horticulturae

MDPI

Article

Joint Action of *Trichoderma atroviride* and a Vegetal Derived-Protein Hydrolysate Improves Performances of Woodland Strawberry in Italy

Lorena Vultaggio [1], Enrica Allevato [2], Beppe Benedetto Consentino [1,*], Pietro Bellitto [1], Simona Napoli [1,*], Claudio Cannata [3], Georgia Ntatsi [4], Sonya Vasto [5], Sara Baldassano [5], Salvatore La Bella [1], Claudio Leto [1] and Leo Sabatino [1]

[1] Dipartimento Scienze Agrarie, Alimentari e Forestali, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy; lorena.vultaggio@unipa.it (L.V.); pietro.bellitto@unipa.it (P.B.); salvatore.labella@unipa.it (S.L.B.); claudio.leto@unipa.it (C.L.); leo.sabatino@unipa.it (L.S.)
[2] Department of Environmental and Prevention Sciences (DiSAP), University of Ferrara, 44121 Ferrara, Italy; enrica.allevato@unife.it
[3] Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, Via S. Sofia, 100, 95123 Catania, Italy; claudio.cannata@phd.unict.it
[4] Laboratory of Vegetable Production, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece; ntatsi@aua.gr
[5] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, 90128 Palermo, Italy; sonya.vasto@unipa.it (S.V.); sara.baldassano@unipa.it (S.B.)
* Correspondence: beppebenedetto.consentino@unipa.it (B.B.C.); simona.napoli@unipa.it (S.N.)

Abstract: Woodland strawberry (*Fragaria vesca* L.) is an underutilized plant species that could benefit from the use of biostimulants to improve crop productivity and fruit quality. The scope of the present study was to appraise the influence of two biostimulants (*Trichoderma atroviride* and plant protein hydrolysate)—used either alone or combined—on the plant performance and economic profitability of two woodland strawberry genotypes ('Alpine' and 'Regina delle Valli'). Overall, data showed that 'Alpine' had the highest productive performances, whereas 'Regina delle Valli' revealed the highest fruit qualitative traits. *T. atroviride* inoculation and V-PH application significantly boosted plant marketable yield (+20.5% for *T. atroviride* and +12.9% for V-PH), total sugars (+1.9% for *Trichoderma* and +1.4% for V-PH) and anthocyanins (+14.1% for *T. atroviride* and +9.8% for V-PH) compared to non-treated plants. Plants supplied with both biostimulants had a higher marketable yield (+34.8%), mean fruit weight (+6.0%), fruit dry matter (+13.8%), total sugars (+3.5%), ascorbic acid (+12.7%), flavonoid (+26.3%) and anthocyanins (+29.9%) compared to non-treated plants. Furthermore, our study revealed that the highest fruit polyphenol concentration was recorded in both genotypes treated with the combination of biostimulants and in 'Regina delle Valli' sprayed with V-PH, whereas the highest antioxidant activity was found in 'Regina delle Valli' fruit when plants were supplied with both biostimulants. Our study pointed out that the application of microbial and non-microbial biostimulants, especially in combination, might be a useful strategy for improving the performances of underutilized species and, therefore, encouraging their cultivation, valorization and economic profitability (+6208.3 EUR /ha when plants were exposed to both biostimulants).

Keywords: *Fragaria vesca* L.; microbial biostimulant; non-microbial biostimulant; biostimulant 2.0; neglected species

check for updates

Citation: Vultaggio, L.; Allevato, E.; Consentino, B.B.; Bellitto, P.; Napoli, S.; Cannata, C.; Ntatsi, G.; Vasto, S.; Baldassano, S.; La Bella, S.; et al. Joint Action of *Trichoderma atroviride* and a Vegetal Derived-Protein Hydrolysate Improves Performances of Woodland Strawberry in Italy. *Horticulturae* **2024**, *10*, 459. https://doi.org/10.3390/horticulturae10050459

Received: 19 March 2024
Revised: 29 April 2024
Accepted: 30 April 2024
Published: 30 April 2024

1. Introduction

Small fruits represent a practical option due to their aptitude to produce prime quality food connected to a specific territorial setting, offering an additional source of income for the farmer. Woodland strawberry (*Fragaria vesca* L.) is one of the most elite small fruits and an imperative source of nutritional and functional components [1,2]. Although the strawberry (*Fragaria* × *ananassa* Duch.) chemical constituents have been deeply examined, little evidence has been reported for *Fragaria vesca* L., an herbaceous species that naturally grows in hilly or low-mountain areas [3]. Woodland strawberry fruits are characterized by a penetrating taste and aroma, are habitually consumed fresh or are employed in pastry-making and handling procedures. Nonetheless, the fruits of woodland strawberry are challenging to find and are to be considered a niche product. Currently, a profitable trade production of *F. vesca* has been established in northernmost Italy (alpine areas of Piedmont and Trentino) in open fields or under mini tunnels [2]. Furthermore, Nin et al. [2] reported that—in Italy—woodland strawberry cultivation is also spread in Lazio (near Rome) and in Campania (Salerno and Avellino) regions.

Woodland strawberry grown area is also located in Sicily, where various genotypes such as 'Alpine' and 'Regina delle Valli' cultivars and an endemic woodland 'Fior di Noto' are mainly cultivated in Marsala (Trapani Province). A local biotype, 'Fragolina di Ribera', a Slow Food Presidia, is also grown in Sciacca and Ribera (Agrigento Province). In this scenario, woodland strawberry is generally cultivated in an open field (in soil cultivation) or under a greenhouse (in soilless cultivation) [4]. Different authors [2,5] highlighted that woodland strawberry soilless cultivation is a very promising growing system since it may reduce harvesting costs, which seriously affect the economic aspect of the crop, and also improve fruit quality. Moreover, woodland strawberry, in Italy, has an interesting market price, ranging from 30.00 (winter period) to 6.00 (spring period) EUR/kg, whereas strawberry (*Fragaria* × *ananassa*) reaches a market price fluctuating from 12.00 to 2.00 EUR/kg, in the winter and spring periods, respectively.

Research has indicated that microbial biostimulants supplied in crop production systems significantly trigger growth and yield, as well as nutritive and functional product quality [6]. Among microbial biostimulants, *Trichoderma* spp. are multipurpose fungi with various valuable functions, making them a promising means to promote agricultural sustainability. These ascriptions may embrace the capacity of *Trichoderma* spp. as biocontrol agents [7], plant growth promoters [8] and biofertilizers [9]. Furthermore, *Trichoderma* could alleviate plant abiotic distresses through the stimulation of endogenous mechanisms managed by phytohormones and the modifications in metabolism of host plants [10]. Within the non-microbial biostimulants panel, vegetal-protein hydrolysates (V-PH) are a promising group of plant biostimulants useful to shift from a resource-intensive to sustainable greenhouse production system, improving crop yield and quality [11]. The eclectic scientific literature concerning the aforesaid effects of V-PH is available, especially on fruiting and leafy vegetables. For instance, contemporary research established that the application of V-PH augmented sweet basil [11] and lettuce yields [12]. V-PH also improved the yield of tomato plants exposed to suboptimal nitrogen doses, upregulating gene expression for amino acid transporter and glutamine synthetase [13].

Despite the important research effort on the effects of biostimulants and natural products on vegetables [14], a boosted understanding concerning the blended supply of microbial and/or non-microbial biostimulants is required to make agriculture more resilient and green [15–17]. In this respect, to the best of our knowledge, there is no evidence on the impact of the combined use of *Trichoderma atroviride*, one of the most versatile species of the genus *Trichoderma*, and of V-PH on the yield and quality traits of woodland strawberry. Accordingly, the current study aimed at elucidating the effects of two biostimulants (*Trichoderma atroviride* and a plant protein hydrolysate)—used alone or in combination—on the performance and economic profitability of two woodland strawberry genotypes ('Alpine' and 'Regina delle Valli').

2. Materials and Methods

2.1. Plant Genetic Resources and Experimental Conditions

The study was conducted near Marsala (TP) in an experimental polyethylene-covered greenhouse (37°44′53″ N; 12°32′41″ E) of the Agricultural, Food and Forestry Science Department—University of Palermo for two cultivation cycles (2018–2019 and 2019–2020).

