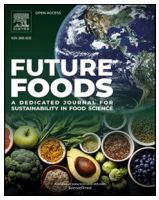
Future Foods 13 (2026) 100991

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo



Changes of nutraceutical traits in baby leaves of sprouting broccoli (*Brassica oleracea* L. var. *italica* Plenck) induced by *Moringa* spp. extracts

Donata Arena [a], Roberto Lo Scalzo [b,*], Hajer Ben Ammar [a], Valentina Picchi [b], Giulia Bianchi [b], Daniela Romano [a], Luca Ciccarello [a], Nicolas Al Achkar [a], Ferdinando Branca [a]

[a] *Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania 95123, Italy*
[b] *CREA Research Centre for Engineering and Agro-Food Processing, via G. Venezian 26, Milano 20133, Italy*

ARTICLE INFO

Keywords:
Brassica crops
Foliar application
Natural extracts
Antioxidant compounds
Glucomoringin
Isothiocyanate derivatives

ABSTRACT

Extracts of *Moringa* spp. are recognized for their healthy properties. The biostimulation of three *Moringa* extracts, *Moringa hildebrandtii* (M1), *Moringa stenopetala* (M2), and *Moringa oleifera* (M3) on baby leaves of a 'Broccolo nero' (*Brassica oleracea* L. var. *italica*) (BR) was studied. The trial compared plants treated with different *Moringa* extracts to a control group. Samples of *Moringa* and 'Broccolo nero' highlighted significant changes in treated plants. Significant increases in fresh weight were observed in BR treated with M3 (BR_M3) compared to the control ($p < 0.001$), while root angle was reduced in all treatments ($p < 0.0001$). BR_M1 enhanced the total phenolic content (459.29 ± 6.45 mg GAE/100 g DW) compared to control (358.00 ± 2.56 mg GAE/100 g DW). BR_M3 exhibited the highest antioxidant activity (126.35 ± 1.80 mg TE/100 g DW) showing an 11.9% increase compared to the control. The treatments with *Moringa* water extracts significantly decreased the content in indolic glucosinolates (i.e., neoglucobrassicin) and enhanced the aliphatic ones (i.e., glucoraphanin and glucoerucin), as along with their derived isothiocyanates. Notably, nitrile and isothiocyanate volatile derivatives varied among treatments, with BR_M2 showing a significant higher amount of phenyl propionitrile (404.48 ± 25.31 nmol/g DW) than the control (an increase of 57.6%). This increase was accompanied by a treatment-related decrease in the two main sulphides (dimethyl disulfide and dimethyl trisulfide). These findings indicate that treatments with leaf water extracts from different *Moringa* species induce significant changes in the phytochemical composition of baby leaves of *Brassica oleracea* var. *italica*.

1. Introduction

Sustainable agriculture has become as a key priority for addressing the challenges of food security and environmental conservation (Çakmakçı et al., 2023), especially for traditional cultivars and small-scale farming-system. In this context, biostimulants - substances capable of enhancing plant growth, improving nutritional quality, and modulating plant metabolism - are gaining increasing attention in both conventional and organic farming systems (Matthews et al., 2025; Mounaimi et al., 2024; Bulgari et al., 2015). Among them, plant-derived biostimulants (PDBs), including extracts obtained from leaves and other plant tissues, offer low-cost and environmentally sustainable tools for improving crop performance and quality (Li et al., 2024; Mandal et al., 2023; Pannacci et al., 2022; Zulfiqar et al., 2020). Nowadays, biostimulants are increasingly applied in the production of novel foods, such as sprouts, microgreens, and baby leaves, which represent a rapidly expanding market segment. These products, widely appreciated by chefs and consumers, owe their popularity to their attractive appearance, vibrant colors, and high content of bioactive compounds with recognized health benefits (Di Gioia et al., 2017). Baby leaves, in particular, are minimally processed, ready-to-eat leaf vegetables that meet consumer demand for fresh, convenient foods. Their appeal lies in the diversity of leaf shapes and colors, as well as their richness in bioactive compounds (Subhasree et al., 2009), which is largely preserved due to the minimal processing. Importantly, leaves harvested at early growth stages contain higher levels of vitamins (C, B9 and K1), carotenoids, phenolics, and antioxidants compared to mature leaves, as observed in spinach (Lester et al., 2010) and lettuce (Oh et al., 2010).

Although the cultivation cycle of baby leaves is still relatively short, production often requires several agronomic interventions, which raise interest in the application of biostimulant to improve sustainability, product quality, and plant tolerance to biotic and abiotic stress (Ruzzi

* Corresponding author.
E-mail address: roberto.loscalzo@crea.gov.it (R. Lo Scalzo).

https://doi.org/10.1016/j.fufo.2026.100991
Received 15 December 2025; Received in revised form 13 March 2026; Accepted 15 March 2026
Available online 16 March 2026

et al., 2024; Saporta et al., 2019; Kaluzewicz et al., 2018).

In addition to their potential effects on plant growth and stress resilience, biostimulants have also been associated with improvements in qualitative traits in several *Brassicaceae* species, particularly those related to nutritional profiles - such as antioxidant activity, ascorbic acid concentration, and photosynthetic pigments – as reported in broccoli (Halshoy et al., 2023), radish (Romano et al., 2022) and rocket (Di Mola et al., 2019). However, adverse effects have also been reported, including reductions in sucrose and total soluble sugars, which may negatively impact sensory attributes such as sweetness and overall flavor (Franzoni et al., 2021). These divergent responses highlight the role of genotypic variability in modulating biochemical outcomes following biostimulant application, as shown by differential reactions of two *Brassica oleracea* L. cultivars, 'Cavolo Laciniato Nero di Toscana' (leaf cabbage) and 'Broccolo nero' (broccoli), treated with *Moringa oleifera* Lam. extracts (Toscano et al., 2021).

Among the PDBs, *Moringa* leaf extracts have shown promising effects on plant growth, development, and nutritional quality.

Native to Asia and Africa, *Moringa* species, particularly *Moringa oleifera* Lam., *M. stenopetala* (Baker f.) Cufod, and *M. hildebrandtii* Engl., are widely cultivated in tropical and subtropical regions (Patil et al., 2022) and are known for their high concentrations of vitamins, minerals, and secondary metabolites such as polyphenols and antioxidants, which contribute to their use in nutrition, medicine, and agriculture (Kumar et al., 2022; Srivastava et al., 2023). Within this framework, *Moringa* extracts have been recognized for their potential as natural biostimulants, promoting plant growth and enhancing nutritional quality in various crops (Culver et al., 2012; Yuniati et al., 2022; Arif et al., 2023; Brockman and Brennan, 2017; Karthiga et al., 2022).

Current climate change trends are projected to expand the potential cultivation area for *Moringa*, given its physiological adaptation to high thermal regimes and drought tolerance. The sustainability of its cultivation, together with its outstanding phytochemical content, contributes to its use in nutrition, medicine, and agriculture (Kumar et al., 2022; Srivastava et al., 2023).

The increase in yield reported in the literature is related to improved plant physiology (Abd El-Mageed et al., 2017), suggesting that *Moringa* extracts can serve as an eco-friendly alternative to synthetic agrochemicals (Mashamaite et al., 2022; Khan et al., 2020). The use of aqueous extracts from *Moringa* as PDBs further enhances their potential for practical agricultural applications due to the simplicity, low cost, and environmental safety of the extraction process (Arif et al., 2023; Hoque et al., 2020; Admane et al., 2023).

Thanks to the high content of proteins, essential amino acids and minerals present in *Moringa* leaves, *Moringa* extracts are also considered a good alternative to inorganic fertilizers (Saini et al., 2016; Yasmeen et al., 2014).

Although the beneficial effects of *M. oleifera* as a biostimulant are relatively well-documented, other congeneric species remain largely underexplored. According to Arif et al. (2023), "among all *Moringa* species only *M. oleifera* has diverse functions" as a plant biostimulant. This limited knowledge underscores the need to investigate the potential of less-studied species. However, previous studies have evidenced substantial diversity among *Moringa* species in terms of their phenotypic traits and phytochemical composition (Hamada et al., 2024).

Hence, the rationale of the present study was that leaf extracts derived from different *Moringa* species may differentially affect the plant growth and the phytochemical profile of Brassica crops. Accordingly, this study aimed to evaluate the effects of leaf water extracts from *Moringa oleifera* Lam., *Moringa stenopetala* (Baker f.) Cufod and *Moringa hildebrandtii* Engl. on plant development and phytochemical traits of the Sicilian broccoli landrace 'Broccolo nero' (*Brassica oleracea* L. var. *italica* Plenck), assessed at the baby leaf growth stage.

2. Materials and methods

2.1. Moringa samples and leaf extract

The *Moringa* seeds were provided by the active genebank of the Department of Agriculture, Food and Environment (Di3A), University of Catania (UNICT). The seeds were sown in cellular trays with 40 cells, using one tray per species (M1 = *Moringa hildebrandtii* Engl., M2 = *Moringa stenopetala* (Baker f.), and M3 = *Moringa oleifera* Lam.). The trays were filled with Brill® Semina Bio organic substrate (Geotec, Italy). The substrate has a fine texture and is composed of blond peat (0–5 mm), Baltic brown peat (0–3 mm), and a simple non-composted plant amendment. According to the manufacturer, the pH is 5.5–6.5 and the electrical conductivity is <0.8 mS cm^{-1} (Sonneveld method, 1:1.5 v/v). The bulk density is 250–280 g L^{-1} and the water retention capacity is 5.5 g^{-1}. The trays were kept in a glasshouse in Catania (37°31′10″ N, 15°04′18″ E; 105 m a.s.l.) in September 2024. The environmental parameters were provided by the local weather station service of Sicilian region (SIAS). The mean temperature was 24.7 °C (Tmax 30.2 ± 3.4 °C; Tmin 19.1 ± 3.0 °C) (Fig. S1). The mean relative humidity was 60.6 ± 7.1%, and mean daily solar irradiance was 17.5 ± 3.0 MJ m^{-2} d^{-1}. The plantlets were grown without fertilizer or pesticide treatments. After one month, the plantlets were transplanted into a greenhouse located in Scicli (RG, Sicily, 36°43′25''N, 14°41′56″E; 37 m) in a single row, with 10 plants per species. Fully developed leaves, harvested 90 days after transplanting, were immediately frozen in liquid nitrogen, stored at −80 °C, then freeze-dried at −55 °C for one week (Heto PowerDry LL3000), and finally finely ground using an IKA-A10 mill (IKA-Werke GmbH & Co. KG, Staufen, Germany). The *Moringa* freeze-dried powders, were stored at −20 °C and subsequently analyzed for the levels of ascorbic acid (AsA), total phenols index (TPC), antioxidant capacity by DPPH, glucomoringin and its desulfo-derivative, moringin, by derivatization and HPLC assay, isobutyl isothiocyanate by GC–MS, (*E*)-2-hexenal, and methyl salicylate as the main VOCs, with the same methods used for the assays on 'Broccolo nero' (see after). The *Moringa* leaf extract (M1, M2, M3) was prepared following the method described by (Pervez et al., 2017), with minor modifications. 50 *g* of the freeze-dried powder was soaked into 200 mL of distilled water and macerated at 25 °C for 48 h on an orbital shaker. After filtration using Whatman qualitative filter paper (Grade 4, 90 mm diameter), the resulting solution was considered a 25% (w/v) stock extract. The dilution ratio (1:30, v/v) was selected to obtain a final concentration of 200 mg L^{-1}, in alignment with (Pervez et al., 2017) protocol.

