Scientific Papers. Series B, Horticulture. Vol. LXX, No. 1, 2026
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF FERTILIZATION REGIMES ON YIELD, BIOACTIVE COMPOUNDS, AND NITRATE CONTENT OF BROCCOLI GROWN UNDER PROTECTED CULTIVATION

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Abstract

Fertilization management is a key factor influencing both yield and nutritional quality of broccoli. This study aimed to evaluate the effects of different fertilization regimes on broccoli yield and on the accumulation of bioactive and quality-related compounds in the main inflorescences. The experiment was conducted in a polyethylene tunnel using three broccoli hybrids. Plants were subjected to different fertilization treatments, including a non-fertilized control, organic fertilization, and mineral fertilization regimes. At harvest, yield parameters were recorded, and biochemical analyses were performed to determine vitamin C, chlorophyll a and b, carotenoids, total phenolic content, antioxidant activity, and nitrate concentration in the broccoli inflorescences. Fertilization significantly influenced broccoli yield and biochemical composition. Nitrate content was also affected by fertilization type, highlighting differences between organic and mineral nutrient management. Results indicate that fertilization strategies play an important role in determining both productivity and nutritional quality of broccoli grown under protected cultivation.

Key words*: broccoli, fertilization, yield, bioactive compounds, antioxidant activity, phenolics, nitrates.*

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica*) has emerged as one of the most economically significant vegetable crops worldwide, with global production exceeding 27 million tonnes annually according to the Food and Agriculture Organization of the United Nations (FAO, 2023). Beyond its commercial importance, broccoli has attracted considerable scientific and consumer interest due to its exceptional nutritional composition and associated health-promoting properties (Jeffery and Araya, 2009; Vasanthi et al., 2009). Increasing public awareness of diet-related health issues has positioned broccoli as a functional food, rich in bioactive compounds such as glucosinolates, phenolic compounds, vitamins, and essential minerals (Moreno et al., 2006; Domínguez-Perles et al., 2010).

The recognized health benefits of broccoli consumption are largely attributed to its diverse array of antioxidant and bioactive constituents. Broccoli inflorescences are particularly abundant in vitamin C (ascorbic acid), which provides strong antioxidant protection (Vallejo et al., 2003). They also contain significant amounts of carotenoids, including β-carotene and lutein, which are essential for visual health and immune function (Schonhof et al., 2007). Chlorophylls present in broccoli contribute additional antioxidant activity (Ferruzzi and Blakeslee, 2007), while phenolic compounds play a critical role in mitigating oxidative stress and reducing the risk of chronic diseases (Podsędek, 2007). Collectively, these compounds contribute to the high antioxidant capacity of broccoli, which has been associated with a decreased risk of cardiovascular diseases, certain cancers, and age-related

disorders (Herr and Büchler, 2010; Vanduchova et al., 2019).

To meet growing consumer demand and ensure consistent supply, protected cultivation systems such as polyethylene tunnels and greenhouses are increasingly employed for year-round broccoli production, particularly in regions characterized by unfavourable climatic conditions (Castilla and Hernández, 2007). These systems provide several agronomic advantages, including extended growing seasons, protection against extreme weather events, improved water use efficiency, enhanced pest and disease control, and the potential for higher yields with improved market quality. However, the controlled environment of protected systems also introduces specific challenges, particularly in nutrient management. The absence of natural rainfall reduces nutrient leaching but can simultaneously promote salt accumulation and nutrient imbalances in the root zone (Gruda, 2009).

Among agronomic practices, fertilization management plays a decisive role in determining both productivity and product quality in vegetable crops (Chen et al., 2018). Broccoli has relatively high nutrient requirements, especially for nitrogen, which strongly influences vegetative growth, head development, and final yield (Everaarts and De Willigen, 1999). Nevertheless, optimizing nitrogen supply is complex. While adequate fertilization enhances yield, excessive nitrogen application may compromise nutritional quality and increase nitrate accumulation in plant tissues (Colla et al., 2018). From an environmental perspective, over-fertilization contributes to nitrate leaching and greenhouse gas emissions, thereby increasing the ecological footprint of production systems (Snyder et al., 2009). Comparative evaluations of fertilizer types have demonstrated significant variations in growth and yield parameters depending on nutrient source and management strategy. For example, Becherescu et al. (2025) reported significant differences in early cabbage performance under different fertilization regimes, highlighting the importance of optimizing fertilizer selection to improve crop outcomes under controlled production systems. These findings support the need for similar investigations in broccoli, particularly under protected cultivation conditions.

The choice between organic and mineral fertilization further complicates nutrient management strategies. Organic fertilizers release nutrients gradually through microbial mineralization processes and may contribute to improved soil structure and long-term soil fertility (Möller and Schultheiß, 2015). In contrast, mineral fertilizers provide nutrients in readily available forms, often resulting in rapid plant uptake but potentially encouraging luxury consumption and higher nitrate accumulation (Worthington, 2001; Herencia et al., 2011). Previous research indicates that fertilization type can significantly influence not only yield but also the concentration of bioactive compounds in brassica vegetables. Organic fertilization has, in some cases, been associated with increased phenolic content and antioxidant activity (Young et al., 2005; Heimler et al., 2017), whereas mineral fertilization may enhance certain vitamin concentrations (Zhao et al., 2008).

Nitrate accumulation represents an additional concern in broccoli production. Excessive dietary nitrate intake has been linked to methemoglobinemia in infants and the potential endogenous formation of carcinogenic N-nitroso compounds (Santamaria, 2006). Brassica species, including broccoli, have a tendency to accumulate nitrates, particularly under conditions of high nitrogen fertilization (Aires et al., 2013). Although the European Union has established maximum nitrate levels for certain leafy vegetables, specific limits for broccoli have not been defined (European Commission, 2011). Consequently, managing nitrogen inputs to ensure both high productivity and safe nitrate levels remains a critical objective in broccoli cultivation.

