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Plant Growth Promoting Bacteria for Aquaponics as a New Strategy That Grants Quality and Nutrient Efficiency in Kohlrabi Cultivation

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Abstract: Consumers are becoming increasingly concerned about eating healthy, and the products they consume are produced in an environmentally friendly way. Therefore, in this work, production techniques such as aquaculture and the use of plant growth-promoting bacteria in kohlrabi cultivation (*Brassica oleracea* L.) were studied. To this end, we applied three types of irrigation treatments (control, mixed water (50% fish effluent/50% drainage water) (mixed water), and mixed water enriched with synthetic fertilizers (mixed water + S)) combined with two formulations of plant growth-promoting bacteria (B1 and B2) in kohlrabi plants. Our data showed that the B1 inoculum combined with control irrigation caused both the increase in dry matter and the diameter of the bulb (17.8% and 8.9%, respectively); moreover, this inoculum increased the concentration of Ca when applied with mixed irrigation solution (water + S), and Zn for the B2 inoculum. The nitrogen utilization efficiency (NUtE) was augmented by the mixed irrigation treatment, with the lowest concentration of nitrates observed in the bulbs. Both inocula increased the total phenolic compounds in the control irrigation, whilst an increase in fructose and sucrose concentrations was only observed with B2.

Keywords: red tilapia; purple kohlrabi; nitrates; organic fertilization; aquaponics system; circular economy framework; antioxidant activity

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1. Introduction

Among the species that are part of the Brassicaceae family, we find kohlrabi (*Brassica oleracea* var. gongylodes), which has a short growing period in early summer and autumn [1]. The most consumed part of kohlrabi is the fleshy, swollen stem (bulb), which can be eaten raw, cooked, or preserved. However, the young leaves can also be consumed in salads or cooked as spinach, so they can be another added product for producers and consumers [1,2]. This vegetable has a high nutritional value for humans since it is a source of antioxidants such as vitamin C, phenolic compounds, glucosinolates, and nutrients such as potassium [3], which grant them antioxidant, anti-inflammatory, and anti-cancer properties [1]. Kohlrabi is available in white, green, and purple varieties, and it has been observed that the latter have antioxidant properties due to the pigments responsible for their color [4].

In recent times, society has become increasingly aware of health and environmental problems, which is why the consumption of foods rich in nutrients and prepared with more environmentally sustainable agricultural techniques has increased [5]. One of these sustainable production techniques is aquaponics. This technique reduces the use of synthetic fertilizers and water by reusing aquaculture products in hydroponic systems and may even reduce/eliminate the discharge of nitrates into the environment [6]. However, not everything is positive in these types of systems, as they could have resulted in deficiencies in some nutrients such as calcium, iron, potassium, manganese, phosphorus, and nitrates [7]. Nitrate (NO_3^-) deficiencies can cause serious problems in the growth and development of kohlrabi since nitrogen (N) intervenes in many processes, such as chlorophyll formation, photosynthesis, stomatal conductance, and the potential quantum efficiency of photosystem II [5].

Authors, such as del Amor and Cuadra-Crespo [8], Consentino et al. [9], and Lastochkina et al. [10], have reported that the use of plant growth-promoting bacteria (PGPB) improves the efficiency of mineral absorption, ameliorating the adverse effect of high NaCl concentrations. Currently, the most important PGPB genera with agricultural benefits are *Arthrobacter*, *Azotobacter*, *Burkholderia*, *Bacilus*, *Azospirirullum*, *Clostridium*, *Pseudomonas*, *Herbaspirillum*, and *Gluconacetobacter* [11]. Both *Azotobacter* and *Azospirillum* are among the most studied genera by the scientific community. They are capable of promoting root growth using different mechanisms, such as the release and biosynthesis of indole-3-acetic acid, gibberellins, cytokinins, amino acids, and polyamines, which favors the absorption of nutrients and water in the plant [12,13]. In addition, they have the ability to make atmospheric N available to plants, which helps reduce mineral fertilizers [14]. Muthukumar and Udaiyan [15] observed that inoculation with *Azospirillum* is more successful when co-inoculated with *Pantoea dispersa*. Therefore, we hypothesized that this could be a strategy to use in combination with aquaponic systems to reduce the harmful impact caused by N deficiency on plants. However, it has been observed that the response of plants to PGPB is associative, as it depends on the genotype and the growth conditions of the plants [9]. For this reason, we consider that it is necessary to study the interaction between bacteria, such as *Azospirillum brasilense*, *Pantoea disperse*, and *Azotobacter salinestris*, and the different doses/mixtures of organic and inorganic N provided, with the aquaponic system for the cultivation of kohlrabi, as there is a lack of research on this. Taking the above into account, the main objective of this work was to evaluate the impact of different levels of N fertilization from an aquaponic system on kohlrabi bulbs inoculated with PGPB to evaluate their yield and quality. Thus, the present trial seeks to find the best fertilization strategy (combination of PGPB with water from aquaponic systems) for kohlrabi plants to attain a greater yield and accumulation of bioactive compounds.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiment was carried out in a greenhouse using a Recirculating Aquaculture System (RAS). The greenhouse (located in Torre Pacheco, Murcia, Spain) is equipped with a computer-regulated drip-irrigation system drip-irrigation system, under controlled environmental conditions. Climate parameters (temperature and humidity) inside the greenhouse during the crop season were monitored with a climate station placed inside the greenhouse. The temperature ranged from 7 to 31 °C and relative humidity from 30% to 80% during the growing season. The RAS produced Nile tilapia (*Oreochromis niloticus*) for sale, and the water was reused for plant cultivation. The culture system consisted of 3 fish tanks of approximately 900 L each, a clarifier, a filter tank, and a drainage water collection tank. The tanks were covered with mesh to prevent the fish from jumping out. In an RAS, the biological process of nitrification, using aerobic bacteria (genera *Nitrobacter* spp. and *Nitrosomonas* spp.), is used to oxidize toxic NH_3 excreted by the fish into NO_2^- and finally into NO_3^-. Each tank contained 71 fish (each weighing ca. 157 g at the beginning of the experiment and ca. 324 g at the end), which were fed daily with an amount of feed equivalent to 2.2−1.9% of the fish weight (the composition of the feed is indicated in Supplementary Table S1).

