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Article

Yield and Quality of Processing Tomato as Improved by Biostimulants Based on *Trichoderma* sp. and *Ascophyllum nodosum* and Biodegradable Mulching Films

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Abstract: Tomato is a great source of bioactive compounds, is important for human health, and is cultivated worldwide. However, the high inputs required for its cultivation must be sustainably managed in order to limit yield losses, thus obtaining high-quality and environmentally friendly production. In this perspective, we compared four biostimulant treatments, i.e., *Ascophyllum nodosum* extract—Bio; microbial biostimulant containing the micro-organism *Trichoderma afroharzianum*—Mic; a combination of both—M-B; not treated—Control) and three mulch treatments (biodegradable film Ecovio—ECO; biodegradable film MaterBi®—NOV; bare soil—BS) and evaluated their effects on yield and quality traits in processing tomato. Both biodegradable films elicited a 27.0% yield increase compared to plants grown on bare soil, and biostimulants determined a 23.7% increase over the Control, with the best performance recorded for M-B (+24.8%). Biodegradable MaterBi® film (NOV) was associated with higher total soluble solids (TSS) and firmness values (average of 4.9 °Brix and 1.30 kg cm^{-2}, respectively), even if a significant effect of biostimulants was observed only for the second element. Carotenoid content was higher in non-treated plants grown on bare soil as well as hydrophilic antioxidant activity (AA), but in this case, no differences between biostimulant treatments were recorded. The lipophilic AA in NOV-treated plants was about six and four times higher than observed in BS and ECO treatments, respectively; NOV also caused a 38.7% increase in ascorbic acid content over the Control but was not different from ECO. All biostimulant treatments elicited a 30% increase in phenol content compared to Control plants. Our findings highlight that microbial biostimulants based on *A. nodosum* extract and *T. afroharzianum* (both applied singularly and combined) can be considered a sustainable tool for increasing yield and improve some quality traits of processing tomato; in addition, we also confirmed the capability of biodegradable mulches, in particular, MaterBi®, to enhance the agronomic performance of tomato.

Keywords: sustainable agricultural practices; beneficial fungi; algal-based product; *Solanum lycopersicum* L.; nutritional quality

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in the world and is grown on all continents, both in the open air and in the greenhouse, destined for fresh or processed consumption. In Europe, Italy leads in open-air processing tomato cultivation, covering 77.150 hectares with 5.6 million tons of produce in 2021, 30% of which was concentrated in southern regions [1].

The limited availability of cultivable land and the huge increase in world population imply a growing food demand as well as the need for optimized crop management techniques [2], including mulching [3].

Plastic films generally improve crop growth because they retain soil moisture, increase soil temperature, suppress weeds, and control pests [4]; however, at the end of their lifetime, the plastic residues of mulching degrade the soil structure and inhibit crop growth [5].

Biodegradable films are a promising alternative to polyethylene (PE) mulches, because they can be buried in the soil at the end of the cropping season and microbially degraded, thus reducing the amount of plastic waste left in the ground [6–9]. Several studies have confirmed the effectiveness of biodegradable films to keep or increase yield and crop quality in tomato, strawberry, and zucchini [10–13]. In particular, the biodegradable films allow the management soil temperature, keeping it cooler or warmer depending on the needs and reducing diurnal temperature variation [14,15]. In addition, the literature reports that biodegradable films: (i) increase soil humus content when buried, improving soil structure; (ii) promote the free exchange of gases between soil and atmosphere (higher soil porosity, lower soil compaction, decreased formation of surface crusts, and better soil drainage) [16]; (iii) reduce soil wind erosion [17]; (iv) promote both the growth and distribution of the crop root system [16]; (v) play a key role in integrated pest management, controlling insect pests [15]. In previous research, Cozzolino et al. [18] compared a biodegradable film to PE and observed that lettuce grown on the first one achieved yields comparable to those obtained using PE film and without negative effects on quality. Di Mola et al. [19] compared a biodegradable film to a PE film, without finding any difference in yield quality and quantity of zucchini grown, both in the open air or in the greenhouse.

Nowadays, one of the main goals of modern agriculture is sustainable food production, and this need pushes research to address various issues, including the identification of new agricultural practices that reduce the use of agrochemicals [20] and thereby the environmental impact of agriculture. From this perspective, biostimulants play a pivotal role; they are products that, based on their composition, can be divided into (i) organic substances; (ii) inorganic compounds; (iii) plant growth-promoting microorganisms or their extracts [21]. These products are not classified as fertilizers or pesticides, but they are able to increase plant growth, yield, and tolerance to abiotic stresses [22]. Among the different categories of biostimulants, seaweed extracts are one of the most widely used in agriculture [21], in particular, brown algae, such as the *Ascophyllum nodosum* (L.) extract, has been reported to promote the growth yield quantity and quality of many crops [23,24]. Among beneficial fungi, *Trichoderma* is one of the most studied, used as a biopesticide and biofertilizer to protect plants and enhance vegetative growth [25–27]. Numerous species are able to suppress pathogens, improve plant growth, and induce disease resistance [25,28]. In the literature, the effects of *Trichoderma* inoculation on plant growth parameters, use efficiency of macro-, oligo- and micronutrients, water use efficiency, photosynthesis, activation of plant secondary metabolism, and accumulation of polyphenols have been observed. In fact, Carillo et al. [29] reported that the contents of lycopene and some amino acids increased in tomato fruits after the application of *T. harzianum*, which also improved crop productivity (+40%) compared to the untreated control. Other works found that *Trichoderma harzianum* T-22 is able to colonize the roots of many different plant species [25,30]. Previous studies demonstrated that the application of selected *Trichoderma* spp. stimulates growth and may improve the nutritional value of lentils [31] and soybean [32], i.e., by increasing mineral content. However, the results are contrasting; in fact, Di Mola et al. [33] reported that the application of *Trichoderma* had no effects on lettuce marketable yield and growth parameters, while improving some quality parameters. Therefore, the response of different crops treated with these fungi in terms of quantity and quality requires further investigation.

