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Yield and Nutritional Quality of Vesuvian Piennolo Tomato PDO as Affected by Farming System and Biostimulant Application

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Abstract: Scientific investigations are being increasingly devoted to biostimulant effects on vegetable yield and quality, with the perspective of sustainable crop management. Two farming systems (conventional or organic) in factorial combination with two biostimulant treatments (tropical plant extract (PE); legume-derived protein hydrolysate (PH)) plus a non-treated control were compared in terms of tomato fruit yield, yield components, mineral composition, functional and nutritional indicators. PE- and PH-based biostimulants resulted in higher plant biomass, PH even in higher leaf area index, compared to non-treated control. Marketable yield was not significantly affected by farming system. PH and PE gave higher yield than non-treated control. PH treatment led to higher fruit number than the control, whereas PE incurred significant increase in yield only under organic farming. The mean fruit weight attained the highest value upon PE application under conventional management. Colour component a* (redness) was higher with the conventional system compared to the organic one, whereas an opposite trend was shown by the organic acids malate, oxalate and isocitrate. Irrespective of the farming system, the soluble solids, fruit brightness (L*) and redness as well as the target organic acids malate, oxalate, citrate and isocitrate were significantly higher than untreated plants by 10.1%, 16.1%, 19.8%, 18.9%, 12.1%, 13.5% and 26.8%, respectively, with no significant differences between the PH- and PE-based biostimulants. Higher lipophilic activity and total ascorbic acid concentration but lower lycopene were recorded under organic management. PE and PH application resulted in higher total phenol and ascorbic acid as well as in lycopene content, and lipophilic antioxidant activity than the non-treated control. Biostimulants proved to be an effective sustainable tool for enhancing tomato fruit yield and functional quality both under conventional and organic vegetable systems.

Keywords: antioxidant activity; functional quality; lycopene; organic farming; protein hydrolysate; *Solanum lycopersicum* L.; tropical plant extract

1. Introduction

Organic horticulture has been increasing worldwide for the past two decades, as a result of rising demand of consumers for healthy and safer food [1], accounting for 3.5 million ha in 2014, which is almost twofold compared to 2008 [2]. Indeed, this farming management is environmentally-friendly due to food production with minimal harm to ecosystems as well as minimal use of inputs in particular fertilizers and pesticides [3]. However, the lower yield compared to conventional agriculture, i.e.

−20% according to Ponisio et al. [4] and −5% to −34% as reported by Seufert et al. [5], represents a disadvantage of organic farming. The latter yield reduction is mainly associated to higher biotic pressure caused by parasites, pests and pathogens [4,6] and to nutrient limitation in particular N and P [7] which limits production in several organic-based systems [8]. In fact, the rate of major minerals such as nitrogen and phosphorus released from organic fertilizers and crop residues do not often meet the crop demand during the highest rate plant growth, leading to significant yield reduction [9].

Within both conventional and organic farming systems, the use of naturally derived plant biostimulants is a promising sustainable approach [10,11], aiming to enhance (i) plant nutrient availability/uptake/assimilation and use efficiency, (ii) abiotic stress tolerance as well as (iii) product quality [12–14]. Within biostimulants, protein hydrolysates (PHs) are mainly made of amino acids, polypeptides and oligopeptides derived from proteins of animal or plant origin upon partial hydrolysis [15] and can be applied to seeds, leaves or soil in several forms (liquid or granular) [12]. Tropical plant extract (PE) and especially legume-derived protein hydrolysates (PHs) obtained from vegetal origin proteins have been drawing interest in world agricultural areas, compared to animal-derived ones, due to both their higher agronomic value [16] and no use constraints in organic farming. Moreover, PE or PH application to leaves and/or roots reportedly elicit physiological processes, thus resulting in enhancement of growth [17,18], production and quality [18,19], tolerance to abiotic stressors, such as drought, soil and water salinity, extreme temperature, nutrient deficiency, soil acidity and alkalinity [11,20–25]. Notably, PE or PHs also encourage plant activity of key enzymes involved either in N or C metabolism [12,24,26,27]. In addition, PH treatment may boost crop performances, by eliciting auxin- and gibberellin-like activities through bioactive peptides [17,28,29]. PE and PHs also exert indirect effects on plants, as they modify the architecture of roots and increase their hair surface expansion, thus enhancing macro- and microelement uptake [26,28,30–32]. However, limited scientific literature are available with regard to the effect of foliar applications of PH or PE in interaction with either conventional or organic farming on agronomical and fruit quality responses of tomato landraces, in particular the long shelf-life cherry tomato landrace 'Pomodorino del Piennolo del Vesuvio' (PPV), a typical niche product of Campania (Italy) horticultural sector.

In the perspective of the above mentioned topics, a two-year experiment was carried out to assess the response of cherry tomato landrace PPV to foliar applications of a vegetal protein based hydrolysate or a tropical plant extract biostimulant in interaction with organic or conventional crop system, in terms of yield, mineral composition, functional and sensorial quality attributes.

2. Materials and Methods

2.1. Growing Conditions and Experimental Protocol

The experimental research was carried out on open field grown tomato (*Solanum lycopersicum* L.) 'Piennolo del Vesuvio D.O.P.' ecotype Riccia, in Portici (Naples), southern Italy characterized by a typical Mediterranean climate, in 2016 and 2017. The soil was sandy-loam having 77% sand, 14.5% silt, 8.5% clay, with soil electrical conductivity of 342 μS cm^{-1}, 1.6% organic matter, 0.94 g kg^{-1} N, 63.9 mg kg^{-1} P_2O_5, 1.8 g kg^{-1} K_2O. The monthly air temperature (day/night) and rainfall recorded at the plant level, expressed as means of the two research years, were the following: 21.6 °C, 7.9 °C and 47.2 mm in April; 24.5 °C, 11.3 °C and 56.3 mm in May; 29.5 °C, 15.8 °C and 23.7 mm in June; 32.2 °C, 17.1 °C and 17 mm in July.