The two most common genotypes of woodland strawberry (*Fragaria vesca* L.) cultivated in Italy were tested: 'Alpine' and 'Regina delle Valli'. These two ever-flowering cultivars are the most productive in the Mediterranean environment and, concomitantly, they are suitable for soilless greenhouse cultivation. Plants were cultivated in an open, soilless growing system. Strawberry plants were grown in a peat moos/coconut fiber (40/60, *v*/*v*)-based substrate mix (FR-LA 21V, Vigorplant, Fombio, Italy) in 3 L plastic baskets, each containing 1 plant. A density of 1.5 plants m^{-2} was adopted. Inside the greenhouse, a data logger was used to record the maximum and minimum air temperatures. Plants were watered, as described by Boztok et al. [18] and Gül and Sevgican [19], depending on solar radiation. Both cultivation cycles started on 12 September and concluded on 30 July. The nutrient solution adopted was that suggested by Vidal [20] with slight modifications: 10 mM of $N\text{-}NO_3$, 1 mM of $N\text{-}NH_4$, 1 mM of $P\text{-}PO_4$, 5.5 mM of K, 3.5 mM of Ca, 1.2 mM of Mg, 2.0 mM of $S\text{-}SO_4$, 20 µM of Fe, 30 µM of B, 1.0 µM of Cu, 5.0 µM of Zn, 10.0 µM of Mn and 1.0 µM of Mo.

2.2. Experimental Setup and Statistical Analysis

Four biostimulant applications (*T. atroviride*, V-PH, *T. atroviride* + V-PH and control) were combined with two woodland strawberry genotypes ('Alpine' and 'Regina delle Valli') in a two-factorial experiment, obtaining 8 treatments. For each treatment, 30 plants were used (3 replicates of 10 plants), resulting in a total of 240 plants organized in a randomized complete block design (RCBD). The trial was replicated for two consecutive cultivation cycles. Statistical analyses were performed using SPSS v. 28 (SPSS Inc., Chicago, IL, USA). The effects of the biostimulant and the genotypes were evaluated using a two-way ANOVA, setting genotype and biostimulant as the main factors. Multiple comparisons were performed using Tukey's test ($p < 0.05$). To evaluate the year effect, an initial three-way ANOVA (genotype × biostimulant × year) was performed (Table S2). To sum up all traits, a heat map was also generated using the online program package clustvis (https://biit.cs.ut.ee/clustvis/ (accessed on 1 February 2024)).

2.3. Microbial and Non-Microbial Biostimulant Treatments

Two biostimulant treatments, a vegetal-derived protein hydrolysate, TYSON® (Mugavero fertilizers, Palermo, Italy) (V-PH), and a *Trichoderma atroviride* AT10 strain (Condor—Atens—Agrotecnologias Naturales S.L, Tarragona, Spain) were applied to assess the single and combined effects on the two different woodland strawberry genotypes tested. The biostimulant TYSON® characteristics are reported in Table S1. Condor is a commercial product that contains 1×10^9 CFU g^{-1} of *Tricoderma atroviride* strain AT10.

Twenty-four hours before transplanting, the woodland strawberry plants were inoculated, soaking the roots for 15 min using a rate of 1 kg ha^{-1} of inoculum (this treatment was conducted only once). From fifteen days after transplant (DAT) to the final harvest, every 20 days, woodland strawberry plants were sprayed with V-PH. Dosages of 0 mL L^{-1} (control) and 3 mL L^{-1} (recommended dose) were supplied, using 100 mL of solution per plant. Control plants were sprayed with only water. To avoid contamination of the V-PH treatment, plants in plastic baskets were spatially isolated during the foliar treatments.

2.4. Yield and Fruit Nutritional Traits

Yield traits were documented for all plants. Marketable yield and mean fruit weight were evaluated. Marketable fruits were defined as fruits not affected by any visible disease or deformity. The marketable yield was expressed as g $plant^{-1}$, while the mean fruit weight was expressed as g.

For the determination of nutritional features, fruits from the 3rd to the 6th harvest were randomly collected. Dry matter values, expressed as a percentage, were determined by drying 80 g of fresh sample in a ventilated oven, set first at 70 °C for 24 h and afterwards at 105 °C for 48 h. To determine the firmness of the fruit, the measurement was carried out with a digital penetrometer to test the resistance to compression. A cylindrical stainlesssteel probe with a diameter of 3 mm was used for the measurement. The fruit firmness value was expressed in Newtons (N). To measure total soluble solids (TSS), woodland strawberry juice was obtained by centrifuging the fruit and filtering. A digital refractometer was used for the analysis, and the data were expressed as °Brix. Total sugar concentration was determined by the method of Serna et al. [21], and results were expressed as mg 100 g^{-1} of fresh weight. The fruit ascorbic acid content was measured using a refractometer with the use of Reflectoquant (RQflex10, Sigma-Aldrich, Saint Louis, MO, USA) and an Ascorbic Acid Test Strip. The results were presented as mg 100 g^{-1} fresh weight.

2.5. Fruit Function Traits and Antioxidant Activity

The total flavonoid content was calculated as in Najda et al. [22]. Briefly, the absorbance was measured at 425 nm via a spectrophotometer, and then the flavonoid content was calculated according to the equation:

$$x = (8.75 \times A)/m$$

where m is the amount of fresh material. The results were expressed as mg g^{-1} of fresh weight. The anthocyanin content was calculated according to the method of Rabino and Mancinelli [23]. The results were expressed as mg of Cya-3-glucoside equivalent per 100 g^{-1} fresh weight. Total polyphenols were analyzed by spectrophotometric assay, as described by Doumett et al. [3]. The results were reported as milligrams of (+)-catechin per 100 g of fresh berries. Antioxidant activity was determined following the Chen and Ho [24] method and expressed as %DPPH.

2.6. Partial Budget Analysis

A partial budget analysis was developed to assess the net economic gains that may promote woodland strawberry cultivation via the application of V-PH and *T. atroviride*, individually or in combination. The economic procedure reported by Giordano et al. [25] was employed. The biostimulants added costs and gross returns were considered. The following formula was applied to evaluate the added net return generated by V-PH, *T. atroviride* and their combination:

$$Added\ net\ return = added\ gross\ return - addedd\ variable\ costs$$

3. Results

3.1. Yield, Qualitative and Nutraceutical Traits of the Fruits

In both years, marketable yield and mean fruit weight were significantly modulated by genotype and biostimulant but not by their interaction (Table 1).

Notwithstanding the biostimulant, 'Alpine' showed a higher marketable yield than the "Regina delle Valli" genotype for both years of cultivation. On the other hand, biostimulants boosted marketable yield; the highest values were noted in woodland strawberry plants treated with *T. atroviride* + V-PH, followed by those inoculated with *T. atroviride*, which in turn showed higher values than V-PH. Control plants had the lowest values. Averaged over the biostimulant, the highest mean fruit weight was recorded in the "Alpine" genotype. Moreover, the plants biostimulated with V-PH or *T. atroviride* + *V*-PH showed the highest mean fruit weight, whereas woodland strawberry plants inoculated with *T. atroviride* or control plants had the lowest ones (Table 1).

As shown in Table 2, statistical analysis for fruit dry matter, fruit firmness, total soluble sugars (TSS), total sugars and ascorbic acid did not expose a significant effect of the interaction biostimulant × genotype in both years.

Irrespective of the biostimulant treatment, the 'Regina delle Valli' genotype showed the highest fruit dry matter content and the highest total sugar and ascorbic acid concentration, whereas fruit firmness and TSS peaked in the 'Alpine' strawberry. Regardless of the genotype, plants treated with *T. atroviride* + V-PH had the highest fruit dry matter content, followed by those treated with *T. atroviride* and those treated with V-PH (Table 2). The lowest dry matter percentage was detected in fruits from control plants. Averaged over genotype, fruits from plants treated with *T. atroviride* displayed the highest firmness, followed by those from plants supplied with *T. atroviride* and V-PH. Plants exposed to V-PH had the lowest values. Without regard to the genotype, fruits from control plants and those from plants inoculated with *T. atroviride* showed the highest TSS, followed by those from plants supplied with V-PH or with *T. atroviride* + V-PH (Table 2). Data on total sugars overlapped with those described for fruit dry matter. Regardless of the genotype, fruits from plants supplied with both biostimulants or with V-PH showed a higher ascorbic acid content than fruits from plants inoculated with *T. atroviride* or control plants (Table 2).

In both years, ANOVA for flavonoids and anthocyanins did not highlight a significant biostimulant × genotype interaction (Table 3).