2.2. Plant materials of 'Broccolo nero'

Seeds of the Sicilian sprouting broccoli 'Broccolo nero' (*Brassica oleracea* L. var. *italica* Plenck, UNICT 4939, BR 354), from the active gene bank of the Di3A, were sown in January 2025 in cellular trays filled with organic Brill® Semina Bio substrate (Geotec, Italy). The trays were placed in a greenhouse in Catania (Sicily, 37°31′10″ N, 15°04′18″ E; 105 m a.s.l.) under natural light (2.65–12.91 MJ m^{-2} d^{-1}) and with mean temperature of 11.7 °C (Tmax 16.1 ± 2.3 °C; Tmin 7.2 ± 1.1 °C). The environmental parameters were provided by the local weather station service of the Sicilian region (SIAS). A completely randomized experimental design was adopted. Specifically, one seed was placed per cell, with three replicates per treatment, each consisting of 300 plants (M1, M2, M3). Foliar applications of the *Moringa* leaf extracts were performed at 15, 30, and 43 days after sowing, following the methodology described by Toscano et al. (2021). Control plants were sprayed with distilled water.

2.3. Plant of 'Broccolo nero': morphometric characterization

The plantlets, following treatment after 43 days, were harvested and carefully cleaned to remove adhering substrate from the roots before

phenotyping. The morphometric measurements were performed by scanning whole seedlings (shoots and roots) with an Epson Perfection V850 Pro scanner (SEIKO EPSON CORPORATION, Nagano-ken, Japan) and analyzing images with WinFolia Regular 2020 software. The fully developed leaves were measured as entire leaves (including petiole and midrib). The main morphometric parameters registered were: fresh weight of 10 individuals (FW), number of leaves (NL), leaf length (LL), leaf width (LW), leaf area (LA), stem length (SL), root length (RL), root angle (RA). After phenotyping, the leaves were deeply frozen at −80 °C, freeze-dried, finely ground by using an IKA-A10 mill (IKA-Werke GmbH & Co.KG, Staufen, Germany) and utilized for the biochemical analysis in triplicate, with different individual plant per sample ($n = 3$).

2.4. Phytochemicals analysis

2.4.1. Soluble solids content (SSC)

SSC was measured in accordance with the protocol by Lamb (1972), with slight modifications. An aqueous extract was prepared by adding 0.5 *g* of freeze-dried powder in 5 mL of distilled water, followed by centrifugation at 1000 *g*. The resulting supernatant was analyzed using a digital bench-top refractometer (Bellingham-Stanley, model RFM 91), and results were expressed in °Brix on a dry weight (DW).

2.4.2. Organic acid profile

The organic acid content was analyzed by HPLC on a Jasco system (Cremella, Italy), following the rationale by Jayaprakasha et al. (2003), adapted and validated in a previous work on Brassica samples (Bianchi et al., 2024). For the extraction, 3 mL of distilled water was added to 100 mg of freeze-dried sample, vortexed at 1000 rpm for 5 min, centrifuged at 10,000 g for 5 min at 4 °C, and the supernatant was filtered by a 0.45 μm nylon membrane. Major detected organic acids, such as citric (retention time 9.3 min), malic (10.6 min), pyruvic (11.7 min), and fumaric (15.8 min), were separated using a Repromer H^+ column (300 × 8 mm, 9 μm; Dr. Maisch, Germany) at 56 °C. The mobile phase consisted of 23 mM H_2SO_4 at 0.5 mL/min, and detection was achieved at 214 nm. Calibration curves using standard solutions were created within concentration ranges of 0.05–1.50 mg/mL for citric, malic, and pyruvic acids, while an interval between 0.002–0.02 mg/mL was used for fumaric acid. Data were reported as mg per 100 *g* DW.

2.4.3. Ascorbic acid (AsA)

The AsA was determined following the procedure by Picchi et al. (2012). 50 mg of freeze-dried powder was homogenized in 1.5 mL of 6% metaphosphoric acid under dark conditions, then centrifuged at 10,000 g for 5 min at 4 °C. The supernatant was filtered through a 0.45 μm nylon membrane and immediately subjected to High Performance Liquid Chromatography (HPLC) analysis. The chromatographic separation was performed using a Supelco Ascentis Phenyl column (250 × 4 mm i.d.) maintained at 44 °C, with 0.02 M orthophosphoric acid as the mobile phase at a flow rate of 0.6 mL/min. The injections of 20 μL were analyzed with UV detection at 254 nm. AsA eluted at 5.9 min. Quantification was based on a calibration curve ($y = 36447x$, $R^2 = 0.997$, range: 2–100 μg/mL) and results were expressed as mg AsA per 100 g of dry weight (DW).

2.4.4. Total phenolic content (TPC)

The TPC was performed following the Folin-Ciocalteu (FC) colorimetric assay, with some modifications (Singleton et al., 1999). The extraction was carried out using EtOH/0.06 N HCl mixture (1:1 v/v), applied in a 10:1 ratio relative to the freeze-dried sample powder. 50 μL of the extract was combined with 3 mL of deionized water and 0.5 mL of FC reagent, then left at room temperature for 5 min. Subsequently, 1 mL of a 10% (w/v) sodium carbonate solution was added, and the reaction mixture was kept in the dark at room temperature for 1 h. The absorbance was recorded at 730 nm. The quantification was performed by a calibration curve generated with standard solutions of gallic acid ($y = 1.84x$, $R^2 = 0.994$; concentration range: 0.003–0.330 mg/mL). The results were expressed as mg gallic acid equivalents (GAE) per 100 *g* of dry weight (DW). Method validation followed the recommendations by Everette et al. (2010), ensuring the exclusion of interfering reducing agents such as ascorbic acid (AsA) and sulfur-containing compounds.

2.4.5. DPPH radical scavenging

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical quenching was evaluated in accordance with the method described by Brand-Williams et al. (1995). 30 mg of freeze-dried powder was extracted with 1 mL of an EtOH/0.02 N HCl (1:1 v/v) solution, homogenized, and centrifuged at 25,000 *g* for 5 min at 4 °C. The control solution contained 0.3 mL methanol, 0.1 mL extracting solution, and 0.1 mL of a 0.5 mM ethanolic DPPH solution. The reaction mixtures were vortexed and transferred to 50 μL capillary tubes for EPR analysis. The measurements were conducted using a MiniScope MS200 EPR spectrometer (Magnettech, Berlin, Germany) and the parameters were: center field 3350 G, scan width 50 G, scan time 120 s, modulation amplitude 2000 mG, microwave attenuation 7 dB, and receiver gain 7 × 10. Spectra were recorded after 1 min of reaction at 25 °C. The results were expressed as mg Trolox equivalents (TE) per 100 g of dry weight (DW).

2.4.6. Glucosinolates determination (GLSs)

The GLSs extraction was carried out following the ISO 9167-1 (1992) method, with slight modifications. Briefly, 200 mg of freeze-dried material were extracted with 5 mL of 70% methanol and incubated at 70 °C for 10 min. The mixture was then centrifuged at 4.000 rpm for 20 min using a Thermo Scientific IEC CL10 centrifuge (France). GLSs desulfation was carried out using a gravity-flow column consisting of a vertically positioned glass Pasteur pipette, packed with a plug of glass wool and 0.5 mL of DEAE Sephadex A-25 resin. The resin had been pre-equilibrated with 0.02 M acetate–pyridine buffer. 2 mL of the clarified extract were applied to the column, followed by 0.5 mL of 0.02 M pyridine solution to maintain a basic pH. For enzymatic desulfation, 15 μL of sulfatase (E.C. 3.1.6.1, Type H1 from *Helix pomatia*, Sigma-Aldrich, St. Louis, MO, USA; CAS No S9626–5KU) were added. The columns were incubated overnight at room temperature. The desulfo-GLSs were then eluted with 1.5 mL of ultrapure water and analyzing by HPLC using an Agilent 1200 Series HPLC system equipped with a diode array detector (DAD) set at 229 nm and a Kinetex C18 column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of solvent A (ultrapure water) and solvent B (acetonitrile:water, 20:80, v/v). The gradient program was as follows: 1% B at 1 min, increased to 99% B by 21 min, held at 99% B until 24 min, returned to 1% B at 29 min, and maintained until 39 min, with a total runtime of 39 min. The flow rate was set at 0.8 mL min^{-1}, and the injection volume was 20 μL. The individual glucosinolates were identified through the retention times (RT) and UV spectra compared to external standards obtained from ChromaDex (Santa Ana, CA, USA): sinigrin (SIN), glucoraphanin (GRA) and neoglucobrassicin (NGBS). Glucomoringin was quantified following the European Commission Regulation (EEC) No 1864/90 of 29 June 1990, which amends Regulation (EEC) No 1470/68 concerning sampling and analytical methods for oilseeds. Glucomoringin levels were calculated as sinigrin equivalents. The results were expressed as micromoles per gram of dry weight (μmol g^{-1} DW).

2.4.7. Analysis of sulforaphane (SRA), moringin and erucin (ERU)

For the analysis of SRA, moringin and erucin, the extraction and purification procedure followed the method described by Pilipczuk et al. (2015). 50 mg of lyophilized powder were incubated with 1.5 mL of 0.1 M sodium phosphate buffer (pH 7.4) at 37 °C for 3 h. The mixture was then vortexed and centrifuged at 10,000 g for 15 min at 4 °C. The supernatant was passed through Bakerbond SPE C18 500-mg cartridges, preconditioned with 3 mL of methanol and 6 mL of water. The stationary phase was washed with 1 mL of sodium phosphate buffer (0.01 M, pH 6.0) and the retained SRA and other ITC were eluted with 1 mL of

methanol. The eluate was filtered using 0.45 μm nylon filters.

Briefly, SRA, moringin and ERU were derivatized with N-acetyl cysteine in sodium bicarbonate (Pilipczuk et al., 2017) and detected at 272 nm. HPLC analysis was then performed following the method described by Lo Scalzo et al. (2024): briefly, the separation was carried out using a Kinetex C18 column (250 mm × 4.6 mm i.d., 5 μm). The flow rate was 0.6 ml/min, the injection volume was 10 μL, and the column temperature was maintained at 43 °C. The mobile phase consisted of solvent A (MeOH 10% in water, with 0.5% HCOOH) and solvent B (CH_3CN/MeOH 9:1, with HCOOH 0.5%). The gradient was as follows: 90% solvent A (5 min isocratic), decreased to 50% solvent A over 20 min, maintained for 5 min, then returned to 90% in 10 min. In these conditions, SRA had a retention time of 9.8 min, moringin eluted at 13.2 min and erucin at 17.8 min. Peak identification was based on literature data and on the comparison of retention times with those of pure reference compounds treated identically to the samples.

2.4.8. Myrosinase activity

Myrosinase activity was determined based on the decomposition of sinigrin by monitoring the decrease in absorbance of the reaction mixture at 230 nm, using a UV–VIS spectrophotometer Jasco model (Cremella, Italy). Aqueous extracts were prepared by weighing 50 mg of lyophilized powder into 1.5 mL of deionized water, followed by vortexing and incubation at 37 °C for 5 h. The mixture was then centrifuged at 10,000 *g* for 15 min. For the determination of myrosinase activity, 200 μL of the extract was added to 1900 μL of 80 mM NaCl and 200 μL of 2.5 mM sinigrin. The sinigrin hydrolysis reaction was carried out at 37 °C for 30 min. Myrosinase activity was calculated as described by Piekarska et al. (2013), using a molar extinction coefficient of 7500 for sinigrin.

2.4.9. Volatile compounds profile

The volatile compounds were extracted using headspace solid-phase microextraction (HS-SPME) and analysed by GC–MS (Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, Italy). Each sample consisted of 200 mg of freeze-dried plant material added to 10 mL of deionised water in a 20 mL glass vial, which was then kept for 4 h at 60 °C to enhance the enzymatic and chemical rearrangements of native sulphur compounds, primarily isothiocyanates and sulphides (Matusheski et al., 2004). Each vial was then closed with an aluminium/silicone-PTFE septum after the addition of 2 g of NaCl. Samples extraction was performed using a 50/30 μm DVB–PDMS–CAR fiber (Supelco, Bellefonte, PA, USA) at 80 °C for 20 min. Volatiles desorption was obtained by exposing the fiber in the GC injector at 200 °C for 5 min.

