Identification of bioactive compounds in broccoli using biofertilizer and aqueous plants compost

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Abstract

A field experiment was conducted in Al-Musaib project area (45 km north of Babylon) to detect and quantify bioactive compounds in the fruits of two broccoli genotypes, using High Performance Liquid Chromatography (HPLC). The experiment included three factors: the first factor was two broccoli genotypes (Max and Azhar), the second factor was the application of aquatic plant compost at three levels (0.00, 2, and 4 kg/unit), and the third factor was soil treatment with different microorganisms (untreated, *Azospirillum* spp. bacteria, and *Trichoderma* spp. fungus). The experiment was designed as a Randomized Complete Block Design (RCBD) with three replications per treatment, each replication consisting of three experimental units. The results showed that the broccoli fruits contained bioactive compounds, and these were affected by the experimental treatments. The Azhar genotype treated with the fungal inoculant and 4 kg/unit of compost showed significantly higher concentrations of Sulforaphane and vitamin C, with average values of 326.04 ppm and 122.55 mg/100 g, respectively. The same genotype, when treated with the fungal inoculant and 2 kg/unit of compost, showed higher levels of glutathione and folic acid in the fruits, reaching 125.6 ppm and 115.80 mg/100 g, respectively. Therefore, we conclude that HPLC is an important technique for detecting bioactive compounds in the fruits of two broccoli genotypes, especially when combined with the application of bio-inoculants and aquatic plant compost.

Keywords: Broccoli florets, HPLC technique, Sulforaphane, aqueous compost, biofertilizers

Introduction

Interest in medicinal plants began as soon as humans became self-aware and started utilizing nature's resources to serve their needs. This interest has grown with the advancement of human knowledge, encompassing various aspects of human needs, including medicinal plants [1].

Broccoli (*Brassica oleracea*) is an annual herbaceous plant belonging to the winter vegetables group. Morphologically, it resembles cauliflower and belongs to the Brassicaceae family, It is not widely cultivated in Iraq. It is grown for its edible flower heads, which are harvested when they are in the green bud stage, along with their tender, fleshy stalks. It is considered one of the most nutritionally rich crops in this family and one of the most used for its therapeutic properties [2]. It contains many vitamins such as A, B1, B2, B6, and B17, as well as minerals such as calcium, sodium, potassium, manganese, zinc, and iron. It is also rich in provitamin A and beta-carotene, and its leaves are a source of polyphenols, fats, and fiber [3]. This plant has not received sufficient attention regarding its nutritional and medicinal value. Furthermore, its thick, leathery leaves make foliar fertilization somewhat difficult, This is compounded by the low organic matter content of Iraqi soils, due to high summer temperatures and low winter rainfall. Therefore, recent research has focused on using safe, non-polluted nutrient sources to meet the plant's nutritional needs, including biofertilizers. These biofertilizers enhance agricultural production by supplying plants with nutrients and improving the physical, chemical, and biological properties of the soil, thus promoting plant growth [2]. It has been shown that these microorganisms play a vital role in the natural cycling of macronutrients, releasing them from organic compounds, they also produce growth regulators, inhibit pathogenic microorganisms and increase plant tolerance to environmental stresses, which are increasingly threatening agricultural production [4]. Organic farming is an ecological system that produces high-quality, healthy nutrients for plants and protects them from diseases and pests. It is a renewable and sustainable system that reduces the use of chemical fertilizers and pesticides. Soils cultivated using organic farming methods exhibit higher biological activity and water retention capacity than those under conventional farming systems, they also play a crucial role in regulating soil pH, which contributes to better nutrient uptake and reduces the absorption of heavy metals [5].

HPLC technology is one of the most important and widely used analytical techniques in analytical and biochemical sciences for the detection, separation, identification, and quantification of bioactive chemical compounds in plant samples, which are difficult to detect using other separation techniques. It is considered one of the best chromatographic separation methods, characterized by its high accuracy under specific conditions of pressure, temperature, and the type of compounds to be separated [6]. Therefore, the current study aims to detect and investigate the bioactive compounds in broccoli fruits under the influence of different levels of aquatic plant compost and various types of microorganisms.