The plant species used in this investigation was purple kohlrabi (*Brassica oleracea* var. *gongylodes*), cv. Ukza, which was acquired from a commercial nursery (Baby plant, S.L., Santomera, Spain). These plants, with a height of 8–10 cm, were planted in 1.2 m long bags filled with coconut fiber (Pelemix, Alhama de Murcia, Murcia, Spain). Each bag contained

3 plants and 3 drippers (2 L h^{-1}). Irrigation management was carried out according to local commercial soilless cultivation, and an excess of nutrient solution was applied to produce a minimum of 30% drainage to avoid salt accumulation and nutrient imbalance in the rhizosphere [16]. Fourteen days after transplant, once the plants had acclimatized, three irrigation treatments were applied, including (1) irrigation water enriched with synthetic fertilizers (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and micronutrient) (control), which is a traditional solution with proportions and concentrations of fertilizers similar to that used by local farmers and with 100% synthetic fertilizers, (2) mixed water (50% fish effluent/50% drainage water) (reused drainage water from the aquaponics plant cultivation system), and (3) this mixed water enriched with nutritional supplementation through the roots (mixed water + S). Table 1 details the nutrient composition of the 3 irrigation treatments used.

Table 1. Nutritional composition of macronutrients of the different irrigation treatments used for kohlrabi plant growth.

Nutrient (mg L^{-1})	Control	Mixed Water	Mixed Water + S
NO_3^-	606.70 ± 54.19	249.74 ± 38.27	550.22 ± 36.31
PO_4^{3-}	367.73 ± 10.50	290.54 ± 8.09	445.75 ± 7.97
SO_4^{2-}	380.46 ± 4.24	338.76 ± 2.19	392.04 ± 4.48
K^+	458.44 ± 12.32	193.32 ± 7.59	429.89 ± 7.15
Ca^{2+}	76.70 ± 3.07	80.61 ± 2.86	81.06 ± 2.84
Mg^{2+}	65.68 ± 0.97	66.20 ± 0.96	68.60 ± 1.29

In addition, before transplanting, 15 g of *Azospirillum brasilense* strain M3 and *Pantoea dispersa* strain C3, immobilized in a solid support, with 10^9 CFU g^{-1} (colony-forming units) (B1: Biopron®, Probelte SA., Murcia, Spain), were added to the substrate per each plant. In the same way, 10 mL of *Azotobacter salinestris*, with 10^7 CFU g^{-1} (B2: Nutribio N®, Ceres Biotics Tech, S.L., San Fernando de Henares, Madrid, Spain) was applied per plant, and the application was repeated every 2 weeks until the end of the experiment. This formulation was applied by syringe to the substrate next to the stem of the plant (coinciding with the root zone). Six plants per treatment were used. Thus, a total of 9 treatments were applied: 3 irrigation treatments (Control, Mixed water, Mixed water + S), and 3 PGPR treatments (Control, B1 and B2) (see Table 2).

Table 2. Irrigation and PGPR treatment applied to kohlrabi plants.

Irrigation	PGPR	Treatments
Control	0	Control
	B1	Control + B1
	B2	Control + B2
Mixed water	0	Mixed water
	B1	Mixed water + B1
	B2	Mixed water + B2
Mixed water + S	0	Mixed water + S
	B1	Mixed water + S + B1
	B2	Mixed water + S + B2

At the end of the experiment (37 days after transplanting), the kohlrabi plants were harvested and weighed to determine the fresh weight (FP) of the whole plants and of the bulbs alone, and their diameter was measured.