Flavonoid concentration peaked in fruits from "Regina delle Valli" plants (Table 3). Moreover, regardless of the genotype, berries from plants supplied with *T. atroviride* + V-PH had the highest flavonoids content, followed by those supplied with V-PH; the lowest values were assessed in control plants or in woodland strawberry plants inoculated with microbial biostimulant. For anthocyanins, data showed that fruits from 'Regina delle Valli' had higher values than those from 'Alpine'. Averaged over genotype, the highest anthocyanin concentrations were found in berries from plants treated with *T. atroviride* + V-PH, followed by those from plants supplied with *T. atroviride* or with V-PH (Table 3). For total polyphenols and antioxidant activity, the two experimental factors significantly interacted in both years (Table 4).

As concerns total polyphenols, the highest values were recorded in fruits from 'Alpine' or 'Regina delle Valli' genotypes treated with *T. atroviride* + V-PH and in fruits from 'Regina delle Valli' plants sprayed with V-PH (Table 4). The lowest values—for fruit total polyphenol concentration—were recorded in 'Alpine' control plants or in those inoculated with *T. atroviride*. For antioxidant activity, the highest values were observed in fruits from 'Regina delle Valli' exposed to the *T. atroviride* + V-PH treatment, followed by those from the 'Regina delle Valli' × V-PH combination. Although 'Alpine' showed a lower value than Regina delle Valli, Alpine also exhibited higher antioxidant activity with *T. atroviride* + V-PH compared to the control. The lowest values were detected in fruits from 'Alpine' control plots.

Table 1. Effect of genotype and biostimulant on marketable yield and mean fruit weight of wild strawberry grown in a soilless cultivation system.

Treatments		Marketable Yield (g plant^{-1})				Mean Fruit Weight (g)			
		I Year		II Year		I Year		II Year	
Genotype (G)									
Alpine		170.6 (19.5)	a	170.2 (19.0)	a	1.2 (0.03)	a	1.2 (0.03)	a
Regina delle Valli		127.2 (15.5)	b	126.8 (15.5)	b	1.1 (0.06)	b	1.1 (0.05)	b
Biostimulant (B)									
Control		127.2 (22.5)	d	127.3 (23.3)	d	1.1 (0.03)	b	1.1 (0.02)	b
T. atroviride		153.3 (27.5)	b	153.0 (27.1)	b	1.1 (0.04)	b	1.1 (0.03)	b
V-PH		143.6 (20.3)	c	142.8 (19.4)	c	1.2 (0.05)	a	1.2 (0.05)	a
T. atroviride + V-PH		171.5 (26.8)	a	170.8 (26.4)	a	1.2 (0.01)	a	1.2 (0.02)	a
Genotype × biostimulant									
Alpine	Control	147.4 (5.0)	a	148.3 (3.2)	a	1.2 (0.03)	a	1.2 (0.03)	a
	T. atroviride	178.2 (3.6)	a	177.7 (3.6)	a	1.2 (0.01)	a	1.2 (0.01)	a
	V-PH	161.3 (7.6)	a	160.0 (7.2)	a	1.2 (0.02)	a	1.2 (0.03)	a
	T. atroviride + V-PH	195.6 (6.5)	a	194.7 (3.7)	a	1.2 (0.02)	a	1.2 (0.02)	a
Regina delle Valli	Control	107.0 (4.4)	a	106.3 (5.2)	a	1.1 (0.03)	a	1.1 (0.02)	a
	T. atroviride	128.3 (4.0)	a	128.3 (2.8)	a	1.1 (0.03)	a	1.1 (0.03)	a
	V-PH	125.9 (5.5)	a	125.7 (3.7)	a	1.2 (0.07)	a	1.2 (0.06)	a
	T. atroviride + V-PH	147.5 (4.1)	a	147.0 (2.8)	a	1.2 (0.02)	a	1.2 (0.02)	a
Significance									
G		***				*			
B		***				***			
G × B		NS				NS			

Results are presented as means (standard deviation). Values within a column followed by different letters are significantly different at $p \leq 0.05$. NS, * and *** are non-significant or significant at 0.05 or at 0.001, respectively. Control: not-treated; *T. atroviride*: inoculated with *Trichoderma atroviride*; V-PH: treated with 'TYSON' vegetal protein hydrolysate; *T. atroviride* + V-PH: treated with *Trichoderma atroviride* and 'TYSON' vegetal protein hydrolysate. I year: 2018–2019; II year: 2019–2020.

Table 2. Effect of genotype and biostimulant on fruit dry matter, fruit firmness, TSS, total sugars and ascorbic acids of wild strawberry grown in a soilless cultivation system.

Treatments		Fruit Dry Matter (%)				Fruit Firmness (N)				TSS (°Brix)				Total Sugars (mg 100 g^{-1} fw)				Ascorbic Acid (mg 100 g^{-1} FW)			
		I Year		II Year		I Year		II Year		I Year		II Year		I Year		II Year		I Year		II Year	
Genotype (G)																					
Alpine		32.3 (1.5)	b	31.6 (2.0)	b	1.13 (0.15)	a	1.13 (0.14)	a	12.1 (0.3)	a	12.2 (0.4)	a	8715.1 (120.4)	b	8719.2 (129.7)	b	18.8 (1.6)	b	18.7 (1.5)	b
Regina delle Valli		32.9 (1.7)	a	32.8 (1.9)	a	1.10 (0.14)	b	1.09 (0.14)	b	10.4 (0.2)	b	10.4 (0.3)	b	8855.7 (120.3)	a	8861.2 (113.4)	a	23.2 (1.2)	a	23.3 (1.4)	a
Biostimulants (B)																					
Control		30.5 (0.6)	c	30.1 (1.0)	c	1.11 (0.05)	c	1.09 (0.04)	c	11.4 (1.0)	a	11.6 (1.0)	a	8638.6 (70.1)	c	8652.4 (80.8)	c	19.7 (2.8)	b	19.7 (2.7)	b
T. atriviride		32.3 (0.4)	b	32.1 (1.1)	b	1.27 (0.03)	a	1.26 (0.02)	a	11.5 (1.0)	a	11.5 (1.0)	a	8805.6 (125.6)	b	8793.8 (124.8)	b	19.9 (2.6)	b	19.9 (2.6)	b
V-PH		32.9 (0.4)	b	31.9 (1.2)	b	0.90 (0.03)	d	0.90 (0.03)	d	11.0 (0.9)	b	10.9 (1.0)	b	8760.5 (96.0)	b	8760.9 (96.8)	b	22.3 (2.5)	a	22.3 (2.7)	a
T. atriviride + V-PH		34.7 (0.7)	a	34.8 (1.0)	a	1.17 (0.03)	b	1.18 (0.03)	b	11.2 (0.9)	b	11.1 (1.1)	b	8937.0 (52.6)	a	8953.7 (45.6)	a	22.2 (2.0)	a	22.3 (2.3)	a
Genotype × biostimulant																					
Alpine	Control	30.3 (0.8)	a	29.6 (0.2)	a	1.13 (0.05)	a	1.13 (0.02)	a	12.3 (0.2)	a	12.6 (0.1)	a	8581.8 (19.4)	a	8593.3 (17.9)	a	17.3 (0.9)	a	17.3 (0.6)	a
	T. atroviride	32.2 (0.2)	a	31.5 (0.6)	a	1.29 (0.03)	a	1.28 (0.01)	a	12.4 (0.1)	a	12.3 (0.4)	a	8698.7 (45.0)	a	8691.5 (54.4)	a	17.5 (0.7)	a	17.5 (0.3)	a
	V-PH	32.5 (0.1)	a	30.9 (0.9)	a	0.91 (0.02)	a	0.93 (0.03)	a	11.8 (0.2)	a	11.7 (0.1)	a	8687.7 (42.8)	a	8673.8 (21.3)	a	20.1 (0.5)	a	19.9 (0.9)	a
	T. atroviride + V-PH	34.2 (0.6)	a	34.4 (1.3)	a	1.19 (0.02)	a	1.20 (0.02)	a	12.0 (0.2)	a	12.1 (0.3)	a	8892.3 (16.4)	a	8918.2 (36.8)	a	20.4 (0.6)	a	20.2 (0.9)	a
Regina delle Valli	Control	30.7 (0.2)	a	30.7 (1.3)	a	1.08 (0.02)	a	1.06 (0.03)	a	10.5 (0.1)	a	10.7 (0.1)	a	8695.3 (49.8)	a	8711.5 (74.5)	a	22.2 (0.7)	a	22.1 (1.2)	a
	T. atroviride	32.5 (0.4)	a	32.6 (1.3)	a	1.26 (0.03)	a	1.24 (0.02)	a	10.5 (0.1)	a	10.6 (0.2)	a	8912.5 (56.1)	a	8896.0 (68.2)	a	22.2 (0.4)	a	22.2 (0.5)	a
	V-PH	33.2 (0.3)	a	32.8 (0.3)	a	0.90 (0.03)	a	0.88 (0.01)	a	10.2 (0.1)	a	10.0 (0.2)	a	8833.3 (72.9)	a	8848.0 (14.2)	a	24.5 (0.4)	a	24.7 (0.4)	a
	T. atroviride + V-PH	35.1 (0.3)	a	35.3 (0.5)	a	1.15 (0.03)	a	1.17 (0.03)	a	10.3 (0.1)	a	10.1 (0.1)	a	8981.7 (25.5)	a	8989.2 (8.4)	a	24.0 (0.7)	a	24.3 (0.5)	a
Significance																					
G		**				*				***				***				***			
B		***				***				***				***				***			
G × B		NS				NS				NS				NS				NS			