Despite extensive research on broccoli agronomy and quality attributes, there remains a need for comprehensive evaluations that integrate yield performance, nutritional quality, and food safety parameters under protected cultivation conditions. Many previous studies have addressed individual aspects - such as yield response, specific bioactive compounds, or nitrate accumulation - without simultaneously examining their interactions

under different fertilization regimes and across hybrid cultivars. Furthermore, limited information is available regarding the combined effects of organic and mineral fertilization strategies on multiple quality indicators within controlled environments.

The present study was designed to comprehensively evaluate the influence of different fertilization regimes (control, organic, and mineral) on broccoli production under protected cultivation. Particular emphasis was placed on yield parameters, the accumulation of bioactive compounds, and nitrate content across different hybrid cultivars. By integrating agronomic performance with nutritional and safety assessments, this research aims to contribute to the development of sustainable fertilization strategies that optimize productivity while maintaining high nutritional value and minimizing potential health and environmental risks.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiment was conducted under protected cultivation conditions in a polyethylene tunnel at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. Cluj County is situated in the north-eastern part of Transylvania, between 23°39'47" – 24°13'46" East longitude and 46°28'44" – 47°28'44" North latitude. The city of Cluj-Napoca lies in the central-north-western part of Romania at an altitude of 345-360 m above sea level, with a mean annual air temperature of 9.52°C. The regional climate is transitional between temperate and continental, characterised by four distinct seasons.

Three commercially available broccoli (*Brassica oleracea* L. var. *italica*) hybrids were used as experimental material: 'Fiesta', 'Marathon', and 'Belstar'. All three hybrids are recommended for early spring production and share resistance to *Fusarium oxysporum*.

'Belstar' is suited for spring and summer culture, reaching physiological maturity approximately 80 days after sowing. It prefers rich, well-moistened soils. The main inflorescence is compact, heavy, and dark green, with characteristics favourable for post-harvest storage.

'Marathon' is recommended for spring production and adapts readily to a wide range of climatic conditions, reaching maturity after approximately 90 days from sowing. The inflorescences are large, heavy, compact, and green, and the hybrid is well suited for freeze preservation.

'Fiesta' is intended for spring and summer growing seasons, maturing approximately 80 days from sowing. It is recommended for dense cropping systems with harvest of the main inflorescence only, excluding secondary lateral shoots. The main head is compact and dark green in colour.

Three organic fertilisers were used: Lignohumate (dose of 1 kg/ha), Alcygol Z2M (dose of 5 l/ha) and Alg Green (dose of 1 l/ha), applied 30 days after transplanting as foliar sprays using a backpack sprayer. Also, one variant was left unfertilised as the control.

Experimental Design and Cultural Practices

The experiment was arranged in a randomised complete block design with three replications. Each experimental unit measured 32 m² (4 m × 8 m). Seeds were sown in late January to produce transplant seedlings. Approximately one month after the sowing date, the seedlings were pricked out into 7 × 7 cm individual pots to allow adequate root development prior to field transplanting.

Seedlings were transplanted into the polyethylene tunnel at a planting density of 50,000 plants ha^{-1}, arranged on two rows per growing bed. Final yield data were obtained by averaging the values recorded across the three replications in order to minimise experimental error. Establishment of broccoli culture was carried out on 15 March (2024). Combining the two experimental factors (cultivar × fertiliser) resulted in 12 experimental variants arranged within the polyethylene tunnel.

During the growing season, the development degree and plant growth (plant height, leaf rosette diameter, number of leaves) and production were determined.

Harvest and Yield Assessment

At commercial maturity, the main inflorescence of each plot was harvested, and inflorescence fresh weight (g) was recorded as the primary yield parameter. Representative fresh samples

of the main inflorescence from each hybrid were immediately transported to the laboratory for biochemical analysis.

Biochemical Analyses

Chlorophyll a and b Determination

Chlorophyll a and b concentrations were determined spectrophotometrically according to the method of Arnon (1949). Inflorescence tissue was finely homogenised using an Ultra-Turrax disperser, and pigments were extracted with 80% (v/v) aqueous acetone under continuous magnetic stirring for two hours in an Erlenmeyer flask protected from light. The extract was vacuum-filtered and the residue re-extracted repeatedly with fresh solvent until the plant material became colourless. The combined extracts were made up to a known volume. Absorbance was measured at 645 nm and 663 nm using a UV-VIS spectrophotometer (Jasco V-530, Tokyo, Japan).

Chlorophyll concentrations were calculated using the following equations:

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = (12.7 \times E663 - 2.69 \times E645) \times v / (1000 \times g)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = (22.9 \times E645 - 4.68 \times E663) \times v / (1000 \times g)$$

where E663 and E645 are the absorbance values at 663 nm and 645 nm, respectively; v is the total volume of the acetone extract (mL); and g is the fresh weight of the extracted tissue (g).

Vitamin C Determination

The ascorbic acid (vitamin C) content was determined by a titrimetric iodometric method. Between 5 and 10 g of fresh inflorescence tissue were weighed, immediately transferred into a mortar containing 20 mL of 2% (v/v) hydrochloric acid to prevent oxidative degradation, and ground to a fine homogenate. The material was transferred quantitatively into a graduated cylinder and the volume was adjusted to 100 mL with distilled water. After thorough mixing and 10 minutes of extraction with periodic stirring, the suspension was filtered through a fluted filter paper into a clean, dry conical flask.

An aliquot of 10 mL of the filtrate was transferred to a conical flask and diluted with 30 mL of distilled water. To this, 5 mL of 1% (w/v) potassium iodide and 0.5 mL of 1% (w/v) starch indicator solution were added. The mixture was titrated with 0.0004 N potassium iodate until a pale blue coloration persisted for at least 30 seconds. Ascorbic acid content was calculated using the formula:

$$\text{mg ascorbic acid/100 g FW} = (100 \times t \times v \times d) / M$$

where t is the titre of the 0.0004 N iodine solution relative to ascorbic acid; v is the volume (mL) of 0.0004 N iodine solution consumed in titration; d is the dilution factor; and M is the mass (g) of the analysed sample.

