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Synergistic Effect of a Plant-Derived Protein Hydrolysate and Arbuscular Mycorrhizal Fungi on Eggplant Grown in Open Fields: A Two-Year Study

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Abstract: Plant biostimulants, such as plant protein hydrolysates (PHs) and arbuscular mycorrhizal fungi (AM), are natural products capable of increasing the yield and quality of crops and decreasing the ecological impact of plant growing cycles. However, there is little research on the mutual application of different categories of biostimulants (microbial and non-microbial). The current study was conducted to examine the effects of “Trainer” PH application (0 or 3 mL L^{-1}) and AM (*R. irregularis*) inoculation on the growth, yield, quality and nitrogen indices of ”Birgah” F_1 eggplant cultivated for two years (2020 and 2021). Results revealed that the combined application of PH and AM significantly enhanced total and marketable yields, average marketable fruit weight and number of marketable fruits by 23.7%, 36.4%, 19.0% and 11.1% compared to non-treated plants (control), respectively. Moreover, biostimulants increased the soluble solids content (SSC), chlorogenic acid, total anthocyanins, K and Mg in the fruits by 16%, 4.6%, 6.4%, 8.6% and 23.9% compared to control plants, respectively. Interestingly, the mutual application of PH and AM improved fruit quality by reducing the glycoalkaloid concentration (−19.8%) and fruit browning potential (−38%). Furthermore, both biostimulants exerted a synergistic action, enhancing nitrogen use efficiency and nitrogen uptake efficiency by 26.7% and 18.75%, respectively. On the other hand, productive and fruit-quality features were significantly influenced by the year due to remarkable differences in terms of maximum temperature between the first and second cultivation cycles. Overall, our research underlined that PH and AM can positively interact to improve the performance of eggplant cultivated in open fields.

Keywords: synergic effect; *Solanum melongena*; post-harvest management; fruit quality

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1. Introduction

Eggplant (*Solanum melongena* L.) is an herbaceous perennial plant belonging to the *Solanaceae* family, appreciated for its nutritive and low-calorie fruits [1]. The global importance of eggplant is evidenced by the large cultivated area and by the total yields obtained [2]. Regarding farming area, China, India and Bangladesh are in the top three positions with about 800,000 ha, 750,000 ha and 53,000 ha, respectively [2]. China is also the top producer worldwide with production of over 37 million tons, followed by India with 128 million tons and Egypt with about 1.3 million tons [2]. In the European Union, Italy is the country with the largest harvested area (9570 ha), followed by Spain (3590 ha) and France (1420 ha) [2]. Italy is also the top European Union eggplant producer with about 300,000 tons, followed by Spain (265,000 tons) and Netherlands (63,000 tons). In Italy, eggplant is mainly grown in open fields (8100 ha), and the most important producer is Sicily (2700 ha), with 700 ha in protected environments [3].

Eggplant is usually grown in intensive monoculture systems, which imply large amounts of inputs [4]. Such systems have a significant impact on the ecosystem; therefore, in recent years, there have been efforts to test eco-friendly tools that could guarantee good plant performance under favourable or unfavourable growing conditions and, at the same time, reduce the impact of the horticultural sector on the environment [5–13].

Among the most recent agriculture innovations, biostimulants are certainly one of the most promising and sustainable [14,15]. As stated in the European Union regulation (1009/2019), biostimulants can be divided into two classes: microbial and non-microbial.

Arbuscular mycorrhizal fungi (AM) are one of the most widely used microbial biostimulants. These fungi establish a mutualistic, symbiotic relationship with the host plant, carrying mineral elements from the soil to the crop via a dense network of hyphae [16,17]. From the host plant, AM obtain sugars that they cannot synthesize since they are chemo-heterotrophic organisms [18]. It has been reported that AM prompted water and mineral uptake through alterations to the root morphology (length, density and number) [19,20]. Likewise, AM may alleviate abiotic distresses, such as salinity, drought or heavy metal contamination [21]. The increase in abiotic stress tolerance is probably linked to the AM's ability to alter host primary and secondary metabolism, stimulating the activation of host defence mechanisms and the biosynthesis of phytochemical compounds [22,23].

As concerns non-microbial biostimulants, plant protein hydrolysates (PHs) are among the most extensively used and appreciated. These biostimulants are obtained through the hydrolysis of vegetable biomass and are composed of a mixture of peptides, amino acids and nitrogen compounds [24]. A direct effect is the increase in nitrogen uptake and assimilation through the regulation of key enzymes involved in nitrogen metabolism [24]. Furthermore, hormone-like activities, chelating and antioxidant effects have been reported [25]. Indirect effects are linked to increases in microbial activity, nutrient availability and overall soil fertility [25]. The positive effects of PHs are related to the enhancements of yield and quality. Moreover, PHs can increase the uptake of mineral elements and reduce the nitrate content in plants by interacting with nitrogen metabolism [25]. The biostimulatory effects of PHs are mainly due to their peptide and amino-acid content. Amino acids are used by plants for different purposes, such as protein biosynthesis, energy production and synthesis of high-biological-activity molecules [26]. Moreover, peptides play important roles in the information exchange between cells, in plant responses to stress conditions and in control of growth and development [25].

During the last decade, many studies on AM and PHs have been conducted to better understand their effects on plants [27]. However, the scientific community agrees that an important branch of biostimulant research is the study of the interaction effects for microbial and non-microbial biostimulants (biostimulant 2.0) [28]. Recently, a study on the interaction effects for *Trichoderma atroviridae* inoculation and seaweed extract application was undertaken with eggplant grown in a protected environment [29]. However, no studies on the interaction between AM and PH have been undertaken. Moreover, it is crucial to study microbial and non-microbial biostimulant interactions in a different growing scenario.

In light of the aforesaid considerations, a two-year study was carried out to appraise the synergistic effects of PH application and AM inoculation on the quality and productive traits of eggplant grown in open fields in a typical Sicilian cultivation area.