2.2. Mineral Content

The NO_3^-, SO_4^{2-}, PO_4^{3-}, and Cl^- concentrations were measured from lyophilized and ground kohlrabi bulbs (0.4 g) with 20 mL of deionized water. The mixture was shaken for 30 min. These ions were determined in an ion chromatograph (METROHM 861 Advanced Compact IC; METROHM 838 Advanced Sampler), and the column used was a

METROHM Metrosep A Supp7—250/4.0 mm. The flow rate was 0.7 mL min^{-1}, and the column temperature was kept at 45 °C. The solvent system consisted of Na_2CO_3 3.6 mM, with an isocratic gradient.

The K, Mg, Na, Ca, Fe, Cu, Mn, Zn, and B concentrations were measured using an ETHOS ONE microwave digestion system (Milestone Inc., Shelton, CT, USA) to extract the cations by acid digestion from lyophilized and ground material (0.1 g). Then, the concentrations of cations were determined using an inductively coupled plasma spectrometer (Varian Vista MPX, Palo Alto, CA, USA). The N content was analyzed from lyophilized and ground kohlrabi bulbs, using a combustion nitrogen/protein determinator (LECO FP-528, Leco Corporation, St. Joseph, MI, USA).

2.3. NUtE Calculation

Nitrogen utilization efficiency (NUtE) is defined as the capacity of the plant to produce biomass of the different organs per unit of N absorbed [17].

$$\text{NUtE} = (\text{g Total Biomass})/(\text{g N absorbed})$$

2.4. Total Phenolic Compounds and Antioxidant Activity (ABTS^{+*})

Total phenolic compounds were extracted from kohlrabi bulb samples frozen at −80 °C, using 5 mL of 80% acetone as the extractant. Then, the samples were centrifuged at 10,000× g at 4 °C for 10 min. To 100 µL of supernatant, 1 mL of Folin–Ciocalteu reagent diluted in Milli-Q water (1:10), 2 mL of Milli-Q water, and 5 mL of sodium carbonate (20%) were added. The mixture was kept in the dark for 30 min. The absorbance was measured at 765 nm, according to the methodology by Kahkonen et al. [18]. The content of total phenols was expressed in equivalents of gallic acid, in mg g^{-1} of dry weight.

Using the ABTS method, the antioxidant activity was determined using the lyophilized bulbs. For this, 0.5 g of bulb was weighed and mixed with 10 mL of acidified MeOH/water solution (80:20 v/v, 1% HCl), sonicated at 20 °C for 15 min, and left for 24 h at 4 °C. The sonicating step was repeated again for another 15 min. Then, the sample was centrifuged at 10,000× g for 10 min. ABTS analyses were performed according to the methods described above [19]. The calibration curve for the ABTS method was prepared with Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid). The results are shown as the mean ± standard error in µM Trolox g^{-1} dry weight (DW).

2.5. Total Soluble Sugars

Sugar extraction was carried out following the method described by Balibrea et al. [20], with slight modifications. Two lyophilized kohlrabi bulb incubations (50 mg) were carried out at a time using 1.5 mL of methane (80%, v/v) for 30 min at 4 °C. During the extraction, 3 shakes were performed. Later, each extract was centrifuged for 15 min at 3500× g, at 4 °C, and the supernatant was filtered through a C18 Sep-Pak cartridge (Waters Associates, Milford, MA, USA), previously activated with 20 mL of methanol/water (80/20). Then, the two supernatants from the double extraction were combined and filtered through a 0.45 µm filter (Millipore, Bedford, MA, USA). In the obtained extracts, the concentration of three sugars (glucose, fructose, and sucrose) was determined by ionic chromatography using an 817 Bioscan system (Metrohm, Herisau, Switzerland) equipped with a pulsed amperometric detector (PAD) and a gold electrode. The column used was a METROHM Metrosep Carb 1–150 IC column (4.6 × 250 mm), which was heated to 32 °C.

2.6. Statistical Analysis

The experimental design was completely random, and 6 repetitions were carried out. Data were analyzed with Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton, VA, USA). First, the data were tested for homogeneity of variance and normality of distribution and then subjected to an analysis of variance (ANOVA) and a separation of means using Duncan's multiple range test at $p \leq 0.05$. Nine combinations of treatments were used, involving 3 irrigation treatments (control, mixed water, and mixed water + S), and 3 PGPR treatments: control (with no addition of bacteria), B1 (*Azospirillum brasilense* strain M3 and *Pantoea dispersa* strain C3), and B2 (*Azotobacter salinestris*).