Results are presented as means (standard deviation). Values within a column followed by different letters are significantly different at $p \leq 0.05$. NS, *, ** and *** are non-significant or significant at 0.05, 0.005 or at 0.001, respectively. Control: not treated; *T. atroviride*: inoculated with *Trichoderma atroviride*; V-PH: treated with 'TYSON' vegetal protein hydrolysate; *T. atroviride* + V-PH: treated with *Trichoderma atroviride* and 'TYSON' vegetal protein hydrolysate. I year: 2018–2019; II year: 2019–2020.

Table 3. Effect of genotype and biostimulant on flavonoids and anthocyanins of wild strawberry grown in a soilless cultivation system.

Treatments		Flavonoids (mg g^{-1} FW)				Anthocyanins (mg 100 g^{-1} FW)			
		I Year		II Year		I Year		II Year	
Genotype (G)									
Alpine		0.63 (0.08)	b	0.63 (0.08)	b	148.1 (18.8)	b	147.5 (18.2)	b
Regina delle Valli		0.66 (0.05)	a	0.66 (0.05)	a	157.3 (12.8)	a	157.0 (12.5)	a
Biostimulant (B)									
Control		0.57 (0.04)	c	0.57 (0.03)	c	134.6 (10.3)	c	135.0 (9.8)	c
T. atroviride		0.61 (0.04)	c	0.61 (0.04)	c	153.6 (6.6)	b	152.9 (5.2)	b
V-PH		0.67 (0.03)	b	0.67 (0.03)	b	148.0 (6.7)	b	147.3 (8.0)	b
T. atroviride + V-PH		0.72 (0.02)	a	0.72 (0.02)	a	174.7 (6.6)	a	173.9 (6.3)	a
Genotype × biostimulant									
Alpine	Control	0.54 (0.02)	a	0.53 (0.02)	a	126.1 (6.1)	a	126.9 (6.0)	a
	T. atroviride	0.58 (0.04)	a	0.58 (0.04)	a	149.6 (7.3)	a	149.5 (5.0)	a
	V-PH	0.65 (0.03)	a	0.65 (0.03)	a	142.7 (4.0)	a	140.8 (4.8)	a
	T. atroviride + V-PH	0.72 (0.03)	a	0.73 (0.02)	a	174.0 (8.1)	a	172.9 (7.7)	a
Regina delle Valli	Control	0.60 (0.03)	a	0.59 (0.02)	a	143.0 (4.0)	a	143.0 (3.1)	a
	T. atroviride	0.63 (0.03)	a	0.63 (0.02)	a	157.7 (2.6)	a	156.3 (2.7)	a
	V-PH	0.69 (0.01)	a	0.69 (0.01)	a	153.2 (3.8)	a	153.7 (3.3)	a
	T. atroviride + V-PH	0.71 (0.02)	a	0.71 (0.02)	a	175.3 (6.4)	a	174.8 (6.0)	a
Significance									
G		**				***			
B		***				***			
G × B		NS				NS			

Results are presented as means (standard deviation). Values within a column followed by different letters are significantly different at $p \leq 0.05$. NS, ** and *** are non-significant or significant at 0.005 or at 0.001, respectively. Control: not-treated; *T. atroviride*: inoculated with *Trichoderma atroviride*; V-PH: treated with 'TYSON' vegetal protein hydrolysate; *T. atroviride* + V-PH: treated with *Trichoderma atroviride* and 'TYSON' vegetal protein hydrolysate. I year: 2018–2019; II year: 2019–2020.

Table 4. Effect of genotype and biostimulant on total polyphenols and antioxidant activity of wild strawberry grown in a soilless cultivation system.

Treatments		**Total Polyphenols (mg Catechin 100 g−1 FW)**				**Antioxidant Activity (%DPPH)**			
		I Year		**II Year**		**I Year**		**II Year**	
Genotype (*G*)									
Alpine		723.7 (93.1)	b	724.6 (81.4)	b	13.7 (1.1)	b	13.7 (1.1)	b
Regina delle Valli		789.2 (55.7)	a	795.2 (48.9)	a	14.4 (1.47)	a	14.4 (1.5)	a
Biostimulants (*B*)									
Control		688.8 (56.4)	c	702.5 (61.3)	c	12.8 (0.5)	c	12.7 (0.5)	c
T. atriviride		688.0 (51.7)	c	703.8 (50.9)	c	12.9 (0.2)	c	12.9 (0.5)	c
V-PH		803.3 (40.9)	b	795.8 (53.3)	b	14.9 (0.5)	b	15.0 (0.4)	b
T. atriviride + V-PH		845.6 (15.7)	a	837.4 (12.4)	a	15.5 (0.8)	a	15.5 (0.9)	a
Genotype × *biostimulant*									
Alpine	Control	639 (17.2)	d	649.8 (30.0)	d	12.5 (0.4)	e	12.4 (0.07)	e
	T. atroviride	641.2 (9.0)	d	658.8 (13.5)	d	12.8 (0.3)	de	12.8 (0.2)	de
	V-PH	767.9 (14.8)	b	752.4 (3.2)	b	14.6 (0.4)	c	14.6 (0.4)	c
	T. atroviride + V-PH	846.6 (23.5)	a	837.3 (16.4)	a	14.8 (0.3)	bc	14.7 (0.3)	bc
Regina delle Valli	Control	738.7 (14.6)	c	745.3 (11.7)	c	13.1 (0.3)	d	13.2 (0.1)	d
	T. atroviride	734.8 (5.9)	c	748.7 (15.3)	c	12.9 (0.2)	d	12.9 (0.7)	d
	V-PH	838.7 (14.0)	a	839.2 (37.9)	a	15.2 (0.4)	b	15.2 (0.1)	b
	T. atroviride + V-PH	844.6 (7.8)	a	837.6 (10.8)	a	16.2 (0.3)	a	16.2 (0.5)	a
Significance									
G		***				***			
B		***				***			
G × B		***				*			

Results are presented as means (standard deviation). Values within a column followed by different letters are significantly different at $p \leq 0.05$. * and *** are significant at 0.05 or at 0.001, respectively. Control: not-treated; *T. atroviride*: inoculated with *Trichoderma atroviride*; V-PH: treated with 'TYSON' vegetal protein hydrolysate; *T. atroviride* + V-PH: treated with *Trichoderma atroviride* and 'TYSON' vegetal protein hydrolysate. I year: 2018–2019; II year: 2019–2020.

3.2. Heat-Map

A heat-map analysis of all tested parameters was performed to summarize the influence of all experimental factors on woodland strawberry plants (Figure 1).