2. Materials and Methods

2.1. Experimental Site and Plant Material

"Birgah" F_1 hybrid eggplant (*Solanum melongena*) plug plants produced by a professional nursery were transplanted to an experimental field of the Department of Agricultural, Food, and Forestry Sciences (SAAF) of the University of Palermo (latitude 38°12′ N, longitude 13°36′ E, altitude 65 m). The study was conducted for two consecutive years (2020 and 2021) in open fields during the spring–summer period (from 4 May to 31 August). Plants were spaced with 0.5 m in each row and 1 m between rows, achieving a density of two plants per m^{-2}. The soil (a typical Rhodoxeralf soil) was mulched with 20 μm thick black polyethylene film and a drip irrigation system was installed. During the cycle, N-P-K supply (250 kg N ha^{-1}, 150 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1}) was provided via fertigation. The doses were calculated considering the theoretical uptake of the crop, the estimated yield and the soil mineral content. All cultivation practices were followed considering the requirements of eggplant grown in open-field Mediterranean climatic conditions [30]. In both years, the preceding crop was cauliflower. Weather parameters (rainfall, maximum and minimum temperature) were recorded using a data logger.

2.2. Plant-Derived Protein Hydrolysate Application and Arbuscular Mycorrhizal Fungi Inoculation

Both microbial and non-microbial biostimulants were tested to evaluate their simple and combined effects. The root systems of the plug plants were inoculated before transplanting with the arbuscular mycorrhizal fungi (AM) *Rhizophagus irregularis* (formerly *Glomus intraradices*), strain CMCCROC7 (Bioplanet, Cesena, Italy), supplying 400 spores per plant. Inoculation was accomplished 24 h before transplantation via the soaking of the root system for 10 min. To assess mycorrhizal colonization, the procedure described by Phillips and Haymann [31] with the slight modification introduced by Torta et al. [32] was used. Mycorrhizal inoculation is presented as the percentage of infection.

Protein hydrolysate treatments were performed using Trainer (Trainer®; Hello Nature Italy SRL, Rivoli Veronese, Verona, Italy), a legume-derived biostimulant produced through enzymatic hydrolysis of vegetable biomass containing organic matter (35.5%), plant amino acids and peptides (31.0%) and organic nitrogen (5.0%). Plant-derived protein hydrolysate (PH) application started 7 days after transplantation (DAT). Every 10 days, the biostimulant solution was administered by foliar spray using the recommended dose (2.5 mL L^{-1}) and a solution equal to 0.5 L m^{-2}. The control plants were sprinkled with water via foliar spray.

2.3. Statistics and Design

The four biostimulant treatments (control, AM, PH and AM + PH) were tested for two consecutive years (2020 and 2021) using a two-factor split-plot experimental design. Each treatment was replicated three times (10 plants per replication), resulting in a total of 120 plants for each cultivation cycle. The statistical analysis was conducted with version 28.0 of SPSS software (StatSoft, Inc., Chicago, IL, USA) using the general linear model (GLM). The impact of each treatment was assessed with a two-way ANOVA analysis, setting the biostimulant and the year as the main factors. Mean separation was obtained using Tukey's HSD test at $p \leq 0.05$.

2.4. Plant Growth and Yield

Growth and yield parameters were measured on all plants. Data on plant height and number of leaves were collected 40 days after transplanting (DAT). Data on total yield, marketable yield and discarded production were recorded at the end of the experiment (31 August, 120 DAT). Total and marketable yield are reported as kg $plant^{-1}$, whereas discarded production is presented as a percentage. The number and average weight of marketable fruits (g $plant^{-1}$) were also determined.

2.5. Fruit Composition

Fruit composition analyses were conducted at the end of the experiment (31 August, 120 DAT) with five fruits randomly selected from each replicate.

Plant dry matter percentage was determined using a ventilated oven, drying 400 g of the sample at a temperature of 80 °C for 12 h until a constant weight was reached. The value is presented as a percentage (%). Firmness was recorded using a Trsnc digital penetrometer (Forlì, Italy). A 6 mm stainless steel cylindrical probe was used to record fruits' resistance to penetration by the penetrometer plunger. The evaluation was carried out on two opposite sides of the fruits' equatorial zone. The values for fruit firmness are reported in newtons (N).

For the estimation of the soluble solids content (SSC), after obtaining the juice from the fruit using a potato masher, it was filtered and a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd., New Taipei, Taiwan) was used for measurement. Values are shown as °Brix.

To determine the browning of the pulp, the pulp brightness (L*) was measured in the central zone of the fruit at two different times using a colorimeter (Chroma-meter CR-400, Minolta Corporation, Ltd., Osaka, Japan). The L* value was immediately recorded after cross-cutting the fruit (L_0) and then again 30 min after the cut (L_{30}). To estimate the potential for oxidation, the method described by Larrigaudiere et al. [33], with minor adjustments as partly suggested by Concellòn et al. [34], was adopted. The value is expressed as $\Delta L_{30} = (L_{30} - L_0)$.

Stommel and Whitakers' [35] procedure was followed with some modifications for the establishment of the chlorogenic acid content of the fruits. The quantification was accomplished via high-performance liquid chromatography (HPLC, Sigma-Aldrich, St. Louis, MO, USA), setting the absorbance to 325 nm. Chlorogenic acid content is indicated as mg 100 g^{-1} of dw. The extraction and analysis of anthocyanins from the skin of fruits was performed following the Mennella method [36], and the analysis was carried out via RP-HPLC using purified D3R (Polyphenols Laboratories AS, Sandnes, Norway) as an external standard.

For the determination of glycoalkaloids, the extraction method reported by Birner [37] was adopted with some adjustments. For the measurement, the method described by Kuronen et al. [38] was used. Thus, determinations were conducted via RP-HPLC using purified solasonine and solamargine as external standards. The detection limit was 0.03 mg 100 g^{-1} dry weight (dw). Values for the glycoalkaloid content are reported as mg 100 g^{-1} dw.

2.6. Mineral Profile and Nitrogen Indices

Analyses for the determination of protein and mineral content were carried out at the end of the experiment (31 August, 120 DAT) with five randomly selected fruits for each replicate. The nitrogen (N) content of the fruit was determined via the Kjeldahl method and, to obtain the protein content, the N value was multiplied by 6.25. The protein content is expressed as g 100 g^{-1} dw. Phosphorus (P) content was estimated colourimetrically, following the method described by Fogg and Wilkinson [39]. Nitrogen use efficiency (NUE) was calculated as the ratio of the yield to the application rate of nitrogen, and it is reported as t kg^{-1}. Nitrogen uptake efficiency (UE) was determined by using the following formula: UE = plant nitrogen content (kg)/nitrogen application (kg). The potassium (K), calcium (Ca) and magnesium (Mg) content of the eggplant fruit were assessed by atomic absorption spectroscopy after wet mineralization [40]. The mineral concentration values are reported as mg 100 g^{-1} dw.

