



Article

Biostimulant Effects of Algae Species, Arbuscular Mycorrhizal Fungi, and Their Combinations on Yield and Quality of Yellow Tomato Landrace Under Different Crop Cycles

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Abstract: Recent agricultural research has prioritized the development of environmentally friendly management strategies to ensure food security, among which the application of biostimulants such as brown algae extracts, arbuscular mycorrhizal fungi (AMF), and their combination are included. The experimental protocol was based on the factorial combination of two planting times (4 May and 1 June) and seven biostimulant treatments (three brown algae species, *Cystoseria tamariscifolia*—C.t.; *Fucus vesiculosus*—F.v.; *Padina pavonica*—P.p.; arbuscular mycorrhizal fungi—AMF; C.t. + AMF; F.v. + AMF; P.p. + AMF) plus an untreated control. The earlier transplant resulted in a higher yield, due to the higher number of fruits per plant, and a higher plant fresh and dry biomass. The treatments with P.p. and F.v. extracts and the combination P.p. + AMF led to the highest yields (56.7 t ha^{−1}), mainly due to the highest fruit number per plant. The earlier planting time led to higher values of dry residue, soluble solids, firmness, and colour component 'a'. The highest values of fruit dry residue were recorded under the F.v. and P.p. extracts, and the combinations F.v. + AMF and P.p. + AMF, the highest soluble solid content with P.p. treatment, and firmness under P.p. + AMF. The highest levels of 'L' and 'a' fruit colour components were obtained under the P.p. extract treatment, of 'b' upon the application of P.p. and F.v. extract, and AMF + P.p. and AMF + F.v. The later planting time led to significantly higher values of the antioxidant parameters, as did the application of the P.p. extract and P.p. + AMF. CAT activity was more intense corresponding to the later tomato crop cycle, P.p. extract, and AMF + P.p. Overall, our study highlights the potential of biostimulants, particularly brown algae extracts and their combination with AMF, to improve tomato yield, antioxidant properties, and biochemical activities.

Keywords: sustainable agriculture; local variety; biostimulants; brown algae extracts; arbuscular mycorrhizal fungi (AMF); fruit colour; antioxidants; catalase activity

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to Solanaceae family, and it is very widespread worldwide due to the remarkable nutritional value associated with the high content of antioxidants such as lycopene, β -carotene, vitamin C, polyphenols [1], and essential trace elements such as copper, manganese, and zinc, which are cofactors of antioxidant enzymes [2,3].

Among the several tomato types, the yellow fruit one is fairly popular, showing distinct quality characteristics also associated with its specific colour [4]. Within the frame of sustainable farming strategies, the application of biostimulants has become increasingly interesting, either as beneficial substances or microorganisms enhancing plant nutrient uptake and metabolism, product quality, and tolerance to stresses [5–8].

Among the main biostimulants, brown algae are rich in nutrients, vitamins, and fatty acids different from those of other plant species [9]. Similarly, arbuscular mycorrhizal fungi (AMF) have shown beneficial effects on plants by reducing dependence on mineral inputs such as fertilizers and pesticides, improving mineral nutrition and stress tolerance in plants [10].

The brown macroalgae belonging to the species *Padina pavonica* are well known for their biostimulant properties, as they encourage plant development by enhancing nutrient absorption and stress tolerance [11]. Indeed, their extracts contain bioactive constituents, such as polysaccharides, particularly algal acid [12], and polyphenols, contributing to boost germination rates, root growth, plant vigor, and yield [11].

The biostimulant effects of the brown seaweed *Fucus vesiculosus* relate to the presence of plant hormones, such as auxins and cytokinins, fucoidans, alginates, and other bioactive substances that improve plant development and stress tolerance [13]. It has been demonstrated that *Fucus vesiculosus* extracts enhance nitrogen absorption and boost plant biomass. In this respect, the application of *Fucus vesiculosus* extracts boosted *Lepidium sativum* (garden cress) development with significant increases in plant length and nutritional content [14].