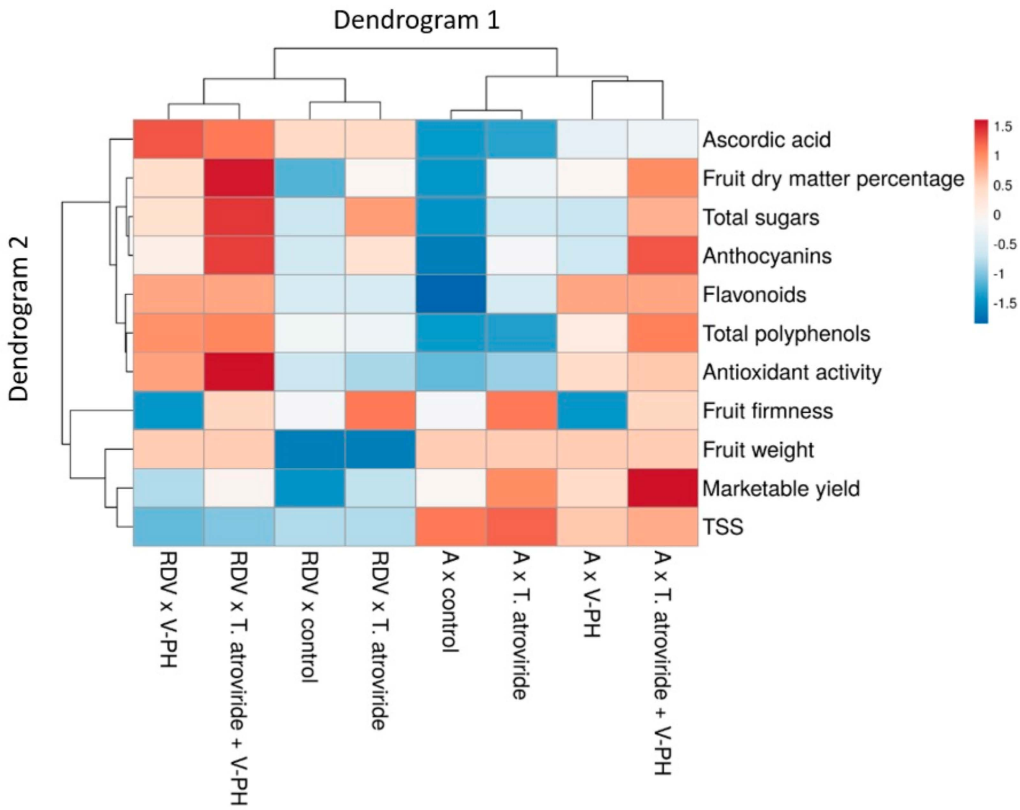

Figure 1. Heat map analysis including all wild strawberry plant traits in response to genotypes and biostimulant treatments. The heat map figure was created using the https://biit.cs.ut.ee/clustvis/online program (accessed on 1 February 2024) package. A: 'Alpine'; RDV: 'Regina delle Valli'.

The graphical analysis shows two dendrograms. The first dendrogram (Dendrogram 1), located on top, contains the 8 treatments. The second dendrogram (Dendrogram 2), located on the left side of the figure, involves the dependent variables, affecting the distribution. The two woodland strawberry cultivars belong to different clusters of Dendrogram 1; however, comparing both in the control state, 'Alpine' (A) had lower ascorbic acid, dry matter percentage, total sugars, anthocyanins, flavonoids, total polyphenols and antioxidant activity than 'Regina delle Valli' (RDV), whereas A showed higher fruit weight, marketable yield and TSS than RDV. Dendrogram 1 showed two main clusters: the group on the left includes the combination with the RDV genotype, whereas the group on the right includes the combination with the A genotype. In particular, the combination RDV × V-PH and RDV × *T. atroviride* + V-PH were separated from those RDV × control and RDV × *T. atroviride*. The latter combinations showed lower values of ascorbic acid, dry matter, total sugars, anthocyanins, flavonoids, total polyphenols and antioxidant activity, mean fruit weight and marketable yield than the RDV × V-PH and RDV × *T. atroviride* + V-PH combinations; however, they revealed higher values in terms of fruit firmness and TSS. The cluster on the left comprised RDV × V-PH and RVD × *T. atroviride* + V-PH combinations. In this group, RDV × V-PH treatment showed the lowest dry matter, total sugar, anthocyanins, antioxidant activity, fruit firmness, marketable yield and TSS values. Meanwhile, the grouping on the right contained the RDV × control and RDV × *T. atroviride* combinations. Within this cluster, the RDV × *T. atroviride* combination was characterized by a higher total sugar, anthocyanins, fruit firmness and marketable yield compared with RDV × control treatment. In the right site of dendrogram 1, two main clusters were documented. Specifically, A × control and A × *T. atroviride* were split from those A × V-PH and A × *T. atroviride* + V-PH. A × control and A × *T. atroviride* revealed lower values of ascorbic acid, dry matter, total sugars, anthocyanins, flavonoids, total polyphenols, antioxidant activity, and marketable yield than A × V-PH and A × *T. atroviride* + V-PH combinations. Nonetheless, A × control and A × *T. atroviride* had higher values in terms of fruit firmness and TSS. The cluster on the left comprised A × control and A × *T. atroviride* combinations. In this group, A × control treatment shows the lowest dry matter, total sugar, anthocyanins, flavonoids, antioxidant activity, fruit firmness and marketable yield. Concomitantly, the cluster on the right enclosed A × V-PH and A × *T. atroviride* + V-PH combinations. Within this cluster, A × *T. atroviride* + V-PH treatment was characterized by a higher dry matter, total sugar, anthocyanins, total polyphenols, antioxidant activity, fruit firmness, marketable yield and TSS compared with the A × V-PH combination.

3.3. Partial Budget Analysis of Biostimulants-Treated Woodland Strawberry Production

The biostimulant effects on yield increase are presented in Table 4. The economic evaluation highlighted a beneficial impact of *T. atroviride* (4282.5 EUR/ha), V-PH (1615.8 EUR/ha) and *T. atroviride* + V-PH (6208.3 EUR/ha), with the maximum added gross return observed in woodland strawberry treated with both biostimulants (Table 5). Among the counted added variable costs, the harvest related to the yield increase was the main cost element, followed by biostimulant treatments (Table 5). Overall, our results underlined that the enhanced net economic benefit was related to the yield increase. Particularly, using both biostimulants (*T. atroviride* + V-PH) was more profitable than using them alone.

Table 5. Added returns of woodland strawberry attained by *T. atroviride*, V-PH, or their combination (*T. atroviride* + V-PH) under greenhouse conditions (soilless system) compared to the untreated control.

Biostimulant	Yield Increase (kg ha^{-1})	Price (EUR kg^{-1})	Added Gross Return (EUR ha^{-1})	Added Variable Cost (EUR ha^{-1})				Added Net Return (EUR ha^{-1})
				Biostimulant Treatment	Application	Harvest	Total	
T. atroviride	391.5	15.0	5872.5	150.0	70.0	1370.0	1590.0	4282.5
V-PH	246.0	15.0	3690.0	614.3	600.0	860.0	2074.3	1615.8
T. atroviride + V-PH	664.5	15.0	9967.5	764.3	670.0	2325.0	3759.3	6208.3

The costs of biostimulants were furnished by suppliers (TYSON® = 10.5 EUR/kg; Condor® = 150.0 EUR/kg); the costs of biostimulant applications were considered reputable based on the information provided by local agricultural contractors.

4. Discussion

This research investigated the response of two woodland strawberry genotypes (‘Alpine’ and ‘Regina delle Valli’) to the application of *Trichoderma atroviride* and vegetal protein hydrolysate, employed alone or in combination. Our study revealed significant differences in terms of productive and qualitative performances between the genotypes. The ‘Alpine’ genotype had a higher marketable yield and mean fruit weight than ‘Regina delle Valli’. These outcomes are corroborated by those of Nin et al. [2], who studied the potential use of alternative growing systems for the cultivation of the woodland strawberry genotypes.

Overall, data suggest that productive performances were higher in ‘Alpine’, whereas most of the qualitative traits were higher in ‘Regina delle Valli’. These data are corroborated by those of Doumett et al. [3], who, by comparing the nutritional and nutraceutical traits of different *Fragaria vesca* genotypes, reported that—overall—‘Regina delle Valli’ had higher qualitative features than ‘Alpine’.

The effect of *Trichoderma* spp. on yield parameters can be attributed to its biofertilizer properties since it has been shown that inoculation increases nutrient availability via the variation of plant root architecture and the production of siderophores and organic acids [26–28]. Moreover, as reported by Kotasthane et al. [29] and Casimiro et al. [30], *Trichoderma* can secrete plant hormones like indol-acetic acid (IAA), which in turn promote plant growth and yield. The positive effect of V-PH on yield can be mainly related to its role in the elicitation of plant primary metabolism [31]. Amino acids contained in V-PH are the main organic nitrogen transporters, and they can also be used for protein synthesis [32]. The outcome on marketable yield can be interpreted as a synergistic effect between *T. atroviride* and V-PH in enhancing plant yield. For mean fruit weight, we may assume that the main increasing effect was prompted by the V-PH application.