A factorial combination of biostimulant application (B) and farming system (F) was applied, based on two biostimulant treatments (PH or PE) plus a non-treated control and two farming systems (organic or conventional). The experimental design was a randomized complete-block design with three replications, yielding 18 experimental units (3 B × 2 F × 3 replications). Each experimental unit consisted of an 8 square meter plot. Tomato seedlings were transplanted on 25 and 24 April in the first and second growing season respectively, at a plant density of 4 plants m^{-2}.

The two commercial PH and PE-based biostimulants 'Trainer'®and 'Auxym'®were kindly provided by Italpollina S.p.A., Rivoli Veronese, Italy. The legume-derived PH biostimulant obtained through enzymatic hydrolysis contains 75% of free amino acids and peptides, 22% of carbohydrates and 3% of mineral nutrients. The detailed aminogram of the product along with the phenolics, flavonoids and elemental composition were reported by Rouphael et al. [31] and Paul et al. [25]. The PE biostimulant obtained by fermentation of tropical plants contains 54% of free amino acids and peptide, 17% carbohydrate, 23% mineral nutrients, 6% vitamins and 0.22% phytohormones as reported in detail by Rouphael et al. [33] and Caruso et al. [32].

Cherry tomato plants were sprayed with a solution containing 3 and 2 ml L^{-1} of PH- or PE-based biostimulant, or with water (non-treated control), four times during the growing season at 7-day intervals, starting in coincidence with the early growth of the first fruit truss.

Organic farming practices were performed in compliance with the EC Regulation 834/2007 and related subsequent updates. Both in conventional and organic systems, the fertilization was carried out with 153 kg ha^{-1} of N, 39 kg ha^{-1} of P_2O_5 and 223 kg ha^{-1} of K_2O. Phosphorus was completely supplied at planting, whereas nitrogen and potassium were given both prior to crop establishment (31% and 55% for N and K_2O respectively) and the remainder on dressing. Under the organic management a 6-5-13 Bioilsa organic-mineral fertilizer (based on hydrolyzed collagen and meat flour), N (11%) and N-K (7%–21%) hydrolyzed protein manure were used; ammonium sulphate, potassium sulphate, potassium nitrate and ammonium nitrate were supplied to the conventionally grown crops. Drip irrigation started when the soil available water capacity decreased to 80%. Crop protection was performed against downy mildew, tomato leaf miner, aphids, whitefly, and red spider.

2.2. Yield, Biometric Assessments and Leaf Color Measurements

Harvests of fully ripe fruits were performed from 14 July to 2 August, as an average of the two research years, and the marketable yield, number of fruits per plant and the mean fruit mass were determined on a sub-plot of 4 m^2. Fruits that were deformed or misshaped were considered unmarketable. The final leaf area was measured on 10 plants in each experimental plot using a Licor 3000 electronic area meter (Licor, Lincoln, NE, USA) and then the leaf area index was calculated. A sample of the fresh material was dried at 70 °C for about 3 days until reaching constant weight, to determine dry aboveground biomass.

Cherry tomato color was measured on the two sides of 10 fruits per experimental unit using Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan) in order to obtain the color space parameters, in particular L* (brightness), a* (redness) and b* (yellowness).

2.3. Juice Total Soluble Solids and Fruit Dry Matter Content

The cherry tomato PPV fruits were homogenized in a blender for 2 min and the homogenate was filtered, then the total soluble solids content was measured using the Bellingham and Stanley digital refractometer (model RFM 81). The tomato fruit dry matter percentage was also determined after drying the fresh material at 70 °C for about 3 days until reaching constant weight. The dried tomato fruit samples were collected for further mineral analysis.

2.4. Mineral and Organic Acids Analysis

The desiccated cherry tomato fruit tissues were ground in a Wiley Mill to pass through an 841 µm screen and used for macro-mineral profile analysis, sodium content and organic acids as described in detail by Rouphael et al. [31] and Kyriacou et al. [34]. Phosphorus, potassium, calcium, magnesium, sulfur, sodium, malate, oxalate, citrate and isocitrate were separated and quantified by ICS-3000 ion chromatography (Dionex, Sunnyvale, CA, USA) coupled to a conductivity detector. Macronutrients, sodium and organic acids concentrations were expressed on a dry weight basis (g kg^{-1} dw).

2.5. Antioxidant Activity Analysis

The lipophylic and hydrophilic antioxidant activities were assessed on extract from freeze-dried cherry tomato PPV fruits (200 mg) added with methanol and distilled water, respectively. The antioxidant activity of the lipophilic and hydrophilic extract fractions were measured with the 2,20-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS [35] and with the N,N-dimethyl-p-phenylenediamine (DMPD) methods [36], respectively. The absorbance of the solutions for LAA and HAA were measured at 734 and 505 nm, respectively. Lipophylic and hydrophilic antioxidant activities were expressed as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchro man-2-carboxylic acid) and mmol ascorbic acid per 100 g of dw [36].

2.6. Antioxidant Molecules Analysis

The total ascorbic acid and polyphenols were assessed spectrophotometrically based on the protocol by Kampfenkel et al. [37] and the Folin–Ciocalteau procedure [38], respectively, after slight modifications [34]. For quantification, ascorbate and gallic acid were used as external standards to build calibration curves both for total ascorbic acid and total polyphenols content. The absorbance of the solutions for total ascorbic acid and total polyphenols were measured at 525 and 765 nm, respectively, and the results were expressed as mg ascorbic acid on 100 g fw and mg gallic acid per 100 g dw. Lycopene content was also assessed spectrophotometrically, based on the protocol by Sadler et al. [39], and for the quantification pure lycopene (Sigma, St. Louis, MO) was used to build the calibration curves. The absorbance of the lycopene hexane solution was measured at 472 nm. Lycopene content was expressed in mg 100 g^{-1} fw.