The effectiveness of *Cystoseira tamariscifolia* extracts as a biostimulant is attributed to their high content of trace elements [15] and amino acids, the increase in phenolic content, antioxidant activity, chlorophyll fluorescence, photosynthetic efficiency, and stress tolerance, which make the plants more resilient to environmental stresses, improve nutrition efficiency, and boost phenological development [16].

Aiming to mitigate the environmental impact of crop management, research was carried out to assess the effects of biostimulants, i.e., extracts from three brown algae species, *Padina pavonica* (P.p.), *Fucus vesiculosus* (F.v.), and *Cystoseira tamariscifolia* (C.t.), as well as in combination with arbuscular mycorrhizal fungi (AMF), on the yield, antioxidant status, and biochemical activities of a tomato landrace in southern Italy.

2. Materials and Methods

2.1. Growing Conditions and Experimental Protocol

Research was carried out on the tomato (*Solanum lycopersicum* L.) landrace ‘Giallo a pera’ grown in an open field at the Department of Agricultural Sciences of the University of Naples Federico II, in 2022 and 2023, in a sandy-loam soil, with 76% sand, 17% silt, and 7% clay, a soil electrical conductivity of $340 \mu\text{S cm}^{-1}$, 1.65% organic matter, $1.1 \text{ g kg}^{-1} \text{ N}$, 169 mg kg^{-1} available phosphorus (Olsen method), $1.8 \text{ g kg}^{-1} \text{ K}_2\text{O}$, a soil exchange capacity of $18.2 \text{ meq } 100 \text{ g}^{-1}$, a soil organic carbon content of 19.7 g kg^{-1} , and a pH of 6.9. The average monthly temperatures and rainfall during the research seasons, in 2022 and 2023, measured at the Meteorological Station located at the Department of Agricultural Sciences, University of Naples Federico II, Portici (Naples), are shown in Figure 1.

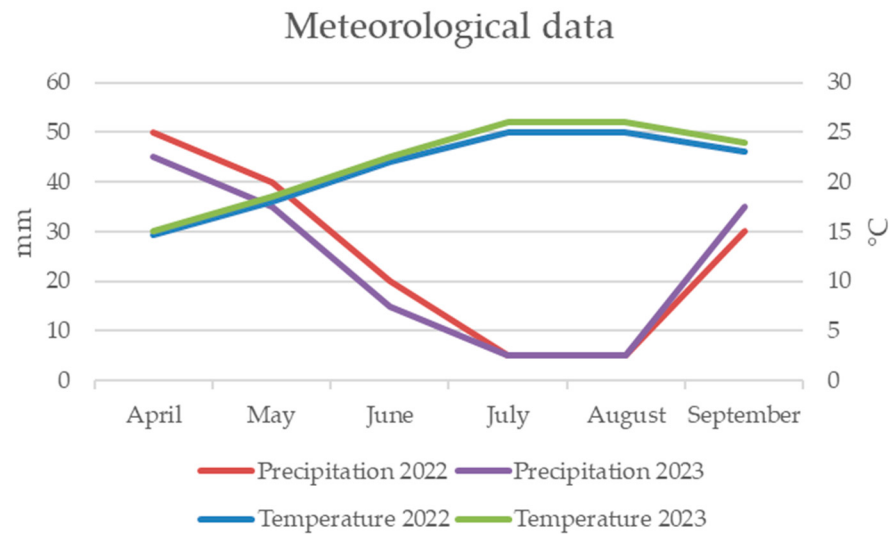


Figure 1. Meteorological data measured during the trial period (2022 and 2023).

The experimental protocol was based on the factorial combination of two planting times (4 May, 1 June) and seven biostimulant treatments (algal extract from *Fucus vesiculosus*—F.v.; algal extract from *Padina pavonica*—P.p.; algal extract from *Cystoseria tamariscifolia*—C.t.; arbuscular mycorrhizal fungi—AMF; *Fucus vesiculosus* + arbuscular mycorrhizal fungi—F.v. + AMF; *Padina pavonica* + arbuscular mycorrhizal fungi—P.p. + AMF; *Cystoseria tamariscifolia* + arbuscular mycorrhizal fungi—C.t. + AMF), plus an untreated control (Ctr), as shown in Figure 2.

