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Microbial and Non-Microbial Biostimulants as Innovative Tools to Increase Macro and Trace Element Mineral Composition of Tomato and Spinach

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Abstract: The use of biostimulants has gained popularity in recent years as a sustainable approach to increase the yield and quality of horticultural crops. However, information is missing concerning their ability to enhance the concentration of some beneficial elements (macro- and microelements) in the edible tissues of plants, which, in turn, are useful for human health. For this reason, we investigated the effects of different microbial and non-microbial biostimulants on the content of essential macro- and micro-nutrients (P, K, Ca, Mg, Cu, Fe, Mn, Zn, Se) in San Marzano and Datterino tomatoes (*Solanum lycopersicum* L.) and spinach (*Spinacia oleracea* L.) by atomic adsorption spectrometry, also estimating the Daily Intake (EDI) and the Nutrient Contribution (NC) of fresh produce. All the biostimulants were able to increase the content of macro- and micro-nutrients in the studied horticultural products. Specifically, compared with control, application of *Trichoderma harzianum* T22 on the Pixel tomato increased Fe, Zn, Cu, Mn and Se contents by 49.66, 38.68, 129.79, 64.03 and 72.72%. In the San Marzano tomato, higher values of Fe (55.16 µg 100 g^{-1} fw), Mn (30.63 µg 100 g^{-1} fw), Zn (20.89 µg 100 g^{-1} fw), Cu (1.91 µg 100 g^{-1} fw) and Se (0.266 µg 100 g^{-1} fw) were obtained after application of a tropical plant extract (TPE) biostimulant. Similarly, compared with control, application of a vegetal-derived protein hydrolysate (VPDH) on spinach increased EDI-Fe, EDI-Zn, EDI-Mn, EDI-Cu and EDI-Se by 98.98, 127.09, 125.93, 68.52 and 230.76%, respectively. Therefore, biostimulants, regardless of their origin and nature, could be an ecological tool for biofortification programs for both fruit and leafy vegetables.

Keywords: *Solanum lycopersicum* L.; *Spinacia oleracea* L.; microelements; biofortification; EDI; *Trichoderma*

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1. Introduction

Recently, a growing awareness of the environmental impact of food production (in terms of pollution, greenhouse gas emissions, soil depletion, biodiversity loss, etc.), has led to the definition of "sustainable food quality" [1]. A more sustainable food production system involves using resources at a pace that can be tolerated and ultimately, completely replenished by our environment. Consequently, a "sustainable food quality" should embrace various issues, including safety, affordability and nutritional and functional values, while controlling the use of chemical fertilizers, herbicides and pesticides by exploiting natural plant defenses and biodiversity [2]. In this perspective, the use of biostimulants is gaining more and more popularity thanks to the possibility of application in more sustainable production systems, which at the same time allows for increasing the yield and quality of the product. Biostimulants can consist of organic substances (e.g., humic acids, algae or plant extracts, protein hydrolysates, chitosan, vitamins), inorganic compounds (e.g., cobalt, silica, selenium) or plant growth promoting microorganisms or their extracts (e.g., fungi and bacteria) [3]. They are not classified as fertilizers or pesticides, but they can increase resource use efficiency, growth and yield and resilience and tolerance to abiotic stresses when applied to plants [4]. Plant biostimulants act mainly on plants by inducing multiple direct and indirect physiological effects, which are linked, just to name a few, to the greater mobility and solubility of mineral nutrients in the soil, changes in the architecture of the root system, better efficiency in water use and ion uptake, mobilization and utilization [5]. Regarding the composition and properties of plant foods, biostimulants can increase the synthesis and accumulation of primary and secondary metabolites, including important categories of antioxidants, such as carotenoids, polyphenols and ascorbic acid [6], thus ultimately improving the nutritional and nutraceutical quality of edible products.