GC–MS analyses were carried out with an Agilent 6890 N GC connected to an Agilent 5973 mass spectrometer and equipped with a DB-1 column (60 *m* × 0.25 mm I.D., film thickness 0.25 μm), in splitless mode, using He as carrier gas (flow 1 mL/min). The column temperature program was: 40 °C for 5 min, 3 °C/min to 180 °C, 8 °C/min to 220 °C for 5 min (duration: 61.7 min). Injector and detector temperatures were 200 and 230 °C, respectively; interconnecting line temperature was 200 °C. The MS settings were as follows: filament voltage, 70 eV; scan range, 39–450 amu; scan speed, 1.4 scan/s. The compounds were identified by comparing their mass spectra with those stored in the Wiley 7n library and analysing authentic standards. Quantification was achieved using a calibration curve obtained from the analysis of standard solutions with known concentration. Concentrations were expressed as nmol/g DW.

2.5. Data analysis

The significance of differences among treatments with different *Moringa* extracts was assessed using one-way analysis of variance (ANOVA). Dunnett's multiple comparisons test was applied to evaluate differences for morphometric traits using R version 4.4.2. (R Core Team, 2024). For the biochemical analyses, ANOVA followed by Tukey's HSD test ($p < 0.05$) was performed using CoStat version 6.451 (CoHort Software, Birmingham, UK). The bar plot, correlation and Principal Component Analysis (PCA) were carried out using the corrplot and ggplot2 packages in R version 4.4.2. Data were given as mean ± standard error ($n = 3$).

3. Results

3.1. Biochemical traits of Moringa leaf extract

3.1.1. Antioxidant traits

A significant variation of the ascorbic acid (AsA) content was detected among the studied *Moringa* species (Table 1). M1 showed the highest concentration of AsA, reaching 1540.1 ± 29.67 mg/100 g DW, which was significantly greater than for the other species ($p < 0.001$); M2 contained a moderate amount (799.5 ± 1.8 mg/100 g DW), while M3 had the lowest one (385.3 ± 2.9 mg/100 g DW). These data indicate species-related differences in AsA, with M1 standing out as the most enriched for this important antioxidant compound.

As regards to TPC, the data evidenced that M1 and M2 exhibited similarly high values (2123.6 ± 38.73 and 2095.2 ± 17.9 mg GAE/100 g DW, respectively), with no significant difference between them (Table 1). In contrast, M3 displayed a markedly lower TPC level (1715.1 ± 66.1 mg GAE/100 g DW). These results suggest a higher phenolic biosynthetic potential in M1 and M2 compared to M3, possibly reflecting species-specific metabolic regulation or adaptation to differing environmental conditions.

The antioxidant capacity of *Moringa* leaves, measured by DPPH radical scavenging activity and expressed as mg Trolox equivalents (TE) per 100 g DW, also varied significantly among the three species (Table 1). M1 exhibited the highest value (575.9 ± 29.72 mg TE/100 g DW), followed by M2 (417.9 ± 17.5 mg TE/100 g DW), with no significant difference between them. In contrast, M3 showed a significantly lower antioxidant activity (218.3 ± 38.6 mg TE/100 g DW). These findings highlight the stronger antioxidant profiles of M1 and M2 compared with M3 and are consistent with their higher AsA and total phenolic contents.

3.1.2. Sulphur active compounds (glucosinolates and ITC) and VOCs profile

Regarding the GLSs profile and content, glucomoringin was the only GLS detected as a desulfo derivative with the applied analytical approach. Its concentration varied among the assayed species, ranging from 36.54 ± 3.22 μmol g^{-1} DW for M2 to 55.70 ± 11.87 μmol g^{-1} DW for M3, while M1 displayed an intermediate level of 46.08 ± 17.28 μmol g^{-1} DW (Table 2). These findings reveal a notable interspecific variation for glucomoringin content, which may be linked to genetic or environmental factors influencing its biosynthesis. As expected, moringin - the corresponding isothiocyanate (ITC) derived from the hydrolysis of the parent glucosinolate - showed a parallel trend, with the lowest concentration for M2 (3.76 ± 0.34 μmol g^{-1} DW), compared with 8.44 ± 0.37 and 6.99 ± 0.07 μmol g^{-1} DW for M1 and M3, respectively (Table 2).

Data on myrosinase activity in the different *Moringa* species are shown in Table 2. Among the species, M1 exhibited the highest activity

Table 1
Antioxidant profile of the three tested *Moringa* species. M1 = *Moringa hildebrandtii* Engl., M2 = *Moringa stenopetala* (Baker f.), and M3 = *Moringa oleifera* Lam. Data are expressed as mean ± standard error.

	AsA (mg/100 g DW)	Folin index (mg GAE/100 g DW)	DPPH index (mg TE/100 g DW)
M1	1540.1 ± 29.67 a	2123.6 ± 38.73 a	575.9 ± 29.72 a
M2	799.5 ± 1.8 b	2095.2 ± 17.9 a	417.9 ± 17.5 b
M3	385.3 ± 2.9 c	1715.1 ± 66.1 b	218.3 ± 38.6 c
	***	***	***

Differences among means (***: $p < 0.001$) are indicated according to Tukey's post-hoc test with different letters.

Table 2
Sulphur active compounds and main VOCs of the three tested species. M1 = *Moringa hildebrandtii* Engl., M2 = *Moringa stenopetala* (Baker f.), and M3 = *Moringa oleifera* Lam. Data are expressed as mean ± standard error.

	Glucomoringin (μmol/g DW)	Moringin (μmol/g DW)	Myrosinase activity (U/g DW)	ITC yield (%)
M1	46.08 ± 17.28	8.44 ± 0.37 b	5.27 ± 4.53	18.3
M2	36.54 ± 3.22	3.76 ± 0.34 a	2.83 ± 1.89	10.3
M3	55.70 ± 11.87	6.99 ± 0.07 c	2.82 ± 0.73	12.5
	n.s.	***	n.s.	

	(*E*)-2-hexenal (nmol/g DW)	**Isobutyl ITC (nmol/g DW)**	**Methyl salicylate (nmol/g DW)**
M1	9.98 ± 1.30 b	9.33 ± 1.50 a	0.27 ± 0.07
M2	20.03 ± 0.90 a	0.23 ± 0.02 b	nd
M3	7.95 ± 0.30 c	0.50 ± 0.07 b	nd
	***	***	

* ITC yield (%) indicates the ratio of the released ITC to the initial glucosinolate concentration.
Differences among means (***: $p < 0.001$) are indicated according to Tukey's post-hoc test with different.
letters.

(5.27 ± 2.53 U/g DW), followed by M2 and M3, which displayed comparable levels (2.83 ± 1.89 and 2.82 ± 0.73 U/g DW, respectively). However, the differences among them were not statistically significant. Interestingly, the ratio of the released ITCs to the initial glucosinolate concentration, expressed as ITC yield (%), was highest for M1 - almost twice that observed for M2 and M3 - consistent with the greater myrosinase activity measured in this species.

The volatile compounds profile of *Moringa* freeze-dried leaves, as determined by GC–MS, revealed distinct differences for the accumulation of key aroma constituents (Table 2), focusing on three representative VOC classes. Specifically, M2 showed the highest value of (*E*)-2 hexenal (20.03 ± 0.90 nmol/g DW), whereas both M1 and M3 showed substantially lower amounts (9.98 ± 1.30 and 7.95 ± 0.30 nmol/g DW, respectively). On the other hand, the content of the isobutyl ITC - a volatile product of glucosinolate hydrolysis - was significantly higher for M1 (9.33 ± 1.50 nmol/g DW), but occurred only in trace for M2 (0.23 ± 0.02 nmol/g DW) and for M3 (0.50 ± 0.07 nmol/g DW). Notably, methyl salicylate, a well-known signaling and defense-related metabolite, was exclusively detected in M1 (0.27 ± 0.07 nmol/g DW).

3.2. The effect of the Moringa extracts on baby leaves of 'Broccolo nero'

3.2.1. Morphometric traits

The results of Dunnett's multiple comparisons test indicate the *Moring* spp. treatment applied to the baby leaves of 'Broccolo nero' did not differ significantly among them (M1, M2, and M3) for most of the measured descriptors, with the exception of fresh weight, leaf area, and root angle (Table 3). The fresh weight of treated plants differed significantly from the control ($p < 0.001$), with the most pronounced increment observed for M3 (Table 3). The morphological descriptors, including leaf number, leaf length and width, and stem length, were not affected by the treatment, as no significant differences were observed compared to the control group ($p > 0.05$). However, the root angle (RA, Table 3) was significantly reduced in all treated samples ($p < 0.0001$), with the most pronounced decrease observed in the comparison between control and BR_M2 (Mean Diff. = 19.00, 95% CI: 15.11 to 22.89). Conversely, leaf area significantly increased for BR_M3 (Mean Diff. = −6.485, 95% CI: −10.38 to −2.590, $p = 0.0003$), indicating that the treatment may have affected the leaf expansion by M3.

3.2.2. Biochemical profile

3.2.2.1. Comparative effects of three Moringa species extracts on soluble solid content and organic acids profile. For soluble solid content (°Brix/

Table 3
The effect of *Moringa* leaf extracts on the morphometric traits of BR baby leaves against samples treated with distilled water (control). M1= *Moringa hildebrandtii* Engl., M2= *Moringa stenopetala* (Baker f.) Cufod, M3= *Moringa oleifera* Lam.

Mean ± standard error (SE, n = 3)	Dunnett's test	Mean Diff.	95.00% CI of diff.	Significance	*p-value*
FW					
19.66±1.11	Control (BR_C)				
21.60±0.69	BR_M1	1.94	−0.540 to 4.420	n.s.	0.2984
23.41±0.98	BR_M2	3.75	1.289 to 6.211	*	0.0139
28.72±0.70	BR_M3	9.06	6.590 to 11.530	***	<0.001
NL					
4.20±0.13	Control (BR_C)				
3.50±0.22	BR_M1	0.7000	−3.195 to 4.595	n.s.	0.9518
4.50±0.17	BR_M2	−0.3000	−4.195 to 3.595	n.s.	0.9958
4.40±0.16	BR_M3	−0.2000	−4.095 to 3.695	n.s.	0.9987
LL					
3.68±0.16	Control (BR_C)				
3.80±0.10	BR_M1	−0.1240	−4.019 to 3.771	n.s.	0.9996
4.08±0.10	BR_M2	−0.4040	−4.299 to 3.491	n.s.	0.9899
4.75±0.19	BR_M3	−1.073	−4.968 to 2.822	n.s.	0.8531
LW					
2.52±0.13	Control (BR_C)				
2.63±0.09	BR_M1	−0.1130	−4.008 to 3.782	n.s.	0.9997
2.82±0.07	BR_M2	−0.3010	−4.196 to 3.594	n.s.	0.9957
3.54±0.15	BR_M3	−1.018	−4.913 to 2.877	n.s.	0.8710
LA					
7.07±0.49	Control (BR_C)				
7.26±0.25	BR_M1	−0.1860	−4.081 to 3.709	n.s.	0.9990
8.79±0.25	BR_M2	−1.719	−5.614 to 2.176	n.s.	0.5952
13.55±0.91	BR_M3	−6.485	−10.38 to −2.590	***	0.0003
SL					
9.42±0.53	Control (BR_C)				
8.90±0.52	BR_M1	0.5230	−3.372 to 4.418	n.s.	0.9787
11.04±0.46	BR_M2	−1.619	−5.514 to 2.276	n.s.	0.6378
9.91±0.38	BR_M3	−0.4860	−4.381 to 3.409	n.s.	0.9827
RL					
12.40±1.26	Control (BR_C)				
10.28±0.69	BR_M1	2.119	−1.776 to 6.014	n.s.	0.4319
12.61±0.60	BR_M2	−0.2100	−4.105 to 3.685	n.s.	0.9985
15.63±1.30	BR_M3	−3.231	−7.126 to 0.6638	n.s.	0.1277
RA					
69.50±1.38	Control (BR_C)				
60.00±2.69	BR_M1	9.500	5.605 to 13.39	***	<0.0001

(*continued on next page*)

Table 3 (*continued*)

Mean ± standard error (SE, n = 3)	Dunnett's test	Mean Diff.	95.00% CI of diff.	Significance	*p-value*
50.50±4.31	BR_M2	19.00	15.11 to 22.89	***	<0.0001
54.00±3.06	BR_M3	15.50	11.61 to 19.39	***	<0.0001

FW= fresh weight of 10 individuals (g), NL= number of leaves (n), LL= leaf length (cm), LW= leaf width (cm), LA= leaf area (cm^2), SL= stem length (cm), RL= root length (cm), RA= root angle (°). n.s. not significant; *** significant at $p < 0.001$.