The present research aimed to assess the agronomic response and qualitative traits of fruits of processing tomatoes grown on different biodegradable mulching films and treated with biostimulants based on *Trichoderma* sp. and/or *Ascophyllum nodosum*.

2. Materials and Methods

2.1. Experimental Design, Growing Conditions, and Tomato Cultivar

The experiment was carried out at the "D'Amore Francesco" private farm located in Frignano (CE–Southern-Italy; 41°02′ N; 14°17′ E; 70 m a.s.l.) during the 2021 Spring–Summer. The trial was conducted on a loamy soil whose physical and chemical characteristics are reported in detail in Table 1.

Table 1. Physical and chemical characteristics of the soil (0–30 cm layer).

Parameters	Measure Unit	Soil
Particle Size Distribution		
Coarse sand	%	16.6
Fine sand	%	31.4
Silt	%	29.0
Clay	%	23.0
N—total (Kjeldahl method)	%	0.118
P_2O_5 (Olsen method)	ppm	161.7
K_2O (Tetraphenylborate method)	ppm	1539.1
Organic matter (Bichromate method)	%	1.39
NO_3-N	ppm	5.0
NH_4-N	ppm	15.1
pH		7.2
Electrical conductivity	dS m^{-1}	0.118

The tested plant was tomato (*Solanum lycopersicum* L.) cultivar 'Heinz 5108 F1' (Agrisem srl Soc., SA, Italy), an early hybrid, with oval-square fruits of good size and consistency.

After manually placing the mulching films, the tomato plants were transplanted on 14 April 2021 at a density of 33,000 plants per hectare (row-to-row spacing within the paired row of 60 cm; spacing between the row pairs of 180 cm; plant-to-plant space on the single row of 33 cm). Ordinary soil fertility management was adopted with background fertilization carried out at two times: before the transplant, with an organ-mineral fertilizer (NP: 10-24), and at the transplant, with diammonium phosphate (NP: 18-46) per a total of 100 kg N ha^{-1}. During the crop cycle, nitrogen was provided in fertigation, as ammonium nitrate (26%) per a total of 200 kg ha^{-1} applied over 7 times. The water losses were determined by the Hargreaves method [34] and fully restored by drip irrigation.

Three different mulches (M) and four biostimulant treatments (B) were compared and arranged in a randomized block design. The mulching treatments were: (i) biodegradable film Ecovio, marketed by BASF (Ludwigshafen, Germany)—ECO; (ii) biodegradable film MaterBi® marketed by Novamont (Novara, Italy)—NOV; (iii) bare soil—BS; both mulching films were black and 15 µm thick. The biostimulant treatments were: (i) Phylgreen® (Trade Corporation International, Madrid, Spain)—Bio; (ii) Trianum-P® (Koppert B. V., Berkel en Rodenrijs, Netherlands)—Mic; (iii) combination of the two—MiBi; (iv) not treated—Control.

All treatments were replicated three times per a total of 36 plots (3M × 4B × 3 replications), each of 3 m^2 (Figure S1).

2.2. Mulch Management and Biostimulant Characteristics and Application

Phylgreen® is a liquid formulation based on an extract of the algae *Ascophyllum nodosum*, obtained through a cold extraction process. It is soluble and with a high content of alginates, vitamins, natural antioxidants, and noble amino acids. It acts as a promoter of photosynthesis and root development, stimulates flowering and setting, and improves the tolerance to abiotic stresses [35].

Trianum-P® is a microbial biostimulant based on an antagonistic strain of the genus *Trichoderma*, containing the micro-organism *Trichoderma afroharzianum* (ex *T. harzianum*) strain Rifai KRL-AG2 (T-22) at a minimum concentration of 1×10^9 CFU g^{-1}. It is formulated as water-dispersible granules, and it is able to increase the plant resistance against different pathogens, to withstand adverse conditions due to nutritional and water deficiencies and climate; in addition, it also facilitates the intake of nutrients by plants, by allowing greater growth and development of plant root and aboveground biomass [36].

The treatments with biostimulants started on 21 April; Bio was applied as a foliar spray at a rate of 3 mL L^{-1} on a bi-weekly basis; Mic was applied to soil on a monthly basis, at a rate of 2.5 kg ha^{-1}. Simultaneously, control plants were sprayed with tap water.

2.3. Soil and Air Temperatures

During the whole cycle, the air and soil temperature were continuously monitored by probes (Vantage Pro2, Hayward, CA, USA, Davis Instruments); the probes for soil temperature were installed at 0–20 cm deep.

2.4. Yield, Morphological Parameters, and Tomato Fruit Colorimetry

The tomato was harvested on July 23 on a 1.5 m^2 sample area per plot; the fruits were separated into marketable, not marketable (rotten), and green and then they were counted and weighed. Then, a sample of marketable fruits was oven-dried at 70 °C until reaching a constant weight, in order to determine the dry matter percentage.

At the harvest, on a sample of ten marketable fruits, the color space parameters (L*: brightness, ranging between 0 (black, no reflection) and 100 (white); a*, chroma parameter ranging between −60 (green) and +60 (red); b*, chroma parameter ranging between −60 (blue) and +60 (yellow)) were determined by a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan) by measuring two sides.