2.7. Statistical Processing

All agronomical and qualitative data were subjected to three-way analysis of variance using the software package SPSS. The means were separated by DMRT test at 0.05 significance level. All the agronomical and quality variables were not significantly affected by the growing season (i.e., year) or its interactions with the two experimental factors applied, and therefore the mean data of the two years were reported.

3. Results and Discussion

3.1. Yield and Morphometric Measurements

As reported in Table 1, the legume-derived protein hydrolysate (PH) resulted in the highest leaf area index and biomass of the vegetative plant parts, though the latter variable was not significantly different from that recorded under the effect of the tropical plant extract (PE).

Marketable yield and its components, fruit number and mean weight, were significantly affected by biostimulant treatment, whereas no differences were recorded between conventional and organic systems. Moreover, fruit number and mean weight were also significantly influenced by the interaction between the two experimental factors (Table 1). The application of PH-based biostimulant resulted in the highest yield but PE biostimulant also gave a significantly higher yield compared to non-treated control (+18.7% and +11.2%, respectively); these outcomes stemmed from the combined effects of fruit number and mean weight (Table 1).

The two latter variables were significantly affected by the interaction between the two studied factors (Table 1). For instance, the fruit number per plant was just connected to the effect of PE-based biostimulant, leading to higher fruit number than the control only under organic system, whereas PH-based biostimulant always showed the best effect. Moreover, the mean fruit weight attained the highest value upon PE application under conventional management, the latter being also higher than that obtained with the organic system, whereas no differences were recorded between the remaining comparisons.

Table 1. Plant growth parameters and yield indicators of 'Piennolo del Vesuvio' cherry tomato as affected by farming system and biostimulant application.

Source of Variance	LAI (m^2 m^{-2})	Aerial Biomass (g dw m^{-2})	Marketable Yield (t ha^{-1})	Marketable Fruits Mean Weight (g per Fruit)	Marketable Fruits Number No. per Plant
Year (Y)	ns	ns	ns	ns	ns
Farming system (F)	ns	ns	ns	ns	ns
Biostimulant (B)	*	*	*	ns	*
Y × F	ns	ns	ns	ns	ns
Y × B	ns	ns	ns	ns	ns
F × B	ns	ns	ns	*	*
Y × F× B	ns	ns	ns	ns	ns
Year (Y)					
2016	4.92	350.3	14.5	15.5	23.1
2017	5.00	375.5	15.0	15.7	24.3
Farming system (F)					
Organic	5.05	368.0	14.7	15.4	24.0
Conventional	4.87	357.8	14.8	15.8	23.3
Biostimulant formulate (B)					
Control	4.54 c	336.4 b	13.4 c	15.4	21.8 b
Legume-derived protein hydrolysate (PH)	5.31 a	385.5 a	15.9 a	15.4	26.0 a
Tropical plant extract (PE)	5.03 b	366.8 a	14.9 b	16.0	23.2 b

dw, dry weight. ns, * nonsignificant or significant at $p \leq 0.05$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

In contrast with the present research findings, in previous investigation [40] conventional management of different vegetable species led to higher yield than organic one. Consistently with our results, Colla et al. [30] detected growth and yield increase of tomato in greenhouse upon PHs application, which is a whole crop cycle extension of the short-time stimulation effect observed on tomato treated with PH extracts [17,41]. Notably, the effects shown by the applied biostimulant on plants is different from the nutritional input elicited by fertilizers [42]. Indeed, in our research tomato plants showed different patterns of yield components response to the applied substances in interaction with the crop system (Figure 1).

Foliar applications of PE and PHs may have triggered in tomato plants a physiological mechanism linked to the enhanced content of signaling molecules which are the prevailing PE and PH components [12]. In this respect, low-sized molecules such as peptides and free amino acids can regulate plant phenological progress upon their easy absorption through leaves and roots by promoting endogenous biosynthesis of phyto-hormones [43]. Consistently, other authors [17,18,31,33,44] reported that plant growth, fruit setting and yield were enhanced by the auxin- and sometimes gibberellin-like activity of the mentioned biostimulants.

PE- and PH-based biostimulants are likely to boost plant development and yield through: (i) stimulating cell proliferation by signaling molecules such as specific amino acids connected to nitrogen metabolism (i.e., glutamic and aspartic acids) and soluble peptides; (ii) vitamin provision targeted to cell protection from oxidation; (iii) encouraging plant metabolism with micronutrients supply ([26] and references cited therein). Moreover, an important increase in cytokinins content was promoted by biostimulant application in *Spinacea oleracea* [45]. An additional action pattern of PE and legume-derived PH consists of enhancing macronutrient uptake and assimilation through modulation of root biomass, density and lateral root number, as well as microbial activity with the consequent higher availability of soil nutrients [13,30].