DW), no significant variations were observed among all *Moringa* species utilized (Fig. 1A). Values ranged from 24.40 ± 1.32 (BR_C) to 26.58 ± 0.91 (BR_M3).

Concerning citrate content (Fig. 1B), BR_M1 showed lower values than the control (1000.28 ± 50.69 vs 1169.61 ± 34.15 mg/100 g DW), while BR_M2 (1406.32 ± 120.70 mg/100 g DW) and BR_M3 (1259.00 ± 44.42 mg/100 g DW) were comparable to BR_C.

For the pyruvate content any significant differences were observed among treatments (Fig. 1C). However, the pyruvate content of the baby leaves showed numerical variations, ranging from 297.55 ± 29.52 mg/100 g DW for BR_C to 591.92 ± 39.19 mg/100 g DW for BR_M2.

3.2.2.2. Comparative effects of three Moringa spp. extracts on the antioxidant profile (AsA, TPC and DPPH index). The AsA content varied from 83.48 ± 3.60 mg/100 g DW for BR_C to 106.37 ± 6.47 mg/100 g DW for BR_M2 (Fig. 2A).

For TPC (Fig. 2B), significant differences were observed between the untreated control and the BR samples treated with different *Moringa* species. In particular, BR_M1 showed the highest TPC level (459.29 ± 6.45 mg GAE/100 g DW), followed by BR_M3 (422.40 ± 0.41 mg GAE/100 g DW) and BR_M2 (402.49 ± 0.47 mg GAE/100 g DW), all of which were significantly higher than BR_C (358.00 ± 2.56 mg GAE/100 g DW). These findings indicate a clear phenolic enrichment resulting from *Moringa* supplementation, particularly by M1.

Regarding DPPH radical scavenging activity (Fig. 2C), BR_M3 showed the highest antioxidant capacity (126.35 ± 1.80 mg TE/100 g DW), but it did not differ significantly from the control (112.90 ± 4.29 mg TE/100 g DW). Conversely, BR_M2 showed significantly lower values (75.81 ± 4.51 mg TE/100 g DW). BR_M1 was comparable to the control.

3.2.2.3. Comparative effects of three Moringa species extracts on GLSs, ITC composition, VOCs and myrosinase activity. The amount of the main GLS compound, neoglucobrassicin (NGBS), decreased significantly by the all moringa extracts applied, as indicated by the highest content observed for BR_C (53.3 μmol/g DW) (Fig. 3). The other two main GLSs detected in the baby leaves of 'Broccolo nero' were glucoraphanin (GRA) and glucoerucin (GER), along with their respective hydrolysis products, the isothiocyanates sulphorafane (SRA) and erucin (ERU). Their content showed an opposite trend compared to NGBS one, as we observed its increment in treated samples in comparison to the control. This pattern is particularly evident for GRA and SRA (Fig. 4A and C), both reaching the highest value in BR_M2, while ERU exhibited its highest content in BR_M1 (Fig. 4D). On the other hand, any significant differences were not observed in GER content, due to the high variability found in samples BR_M2 and BR_M3 (Fig. 4B).

The myrosinase activity (Fig. 5) ranged from 6.16 U/g DW (BR_M1) to 12.51 U/g DW (BR_M2). The treatments were statistically comparable to the control (BR_C: 7.78 U/g DW). BR_M1 and BR_M3 showed similar values (6.16 and 6.47 U/g DW, respectively).

Regarding to the volatile compounds profile (Fig. 6A), the isothiocyanates 3-butenyl ITC (3BTC) (2.47 ± 1.32 nmol/g DW) and benzyl ITC (BITC) (0.12 ± 0.01 nmol/g DW) were detected exclusively for BR_M3, and they were not detected for BR_C, BR_M1, and BR_M2. Moreover, beta-phenethyl ITC (PEITC) was detected in all samples except for BR_M1. The highest concentration was observed for BR_M3 (0.58 ± 0.02 nmol/g DW), followed by BR_C (0.22 ± 0.01 nmol/g DW) and BR_M2 (0.22 ± 0.04 nmol/g DW).

With regards to the sulfur volatiles (Fig. 6B), BR_C exhibited the highest content of dimethyl disulfide (DMDS) (127.91 ± 7.77 nmol/g DW), followed by BR_M2 (69.65± 17.20 nmol/g DW) and BR_M1 (51.93 ± 4.58 nmol/g DW), whereas BR_M3 showed no detectable levels. A similar trend was observed for dimethyl trisulfide (DMTS), with the control group showing the highest values (104.66 ± 4.74 nmol/g DW), followed by BR_M2 (61.39 ± 11.11 nmol/g DW), BR_M1 (44.60 ± 6.63 nmol/g DW), and lower amount were detected for BR_M3 (12.53 ± 1.76 nmol/g DW). For the aldehyde (*E*)-2-hexenal (E2H), commonly linked to lipid peroxidation and green leaf volatiles, BR_M2 showed the highest content (25.16 ± 2.79 nmol/g DW), followed by BR_M1 (14.34 ± 2.27 nmol/g DW) and BR_C (12.32 ± 0.36 nmol/g DW), while this compound was slightly detected for BR_M3. The indole (IND) was abundant for BR_M3 (82.00 ± 6.58 nmol/g DW) and BR_M2 (80.12 ± 6.61 nmol/g DW), followed by the BR_C (62.98 ± 0.15 nmol/g DW) and significantly low in BR_M1 (34.70 ± 3.51 nmol/g DW); that suggests the treatment activate a specific modulation of the indole biosynthesis pathway consequent to the degradation of native indole GLSs. Finally, for the nitrile derivatives, phenyl propionitrile (PPN) was significantly much most abundant for BR_M2 (404.48 ± 25.31 nmol/g DW), followed by BR_M3 (173.05 ± 4.66 nmol/g DW), BR_C (171.89 ± 5.08 nmol/g DW), and BR_M1 (126.78 ± 35.83 nmol/g DW). These results mainly indicate an enhancement of nitrile formation pathways in the case of specific *Moringa* treatments, especially by M2, and a concomitant decrease of the sulphides content (DMDS and DMTS).

4. Discussion

The biochemical profiles of leaf extracts from three *Moringa* species revealed marked interspecific variations for the key phytochemical and

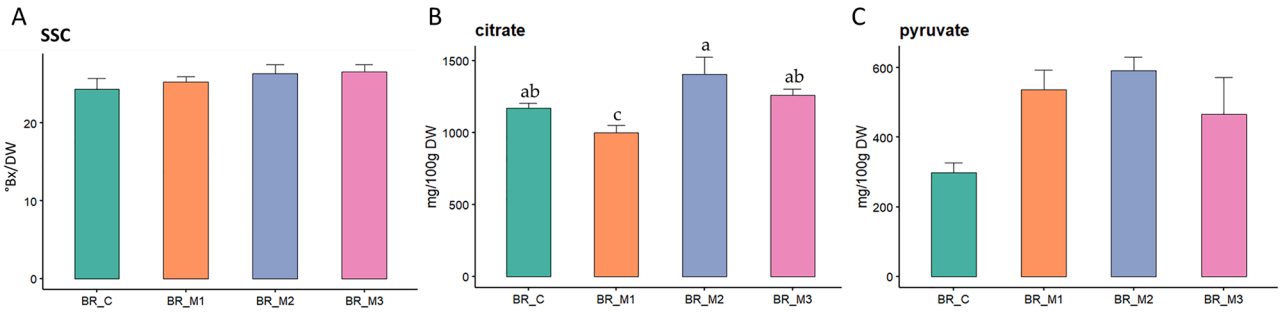


Fig. 1. Soluble solid (A), citrate (B) and pyruvate (C) content of BR baby leaves sprayed with three different extracts of *Moringa* leaves. *C*= control, M1= *Moringa hildebrandtii* Engl., M2= *Moringa stenopetala* (Baker f.) Cufod, M3= *Moringa oleifera* Lam. Values are expressed as means ± standard error (SE, n = 3).

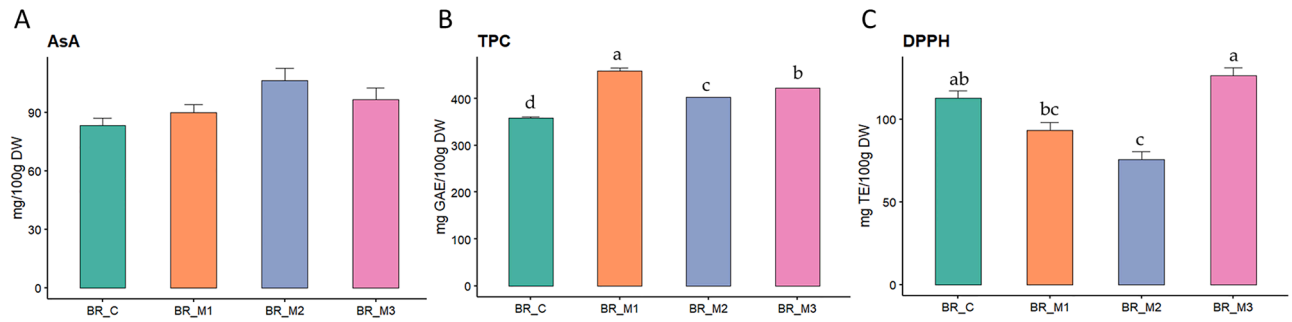


Fig. 2. Effect of the three different *Moringa* extract on AsA (A), TPC (B) and DPPH index (C) of BR baby leaves. *C*= control, M1= *Moringa hildebrandtii* Engl., M2= *Moringa stenopetala* (Baker f.) Cufod, M3= *Moringa oleifera* Lam. Values are expressed as means ± standard error (SE, $n = 3$). Different letters indicate statistically significant differences as determined by Tukey's HSD test ($p < 0.05$).

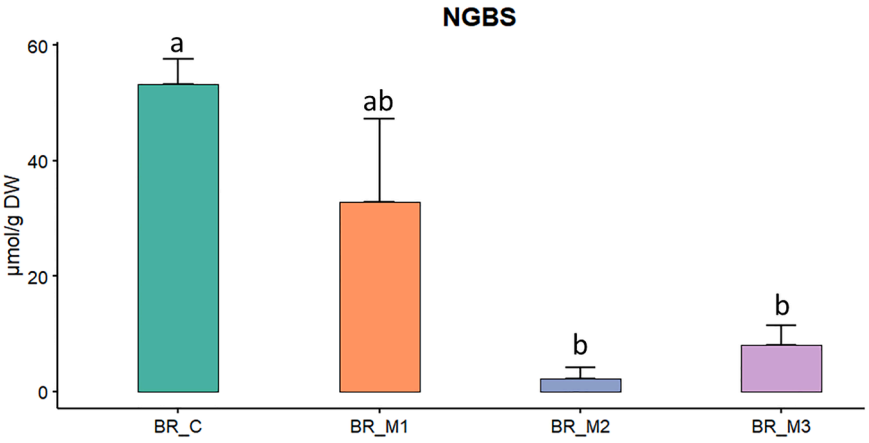


Fig. 3. Levels of neoglucobrassicin (NGBS), the main glucosinolate detected in 'Broccolo nero' in control (BR_C) and in the treated plants with different *Moringa* leaves' extracts (BR_M1, BR_M2 and BR_M3). BR_M1= *Moringa hildebrandtii* Engl., BR_M2= *Moringa stenopetala* (Baker f.) Cufod, BR_M3= *Moringa oleifera* Lam. Values are expressed as means ± standard error (SE, $n = 3$).

enzymatic traits, providing valuable insights for their uses as bioactive inputs for sustainable vegetable production. The data acquired support the interpretation of how the biostimulants influence the physiological and the metabolic responses of the baby leaves of 'Broccolo nero' (*Brassica oleracea* var. *italica*). Notably, the three *Moringa* species used for this experiment trials stimulated distinct effects on the biochemical traits of the 'Broccolo nero' baby leaves.

