progressive increases as the concentration of the natural biostimulant was increased; the biomass increased by ~38% and ~17%, respectively, for the 1:30 and 1:40 leaf treatments ($p < 0.001$). Dry biomass did not show a significant difference among treatments (Table 1).

Table 1. Effect of *Moringa oleifera* leaf extract (MLE) foliar application on fresh and dry biomass (FW, g plant^{-1}), total leaf area (cm^2 plant^{-1}), unit leaf area (cm^{-2}), and land leaf dimension (Wx and Wy) (cm) of radish baby leaf. MLE extract was sprayed 15 and 30 days after planting and 2 days before harvesting (45 days after planting).

Treatments	Fresh Biomass (FW, g plant^{-1})	Dry Biomass (DW, %)	Total Leaf Area (cm^2 plant^{-1})	Unit Leaf Area (cm^2)	Wx (cm)	Wy (cm)
Control	0.514 ± 0.016 ^{c}	15.640 ± 0.339	12.601 ± 0.516 ^{c}	4.475 ± 0.118 ^{c}	1.861 ± 0.039 ^{c}	3.731 ± 0.052 ^{c}
1:40	0.602 ± 0.020 ^{b}	16.208 ± 0.321	14.935 ± 0.569 ^{b}	5.301 ± 0.125 ^{b}	2.058 ± 0.030 ^{b}	3.970 ± 0.061 ^{b}
1:30	0.800 ± 0.022 ^{a}	16.796 ± 0.321	20.460 ± 0.652 ^{a}	6.781 ± 0.219 ^{a}	2.358 ± 0.049 ^{a}	4.556 ± 0.073 ^{a}
Significance	***	ns	***	***	***	***

Values (means ± *se*) within the same column, followed by the same letter, do not significantly differ at $p < 0.05$ according to Tukey's test; ns, not significant; significant at p 0.001 (***). Three biological replicates of 12 plants each were used for measurements ($n = 3$).

The total leaf area was progressively enhanced as the concentration of the natural biostimulant increased; the total leaf area increased by ~30% and ~15%, respectively, for the 1:30 and 1:40 leaf treatments ($p < 0.001$). A similar trend was observed for the unit leaf area ($p < 0.001$) (Table 1).

The length and width increased significantly from 1.9 to 2.4 cm and from 3.7 to 4.6 cm, respectively, for the control and the 1:30 treatments ($p < 0.001$) (Table 1).

Referring to the chromatic parameters, the MLE foliar applications influenced only a* ($p < 0.01$) and b* ($p < 0.05$), while L* did not show any statistically significance (Table 2).

Table 2. Effect of *Moringa oleifera* leaf extract (MLE) foliar application on chromatic coordinate (L*, a*, and b*), minimum fluorescence (F0), maximal fluorescence (Fm), and maximum quantum efficiency of PSII (Fv/Fm) of radish baby leaf. MLE extract was sprayed 15 and 30 days after planting and 2 days before harvesting (45 days after planting).

Treatments	L*	a*	b*	F0	Fm	Fv/Fm
Control	54.390 ± 0.449	−21.008 ± 0.248 ^{a}	34.031 ± 0.660 ^{b}	444.000 ± 18.369	1779.889 ± 57.664	0.748 ± 0.012 ^{b}
1:40	55.082 ± 0.450	−21.362 ± 0.158 ^{a}	35.107 ± 0.567 ^{ab}	428.556 ± 33.311	1935.000 ± 147.016	0.778 ± 0.002 ^{a}
1:30	55.470 ± 0.482	−22.001 ± 0.197 ^{b}	36.403 ± 0.733 ^{a}	433.778 ± 11.201	2037.222 ± 59.715	0.786 ± 0.004 ^{a}
Significance	ns	**	*	ns	ns	**

Values (mean ± *se*) within each column, followed by the same letter do not significantly differ at $p < 0.05$ according to Tukey's test; ns, not significant; significant at $p < 0.05$ (*) and 0.01 (**). Three biological replicates of 12 plants each were used for measurements ($n = 3$).

No significant differences were observed for F0 and the Fm among the treatments (Table 2). The Fv/Fm ratio showed a significant increase in response to MLE treatments (0.79 and 0.78 for the 1:30 and 1:40 treatments, respectively) as compared with control plants (0.74).

3.2. Chlorophyll a, b, and Total, and Carotenoids

Chlorophyll *a* content showed significant differences in both MLE treatments as compared with control plants ($p < 0.001$). As a result, the same pattern was found for chlorophyll *b* and total chlorophyll ($p < 0.001$).