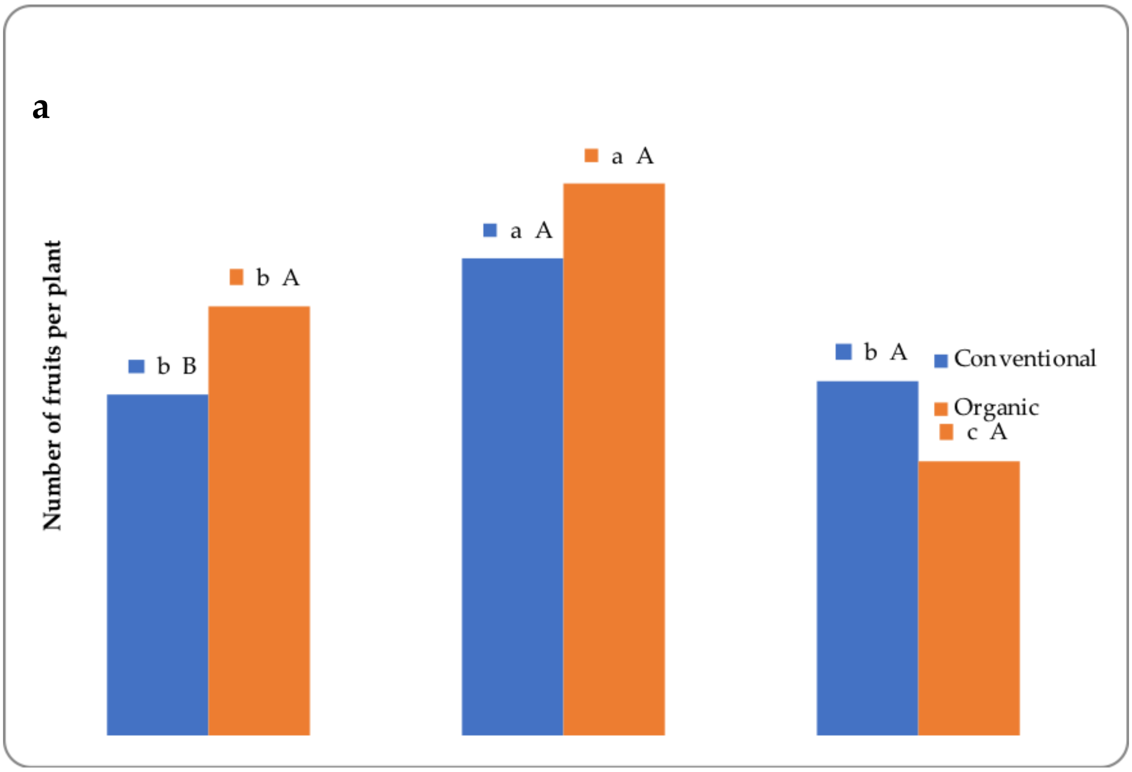

Figure 1. *Cont.*

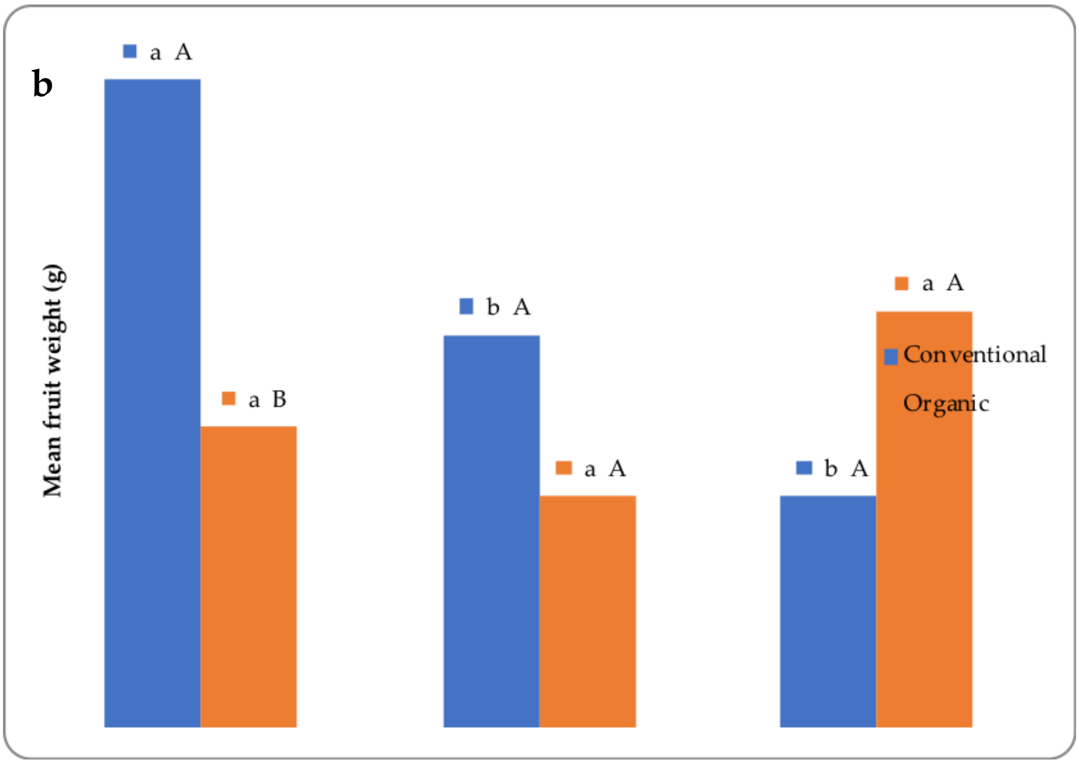

Figure 1. Interaction between farming system and biostimulant application on 'Piennolo del Vesuvio' cherry tomato fruit number per plant (**a**) and mean weight (**b**). Different letters mean significant difference according to Duncan's multiple range test at $p \leq 0.05$. Lowercase letters refer to the comparison between biostimulants, whereas capital letters to the comparison between farming systems within each biostimulant application.

In other experiments, *Lactuca sativa* L. sprayed with PE or PH showed a 11% higher biomass than non-treated control, which may be as a consequence of both the stimulation exerted by the most represented substances such as amino acids and key peptides and of enhancement of cultivable epiphytic bacteria as well as their species richness and diversity [46]. Overall, the direct and/or indirect mode of actions of the applied biostimulants may have boosted both growth and crop productivity of treated cherry tomato plants compared to the non-treated control treatment.

3.2. Fruit Colorimetry, Nutritional Quality and Mineral Profile

Farming system significantly affected some target indicators of tomato fruit colorimetry and nutritional quality as well as mineral composition (Tables 2 and 3). Two out of the three variables characterizing the colour (a* and b*) were higher under conventional management compared to the organic one; conversely, the organic acids malate, oxalate and isocitrate attained higher concentrations in the organically grown berries (Table 2). In the present research, both tomato fruit dry matter percentage and soluble solids were not significantly affected by farming management, whereas in previous investigations asparagus spears [47] and leek pseudo-stems [48] organically grown in southern or northern Europe respectively showed higher dry matter and sugar content than those managed conventionally.