Ascorbic acid and phenols levels varied significantly among the *Moringa* spp. extracts, contributing further to their antioxidant capacity; M1 showed nearly twice of its concentration for M2. Vitamin C plays an important role for the cellular redox homeostasis, for the regeneration of other antioxidants, and for the modulation of the stress-responsive signaling networks (Akram et al., 2017; Ahmed et al., 2016). The amount of the AsA in the samples of *Moringa oleifera* (Table 1) is in accordance with previous reports, ranging from 50 to 150 mg/100 g FW (Thippeswamy et al., 2020;Khondoker et al., 2016) meaning around 650–1800 mg/100 g DW (Madukwe et al., 2013). The high AsA content observed for M1 enhances its antioxidant profile and supports its potential use for biostimulant formulations aimed at enhancing plant stress resilience (Gopalakrishnan et al., 2016). The levels of phenols measured in this study averaged approximately about 2000 mg/100 g DW. Previous reports describe highly variable amounts, limited to samples of *Moringa oleifera*, ranging from about 2000 to 4000 mg/100 g DW (Sreelatha and Padma, 2009; Vongsak et al., 2013). Interestingly, very similar levels of total phenols and AsA were found for *M. oleifera* and *M. stenopetala* samples of leaves in a previous comparative study (Hamada et al., 2024). Generally, these findings evidenced a clear agreement with the data presented here, further confirming the strong correlation with antioxidant capacity.

Samples M1 and M2 exhibited significantly higher total phenolic content (TPC) and DPPH radical scavenging activity compared to M3, highlighting their antioxidant potential. The strong correlation between AsA, TPC and DPPH values, with r values ranging from 0.8 to 0.9, is consistent with the established role of AsA and polyphenols as primary antioxidants in plant systems (Sreelatha and Padma, 2009). This biochemical capacity underpins their nutraceutical value and potential as crop enhancers (Pareek et al., 2023; Lobo et al., 2010). However, the secondary metabolites of *Moringa* spp. extend beyond antioxidants to encompass sulfur-containing compounds derived from GLSs metabolism. A recent survey of the literature on glucomoringin quantification of the *M. oleifera* leaves reported substantial heterogeneity, from 16 to 112 µmol/g DW (Lopez-Rodriguez et al., 2020). In this context, the profiling of GLSs and their hydrolysis products revealed species-specific differences. The M3 showed the highest concentration of glucomoringin - the predominant GLS identified in *Moringa* leaves (Lopez-Rodriguez et al., 2020), but its corresponding isothiocyanate (moringin) was observed for M1. This result fully corresponded to the highest GLS-to-ITC bioconversion efficiency of M1 in comparison to M3, which can be attributed to the related myrosinase activities (Table 2). In the present work, moringin levels ranged from 3.8 to 8.4 µmol/g DW. Establishing an exact quantitative reference for moringin concentrations of *Moringa* leaves and seeds remains challenging, given the variability across studies. Engsuwan et al. (2017), employing a derivatization approach comparable to that applied here, reported isothiocyanate concentrations of *Moringa* leaves ranging from 0.7 – 7.5 mg/g DW of phenethyl isothiocyanate equivalents, corresponding to 2.2 to 24.1 µmol/g DW of moringin equivalents. The ranges detected in the present work fall within this reference interval.

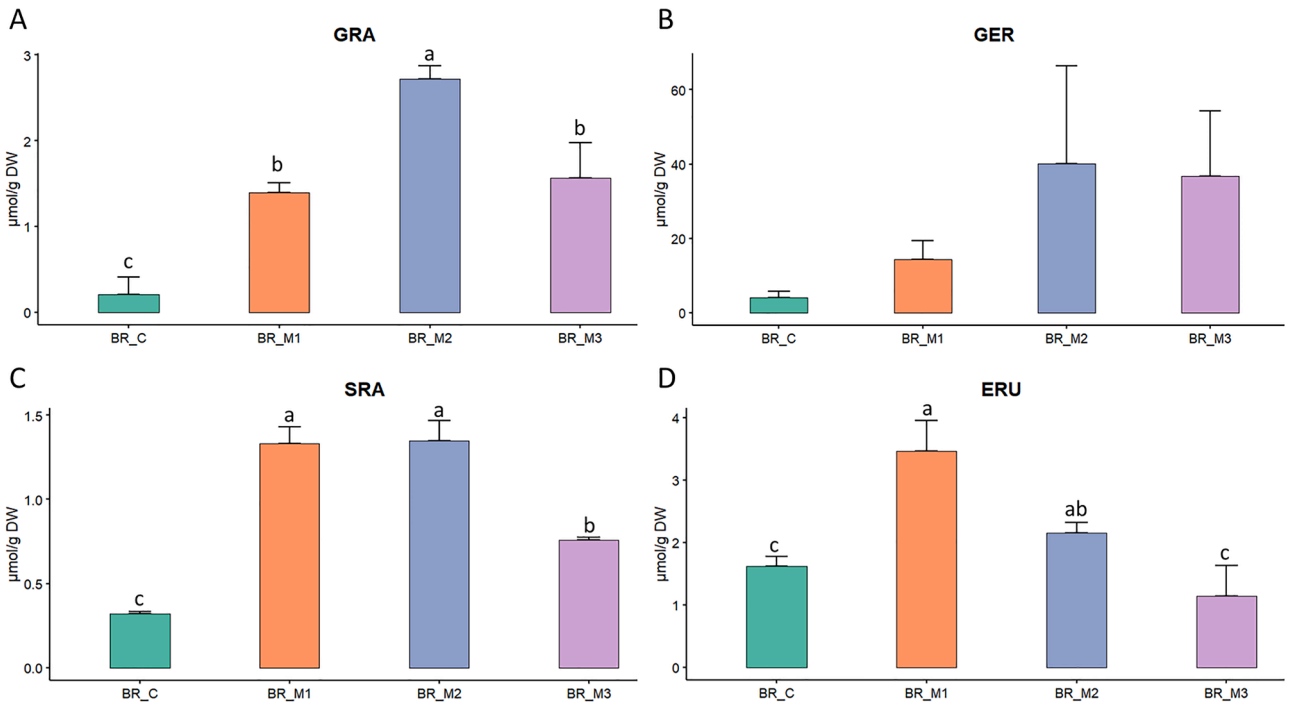


Fig. 4. Levels of glucoraphanin (GRA, A), its derived ITC, glucoerucin (GER, B), sulphoraphane (SRA, C), and its derived ITC, erucin (ERU, D) in 'Broccolo nero' in control (BR_C) and in the treated plants with different Moringa leaves' extracts (BR_M1, BR_M2 and BR_M3). BR_M1= Moringa hildebrandtii Engl., BR_M2= Moringa stenopetala (Baker f.) Cufod, BR_M3= Moringa oleifera Lam. Values are expressed as means ± standard error (SE, $n = 3$).

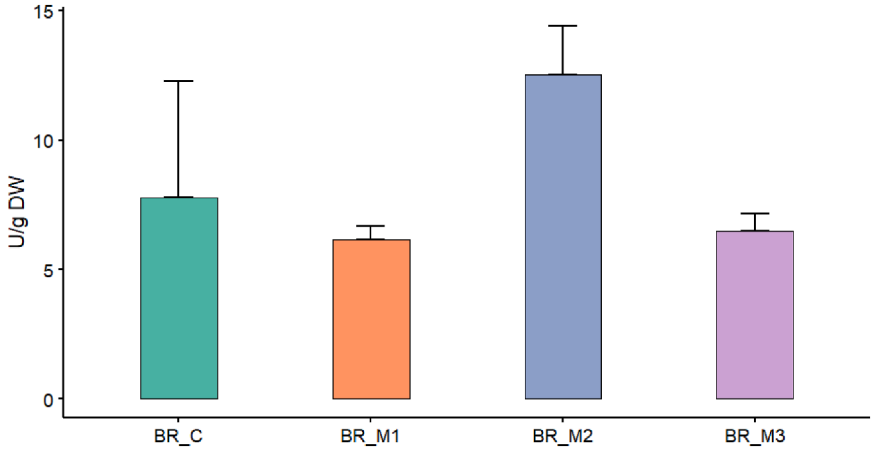


Fig. 5. Myrosinase activity of BR baby leaves sprayed with three Moringa leaf extract (U/g DW). BR_C= control, BR_M1= Moringa hildebrandtii Engl., BR_M2= Moringa stenopetala (Baker f.) Cufod, BR_M3= Moringa oleifera Lam. Values are expressed as means ± standard error (SE, $n = 3$).

This study indicates that *Moringa* leaves water extracts act as biostimulant primarily through modulation of the metabolism of the secondary metabolites accumulation (Admane et al., 2023). Morphometric analysis showed that foliar application of *Moringa* leaf extracts (BR_M1, BR_M2, BR_M3) did not significantly affect overall the plant traits analyzed, except for the significant increment of the fresh weight for BR_M2 and BR_M3, of the leaf area for BR_M3, and the significant reduction in root angle for the for all treatment plants in comparison to the control ones (Table 3). Leaf number, leaf dimensions, and stem and root length did not differ significantly from the control ($p > 0.05$), indicating that these plant traits were largely unaffected by treatment (Table 3).

In contrast to the morphometric data, the biochemical profiles revealed pronounced differences, with clear implications for the nutraceutical potential of 'Broccolo nero'.

In baby leaves of 'Broccolo nero', AsA values show a slight increase for BR_M2, although the difference is not statistically significant (Fig. 2A). Total phenolic content was significantly increased by all the baby leaves treated by *Moringa* extracts, particularly by *M. hildebrandtii* (BR_M1). These results are consistent with literature reports highlighting their rich flavonoid and phenolic acid composition (Pareek et al., 2023).

The biochemical traits analyzed enhance the value of the *Moringa* water extracts as functional agents for priming antioxidant defenses of high-value vegetable crops as reported in the study of Hafeez et al. (2022).

A major finding of this study is that the three water extracts from different *Moringa* species strongly affected the GLSs levels, and their breakdown products (ITC), of the baby leaves of 'Broccolo nero'. The main glucosinolates detected belong to the indolic class, and it is