3. Results

3.1. Physical Characterization of Kohlrabi Bulb

Kohlrabi bulbs were affected by irrigation with the mixed water treatment, as can be observed in the values of bulb dry matter and diameter, which were reduced (Figure 1A,B). These reductions were 50.5% in the case of the dry matter of the bulb with respect to the control and 26.8% in the case of the bulb diameter (Figure 1A,B). However, when the plants were irrigated with a mixture of water enriched with synthetic fertilizers (Mixed water + S), no significant effects were observed with respect to the control plants (Figure 1A,B).

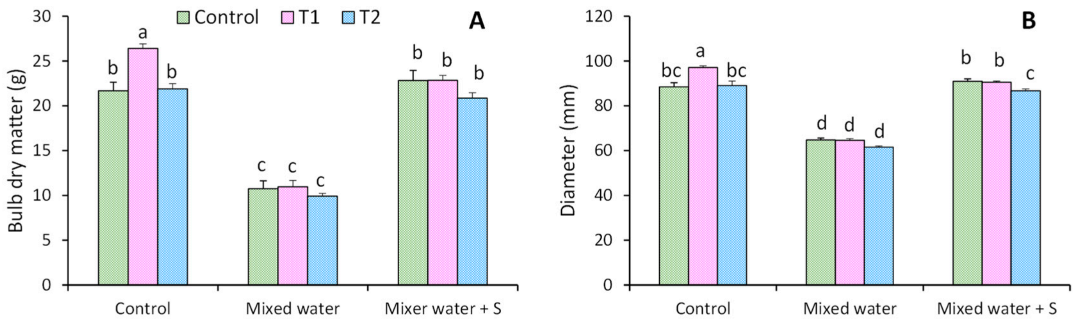

Figure 1. Bulb dry matter (**A**) and diameter (**B**) of kohlrabi plants irrigated with control solution, mixed water, and mixed water + S; and inoculated with two formulations of plant growth-promoting bacteria (Control, B1 and B2). Data are presented as treatment means ± SE (n = 6). Different letters indicate significant differences ($p \leq 0.05$) between treatments.

Regarding the inoculation with bacteria, the only significant effect was observed with the application of the B1 inoculum combined with the control irrigation. This treatment increased both the dry matter and the diameter of the bulb (17.8% and 8.9%, respectively).

3.2. Mineral Content

Table 3 shows the content of cationic macro and microelements in the kohlrabi bulb. Potassium (K) was the most abundant cation of the measured macronutrients, while iron (Fe) was the most abundant of the micronutrients (Table 3). The kohlrabi bulbs irrigated with both mixed water treatments had a reduced cation content (K, P, Fe, Cu, and B), with the greatest reduction being due to the unenriched mixed water treatment (mixed water) (Table 3). And on the contrary, the calcium (Ca) content increased (Table 3).

However, the combination of the mixed water + S treatment with both PGPR inocula attenuated the negative effects of irrigation on K and phosphorus (P), with both reaching the values of control plants (Table 3). In addition, in the case of the B1 inoculum, the content of Ca and Mg increased, as well as that of Zn in the case of B2 (Table 3). On the contrary, when both inoculants were applied in combination with control irrigation, a reduction in cations, such as K, P, Fe, Cu, Mn, and B, was observed (Table 3).

The most important anions determined in the kohlrabi bulb were chloride, nitrate, phosphates, and sulfates. Of these anions, the most abundant were phosphates, and the least abundant were chlorides (Table 4). Of the irrigation treatments, the only one that negatively affected the anion concentration was mixed water, as it reduced nitrates and phosphates, while it increased chlorides (Table 4). The other irrigation treatments did not have significant effects on the anions (Table 4). Regarding the effect of bacteria, the B1 treatment combined with mixed water increased the sulfate content, while the B2 combined with mixed water + S reduced phosphates (Table 4). None of the inocula presented significant effects when they were applied with control irrigation (Table 4).

The N content in kohlrabi bulbs varied between 2.13 and 3.46% on a dry weight basis (Figure 2A). The N content was only affected by irrigation with mixed water, which caused a reduction of 38.0% compared to the control (Figure 2A).

NUtE, which indicates the nitrogen use efficiency of the plant, increased in both mixed water irrigation treatments by 31.5% in the case of mixed water alone and 17.8% in the mixed water + S (Figure 2B) treatment. The bacteria had an opposite effect in this case, as the B1 inoculum, when combined with the mixed water, reduced the NUtE by 21.6% compared to the same treatment without bacteria and by 17.4% in combination with the mixed water + S (Figure 2B). On the other hand, B2 also reduced NUtE by 21.6% when combined with mixed water + S (Figure 2B).

Table 3. Mean concentrations of cations (mg g^{-1} DW) of kohlrabi bulb affected by different irrigation treatments (control, mixed water, and mixed water + S) and subjected to inoculation with two formulations of plant growth-promoting bacteria (control, B1 and B2).