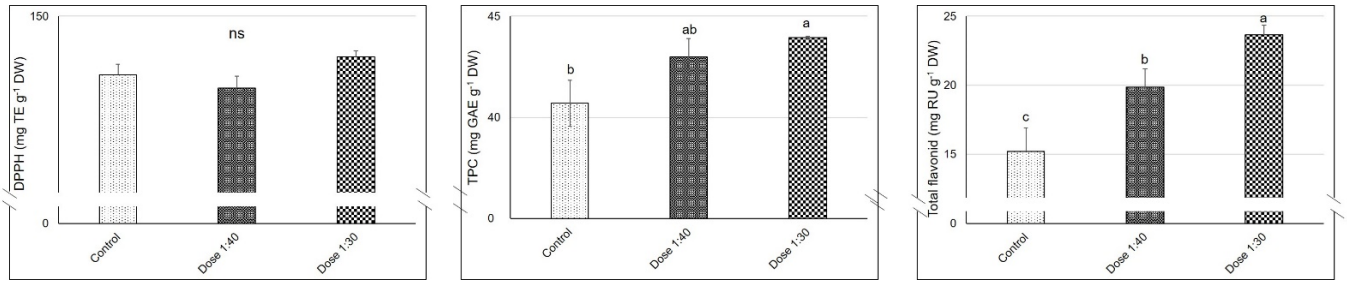
Carotenoids accumulated in a larger amount in the 1:40 leaf treatment (0.17 μg g^{-1} FW, against 0.10 μg g^{-1} in control plants, $p \leq 0.001$). Treatment with MLE showed a higher chlorophyll *a*/*b* ratio than the value observed in the control plants. The same trend was observed in the results for total chlorophyll content and carotenoid content (Table 3).

Table 3. Effect of *Moringa oleifera* leaf extract (MLE) foliar application on chlorophyll *a* and *b* content, chlorophyll *a*/*b* ratio (Chl *a*/Chl *b*), total chlorophyll, carotenoid content, and chlorophyll/carotenoids ratio (Chl/Car) of radish baby leaf. MLE extract was sprayed 15 and 30 days after planting and 2 days before harvesting (45 days after planting).

Treatments	Chl *a* (μg mg^{-1} FW)	Chl *b* (μg mg^{-1} FW)	Total Chl (μg mg^{-1} FW)	Carotenoids (μg mg^{-1} FW)	Chl *a*/Chl *b*	Chl/Car
Control	0.410 ± 0.021 ^{b}	0.155 ± 0.010 ^{c}	0.572 ± 0.041 ^{b}	0.097 ± 0.003 ^{c}	2.654 ± 0.002 ^{b}	3.284 ± 0.187 ^{b}
1:40	0.699 ± 0.023 ^{a}	0.229 ± 0.016 ^{a}	0.923 ± 0.036 ^{a}	0.173 ± 0.004 ^{a}	3.051 ± 0.005 ^{a}	4.923 ± 0.191 ^{a}
1:30	0.682 ± 0.014 ^{a}	0.208 ± 0.00 ^{b}	0.826 ± 0.016 ^{a}	0.152 ± 0.008 ^{b}	2.983 ± 0.004 ^{a}	4.453 ± 0.112 ^{a}
Significance	***	***	***	***	***	**

Values (means ± *se*) within the same column, followed by the same letter, do not significantly differ at $p < 0.05$ according to Tukey's test; ns, not significant; significant at $p < 0.01$ (**) and 0.001 (***). Three biological replicates were used for measurements ($n = 3$).

DPPH was used to determine free radical scavenging activity. The DPPH was not influenced by treatments (Figure 1a). The treatment did not exert any effect on this parameter, while significant differences were observed for TPC (Figure 1b). Treatment with MLE increased the TPC by 8% in 1:30 as compared with the control plants. The total flavonoids accumulated in a larger amount in the 1:30 and 1:40 leaf treatment (23.6 and 19.5 mg RUg^{-1} FW in the 1:30 and 1: 40 leaf treatments, respectively, against 15.2 mg RU g^{-1} in control plants) (Figure 1c).

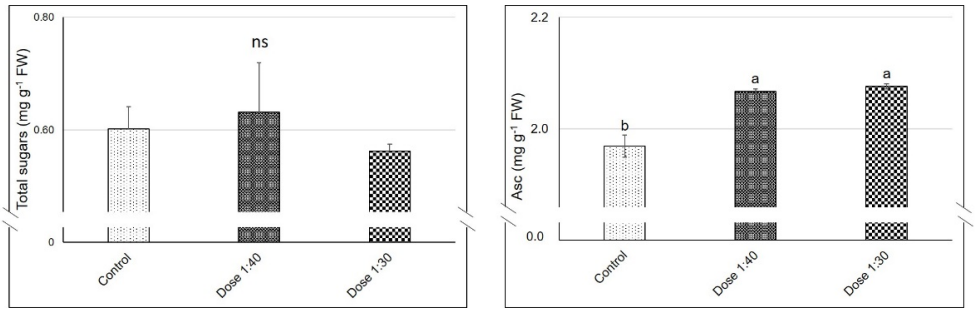


(**a**) (**b**) (**c**)

Figure 1. Effect of *Moringa oleifera* leaf extract (MLE) foliar application on the antioxidant activity (**a**) (DPPH, mg TE g^{-1} FW), TPC (**b**) (mg GAE g^{-1} FW), and total flavonoid content (**c**) (mg RU g^{-1} FW) of radish baby leaf. Data are means ± standard error ($n = 3$). Three biological replicates were used for the analysis. Different letters indicate significance at $p < 0.05$ according to Tukey's test; ns, not significant.