For the treatment distribution in the field, a split-plot design was used, assigning the main plots to the planting time and the sub-plots to the biostimulant treatments, with three replications. Each plot had a 5 m² surface area and contained 12 plants.

Transplant of tomato seedlings was practiced according to 35 cm spacing along the rows which were 120 cm apart. The harvests were performed in the first ten days of August, corresponding to the first planting time, and in the last week of August and first three days of September for the later transplant.

The AMF were applied at the transplant by fertigation at the dose of 0.17 g m⁻², using a commercial consortium (MycoApply DR, Sumitomo Chemical Italia, Milan, Italy) consisting of fungi (*Glomus intraradices*, *Glomus aggregatum*, *Glomus mosseae*, *Glomus etunicatum*) at 1% overall concentration, and rhizosphere bacteria (*Bacillus subtilis*, *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Rhizobium* spp.) at 2,180,000 CFU g⁻¹.

The algae samples were collected manually at the fishing port of Menzel Abdrahmen in Bizerte. They were washed in seawater to remove any debris adhering to their thallus, and then placed in airtight plastic bags containing seawater. In the laboratory, the samples were rinsed again with tap water and then with distilled water to remove any particle influencing the assessment of biological activity. The algae were then placed in the dark at room temperature until complete dehydration. After drying, the samples were ground, and the powder obtained was stored in glass vials until further use.

For the water extraction, one gram of finely ground raw material was added with 10 mL distilled water, and then the mixture was placed in a stirrer at room temperature, filtered under pressure after 24 h and, finally, stored in a dark place until use. A solution of distilled water with 4% diluted seaweed powder was used to prepare the biostimulant solution, applied every two weeks to the single plants by root application (2 L of solution).