Finally, at the harvest, another sample of marketable fruits per each treatment and replicate was frozen at −80 °C and then lyophilized with a lyophilizer Crist, Alpha 1–4 (Osterode, Germany) for the determination of some qualitative characteristics, as reported in the two following paragraphs.

2.5. Lipophilic and Hydrophilic Antioxidant Activity and Total Ascorbic Acid Analysis

On 200 mg freeze-dried material, the lipophylic antioxidant activity (LAA) was determined spectrophotometrically (Hach DR 2000, Hach Co., Loveland, CO, USA) at 734 nm, after extraction with methanol, according to the method of Re et al. [37]. It was expressed as mmol of Trolox per 100 g of dry weight (dw).

As regards the hydrophilic antioxidant activity (HAA), the sample was extracted with distilled water; then, according to the N, N-dimethyl-p-phenylenediamine (DMPD) method [38], the HAA was assessed spectrophotometrically at 505 nm, and the values were expressed as mmol ascorbic acid 100 g^{-1} on dry weight (dw).

The total ascorbic acid (TAA) was measured by a spectrophotometer according to the procedure detailed by Kampfenkel et al. [39], and the solution absorbance was measured at 525 nm.

2.6. Carotenoid Analysis and Nitrate Content

Carotenoid content was spectrophotometrically assessed on 1 g of fresh fruit after the extraction with ammoniacal acetone according to the method stated by Wellburn [40], and it was express as mg g^{-1} fresh weight (fw).

Nitrate content was assessed on an oven-dried sample of marketable fruits according to the protocol of Sah [41]; the solution absorbance was measured at 550 nm, and the results were express as mg kg^{-1} (fw).

2.7. Total Soluble Solids Content and Firmness Determination

On fresh fruit juice, total soluble solid (TSS) content was measured by a digital refractometer (Sinergica Soluzioni, DBR35, Pescara, Italy), and it was expressed as °Brix.

Firmness was measured by a digital penetrometer (T.R. Turoni srl, Forlì, Italy) equipped with an 8 mm diameter probe; the measurements were performed on the two opposite sides of five fruits per replicate. Results were expressed in kg cm^{-2}.

2.8. Statistical Processing

All data were subjected to variance analysis (two-way ANOVA) using the SPSS 21 software package, version 22 (Chicago, IL, USA). The means were separated using Tukey's Test at $p \leq 0.05$.

3. Results

3.1. Soil Temperatures as Affected by Mulching Films

The minimum soil temperatures showed an increasing trend, and they always were higher than air temperature; as expected, the average temperature under the two mulching films was higher than that of bare soil: 19.9 vs. 18.6 °C, respectively (Figure 1A). Instead, the differences between the maximum temperatures of covered and not covered soil were greatly reduced from the second ten days of May; on average, the two biodegradable mulching films elicited only a 0.5 °C increase over the bare soil (Figure 1B).

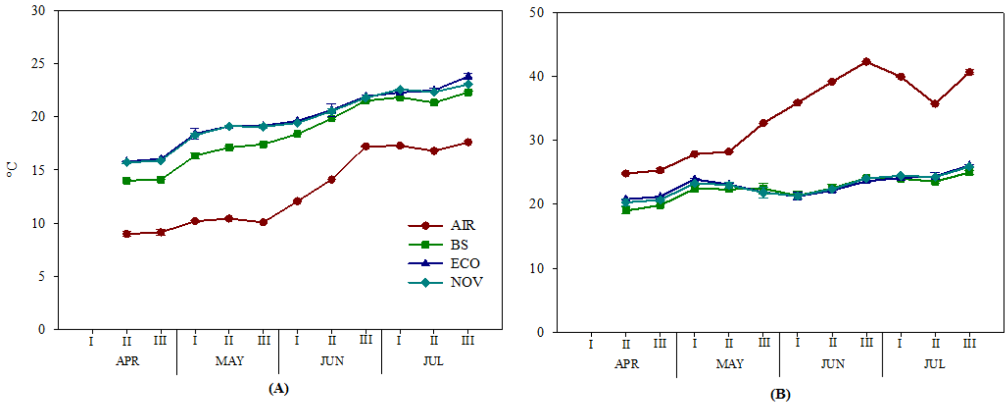

Figure 1. Pattern of the soil minimum (**A**) and maximum (**B**) average temperatures as affected by mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) compared to air temperature (Air). Vertical bars indicate standard error (n = 3).

3.2. Yield and Yield Components of Tomato Fruits

The marketable yield was significantly affected only by the main effect of the experimental factors (Figure 2). Notably, both biodegradable films were similar and elicited a 27.0% yield increase compared to plants grown on bare soil (Figure 2). Biostimulant application also boosted yield, irrespective of the type of biostimulant, with an average 23.7% yield increase over the control and an average yield of 135.0 t ha^{-1} (Figure 2). Specifically, microbial and plant biostimulants, when applied alone, increased yield by 23.7% and 22.5%, respectively, while the combined application resulted in a 24.8% yield increase as compared to untreated plants (Figure 2).

The M × B interaction did not affect any yield component, while mulching statistically affected only the number of marketable fruits per square meter, which was also influenced by the biostimulant application, as well as their average weight, production (t ha^{-1}), and number of green fruits (Table 2). Both mulching films elicited a greater number of marketable fruits (+22.2% increase) compared to BS treatment (Table 2). The treatments with biostimulants, alone or in combination, elicited an increase for all considered parameters, even if not always significantly different from the control. All tested biostimulants (Mic, Bio, M-B) significantly increased the number of marketable fruits as compared to the control without differences between formulations; conversely, for the average weight of marketable fruits, the combined application (M-B) was not different from the control, while Mic and Bio applications determined the highest values (Table 2). Finally, for the production and number per square meter of green fruits, only the microbial biostimulant (Mic) was different from the control, eliciting a two-fold increase; nevertheless, the three biostimulants were not different, and the mean increases of the other two biostimulants over the control were 29.9% and 29.1%, respectively (Table 2).