Irrigation	PGPR	K	Ca	Mg	P	Fe	Cu	Mn	Zn	B
		g Kg^{-1} DW				mg Kg^{-1} DW				
Control	Control	48.9 ± 1.4 a	2.51 ± 0.1 de	2.49 ± 0.1 bcd	6.7 ± 0.1 b	46.9 ± 4.2 a	3.7 ± 0.1 a	24.16 ± 1.1 a	27.7 ± 1.0 bcd	24.92 ± 1.0 a
	B1	42.6 ± 1.1 b	2.20 ± 0.1 e	2.16 ± 0.1 d	6.3 ± 0.1 c	34.9 ± 1.0 b	3.0 ± 0.1 bc	21.42 ± 0.8 ab	26.6 ± 1.3 cd	23.05 ± 1.3 cd
	B2	40.0 ± 0.5 b	2.21 ± 0.1 e	2.15 ± 0.1 d	5.9 ± 0.0 d	34.6 ± 1.1 b	2.5 ± 0.2 c	19.30 ± 1.1 b	25.5 ± 1.0 d	21.20 ± 1.0 d
Mixed water	Control	40.1 ± 1.0 b	2.97 ± 0.1 abc	2.72 ± 0.1 abc	6.0 ± 0.1 cd	31.6 ± 0.5 bc	3.0 ± 0.2 bc	22.42 ± 0.5 a	28.1 ± 0.8 bcd	23.40 ± 0.8 bcd
	B1	42.5 ± 1.4 b	3.29 ± 0.1 a	2.95 ± 0.1 a	6.0 ± 0.1 cd	32.4 ± 1.6 bc	3.4 ± 0.2 ab	23.00 ± 0.9 a	26.9 ± 0.9 cd	24.55 ± 0.9 ab
	B2	41.2 ± 1.1 b	2.79 ± 0.1 bcd	2.55 ± 0.1 bc	6.1 ± 0.1 cd	28.9 ± 1.2 c	3.4 ± 0.2 ab	21.37 ± 0.4 ab	26.1 ± 1.0 cd	22.30 ± 1.0 cd
Mixed water + S	Control	42.7 ± 1.4 b	2.66 ± 0.2 bc	2.45 ± 0.2 cd	6.3 ± 0.1 c	34.9 ± 1.0 b	2.8 ± 0.1 c	22.53 ± 0.7 a	29.0 ± 0.7 bc	23.65 ± 0.7 abc
	B1	48.2 ± 0.4 a	3.14 ± 0.2 a	2.84 ± 0.1 ab	6.9 ± 0.1 ab	35.8 ± 0.7 b	2.6 ± 0.1 c	22.42 ± 1.3 a	30.1 ± 0.1 ab	24.86 ± 0.1 a
	B2	47.8 ± 0.8 a	3.09 ± 0.1 ab	2.68 ± 0.1 abc	7.2 ± 0.2 ab	30.5 ± 1.4 bc	1.9 ± 0.2 d	21.87 ± 0.6 ab	32.6 ± 0.8 a	24.78 ± 0.8 a

Data are mean ± SE (n = 6). Different letters within a column indicate significant ($p \leq 0.05$) differences between treatments.

Table 4. Mean concentrations of anions (mg g^{-1} DW) of kohlrabi bulb affected by different irrigation treatments (control, mixed water, and mixed water + S) and subjected to inoculation with two formulations of plant growth-promoting bacteria (Control, B1 and B2).

Irrigation	PGPB	Chloride	Nitrate	Phosphate	Sulfate
Control	Control	3.55 ± 0.28 bc	16.39 ± 0.81 a	21.02 ± 0.60 abc	11.32 ± 0.79 bcd
	B1	3.55 ± 0.41 bc	16.08 ± 1.87 a	21.31 ± 0.34 ab	10.35 ± 0.63 cd
	B2	3.20 ± 0.17 c	17.52 ± 1.28 a	21.89 ± 0.41 a	10.18 ± 0.41 cd
Mixed water	Control	4.74 ± 0.29 a	4.68 ± 0.55 b	18.78 ± 0.62 d	11.32 ± 0.29 bc
	B1	5.15 ± 0.33 a	5.18 ± 0.80 b	19.89 ± 0.49 bcd	13.10 ± 0.52 a
	B2	4.34 ± 0.25 ab	5.23 ± 0.81 b	20.14 ± 0.61 abcd	11.94 ± 0.24 ab
Mixed water + S	Control	2.94 ± 0.31 c	17.47 ± 1.92 a	21.37 ± 0.56 ab	9.56 ± 0.43 d
	B1	3.60 ± 0.20 bc	18.77 ± 0.78 a	21.73 ± 0.39 a	10.80 ± 0.29 bcd
	B2	3.37 ± 0.16 c	18.42 ± 0.76 ab	19.52 ± 0.87 cd	10.50 ± 0.27 bcd

Data are mean ± SE (n = 6). Different letters within a column indicate significant ($p \leq 0.05$) differences between treatments.