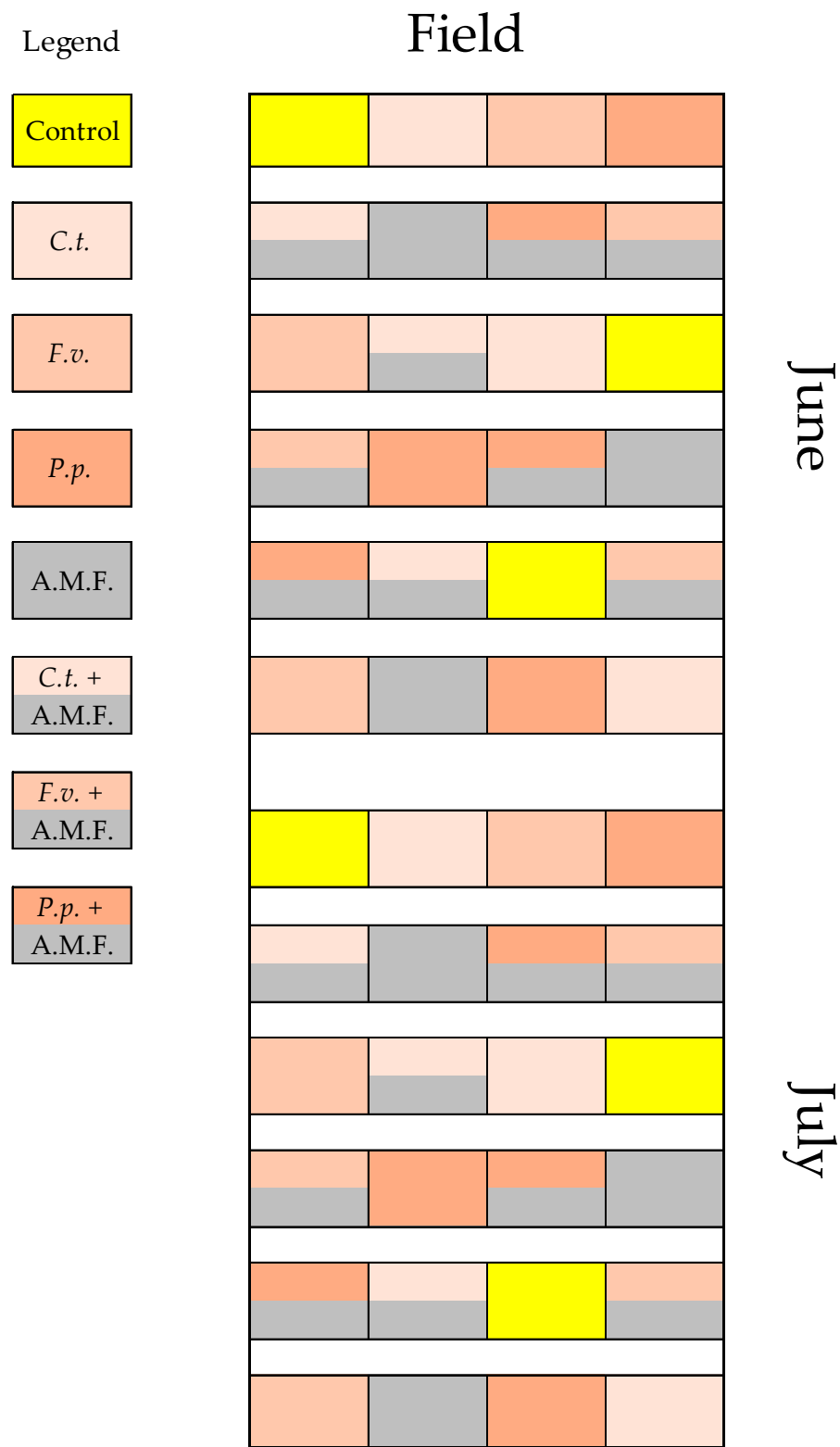


Figure 2. Experimental design applied for the distribution of the treatments in the field. Legend: Control: untreated; C.t.: *Cystoseria tamariscifolia*; F.v.: *Fucus vesiculosus*; P.p.: *Padina pavonica*; AMF: arbuscular mycorrhizal fungi.

2.2. Yield and Quality Determinations

At harvest, performed when the fruits were ripe, random samples of 50 fruits per plot were collected to determine yield, fruit number and mean weight, fruit quality (i.e., soluble

solids, firmness, colour components), and antioxidants (i.e., vitamin C and polyphenol content, antioxidant activity). From each of the mentioned samples, 5 sub-samples were used for oxidative stress analyses (i.e., Glutathione peroxidase—GPOX; Catalase—CAT).

Moreover, five plants per plot were randomly selected to measure their fresh weight, as well as the dry weight, after staying in a forced-air oven at 60 °C until constant weight.

The total soluble solids (TSSs) content was assessed using a portable digital refractometer, model DBR 35 (Sinergica Soluzioni s.r.l., Pescara, Italy), and expressed in °Brix.

Firmness was measured on both sides of the equatorial zone of five fruits per replication, using a digital penetrometer (T.R. Turoni s.r.l., Forlì, Italy) fitted with an 8 mm tip. The force applied for a 4 mm penetration was expressed in kg m^{−2}.

Colour measurements were made using a Minolta CR-300 colorimeter (Minolta Camera Co., Ltd., Osaka, Japan), referring to the CIELAB colour space including the components L*, a*, and b*.

2.3. Antioxidant Determinations

At harvest, fresh tomato fruit samples were collected in each plot and immediately analyzed to determine total ascorbic acid (TAA). Another fruit sample per plot was freeze-dried and stored at −80 °C, to measure hydrophilic antioxidant activity (HAA), lipophilic antioxidant activity (LAA), and total polyphenols. Spectrophotometric methods were used to determine TAA, HAA, and LAA, measuring the solution absorbance at 505 and 734 nm for HAA and LAA, respectively [17,18], and the total phenolic content at 765 nm [19].

2.4. Biochemical Determinations

The catalase activity was determined spectrophotometrically [20], measuring the absorbance decrease with a Jenway 6300 spectrophotometer (London, UK) at a 240 nm optical wavelength and a molecular extinction coefficient of $\epsilon = 39,400 \text{ M}^{-1} \text{ L cm}^{-1}$. The reaction mixture contained 100 mL crude enzyme extract, 50 mL 0.3% hydrogen peroxide H₂O₂, and 2850 mL phosphate buffer (50 mM, pH = 7.2), to achieve a final 3 mL volume.