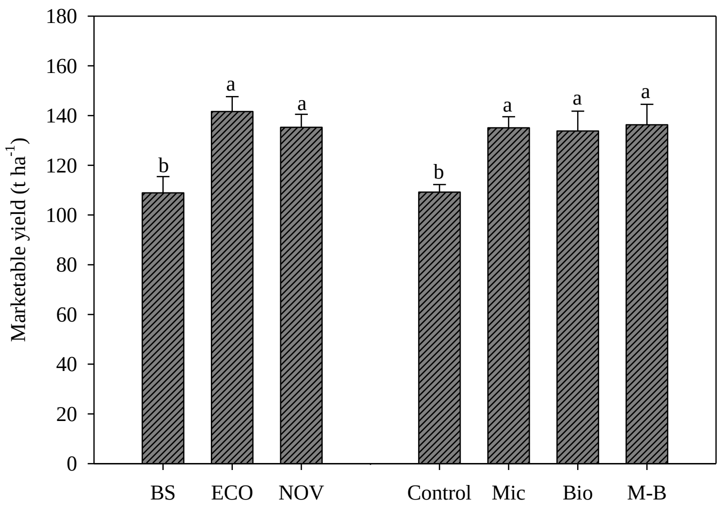

Figure 2. Tomato marketable yield as affected by mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Control: not treated). Each column indicates the mean value of the three replicates. Vertical bars indicate standard error; different letters indicate significant differences according to Tukey's test ($p < 0.05$).

Table 2. Number per square meter and average weight of marketable tomato fruits, and production, number per square meter, and average weight of green, and rotten tomato fruits as affected by mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Control: not treated).

Treatment	**Fruits**							
	Marketable			**Green**			**Rotten**	
	n m^{-2}	g fruit^{-1}	t ha^{-1}	n m^{-2}	g fruit^{-1}	t ha^{-1}	n m^{-2}	g fruit^{-1}
Mulching								
BS	258.3 ± 23.5 b	42.5 ± 1.6	24.1 ± 9.2	72.1 ± 23.4	31.0 ± 4.6	0.84 ± 0.58	2.5 ± 0.9	23.1 ± 10.6
ECO	320.4 ± 10.9 a	44.3 ± 2.3	30.2 ± 8.3	88.2 ± 21.2	32.9 ± 1.6	0.89 ± 0.50	3.0 ± 1.0	24.3 ± 7.5
NOV	311.0 ± 12.3 a	43.5 ± 1.1	26.9 ± 6.2	73.3 ± 14.7	35.8 ± 4.6	0.86 ± 0.24	3.0 ± 0.8	29.1 ± 6.3
Biostimulant								
Control	266.6 ± 14.9 b	41.2 ± 2.0 b	19.6 ± 6.8 b	57.8 ± 15.4 b	32.4 ± 3.4	0.65 ± 0.46	2.8 ± 0.9	17.4 ± 7.5
Mic	299.1 ± 11.5 a	45.3 ± 1.8 a	37.6 ± 12.4 a	104.4 ± 28.9 a	33.8 ± 4.1	0.68 ± 0.22	2.1 ± 0.8	31.1 ± 5.8
Bio	298.8 ± 22.2 a	45.0 ± 1.6 a	21.8 ± 5.4 ab	66.8 ± 15.8 ab	32.3 ± 2.3	1.46 ± 0.84	4.2 ± 1.1	29.0 ± 9.3
M-B	321.7 ± 13.5 a	42.3 ± 1.3 ab	29.2 ± 7.4 ab	82.5 ± 19.0 ab	34.5 ± 4.5	0.66 ± 0.23	2.2 ± 0.8	24.5 ± 9.9
Significance								
Mulching (M)	**	ns	ns	ns	ns	ns	ns	ns
Biostimulant (B)	**	*	*	*	ns	ns	ns	ns
M × B	ns	ns	ns	ns	ns	ns	ns	ns

ns, *, **: non-significant or significant at $p < 0.05$ and 0.01, respectively. Different letters within each column indicate significant differences according to Tukey's test at $p \leq 0.05$.

3.3. Color Parameters and Carotenoids of Tomato Fruits

All three color parameters were affected by the main effect of both experimental factors, while their interaction was never significant (Table 3). The brightness was significantly higher in ECO mulching, which determined a lower value of a* parameter (fruits were less red); BS reached higher values of a* and b* parameters, which were different from both mulching films (Table 3). As for the application of biostimulants, the use of *Trichoderma*, both alone and in combination with *Ascophyllum*, improved the brightness of tomato fruits (+9.4% over the mean value of Control and Bio) but had an opposite effect on a* and b*parameters, for which the higher values were reached by the control and Bio samples (Table 3).

Table 3. Color parameters (L*: brightness; a*: green intensity, chroma component ranging from green (–60) to red (+60); b*: chroma component ranging from blue (–60) to yellow (+60)) as affected by mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Control: not treated).