Materials and Methods:

A field experiment was conducted in Al-Maysab project area during the period from October 5, 2024, to February 15, 2025, to detect and investigate the bioactive compounds in two broccoli hybrids under the influence of aquatic plant compost and biofertilizers. Broccoli seeds of the hybrid varieties Max F1 (produced by the Dutch company Horti) and Azhar F1 (produced by the Dutch company Delta) were sown on August 20, 2024, in 209-cell polystyrene trays filled with peat moss, with 2 seeds per cell, for seedling production. The trays were placed in a plastic greenhouse to protect the seedlings from any sudden environmental changes that could affect germination rates. After seedling emergence and when the seedlings had reached the appropriate size (5-6 true leaves), plants with as uniform growth as possible were selected, spaced, and transplanted to the field on October 5, 2024, for both hybrids and both experiments. All necessary field management practices were followed during the course of the experiments [7] After preparing the planting area (clearing, marking, and tilling), it was divided into 3 replicates, each consisting of 3 rows, 2 meters long, with 15 plants per row. The spacing between plants within a row was 40 cm, and the spacing between rows was 75 cm. The spacing between experimental units was 50 cm, and the spacing between replicates was 1.5 meters.

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Table (1): Some chemical and physical properties of the experimental soil.

Soil texture	Particle size distribution of soil fractions(%)			N. P. K. (mg Kg^{-1})			Organic matter (%)	E.C ds. m^{-1}	pH
	sand	silt	clay	K	P	N			
Sandy loam	52	21	27	173.4	11.7	17.4	6.5	1.6	7.4

prepared from aquatic plants collected on May 2, 2024, from three different locations along the Euphrates River.

These plants included *Ceratophyllum demersum* and *Eichhornia crassipes*, along with green algae. The compost was then subjected to drying, grinding, composting, fermentation, and storage. Prior to application, its chemical and physical properties were measured at the Ministry of Science and Technology laboratories, as shown in Table 2 below.

Soil analysis was conducted at the Soil and Water Laboratory of the Babylon Directorate of Agriculture. The research experiment utilized three factors, arranged according to the experimental design, as follows:

First/ genetic type: Two broccoli genotypes were used: Max and Azhar.

Second/ aquatic plants compost: The experimental plots were treated with three levels of aquatic plant compost: control (no treatment), 2 kg, and 4 kg. The compost was

Table 2: Chemical Composition of the Fermented Compost

Compound	Value	Unit	Compound	Value	Unit	Compound	Value	Unit
Nitrogen	16.95	%	GA_3	45.75	ppm	Vitamins	763.33	ppm
Potassium	23.46	%	IAA	156.83	ppm	Glutamic	66.05	ppm
Phosphor	6.18	%	Cytokinin	206.58	ppm	Tryptophane	79.28	ppm
Thiamine	13.42	ppm	Valine	398.66	ppm	Phenylalanine	304.51	ppm
Glycine	197.61	ppm	Serine	1095.91	ppm	Threonine	2548.96	ppm

Technology Department at Al-Maseeb Technical College. These were the bacterial fertilizer *Azospirillum* spp. and the fungal fertilizer *Trichoderma* spp.

ground and stored in perforated plastic bags in the refrigerator until analysis [8]. The sample solution was prepared by weighing 0.25 g of the ground sample using a sensitive balance. It was then crushed and ground using a mortar and pestle to obtain a fine powder, and dissolved in 10 ml of alkaline methanol using KOH on a water bath with automatic shaking for 3 hours. The solution was then removed from the water bath and allowed to dry

Third/ Biofertilizers: The plant soil was treated with two types of biofertilizers, which were cultivated and propagated in the laboratories of the Bioremediation

Determination of the fruit's content of bioactive compounds using High Performance Liquid Chromatography (HPLC):