Carotenoid Determination

Total carotenoid content was determined by a combined spectrophotometric and HPLC approach. For extraction, 10 g of fresh inflorescence tissue were homogenised with a mixture of methanol, ethyl acetate, and petroleum ether (1:1:1, v/v/v) and allowed to extract for four hours. After vacuum filtration, the residue was re-extracted twice with the same solvent system. The pooled extracts were sequentially washed in a separating funnel with water, diethyl ether, and saturated NaCl solution. The ethereal phase was evaporated to dryness under reduced pressure using a rotary evaporator at 35°C. The resulting oleoresin was dissolved in a known volume of diethyl ether and total carotenoid content was estimated spectrophotometrically at 450 nm using an absorption coefficient of A1%1cm = 2500, with a UV-VIS spectrophotometer (Jasco V-530, Tokyo, Japan), according to the formula:

$$X\ (\text{mg carotenoids g}^{-1}\text{ FW}) = (A450 \times V \times 1000) / (2500 \times W \times 100)$$

where A450 is the absorbance at 450 nm; V is the volume of the extract (mL); 2500 is the percentage extinction coefficient of carotenoids (A1%1cm); and W is the fresh weight of the sample (g).

Individual carotenoid profiling was performed by HPLC using a Shimadzu LC-20AT system equipped with an SPD-M20A photodiode array (PDA) detector and a YMC C30 column (250 mm × 4.6 mm, 5 µm). The mobile phases were: Solvent A - methanol:methyl tert-butyl ether (MTBE): water (81:15:4, v/v/v) and Solvent B - MTBE: methanol:water (90:7:3, v/v/v). Gradient elution was applied as follows: 0-50 min, 0-55% B; 50-51 min, 55-60% B; 51-52 min, 60% B isocratic; 52-54 min, 60-1% B; followed by re-equilibration. Carotenoids were identified by comparing chromatographic retention times

with those of authentic reference standards and by matching their UV-VIS absorption spectra recorded by the PDA detector; cis-isomers of α- and β-carotene, for which no standards were available, were identified based on their characteristic spectral profiles.

Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu (F-C) colorimetric method as described by Singleton and Rossi (1965) and subsequently adapted for plant tissue by Ainsworth and Gillespie (2007). Briefly, a methanolic extract of broccoli inflorescence tissue was prepared and reacted with the F-C reagent under alkaline conditions (sodium carbonate solution, pH ~10). The resulting blue chromophore, formed through electron transfer from phenolic hydroxyl groups to the phosphomolybdic–phosphotungstic acid complexes, was measured spectrophotometrically at 765 nm. Results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight (mg GAE 100 g^{-1} FW), using a gallic acid calibration curve.

Antioxidant Activity

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to Brand-Williams et al. (1995). Methanolic broccoli extracts were mixed with a DPPH radical solution in methanol, and the decrease in absorbance at 515-517 nm was monitored after a defined reaction period. The radical scavenging capacity was expressed as the effective concentration causing 50% reduction of the initial DPPH absorbance (EC50, mg mL^{-1}) or as Trolox equivalent antioxidant capacity (TEAC, μmol Trolox g^{-1} FW). To complement the DPPH assay, antioxidant activity was additionally evaluated by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorisation assay (Re et al., 1999), in which the pre-formed ABTS•+ radical was generated by oxidation with potassium persulfate and the decrease in absorbance at 734 nm upon addition of the extract was used to calculate TEAC values relative to Trolox.

Nitrate Concentration

Nitrate concentration in broccoli inflorescences was determined by ion chromatography (IC) with conductivity detection, following the approach described by Cavazos-Garduño et al. (2020) and adapted from the methods validated by the Association of Official Analytical Chemists (AOAC). Briefly, fresh tissue was extracted in hot deionised water (70°C), the extract was filtered and diluted appropriately, and anions were separated on an anion-exchange column using a potassium hydroxide eluent with suppressed conductivity detection. Results were expressed as milligrams of nitrate (NO_3^-) per kilogram of fresh weight (mg kg^{-1} FW). As an additional verification, selected samples were analysed by a visible spectrophotometric (colorimetric) method using N-(1-naphthyl) ethylenediamine dihydrochloride and sulfanilic acid as chromogenic reagents, with absorbance measured at 540 nm (Nerdy, 2018), to confirm the accuracy of the IC determinations.

Statistical Analysis

All biochemical analyses were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Data were analysed using ANOVA. Means were separated using Duncan's multiple range test at the significance level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Broccoli hybrids showed comparable vegetative growth (Table 1), with plant heights ranging from 20.50 cm (Belstar) to 22.00 cm (Fiesta), leaf numbers from 7.20 (Belstar) to 7.80 (Marathon), and total fresh weights from 10.40 g (Belstar) to 12.80 g (Marathon). The relatively homogeneous initial conditions across genotypes are important for minimising pre-existing variability and enabling subsequent growth and yield differences to be attributed to hybrid identity and fertilizer treatment. Differences in biomass were not high: Marathon produced the highest leaf fresh weight (8.40 g), while Fiesta showed the greatest stem mass (2.50 g), and Belstar the lowest total biomass (10.40 g), differences likely reflecting inherent genotypic variation in early seedling vigour. The importance of uniform transplant quality for reliable broccoli field experiments has been emphasised by Thapa and Rai (2012), who noted that standardised seedling characteristics at the time

of planting are a precondition for valid comparisons of cultivar performance. The morphological variation observed among hybrids at this stage is consistent with the germplasm-level diversity documented in *Brassica oleracea* var. *italica*, where even closely related commercial hybrids can differ significantly in seedling architecture due to differences in germination rate, cotyledon area, and hypocotyl growth. (Farnham et al., 2000).