No significant differences were observed for the total sugar content (Figure 2a). The highest concentration of ascorbic acid was found in both MLE treatments; the plants treated with *Moringa oleifera* leaf extract showed an increase in total sugar content of 5% as compared with the control plants (Figure 2b).

The nitrate content in plants treated with *Moringa oleifera* leaf extract did not show any significant differences compared to the control plants (Figure 3).



(**a**) (**b**)

Figure 2. Effect of *Moringa oleifera* leaf extract (MLE) foliar application on total sugars (**a**) (mg g^{-1} FW) and ascorbic acid (Asc) (**b**) (mg g^{-1} FW) of radish baby leaf. Data are means ± standard error (n = 3). Three biological replicates were used for the analysis. Different letters indicate significance at $p < 0.05$ according to Tukey's Test; ns, not significant.

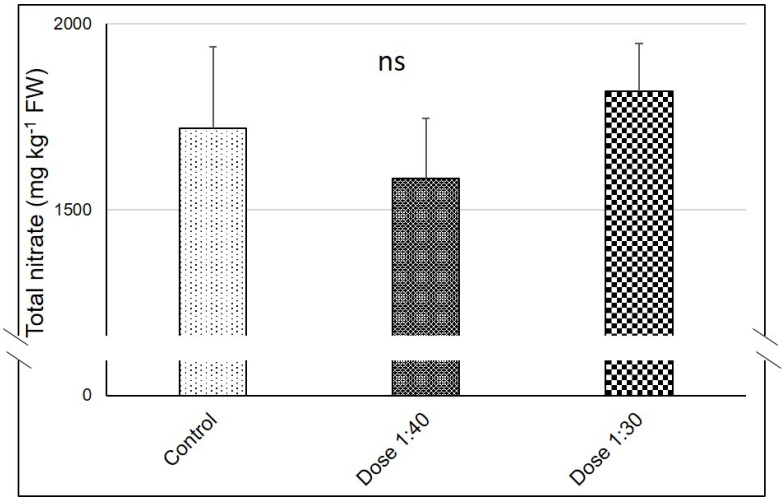


Figure 3. Effect of *Moringa oleifera* leaf extract (MLE) foliar application on nitrate content (mg kg^{-1} FW) of radish baby leaf. Data are means ± standard error (n = 3). Three biological replicates were used for the analysis. Different letters indicate significance at $p < 0.05$ according to Tukey's test; ns, not significant.

4. Discussion

Our experimental results on the use of a plant extract as a possible substitute for widespread agrochemicals agree with those of other studies on more environmentally friendly agricultural practices [30–32]. Therefore, foliar treatments with the use of biostimulants could represent an alternative method for improving plant growth and yield [33]. Numerous biostimulants have been derived from the tissues and cells extracted from plant material and have been applied to improve both crop yield and quality, and to enhance plants' resistance to abiotic stresses [34].

Nowadays, farmers and researchers are frequently using biostimulant plant extracts to improve crops in normal and stress conditions [35–37]. Among them, the components of *Moringa oleifera* leaf extract have resulted in interesting effects on enhancing plant growth and/or secondary metabolite synthesis [23,38–40].

The results of our study showed that the different treatments with MLE improved both plant growth and the nutraceutical properties of radish. The significant enhancement of the fresh weight of radish baby leaf was recorded with both MLE treatments. According to Scaglia et al. [41], biostimulant applications enhance plant growth by stimulating nutrient uptake and efficiency, improving tolerance to environmental stressor and enhanc-

ing the overall crop quality. The high contents of crude protein (43.5%) in leaf and twig *Moringa oleifera* extracts have been shown to increase the growth rate and both auxin and cytokinin contents [42,43]. As recent as two decades ago, Foidll et al. [44] reported faster growth in basil young plants by detecting an increase of stem diameter and larger leaves using *Moringa oleifera* extract foliar application as compared with a control. According to his results, elevated concentrations of MLE were directly related to an increase in shoot growth. The data were in line with Abdalla [39] and Hassanein et al. [45], who used MLE as organic fertilizer applied in fertigation.

Our results showed significant differences in terms of morphobiometric characteristics in seedlings treated with *Moringa oleifera* extracts as compared with control plants; this was in agreement with a number of authors who showed significantly better growth parameters (plant height, weight of shoot, leaf number, etc.) in diverse vegetables and legume crops, such as okra, pepper, snap bean, common bean, eggplant, and tomato [46–51].