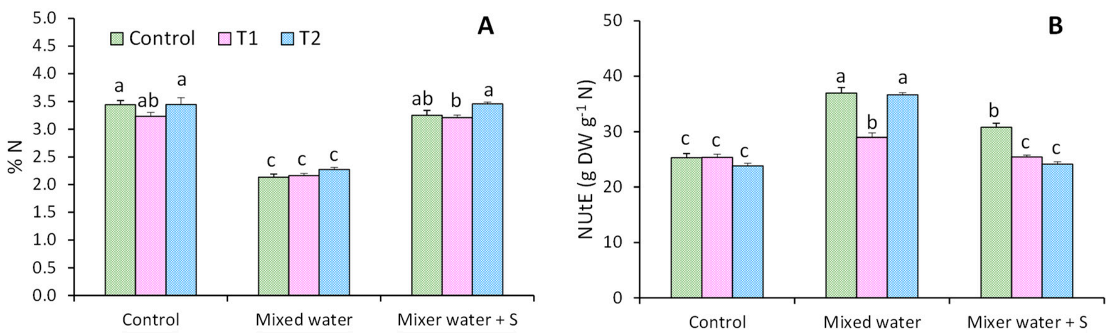

Figure 2. Nitrogen percent (**A**) and nitrogen utilization efficiency (NUtE) (**B**) of kohlrabi plants irrigated with control solution, mixed water, and mixed water + S; and inoculated with two formulations

of plant growth-promoting bacteria (control, B1 and B2). Data are presented as treatment means ± SE (n = 6). Different letters indicate significant differences ($p \leq 0.05$) between treatments.

3.3. Total Phenolic Compounds and Antioxidant Activity (ABTS^{+*})

The irrigation treatments increased the concentrations of both, although significant differences were only observed with the mixed water + S treatment (Figure 3A). On the other hand, the B2 inoculum combined with the control irrigation increased the concentration of total phenolic compounds in the kohlrabi plants with respect to the control (24.4%), while in combination with the mixed water + S treatment, an opposite effect was observed (Figure 3A), as shown by a reduction of 49.1% (Figure 3A).

Kohlrabi bulbs showed statistically different antioxidant activity values, ranging from 535 to 2560 μM Trolox g^{-1} DW (Figure 3B). The antioxidant activity showed opposite behaviors according to the irrigation treatment applied (Figure 3B). The mixed water treatment caused a 22.7% reduction with respect to the control, while the mixed water + S treatment increased the values of antioxidant activity by 16.7% with respect to the values of the control plants.

Regarding the effect of the inoculation with bacteria, Figure 3B shows that the antioxidant activity was lower when both types of inocula were applied in combination with the mixed water + S treatment (21.9% and 15.9%, respectively).

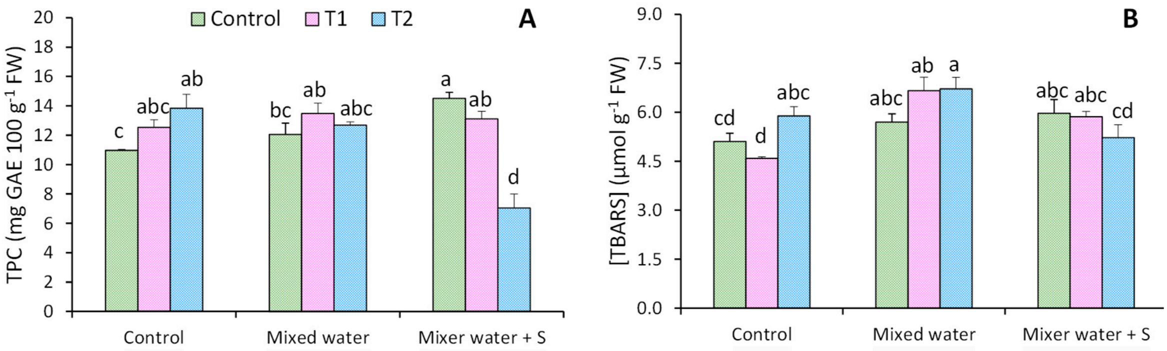

Figure 3. Total phenol compounds (TPC) (**A**) and antioxidant activity (ABTS^{+*}) (**B**) of kohlrabi plants irrigated with control solution, mixed water, and mixed water + S; and inoculated with two formulations of plant growth-promoting bacteria (control, B1 and B2). Data are presented as treatment means ± SE (n = 6). Different letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

3.4. Total Soluble Sugars

Table 5 shows the three free sugars in the kohlrabi bulb (glucose, fructose, and sucrose). The total content of free sugars varied from 830.3 to 1207.6 mg g^{-1} DW, with a reduction observed due to the application of both irrigation treatments (Table 5). When studying the different sugars measured individually, it was observed that glucose was the predominant one (Table 5). The application of the mixed water treatment caused a 20.0% reduction in glucose; however, it led to a strong increase in sucrose, which was 52.8% compared to the control (Table 5). On the other hand, irrigation with mixed water + S resulted in a general reduction in all sugars (Table 5).