Figure 1 illustrates representative broccoli plants from the experiment at the time of transplanting into the polyethylene tunnel. The photographs document the general morphology and seedling development stage, showing well-developed root systems, compact rosette architecture, and uniformly expanded leaves characteristic of high-quality broccoli seedling produced under controlled nursery conditions. Plants displayed dark green foliage with no visible symptoms of nutrient deficiency, pathogen infection, or mechanical damage, confirming the suitability of the transplant material for the experimental trial. The visual uniformity among transplants across the three cultivars supports the interpretation that no systematic bias in initial plant quality was introduced into the experiment.

Table 1. Development of broccoli plants at transplanting

Variant	Height (cm)	No. Of Leaves	Plant weight (g)			
			Total wt (g)	Leaf wt (g)	Stem wt (g)	Root wt (g)
Fiesta	22.00	7.60	12.60	8.00	2.50	2.10
Marathon	21.25	7.80	12.80	8.40	2.00	2.40
Belstar	20.50	7.20	10.40	6.60	2.00	1.80



Figure 1. Details of broccoli plants from the experiment

Vegetative growth parameters of the three broccoli hybrids recorded 30 days after transplanting are presented in Table 2, prior to the application of organic fertilizers. No statistically significant differences were detected among hybrids for plant height (44.67-46.25 cm), rosette diameter (43.84-45.86 cm), or leaf number (11.00-13.30 leaves), as all means were assigned the same Duncan grouping (a). The absence of significant inter-cultivar differences at this stage confirms that growth conditions within the polyethylene tunnel were uniform and that no systematic competitive advantage existed among genotypes before the fertilizer treatments were imposed. These findings provide a sound baseline for interpreting the significant differences observed at 60 days (Table 3) as responses to the combined effect of hybrid genotype and fertilizer treatment rather than pre-existing growth differentials. The plant height values recorded (44.67-46.25 cm) are comparable with those reported by Thapa and Rai (2012) for broccoli cultivars under polyhouse conditions at a similar growth stage (30.78-41.69 cm), with the somewhat higher values in the present study likely reflecting the warmer microclimate of spring production in Cluj-Napoca and the more favourable transplant size achieved by the 7×7 cm pot-grown seedlings. Damato and Trotta (2002) also noted that early vegetative growth in broccoli under protected cultivation is strongly influenced by light interception and air temperature, with tunnel-grown plants generally developing faster than open-field counterparts at equivalent calendar dates during spring.

Table 2. Plant growth before fertilizer application (30 days after planting)

Hybrid	Plant height (cm)		Plant diameter (cm)		Number of leaves	
Fiesta	44.80	a	43.84	a	11.70	a
Marathon	46.25	a	45.86	a	13.30	a
Belstar	44.67	a	45.42	a	11.00	a
LSD P = .05 Standard Deviation	LSD=3.081 SD=0.877		LSD=3.732 SD=1.062		LSD=4.142 SD=1.179	
Means followed by the same letter or symbol do not significantly differ (P = .05, Duncan's New MRT)						

The combined effects of cultivar and organic fertilizer on broccoli plant growth at 60 days after transplanting, a stage corresponding to active head development and close to the onset

of commercial maturity, are presented in Table 3. Statistically significant differences were recorded among all 12 treatment combinations for plant height, rosette diameter, and leaf number, indicating that both hybrid and fertilizer treatments, and their interaction, significantly influenced vegetative development at this stage.

Within hybrid factor, Marathon consistently obtained higher plant height under fertilized conditions (up to 80.27 cm with Lignohumate), while Belstar produced the highest leaf number (up to 20.20 with Lignohumate). Fiesta showed intermediate responses for most parameters. These cultivar-specific patterns are consistent with the genetic diversity in vegetative architecture documented for commercial broccoli hybrids: cultivars adapted to spring production tend to differ in canopy geometry, with some prioritising lateral leaf expansion (associated with higher leaf number and rosette diameter) and others favouring vertical growth (associated with greater plant height). Farnham et al. (2000) observed significant cultivar-by-environment interactions for vegetative growth traits in broccoli and emphasised that the expression of these traits is strongly modulated by growing conditions and applied inputs.

Among fertilizer treatments, Lignohumate produced the greatest growth stimulus across all three hybrids and all three measured parameters, followed in most cases by Alcygol Z2M and Alg Green, with unfertilized controls consistently recording lower values. The growth-promoting effects of humic acid-based products, including Lignohumate, are well documented in the Brassica literature. Selim and Mosa (2012) demonstrated that humic substance applications significantly increased plant height, number of leaves, and leaf area in broccoli grown under sandy soil conditions, attributing these effects to enhanced nutrient uptake, stimulation of root growth, and auxin-like activity of humic molecules that promotes cell elongation. Ameri and Tehranifar (2012) similarly reported that potassium humate treatments increased broccoli plant height and leaf number relative to untreated controls, with effects comparable in magnitude to those observed in the present study. The broader physiological basis for these responses has been reviewed by Canellas et al. (2021) and Yakhin et al. (2017), who described the multiple mechanisms through which humic substances act as biostimulants, including the promotion of H+-ATPase activity, enhancement of lateral root formation, and chelation of micronutrients such as iron and zinc, which are essential for chlorophyll synthesis and enzyme function. The seaweed-based biostimulants Alcygol Z2M and Alg Green also produced significant growth increments relative to the unfertilized controls, consistent with the growing body of evidence supporting the use of macroalgae-derived extracts in Brassica production.

Craigie (2011) reviewed the biostimulant activity of seaweed extracts and documented their content of natural cytokinins, auxins, betaines, and polyamines, all of which contribute to enhanced cell division and elongation.