In this study, we focused on three types of biostimulants, two of which are of plant origin, (a tropical plant extract and a commercial legume derived-plant hydrolysate) and a microbial-based biostimulant containing *Trichoderma harzianum* strain T22, an endophytic fungus, was applied to two varieties of tomato (San Marzano and Datterino) and spinach. Both tomato (*Solanum lycopersicum* L.) and spinach (*Spinacia oleracea* L.) are among the most widely grown vegetables, are globally consumed both fresh and in a variety of processed products, and are considered a powerhouse for nutrients and are low in calories [7]. The San Marzano tomato is a traditional variety grown in the Campania region in southern Italy; indigenous tomato varieties may have interesting traits such as stress resistance and high-quality fruit [8]. Spinach is a vegetable with a high biological value, but is prone to the accumulation of nitrates, which is considered potentially dangerous because they are related to methemoglobinemia in children (blue baby syndrome) and the synthesis of N-nitroso compounds (NOCs), classified as "probably carcinogenic to humans" [9]. Biostimulants may also lower the nitrate content because they are able to improve their reductive assimilation, thus limiting their accumulation in the leaves. In many experimental tests, the use of biostimulants has allowed for keeping nitrate below the maximum residue levels (MRLs) [10].

Many studies have focused their attention on the capacity of biostimulants to decrease the nitrate content in leafy vegetables or increase the content of beneficial such as K, Ca, and Mg; however, this study, for the first time, investigated the effects of different biostimulants on the concentration of essential macro- and micronutrients (P, K, Ca, Mg, Cu, Fe, Mn, Zn, Se) in San Marzano and Datterino tomatoes and spinach.

2. Materials and Methods

2.1. Greenhouse Experiment of Mini Plum Tomato Pixel Variety F1 under Trichoderma harzianum T22

Mini plum tomato (*Solanum lycopersicum* L.) seedlings variety Pixel F1 (indeterminate growth; ISI Sementi SpA, Fidenza, Italy) were transplanted in a greenhouse located at the University of Naples Federico II, Portici (NA), south Italy (40°49′ N, 14°15′ E; 72 m a.s.l.) on April 2019 [11]. Before transplant, 1.4 kg m^{-2} of manure (32-7-2) was supplied to the greenhouse soil. This latter was classified as sandy loam texture soil (sand, silt, and clay: 75%, 18%, and 6%), with electrical conductivity (EC) 0.4 dS m^{-1}, pH 7.1, total N 0.10%, organic matter 1.6% (*w/w*), and Olsen phosphorus and exchangeable potassium were 31 and 1012 mg kg^{-1}, respectively, NO_3-N and NH_4-N at 90 and 10 mg kg^{-1}, respectively. *Trichoderma harzianum* T22 (T22) (10^7 spore mL^{-1}; commercial formulation of Trianum, kindly provided by Koppert Biological Systems, Rotterdam, the Netherlands) was applied before transplant and occurred on 4 April 2019, dipping the seedling roots in a solution containing the inoculum whereas the control was treated with water. The application of T22 was repeated at 48, 72 and 89 days after transplanting (DAT) by manually watering each plant with the inoculum at 25, 100 and 250 mL, respectively. Increasing quantities of inoculum were used according to the increase in plant growth in the subsequent phenological phases. Plants were fertigated with 2.5 kg of calcium nitrate three times in two-week intervals, starting on 59 DAT; foliar was treated with calcium and magnesium between 75 and 90 DAT, and four times with *Bacillus thuringiensis* vr. *kurstaki*, every 10-days starting from 60 DAT [11]. Harvesting was performed once weekly and starting on 12 July.

2.2. Greenhouse Experimental of Pomodoro San Marzano DOP under Plant Extract-Based Biostimulant

The experiment was carried out on a "Pomodoro San Marzano dell'Agro Sarnese-nocerino DOP" (*Solanum lycopersicum* L.) landrace in the 2017 summer growing season, in a greenhouse on the experimental pilot farm "Torre Lama" (Bellizzi, SA, Italy) of the Department of Agricultural Sciences (40° 37′ N, 14° 57′ E, 60 m a.s.l.). Clay loam texture soil was used (sand, silt, and clay: 47%, 25%, and 28%), with electrical conductivity (EC) 0.15 dS m^{-1}, pH 7.8, total nitrogen 0.11%, organic matter 1.23% (w/w) and Olsen phosphorus and exchangeable potassium being 85 and 889 mg kg^{-1}, respectively. Plants were fertigated once per day with a nutrient solution at EC 1.9 dS m^{-1} and pH 6.4 as detailed in [12]. The tomato plants were treated by foliar application of a commercial tropical plant extract (TPE) Auxym® (Italpollina, Rivoli Veronese, Italy), produced through water extraction and fermentation of tropical plant biomasses as detailed in [2]. Tomato plants were sprayed uniformly with fertilizer containing 2 mL L^{-1} of Auxym® or only with fertilizer for control plants, starting from the 35th day after the transplant (DAT). Harvesting starting at 60th DAT (1 July) and continued until the end of the experiment (1 August; 90th DAT).