Regarding the treatment with bacteria, it was observed that the application of the B1 inoculum with the control irrigation had no significant effects on any of the sugars measured, while the B2 inoculum caused a reduction of 20.5%, 23.3%, and 45.5% in glucose, fructose, and sucrose, respectively (Table 5). However, when the application of the B2 inoculum was performed in combination with the irrigation treatments, an opposite behavior was observed, as shown by the reduction in the negative effect of the irrigation treatments on the sugar concentration (Table 5). Specifically, the combined application of the mixed water treatment and the B2 inoculum increased the concentration of sugars, reaching values similar to those of the control plants in the case of total sugars.

Table 5. Mean concentrations of sugars (mg g^{-1} DW) of kohlrabi bulb affected by different irrigation treatments and subjected to inoculation with plant growth promoting bacteria (PGPB).

Irrigation	PGPB	Glucose	Fructose	Sucrose	Total Free Sugars
Control	Control	790.4 ± 53.8 a	280.6 ± 25.7 ab	39.4 ± 6.3 bc	1110.4 ± 77.3 abc
	B1	817.8 ± 42.1 a	271.4 ± 8.6 ab	42.3 ± 6.2 bc	1131.5 ± 46.1 ab
	B2	628.2 ± 17.8 b	215.3 ± 11.3 c	21.5 ± 1.9 d	864.9 ± 28.1 de
Mixed water	Control	633.6 ± 20.7 b	245.1 ± 7.3 bc	83.5 ± 4.6 a	961.1 ± 25.5 de
	B1	675.1 ± 13.0 b	282.9 ± 20.2 ab	46.0 ± 4.3 a	1004.0 ± 27.6 bcd
	B2	816.8 ± 42.7 a	316.2 ± 24.0 a	74.6 ± 3.4 b	1207.6 ± 64.8 a
Mixed water + S	Control	611.4 ± 25.9 b	195.7 ± 8.5 c	23.1 ± 2.7 d	830.3.0 ± 33.4 e
	B1	648.7 ± 42.7 b	207.2 ± 14.4 c	32.0 ± 3.0 cd	888.0 ± 55.7 de
	B2	629.8 ± 18.4 b	316.3 ± 7.9 a	21.4 ± 0.8 d	967.5 ± 18.1 cde

Data are means ± SE (n = 6). Different letters within a column indicate significant ($p \leq 0.05$) differences between treatments.

4. Discussion

4.1. Physical Characterization of Kohlrabi Bulb

To our knowledge, this may be the first study on growing purple kohlrabi in an aquaponic system. In view of our results, the fact that aquaponic systems provide a lower amount of certain nutrients, such as potassium, phosphates, and mainly nitrates, negatively affects the growth and development of kohlrabi bulbs. These results coincide with the findings by Jamil [21] and Al-Azzawi et al. [22] who observed a reduction in both dry matter and diameter of kohlrabi plants due to the application of deficient doses of nitrogen, phosphorus, and potassium, even observing that the combined application of organic and inorganic fertilizers increased these parameters. In our case, the extra contribution of inorganic fertilizers in the mixed water + S treatment provided concentrations of those nutrients that were quite similar to those of the control irrigation, diminishing the negative effects of the deficient treatment (mixed water). As can be observed in Figure 1A,B, both the dry matter and the diameter of the kohlrabi bulbs were similar in the plants grown with the control solution and with the mixed water + S treatment.

On the other hand, the B1 inoculum in combination with the control solution increased the dry matter and diameter of the kohlrabi plants. This increase could be responsible for the finding that no major effects were observed in the other measured parameters due to a dilution effect derived from the greater biomass.

4.2. Mineral Content

As can be observed in Table 3, purple kohlrabi cv. Ukza is rich in macro and micronutrients. Thus, in comparison with other studies carried out on the Azur, Videnska Synia, Videnska Bila, and Hihant varieties, cv. Ukza had higher contents of these nutrients [23]. Of the macro and micronutrients, the K content stands out, which was the predominant nutrient, with its content being 13-fold higher than those reported in previous studies [23].

Regarding nutrient absorption, as expected, in the case of irrigation treatments, a clear relationship was observed between the concentration provided through irrigation and the amount absorbed. However, in the case of inoculation with bacteria, both B1 and B2 combined with the mixed water + S treatment increased the absorption of all macronutrients, equaling, in most cases, the values of control plants (Table 3). These findings are in harmony with those reported by Soltan et al. [1], who observed increases in N, P, K, and Mg, as the bacteria increased the availability of nutrients required by the plants. The nutritional effect of *Pantoea* can be ignored, as it does not affect the absorption of N and P [8]. On the contrary, when the inocula were applied in combination with the control treatment, an even greater nutrient reduction occurred than with the irrigation treatments (Table 3). B1 caused reductions in K, P, Fe, Cu, and B, while B2 reduced B, in addition to the aforementioned (Table 3). Other authors related this greater absorption of nutrients with a greater production of growth-promoting substances (ABA, IAA, and several gibberellins) by the PGPB of the genera *Pseudomonas, Azospirillum*, *Azotobacter*, *Burkholderia*, and *Bacillus* [24–26]. These substances promote root growth and, therefore, increase the absorption of both water and nutrients.