Treatments	L*	a*	b*
Mulching			
BS	25.7 ± 0.7 c	38.4 ± 0.7 a	44.3 ± 0.9 a
ECO	32.7 ± 1.2 a	32.6 ± 0.9 c	36.9 ± 2.3 b
NOV	29.7 ± 1.0 b	35.6 ± 1.0 b	39.6 ± 1.7 b
Biostimulant			
Control	27.7 ± 0.7 b	36.5 ± 0.9 a	42.9 ± 1.5 a
Mic	30.7 ± 1.1 a	34.1 ± 1.1 b	38.1 ± 1.7 b
Bio	28.4 ± 0.9 b	36.2 ± 0.7 a	43.2 ± 1.5 a
M-B	30.7 ± 1.0 a	35.4 ± 0.8 ab	36.9 ± 1.9 b
Significance			
Mulching (M)	**	**	**
Biostimulant (B)	**	*	**
M × B	ns	ns	ns

ns, *, **: non-significant or significant at $p < 0.05$ and 0.01, respectively. Different letters within each column indicate significant differences according to Tukey's test at $p \leq 0.05$.

Carotenoid content in tomato fruits was significantly affected by the interaction between the two experimental factors (mulching and biostimulants) (Figure 3). The trend was similar to that observed for the a* color parameter, which is indicative of the red color of the fruit; in fact, irrespective of mulching, on average, the higher values were recorded in fruits produced by untreated plants. In particular, the highest value was recorded in fruits from untreated plants grown on bare soil, but it was not different from the value of M-B (Figure 3).

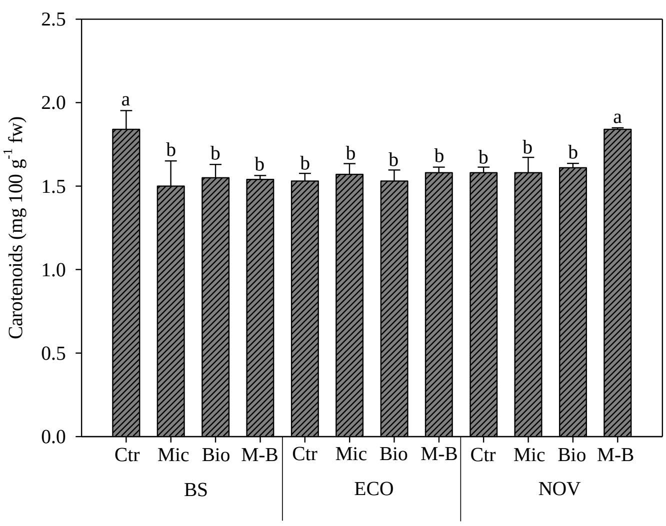

Figure 3. Carotenoid content of tomato fruits as affected by the interaction mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Ctr: not treated). Each column indicates the mean value of the three replicates. Vertical bars indicate standard error; different letters indicate significant differences according to Tukey's test ($p < 0.05$).

3.4. Total Soluble Solids Content and Firmness of Tomato Fruits

The mulching by biostimulant interaction significantly affected the total soluble solids (TSS) content and firmness of tomato fruits (Figures 4 and 5). As regards TSS content, all the applied biostimulant treatments combined with NOV reached higher values (averagely 4.9 °Brix), without differences between them. Interestingly, ECO mulch film reached the same level of NOV only when the microbial and vegetal biostimulant mix was applied (Figure 4). On the other hand, the lowest values were recorded for plants grown on bare soil (4.15 °Brix) and, in absence of mulching, only Bio and M-B treatments were able to significantly raise up TSS values (Figure 4). A similar trend was found for dry matter (DM) percentage, even if neither the main effect nor the interaction of factors was significant; the lowest value was recorded in BS (5.3%) while mulching reached higher values (5.6%, mean value) (data not reported). The biostimulants application elicited a 7.5% increase in DM percentage compared to the Control, except for the BIO treatment.

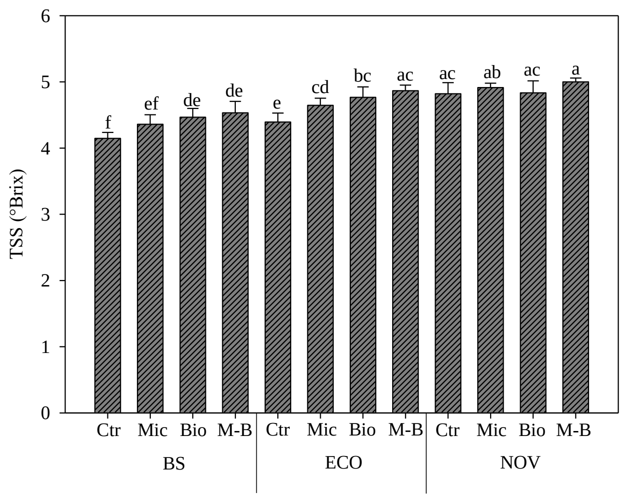

Figure 4. Total Soluble Solid (TSS) content of tomato fruits as affected by interaction mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Ctr: not treated). Each column indicates the mean value of the three replicates. Vertical bars indicate standard error; different letters indicate significant differences according to Tukey's test ($p < 0.05$).

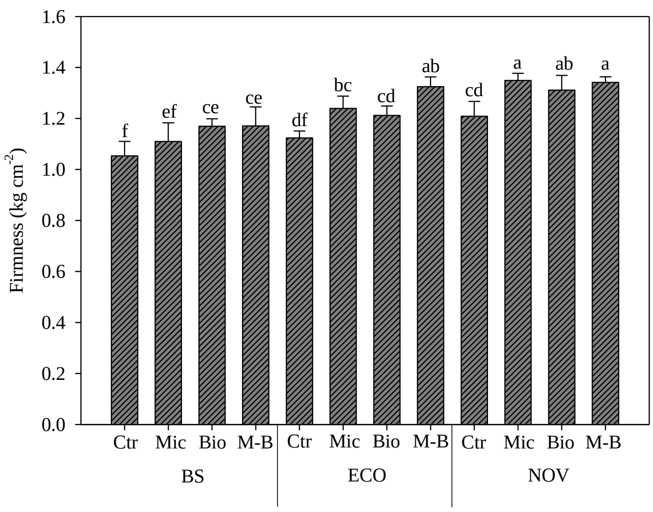

Figure 5. Firmness of tomato fruits as affected by the interaction mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Ctr: not treated). Each column indicates the mean value of the three replicates. Vertical bars indicate standard error; different letters indicate significant differences according to Tukey's test ($p < 0.05$).