Table 2. Flavor compounds and fruit colorimetry of 'Piennolo del Vesuvio' cherry tomato as affected by farming system and biostimulant application.

Source of Variance	Dry Matter (%)	TSS (°Brix)	Fruit Colorimetry			Organic Acids (g kg^{-1} dw)			
			L *	A *	B *	Malate	Oxalate	Citrate	Isocitrate
Farming system	ns	ns	ns	*	*	*	*	ns	*
Biostimulant	ns	*	*	*	ns	*	*	*	*
F × B	ns	ns	ns	ns	ns	ns	ns	ns	ns
Farming system									
Organic	8.6	7.3	40.8	31.3	20.4	13.4	1.25	43.8	0.54
Conventional	8.9	7.5	43.6	34.4	23.5	10.9	1.07	40.5	0.43
Biostimulant formulate									
Control	8.4 b	6.9 b	38.1 b	29.0 b	21.1 b	10.8 b	1.07 b	38.7 b	0.41 b
Legume-derived protein hydrolysate	8.9 a	7.6 a	44.9 a	35.1 a	22.1 a	12.4 a	1.22 a	43.3 a	0.53 a
Tropical plant extract	9.0 a	7.6 a	43.6 a	34.4 a	22.6 a	13.3 a	1.18 a	44.6 a	0.51 a

TSS, total soluble solids. ns, * nonsignificant or significant at $p \leq 0.05$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Table 3. Fruit mineral composition of 'Piennolo del Vesuvio' cherry tomato as affected by farming system and biostimulant application.

Source of Variation	Mineral Composition (g kg^{-1} dw)					
	P	K	S	Ca	Mg	Na
Farming system	ns	ns	*	ns	ns	ns
Biostimulant	*	*	*	ns	*	*
F × B	ns	ns	ns	ns	ns	ns
Farming system						
Organic	0.87	36.43	0.76 a	5.51	1.44	0.31
Conventional	0.93	35.01	0.68 b	6.02	1.52	0.29
Biostimulant formulate						
Control	0.82 b	33.83 b	0.62 c	5.60	1.30 b	0.28 b
Legume-derived protein hydrolysate	1.00 a	36.66 a	0.83 a	5.72	1.59 a	0.30 ab
Tropical plant extract	0.87 ab	36.68 a	0.72 b	5.92	1.56 a	0.32 a

ns, * nonsignificant or significant at $p \leq 0.05$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Regardless of the farming system, the soluble solids, fruit brightness and redness as well as the target organic acids malate, oxalate, citrate and isocitrate were significantly higher than untreated plants by 10.1%, 16.1%, 19.8%, 18.9%, 12.1%, 13.5% and 26.8%, respectively, with no significant differences between the PH- and PE-based biostimulants (Table 2). The highest fruit juice soluble solids and organic acids obtained in biostimulant-treated plants independently on the formulate could be considered important key quality attributes for consumer satisfaction [49]. Consistently with our findings, Rouphael et al. [31,33], Colla et al. [18] and Ertani et al. [19] reported the increased content of soluble solids, glucose and fructose in greenhouse grown *Solanum lycopersicum* and *Capsicum chinensis* fruits upon the treatment with biostimulants, derived from tropical plant extract fermentation, enzymatic hydrolysis of legume and alfalfa plants or by extraction of red grapes.

Minerals content is essential for the quality of fruit vegetables including tomato. Based on two surveys carried out in Finland and the USA, Levander [50] demonstrated that the contribution of vegetables to dietary intake of phosphorus, potassium, calcium, magnesium and sodium is 7–11%, 31–35%, 5–7%, 18–24% and 11%, respectively. The present work has generated important information regarding the relative abundance of minerals in cherry tomato landrace and its variation range across farming system and biostimulant application. In this respect, K was found by far the most abundant mineral, followed by Ca, P, Mg, S and Na (Table 2).

For all measured minerals no significant interaction between farming system and biostimulant application was observed (Table 3). Neither farming system nor biostimulant application had significant effect of Ca content in fruit (average 5.7 g kg^{-1}). The effect of biostimulant application on tomato fruit mineral profile was much more pronounced than the farming system. K and Mg were positively affected by both biostimulants compared to non-treated control, with no significant difference between them. PH-based biostimulant exhibited a higher content of P; in addition, both commercial biostimulants had a better effect on S content compared to the untreated control, with PH showing the highest values (Table 3). In other investigations, compared to non-treated plants the application of a PH-based biostimulant resulted in better nutritional status: higher K and Mg content in tomato [18,31] and in spinach [33] grown under protected cultivation.

In the present research, the increased concentration of cherry tomato fruit K and Mg induced by the application PH-based biostimulant might have been mediated through several direct/indirect mechanisms involving: (i) enhanced mineral uptake promoted by root growth stimulation encouraging absorption, translocation and accumulation of nutrients [17,51]; (ii) higher nutrient transporter expression in cell membranes [24,52]; (iii) the action of PH biostimulant bioactive compounds (soluble peptides, carbohydrates and free amino acids) in strengthening the sink effect and therefore the movement of nutrients within the plant [42].

3.3. Antioxidant Activity and Bioactive Content

Fruit vegetables in particular tomato are considered good sources of lipophilic and hydrophilic antioxidant molecules such as lycopene, total ascorbic acid and polyphenols. The influence of farming system and biostimulant application on antioxidant activities and bioactive compounds are reported in Table 4. Neither farming system nor biostimulant application had a significant effect on hydrophilic antioxidant activity (average 10.9 mmol ascorbate eq. 100 g^{-1} dw). When averaged over biostimulant application, higher lipophilic activity and total ascorbic acid concentration but lower lycopene were recorded under organic management compared to the conventional one. Moreover, no significant differences between the two farming systems arose with regard to hydrophilic antioxidant activity and phenols content (Table 4). Consistently with our results, in previous research carried out on strawberry in southern Italy [53], organic farming resulted in higher fruit ascorbic acid than the conventional management. As for the biostimulant application, both the PH and PE biostimulants resulted in higher lipophilic antioxidant activity as well as phenols, ascorbic acid and lycopene concentration than non-treated control, with no significant differences between the two commercial biostimulants used (Table 4).