Guaiacol peroxidase (GPOX) activity was determined using a spectrophotometer at 470 nm [21]. The linear molar extinction coefficient $\epsilon = 26.6 \text{ mM cm}^{-1}$ was used. To reach a final 3 mL volume, the reaction mixture contained 100 µL of enzyme extract, 2700 µL phosphate buffer (100 mM, pH 6.5), 100 µL guaiacol (18 mM), and 100 µL H₂O₂. The GPOX activity is expressed in µmoles oxidized min^{−1} g^{−1} MF, calculated by the following formula:

$$\text{Act} = (\Delta A \times Vt) / (\epsilon \times \Delta t \times L \times Ve \times P)$$

Act: enzymatic activity in moles min^{−1} mg^{−1} protein.

ϵ : molar linear extinction coefficient in M.

ΔA : mean difference in absorbance.

2.5. Statistical Analysis

The data were processed by two-way analysis of variance (ANOVA), and the mean separations were performed using the Duncan's multiple range test, with reference to the 0.05 probability level, using the SPSS software version 29. The data expressed as percentages were subjected to angular transformation before processing.

3. Results and Discussion

3.1. Yield Parameters

In the present study, no significant differences arose between the two research years and, therefore, only the average results of 2022 and 2023 related to the examined variables are presented. Furthermore, no interactions were recorded between the two factors (planting time and biostimulant) applied, so that only the main effects of the applied experimental factors are presented.

As shown in Table 1, the earlier transplant resulted in a higher yield, due to the higher number of fruits per plant, and the higher plant fresh and dry biomass. As for biostimulant

comparison, the highest yields were recorded under the treatments with P.p. and F.v. extracts and the combination P.p. + AMF (56.7 t ha^{-1}), mainly due to the highest fruit number per plant. The control showed the lowest mean weight of tomato fruits, whereas no significant differences arose between the biostimulant treatments (Table 1). The P.p. extract and the AMF + P.p. combination gave the highest fresh and dry biomass of tomato plants, 1.6 and 1.9 times higher than the untreated control on average, respectively (Table 1).

Table 1. Effects of planting time and biostimulant type on tomato yield and growth parameters.

Treatment	Yield t ha^{-1}	Mean Fruit Weight (g)	No. Fruits Per Plant	Plant Fresh Biomass (g)	Plant Dry Biomass (g)
Planting time					
4 May	56.5 ± 2.3	89.3 ± 2.5	21.1 ± 1.2	1156.8 ± 45.7	78.2 ± 3.1
1 June	43.7 ± 1.9	89.0 ± 2.4	16.4 ± 1.0	734.4 ± 35.2	40.4 ± 2.0
	*	n.s.	*	*	*
Biostimulant					
Control	$42.7 \pm 1.8 \text{ c}$	$84.0 \pm 2.0 \text{ b}$	$17.0 \pm 0.8 \text{ c}$	$774.8 \pm 30.9 \text{ d}$	$44.6 \pm 1.9 \text{ d}$
C.t.	$47.6 \pm 2.1 \text{ b}$	$89.8 \pm 2.1 \text{ a}$	$17.7 \pm 0.9 \text{ c}$	$882.9 \pm 33.2 \text{ c}$	$59.2 \pm 2.4 \text{ c}$
F.v.	$55.3 \pm 2.3 \text{ a}$	$90.0 \pm 2.2 \text{ a}$	$20.5 \pm 1.0 \text{ a}$	$981.5 \pm 38.4 \text{ b}$	$57.5 \pm 2.3 \text{ b}$
P.p.	$57.4 \pm 2.4 \text{ a}$	$94.4 \pm 2.3 \text{ a}$	$20.3 \pm 1.0 \text{ a}$	$1211.0 \pm 45.1 \text{ a}$	$89.4 \pm 3.6 \text{ a}$
AMF	$49.2 \pm 2.0 \text{ b}$	$88.2 \pm 2.1 \text{ ab}$	$18.6 \pm 0.9 \text{ bc}$	$852.0 \pm 32.1 \text{ cd}$	$46.9 \pm 2.0 \text{ cd}$
AMF + C.t.	$50.7 \pm 2.2 \text{ b}$	$91.6 \pm 2.2 \text{ a}$	$18.5 \pm 0.9 \text{ bc}$	$877.3 \pm 33.7 \text{ c}$	$53.1 \pm 2.3 \text{ c}$
AMF + F.v.	$54.0 \pm 2.3 \text{ ab}$	$89.7 \pm 2.2 \text{ a}$	$20.1 \pm 1.0 \text{ ab}$	$967.7 \pm 38.0 \text{ b}$	$67.1 \pm 2.7 \text{ b}$
AMF + P.p.	$57.3 \pm 2.4 \text{ a}$	$93.5 \pm 2.3 \text{ a}$	$20.4 \pm 1.0 \text{ a}$	$1217.6 \pm 45.3 \text{ a}$	$80.0 \pm 3.2 \text{ a}$
Source of variance					
Planting time (P)	*	n.s.	*	*	*
Biostimulant (B)	*	*	*	*	*
P $\times$ B	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.: not significant; *: significant at $p < 0.05$. Within each column, values with the same letters do not significantly differ according to Duncan's multiple range test at $p < 0.05$. Values followed by $\pm$ sign are the standard errors of the mean.