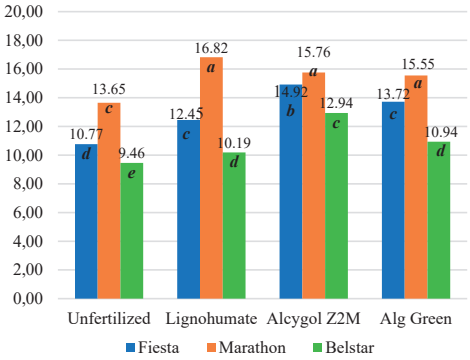
Table 3. Influence of cultivar and fertilizer on broccoli plant growth (60 days after planting)

Variant		Plant height (cm)		Plant diameter (cm)		Number of leaves	
Hybrid	Fertilizer						
Fiesta	Unfertilized	70.77	d	62.67	j	16.00	*g*
	Lignohumate	77.46	c	71.10	b	18.00	*c*
	Alcygol Z2M	75.43	d	69.80	g	16.40	*f*
	Alg Green	73.72	d	66.35	a	16.00	*g*
Marathon	Unfertilized	73.65	e	64.00	j	16.50	*f*
	Lignohumate	80.27	d	75.60	f	19.80	*b*
	Alcygol Z2M	79.48	a	68.80	c	18.00	*c*
	Alg Green	75.55	f	68.25	i	17.40	*d*
Belstar	Unfertilized	71.46	g	58.53	j	17.50	*d*
	Lignohumate	79.47	g	74.80	e	20.20	*a*
	Alcygol Z2M	75.88	e	65.65	h	18.60	*c*
	Alg Green	75.94	b	62.05	d	18.50	*c*
LSD P=.05 Standard Deviation		LSD=4.953 SD=3.061		LSD=8.576 SD=5.129		LSD=1.869 SD=1.392	
Means followed by the same letter or symbol do not significantly differ (P=.05, Duncan's New MRT)							

Zodape et al. (2011) reported increased plant height and leaf area index in cabbage (Brassica oleracea var. capitata) treated with seaweed extract at application rates similar to those used in the present study, while Bulgari et al. (2019) reviewed multiple studies demonstrating that seaweed-based biostimulants improve vegetative growth in leafy Brassica crops by improving mineral nutrition and stimulating endogenous phytohormone activity. The ranking of Lignohumate > seaweed extracts > control observed in the present study for most growth parameters aligns with the comparative biostimulant efficiency reported by Yakhin et al. (2017), who found that humate-based

products generally elicit stronger vegetative growth responses than seaweed-based products when applied as foliar sprays or soil drenches to brassicaceous crops.

Total broccoli production (t ha^{-1}) recorded across all hybrid and fertilizer treatment combinations, allowing a direct visual comparison of productive performance, is presented in Figure 2. The chart clearly illustrates the differences between cultivars, with Marathon consistently achieving the higher yields, regardless of fertilizer treatment, followed by Fiesta, and Belstar recording the lowest production values across all treatments. Within each cultivar, the progressive increase in yield from the unfertilized control through the seaweed-based treatments (Alcygol Z2M, Alg Green) to the Lignohumate-treated variant is visually apparent, reinforcing the conclusion that all three biostimulants exerted a positive effect on production, with Lignohumate being the most effective.



Means followed by the same letter or symbol do not significantly differ (P=.05, Duncan's New MRT)
LSD P=.05 0.601
Standard Deviation 0.414

Figure 2. Broccoli production influenced by hybrid and fertilizer

Yield range depicted in Figure 2 (9.46-16.82 t ha^{-1}) is similar with data reported in the literature for spring broccoli production. Thapa and Rai (2012) recorded yields of 6.75-14.55 t ha^{-1} for broccoli cultivars grown under polyethylene tunnel conditions in India, with cultivar differences similar to those visible in Figure 2. Sace and Genuino (2016) documented spring broccoli yields of 11.5-15.8 t ha^{-1} under conventional nitrogen fertilization regimes in the Philippines, values that overlap substantially with the fertilized treatments in the present study, suggesting that the organic biostimulants evaluated here are capable of matching the yield-promoting effect of standard mineral nitrogen inputs. Damato and Trotta (2002) reported head fresh weights corresponding to field yields in the range of 10-18 t ha^{-1} for broccoli cultivars grown under protected cultivation in southern Italy, confirming that the production levels achieved in the present experiment are well within the upper range documented for spring broccoli under tunnel conditions in temperate European climates. The differences between the unfertilized Belstar treatment (9.46 t ha^{-1}) and the best-performing Marathon + Lignohumate combination (16.82 t ha^{-1}) represent a yield difference of approximately 78%, which can be attributed to the combined advantage of superior hybrid productive potential and the growth-promoting activity of humic acid-based biostimulants (Selim and Mosa, 2012; Yakhin et al., 2017).

Table 4. Influence of cultivar and organic fertilizers on chlorophyll, carotenoid and vitamin C content of broccoli inflorescences (2024)