Extraction and separation of bioactive compounds: Plant samples of the flower discs were taken at full maturity, specifically on January 30, 2025, from three replicates. After drying at room temperature until constant weight was achieved, the plant samples were compounds and the sample solution. Standard solutions of the four compounds were prepared, and the concentrations of these compounds in the samples were determined by comparing the peak areas of the unknown sample with the known peak areas of the corresponding standard. This procedure was repeated for all analyzed samples, using the appropriate separation conditions for each compound [10]. By comparing the unknown peak areas with those of the known standard, the four compounds Sulforaphane, glutathione, vitamin C, and folic acid were identified in all samples. The concentrations of the compounds in the plant samples were calculated using the following equation:

Sample concentration (ppm) = (Sample peak area × Standard concentration) / (Standard peak area) × Dilution factor

completely. The sample was then dissolved in 1 ml of methanol, filtered through filter paper, and then filtered again through a 0.2 µm PTFE filter. The filtrate was then analyzed using HPLC (High Performance Liquid Chromatography) using standard solutions of the bioactive compounds found in the plant's flower disc [9]. HPLC Separation Conditions: The chemical compounds in broccoli plants were analyzed in the laboratories of the Materials Analysis Department at the Ministry of Science and Technology. High-performance liquid chromatography (HPLC) was used to determine the concentration of the four compounds. A modern Shimadzu LC-10AV liquid chromatograph, equipped with a dual-pump injector (LC-10V), was used to determine the retention time and peak area of both the standard solution of the active

Table (3): Retention time and peak area of the biologically active compounds in broccoli

seq	Subject	Retention time minute	area
1	Sulforaphane	6.04	1458.90
2	Glutathione	7.72	1698.07
3	Vitamin C	4.17	1523.65
4	Folic acid	6.30	2965.07

Experimental Design

A factorial experiment (2×3×3) was conducted using a Randomized Complete Block Design with three replications per treatment. Each replication consisted of 18 experimental units, with three rows within each replication. Statistical analysis of the studied traits was performed using GenStat 2012 software. The results between treatments were compared using the Least Significant Difference (LSD) test at a 5% probability level to determine statistical significance [11].

Results and Discussion

First/ Sulforaphane Content (ppm)

The results of the statistical analysis in Table 4 show no significant differences in the Sulforaphane content of the broccoli heads due to the different genotypes. The Azhar genotype had the highest average content of 293.046 ppm, compared to 291.964 ppm for the Max variety. The application of compost to the plants had a significant effect on Sulforaphane content, with the 4 kg/m² treatment achieving the highest average of

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hybrid variety and the application of compost had a significant effect on this trait. The treatment combining the Azhar and Max hybrid varieties with 4 kg of compost showed a significantly higher average (309.883 and 309.633 ppm, respectively), with no significant difference between the two. The lowest average was recorded for the Azhar hybrid variety without compost application (261.370 ppm). The same table also shows significant differences in this trait due to the interaction between genotype and biofertilizer. 309.758 ppm, compared to the control treatment (no compost) which recorded the lowest average of 263.823 ppm. The application of biofertilizer also had a significant effect on Sulforaphane content; the treatment with *Trichoderma* spp. fungus in the soil. The highest average value was 306.288 ppm, compared to the control treatment which gave the lowest average of 275.945 ppm. The interaction between the experimental factors had a significant effect on the Sulforaphane content of the fruits. Specifically, the interaction between the

Table (4): Effect of aqueous plants compost, bio-fertilizer, and their interactions on Sulforaphane content (ppm) in two broccoli cultivars.