Table 4. Antioxidant activity and bioactive content of 'Piennolo del Vesuvio' cherry tomato as affected by farming system and biostimulant application.

Source of Variation	Antioxidant Capacity		Lycopene	Total Phenols	Total Ascorbic Acid
	Lipophilic (mmol Trolox eq. $100g^{-1}$ dw)	Hydrophilic (mmol Ascorbate eq. $100g^{-1}$ dw)	(mg $100g^{-1}$ fw)	(mg Gallic Acid eq. $100g^{-1}$ dw)	(mg $100g^{-1}$ fw)
Farming system	*	ns	*	ns	*
Biostimulant	*	ns	*	*	*
F × B	ns	ns	ns	ns	ns
Farming system					
Organic	8.1 a	11.0	171.0 b	1.9	23.9 a
Conventional	7.7 b	10.8	188.2 a	1.9	18.5 b
Biostimulant formulate					
Control	5.8 b	10.7	150.2 b	1.8 b	14.5 c
Legume-derived protein hydrolysate	9.1 a	11.1	196.3 a	2.0 a	29.9 a
Tropical plant extract	8.7 a	10.9	192.0 a	2.0 a	19.2 b

ns, * nonsignificant or significant at $p \leq 0.05$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

The phytochemical homeostasis requires enzymatic activities leading to a stabilization of the concentration of antioxidants which show an increase both in response to free radical production [19,31] and when K and Mg in the tissues are high [31]. In this respect, the protection against oxidative stresses in maize plants was primed by both protein hydrolysate and plant extract based biostimulant through the expression of superoxide dismutases activity-regulating genes [54], which catalyze the enzymatic dismutation of superoxide to H_2O_2 [55]. The application of protein hydrolysates in greenhouse conditions encouraged the synthesis of ascorbate, p-coumaric, chlorogenic acid, capsaicin and antioxidant activity in *Capsicum chinensis* L. fruits [19], as well vitamin C in tomato fruits [18,31]. Similarly, *Spinacia oleracea* phenolic acids production was enhanced by biostimulant application [45], through the phenylalanine ammonia lyase pathway [56]. Therefore, the foliar application of plant biostimulants such as PH or PE can be instrumental in satisfying increasing consumer standards for the functional quality aspects of fresh cherry tomato PPV landrace [57,58].

4. Conclusions

From research carried out in southern Italy on tomato landrace 'Piennolo del Vesuvio D.O.P.' the effective application of plant biostimulants based on tropical plant extract or legume-derived protein hydrolysate on fruit yield, nutritional and functional attributes arose. Indeed, both formulates overall enhanced production, quality, mineral and antioxidant indicators either under organic or conventional farming systems. Controversial outcomes stemmed from the comparison between the two crop managements, as conventional farming resulted in better colored and lycopene richer fruits, but higher organic acids, ascorbic acid content and lipophilic antioxidant activity was recorded when organic procedures were applied. The present study allows us to draw important conclusions relevant to the significant contribution of biostimulant application in making sustainable even a conventional tomato farming system.

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References

1. Dorais, M.; Alsanius, B. Recent advances in organic horticulture technology and management. *Sci. Hortic.* **2016**, *208*, 1–2. [CrossRef]
2. Willer, H.; Lernoud, J. The World of Organic Agriculture—Statistics and Emerging Trends 2016. FiBL-IFOAM Rep. Bonn. Available online: https://shop.fibl.org/fileadmin/documents/shop/1698-organicworld-2016.pdf (accessed on 23 August 2019).
3. Dorais, M. Organic production of vegetables: State of the art and challenges. *Can. J. Plant Sci.* **2007**, *85*, 1055–1066. [CrossRef]
4. Ponisio, L.C.; M'Gonigle, L.K.; Mace, K.C.; Palomino, J.; de Valpine, P.; Kremen, C. Diversification practices reduce organic to conventional yield gap. *Proc. R. Soc. B* **2015**, *282*, 20141396. [CrossRef] [PubMed]
5. Seufert, V.; Ramankutty, N.; Foley, J.A. Comparing the yields of organic and conventional agriculture. *Nature* **2012**, *485*, 229–232. [CrossRef] [PubMed]
6. Orsini, F.; Maggio, A.; Rouphael, Y.; De Pascale, S. 'Physiologically quality' of organically grown vegetables. *Sci. Hortic.* **2016**, *208*, 131–139. [CrossRef]
7. De Ponti, T.; Rijk, B.; Van Ittersum, M.K. The crop yield gap between organic and conventional agriculture. *Agric. Syst.* **2012**, *108*, 1–9. [CrossRef]