2.3. Greenhouse Experiment of Spinacia oleracea L. under Protein Hydrolysate-Based Biostimulant

The experiment on "baby spinach" (*Spinacia oleracea* L. cv. Platypus RZ F1, RijkZwaan, Bologna, Italy) was conducted from January to March 2018 in a greenhouse in the same location and soil composition as the Pixel experiments [2]. The commercial vegetal-derived protein hydrolysate (VPDH) biostimulant Trainer® was provided by Italpollina S.p.A. (Rivoli Veronese, Italy). It was obtained through enzymatic hydrolysis and its composition is described in detail by [13]. No detectable phytohormones in the commercial PH have been recorded. The baby spinach was planted on 19 January, supplied with 45 kg ha^{-1} of N, applied as NH_4NO_3 (34%) by an overhead irrigation system, in 3 weekly applications starting 7 days after sowing (DAS). It was foliar sprayed uniformly or not (control) with 4 mL L^{-1} of PH at 25, 32, 39, 46 and 53 DAS. Plants were harvested on 14 March (55 DAS).

2.4. Macro- and Micronutrients Analyses

All samples were lyophilized and then they were ground into a powder by a Fritsch pulverisette 6 (Fritsch Pulverisette type 00.502. Oberstein, Germany) with an agate mortar to prevent element contamination. To evaluate the total K, Ca, Ca, Mg, Fe, Zn, Cu, Cr, Mn and Se concentration, samples were mineralized (250 mg) using a combination of hydrogen peroxide and nitric acid (H_2O_2 50% *v/v*: HNO_3 65% *v/v* = 1:3) in a microwave oven (Milestone—mls 1200—Microwave Laboratory Systems). After digestion, the solutions were diluted by deionised water to a final volume of 50 mL. The concentration of each element was measured by atomic adsorption spectrometry (SpectrAA 20 Varian) via graphite furnace and flame. Accuracy was checked by concurrent analysis of standards (Resource© by PSP Technology Corporation, Laramie, WY, USA) and the recovery was in a range of 90–110% for each element [14,15].

2.5. Estimated Daily Intake (EDI) and Recommended Daily Allowance (RDA) Percentage of Macro- and Micronutrients from Tomato and Spinach

The EDI (expressed as mg per day) and RDA (expressed as percentage) were calculated based on the daily portion of fresh tomato or spinach consumed per capita (22.5 g) for adults [16]) and the recommended daily intake for each mineral. EDI was calculated as the product of the macro- or micronutrients concentration and the average daily consumption of fresh produce (22.5 g). In accordance with the scientific literature, the recommended daily intakes for the respective macro- and micronutrients were: 3500 mg K, 1000 mg P, 1000 mg Ca, 350 mg Mg, 15 mg Fe, 15 mg Zn, 5 mg Mn, 3 mg B, 2 mg Cu, 120 µg Cr, 75 µg Mo and 55 µg Se [17–19].

2.6. Data Analyses

For each experiment, the data were subjected to analysis of variance (ANOVA) and the means were compared with Student's *t* test. The SPSS 20 software package (IBM Corp., Armonk, NY, USA) was used. Data represent the mean ± standard error of 3 replications (n = 3).

3. Results and Discussion

The macro- and micronutrients concentration in horticultural crops is influenced by genetic and environmental factors, whose interaction drives variation in plant traits [20]. Recent studies have proven that plant biostimulants (PB), including plant-derived extracts or hydrolysates and microbial biostimulants, can improve nutrient use efficiency (NUE) in plants [21–24]. In particular, PB may boost macro- and micronutrients' uptake and assimilation, mainly because of changes in root architecture, including increases in length, density, surface area, number of lateral roots and root exudation of low-molecular-weight metabolites able to promote the formation of micro-aggregates enhancing microbial activity and, in particular, nitrogen and phosphorus uptake [25,26]. In our previous studies, we focused our attention on the capacity of biostimulants to increase the content of beneficial ions such as K, Ca and Mg or decrease the content of the antinutrient nitrate in leafy vegetables [2,27].