Hybrid	Compost Kg h^{-1}	Bio-fertilizer			Hybrid × Compost
		Non	*Azos.*	*Trich.*	
Azhar	0.00	238.690	262.790	282.630	261.370
	2.00	291.600	306.900	325.150	307.883
	4.00	292.180	311.430	326.040	309.883
Max	0.00	250.380	275.540	272.910	266.277
	2.00	290.960	301.230	307.760	299.983
	4.00	291.860	313.800	323.240	309.633
L.S.D. $_{0.05}$		7.420			4.283
Hybrid × Bio-fertilizer					Hybrid
Azhar		274.157	293.707	311.273	293.046
Max		277.733	296.857	301.303	291.964
L.S.D. $_{0.05}$		4.284			*N.S.*
Compost × Bio-fertilizer					Compost
0.00		244.535	269.165	277.770	263.823
2.00		291.280	304.065	316.455	303.933
4.00		292.020	312.615	324.640	309.758
L.S.D. $_{0.05}$		5.247			3.029
average Bio-fertilizer		275.945	295.282	306.288	
L.S.D. $_{0.05}$		3.029			

(324.640 ppm), compared to the control treatment (244.535 ppm). The same table also shows significant differences resulting from the combined effect of all three factors (hybrid variety, compost, and bio-fertilizer). The treatment combining the Azhar hybrid variety with 2 kg of compost and *Trichoderma* spp. gave the highest average Sulforaphane content (325.15 ppm), The Azhar hybrid treated with *Trichoderma* spp. fungus had the highest average (311.273 ppm), compared to the untreated Azhar hybrid (274.157 ppm). The same trend was observed when plant compost was combined with the bio-fertilizer. The treatment combining 4 kg of compost with *Trichoderma* spp. consistently showed significantly higher levels of Sulforaphane in the broccoli heads ppm for the Max hybrid. Regarding the application of compost to the soil, the same table showed a significant effect on glutathione content. The 2 kg/m² compost treatment gave the highest average of 113.8 ppm, which was not significantly different from the 4 kg/m² treatment (110.1 ppm), compared to the control treatment (98.5 ppm). The bio-fertilizer also had a significant effect on glutathione content; the *Trichoderma* spp. treatment gave the highest average of 115.0 ppm, while the lowest average was 99.6 ppm in the control treatment.

surpassing all other combinations. The lowest average was observed in the control treatment (Azhar hybrid without compost or bio-fertilizer), at 238.690 ppm.

Second: Glutathione content (ppm)

The results of the statistical analysis in Table 5 showed that the genetic makeup of the broccoli plants did not have a significant effect on the glutathione content of the heads. The Azhar hybrid had the highest average content at 108.00 ppm, compared to 106.9

Table (5): Effect of aqueous plants compost, bio-fertilizer, and their interactions on the glutathione content (ppm) of two broccoli cultivars

Hybrid	Compost Kg h^{-1}	Bio-fertilizer			Hybrid × Compost
		Non	*Azos.*	*Trich.*	
Azhar	0.00	81.8	102.7	110.6	98.4
	2.00	111.5	111.7	125.6	116.3
	4.00	106.6	109.9	111.4	109.3
Max	0.00	84.8	104.8	106.1	98.6
	2.00	105.6	106.5	121.7	111.3
	4.00	107.4	110.5	114.8	110.9
L.S.D. $_{0.05}$		15.23			8.79
variety × Bio-fertilizer					Hybrid
Azhar		100.0	108.1	115.9	108.0
Max		99.2	107.3	114.2	106.9
L.S.D. $_{0.05}$		8.79			*N.S.*
Compost × Bio-fertilizer					Compost
0.00		83.3	103.7	108.3	98.5
2.00		108.5	109.1	123.7	113.8
4.00		107.0	110.2	113.1	110.1
L.S.D. $_{0.05}$		10.77			6.22
average Bio-fertilizer		99.6	107.7	115.0	
L.S.D. $_{0.05}$		6.22			

hybrids treated with *Trichoderma* spp.) gave the highest average values of (115.9 and 114.2) ppm, respectively, without significant difference between them, thus outperforming other treatments. The interaction of (Max hybrid without bio-fertilizer treatment) recorded the lowest average value of 99.2 ppm. The interaction of compost with the bio-fertilizer also had a significant effect on this trait, with the interaction of (2 kg/m² compost with *Trichoderma* spp.) yielding the highest