The firmness, a parameter indicative of the maturation degree of tomato fruits and of their suitability for transformation, showed a similar trend as compared to TSS content (Figure 5). In fact, higher values were recorded in NOV treatments (1.30 kg cm^{-2}) and were increased by all biostimulant treatments (regardless of their formulation); ECO reached the same level only when M-B was applied (Figure 5). For this parameter, the plants grown on bare soil and not treated showed the lowest value of firmness, which was not different from the BS-Mic and ECO-Control (Figure 5). Generally, the biostimulants have always improved the firmness of tomato fruits compared to the corresponding Control plants as well as the mulching compared to bare soil (Figure 5).

3.5. Antioxidant Activity and Compounds in Tomato Fruits

The lipophilic antioxidant activity (LAA) and the content of ascorbic acid were affected by mulching, while the content of total phenols varied only according to the biostimulant application (Table 4), and the Mx B interaction significantly affected the hydrophilic antioxidant activity (HAA) (Figure 6). The highest values of HAA were recorded in plants grown on bare soil, with 13.92 vs. 11.95 and 9.17 mmol AA 100 g^{-1} dw of the NOV and ECO, respectively (Figure 6). No significant differences were recorded between all biostimulant treatments for the plants grown on BS, which were also not different from the control and Mic of the NOV and the M-B of the ECO treatment (Figure 6). The lowest values were found in the control, Bio, and Mic treatments of ECO, but this last one was not different from the Bio and M-B of NOV.

Table 4. Tomato plant lipophilic antioxidant activity (LAA), phenols, and total ascorbic acid content (AsA) at harvest in relation to mulching biofilms and biostimulant application.

Treatments	LAA	Phenols	AsA
	mM Trolox 100 g^{-1} dw	mg gallic acid g^{-1} dw	mg 100 g^{-1} fw
Mulching			
BS	5.52 ± 0.63 c	1.64 ± 0.09	21.20 ± 0.81 b
ECO	8.45 ± 0.71 b	1.74 ± 0.06	29.74 ± 1.52 a
NOV	32.92 ± 1.07 a	1.73 ± 0.07	29.06 ± 1.23 a
Biostimulant			
Control	14.03 ± 4.67	1.38 ± 0.06 b	26.21 ± 2.34
Mic	16.82 ± 4.44	1.83 ± 0.04 a	28.17 ± 2.32
Bio	16.73 ± 4.30	1.88 ± 0.07 a	27.11 ± 1.53
M-B	14.96 ± 4.30	1.74 ± 0.06 a	25.17 ± 1.39
Significance			
Mulching (M)	**	ns	**
Biostimulant (B)	ns	**	ns
M × B	ns	ns	ns

ns, **: non-significant or significant at $p < 0.01$. Different letters within each column indicate significant differences at $p \leq 0.05$.

Both mulching films elicited an increase in the LAA and total ascorbic acid content (AsA); however, for the LAA, all mulching treatments were significantly different, and the increase in NOV was about six and four times higher than the values observed in bare soil and ECO, respectively (Table 4). As regards the ascorbic acid, the NOV treatment was not different from ECO and, on average, they elicited a 38.7% increase over the control (Table 4).

Finally, all biostimulant treatments boosted the content of total phenols, with an average increase of 30% compared to the control plants (Table 4).

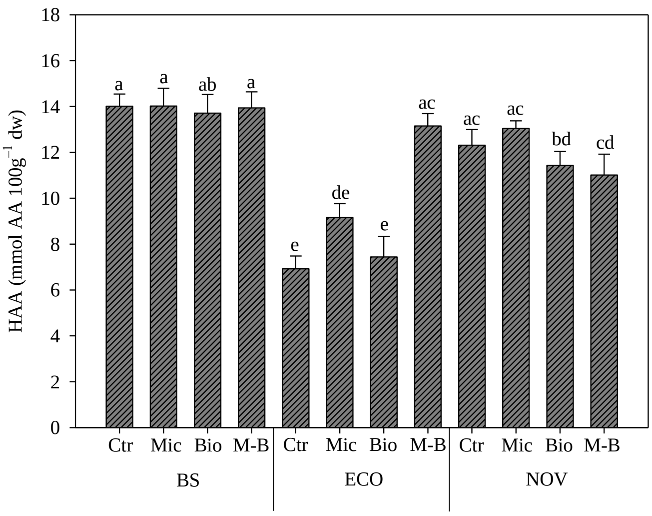

Figure 6. Hydrophilic antioxidant activity (HAA) in tomato fruits as affected by the interaction mulching (ECO: biodegradable film Ecovio; NOV: biodegradable film MaterBi®; BS: bare soil) and biostimulant application (Bio: treated with Phylgreen®; Mic: treated with Trianum-P®; M-B: combination of Bio and Mic; Control: not treated). Each column indicates the mean value of the three replicates. Vertical bars indicate standard error; different letters indicate significant differences according to Tukey's test ($p < 0.05$).