In this study, for the first time, we show preliminary results on the effects of different biostimulants not only on the content of essential macronutrients (P, K, Ca and Mg) but also on micro-nutrients (Cu, Fe, Mn, Zn and Se) in tomato fruits and spinach.

In particular, the analysis of microelements in Pixel cherry tomato fruits (Table 1) showed that, regardless of treatment, Fe was the most abundant in micronutrients (122.30 μg 100g^{-1} fw, on average), followed by Zn (16.93 μg 100g^{-1} fw, on average), Cu (9.46 μg 100g^{-1} fw, on average), Mn (8.51 μg 100g^{-1} fw, on average) and Se (0.015 μg 100g^{-1} fw, on average). The application of the T22 biostimulant significantly increased, compared to control, the concentration of all micronutrients analyzed by ICP-OES. Specifically, an increase of 49.66, 38.68, 129.79, 64.03 and 72.72% was recorded for Fe, Zn, Cu, Mn and Se, respectively. Similar results were also observed in *Lens culinaris* [28], *Triticum aestivum* [29], *Lupinus* L [30] and *Cucurbita pepo* [31] treated with *Trichoderma*, which, as supported by [32] through the mechanisms of chelation and reduction, would have allowed for better solubilization of these mineral compounds, including Fe_2O_3, MnO_2 and Zn.

Table 1. Macro- and micronutrients' content of Pixel tomato fruits under control or *T. harzianum* strain T22 biostimulant treatments.

Treatment	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Se
	(mg 100 g^{-1} fw)				(μg 100 g^{-1} fw)				
Control	1.59 ± 0.15	15.82 ± 1.62	2.70 ± 0.20	1.87 ± 0.18	5.74 ± 0.48	97.97 ± 8.84	6.45 ± 0.58	14.19 ± 1.32	0.011 ± 0.001
T22	2.32 ± 0.14	25.61 ± 1.02	8.24 ± 0.33	3.40 ± 0.18	13.19 ± 0.50	146.63 ± 6.55	10.58 ± 0.45	19.68 ± 0.72	0.019 ± 0.001
Significance	**	**	***	***	**	**	***	*	***

*, **, ***: Significant at $p \leq 0.05$, 0.01, or 0.001, respectively, according to the Student's *t*-test. All data are mean ± standard error, n = 3.

T22 biostimulant treatment resulted in significant differences in EDI and NC for all micronutrients analyzed (Table 2). Regardless of treatment, Fe showed the highest EDI and NC values, which averaged 244.6 μg die^{-1} and 1.63%, respectively. Compared to control, biostimulant application increased the EDI values in all microelements. The NC values of Fe, Zn, Cu, Mn and Se increased by 49.61, 36.84, 131.57, 61.53 and 70%, respectively.

Table 2. Estimated Daily Intake (EDI) and Nutrient Contribution (NC) of Pixel tomato fruits under control or *T. harzianum* strain t22 biostimulant treatments.

Mineral Element		Control		T22		Sig.	
		EDI	NC	EDI	NC	EDI	NC
P	(mg die^{-1})—(%)	3.17 ± 0.30	0.32 ± 0.03	4.65 ± 0.28	0.46 ± 0.03	**	**
K	(mg die^{-1})—(%)	31.65 ± 3.24	0.90 ± 0.09	51.22 ± 2.04	1.46 ± 0.06	**	**
Ca	(mg die^{-1})—(%)	5.39 ± 0.39	0.54 ± 0.04	16.49 ± 0.65	1.65 ± 0.07	***	***
Mg	(mg die^{-1})—(%)	3.75 ± 0.35	1.07 ± 0.10	6.80 ± 0.35	1.94 ± 0.10	***	***
Cu	(μg die^{-1})—(%)	11.49 ± 0.96	0.57 ± 0.05	26.39 ± 0.99	1.32 ± 0.05	**	**
Fe	(μg die^{-1})—(%)	195.9 ± 17.7	1.31 ± 0.12	293.3 ± 13.1	1.96 ± 0.09	**	**
Mn	(μg die^{-1})—(%)	12.90 ± 1.16	0.26 ± 0.02	21.17 ± 0.90	0.42 ± 0.02	***	***
Zn	(μg die^{-1})—(%)	28.38 ± 2.63	0.19 ± 0.02	39.36 ± 1.44	0.26 ± 0.01	*	*
Se	(μg die^{-1})—(%)	0.022 ± 0.001	0.040 ± 0.003	0.037 ± 0.002	0.068 ± 0.004	***	***

*, **, ***: Significant at $p \leq 0.05$, 0.01, or 0.001, respectively, according to the Student's *t*-test. All data are mean ± standard error, n = 3.