As reported in the study conducted by Snapp [22] on tomato crop for fresh consumption, an early transplant could result in a 140% higher root volume increase, compared with a later transplant, with the consequence of a more efficient uptake of mineral elements, leading to higher fruit yield due to the higher number of fruits.

The use of biostimulants derived from algae can improve crop productivity, decrease reliance upon synthetic fertilizers, and reduce the ecological impact of agriculture [23]. Biostimulants enhance soil health by stimulating beneficial microbial activity, optimizing nutrient cycling, and improving soil structure [24], thus encouraging both the quantity and quality of crop production, resulting in increased profits for growers [25]. Additionally, biostimulants derived from algae improve the efficiency of nutrient use and help plants cope with abiotic stressors such as drought and salt [26].

Plant biostimulants represent an effective tool to augment production [5,27,28]. Previous research reported that signaling molecules, including polysaccharides, soluble peptides, oligopeptides, and free amino acids representing between 30 and 40% of the total content of commercial algae extracts, could contribute to enhancing tomato plant productivity [29,30]. The mentioned substances support the endogenous production of phytohormones and control plant growth and development [31]. Notably, by promoting endogenous homeostasis, polysaccharide-rich algae extracts present in biostimulants enhance plant productivity [32].

In this study, tomato yield was significantly affected by the type of treatment (algae extract, arbuscular mycorrhizal fungi, or their combination) and, particularly, P.p. and F.v. elicited a higher yield than the combination AMF + P.p. Differently from AMF, receiving photosynthetic carbohydrates from host plant roots upon supplying them with nutrients and water through their extensive hyphal network [33,34], algal extract provides growth-critical nutrients, such as polysaccharides, amino acids, vitamins, phytohormones,

and antioxidants, stimulating plant growth and increasing yield and quality [35]. In this respect, the combination of algae extracts and mycorrhizae may result in synergistic effects. El Chami et al. [36] found that the use of seaweed extracts in pear production significantly reduced total fertilization by 13% and improved quantitative and qualitative fruit performances.

Some studies have highlighted the beneficial effect of the application of brown algae extract as a biostimulant on different crops, like the work conducted by Hernandez-Herrera et al. [37] where seaweed extracts, including *Padina* sp., significantly enhanced the germination, plant height, and root length of tomato plants. Moreover, in the research conducted by Cozzolino et al. [38], the application of *Ecklonia maxima* improved the marketable yield, nitrogen use efficiency, and the overall quality of tomato fruits.