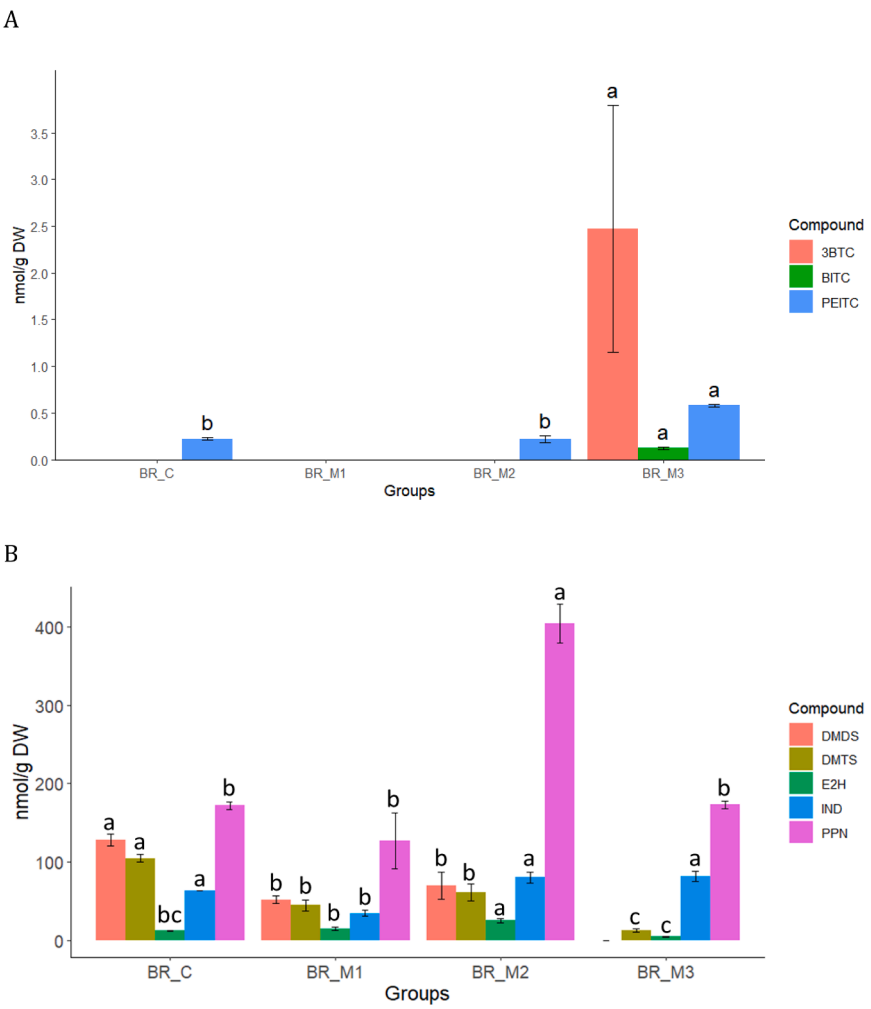


Fig. 6. Volatile compounds of BR baby leaves sprayed with three different *Moringa* extract (nmol/g DW). BR_C= control, BR_M1= *Moringa hildebrandtii* Engl., BR_M2= *Moringa stenopetala* (Baker f.) Cufod, BR_M3= *Moringa oleifera* Lam. Values are expressed as means ± standard error (SE, $n = 3$). Different letters indicate statistically significant differences as determined by Tukey's HSD test ($p < 0.05$).
A: 3BTC= 3-butenyl ITC, BITC= benzyl ITC, PEITC= beta-phenethyl ITC.
B: DMDS= dimethyl disulfide, DMTS= dimethyl trisulfide, E2H= (E)-2-hexenal, IND= indole, PPN= phenyl propionitrile.

represented by the neoglucobrassicin (NGBS) identified as the predominant compound (Fig. 3). NGBS was detected at high levels for the control, but significantly decreased by all the *Moringa* water extracts applied, particularly for the baby leaves of BR_M2. Alongside the decrease of the indolic GLSs, we observed a significant increment of the amount of several aliphatic GLSs, such as glucoerucin (GER) and glucoraphanin (GRA) (Fig. 4A and B). The presence and the concentrations of the indolic and aliphatic GLSs in the baby leaves of 'Broccolo nero' have been previously reported and are consistent with those observed in the present study (Branca et al., 2018a, 2018b; Arena et al., 2024, 2025). Indeed, aliphatic and indolic glucosinolates (GLSs) have been reported to vary in *Brassica* species depending on the genetic background and on the grown conditions (Martínez-Ballesta et al., 2015; Shawon et al., 2020; Schonhof et al., 2004).

This difference between the GLS availability and the content of their hydrolysis products highlights the importance of their enzymatic activation, particularly by the regulation of endogenous myrosinase and some other specifier proteins (Sugiyama and Hirai, 2019). Interestingly, the VOC analysis revealed the baby leaves of BR_M2 showed a preferential formation of nitriles, such as phenyl propionitrile (PPN), indicating a possible redirection of hydrolysis through the action of nitrile-specifier proteins (NSPs). In contrast, the baby leaves of BR_M3 favored isothiocyanate production, potentially due to the suppression of NSP activity or the activation of the classical myrosinase pathway. These divergent responses suggest that each *Moringa* spp. extract selectively modulates GLS breakdown, with potential applications in tailoring extract use to achieve desired phytochemical outcomes (e.g., enhancing health-promoting ITCs or influencing plant-insect interactions). This is evident for the baby leaves of BR_M1 and BR_M2 for the enhanced presence of both SRA and ERU (Fig. 4C and D). In this context, further evidence from studies using molecular approaches are ongoing as they strongly encouraged to confirm these findings.

Volatile profiling highlighted other differences among treated samples considering the differentiated levels of (*E*)-2-hexenal, indole, and sulfur volatiles. (*E*)-2-hexenal resulted higher in BR_M2, while the trend of indole levels under both BR_M2 and BR_M3, concomitant with the strong decrease of parent GLSs (Fig. 3), suggests the possible influence of these extracts in modulating both GLSs and VOC profiles.

The decrease of DMDS and DMTS in BR_M3, suggests a differentiated allocation of sulfur resources under elicitor input (Malhotra et al., 2023). Specifically, sulphides are breakdown products of S-methyl-L-cysteine sulphoxide (MCSO) (Edmands et al., 2013). Since MCSO catabolism involves the production of pyruvic acid, the tendency towards an increase of this compound by the *Moringa* spp. treatments (Fig. 1) is unexpected and may be attributable to other factors. One plausible explanation is that DMDS and DMTS might originate from

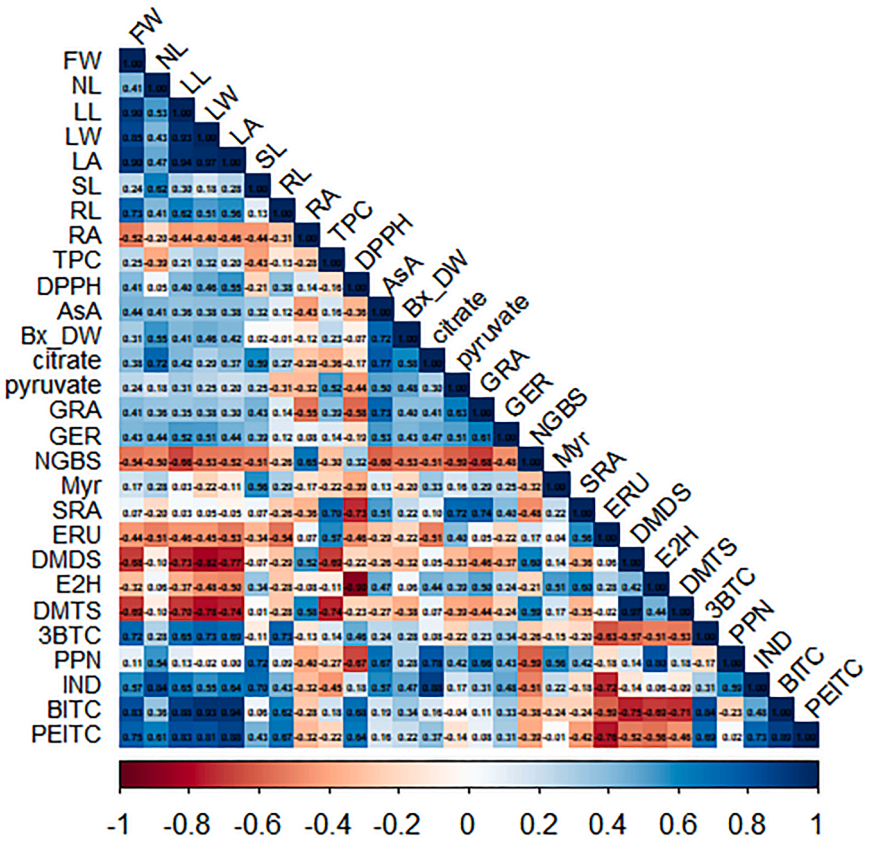


Fig. 7. Pearson's correlation analysis among the studied traits of 'Broccolo nero' baby leaves sprayed with *Moringa* leaf extracts. Abbreviations: FW= fresh weight of 10 individuals, NL= number of leaves, LL= leaf length, LW= leaf width, LA= leaf area, SL= stem length, RL= root length, RA= root angle, TPC= Total phenolic content, DPPH=1,1-diphenyl-2-picrylhydrazil scavenging, AsA= ascorbic acid, Bx_DW= soluble solid content, SRA= sulforaphane, ERU= erucin, DMDS= dimethyl disulfide, E2H= (E)-2-hexenal, DMTS= dimethyl trisulfide, 3BTC= 3-butenyl ITC, PPN= phenyl propionitrile, IND= indole, BITC= benzyl ITC, PEITC= beta-phenethyl ITC, GRA= glucoraphanin, GER= glucoerucin, NGBS= neoglucobrassicin, Myr= Myrosinase.

sulphur-containing precursors other than MCSOs - such as cystine, cysteine and S-methyl-L-cysteine - which were not detected in the present work (Derbali et al., 1998).

The Pearson's correlation analysis under *Moringa oleifera* extract treatments revealed a coherent pattern of enhanced plant growth and development, evidenced by strong positive correlations between fresh weight (FW) and key morphological traits including leaf length (LL), width (LW), area (LA), and root length (RL), with r values between 0.73 and 0.90, indicating a systemic growth-promoting effect likely mediated by *Moringa* extract derived bioactives.

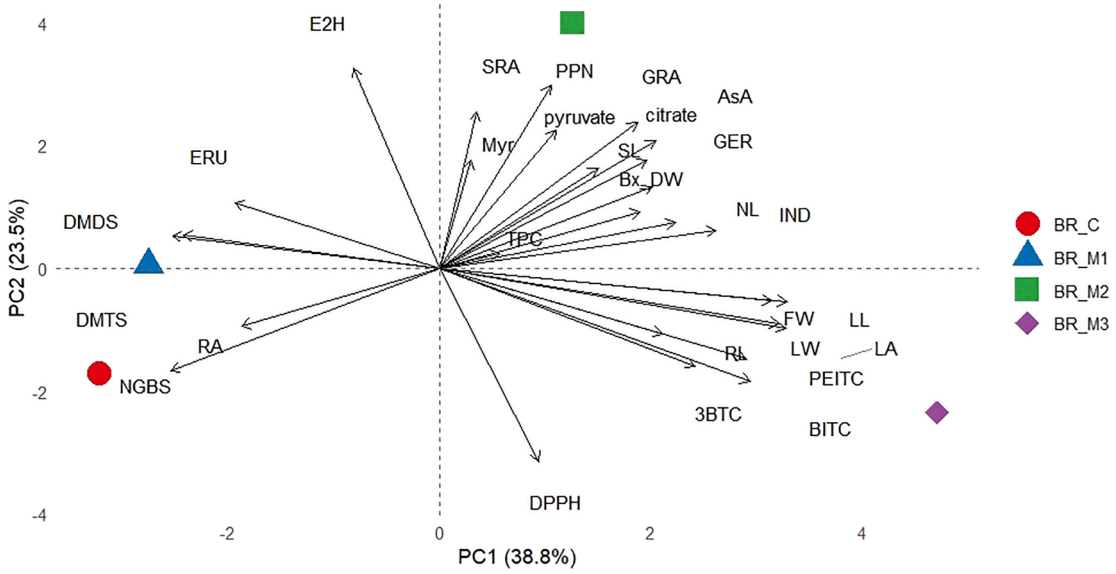


Fig. 8. PCA of the tested parameters. BR_C= control, BR_M1= *Moringa hildebrandtii* Engl., BR_M2= *Moringa stenopetala* (Baker f.) Cufod, BR_M3= *Moringa oleifera* Lam. See caption of Fig. 7 for abbreviations.

Notably, isothiocyanates such as benzyl isothiocyanate (BITC), phenylethyl isothiocyanate (PEITC), and 3-butenyl isothiocyanate (3BTC) were positively associated with FW and morphological traits (r = 0.72–0.94), suggesting that these compounds, either directly supplied or endogenously induced, contribute to growth enhancement.

Positive correlations between citrate, ascorbic acid, and biomass parameters (r = 0.72 – 0.77) further emphasize the integration of metabolism and antioxidant capacity in sustaining the growth-stimulating effects of *Moringa* treated samples. In support of previous findings, it is worth highlighting the significant negative correlations between GRA and NGBS (r = - 0.68) and between GER and NGBS (r = - 0.48), as well as the positive relationship between GRA and SRA (r = 0.74).