A significant effect was observed due to the interaction of the two factors. The interaction of (Azhar hybrid with 2 kg/m² of green compost) gave the highest average value of 116.3 ppm, surpassing most other treatments. In contrast, the interaction of (Azhar hybrid without compost addition) recorded the lowest average value of 98.4 ppm. The interaction of the hybrid with the bio-fertilizer also had a significant effect on this trait. The interaction of (Azhar and Max

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The results in the same table indicate that the interactions between the experimental factors had significant effects on the vitamin C content of the fruits. Specifically, this parameter was significantly affected by the interaction between the hybrid variety and green compost. The combination of the 'Max' hybrid variety with 2 kg/m² of compost gave the highest average vitamin C content (119.12 mg/100g), surpassing most other treatments. In contrast, the 'Max' hybrid variety without compost application recorded the lowest average (102.79 mg/100g). The interaction between the hybrid variety and the bio-fertilizer also significantly affected this trait. The 'Azhar' hybrid variety treated with *Trichoderma* spp. achieved the highest average (120.15 mg/100g), while the 'Max' hybrid variety without bio-fertilizer treatment had the lowest average (104.00 mg/100g). Significant differences in vitamin C content were also observed due to the interaction between green compost and the bio-fertilizer, as shown in the same table. The combination of 2 kg/m² of compost with *Trichoderma* spp. resulted in the highest average (122.80 mg/100g), compared to the control treatment (no organic or biological amendment), which gave the lowest average (96.72 mg/100g). The results in the table also showed that the three-way interaction had a significant effect on the aforementioned trait. The treatment (Max hybrid plants treated with 2 kg/m² of green compost and the fungus *Trichoderma* spp.) gave the highest average value for this trait, at 123.0, while the lowest average was recorded for the control treatment (Max hybrid plants without compost or biofertilizer), with an average of 95.75 mg/100g.

average of 123.7 ppm, compared to 83.3 ppm for the interaction of (no compost and no bio-fertilizer added to the soil). The results in the same table also showed that the interaction of all three experimental factors had a significant effect on the glutathione content of the fruit. The interaction of (Azhar hybrid treated with 2 kg/m² compost and *Trichoderma* spp.) gave the highest average value of 125.6 ppm, while the lowest average was recorded for the interaction of (Azhar hybrid without bio-fertilizer and compost treatment) at 81.8 ppm.

Thirdly/ Vitamin C (mg/100g)

Table (6) shows that the genetic makeup did not significantly affect the vitamin C content of broccoli. The Azhar hybrid plants recorded the highest average content of 113.02 mg/100g, while the other hybrid plants recorded an average of 112.98 mg/100g. The same table also shows a significant effect of treating the soil with aquatic plant compost. The highest average for this indicator was recorded at the 2 kg level, reaching 118.37 mg/100g, which was not significantly different from the 4 kg level, which achieved an average of 116.07 mg/100g. The control treatment gave the lowest average at 104.56 mg/100g. The significant effect of vitamin C in broccoli continued with the treatment of the soil with different types of microorganisms. Soil treated with *Trichoderma* spp. fungus gave the highest average of 118.45 mg/100g, which was not significantly different from the treatment with the bacterial fertilizer *Azospirillum* spp., which gave an average of 115.29 mg/100g. The control treatment recorded the lowest average at 105.26 mg/100g.

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Table (6): Effect of aqueous plants compost, biofertilizer, and their interactions on the vitamin C content (ppm) of two broccoli cultivars

Hybrid	Compost Kg h^{-1}	Bio-fertilizer			Hybrid × Compost
		Non	*Azos.*	*Trich.*	
Azhar	0.00	97.68	105.91	115.40	106.33
	2.00	108.46	121.90	122.50	117.62
	4.00	105.85	116.56	122.55	114.99
Max	0.00	95.75	104.63	107.99	102.79
	2.00	111.54	122.72	123.09	119.12
	4.00	112.28	120.00	119.16	117.15
L.S.D. $_{0.05}$		10.296			5.945
Hybrid × Bio-fertilizer					Hybrid
Azhar		104.00	114.79	120.15	112.98
Max		106.52	115.79	116.75	113.02
L.S.D. $_{0.05}$		5.945			*N.S.*
Compost × Bio-fertilizer					Compost
0.00		96.72	105.27	111.69	104.56
2.00		110.00	122.31	122.80	118.37
4.00		109.06	118.28	120.85	116.07
L.S.D. $_{0.05}$		7.281			4.203
average Bio-fertilizer		105.26	115.29	118.45	
L.S.D. $_{0.05}$		4.203			

other treatments, the lowest average was 82.55 mg/100g in the control treatment.