4. Discussion

Modern agriculture has multiple goals, among which one of the most important is to obtain high-quality and environmentally friendly products, obviously without decreasing yield. In this perspective, in recent years, the application of microbial biostimulants (e.g., *Trichoderma* spp.) has become increasingly widespread thanks to their positive effects on plant disease control [42,43], plant growth stimulation, and production of bioactive compounds with antioxidant activity [44–47].

The present research addressed improving the agro techniques of a processing tomato cultivar by assessing the effect of different biostimulants (vegetal-based, microbial-based, or a mix of both) in combination with biodegradable mulching films on agronomic crop response (yield and some qualitative traits of fruits).

It is well known that mulching elicits a yield increase thanks to the increase in soil temperature, the preservation of humidity, the reduction of weeds and pests, and a more efficient use of soil nutrients [48,49]. Although the black PE plastic films are still the most used in agriculture, they require about 100 years to degrade [50]; thus, they are often replaced by biodegradable films, thanks to their characteristic to overcome the removal and disposal problems typical of traditional PE films [51]. Our results highlighted a 27.0% increase in the yield of tomato plants grown on both types of biodegradable films compared to the production obtained on bare soil (BS). In particular, the greater marketable yield is mainly due to a greater number of ripe fruits at the harvest (+22.2% compared to BS), while no significant increase in the average weight of tomato fruits was recorded. On the other hand, many previous studies have already confirmed that, similarly to PE, biodegradable films are equally able to increase crop yield and/or quality of products (tomato [52,53]; lettuce [18,54]; zucchini [19]; melon [55]; strawberry [56]; and other vegetable crops [7,57]).

We assume that the increase elicited by mulching films was due primarily to the increase in soil temperature and presumably also to the greater water availability. In fact, both films increased soil minimum temperature in the top layer, at 1.3 °C higher than in bare soil; otherwise, the maximum temperatures showed less marked differences (+0.5 °C). Our findings are consistent with those reported by Acharya et al. [16], which highlighted that organic mulches improve the soil temperature, particularly in the night and early morning hours.

As expected, the application of biostimulants boosted yield compared to the untreated control by about 23.7%, with the best performance recorded for the microbial biostimulant (the commercial strain T22 of *Trichoderma afroharzianum*) used both alone (+23.7%) or in combination with the vegetable biostimulant Phylgreen®, based on pure algae extracts (*Ascophyllum nodosum*) (+24.8%). Our data agree with the results of Caruso et al. [58] and Fiorentino et al. [59] on leafy vegetables. Di Mola et al. [33], in lettuce treated with a microbial biostimulant containing *Trichoderma* spp., did not find a positive effect on yield, suggesting that the response to *Trichoderma*-based products could be species-specific and strongly linked to the duration of crop cycles. Indeed, longer cycles could give more time to the fungi for better establishing in the soil/roots complex, boosting the colonies and thus their activity. The beneficial effects of *Trichoderma* spp. on different crops are well known and include increase in root development, leaf area, yield, as well as the induction of plant defense mechanisms against numerous pathogens (systemic resistance induction, ISR) [60]. Specifically, in our research, the increase in yield was due to the increase in both the number and the average weight of marketable fruits compared to the untreated plants: +15.0%, and +7.3%, respectively. Recent studies have highlighted the involvement of secondary metabolites produced by these beneficial fungi in interactions with plants [61]. Vinale et al. [62] showed that secondary metabolites isolated by *T. harzianum* M10 and *T. atroviride* P1 (harzianic acid and 6 pentyl-α-pyrone, respectively) significantly stimulate tomato growth. However, the effectiveness of treatments with mixtures based on microorganisms is certainly conditioned by environmental parameters that limit their applicability [63–65] and therefore by abiotic factors, but also by interaction with the microorganisms already naturally present in the soil (biotic factors). As regards the specific effect of the *Ascophyllum nodosum*-based biostimulant (Phylgreen®), Fleming et al. [66] demonstrated how this product has a growth-promoting effect on *Arabidopsis thaliana* 7 days after the first treatment, even in conditions of a major drought, both in single applications and in combination with the microbial biostimulant, similar to our findings.

Interestingly, all three biostimulants elicited an increase in production and number per square meter of green fruits; this result gives us good reason to assume that these bio-formulates are also able to increase the productive potentiality of our processing tomato cultivar both in terms of quantity and duration of productive phase (more green fruits).

The quality parameters of the tested processing tomato were affected by the experimental factors; in particular, we found that fruits obtained from plants grown on bare soil and not treated with biostimulants showed a lower content of soluble solids (TSS) than those grown on soil covered by biodegradable films, irrespective of the type, and treated with biostimulants, mainly with the mix M-B. Acharya et al. [16] report that organic mulches improve soil chemical properties due to the addition of organic matter and plant nutrients to the soil upon degradation and also thanks to the ability of mulching to reduce nitrogen losses by volatilization and leaching. Therefore, we suggest that, under mulches, there was a greater availability of nutrients, mainly due to the reduced losses, which probably caused a greater accumulation of TSS. In addition, Ali et al. [67] on tomato plants treated with an *A. nodosum* seaweed extract at 0.2% and 0.5%, found that the total soluble solids were higher in treated plants, and they increased with the increase of the seaweed concentration, showing values similar to ours (4.72, mean value of the two treatments, and 4.69, mean value of Bio treatments). However, the literature also reports different results than ours; in particular, Carillo et al. [29], in tomato fruits, did not find a significant effect of *Trichoderma harzianum* T22 on total soluble solids, while Ruiz-Cisneros et al. [68] found that tomato treated with *T. harzianum* showed lower values of TSS.