8. Berry, P.M.; Sylvester-Bradley, R.; Philipps, L.; Hatch, D.J.; Cuttle, S.P.; Rayns, F.W.; Goslin, P. Is the productivity of organic farms restricted by the supply available nitrogen? *Soil Use Manag.* **2002**, *18*, 248–255. [CrossRef]
9. Lester, G.E.; Saftner, R.A. Organically versus conventionally grown produce: Common production inputs, nutritional quality, and nitrogen delivery between the two systems. *J. Agric. Food. Chem.* **2011**, *59*, 10401–10406. [CrossRef]
10. Colla, G.; Rouphael, Y. Biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 1–2. [CrossRef]
11. Rouphael, Y.; Cardarelli, M.; Bonini, P.; Colla, G. Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. *Front. Plant Sci.* **2017**, *8*, 131. [CrossRef]
12. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini, L.; Canaguier, R.; Rouphael, Y. Protein hydrolysates as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 28–38. [CrossRef]
13. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
14. Rouphael, Y.; Cardarelli, M.; Colla, G. Role of arbuscular mycorrhizal fungi in alleviating the adverse effects of acidity and aluminium toxicity in zucchini squash. *Sci. Hortic.* **2015**, *188*, 97–105. [CrossRef]
15. Schaafsma, G. Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. *Eur. J. Clin. Nutr.* **2009**, *63*, 1161–1168. [CrossRef] [PubMed]
16. Cerdán, M.; Sánchez-Sánchez, A.; Jordá, J.D.; Juárez, M.; Sánchez-Andreu, J. Effect of commercial amino acids on iron nutrition of tomato plants grown under lime-induced iron deficiency. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 859–866. [CrossRef]
17. Colla, G.; Rouphael, Y.; Canaguier, R.; Svecova, E.; Cardarelli, M. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. *Front. Plant Sci.* **2014**, *5*, 448. [CrossRef]
18. Colla, G.; Cardarelli, M.; Bonini, P.; Rouphael, Y. Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato. *Hort. Sci.* **2017**, *52*, 1214–1220. [CrossRef]
19. Ertani, A.; Pizzeghello, D.; Francioso, O.; Sambo, P.; Sanchez-Cortes, S.; Nardi, S. *Capsicum chinensis* L. growth and nutraceutical properties are enhanced by biostimulants in a long-term period: Chemical and metabolomic approaches. *Front. Plant Sci.* **2014**, *5*, 375. [CrossRef]
20. Botta, A. Enhancing plant tolerance to temperature stress with amino acids: An approach to their mode of action. *Acta Hortic.* **2013**, *1009*, 29–35. [CrossRef]
21. Colla, G.; Svecova, E.; Rouphael, Y.; Cardarelli, M.; Reynaud, H.; Canaguier, R.; Planques, B. Effectiveness of a plantderived protein hydrolysate to improve crop performances under different growing conditions. *Acta Hortic.* **2013**, *1009*, 175–179. [CrossRef]
22. Ertani, A.; Schiavon, M.; Muscolo, A.; Nardi, S. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant Soil* **2013**, *364*, 145–158. [CrossRef]
23. Lucini, L.; Rouphael, Y.; Cardarelli, M.; Canaguier, R.; Kumar, P.; Colla, G. The effect of a plant-derived protein hydrolysate on metabolic profiling and crop performance of lettuce grown under saline conditions. *Sci. Hortic.* **2015**, *182*, 124–133. [CrossRef]
24. Sestili, F.; Rouphael, Y.; Cardarelli, M.; Pucci, A.; Bonini, P.; Canaguier, R.; Colla, G. Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation. *Front. Plant Sci.* **2018**, *9*, 1233. [CrossRef] [PubMed]
25. Paul, K.; Sorrentino, M.; Lucini, L.; Rouphael, Y.; Cardarelli, M.; Bonini, P.; Reynaud, H.; Canaguier, R.; Trtílek, M.; Panzarová, K.; et al. Understanding the biostimulant action of vegetal-derived protein hydrolysates by high-throughput plant phenotyping and metabolomics: A case study on tomato. *Front. Plant Sci.* **2019**, *10*, 47. [CrossRef] [PubMed]
26. Colla, G.; Hoagland, L.; Ruzzi, M.; Cardarelli, M.; Bonini, P.; Canaguier, R.; Rouphael, Y. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Front. Plant Sci.* **2017**, *8*, 2202. [CrossRef] [PubMed]
27. Schiavon, M.; Ertani, A.; Nardi, S. Effects of an alfalfa protein hydrolysate on the gene expression and activity of enzymes of TCA cycle and N metabolism in *Zea mays* L. *J. Agric. Food Chem.* **2008**, *56*, 11800–11808. [CrossRef] [PubMed]