3. Results

3.1. Weather Data and Mycorrhizal Inoculation

Data on weather (rainfall, maximum and minimum temperatures) recorded during the two years at the experimental field are presented in Figure 1.

The total rainfall recorded during the two years was similar: 84 mm and 81 mm of rain fell during the 2020 and 2021 growing cycles, respectively. Regarding the maximum temperatures, with the exception of the month of May in 2020, 2021 was warmer than 2020, especially during June and July. On the other hand, minimum temperatures were similar between the two growing cycles (Figure 1).

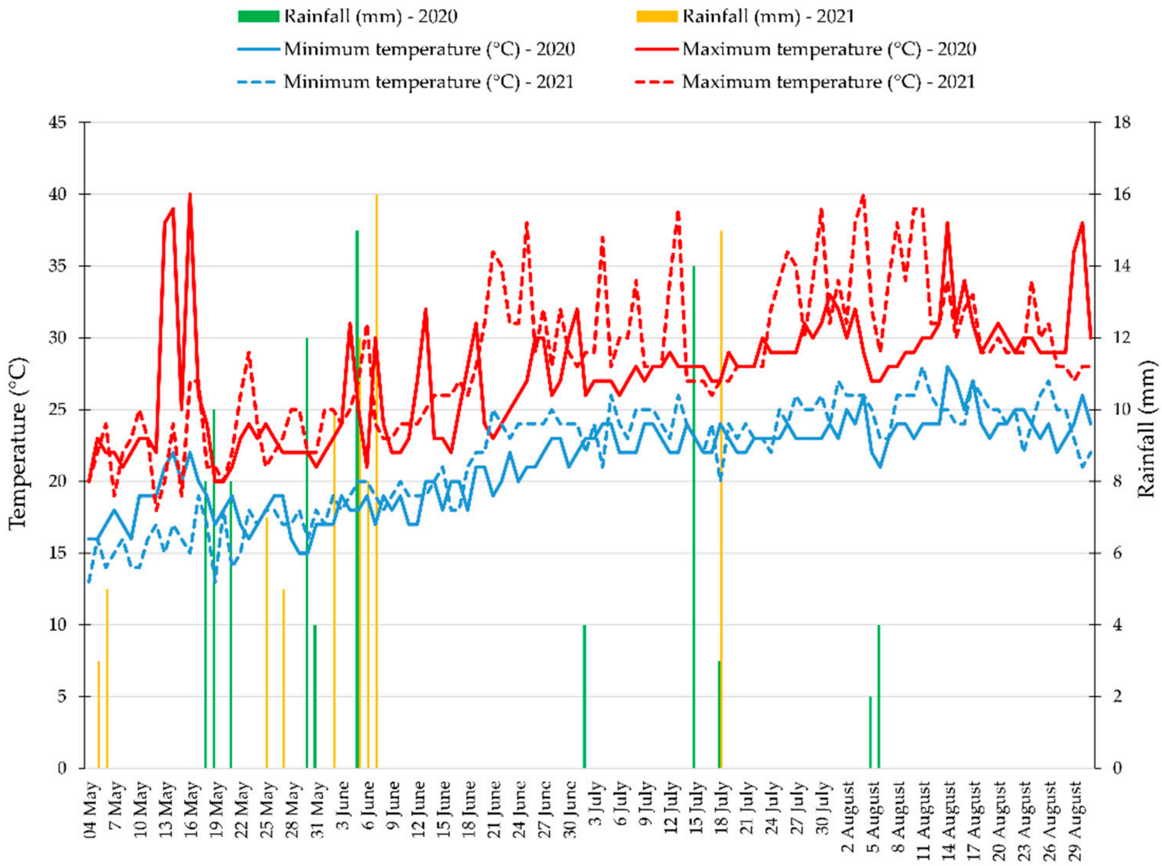

Figure 1. Rainfall and maximum and minimum temperatures recorded at the experimental field from 4 May to 31 August in 2020 and 2021.

Mycorrhizal inoculation percentage was significantly affected by the interaction between biostimulant and year (Figure S1). The highest inoculation values were recorded in plants cultivated during 2021 and supplied with AM or AM + PH, followed by those subjected to the same treatments but grown during the first year. The lowest values were recorded in control plants and those treated with PH cultivated in the first or second years (Figure S1).

3.2. Plant Growth and Yield

For plant growth traits (plant height 40 DAT and number of leaves 40 DAT) and yield features (total yield, marketable yield, average marketable fruit weight, number of marketable fruits and discarded production), ANOVA did not underline a significant effect for the interaction B × Y; consequently, the factors were judged to act independently of each other (Figure 2 and Table 1). Thus, the biostimulants performed equally in different years of the experiment. In particular, biostimulants had significant effects on all of these traits, while the year did not have a significant effect on discarded production.

When averaged over the year, plants treated with both biostimulants had the highest height at 40 DAT and number of leaves 40 DAT (Figure 2A,B), whereas the lowest values were recorded in control plants. When averaged for each biostimulant treatment, plants cultivated in the second year showed values for plant height and number of leaves higher than those grown in the first year.

Regardless of the year, plants treated with AM + PH had the highest total yield, marketable yield, average marketable fruit weight and number of marketable fruits (Table 1). The lowest total yield, marketable yield and average marketable fruits weight were recorded in control plants, whereas the lowest number of marketable fruits were found in control plants and in those treated with AM or PH (Table 1). Control plants had the highest discarded production, followed by those inoculated with AM, while plants treated with both biostimulants had the lowest discarded production (Table 1). Disregarding the biostimulant treatments, plants cultivated in the second year produced a higher total yield, marketable yield, average marketable fruit weight and number of marketable fruits than those grown in the first year (Table 1). In contrast, discarded production was not affected by the year.