Among the quality parameters, firmness is an important index of determining maturity and suitable postharvest processing quality, and its variations during the maturation process involve changes in the chemical composition of the cell [69]. For this parameter, we recorded behavior similar to that of TSS; in fact, the higher values were recorded in tomato fruits of plants grown on NOV film and treated with biostimulants, among which the microbial one used alone or mixed showed the best performance.

Our data confirm the results reported in the research carried out by Ruiz-Cisneros et al. [68], in which the application of *T. harzianum* increased the consistency of fruits by 1.2 times compared to that of the untreated plants. This increase can be attributed to the induction of the biosynthesis of phytohormones following the activation of the defense mechanisms of the plant, with particular reference to ethylene, a chemical compound responsible for the expression of plant maturity [70]. Our findings are also consistent with the results of Ali et al. [67], which reported that the values of firmness were higher (about +34% compared to control plants) in plants treated with *A. nodosum* extracts. Cozzolino et al. [71], on tomato plants treated with three different biostimulants (seaweed extracts, legume-derived protein hydrolizate, and tropical plant extracts), found a significant increase in firmness as well as in total soluble solids.

The color of the tomato fruit is the most important external characteristic in evaluating ripeness and is an important discriminant both for consumers and for industries. During maturation, important changes occur in the content of chlorophyll (green), lycopene (red), and beta-carotene (orange) of the fruit [72], and various stages of development can be differentiated according to the external color [73–75]. In our study, higher values of brightness (L*) were found in tomatoes grown on covered soil (in particular ECO), while the red intensity was higher in fruits obtained on bare soils (high values of a* parameter). This may be due to the fact that black films, like those used in these experiments, reflect less sunlight than white or bare ground [76], thus causing less production of colored pigments in the fruits. On the other hand, for carotenoids, which include lycopene and beta-carotene, responsible for the red/orange color of fruits, we found higher values in fruits of plants grown on bare soil and not treated, but not different from the values of fruits obtained on NOV film and treated with M-B. In the literature, the results regarding the effects of biostimulants on color parameters are contrasting; in fact, Ruiz-Cisneros et al. [68] reported that the tested microorganisms, including *T. harzianum*, did not modify the color of tomato fruits; Ali et al. [67] found that the brightness of tomato fruits treated with *A. nodosum* extracts decreased while a* and b* parameters increased.

In addition to β-carotene and lycopene, the tomato is generally rich in numerous other compounds important for nutrition and human health, including, flavonoids, organic acids, phenolics, as well as ascorbic acid and chlorophyll [77], compounds known for their antioxidant properties. It has been observed that β carotene participates in the processes of prevention and neutralization of free radical reactions, and ascorbic acid helps to eliminate reactive oxygen compounds, such as superoxide, hydrogen peroxide, and singlet oxygen, involved in oxidation reactions [78,79]. The antioxidant activity of these molecules is one of the most important features in determining the nutritional quality of many foods, including tomatoes. Compounds with these properties, such as ascorbic acid and phenols, have beneficial effects on human health because they play a key role in delaying the damage caused by oxidative processes and therefore prevent various pathologies related to them [80,81]. The antioxidant activity can be distinguished as lipophilic and hydrophilic, depending on the nature of the antioxidant compounds examined; in tomato fruits, it mainly depends on the total content of polyphenols and lycopene [82]. In our research, mulching positively influenced the lipophilic antioxidant activity (LAA) as well as the content of ascorbic acid, with the higher values recorded in fruits of plants grown on NOV film (about six times and 37.0% more than BS values, respectively). Contrarily, higher values of hydrophilic antioxidant activity (HAA) were recorded in fruits of plants grown on bare soils, irrespective of the biostimulant application; however, this was not different from the NOV-Control and NOV-Mic.

Previous works on tomato [83,84] reported a greater amount of ascorbic acid in the fruits obtained from plants grown on covered soil than the bare soil; it can therefore be assumed that the solar radiation [85,86] and the increased availability of water [87], due to the mulching films, are able to ensure a greater accumulation of ascorbic acid.

Finally, the application of biostimulants, both alone and in combination, led to a significant increase (>30%) in the total phenol content in treated berries compared to the control. Cozzolino et al. [71], in tomato, did not find a significant effect of biostimulant treatments (seaweed extracts, legume-derived protein hydrolysate, and tropical plant extracts) on the content of phenols in tomato fruits; similarly, Di Mola et al. [33], in lettuce, did not find a significant effect of treatment with *Trichoderma* on phenol content, but they reported a positive effect of the interaction of black polyethylene and treatment with *Trichoderma* both on LAA and HAA.

5. Conclusions

The findings of our research indicate that the application of biostimulants, based on either *Trichoderma afroharzianum* or *Ascophyllum nodosum*, improved the marketable yield of the processing tomato and also increased the yield of green fruits, thus suggesting an improved productive potentiality. Moreover, they also enhanced the phenol content.

As regards the use of biodegradable mulching films, our results confirmed, as already reported in the literature for other crops, their ability to increase yield, for both the Materbi® and Ecovio films; furthermore, they also improved the lipophilic antioxidant activity and ascorbic acid content of tomato fruits.

Finally, interestingly, we highlighted a combined effect of both experimental factors (mulches and biostimulants) on two very important parameters for processing tomatoes, firmness and total soluble solids, which were increased in plants grown on mulched soils and treated with biostimulants.

Therefore, both agricultural practices seem to be effective eco-sustainable tools for improving yield and quality of the processing tomato, and their combined effects on some quality traits should be further developed in future research and applied to other crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030901/s1, Figure S1. Layout of the experimental design.

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