Leaf area is one of the principal growth indices and can be used able to describe the economic yield of crops because the leaf area index (LAI) is naturally connected with leaf area [52]. MLE treatments determined an increase in total leaf area and unit leaf area. The positive effect could be due to the cumulative effect of growth hormones as well micro- and macronutrients present in this natural biostimulant [53]. The quantum yield of PSII expresses the primary photochemistry of PSII capacity, which is highly sensitive to different abiotic stresses [54]. Our results reveal no stress status in the radish plants treated with MLE; in fact, the treatments with MLE did not damage the photosynthetic apparatus efficiency, and vice versa, the treatments revealed a significant increase as compared with the control plants.

Leaf color is considered to be an index to indicate the health of the seedlings. Our results showed a little (a* and b*) or no significant (L*) variation between control and treated seedlings.

The results of our experiment showed an increase in chlorophyll *a* and chlorophyll *b* content, total chlorophyll, and carotenoid content. Therefore, the positive effect of MLE on photosynthetic pigments could be correlated to the substantial amounts of chlorophyll in MLE, as reported by Toscano et al.'s [20] analysis of MLE extract (chlorophyll *a* 1.2 μg mg^{-1} FW and chlorophyll *b* 1.4 μg mg^{-1} FW). Leaf chlorophyll concentration is an important quality parameter directly linked with the visual appearance of vegetables [55]. One of the characteristic responses to biostimulants is an increase in chlorophyll content in treated plants. Previous studies have shown that *Moringa oleifera* leaf extract (MLE) could improve photosynthetic pigments under normal and stress environmental conditions [56].

In our study, the chlorophyll and carotenoid contents increased with the treatments. These results were in line with previous data reported by other authors who showed that MLE foliar application increased the content of phenolic compounds, other biochemicals, and total chlorophyll in spinach (*Spinacia oleracea* L.) leaves [57].

The increased photosynthetic pigment in treated plants could be imputed to nutrients such as nitrogen and magnesium present in biostimulants, which are fundamental for chlorophyll synthesis [58]. The results of our trial showed that the different LME treatments increased the phenol and flavonoid contents of radish baby leaf. The total flavonoid content (TFC) represented 40% of the TPC. The higher content of phenols and total polyphenols in plants treated with MLE 1:30 may be due to the content of phenols, flavonoids, and phytohormones in the leaves of *Moringa oleifera*. Similar results were also observed by Nasir et al. [59] and Mehmood et al. [40] in plants of 'Kinnow' mandarin and black cumin, who demonstrated that the phenolic content increased with the MLE application.

Reductions in sugars after biostimulant applications have been found in different vegetables. As reported by Bulgari et al. [60], lower sucrose and reducing sugars were found in endive and lettuce vegetables treated with a commercial biostimulant (ONE®). In our study, instead, the MLE treatments did not modify the total sugar content.

In our experiment, the ascorbic acid increased in plants treated with *Moringa oleifera* leaf spray as compared with the control, in agreement with Yasmeen et al. [61], where

Moringa oleifera leaf extract (30 times diluted) enhanced the ascorbic acid content in wheat grown under high salt conditions.

Another essential aspect, predominant in leafy vegetables, is the accumulation of nitrates. Nitrate accumulation is an essential aspect of leafy vegetables; for this parameter, threshold values have been indicated above the maximum allowed, which are considered to be potentially dangerous for human health. Our results showed no significant differences among the treatments for this parameter. The samples treated with *Moringa oleifera* leaf extract, indeed, did not show an increase in nitrate content, which remained below the maximum allowed threshold according to the European Commission Regulation.

We plan to conduct future research to analyze whether the response is species-specific, to identify the optimal dose using a wider range of MLE treatments, and to identify the limit dose. The effect of repeated treatments over time should be the focus of future research to understand the influence of the additive effect on the nutraceutical properties of plants.

5. Conclusions

Overall, the results of this study showed that aqueous *Moringa oleifera* leaf extract (MLE) can modify the growth indices and some nutraceutical properties of radish baby leaves. In particular, a 1:30 dose was able to improve the growth (fresh biomass, total and unit leaf area, and land leaf dimension) and the total flavonoid and carotenoid contents. For other parameters, i.e., Fv/Fm ratio, chlorophyll *a* and carotenoid content, and TPC ascorbic acid content, the effect of MLE was evident for all doses adopted. The increases in the morphological parameters suggest that the most concentrated dose is the most effective. Further research is necessary to better individuate the action mechanisms and to define the dose and modality of *Moringa oleifera* applications. Nevertheless, this natural biostimulant appears to be a very promising application for increasing the sustainability of the production processes of studied radish baby leaves.

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