Variant		Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Vitamin C (mg 100g^{-1} FW)
Hybrid	Fertilizer				
Fiesta	Unfertilized	0.82±0.04 ijk	0.31±0.02 ij	0.048±0.002 ghijk	68.5±2.1 ijk
	Lignohumate	0.91±0.03 bcd	0.35±0.02 bcd	0.054±0.003 bcd	75.2±2.4 cd
	Alcygol Z2M	0.86±0.04 fgh	0.33±0.02 efg	0.051±0.003 defgh	71.8±2.7 efgh
	Alg Green	0.93±0.05 bc	0.36±0.02 ab	0.056±0.004 ab	77.4±2.9 bc
Marathon	Unfertilized	0.79±0.03 kl	0.29±0.01 l	0.045±0.002 kl	65.3±2.0 l
	Lignohumate	0.88±0.04 fghi	0.33±0.02 efgh	0.052±0.003 defg	72.6±2.5 efg
	Alcygol Z2M	0.84±0.03 hij	0.31±0.02 ij	0.049±0.002 efghij	69.7±2.3 hij
	Alg Green	0.90±0.04 cde	0.34±0.02 de	0.053±0.003 bcde	74.1±2.6 de
Belstar	Unfertilized	0.85±0.04 ijk	0.32±0.02 ghi	0.050±0.003 defghi	70.2±2.2 hi
	Lignohumate	0.94±0.04 ab	0.36±0.02 ab	0.056±0.003 abc	78.0±2.8 ab
	Alcygol Z2M	0.89±0.04 def	0.34±0.02 de	0.053±0.003 bcde	73.4±2.5 def
	Alg Green	0.97±0.05 abc	0.37±0.02 a	0.058±0.004 a	80.1±3.0 a
LSD	0.032	0.018	0.004	2.31	

Variant		Chlorophyll a (mg g⁻¹ FW)	Chlorophyll b (mg g⁻¹ FW)	Carotenoids (mg g⁻¹ FW)	Vitamin C (mg 100g⁻¹ FW)
Hybrid	Fertilizer				
P=0.05					
Std. Dev.	0.022	0.013	0.003	1.59	

Means followed by the same letter within a column do not significantly differ (P ≤ 0.05, Duncan's New Multiple Range Test). FW = Fresh Weight.

Concentrations of chlorophyll a, chlorophyll b, total carotenoids, and vitamin C in broccoli inflorescences are presented in Table 4. All four biochemical parameters showed statistically significant variation among treatment combinations, confirming that both hybrid genotype and fertilizer regime meaningfully influence the nutritional quality of broccoli inflorescences at harvest.

Chlorophyll a concentration ranged from 0.79 mg g^{-1} FW (Marathon unfertilized) to 0.97 mg g^{-1} FW (Belstar + Alg Green), and chlorophyll b from 0.29 to 0.37 mg g^{-1} FW. These values are consistent with the range reported by Murcia et al. (2000), who determined chlorophyll contents in raw broccoli inflorescences of 0.10-0.14 mg g^{-1} FW for chlorophyll a and 0.04-0.05 mg g^{-1} FW for chlorophyll b, and by Vallejo et al. (2003), who documented chlorophyll a values up to 0.93 mg g^{-1} FW across a range of *Brassica oleracea* genotypes. The consistent pattern in the present study, with Belstar accumulating the highest chlorophyll concentrations and Marathon the lowest, suggests a genotypic difference in pigment biosynthesis or retention capacity. Among fertilizer treatments, Alg Green and Lignohumate systematically produced the highest chlorophyll a and b concentrations within each hybrid, with statistically significant differences from the unfertilized controls. The positive effect of humic acid-based products on chlorophyll accumulation has been attributed to their ability to enhance iron bioavailability and stimulate the biosynthesis of delta-aminolevulinic acid, a key precursor in the chlorophyll biosynthetic pathway (Ameri and Tehranifar, 2012; Canellas et al., 2021). The stimulatory effect of seaweed extracts (Alg Green, Alcygol Z2M) on leaf and inflorescence greenness is consistent with their content of natural cytokinins, which delay chlorophyll degradation by retarding senescence-associated protease and chlorophyllase activity (Craigie, 2011). Abdel-Mawgoud et al. (2012) confirmed these mechanisms in a study of broccoli treated with bio-organic fertilizers, reporting that chlorophyll concentrations in inflorescences were significantly higher in organically fertilized plants than in controls, with variation among cultivars comparable to that observed in the present experiment.

Total carotenoid concentrations ranged from 0.045 mg g^{-1} FW (Marathon unfertilized) to 0.058 mg g^{-1} FW (Belstar + Alg Green). These values are similar to those obtained by Liu et al. (2018) for broccoli inflorescences (approximately 0.04-0.07 mg g^{-1} FW), with the variation among treatments attributable to differences in genotype, fertilizer input, and potentially microenvironmental conditions within the tunnel. Carotenoids in broccoli inflorescences are predominantly represented by β-carotene and lutein, both of which contribute to provitamin A activity, blue-light photoprotection, and antioxidant capacity (Kurilich et al., 2002; Farnham and Kopsell, 2006). The systematic enhancement of carotenoid content by Alg Green treatment across all three hybrids is consistent with the algae-derived xanthophyll precursors present in seaweed extracts and with the improved photosynthetic metabolism associated with cytokinin-containing biostimulants, which promote the expression of carotenoid biosynthetic genes such as phytoene synthase and lycopene cyclase (Craigie, 2011).

Vitamin C (ascorbic acid) content ranged from 65.3 mg 100 g^{-1} FW (Marathon unfertilized) to 80.1 mg 100 g^{-1} FW (Belstar + Alg Green). These values exceed the commonly cited reference value of approximately 55 mg 100 g^{-1} FW for raw broccoli and fall within the range of 57.4–131.4 mg 100 g^{-1} FW documented by Kurilich et al. (2009) across commercial broccoli samples. The superior vitamin C content under fertilized treatments, particularly Alg Green and Lignohumate, relative to unfertilized controls is consistent with the hypothesis that organic nitrogen sources, by promoting gradual amino acid synthesis rather than rapid nitrate accumulation, allow a greater proportion of photosynthetically fixed carbon to be allocated to secondary metabolite pathways including ascorbate biosynthesis (Nguyen et al., 2025). The consistently higher vitamin C values recorded for Belstar relative

to Marathon and Fiesta across all fertilizer treatments confirm that cultivar genotype is a primary determinant of ascorbic acid accumulation in broccoli, a finding corroborated by Vallejo et al. (2003), who observed over twice as much in vitamin C content among nine *Brassica oleracea* genotypes grown under identical conditions.