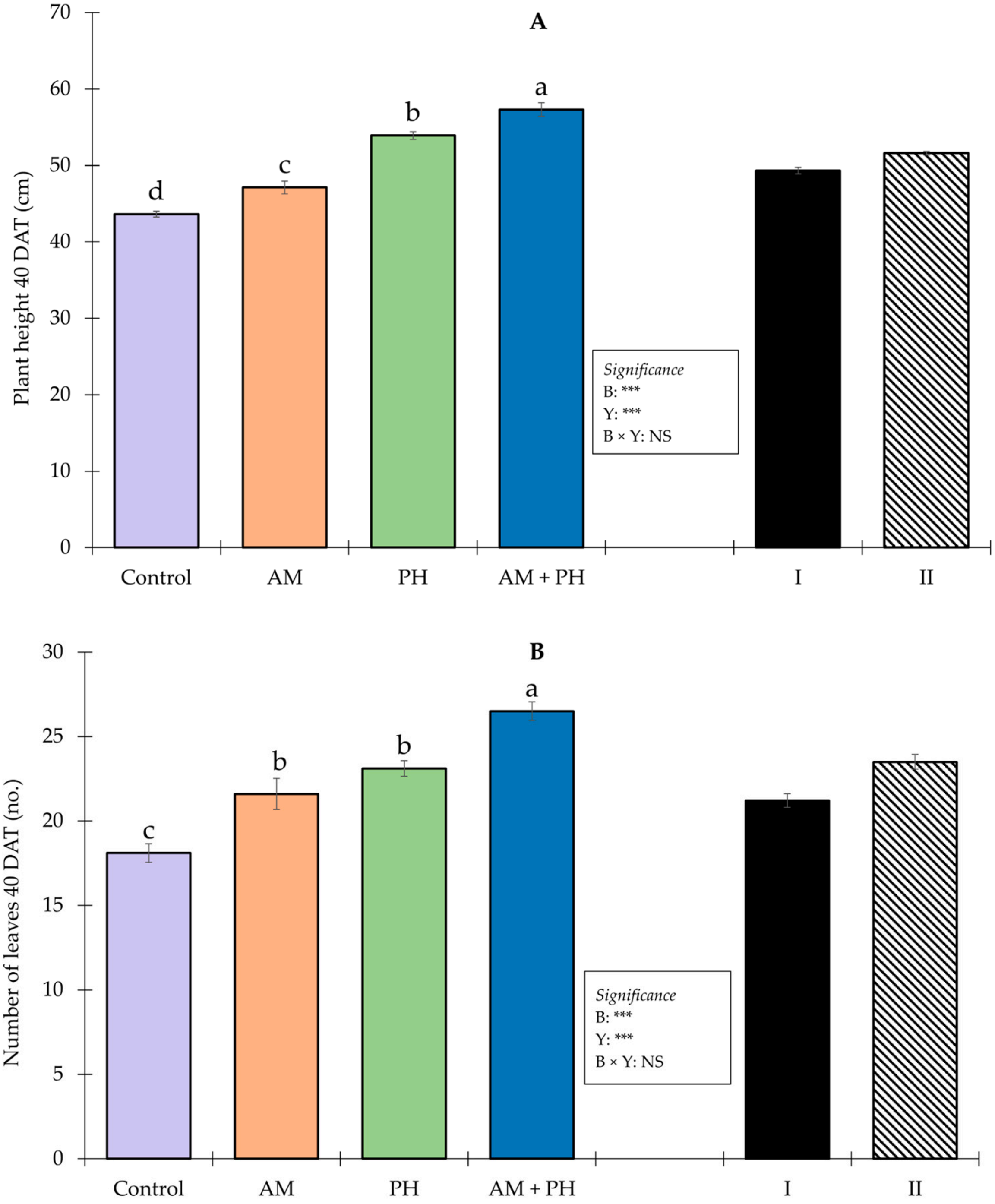

Figure 2. Eggplant plant height 40 DAT (**A**) and number of leaves 40 DAT (**B**) as affected by biostimulant and year of cultivation. Data are presented as means ± SE. Means with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021; DAT: days after transplant.

Table 1. Total yield, marketable yield, average marketable fruit weight, number of marketable fruits and discarded production for eggplant as affected by biostimulant and year of cultivation. Data were collected at the end of the experiment (31 August, 120 DAT).

Treatments	Total Yield (kg Plant^{-1})		Marketable Yield (kg Plant^{-1})		Average Marketable Fruit Weight (g)		No. of Marketable Fruits (no.)		Discarded Production (%)	
Biostimulant (B)										
Control	3.8	d	3.3	d	373.3	d	9.0	b	11.0	a
AM	4.0	c	3.7	c	396.2	c	9.5	b	5.8	c
PH	4.4	b	4.0	b	430.0	b	9.4	b	8.3	b
AM + PH	4.7	a	4.5	a	444.2	a	10.0	a	6.3	c
Year (Y)										
I	4.3		4.0		417.0		9.6		7.3	
II	4.2		3.8		404.8		9.4		8.3	
Significance										
B	***		***		***		***		***	
Y	***		***		**		*		NS	
B × Y	NS		NS		NS		NS		NS	

Values with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysates; I: 2020; II: 2021.

3.3. Fruit Quality and Browning

The two factors (biostimulant and year) independently influenced fruit dry matter, firmness, SSC, chlorogenic acid and glycoalkaloids (Figure 3 and Table 2). In particular, ANOVA showed that the biostimulant significantly affected the aforesaid parameters, whilst the year only had a significant effect for chlorogenic acid.