The results also showed significant interactions between the different factors. Specifically, the interaction between hybrid type and compost application was significant. The treatment combining the Max hybrid plants with 4 kg/m² of compost showed the highest average folic acid content of 107.20 mg/100g, surpassing most other interaction treatments. In contrast, the treatments involving the Azhar and Max hybrid plants without compost application showed the lowest averages of 76.35 and 77.27 mg/100g, respectively, with no significant difference between them. The interaction between genotype and type of biofertilizer also had a significant effect on this trait. The interaction between (Azhar and Max hybrid plants treated with *Trichoderma* spp.) gave the highest average value (103.14 and 102.67 mg/100g, respectively), which was not significantly different from each other and outperformed

Fourthly/ Folic acid (mg/100g)

The results in Table (7) indicate no significant differences in the folic acid content of broccoli due to genetic variation. The Max hybrid plants showed the highest average content of 95.54 mg/100g, compared to 94.31 mg/100g for the Azhar hybrid plants. Regarding the application of green compost to hydroponically grown plants, the 4 kg/m² treatment showed a significant increase in folic acid content, reaching an average of 105.78 mg/100g, which was not significantly different from the 2 kg/m² treatment (102.19 mg/100g). Both of these treatments were superior to the control treatment (76.81 mg/100g). Regarding the type of bio-fertilizer, the *Trichoderma* spp. treatment resulted in the highest average folic acid content of 102.90 mg/100g, outperforming the

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Trichoderma spp.) yielding the highest average of 114.81 mg/100g, surpassing most other treatments. The lowest average was observed in the interaction treatment (no compost or biofertilizer application) with an average of 65.70 mg/100g. Significant differences were also observed in the three-way interaction effect on the folic acid content of the fruit, as shown in the same table.

in the three-way interaction treatment (Azhar and Max hybrid plants without organic or biofertilizer treatment) with averages of (65.59 and 65.80) mg/100g, respectively, without significant differences between them.

most other treatments. The lowest average value was observed in the interaction treatments (Azhar and Max hybrid plants without biofertilizer treatment) with averages of (81.33 and 83.78) mg/100g, respectively, without significant differences between them. The interaction between green compost and the type of biofertilizer also had a significant effect on the same trait, with the interaction treatment (2 kg compost level with

The interaction treatment (Azhar hybrid plants treated organically with 2 kg compost and biologically with *Trichoderma* spp.) gave the highest average for this trait (115.80 mg/100g), exceeding most other treatments. The lowest average was recorded

Table (7): Effect of aqueous plants compost, bio-fertilizer, and their interactions on the folic acid content (ppm) of the fruit in two broccoli varieties

Hybrid	Compost Kg h^{-1}	Bio-fertilizer			Hybrid × Compost
		Non	*Azos.*	*Trich.*	
Azhar	0.00	65.80	78.56	84.70	76.35
	2.00	79.70	111.20	115.80	102.23
	4.00	98.48	105.67	108.92	103.36
Max	0.00	65.59	81.23	84.98	77.27
	2.00	81.52	111.10	113.82	102.15
	4.00	104.23	108.18	109.19	107.20
L.S.D. $_{0.05}$		7.40			4.27
Hybrid × Bio-fertilizer					Hybrid
Azhar		81.33	98.48	103.14	94.31
Max		83.78	100.17	102.67	95.54
L.S.D. $_{0.05}$		4.27			*N.S.*
Compost × Bio-fertilizer					Compost
0.00		65.70	79.89	84.84	76.81
2.00		80.61	111.15	114.81	102.19
4.00		101.36	106.93	109.06	105.78
L.S.D. $_{0.05}$		5.23			3.02
average Bio-fertilizer		82.55	99.32	102.90	
L.S.D. $_{0.05}$		3.02			

particularly the macronutrients N, P, K, and S (Table 2), which are released in a mineral form and become readily available for plant absorption. Organic matter acts as a reservoir of these essential nutrients, positively impacting the content of secondary metabolites produced by the plant during its