Regardless of the treatment, among the five micronutrients quantified in San Marzano tomato fruits (Table 3), the most abundant were Fe, followed by Mn, Zn, Cu and Se (39.63 μg 100 g^{-1} fw > 21.24 μg 100 g^{-1} fw > 14.15 100 g^{-1} fw, 1.39 μg 100 g^{-1} fw, and 0.19 μg 100 g^{-1} fw, on average, respectively), unlike what was observed in Tomato Pixel (Table 2), highlighting the effect of genotypes on mineral bioaccumulation in fruits [13]. For all micronutrients, the application of the TPE biostimulant resulted in a significant difference with $p \leq 0.01$. Specifically, the concentrations of Fe, Mn, Zn, Cu and Se in tomato fruits treated with the biostimulant were 128.87, 158.26, 181.91, 119.54 and 131, 30% higher than in control, similar to the findings of a previous study by [33].

Table 3. Macro- and micronutrients content of San Marzano tomato fruits under control or tropical plant extract (TPE) treatments.

Treatment	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Se
	(mg 100 g^{-1} fw)				(μg 100 g^{-1} fw)				
Control	1.69 ± 0.10	16.51 ± 0.66	0.27 ± 0.01	0.74 ± 0.07	0.87 ± 0.02	24.10 ± 0.70	11.86 ± 0.32	7.41 ± 0.23	0.115 ± 0.01
TPE	2.78 ± 0.08	30.54 ± 0.73	1.54 ± 0.03	2.44 ± 0.09	1.91 ± 0.06	55.16 ± 1.16	30.63 ± 0.52	20.89 ± 0.72	0.266 ± 0.01
Significance	*	**	***	**	**	**	**	**	**

*, **, ***: Significant at $p \leq 0.05$, 0.01, or 0.001, respectively, according to the Student's *t*-test. All data are mean ± standard error, n = 3.

The same authors [33] assessed that the positive effects of plant biostimulants on the mineral profile are not attributable to the micronutrients in commercial formulations (present in low concentrations) but to their role as direct promoters of root uptake. It is possible that biostimulants may influence active soil microorganisms [34], thus changing the soil texture and structure, which influence the nutrients' availability from soil to plant roots [35]. Billard and coworkers [36] suggest that such positive effects on the bioaccumulation of the mineral profile in leaves may also be attributable to the improved translocation of minerals from roots to shoots. This latter finding is confirmed by the fact that nutrient concentrations in plant tissues are known to fluctuate together [37], therefore a biostimulation of translocation capacity may improve in general the use efficiency of most of the nutrients. In addition, since Zn has limited mobility in leaves [38], the fact that it increased in tomato fruits means that translocation from leaves to fruit was also enhanced.

The results reported in Table 4 show that the EDI and NC of all micronutrients were significantly affected by the biostimulant treatment. Compared with control, the application of biostimulant on tomato fruits increased EDI-Fe by 128.87%, EDI-Mn by 158.26%, EDI-Zn by 181.91%, EDI-Cu by 119.54% and EDI-Se by 131.30%. The same trend was observed for the NC of all micronutrients. Specifically, biostimulant treatment resulted in the highest values of NC-Mn (1.23%), NC-Se (0.97%), NC-Fe (0.74%), NC-Zn (0.28%) and NC-Cu (0.19%).

Table 4. Estimated Daily Intake (EDI) and Nutrient Contribution (NC) of San Marzano tomato fruits under control or tropical plant extract (TPE) treatments.