Total phenolic content (TPC), DPPH radical scavenging activity (EC_{50}), and nitrate concentration in broccoli inflorescences are summarized in Table 5, as affected by cultivar and organic fertilizer treatment. All three parameters varied significantly among the 12 treatment combinations, demonstrating that both genotype and biostimulant application exert meaningful effects on the phytochemical profile and food safety attributes of broccoli inflorescences.

Total phenolic content ranged from 19.8 mg GAE 100 g^{-1} FW (Marathon unfertilized) to 30.2 mg GAE 100 g^{-1} FW (Belstar + Alg Green).

Table 5. Influence of cultivar and organic fertilizers on total phenolic content, antioxidant activity and nitrate concentration of broccoli inflorescences (2024)

Variant		Total Phenolic Content (mg GAE 100g^{-1} FW)	DPPH EC_{50} (mg mL^{-1})	Nitrate (mg kg^{-1} FW)
Hybrid	Fertilizer			
Fiesta	Unfertilized	21.4±0.8 k	0.85±0.04 ijk	342±18 bc
	Lignohumate	27.3±1.1 de	0.96±0.05 abcd	318±15 ghi
	Alcygol Z2M	24.6±0.9 gh	0.91±0.04 defgh	335±16 cdef
	Alg Green	29.1±1.2 ab	0.98±0.06 ab	310±14 hijk
Marathon	Unfertilized	19.8±0.7 l	0.80±0.03 kl	358±19 a
	Lignohumate	25.9±1.0 f	0.93±0.05 bcdef	325±16 efg
	Alcygol Z2M	23.2±0.9 i	0.88±0.04 fghi	341±17 bcd
	Alg Green	27.7±1.1 cd	0.95±0.05 bcde	319±15 gh
Belstar	Unfertilized	22.6±0.9 ij	0.87±0.04 ghij	350±18 ab
	Lignohumate	28.5±1.1 bc	0.97±0.05 abc	312±14 hij
	Alcygol Z2M	25.4±1.0 fg	0.92±0.05 cdefg	337±16 cde
	Alg Green	30.2±1.3 a	1.01±0.06 a	305±13 jkl
LSD P=0.05		1.12	0.052	12.4
Std. Dev.		0.77	0.036	8.51

Means followed by the same letter within a column do not significantly differ ($P \leq 0.05$, Duncan's New Multiple Range Test). FW = Fresh Weight.
GAE = gallic acid equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity expressed as EC_{50}.

These values are consistent with the range of 17-42 mg GAE 100 g^{-1} FW reported for fresh broccoli inflorescences by Naguib et al. (2012) using the Folin-Ciocalteu method under comparable extraction conditions, and they overlap with the values documented by Abdel-Mawgoud et al. (2012) for organically fertilized broccoli (20-32 mg GAE 100 g^{-1} FW). The highest TPC values were consistently observed under Alg Green treatment across all three cultivars, suggesting that the phenolic catalyst activity of algae-derived compounds, including oligosaccharides and polysaccharide fragments released during extract preparation, may actively stimulate the phenylpropanoid pathway in broccoli inflorescences. Comparable seaweed-induced enhancement of phenolic content in *Brassica* crops has been reported by Zodape et al. (2011) and reviewed by Bulgari et al. (2019), who attributed this effect to a mild oxidative stress response triggered by algae-derived elicitors that up-regulates the expression of phenylalanine ammonia-lyase (PAL) and downstream biosynthetic enzymes. Lignohumate also significantly enhanced TPC relative to unfertilized controls in all hybrids, consistent with the findings of Selim and Mosa (2012) and with the proposed role of humic substances as indirect triggers of secondary metabolism through their effects on soil microbial activity and root exudate composition.

DPPH EC_{50} values ranged from 0.80 mg mL^{-1} (Marathon unfertilized) to 1.01 mg mL^{-1} (Belstar + Alg Green). In the DPPH assay, higher EC_{50} values indicate that a greater extract concentration is required to achieve 50% radical inhibition; therefore, Marathon unfertilized exhibited the lowest antioxidant capacity per unit mass, while Belstar + Alg Green required the highest extract concentration. This apparent paradox - in which the treatment with the highest TPC also shows the highest EC_{50} (lowest efficiency per mg) - may reflect qualitative shifts in the phenolic profile rather than a simple quantitative increase. As noted by Gülçin et al. (2004), the DPPH assay is sensitive to the chemical structure of individual phenolics, with hydroxycinnamic acids and flavonols being more reactive than hydroxybenzoic acid derivatives; if biostimulant treatment promotes

the accumulation of less-reactive structural phenolics (e.g., cell wall-bound hydroxycinnamic acid esters), TPC may increase without a proportional improvement in DPPH scavenging efficiency. This interpretation is consistent with the observation by Slávińska et al. (2025) that the relationship between TPC and antioxidant capacity in broccoli is non-linear and highly dependent on the relative proportions of individual phenolic classes. The moderate range of EC_{50} variation among treatments (0.80-1.01 mg mL^{-1}) indicates that all combinations maintained substantial antioxidant capacity, confirming broccoli as a valuable dietary source of antioxidants regardless of cultivar or fertilizer treatment.