The significant increase in the content of bioactive compounds in broccoli fruits, as shown in Tables 4-7, may be attributed to the effect of the organic matter added to the soil, specifically the compost made from fermented aquatic plants. This compost contains essential nutrients for plant growth,

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concentration of active compounds produced by the plant during its growth period, especially when the soil was treated with the fungus *Trichoderma*. This fungus positively influenced the levels of secondary metabolites, likely due to its beneficial effects on the plant. It participates in nutrient cycling in the soil by supplying and fixing nitrogen, phosphorus, and sulfur, which directly affect the synthesis and composition of the plant's chemical compounds [21 and 22]. It also converts insoluble nitrogen into soluble forms, facilitating absorption by the roots, such as the conversion of nitrates to ammonia, and contributes to the formation of root nodules. Furthermore, it has the ability to solubilize many poorly soluble nutrients, such as zinc, iron, and copper, due to the production of large quantities of biochemical compounds that are responsible for this solubilization [23 and 24]. This was further demonstrated by [25] who found that Trichoderma spp. isolates are efficient at solubilizing phosphate and increasing phosphorus availability through the production of the enzyme phosphatase. This was confirmed in the study by [26] which indicated that the biofertilizer Trichoderma spp. is capable of supplying phosphorus and some micronutrients. [27] also concluded that the metabolites produced by Trichoderma spp. It is responsible for the chelation of iron, as it enhances its reduction and conversion into a form readily available for absorption. It also plays a role in the degradation of chlorinated organic compounds and contributes to increased production of the plant hormone IAA by oxidizing the amino acid tryptophan, which is secreted by the roots. This, in turn, promotes root hair growth, ultimately leading to an increase in the concentration of bioactive compounds produced by the plant.

The study concluded that the application of environmentally friendly agents used in the experiment had a significant positive effect on increasing the concentration of bioactive compounds in the fruits of two life cycle [12, 13 and 14]. Alternatively, the significant increase in bioactive compounds in broccoli fruits may be due to the compost's ability to address soil problems such as low organic matter content, poor fertility, low moisture retention, high soil temperatures in summer, and poor aeration. The compost also supplies nutrients through the mineralization of its organic matter and provides energy for beneficial microorganisms [15]. Furthermore, it provides various essential nutrients, especially macronutrients, and produces a range of organic acids, amino acids, vitamins, and other compounds that plants need during their life cycle, which consequently leads to an increase in their secondary metabolites [16 and 17]. This was also evident in the study by [18] which concluded that adding green compost to the soil was a suitable alternative for increasing its fertility as a source of organic matter, and significantly improved its properties due to its chemical and biological characteristics, which contribute to positive effects on plant growth. It was also considered an effective tool for supplying essential nutrients to plants, in addition to its high capacity to provide and exchange important cations, increase the number of beneficial microorganisms in the soil, and reduce the population of pathogenic microorganisms. All of this positively impacts the production of plant compounds, including secondary metabolites. Furthermore, amino acids play an important role in stimulating cell division and elongation, and in various processes related to protein synthesis, as shown in Table 2. Moreover, amino acids are the primary precursors of natural plant components, including carbohydrates, hormones, pigments, and secondary metabolites. The presence of phenylalanine in green compost may also have a significant effect on increasing the concentration of secondary metabolites, as it is the precursor compound in the synthesis of these metabolites during glycolysis, a process that occurs in plants during their growth and development [19 and 20]. The biofertilizer also played a significant role in increasing the

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differences in the studied parameters under the experimental conditions.

broccoli hybrids. Furthermore, the genetic differences between the Azhar and Max hybrids did not show statistically significant

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