Mineral Element		Control		TPE		Sig.	
		EDI	NC	EDI	NC	EDI	NC
P	(mg die^{-1})—(%)	3.38 ± 0.21	0.34 ± 0.02	5.56 ± 0.15	0.56 ± 0.02	*	*
K	(mg die^{-1})—(%)	33.02 ± 1.32	0.94 ± 0.04	61.09 ± 1.46	1.75 ± 0.04	**	**
Ca	(mg die^{-1})—(%)	0.53 ± 0.02	0.05 ± 0.01	3.08 ± 0.06	0.31 ± 0.01	***	***
Mg	(mg die^{-1})—(%)	1.49 ± 0.15	0.43 ± 0.04	4.88 ± 0.18	1.40 ± 0.05	**	**
Cu	(μg die^{-1})—(%)	1.74 ± 0.03	0.09 ± 0.00	3.82 ± 0.12	0.19 ± 0.01	**	**
Fe	(μg die^{-1})—(%)	48.20 ± 1.40	0.32 ± 0.01	110.31 ± 2.33	0.74 ± 0.02	**	**
Mn	(μg die^{-1})—(%)	23.72 ± 0.64	0.47 ± 0.01	61.26 ± 1.04	1.23 ± 0.02	**	**
Zn	(μg die^{-1})—(%)	14.82 ± 0.46	0.10 ± 0.01	41.78 ± 1.43	0.28 ± 0.01	**	**
Se	(μg die^{-1})—(%)	0.230 ± 0.01	0.419 ± 0.02	0.532 ± 0.01	0.968 ± 0.02	**	**

*, **, ***: Significant at $p \leq 0.05$, 0.01, or 0.001, respectively, according to the Student's *t*-test. All data are mean ± standard error, $n = 3$.

The results for the concentration of the five micronutrients in spinach leaves, reported in Table 5, show that, regardless of biostimulant treatment, the most abundant microelement was Fe, followed by Zn, Mn, Cu and Se, with average values of 346.15, 194.3, 117.43, 21.22 and 0.176 μg 100 g^{-1} fw, respectively. In line with other studies [21,39,40] reporting that the VDPD improves the uptake of macro- and micronutrients by plants, compared with control, the foliar application of VDPH increased the concentration of all micronutrients which are all essential for human health [41,42]. Specifically, the highest percentage increase was recorded for Se (224.09%) followed by Zn (127.10%), Mn (125.90%), Fe (102.70%) and Cu (68.50%).

Table 5. Macro- and micronutrients content of spinach plants under control or vegetal-derived protein hydrolysate (VPDH) treatments.

Treatment	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Se
	(mg 100 g^{-1} fw)				(μg 100 g^{-1} fw)				
Control	4.91 ± 0.31	46.88 ± 2.32	7.10 ± 0.35	6.76 ± 0.38	15.81 ± 0.91	240.6 ± 13.60	72.06 ± 2.90	118.8 ± 5.55	0.083 ± 0.01
PDPH	8.39 ± 0.25	66.68 ± 3.18	13.50 ± 0.67	19.43 ± 0.73	26.64 ± 0.86	478.7 ± 25.09	162.8 ± 4.66	269.8 ± 10.37	0.269 ± 0.01
Significance	**	**	**	***	***	**	***	***	***

, *: Significant at $p \leq 0.01$ or 0.001, respectively, according to the Student's *t*-test. All data are mean ± standard error, $n = 3$.

Remarkably, the latter element (Cu) plays a key role in the course of infectious disease caused by the SARS-CoV-2 virus (COVID-19) [43].

As shown in Table 6, VDPH significantly influenced spinach's EDI and NC of micronutrients. Compared to control, spinach plants treated with the biostimulant increased EDI-Fe, EDI-Zn, EDI-Mn, EDI-Cu and EDI-Se by 98.98, 127.09, 125.93, 68.52 and 230.76%, respectively. Biostimulant treatment resulted in the highest values in NC-Fe (5.11%), NC-Zn (2.88%), NC-Mn (5.21%), NC-Cu (2.13%) and NC-Se (0.784%).

Table 6. Estimated Daily Intake (EDI) and Nutrient Contribution (NC) of spinach plants under control or vegetal-derived protein hydrolysate (VDPH) treatments.