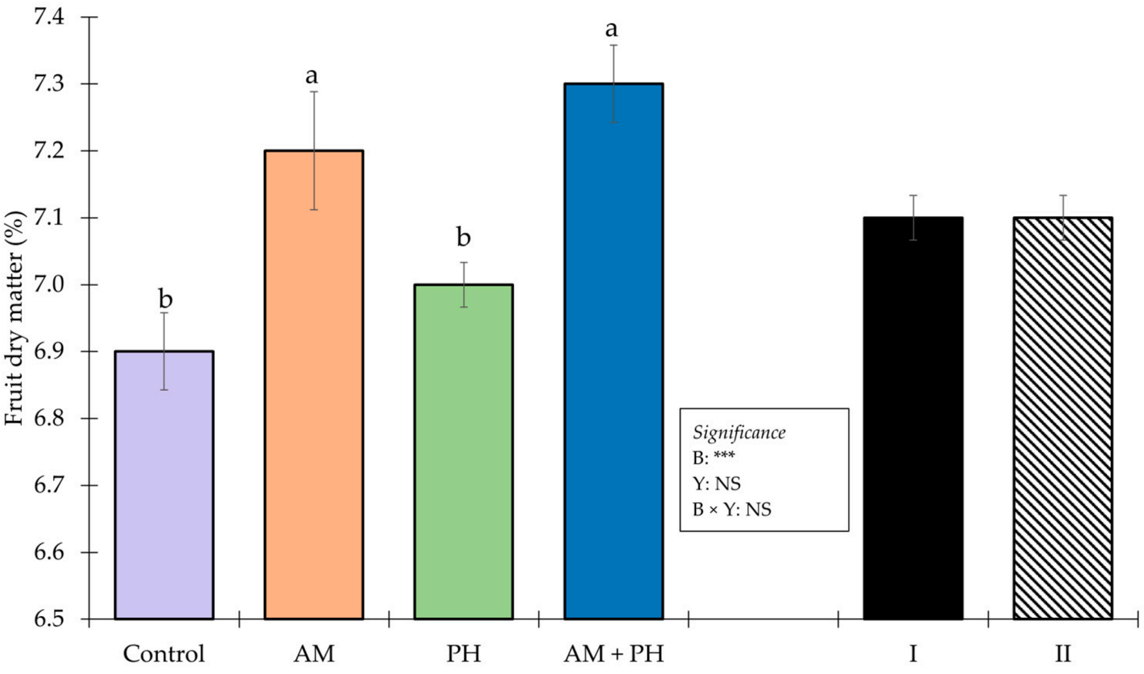

Figure 3. Eggplant fruit dry matter (%) as affected by biostimulant and year of cultivation. Data are presented as means ± SE. Means with different letters are statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

Table 2. Eggplant firmness, SSC, chlorogenic acid and glycoalkaloids as affected by biostimulant and year of cultivation.

Treatments	Firmness (N)		SSC (°Brix)		Chlorogenic Acid (mg 100 g^{-1} of dw)		Glycoalkaloids (mg 100 g^{-1} of dw)	
Biostimulant (B)								
Control	−40.9	b	4.4	c	763.4	c	90.3	a
AM	−44.1	c	4.8	b	778.5	b	78.1	b
PH	−38.2	a	4.7	b	776.2	b	72.2	c
AM + PH	−44.2	c	5.1	a	798.7	a	72.4	c
Year (Y)								
I	−41.9		4.7		772.0		79.0	
II	−41.8		4.7		786.2		77.5	
Significance								
B	***		***		***		***	
Y	NS		NS		***		NS	
B × Y	NS		NS		NS		NS	

Values with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

For fruit dry matter, the highest values were recorded in fruits from plants treated with AM or AM + PH, followed by those from control plants or plants supplied with PH (Figure 3).

When averaged for each year, fruits from plants treated with PH revealed the highest firmness values, followed by those from control plants. The lowest firmness values were recorded in plants treated with AM or AM + PH (Table 2). Irrespective of the year, the highest SSC and chlorogenic acid values were found in fruits from plants treated with both biostimulants, followed by those from plants treated with AM or PH. In contrast, the control plants produced fruits with the lowest SSC and chlorogenic acid values (Table 2). Regarding the fruit glycoalkaloid concentration, the highest values were recorded in fruits from control plants, whereas the lowest ones were found in fruits from plants treated with PH or AM + PH (Table 2). When not considering the biostimulant application, the year did not significantly influence the fruit dry matter, firmness, SSC or glycoalkaloids of the eggplant fruits, but plants grown in the second year had a higher chlorogenic acid concentration than those cultivated in the first year (Table 2).

For total anthocyanins, the two factors acted differently in different years of the experiment. This finding was related to the AM effect, which was approximately the same in both years, generating a significant interaction between B and Y (Figure 4). Fruits from plants cultivated in the second year and treated with both biostimulants had the highest anthocyanin content, followed by those collected from plants grown in the second year and supplied with PH, which, in turn, showed higher values than fruits from plants cultivated in the first year and treated with AM + PH. The lowest values were found in control plants cultivated in the first year (Figure 4).

ANOVA for ΔL_{30} did not reveal a significant interaction between the biostimulant treatment and the year, indicating that the two factors behaved separately (Figure 5). Regardless of the year, the highest ΔL_{30} value was recorded in fruits from control plants, followed by those from AM-inoculated plants. The lowest values were recorded in fruits from plots treated with both biostimulants. In contrast, when averaged for each biostimulant treatment, the year did not significantly influence fruit pulp browning.

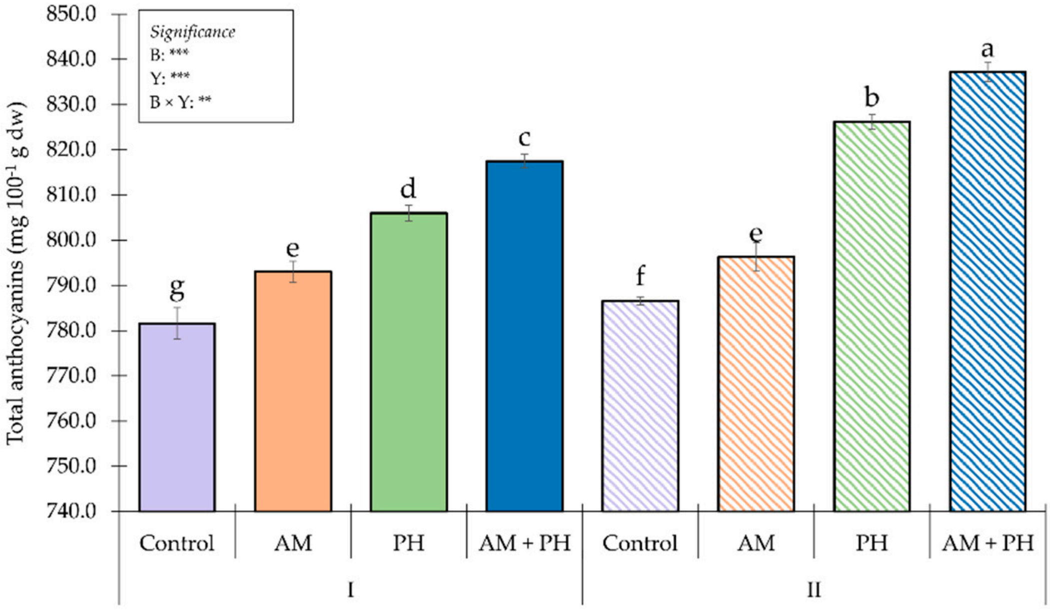

Figure 4. Eggplant total anthocyanins as affected by biostimulant and year of cultivation. Data are presented as means ± SE. Means with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