A more integrated understanding of the phenomena associated with the changes induced by different *Moringa* extract treatments can be inferred from the PCA (Fig. 8), where the first two principal components (PC1 and PC2) together accounted for 62.3% of the total system variability considered. It is evident that the different treatments produced clearly distinguishable associations. The control plot was strongly associated with negative PC1 values and the NGBS vector, whereas BR_M1 correlated with DMDS, DMTS and ERU. On the other hand, BR_M2 showed the opposite trend, being associated with GRA, SRA, AsA, PPN, citrate and pyruvate, while BR_M3 clustered near the vectors 3BTC, BITC, PEITC, FW, and LW.

Among the tested species, *Moringa hildebrandtii* (M1) and *Moringa stenopetala* (M2) enhanced aliphatic glucosinolate accumulation. BR_M2 showed the highest myrosinase activity and higher hydrolysis products (isothiocyanates and nitriles), whereas *M. oleifera (*M3) highlighted a different isothiocyanate and volatile profile.

The results of this study offer promising insights into the use of *Moringa* leaf extracts as sustainable biostimulants for modulating both plant morphology and secondary metabolism of the *Brassica oleracea* L. crops. It also underscores the importance of considering species-specific genetic differences among the three Moringa species utilised, as biochemical responses were not uniform across treatments.

5. Conclusion

This study provides novel insights for the biostimulants potential of the leaf water extract of three different species of *Moringa*, highlighting their differential ability to modulate the secondary metabolism and the volatile compound profiles of the Sicilian landraces of 'Broccolo nero' (*Brassica oleracea* var. *italica*. Distinctive biochemical differences were observed, particularly for the native glucosinolates, for their hydrolysis products, and for the sulfur-nitrogen volatile compounds.

The targeted enhancement of the health-promoting glucosinolates, and of their derivatives, may contribute to improved crop nutritional quality and the development of functional food, aligning with circular and low-input agronomic strategies.

From a horticultural perspective, these findings underscore the practical potential of *Moringa* water extracts as natural biostimulants and highlight the importance of developing standardized, and species-specific formulations, for future applications. Future research should focus on transcriptomic validation, dose–response optimization, and field trials to refine the water extract formulations and the application strategies under diverse environmental conditions.

Funding

This research was supported by ALSignOX – "Molecular signatures di stress ossidativo nella Sclerosi Laterale Amiotrofica" (PIA.CE.RI. 2024–2026).

Ethical statement

The corresponding Author, on behalf of all co-Authors, declare that this article does not contain any studies with human or animal participants.

CRediT authorship contribution statement

Donata Arena: Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Roberto Lo Scalzo:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Hajer Ben Ammar:** Methodology, Investigation. **Valentina Picchi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Giulia Bianchi:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Daniela Romano:** Validation, Supervision. **Luca Ciccarello:** Validation, Methodology, Formal analysis. **Nicolas Al Achkar:** Methodology, Investigation, Formal analysis. **Ferdinando Branca:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2026.100991.

Data availability

Data will be made available on request.

References

Abd El-Mageed, T.A., Semida, W.M., Rady, M.M., 2017. Moringa leaf extract as biostimulant improves water use efficiency, physio-biochemical attributes of squash plants under deficit irrigation. Agric. Water. Manage 193, 46–54. https://doi.org/10.1016/j.agwat.2017.08.004.

Admane, N., Cavallo, G., Hadjila, C., Cavalluzzi, M.M., Rotondo, N.P., Salerno, A., Cannillo, J., Difonzo, G., Caponio, F., Ippolito, A., Lentini, G., Sanzani, S.M., 2023. Biostimulant formulations and *moringa oleifera* extracts to improve yield, quality, and storability of hydroponic lettuce. Molecules 28, 373. https://doi.org/10.3390/molecules28010373.

Ahmed, K., Banik, R., Hossain, M., Jahan, I., 2016. Vitamin C (L-ascorbic Acid) content in different parts of *moringa oleifera* grown in Bangladesh. ACSJ 11, 1–6. https://doi.org/10.9734/ACSJ/2016/21119.

Akram, N.A., Shafiq, F., Ashraf, M., 2017. Ascorbic acid-A potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front. Plant Sci. 8, 613. https://doi.org/10.3389/fpls.2017.00613.

Arena, D., Ben Ammar, H., Rodriguez, V.M., Velasco, P., Calì, R., Ciccarello, L., Branca, F., 2025. The role of melatonin in modulating morphometric parameters and bioactive compounds of *brassica oleracea* L. var. *Italica* plenck under drought stress. Agronomy 15, 279. https://doi.org/10.3390/agronomy15020279.

Arena, D., Ben Ammar, H., Rodriguez, V.M., Velasco, P., Garcia, G., Calì, R., Branca, F., 2024. Exogenous melatonin affects the morphometric characteristics and glucosinolates during the initial growth stages of broccoli. Agronomy 14, 286. https://doi.org/10.3390/agronomy14020286.

Arif, Y., Bajguz, A., Hayat, S., 2023. Moringa oleifera extract as a natural plant biostimulant. J. Plant Growth Regul. 42, 1291–1306. https://doi.org/10.1007/s00344-022-10630-4.

Bianchi, G., Picchi, V., Tava, A., Doria, F., Walley, P.G., Dever, L., Di Bella, M.C., Arena, D., Ben Ammar, H., Lo Scalzo, R., Branca, F., 2024. Insights into the phytochemical composition of selected genotypes of organic kale (*Brassica oleracea* L. var. *acephala*). J. Food Compos. Anal. 125, 105721. https://doi.org/10.1016/j.jfca.2023.105721.

Branca, F., Chiarenza, G.L., Cavallaro, C., Gu, H., Zhao, Z., Tribulato, A., 2018a. Diversity of Sicilian broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis*) landraces and their distinctive bio-morphological, antioxidant, and genetic traits. Genet. Resour. Crop. Evol. 65, 485–502. https://doi.org/10.1007/s10722-017-0547-8.

Branca, F., Picchi, V., De Barros, A., Bifera, R., Donzella, E., Tribulato, A., Rosa, E., Lo Scalzo, R., 2018b. Phytochemical content of the wild and cultivated *Brassica* (n =9) collection of the ECPGR "COCHEVA BRAS" project. Acta Hortic. 33–38. https://doi.org/10.17660/ActaHortic.2018.1202.5.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Brockman, H.G., Brennan, R.F., 2017. The effect of foliar application of Moringa leaf extract on biomass, grain yield of wheat and applied nutrient efficiency. J. Plant Nutr. 40, 2728–2736. https://doi.org/10.1080/01904167.2017.1381723.

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., Ferrante, A., 2015. Biostimulants and crop responses: a review. Biol. Agric. Hortic. 31, 1–17. https://doi.org/10.1080/01448765.2014.964649.

Culver, M., Fanuel, T., Chiteka, A.Z., 2012. Effect of moringa extract on growth and yield of tomato. Green. J. Agri. Sci. 2 (5), 207–211.

Çakmakçı, R., Salık, M.A., Çakmakçı, S., 2023. Assessment and principles of environmentally sustainable food and agriculture systems. Agriculture 13, 1073. https://doi.org/10.3390/agriculture13051073.

Derbali, E., Makhlouf, J., Vezina, L.-P., 1998. Biosynthesis of sulfur volatile compounds in broccoli seedlings stored under anaerobic conditions. Postharvest Biol. Technol. 13, 191–204. https://doi.org/10.1016/S0925-5214(98)00016-7.

Di Gioia, F., Renna, M., Santamaria, P., 2017. Sprouts, Microgreens and "Baby Leaf" Vegetables. In: Yildiz, F., Wiley, R. (Eds.), Minimally Processed Refrigerated Fruits and Vegetables. Food Engineering Series. Springer, Boston, MA. https://doi.org/10.1007/978-1-4939-7018-6_11.

Di Mola, I., Ottaiano, L., Cozzolino, E., Senatore, M., Giordano, M., El-Nakhel, C., Sacco, A., Rouphael, Y., Colla, G., Mori, M., 2019. Plant-based biostimulants influence the agronomical, physiological, and qualitative responses of baby rocket leaves under diverse nitrogen conditions. Plants 8, 522. https://doi.org/10.3390/plants8110522.

Edmands, W.M.B., Gooderham, N.J., Holmes, E., Mitchell, S.C., 2013. S-methyl- L -cysteine sulphoxide: the Cinderella phytochemical? Toxicol. Res. 2, 11–22. https://doi.org/10.1039/C2TX20030A.

Engsuwan, J., Waranuch, N., Limpeanchob, N., Ingkaninan, K., 2017. HPLC methods for quality control of *Moringa oleifera* extract using isothiocyanates and astragalin as bioactive markers. ScienceAsia 43, 169. https://doi.org/10.2306/scienceasia1513-1874.2017.43.169.

Everette, J.D., Bryant, Q.M., Green, A.M., Abbey, Y.A., Wangila, G.W., Walker, R.B., 2010. Thorough study of reactivity of various compound classes toward the Folin−Ciocalteu reagent. J. Agric. Food Chem. 58, 8139–8144. https://doi.org/10.1021/jf1005935.

Franzoni, G., Bulgari, R., Ferrante, A., 2021. Maceration time affects the efficacy of borage extracts as potential biostimulant on rocket salad. Agronomy 11, 2182. https://doi.org/10.3390/agronomy11112182.

Gopalakrishnan, L., Doriya, K., Kumar, D.S., 2016. *Moringa oleifera*: a review on nutritive importance and its medicinal application. Food Sci. Hum. Wellness 5, 49–56. https://doi.org/10.1016/j.fshw.2016.04.001.

Hafeez, A., Tipu, M.I., Saleem, M.H., Al-Ashkar, I., Saneoka, H., Sabagh, A.E., 2022. Foliar application of moringa leaf extract (MLE) enhanced antioxidant system, growth, and biomass related attributes in safflower plants. South Afr. J. Bot. 150, 1087–1095. https://doi.org/10.1016/j.sajb.2022.09.021.

Halshoy, H., Mahmood, A., Tofiq, G., 2023. Effect of plant biostimulants on growth, yield and some mineral composition of broccoli plants (Brassica oleracea var. Italica). TJAS 23, 130–140. https://doi.org/10.25130/tjas.23.1.16.

Hamada, F.A., Sabah, S.S., Mahdy, E.M.B., El-Raouf, H.S.A., El-Taher, A.M., El-leel, O.F. A., Althobaiti, A.T., Ghareeb, M.A., Randhir, R., Randhir, T.O., 2024. Genetic, phytochemical and morphological identification and genetic diversity of selected Moringa species. Sci. Rep. 14, 30476. https://doi.org/10.1038/s41598-024-79148-x.

Hoque, T.S., Rana, M.S., Zahan, S.A., Jahan, I., Abedin, M.A., 2020. Moringa leaf extract as a bio-stimulant on growth, yield and nutritional improvement in cabbage. Asian J. Med. Biol. Res. 6, 196–203. https://doi.org/10.3329/ajmbr.v6i2.48050.

ISO 9167-1:1992; Rapeseed-Determination of Glucosinolates Content-Part 1: Method Using High-Performance Liquid Chromatography. International Standard Organization: Geneva, Switzerland, 1992; Volume 9167, pp. 1–9.

Jayaprakasha, G.K., Jena, B.S., Sakariah, K.K., 2003. Improved liquid chromatographic method for determination of organic acids in leaves, pulp, fruits, and rinds of garcinia. J. AOAC Int. 86, 1063–1068. https://doi.org/10.1093/jaoac/86.5.1063.

Kaluzewicz, A., Bosiacki, M., Spizewski, T., 2018. Influence of biostimulants on the content of macro-and micronutrients in broccoli plants exposed to drought stress. J. Elementol. 23 (1). https://doi.org/10.2478/fhort-2018-0012.

Karthiga, D., Chozhavendhan, S., Gandhiraj, V., Aniskumar, M., 2022. The effects of Moringa oleifera leaf extract as an organic bio-stimulant for the growth of various plants: review. Biocatal. Agric. Biotechnol. 43, 102446. https://doi.org/10.1016/j.bcab.2022.102446.