28. Ertani, A.; Cavani, L.; Pizzeghello, D.; Brandellero, E.; Altissimo, A.; Ciavatta, C.; Nardi, S. Biostimulant activities of two protein hydrolysates on the growth and nitrogen metabolism in maize seedlings. *J. Plant. Nutr. Soil. Sci.* **2009**, *172*, 237–244. [CrossRef]
29. Matsumiya, Y.; Kubo, M. Soybean. In *Soybean and Nutrition*; El-Shemy, H., Ed.; InTech Europe: Rijeka, Croatia, 2011; pp. 215–230. [CrossRef]
30. Colla, G.; Rouphael, Y.; Di Mattia, E.; El-Nakhel, C.; Cardarelli, M. Co-inoculation of *Glomus intraradices* and *Trichoderma atroviride* acts as a biostimulant to promote growth, yield and nutrient uptake of vegetable crops. *J. Sci. Food Agric.* **2015**, *95*, 1706–1715. [CrossRef] [PubMed]
31. Rouphael, Y.; Colla, G.; Giordano, M.; El-Nakhel, C.; Kyriacou, M.C.; De Pascale, S. Foliar applications of a legume-derived protein hydrolysate elicit dose dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. *Sci. Hort.* **2017**, *226*, 353–360. [CrossRef]
32. Caruso, G.; Stoleru, V.; De Pascale, S.; Cozzolino, E.; Pannico, A.; Giordano, M.; Teliban, G.; Cuciniello, A.; Rouphael, Y. Production, leaf quality and antioxidants of perennial wall rocket as affected by crop cycle and mulching type. *Agronomy* **2019**, *9*, 194. [CrossRef]
33. Rouphael, Y.; Giordano, M.; Cardarelli, M.; Cozzolino, E.; Mori, M.; Kyriacou, M.; Bonini, P.; Colla, G. Plant and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. *Agronomy* **2018**, *8*, 126. [CrossRef]
34. Kyriacou, M.C.; El-Nakhel, C.; Graziani, G.; Pannico, A.; Soterious, G.A.; Giordano, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chem.* **2019**, *277*, 107–118. [CrossRef] [PubMed]
35. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Rad. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
36. Fogliano, V.; Verde, V.; Randazzo, G.; Ritieni, A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J. Agric. Food Chem.* **1999**, *47*, 1035–1040. [CrossRef] [PubMed]
37. Kampfenkel, K.; Van Montagu, M.; Inzé, D. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Anal. Biochem.* **1995**, *225*, 165–167. [CrossRef] [PubMed]
38. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
39. Sadler, G.; Davis, J.; Dezman, D. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *J. Food Sci.* **1990**, *55*, 1460–1461. [CrossRef]
40. Caruso, G.; Conti, S.; La Rocca, G. Influence of crop cycle and nitrogen fertilizer form on yield and nitrate content in dierent species of vegetables. *Adv. Hortic. Sci.* **2011**, *25*, 81–89.
41. Parrado, J.; Bautista, J.; Romero, E.J.; García-Martínez, A.M.; Friaza, V.; Tejada, M. Production of a carob enzymatic extract: Potential use as a biofertilizer. *Bioresour. Technol.* **2008**, *99*, 2312–2318. [CrossRef]
42. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
43. Cavani, L.; Ciavatta, C. Attività biostimolante negli idrolizzanti proteici. *L'Informatore Agrario* **2007**, *44*, 46–52.
44. Carillo, P.; Colla, G.; El-Nakhel, C.; Bonini, P.; D'Amelia, L.; Dell'Aversana, E.; Pannico, A.; Giordano, M.; Sifola, M.I.; Kyriacou, M.C.; et al. Biostimulant application with a tropical plant extract enhances *Corchorus olitorius* adaptation to sub-optimal nutrient regimens by improving physiological parameters. *Agronomy* **2019**, *9*, 249. [CrossRef]
45. Kulkarni, M.G.; Rengasamy, K.R.R.; Pendota, S.C.; Gruz, J.; Plaˇcková, L.; Novák, O.; Doležal, K.; Van Staden, J. Bioactive molecules derived from smoke and seaweed *Ecklonia maxima* showing phytohormone-like activity in *Spinacia oleracea* L. *New Biotechnol.* **2019**, *48*, 83–89. [CrossRef] [PubMed]
46. Luziatelli, F.; Ficca, A.G.; Colla, G.; Baldassarre Švecová, E.; Ruzzi, M. Foliar application of vegetal-derived bioactive compounds stimulates the growth of beneficial bacteria and enhances microbiome biodiversity in lettuce. *Front. Plant. Sci.* **2019**, *10*, 60. [CrossRef] [PubMed]
47. Caruso, G.; Villari, G.; Borrelli, C.; Russo, G. Effects of crop method and harvest seasons on yield and quality of green asparagus under tunnel in southern Italy. *Adv. Hort. Sci.* **2012**, *26*, 51–58.

48. Golubkina, N.A.; Seredin, T.M.; Antoshkina, M.S.; Kosheleva, O.V.; Teliban, G.C.; Caruso, G. Yield, quality, antioxidants and elemental composition of new leek cultivars under greenhouse organic or conventional system. *Horticulturae* **2018**, *4*, 39. [CrossRef]
49. Kader, A.A. *Postharvest Technology of Horticultural Crops*; University of California, Division of Agriculture and Natural Resources Publication: Davis, CA, USA, 2002; Volume 3311, p. 535.
50. Levander, O.A. Fruit and vegetable contributions to dietary mineral intake in human health and disease. *Hort. Sci.* **1990**, *25*, 1486–1488.
51. Lucini, L.; Rouphael, Y.; Cardarelli, M.; Bonini, P.; Baffi, C.; Colla, G. A vegetal biopolymer-based biostimulant promoted root growth in melon while triggering brassinosteroids and stress-related compounds. *Front. Plant Sci.* **2018**, *9*, 472. [CrossRef]
52. Ertani, A.; Schiavon, M.; Nardi, S. Transcriptome-wide identification of differentially expressed genes in *Solanum Lycopersicon* L. in response to an alfalfa-protein hydrolysate using microarrays. *Front. Plant Sci.* **2017**, *8*, 1159. [CrossRef]
53. Conti, S.; Villari, G.; Faugno, S.; Melchionna, G.; Somma, S.; Caruso, G. Effects of organic vs. conventional farming system on yield and quality of strawberry grown as an annual or biennial crop in southern Italy. *Sci. Hortic.* **2014**, *180*, 63–71. [CrossRef]
54. Trevisan, S.; Manoli, A.; Quaggiotti, S.A. Novel biostimulant, belonging to protein hydrolysates, mitigates abiotic stress effects on maize seedlings grown in hydroponics. *Agronomy* **2019**, *9*, 28. [CrossRef]
55. Wang, W.; Xia, M.X.; Chen, J.; Yuan, R.; Deng, F.N.; Shen, F.F. Gene expression characteristics and regulation mechanisms of superoxide dismutase and its hysiological roles in plants under stress. *Biochem.* **2016**, *81*, 465–480. [CrossRef]
56. Hyun, M.W.; Yun, Y.H.; Kim, J.Y.; Kim, S.H. Fungal and plant phenylalanine ammonialyase. *Mycobiol.* **2011**, *39*, 257–265. [CrossRef] [PubMed]
57. Rouphael, Y.; Kyriacou, M.C.; Petropoulos, S.A.; De Pascale, S.; Colla, G. Improving vegetable quality in controlled environments. *Sci. Hortic.* **2018**, *234*, 275–289. [CrossRef]
58. Kyriacou, M.C.; Rouphael, Y. Towards a new definition of quality for fresh fruits and vegetables. *Sci. Hortic.* **2018**, *234*, 463–469. [CrossRef]