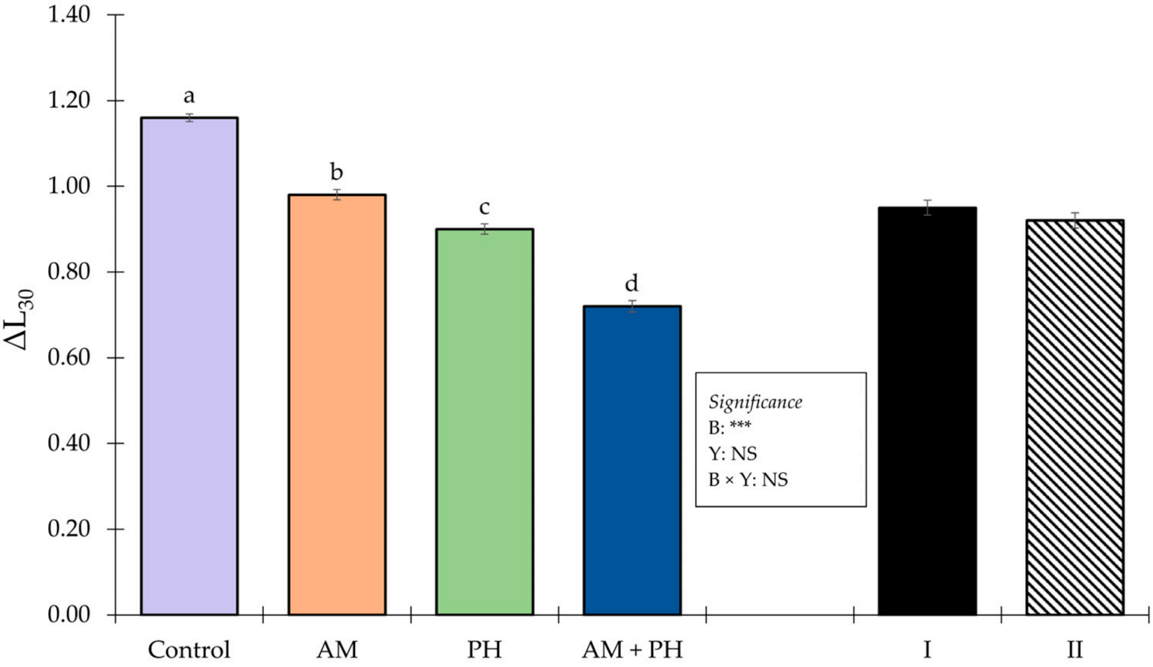

Figure 5. Eggplant ΔL_{30} as affected by biostimulant and year of cultivation. Data are presented as means ± SE. Means with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

3.4. Fruit Proteins and Mineral Profile

Biostimulant and year independently influenced proteins, P, K, Ca and Mg, as reported in Table 3.

Table 3. Eggplant protein, P, K, Ca and Mg concentrations as affected by biostimulant and year of cultivation.

Treatments	Proteins (g 100 g^{-1} dw)		P (mg 100 g^{-1} dw)		K (mg 100 g^{-1} dw)		Ca (mg 100 g^{-1} dw)		Mg (mg 100 g^{-1} dw)	
Biostimulant (B)										
Control	9.66	a	558.0	a	346.3	c	112.6	a	18.51	c
AM	9.66	a	559.1	a	346.4	c	112.8	a	20.71	b
PH	8.68	b	558.9	a	368.9	b	112.6	a	18.50	c
AM + PH	8.68	b	558.6	a	376.0	a	112.7	a	22.93	a
Year (Y)										
I	9.10		558.4		335.6		112.6		19.92	
II	9.25		558.9		363.2		112.7		20.40	
Significance										
B	***		NS		***		NS		***	
Y	***		NS		***		NS		*	
B × Y	NS		NS		NS		NS		NS	

Values with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

The highest protein concentration was found in fruits from control plants and from those treated with AM, whereas the lowest values were recorded in fruits from PH- and AM + PH-treated plants (Table 3). The highest K values were documented in fruits harvested from plots treated with both biostimulants, followed by those from PH-treated plants. The lowest K values were recorded in control plants or in those treated with AM (Table 3). The highest Mg concentration was detected in fruits from plants supplied with both biostimulants, followed by those harvested from untreated plants (control) and those treated with PH. Biostimulant application did not have a significant effect on P and Ca. Averaged for each biostimulant treatment, fruits from plants cultivated in the second year had the highest protein, K and Mg concentrations. The year had no significant effect on P and Ca concentrations (Table 3).

3.5. Nitrogen Indices

As shown in Table 4, the two experimental factors separately influenced NUE and UE (Table 4).

Table 4. Eggplant nitrogen use efficiency (NUE) and uptake efficiency (UE) as affected by biostimulant and year of cultivation.

Treatments	NUE (t/kg)		UE (kg/kg)	
Biostimulant (B)				
Control	0.30	d	0.32	c
AM	0.32	c	0.36	b
PH	0.35	b	0.34	b
PH + AM	0.38	a	0.38	a
Year (Y)				
I	0.34		0.34	
II	0.33		0.36	
Significance				
B	***		***	
Y	***		***	
B × Y	NS		NS	

Values with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. NS: not significant; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

As concerns the NUE, regardless of the year, the highest values were obtained from plants treated with both biostimulants, followed by those supplied with PH, which, in turn, showed higher values than those inoculated with AM. Control plants showed the lowest NUE values. Averaged for B, plants cultivated during the first year had the highest NUE values. Irrespective of Y, plants subjected to both biostimulants had the highest UE values, followed by those supplied with AM or PH (Table 4), whereas, without regard for B, plants from the second year showed the highest UE values.

4. Discussion

Concern regarding the environmental impact of intensive crop systems has led to the development of sustainable agronomic means, such as biostimulants. These products, although they do not contain high quantities of macro- or microelements, have positive effects on crop yield and quality, as well as stress tolerance. In this study, the effects of two biostimulants (microbial and non-microbial) on the yield and qualitative parameters of eggplant grown in open fields were investigated.

At the end of the cultivation cycle, the mycorrhizal colonization percentage detected in inoculated plants was significantly higher than that detected in non-inoculated plants. Specifically, the colonization percentages were 70.7% (first year × AM), 70.0% (first year × AM + PH), 73.7% (second year × AM) and 74.3% (second year × AM + PH). Remarkably, the year also had a significant effect on the colonization percentages of the inoculated plants. Indeed, plants grown in the first year showed a slight reduction in the mycorrhizal colonization percentage. These results could be linked to the high temperatures recorded during the first part of the 2020 growing cycle, which could have slowed down the development of the AM.