Mineral Element		Control		VDPH		Sig.	
		EDI	NC	EDI	NC	EDI	NC
P	(mg die^{-1})—(%)	7.85 ± 0.49	0.78 ± 0.05	13.42 ± 0.41	1.34 ± 0.04	**	**
K	(mg die^{-1})—(%)	75.01 ± 3.71	2.14 ± 0.11	106.7 ± 5.09	3.05 ± 0.15	**	**
Ca	(mg die^{-1})—(%)	11.37 ± 0.56	1.14 ± 0.06	21.60 ± 1.08	2.16 ± 0.11	**	**
Mg	(mg die^{-1})—(%)	10.81 ± 0.60	3.09 ± 0.17	31.09 ± 1.17	8.88 ± 0.34	***	***
Cu	(μg die^{-1})—(%)	25.29 ± 1.45	1.26 ± 0.07	42.62 ± 1.37	2.13 ± 0.07	***	***
Fe	(μg die^{-1})—(%)	384.9 ± 21.77	2.57 ± 0.15	765.9 ± 40.14	5.11 ± 0.27	**	**
Mn	(μg die^{-1})—(%)	115.3 ± 4.64	2.31 ± 0.09	260.5 ± 7.45	5.21 ± 0.15	***	***
Zn	(μg die^{-1})—(%)	190.1 ± 8.87	1.27 ± 0.06	431.7 ± 16.60	2.88 ± 0.11	***	***
Se	(μg die^{-1})—(%)	0.134 ± 0.01	0.243 ± 0.01	0.431 ± 0.01	0.784 ± 0.02	***	***

, *: Significant at $p \leq 0.05$, 0.01, or 0.001, respectively, according to the Student's *t*-test. All data are mean ± standard error, $n = 3$.

As shown by the heat map analysis (Figure 1), all the biostimulant treatments induced an increase of the content of the macro- and micronutrients. In particular, San Marzano under TPE and spinach under VDPH treatment underwent the most noteworthy increase of micronutrients, except for Cu, in addition to Mg, whereas Ca and Cu highly increased under T22 and TPE treatments.

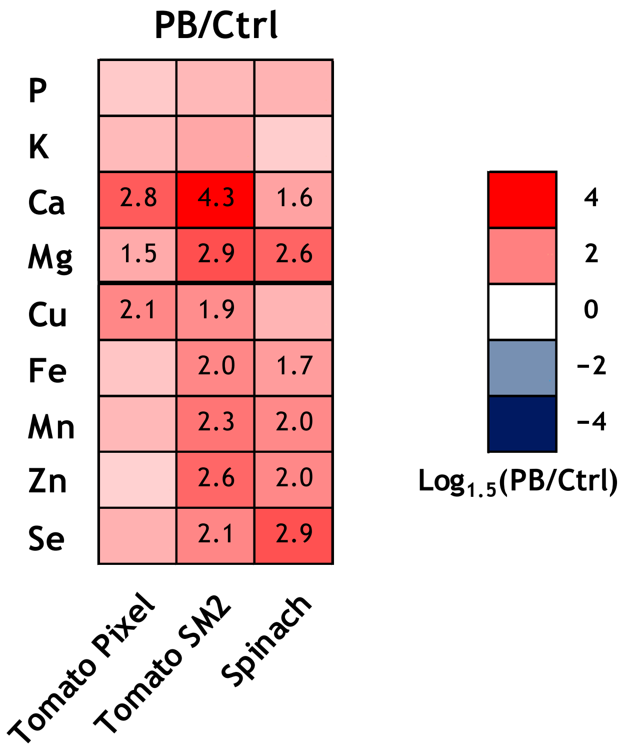

Figure 1. Heat map analysis summarizing macro- and micronutrients variations in Pixel and San Marzano tomato fruits, and spinach plants under *T. harzianum* strain T22, tropical plant extract or vegetal-derived protein hydrolysate biostimulant treatments vs. respective controls. Results were calculated as Logarithm base 1.5 ($Log_{1.5}$) of plant biostimulants treatments/control values (PB/Ctrl). Results were visualized using a false color scale, with red indicating an increase and blue a decrease of values relative to those in control condition. No differences were visualized by white squares.

4. Conclusions

This study provided evidence on the ability of the different biostimulants to sustainable improve the mineral nutrients use efficiency of the treated species and the nutritional quality of food products. Indeed, the application of biostimulants in horticulture may be a useful tool for achieving a high level of sustainability through the reduction of fertilizing elements and therefore of environmental pollution. At the same time, they allow for increasing the productive and qualitative parameters of crops, limiting the negative influences of environmental stress and allowing plants to express their full potential. Therefore, biostimulants represent a promising strategy for boosting agri-food systems while addressing the sustainable development goals of the 2030 Agenda for better production and better nutrition.

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