Khan, S., Basra, S.M.A., Nawaz, M., Hussain, I., Foidl, N., 2020. Combined application of Moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum* L.). South Afr. J. Bot. 129, 74–81. https://doi.org/10.1016/j.sajb.2019.01.007.

Khondoker, S.A., Rajib, B.M., Hemayet, H., Ismetara, J., 2016. Vitamin C (L-ascorbic Acid) Content in Different Parts of Moringa oleifera Grown in Bangladesh. ACSJ 211, 19–21.

Kumar, M., Selvasekaran, P., Kapoor, S., Barbhai, M.D., Lorenzo, J.M., Saurabh, V., Potkule, J., Changan, S., ElKelish, A., Selim, S., Sayed, A.A.S., Radha, Singh, S., Senapathy, M., Pandiselvam, R., Dey, A., Dhumal, S., Natta, S., Amarowicz, R., Kennedy, J.F., 2022. *Moringa oleifera* Lam. Seed proteins: extraction, preparation of protein hydrolysates, bioactivities, functional food properties, and industrial application. Food Hydrocoll. 131, 107791. https://doi.org/10.1016/j.foodhyd.2022.107791.

Lamb, F.C., 1972. Modification of AOAC method for determination of soluble solids in tomato products. J. AOAC Int. 55, 809–810. https://doi.org/10.1093/jaoac/55.4.809.

Lester, G.E., Makus, D.J., Hodges, D.M., 2010. Relationship between fresh-packaged spinach leaves exposed to continuous light or dark and bioactive contents: effects of cultivar, leaf size, and storage duration. J. Agric. Food Chem. 58, 2980–2987. https://doi.org/10.1021/jf903596v.

Li, J., Lardon, R., Mangelinckx, S., Geelen, D., 2024. A practical guide to the discovery of biomolecules with biostimulant activity. J. Exp. Bot. 75, 3797–3817. https://doi.org/10.1093/jxb/erae156.

Lo Scalzo, R., Bianchi, G., Picchi, V., Campanelli, G., Ficcadenti, N., Treccarichi, S., Arena, D., Sestili, S., Branca, F., 2024. Compositional traits of hybrid populations of *brassica oleracea* L. var. *Italica* (broccoli) and *brassica oleracea* L. var. *Botrytis* (cauliflower) during four organic breeding cycles. J. Food Compos. Anal. 131, 106209. https://doi.org/10.1016/j.jfca.2024.106209.

Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: impact on human health. Phcog. Rev. 4, 118. https://doi.org/10.4103/0973-7847.70902.

Lopez-Rodriguez, N.A., Gaytán-Martínez, M., De La Luz Reyes-Vega, M., Loarca-Piña, G., 2020. Glucosinolates and isothiocyanates from Moringa oleifera: chemical and biological approaches. Plant Foods Hum. Nutr. 75, 447–457. https://doi.org/10.1007/s11130-020-00851-x.

Madukwe, E.U., Ugwuoke, A.L., Ezeugwu, J.O., 2013. .Effectiveness of dry Moringa oleifera leaf powder in treatment of anaemia. Int. J. Med. Med. Sci. https://doi.org/10.5897/IJMMS2013.0884.

Malhotra, B., Kumar, P., Bisht, N.C., 2023. Defense versus growth trade-offs: insights from glucosinolates and their catabolites. Plant Cell Environ. 46, 2964–2984. https://doi.org/10.1111/pce.14462.

Mandal, S., Anand, U., López-Bucio, J., Radha, Kumar, M., Lal, M.K., Tiwari, R.K., Dey, A., 2023. Biostimulants and environmental stress mitigation in crops: a novel and emerging approach for agricultural sustainability under climate change. Environ. Res. 233, 116357. https://doi.org/10.1016/j.envres.2023.116357.

Martínez-Ballesta, M., Moreno-Fernández, D.A., Castejón, D., Ochando, C., Morandini, P. A., Carvajal, M., 2015. The impact of the absence of aliphatic glucosinolates on water transport under salt stress in Arabidopsis thaliana. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.00524.

Mashamaite, C.V., Ngcobo, B.L., Manyevere, A., Bertling, I., Fawole, O.A., 2022. Assessing the usefulness of *moringa oleifera* leaf extract as a biostimulant to supplement synthetic fertilizers: a review. Plants 11, 2214. https://doi.org/10.3390/plants11172214.

Matthews, S., Siddiqui, Y., Ali, A., 2025. Unleashing the power of bio-stimulants for enhanced crop growth, productivity, and quality: a comprehensive review. J. Plant Nutr. 48, 703–725. https://doi.org/10.1080/01904167.2024.2412736.

Matusheski, N.V., Juvik, J.A., Jeffery, E.H., 2004. Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. Phytochemistry 65, 1273–1281. https://doi.org/10.1016/j.phytochem.2004.04.013.

Mounaimi, S., Hamim, A., El Boukhari, M.E.M., Elarroussi, H., Lyamlouli, K., 2024. A comprehensive review of integrating biostimulants and biopesticides for organic berry farming: exploring challenges and opportunities for Africa. Front. Sustain. Food Syst. 8, 1452823. https://doi.org/10.3389/fsufs.2024.1452823.

Oh, M.-M., Carey, E.E., Rajashekar, C.B., 2010. Regulated water deficits improve phytochemical concentration in lettuce. J. Amer. Soc. Hort. Sci. 135, 223–229. https://doi.org/10.21273/JASHS.135.3.223.

Pannacci, E., Baratta, S., Falcinelli, B., Farneselli, M., Tei, F., 2022. Mugwort (*Artemisia vulgaris* L.) aqueous extract: hormesis and biostimulant activity for seed germination and seedling growth in vegetable crops. Agriculture 12, 1329. https://doi.org/10.3390/agriculture12091329.

Pareek, A., Pant, M., Gupta, M.M., Kashania, P., Ratan, Y., Jain, V., Pareek, A., Chuturgoon, A.A., 2023. Moringa oleifera: an updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. IJMS 24, 2098. https://doi.org/10.3390/ijms24032098.

Patil, S.V., Mohite, B.V., Marathe, K.R., Salunkhe, N.S., Marathe, V., Patil, V.S., 2022. Moringa tree, gift of nature: a review on nutritional and industrial potential. Curr. Pharmacol. Rep. 8, 262–280. https://doi.org/10.1007/s40495-022-00288-7.

Pervez, K., Ullah, F., Mehmood, S., Khattak, A., 2017. Effect of Moringa oleifera Lam. leaf aqueous extract on growth attributes and cell wall bound phenolics accumulation in maize (Zea mays L.) under drought stress. Kuwait J. Sci. 44 (4).

Picchi, V., Migliori, C., Lo Scalzo, R., Campanelli, G., Ferrari, V., Di Cesare, L.F., 2012. Phytochemical content in organic and conventionally grown Italian cauliflower. Food Chem. 130, 501–509. https://doi.org/10.1016/j.foodchem.2011.07.036.

Piekarska, A., Kusznierewicz, B., Meller, M., Dziedziul, K., Namieśnik, J., Bartoszek, A., 2013. Myrosinase activity in different plant samples; optimisation of measurement conditions for spectrophotometric and pH-stat methods. Ind. Crops Prod. 50, 58–67. https://doi.org/10.1016/j.indcrop.2013.06.048.

Pilipczuk, T., Dawidowska, N., Kusznierewicz, B., Namieśnik, J., Bartoszek, A., 2015. Simultaneous determination of indolic compounds in plant extracts by solid-phase extraction and high-performance liquid chromatography with UV and fluorescence detection. Food Anal. Methods 8, 2169–2177. https://doi.org/10.1007/s12161-015-0106-x.

Pilipczuk, T., Kusznierewicz, B., Chmiel, T., Przychodzeń, W., Bartoszek, A., 2017. Simultaneous determination of individual isothiocyanates in plant samples by HPLC-DAD-MS following SPE and derivatization with N -acetyl- l -cysteine. Food Chem. 214, 587–596. https://doi.org/10.1016/j.foodchem.2016.07.125.

R Core Team, 2024. R: A language and environment for statistical computing (Version 4.4. 2)[Computer software]. R Foundation for Statistical Computing.

Romano, D., La Fornara, G., Tribulato, A., Toscano, S., 2022. Can Moringa leaf spray treatment increase the nutraceutical properties of radish baby leaf? Horticulturae 8, 671. https://doi.org/10.3390/horticulturae8080671.

Ruzzi, M., Colla, G., Rouphael, Y., 2024. Editorial: biostimulants in agriculture II: towards a sustainable future. Front. Plant Sci. 15, 1427283. https://doi.org/10.3389/fpls.2024.1427283.

Saini, R.K., Sivanesan, I., Keum, Y.-S., 2016. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. 3 Biotech 6, 203. 10.1007/s13205-016-0526-3.

Saporta, R., Bou, C., Frías, V., Mulet, J.M., 2019. A method for a fast evaluation of the biostimulant potential of different natural extracts for promoting growth or tolerance against abiotic stress. Agronomy 9, 143. https://doi.org/10.3390/agronomy9030143.

Schonhof, I., Krumbein, A., Brückner, B., 2004. Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. Nahrung 48, 25–33. https://doi.org/10.1002/food.200300329.

Shawon, R.A., Kang, B.S., Lee, S.G., Kim, S.K., Ju Lee, H., Katrich, E., Gorinstein, S., Ku, Y.G., 2020. Influence of drought stress on bioactive compounds, antioxidant enzymes and glucosinolate contents of Chinese cabbage (Brassica rapa). Food Chem. 308, 125657. https://doi.org/10.1016/j.foodchem.2019.125657.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

Sreelatha, S., Padma, P.R., 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum. Nutr. 64, 303–311. https://doi.org/10.1007/s11130-009-0141-0.

Srivastava, S., Pandey, V.K., Dash, K.K., Dayal, D., Wal, P., Debnath, B., Singh, R., Dar, A. H., 2023. Dynamic bioactive properties of nutritional superfood *Moringa oleifera*: a comprehensive review. J. Agric. Food Res. 14, 100860. https://doi.org/10.1016/j.jafr.2023.100860.

Subhasree, B., Baskar, R., Laxmi Keerthana, R., Lijina Susan, R., Rajasekaran, P., 2009. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chem. 115, 1213–1220. https://doi.org/10.1016/j.foodchem.2009.01.029.

Sugiyama, R., Hirai, M.Y., 2019. Atypical myrosinase as a mediator of glucosinolate functions in plants. Front. Plant Sci. 10, 1008. https://doi.org/10.3389/fpls.2019.01008.

Thippeswamy, T.G., Shreedhar, M.V., Murty, B.R.S., Thejaswi, N., 2020. Ascorbic acid and mineral content in Moringa oleifera leaves: a study of ascorbic acid stability. J. Pharma. Sci. Res. 12 (7), 978–986.

Toscano, S., Ferrante, A., Branca, F., Romano, D., 2021. Enhancing the quality of two species of baby leaves sprayed with moringa leaf extract as biostimulant. Agronomy 11, 1399. https://doi.org/10.3390/agronomy11071399.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., 2013. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind. Crops Prod. 44, 566–571. https://doi.org/10.1016/j.indcrop.2012.09.021.

Yasmeen, A., Nouman, W., Basra, S.M.A., Wahid, A., Hafeez-ur-Rehman, Hussain, N., Afzal, I., 2014. Morphological and physiological response of tomato (*Solanum lycopersicum* L.) to natural and synthetic cytokinin sources: a comparative study. Acta Physiol. Plant 36, 3147–3155. https://doi.org/10.1007/s11738-014-1662-1.

Yuniati, N., Kusumiyati, K., Mubarok, S., Nurhadi, B., 2022. The role of moringa leaf extract as a plant biostimulant in improving the quality of agricultural products. Plants 11, 2186. https://doi.org/10.3390/plants11172186.

Zulfiqar, F., Casadesús, A., Brockman, H., Munné-Bosch, S., 2020. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. Plant Sci. 295, 110194. https://doi.org/10.1016/j.plantsci.2019.110194.