The effectiveness of the inoculants was also studied by analyzing the accumulation of total N, and in no case were changes observed with respect to the plants grown without the inoculants. This finding coincides with that observed by Masood et al. [27], who did not observe any effects when PGPB was applied in N-limited or N-free environments. The fact that inoculation with bacteria does not have a more significant effect could be due both to the properties of the substrate (chemical and physical), the special characteristics of irrigation management in soilless crops (very high frequency of irrigation), and the specific response of this crop (species/variety) to this inocula. On the other hand, the application of low concentrations of nitrates in the irrigation water led to a lower N accumulation in the kohlrabi bulb tissues. However, both N assimilation and absorption are of great importance for the growth, development, and adaptation of plants to environmental stresses, as N is closely related to photosynthesis, photorespiration, and secondary metabolism [28].

When we studied the efficiency in the use of absorbed N (NUtE), it was observed that plants irrigated with a lower concentration of nitrates increased their NUtE, which indicates that plants in these conditions have a greater capacity to synthesize more biomass per unit of N absorbed. Authors, such as Qin et al. [29], observed similar results in tomato plants subjected to stress due to N deficiencies. These plants had reduced growth and biomass accumulation due to low N supply. However, the NUE increased, as a result of an increase in N uptake efficiency (NUpE) and NUtE. What this demonstrates is that plants have a defense system that allows them to survive under N deficiency stress conditions by enhancing nutrient uptake by the roots and energy use for N assimilation.

4.3. Total Phenolic Compounds and Antioxidant Activity ($ABTS^{+*}$)

It is known that kohlrabis are vegetables with potent antioxidant activity, which may be due to the presence of phenolic compounds (such as myricetin, luteolin, kaempferol, and apigenin) or their phytochemical components [30]. In the case of purple kohlrabi, the concentration of phenolic compounds is even higher, as they are responsible for giving said pigmentation to these varieties. Authors such as Jung et al. [30] made a comparison of the content of phenolic compounds in green and purple kohlrabi, and the concentration of total phenolic compounds was twice as high in purple kohlrabi. Regarding the increase in total phenolic compounds due to the N content provided in the irrigation water, different behaviors have been described in the literature. Authors such as Nieto-Ramírez et al. [31] observed a higher content in *L. graveolens* irrigated with aquaculture wastewater than in those irrigated with a Steiner solution. Similar results were also observed in *Origanum vulgare L.* ssp. Hirtum, when fertilized with different levels of nitrogen (0.150 kg of N ha^{-1}), as the highest content of total phenols was obtained in unfertilized plants. On the contrary, other authors did not see significant differences in the content of phenolic compounds of *Lactuca sativa* L., neither by the application of different nitrogen concentrations nor by the application of PGPB [32]. However, as we observed in our plants irrigated with a water + S mixture, Shams [33] and Al-Azzawi et al. [22] observed an increase in total phenolic compounds in kohlrabi plants when applying combined mineral and organic N. This higher content of total phenolic compounds is partly responsible for granting them a greater antioxidant potential, as these compounds protect against oxidative damage and play an important role in human nutrition. Therefore, this increase in total phenolic compounds is of great interest, as it has been observed that phenols act as excellent anti-inflammatory agents, preventing diabetes mellitus and its possible complications [30].

On the other hand, in the case of the inocula, the only one that had significant effects was B2, but the behavior was completely different depending on the irrigation treatment with which it was combined. Authors such as Benedetto et al. [34] and Cappellari et al. [35] stated that inoculation with PGPB improves the production of secondary metabolites, such as phenolic compounds. However, Ottaiano et al. [36], in a study of the impact of PGPB supply under different N regimes on the quality and yield of lettuce, found that the content of ascorbic acid and total phenolics decreased as the N level increased.

4.4. Total Soluble Sugars

It has been previously observed that the content of free sugars in leaves is positively influenced by abiotic stresses such as high temperatures and nutrient deficiency, among other factors [37,38]. The accumulation of osmolytes, such as soluble sugars, is one of the defense mechanisms that plants have to reduce damage from different abiotic stresses [39]. On the contrary, the data from the present work showed that in the case of kohlrabi bulbs, a reduction in the concentration of total soluble sugars occurred, which is in agreement with the results obtained in another study on cauliflower florets subjected to high temperatures [40].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9121299/s1, Table S1: The fish feed composition.

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