The findings revealed that both biostimulants were useful for increasing plant growth and yield traits, demonstrating a reduction in terms of discarded production. These data are fully in line with those published by Sabatino et al. [41], who, studying the effect of grafting and arbuscular mycorrhizal fungi inoculation on eggplant cultivated in a protected environment, found a beneficial effect from AM on plant growth and yield features. These findings also concur with those of Oztekin et al. [42], who found an increase in the total yield, marketable yield and average fruit weight of AM-inoculated plants. As reported by De Pascale et al. [43] and Rouphael et al. [21], the valuable effects of AM inoculation on yield and growth traits could be linked to increases in mineral uptake and translocation, as well as to the enhancement of the root system expansion of the inoculated plants. The data are also in line with those reported by Rouphael et al. [44], who found increases in plant height and number of leaves in PH-treated plants. Furthermore, the results agree with those of Consentino et al. [45], who found significant increases in the yield parameters of celery plants treated with PH. As reported by Colla et al. [46], the positive effects of PH application on yield and growth traits could be attributed to its influence on root vigour traits, which, in turn, results in better uptake of water and nutrients. Indeed, the authors observed an increase in root growth in plants supplied with PH compared to untreated plants. Remarkably, plants treated with both biostimulants performed better than those supplied with only one. Thus, in agreement with Rouphael and Colla [28], we may speculate that there was a synergistic effect between arbuscular mycorrhizal fungi inoculation and plant protein hydrolysate application. Regarding the cultivation year, data revealed that plants grown in 2020 performed better in terms of yield and growth parameters. This significant increase could have been linked to the optimal temperatures recorded during the whole growing cycle, with the only exception occurring during 13–16 May.

Arbuscular mycorrhizal inoculation significantly boosted fruit dry matter percentage compared to the control. This result is in accordance with the findings of Sharma et al. [47], who, studying the effects of different microbial biostimulants on eggplant, revealed that AM application increased fruit dry matter. However, this is in contrast with the findings of Sabatino et al. [41], who reported no significant effect from AM on fruit dry matter percentage, although an increasing trend was noted. The results for PH applications revealed no significant effect on fruit dry matter. This finding is in contrast with that of Consentino et al. [45], who found a significant decrease in the dry matter of PH-treated plants compared to the control. Moreover, Lucini et al. [48] found an opposite trend, describing a significant increase in dry biomass in lettuce plants treated with PH. These differences could be related to the different growing conditions (protected environment vs. open field) and to the different species (fruiting vs. leafy vegetable). Interestingly, the combined application of both biostimulants also gave the highest values in terms of fruit dry matter percentage. We can, therefore, hypothesize that the two biostimulants acted synergistically to increase the fruit dry matter of eggplant.

Both treatments had positive effects on fruit firmness. The data agree with those reported by Sabatino et al. [41] for eggplant and by Miceli et al. [49] for watermelon but disagree with those reported by Maboko et al. [50] for tomato, where no significant differences between control and AM-inoculated plants were found. The differences could be related to the different cultivation systems adopted by the authors. Indeed, the studies by the first two teams were conducted in soil conditions, whereas the last one was performed in a soilless system. Furthermore, PH application had a negative effect on fruit firmness. This outcome did not agree with Cozzolino et al. [51], who, investigating the effects of plant-based biostimulants on tomato, found a significant firmness increase in fruit from PH-treated plants. In contrast, Soteriou et al. [52], studying the effect of a vegetal protein hydrolysate on watermelon, revealed no significant variation in fruit firmness. The contrasting results obtained could be linked to the different plants' responses to PH (eggplant, tomato and watermelon), which seem to be species-related traits. Another important outcome was that the combined application of biostimulants had a better effect on firmness than PH alone, which had the lowest values. Consequently, we can assume that, even for fruit firmness, microbial and non-microbial biostimulants positively interacted with each other.

Both biostimulants (PH and AM) significantly boosted the SSC of eggplant fruits compared to the control. The findings concur with those of Sabatino et al. [41] and Sharma et al. [47] but disagree with those of Oztekin et al. [42], who found no AM effect on tomato fruit SSC. Moreover, the data are in accordance with those reported by Ordookhani and Zare [53], who, studying the application of different microbial biostimulants on tomato, revealed that the application of AM significantly boosted soluble solids content. The findings are also in line with those of Cozzolino et al. [51], who reported a significant increase in SSC in fruits from PH-treated plants. Furthermore, the data agree with Rouphael et al. [54], who, studying the influence of a plant protein hydrolysate on different tomato cultivars, underlined an increase in SSC in fruits harvested from PH-treated plants. Interestingly, the mutual application of both biostimulants gave the highest values in terms of fruit SSC. These results may be related to the positive effects that both biostimulants have on primary metabolism [25].

Both biostimulants significantly improved chlorogenic acid concentration in eggplant fruits. The results are in line with those reported by Sabatino et al. [41], who found an overall increase in chlorogenic acid in eggplant fruits from AM-inoculated plants. Moreover, the data are in line with those reported by Ertani et al. [55], who, conducting a greenhouse experiment with protein hydrolysates on *Capsicum chinensis* nutraceutical traits, found that pepper fruits treated with PH had higher levels of chlorogenic acid than the control. We found that the highest chlorogenic acid concentration was recorded in fruits from plants treated with both biostimulants. As reported by Sbrana et al. [22], AM symbiosis is capable of changing host-plant secondary metabolism, triggering the biosynthesis of phytochemicals. These physiological effects could be related to the activation of host defence mechanisms in colonised plants [23]. On the other hand, a similar mechanism was described for PH that increases antioxidant enzyme activity and the production of secondary metabolites [25]. Moreover, fruits from plants grown in 2021 had a higher chlorogenic acid concentration than those harvested from plants cultivated in 2020. This outcome could be explained by the higher temperatures recorded in 2021 as compared with those of 2020. Considering that (i) the optimal temperature range for eggplant is 22–26 °C [4], (ii) plants cultivated during 2021 were more stressed than those grown in 2020 and (iii) stressed plants increase the production of total polyphenols [56], we may assume that the higher chlorogenic acid concentrations recorded were related to the higher stress affecting the plants.