Data on fruit dry matter percentage concur with those of Colla et al. [33], who found an increase in the dry matter of several vegetable crops when inoculated with *Trichoderma*. Moreover, the data overlapped with those of Chen et al. [34], who reported that Pakchoi plants (*Brassica chinensis* L.) treated with two *Trichoderma* strains had a higher dry weight than non-treated ones. The increasing effect recorded can be attributed to the role of *Trichoderma* on plant mineral nutrition, with particular reference to nitrogen and phosphorous uptake [26]. Moreover, as stated by Colla et al. [32], the enhancement of dry matter recorded in PH-treated plants can be related to the amino acids contained in V-PH, which influence nitrogen metabolism via the modulation of N accumulation and transport. Since the highest fruit dry matter content was recorded in plants treated with both biostimulants (*T. atroviride* + V-PH), we may assume that the two biostimulants interacted synergistically.

Fruit firmness is a notable qualitative trait of woodland strawberry fruit, influencing transport and consumer appreciation. Results on fruit firmness differ from those of Cozzolino et al. [35], who found a significant increase in fruit firmness when tomato plants were supplied with plant protein hydrolysate. Moreover, Soteriou et al. [36] found that plant protein hydrolysate did not influence watermelon fruit firmness. Consequently, due to these contrasting results, we can speculate that the different plant responses to V-PH depended on plant species (strawberry, watermelon and tomato). Conversely, the positive effect of *Trichoderma* on fruit firmness can be related to its ability to solubilize calcium phosphate [37] and, consequently, increase fruit firmness [38]. The mutual application of biostimulants increases fruit firmness compared to the control; however, in this case, it seems that the biostimulants did not act synergistically.

Results on total soluble solids (TSS) are coherent with those of Fernando et al. [39], who, by testing the biostimulant activity of *Trichoderma* on melon, revealed no significant effect of the inoculation on TSS. However, the findings were in contrast with those of Apostol et al. [40], who reported that *Trichoderma* inoculation modulates pepper fruit TSS depending on genotype. Moreover, the findings are in contrast with those of Colla et al. [41], who reported an increase in tomato TSS when plants were exposed to PH. Furthermore, Soppelsa et al. [42] reported no significant effect of PH on strawberry (*Fragaria* × *ananassa*) fruit TSS. The contrasting results obtained can be explained as a different plant response to V-PH and *Trichoderma* application, suggesting its genotype dependence.

Data on total sugars concur with those of Sani et al. [43], who observed an enhancement in terms of total sugars in tomato fruits when plants were inoculated with *Trichoderma*. Also, our results agree with those of Sabatino et al. [12], who found an increase in total sugars in lettuce plants treated with PH. Since sugars were produced via photosynthesis, and considering that this process is directly related to plant primary photochemical reactions [44], we may assume that both biostimulants enhanced woodland strawberry plant photosynthesis capacity. Interestingly, the highest total sugar values were assessed in plants supplied with both biostimulants, suggesting a synergistic effect between them.

Outcomes on ascorbic acid agreed with Rouphael et al. [45], who, assessing the impact of a plant PH on tomatoes, reported an improvement in fruit ascorbic acid concentration when plants were treated with V-PH. Moreover, results concurred with those of other authors [46,47] on tomato and pepper. The effect of V-PH on ascorbic acid can be linked to the modulation of plant secondary metabolism via enzymes involved in phytochemical homeostasis and in the variation of plant nutritional status [11]. Moreover, V-PH stimulates the biosynthesis of some amino acids like phenylalanine and tyrosine, precursors of ascorbic acid synthesis [32]. However, considering that the highest ascorbic acid value was found in berries from plants treated with V-PH or exposed to both biostimulants, we can affirm that *Trichoderma* did not have an antagonistic effect when combined with V-PH.

The findings on flavonoids tied well with those of Ertani et al. [48], who, by conducting a study on the effect of PH on maize, revealed that the application of PH elicited flavonoids biosynthesis. The flavonoid increase is related to the variation of some enzyme, such as phenylalanine ammonia lyase (PAL), which is comprised in phenyl-propanoid production [32]. Indeed, it was shown that the gene encoding for PAL can be encouraged by biostimulant applications [48,49]. Remarkably, we found the highest flavonoid values in fruits from plants subjected to both biostimulants. This outcome was also detected by Rouphael et al. [50] on lettuce. Moreover, since *Trichoderma* grows thanks to the sugars taken up by the plants [32], and considering that the combination of the two biostimulants revealed the highest total sugar content, we may hypothesize that *Trichoderma's* effect on flavonoids was boosted.

Data on anthocyanins agree with those obtained by Lombardi et al. [51], who reported an increase in anthocyanins when strawberry plants were inoculated with *Trichoderma*. The *Trichoderma* mechanism of action is mainly connected to PAL and chorismate synthase production and, consequently, the phenylpropanoid pathway, which in turn is related to anthocyanin biosynthesis [51]. Interestingly, fruits from plants treated with both biostimulants had the highest anthocyanin values, revealing a synergistic effect between *Trichoderma* and V-PH.

Outcomes on total polyphenols are in line with those of Soppelsa et al. [42], who, by studying the influence of biostimulants to enhance growth, yield and fruit quality of strawberry (*Fragaria* × *ananassa* Duch.) plants cultivated under nutrient limitation, found that the application of a vegetal protein hydrolysate boosted fruit polyphenol concentration. The data also agree with Parrado et al. [52] and with Gurav and Jadhav [53]. Furthermore, as reported by Zhou et al. [54], the increase in phenolic components can be directly related to the "ex novo" synthesis of phenolic compounds via the activation of plant defense genes, such as PAL. Phenolic production could also be related to the amino acids contained in the V-PH (phenylalanine and tyrosine), which stimulate phenolic biosynthesis. Moreover, as stated by Zhou et al. [55], the nitrogen metabolism—modulated by V-PH application—is related to phenolic production. Moreover, 'Alpine' revealed the highest values when treated with both biostimulants, whereas 'Regina delle Valli' had the highest values when treated with V-PH or with *T. atroviride* + V-PH.

Outcomes on antioxidant activity are in line with those of Nzanza et al. [56], who found no significant effect of *Trichoderma* on tomato antioxidant activity. However, Lombardi et al. [53], who, evaluating the effect of three *Trichoderma* strains on strawberry, found that two out of three strains increased antioxidant activity, whereas the GV41 *Trichoderma* strain reduced antioxidant activity compared with the control. Moreover, our study is in accordance with Caruso et al. [57], Ertani et al. [46] and Colla et al. [41] on *Diplotaxis tenuifolia*, pepper and tomato, respectively, who reported an increase in antioxidant activity in PH-treated plants. Moreover, the increase in antioxidant activity can be linked to the activation of vital enzymes involve in cell antioxidant homeostasis [57]. The antioxidant activity was significantly influenced by the mutual application of biostimulants, underlining a synergistic effect, especially in the 'Regina delle Valli' genotype.

5. Conclusions

This study investigated the impact of two biostimulants (*Trichoderma atroviride* and plant protein hydrolysate)—used alone or in combination—on the performance of two woodland strawberry genotypes. Our research underlined that the highest marketable yield, mean fruit weight, fruit dry matter, total sugars, ascorbic acid, flavonoids, anthocyanins, total polyphenols and antioxidant activity were observed when woodland strawberry plants were simultaneously exposed to both biostimulants. Our study underlined that the use of biostimulants could be an effective strategy to increase the plant performance and economic profitability of woodland strawberry and, concomitantly to enhance the sustainability and resilience of its greenhouse cultivation system.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10050459/s1, Table S1: Composition and aminogram of Tyson® protein hydrolysate; Table S2: Significance of the ANOVA for all recorded parameters.

Author Contributions: Conceptualization, L.V., B.B.C. and L.S.; methodology, L.V., E.A., B.B.C., S.N., S.V. and S.B.; software, L.V., B.B.C., P.B. and L.S.; validation, L.S., G.N., S.L.B. and C.L.; formal analysis, L.V., E.A., P.B., S.V., S.B. and C.C.; investigation, L.V., E.A., B.B.C., P.B., S.N., S.L.B. and C.L.; resources, L.S.; data curation, L.V., B.B.C. and L.S.; writing—original draft preparation, L.V., B.B.C., G.N. and L.S.; writing—review and editing, L.V., B.B.C., C.C., G.N. and L.S.; visualization, L.V. and L.S.; supervision, L.S. and G.N.; project administration, L.S.; funding acquisition, L.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy reason.