Nitrate concentrations in inflorescences ranged from 305 mg kg^{-1} FW (Belstar + Alg Green) to 358 mg kg^{-1} FW (Marathon unfertilized). All recorded values fall well below the maximum limits established by European Union Regulation (EC) No 1258/2011 for high-accumulating vegetables (1000-3000 mg kg^{-1} FW for leafy vegetables), confirming the food safety of the harvested inflorescences under all treatment conditions. The relatively low nitrate concentrations in broccoli inflorescences relative to leaf tissue are consistent with the species-level data reviewed by Santamaria (2006) and Bian et al. (2020), who classified Brassica inflorescences as moderate-to-low nitrate accumulators compared to spinach, lettuce, or celery. A reverse relationship between organic fertilizer application and nitrate concentration was observed in the present study: plants receiving Alg Green and Lignohumate accumulated significantly less nitrate than unfertilized controls in all three hybrids. This finding is consistent with the mechanism proposed by Colla et al. (2018), whereby organic nitrogen sources promote the gradual mineralisation of nitrogen and its rapid assimilation into amino acids and proteins via enhanced glutamine synthetase activity, reducing the pool of free nitrate remaining in inflorescence tissue. Nguyen et al. (2025) confirmed a similar nitrate-diluting effect of organic fertilizers in leafy Brassica crops, attributing it to both the slow-release nitrogen supply and the stimulation of nitrate reductase activity by humate and seaweed-derived compounds. From a food quality perspective, the reduction in nitrate content under organic fertilization, combined with concurrent increases in vitamin C and phenolic content, represents a favourable nutritional outcome that adds value to organically produced broccoli.

CONCLUSIONS

The present study investigated the vegetative growth, yield, and biochemical quality of three broccoli hybrids (Fiesta, Marathon, and Belstar) cultivated under polyethylene tunnel conditions in Cluj-Napoca, Romania, in response to three organic fertilizer treatments - Lignohumate (1 kg ha^{-1}), Alcygol Z2M (5 L ha^{-1}), and Alg Green (1 L ha^{-1}) - applied 30 days after transplanting, alongside an unfertilized control. The results consistently demonstrated that both cultivar and fertilizer type independently and significantly influenced every parameter assessed.

Regarding vegetative development, all three hybrids showed homogeneous growth at 30 days after transplanting, prior to fertilizer application, confirming the validity of the experimental starting point. By 60 days after transplanting, statistically significant differences were found among all 12 hybrid-fertilizer combinations for plant height, rosette diameter, and number of leaves. Lignohumate consistently promoted the greatest vegetative growth across all cultivars, recording the highest plant heights in Marathon (80.27 cm) and Belstar (79.47 cm) and the greatest leaf number in Belstar (20.20 leaves). Seaweed-based biostimulants (Alcygol Z2M and Alg Green) produced intermediate but significant growth increments relative to the unfertilized controls.

Total inflorescence yield ranged from 9.46 t ha^{-1} (Belstar unfertilized) to 16.82 t ha^{-1} (Marathon + Lignohumate), with Marathon consistently outperforming Fiesta and Belstar across all fertilizer treatments. Lignohumate application increased yield by 7.7% in Belstar, 15.6% in Fiesta, and 23.2% in Marathon relative to their respective unfertilized controls, demonstrating that the humate-based biostimulant not only elevated absolute yield but also amplified genotype-specific productive potential. The yield levels recorded for

fertilized treatments compare favourably with spring broccoli production data reported in the international literature under both protected and open-field conditions, confirming the agro-climatic suitability of the Cluj-Napoca polyethylene tunnel environment for high-yielding spring broccoli cultivation.

Biochemical analyses of inflorescence tissue revealed that organic fertilization significantly enhanced nutritional quality across all measured parameters. Chlorophyll a content ranged from 0.79 to 0.97 mg g^{-1} FW, chlorophyll b from 0.29 to 0.37 mg g^{-1} FW, and total carotenoids from 0.045 to 0.058 mg g^{-1} FW, with Belstar and Alg Green treatments consistently producing the highest pigment concentrations. Vitamin C content ranged from 65.3 mg 100 g^{-1} FW (Marathon unfertilized) to 80.1 mg 100 g^{-1} FW (Belstar + Alg Green), exceeding cited reference values for commercial broccoli and confirming the superior nutritional quality achievable through organic biostimulant application. Total phenolic content ranged from 19.8 to 30.2 mg GAE 100 g^{-1} FW, with Alg Green treatment eliciting the highest accumulation of phenolics across all three cultivars, likely through the elicitor activity of algae-derived oligosaccharides on the phenylpropanoid biosynthetic pathway. DPPH radical scavenging capacity (EC_{50}: 0.80-1.01 mg mL^{-1}) did not show a direct linear relationship with total phenolic content, indicating that antioxidant capacity is influenced not only by the total amount of phenolics but also by their specific chemical composition and reactivity.

Nitrate concentrations in broccoli inflorescences were low across all treatments (305-358 mg kg^{-1} FW), well below the European Union regulatory limits for vegetables. Plants treated with Alg Green and Lignohumate consistently accumulated less nitrate than unfertilized controls. This result is attributable to the slow-release organic nitrogen supply and the stimulation of nitrate reductase activity associated with humate- and seaweed-derived compounds.

From a practical standpoint, the results of this study support the following recommendations for spring broccoli production under protected cultivation in temperate climates: (i) Marathon hybrid should be preferred where maximising total inflorescence yield is the primary objective; (ii) Belstar hybrid is recommended where superior biochemical quality - particularly vitamin C, chlorophyll, and phenolic content - is the priority; (iii) Lignohumate at 1 kg ha^{-1}, applied 30 days after transplanting, represents the most effective single biostimulant treatment for simultaneously improving both vegetative growth and yield; (iv) Alg Green at 1 L ha^{-1} is the preferred option for maximising nutritional quality parameters, particularly total phenolic content and vitamin C, while simultaneously minimising nitrate accumulation.

In conclusion, this study demonstrates that the combined selection of an appropriate broccoli hybrid and a well-matched organic biostimulant can substantially improve both the agronomic and nutritional performance of spring broccoli grown under polyethylene tunnel conditions. The consistent superiority of Lignohumate for growth and yield promotion, and of Alg Green for biochemical quality enhancement, provides a sound evidence base for the development of integrated, organic-compatible production strategies for broccoli in the north-western Romania climate. Future research should investigate multi-season reproducibility of these results, the effects of combined biostimulant applications, and the potential interaction between organic fertilization and post-harvest storage quality of the inflorescences.

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