Both biostimulants significantly decreased glycoalkaloid concentration in the fruits. These data agree with those presented by Sabatino et al. [41], who reported a reduction in glycoalkaloids when plants were inoculated with AM. Moreover, the mutual application of PH and AM positively influenced the glycoalkaloid concentration. Since (i) the study was accomplished in an open field, exposing plants to non-optimal conditions; (ii) non-optimal growing conditions increase glycoalkaloid concentration in plants [57]; and (iii) microbial and non-microbial biostimulants enhance the tolerance to mild or severe stresses [58], the reduction in glycoalkaloids recorded in biostimulated plants could have been the result of the plants being less stressed.

Control plants and those inoculated with AM produced fruits with the highest protein content, whereas plants treated with PH or with AM + PH produced fruits with the lowest protein content. Since protein concentration is strictly related to the presence of N, these findings concur with those of Consentino et al. [45] for celery and Amr and Hadidi [59] for leafy green vegetables showing reductions in N and nitrate content, respectively. As reported by Calvo et al. [60] and Colla et al. [25], PH's ability to limit the accumulation of N in plant tissues can be attributed to the regulation of various metabolic pathways involved in N metabolism. In addition, Colla et al. [25] showed that the high concentration of amino acids in protein hydrolysates reduces N uptake by plant roots. Moreover, data revealed that proteins were significantly influenced by the year. As stated by Ma et al. [61], high temperatures stimulate the accumulation of protein in plants. Consequently, the higher protein concentration recorded in fruits from plants cultivated in 2021 (the warmest year) was a consequence of the climatic trend.

Findings regarding the mineral profile revealed that neither treatment had a significant effect on P or Ca, whereas the mutual application of PH and AM gave the highest values in terms of fruit K and Mg concentrations. Overall, these data agree with those reported by Sabatino et al. [41], Consentino et al. [45] and Sharma et al. [47], who found that both PH and AM could increase plant mineral concentration. The positive effect of AM on mineral uptake is well-documented [41,47,62]. Indeed, AM create a dense network of hyphae, which can help plants take up nutrients and water from the soil [21]. Concomitantly, the positive effect of PH can be linked to its carbohydrate, phenol and phyto-hormone contents, which stimulate plant mineral uptake and assimilation [25]. Interestingly, the mutual application of biostimulants was more effective than their single use. Consequently, we can speculate that these two biostimulants synergistically act in increasing K and Mg fruit concentrations via the modulation of mineral uptake and accumulation.

Data on nitrogen indices revealed that the NUE and UE were significantly increased by the mutual application of biostimulants compared to the control. These outcomes are in concordance with those of Sabatino et al. [41] and Zhu et al. [63]. Moreover, the findings are in line with those of Di Mola et al. [64], who, investigating the effects of PH application on baby spinach and lamb's lettuce under different N regimes, found a significant increase in NUE and UE. The same results were obtained by Cozzolino et al. [51] for tomato and by Sabatino et al. [27] for lettuce. Since N indices are calculated from the yield, the fruit nitrogen concentration and the nitrogen application rate, and considering that the combined application of biostimulants significantly modulated yield parameters and fruit N content, the obtained data show the results of these biostimulatory effects.

Results for anthocyanins showed the positive effect of the treatments. This was in agreement with the study by Sabatino et al. [41], who reported an increase in anthocyanins in fruits from AM-inoculated plants. Furthermore, the data are in concordance with those of other authors [65–67], who, evaluating the effects of AM on the phytochemical production in *Ocimum basilicum*, found that the inoculation increased antioxidant concentration. Baslam et al. [68] underlined that lettuce plants inoculated with biostimulants had higher anthocyanin concentrations than control plants. Moreover, it has been reported that strawberry plants treated with AM produced fruits with high anthocyanin cyanidin-3-glucoside levels [69]. Protein hydrolysate application significantly increased anthocyanin concentration in fruits. These findings are in concordance with those reported by Di Mola et al. [64], who showed that PH application was able to boost plant secondary metabolism in lamb's lettuce and baby spinach plants. The findings also revealed that total anthocyanins were higher in the second year of cultivation. This could be explained by the plant defence mechanism against thermal stress. Indeed, plants react to temperature stress by biosynthesizing flavonoids, such as anthocyanins, which have a high antioxidant capability [70].

S. melongena is among the top ten vegetables in terms of antioxidant activity; thus, the browning potential of eggplant fruits could be estimated [71,72]. The browning of eggplant pulp starts upon slicing the fruit with the release of the polyphenol oxidase enzyme. This enzyme, in the presence of oxygen, oxidizes phenolics and polymerizes o-quinones, generating brown pigments. In our study, the browning potential of fruits was reduced by application of biostimulants. Thus, we can assume that the biostimulant application significantly reduced the oxidative potential of the fruit pulp. Indeed, as already reported, biostimulants interact with plant secondary metabolism and with enzymes involved in defence against oxidative stress [21,25].

5. Conclusions

In the current research, we studied the effects of two biostimulants (microbial and non-microbial)—used alone or combined—on the yield and quality of eggplant plants cultivated in open-field conditions during two consecutive years. The findings revealed that PH and AM significantly enhanced growth and yield parameters, especially when combined. Moreover, the mutual application of both biostimulants significantly decreased the discarded production. Overall, the combined application of PH and AM boosted qualitative fruit parameters, such as SSC and chlorogenic acid, protein, mineral and anthocyanin concentrations. At the same time, the combination of biostimulants significantly reduced the glycoalkaloid content in fruits, making them more nutritious and also decreasing the browning potential. Furthermore, biostimulant application enhanced NUE and UE indices, improving the efficiency of N plant nutrition. Our study also showed that the different weather patterns between 2020 and 2021 affected yield, growth and quality parameters. These results were due to the higher temperatures recorded in the experimental field during 2021. Our research underlined that the combined use of PH and AM can be a useful agronomic tool to improve the yield and qualitative traits of eggplant, including under optimal or sub-optimal growing conditions. Furthermore, the current study represents a preliminary agronomic approach and, consequently, it deserves specific attention in future omics research activities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9050592/s1, Figure S1: Mycorrhizal inoculation percentage as affected by biostimulant and year of cultivation. Data are presented as means ± SE. Means with different letters were statistically dissimilar according to the Tukey HSD test at $p \leq 0.05$. **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$. AM: arbuscular mycorrhizal fungi; PH: protein hydrolysate; I: 2020; II: 2021.

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