Acknowledgments: The authors thank Mugavero fertilizers for providing the TYSON® vegetal derived-protein hydrolysate.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Del Bubba, M.; Giordani, E.; Ancillotti, C.; Petrucci, W.A.; Ciofi, L.; Morelli, D.; Marinelli, C.; Checchini, L.; Furlanetto, S. Morphological, Nutraceutical and Sensorial Properties of Cultivated *Fragaria vesca* L. Berries: Influence of Genotype, Plant Age, Fertilization Treatment on the Overall Fruit Quality. *Agric. Food Sci.* **2016**, *25*, 187–201. [CrossRef]
2. Nin, S.; Petrucci, W.A.; Giordani, E.; Marinelli, C. Soilless Systems as an Alternative to Wild Strawberry (*Fragaria vesca* L.) Traditional Open-Field Cultivation in Marginal Lands of the Tuscan Apennines to Enhance Crop Yield and Producers' Income. *J. Hortic. Sci. Biotechnol.* **2018**, *93*, 323–335. [CrossRef]
3. Doumett, S.; Fibbi, D.; Cincinelli, A.; Giordani, E.; Nin, S.; Del Bubba, M. Comparison of Nutritional and Nutraceutical Properties in Cultivated Fruits of *Fragaria vesca* L. Produced in Italy. *Food Res. Int.* **2011**, *44*, 1209–1216. [CrossRef]
4. Caracciolo, G.; D'Anna, E.; Moncada, A.; D'Anna, F. Evaluation of the Quality and Antioxidant Capacity of Woodland Strawberry Biotypes in Sicily. *J. Food Agric. Environ.* **2013**, *11*, 522–525.
5. Caruso, G.; Villari, G.; Melchionna, G.; Conti, S. Effects of Cultural Cycles and Nutrient Solutions on Plant Growth, Yield and Fruit Quality of Alpine Strawberry (*Fragaria vesca* L.) Grown in Hydroponics. *Sci. Hortic.* **2011**, *129*, 479–485. [CrossRef]
6. Consentino, B.B.; Aprile, S.; Rouphael, Y.; Ntatsi, G.; De Pasquale, C.; Iapichino, G.; Alibrandi, P.; Sabatino, L. Application of PGPB Combined with Variable N Doses Affects Growth, Yield-Related Traits, N-Fertilizer Efficiency and Nutritional Status of Lettuce Grown under Controlled Condition. *Agronomy* **2022**, *12*, 236. [CrossRef]
7. Sachdev, S.; Singh, R.P. Isolation, Characterisation and Screening of Native Microbial Isolates for Biocontrol of Fungal Pathogens of Tomato. *Clim. Chang. Environ. Sustain.* **2018**, *6*, 46. [CrossRef]
8. Sachdev, S.; Singh, A.; Singh, R.P. Optimization of Culture Conditions for Mass Production and Bio-Formulation of Trichoderma Using Response Surface Methodology. *3 Biotech* **2018**, *8*, 360. [CrossRef] [PubMed]
9. Sachdev, S.; Singh, R.P. Trichoderma: A Multifaceted Fungus for Sustainable Agriculture. In *Ecological and Practical Applications for Sustainable Agriculture*; Bauddh, K., Kumar, S., Singh, R.P., Korstad, J., Eds.; Springer: Singapore, 2020; pp. 261–304, ISBN 9789811533723.
10. Macías-Rodríguez, L.; Contreras-Cornejo, H.A.; Adame-Garnica, S.G.; del-Val, E.; Larsen, J. The Interactions of *Trichoderma* at Multiple Trophic Levels: Inter-Kingdom Communication. *Microbiol. Res.* **2020**, *240*, 126552. [CrossRef] [PubMed]
11. Consentino, B.B.; Vultaggio, L.; Sabatino, L.; Ntatsi, G.; Rouphael, Y.; Bondì, C.; De Pasquale, C.; Guarino, V.; Iacuzzi, N.; Capodici, G.; et al. Combined Effects of Biostimulants, N Level and Drought Stress on Yield, Quality and Physiology of Greenhouse-Grown Basil. *Plant Stress* **2023**, *10*, 100268. [CrossRef]
12. Sabatino, L.; Consentino, B.B.; Rouphael, Y.; De Pasquale, C.; Iapichino, G.; D'Anna, F.; La Bella, S. Protein Hydrolysates and Mo-Biofortification Interactively Modulate Plant Performance and Quality of 'Canasta' Lettuce Grown in a Protected Environment. *Agronomy* **2021**, *11*, 1023. [CrossRef]
13. 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*, 364202. [CrossRef] [PubMed]

14. Vultaggio, L.; Sabatino, L.; Rouphael, Y.; De Pasquale, C.; La Bella, S.; Consentino, B.B. Configuration of Strawberry Yield, Nutritional and Functional Traits in Response to LPE Application in a Two-Year Study. *Agronomy* **2023**, *13*, 1266. [CrossRef]
15. Rouphael, Y.; Lucini, L.; Miras-Moreno, B.; Colla, G.; Bonini, P.; Cardarelli, M. Metabolomic Responses of Maize Shoots and Roots Elicited by Combinatorial Seed Treatments With Microbial and Non-Microbial Biostimulants. *Front. Microbiol.* **2020**, *11*, 532306. [CrossRef] [PubMed]
16. Sabatino, L.; Consentino, B.B.; Ntatsi, G.; La Bella, S.; Baldassano, S.; Rouphael, Y. Stand-Alone or Combinatorial Effects of Grafting and Microbial and Non-Microbial Derived Compounds on Vigour, Yield and Nutritive and Functional Quality of Greenhouse Eggplant. *Plants* **2022**, *11*, 1175. [CrossRef] [PubMed]
17. Di Miceli, G.; Vultaggio, L.; Sabatino, L.; De Pasquale, C.; La Bella, S.; Consentino, B.B. Synergistic Effect of a Plant-Derived Protein Hydrolysate and Arbuscular Mycorrhizal Fungi on Eggplant Grown in Open Fields: A Two-Year Study. *Horticulturae* **2023**, *9*, 592. [CrossRef]
18. Boztok, K.; Sevgican, A.; Yoltas, T. Solar radyasyon esas alinarak yapilan farkly seviyelerde sulamanin sera domates (Lycopersicon esculentum Mill.) yetistiriciliginde ürüne etkileri. *Ege Ün. Ziraat Fak Derg.* **1984**, *21*, 19–24.
19. Gül, A.; Sevgican, A. Effect of growing media on glasshouse tomato yield and quality. *Acta Hortic.* **1992**, *3030*, 145–150. [CrossRef]
20. Vidal, P. *Fertirrigación: Desde la Teoría a la Práctica*; Trama Impresores S.A.: Chillan, Cile, 2019.
21. Serna, M.; Hernández, F.; Coll, F.; Coll, Y.; Amorós, A. Effects of Brassinosteroid Analogues on Total Phenols, Antioxidant Activity, Sugars, Organic Acids and Yield of Field Grown Endive (*Cichorium endivia* L.). *J. Sci. Food Agric.* **2013**, *93*, 1765–1771. [CrossRef] [PubMed]
22. Najda, A.; Dyduch-Sieminska, M.; Dyduch, J.; Gantner, M. Comparative Analysis of Secondary Metabolites Contents in Fragaria Vesca L. Fruits. *Ann. Agric. Environ. Med.* **2014**, *21*, 339–343. [CrossRef] [PubMed]
23. Rabino, I.; Mancinelli, A.L. Light, Temperature, and Anthocyanin Production 1. *Plant Physiol.* **1986**, *81*, 922–924. [CrossRef] [PubMed]
24. Chen, J.H.; Ho, C.-T. Antioxidant Activities of Caffeic Acid and Its Related Hydroxycinnamic Acid Compounds. *J. Agric. Food Chem.* **1997**, *45*, 2374–2378. [CrossRef]
25. Giordano, M.; El-Nakhel, C.; Caruso, G.; Cozzolino, E.; De Pascale, S.; Kyriacou, M.C.; Colla, G.; Rouphael, Y. Stand-Alone and Combinatorial Effects of Plant-Based Biostimulants on the Production and Leaf Quality of Perennial Wall Rocket. *Plants* **2020**, *9*, 922. [CrossRef] [PubMed]
26. López-Bucio, J.; Pelagio-Flores, R.; Herrera-Estrella, A. *Trichoderma* as Biostimulant: Exploiting the Multilevel Properties of a Plant Beneficial Fungus. *Sci. Hortic.* **2015**, *196*, 109–123. [CrossRef]
27. Samolski, I.; Rincón, A.M.; Pinzón, L.M.; Viterbo, A.; Monte, E. The Qid74 Gene from *Trichoderma harzianum* Has a Role in Root Architecture and Plant Biofertilization. *Microbiology* **2012**, *158*, 129–138. [CrossRef] [PubMed]
28. Zhao, L.; Wang, F.; Zhang, Y.; Zhang, J. Involvement of *Trichoderma asperellum* Strain T6 in Regulating Iron Acquisition in Plants. *J. Basic Microbiol.* **2014**, *54*, S115–S124. [CrossRef] [PubMed]
29. Kotasthane, A.; Agrawal, T.; Kushwah, R.; Rahatkar, O.V. In-Vitro Antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and Their Response towards Growth of Cucumber, Bottle Gourd and Bitter Gourd. *Eur. J. Plant Pathol.* **2015**, *141*, 523–543. [CrossRef]
30. Casimiro, I.; Marchant, A.; Bhalerao, R.P.; Beeckman, T.; Dhooge, S.; Swarup, R.; Graham, N.; Inzé, D.; Sandberg, G.; Casero, P.J.; et al. Auxin Transport Promotes Arabidopsis Lateral Root Initiation. *Plant Cell* **2001**, *13*, 843–852. [CrossRef] [PubMed]
31. Zuluaga, M.Y.A.; Monterisi, S.; Rouphael, Y.; Colla, G.; Lucini, L.; Cesco, S.; Pii, Y. Different Vegetal Protein Hydrolysates Distinctively Alleviate Salinity Stress in Vegetable Crops: A Case Study on Tomato and Lettuce. *Front. Plant Sci.* **2023**, *14*, 1077140. [CrossRef] [PubMed]
32. 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]
33. 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]
34. Chen, D.; Hou, Q.; Jia, L.; Sun, K. Combined Use of Two Trichoderma Strains to Promote Growth of Pakchoi (*Brassica chinensis* L.). *Agronomy* **2021**, *11*, 726. [CrossRef]
35. Cozzolino, E.; Mola, I.D.; Ottaiano, L.; El-Nakhel, C.; Rouphael, Y.; Mori, M. Foliar Application of Plant-Based Biostimulants Improve Yield and Upgrade Qualitative Characteristics of Processing Tomato. *Ital. J. Agron.* **2021**, *16*. [CrossRef]
36. Soteriou, G.A.; Rouphael, Y.; Emmanouilidou, M.G.; Antoniou, C.; Kyratzis, A.C.; Kyriacou, M.C. Biostimulatory Action of Vegetal Protein Hydrolysate and the Configuration of Fruit Physicochemical Characteristics in Grafted Watermelon. *Horticulturae* **2021**, *7*, 313. [CrossRef]
37. Rudresh, D.L.; Shivaprakash, M.K.; Prasad, R.D. Tricalcium Phosphate Solubilizing Abilities of *Trichoderma* spp. in Relation to P Uptake and Growth and Yield Parameters of Chickpea (*Cicer srietinum* L.). *Can. J. Microbiol.* **2005**, *51*, 217–222. [CrossRef] [PubMed]
38. Sabatino, L.; D'Anna, F.; Prinzivalli, C.; Iapichino, G. Soil Solarization and Calcium Cyanamide Affect Plant Vigor, Yield, Nutritional Traits, and Nutraceutical Compounds of Strawberry Grown in a Protected Cultivation System. *Agronomy* **2019**, *9*, 513. [CrossRef]

39. Fernando, D.; Milagrosa, S.; Francisco, C.; Francisco, M. Biostimulant Activity of *Trichoderma saturnisporum* in Melon (*Cucumis melo*). *HortScience* **2018**, *53*, 810–815. [CrossRef]
40. Apostol, F.-D.; Dinu, M.; Dumitru, M.G.; Maracineanu, C.; Josceanu, A.M.; Giugea, N. Influence of fertilization with *trichoderma atroviride* and fulvic acids upon the nutritive constituents in long pepper fruits. *UPB Sci. Bull. B Chem. Mater. Sci.* **2022**, *84*, 83–98.
41. 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. *HortScience* **2017**, *52*, 1214–1220. [CrossRef]
42. Soppelsa, S.; Kelderer, M.; Casera, C.; Bassi, M.; Robatscher, P.; Matteazzi, A.; Andreotti, C. Foliar Applications of Biostimulants Promote Growth, Yield and Fruit Quality of Strawberry Plants Grown under Nutrient Limitation. *Agronomy* **2019**, *9*, 483. [CrossRef]
43. Sani, M.N.H.; Islam, M.N.; Uddain, J.; Chowdhury, M.S.N.; Subramaniam, S. Synergistic Effect of Microbial and Nonmicrobial Biostimulants on Growth, Yield, and Nutritional Quality of Organic Tomato. *Crop Sci.* **2020**, *60*, 2102–2114. [CrossRef]
44. Ciriello, M.; Formisano, L.; El-Nakhel, C.; Corrado, G.; Rouphael, Y. Biostimulatory Action of a Plant-Derived Protein Hydrolysate on Morphological Traits, Photosynthetic Parameters, and Mineral Composition of Two Basil Cultivars Grown Hydroponically under Variable Electrical Conductivity. *Horticulturae* **2022**, *8*, 409. [CrossRef]
45. Rouphael, Y.; Colla, G.; De Pascale, S. Protein Hydrolysate-Based Biostimulant Improves Yield and Fruit Quality of Greenhouse Fresh Tomato. *Acta Hortic.* **2020**, 335–342. [CrossRef]
46. 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] [PubMed]
47. 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. Hortic.* **2017**, *226*, 353–360. [CrossRef]
48. Ertani, A.; Francioso, O.; Tugnoli, V.; Righi, V.; Nardi, S. Effect of Commercial Lignosulfonate-Humate on *Zea mays* L. Metabolism. *J. Agric. Food Chem.* **2011**, *59*, 11940–11948. [CrossRef] [PubMed]
49. Schiavon, M.; Pizzeghello, D.; Muscolo, A.; Vaccaro, S.; Francioso, O.; Nardi, S. High Molecular Size Humic Substances Enhance Phenylpropanoid Metabolism in Maize (*Zea mays* L.). *J. Chem. Ecol.* **2010**, *36*, 662–669. [CrossRef] [PubMed]
50. 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*, 246448. [CrossRef] [PubMed]
51. Lombardi, N.; Caira, S.; Troise, A.D.; Scaloni, A.; Vitaglione, P.; Vinale, F.; Marra, R.; Salzano, A.M.; Lorito, M.; Woo, S.L. Trichoderma Applications on Strawberry Plants Modulate the Physiological Processes Positively Affecting Fruit Production and Quality. *Front. Microbiol.* **2020**, *11*, 522584. [CrossRef] [PubMed]
52. Parrado, J.; Escudero-Gilete, M.L.; Friaza, V.; García-Martínez, A.; González-Miret, M.L.; Bautista, J.D.; Heredia, F.J. Enzymatic Vegetable Extract with Bio- Active Components: Influence of Fertiliser on the Colour and Anthocyanins of Red Grapes. *J. Sci. Food Agric.* **2007**, *87*, 2310–2318. [CrossRef]
53. Gurav, R.G.; Jadhav, J.P. A Novel Source of Biofertilizer from Feather Biomass for Banana Cultivation. *Environ. Sci. Pollut. Res.* **2013**, *20*, 4532–4539. [CrossRef] [PubMed]
54. Zhou, W.; Zheng, W.; Lv, H.; Wang, Q.; Liang, B.; Li, J. Foliar Application of Pig Blood-Derived Protein Hydrolysates Improves Antioxidant Activities in Lettuce by Regulating Phenolic Biosynthesis without Compromising Yield Production. *Sci. Hortic.* **2022**, *291*, 110602. [CrossRef]
55. Zhou, W.; Liang, X.; Li, K.; Dai, P.; Li, J.; Liang, B.; Sun, C.; Lin, X. Metabolomics Analysis Reveals Potential Mechanisms of Phenolic Accumulation in Lettuce (*Lactuca sativa* L.) Induced by Low Nitrogen Supply. *Plant Physiol. Biochem.* **2021**, *158*, 446–453. [CrossRef] [PubMed]
56. sNzanza, B.; Marais, D.; Soundy, P. Yield and Nutrient Content of Tomato (*Solanum lycopersicum* L.) as Influenced by *Trichoderma harzianum* and *Glomus mosseae* Inoculation. *Sci. Hortic.* **2012**, *144*, 55–59. [CrossRef]
57. Caruso, G.; El-Nakhel, C.; Rouphael, Y.; Comite, E.; Lombardi, N.; Cuciniello, A.; Woo, S.L. *Diplotaxis tenuifolia* (L.) DC. Yield and Quality as Influenced by Cropping Season, Protein Hydrolysates, and Trichoderma Applications. *Plants* **2020**, *9*, 697. [CrossRef] [PubMed]