3.2. Quality Parameters

The earlier planting time led to higher values of dry residue, soluble solids, firmness, and colour component 'a*' (green–magenta axis). The biostimulant application significantly increased the dry residue in tomato fruits (Table 2) in most cases, with the highest values recorded under the F.v. and P.p. extracts as well as the combinations F.v. + AMF and P.p. + AMF, 13.8% higher than the untreated control. The soluble solids content was the highest for P.p. treatment (11.9% higher than the control). The highest firmness value was recorded in tomato fruits treated with the combination P.p. + AMF (19.3% higher than the untreated control; Table 2).

Table 2. Effects of planting time and biostimulant type on quality parameters of tomato fruits.

Treatment	Dry Residue (%)	Soluble Solids (°Brix)	Firmness (kg)	L*	a*	b*
Planting time						
4 May	4.8 ± 0.2	4.2 ± 0.1	1.27 ± 0.05	58.0 ± 1.5	−5.8 ± 0.3	33.9 ± 0.8
1 June	5.3 ± 0.2	4.6 ± 0.2	1.39 ± 0.06	57.5 ± 1.4	−4.7 ± 0.3	34.2 ± 0.8
	*	*	*	n.s.	*	n.s.
Biostimulant						
Control	4.7 ± 0.2 c	4.2 ± 0.1 c	1.19 ± 0.05 c	55.6 ± 1.4 c	−7.4 ± 0.4 f	31.4 ± 0.8 b
C.t.	4.8 ± 0.2 bc	4.4 ± 0.2 bc	1.24 ± 0.05 bc	57.6 ± 1.5 bc	−6.3 ± 0.3 e	33.5 ± 0.8 ab
F.v.	5.3 ± 0.2 a	4.6 ± 0.2 ab	1.33 ± 0.06 ab	59.0 ± 1.5 bc	−5.0 ± 0.3 c	34.6 ± 0.9 a
P.p.	5.4 ± 0.2 a	4.7 ± 0.2 a	1.40 ± 0.06 ab	61.5 ± 1.6 a	−3.0 ± 0.2 a	35.2 ± 0.9 a
AMF	4.9 ± 0.2 bc	4.4 ± 0.2 bc	1.27 ± 0.05 bc	58.7 ± 1.5 bc	−5.6 ± 0.3 d	33.9 ± 0.8 ab
AMF + C.t.	5.0 ± 0.2 b	4.4 ± 0.2 bc	1.29 ± 0.05 bc	58.0 ± 1.5 bc	−6.1 ± 0.3 e	33.9 ± 0.8 ab
AMF + F.v.	5.4 ± 0.2 a	4.5 ± 0.2 ab	1.32 ± 0.06 ab	59.2 ± 1.5 bc	−5.3 ± 0.3 c	34.3 ± 0.9 a
AMF + P.p.	5.3 ± 0.2 a	4.6 ± 0.2 ab	1.42 ± 0.06 a	61.2 ± 1.6 ab	−3.8 ± 0.2 b	35.4 ± 0.9 a
Source of variance						
Planting time (P)	*	*	*	n.s.		n.s.
Biostimulant (B)	*	*	*	*		*
P × B	n.s.	n.s.	n.s.	n.s.		n.s.

n.s.: not significant; *: significant at $p < 0.05$. Within each column, values with the same letters do not significantly differ according to Duncan's multiple range test at $p < 0.05$. Values followed by ± sign are the standard errors of the mean.

The brightest ('L*' component) fruits, also tending more towards a red color, were obtained under the P.p. extract treatment (10.6 and 59.4% higher than the untreated control, respectively); the component 'b*' (blue–yellow axis) showed the highest levels upon the application of P.p. and F.v. extract as well as the combination of AMF + P.p. and AMF + F.v., with a 11.1% average increase compared to the untreated control.

As reported in previous studies, an accumulation of dry residue and soluble solids in tomato could be linked to a soil water deficit condition, more present in late-transplanted crops, that could lead to both an accumulation of sugars and synthesis of other solutes in the fruit, as result of a high transpiration rate [39,40].

In the present research, P.p. brown seaweed extract significantly improved the soluble solids content in tomato fruits, consistently with the results of Mannino et al. [41]. Mzibra et al. [42] found that polysaccharide-enriched extracts (PEEs) of six Moroccan algae positively affected fruit quality parameters, such as total soluble solids content due to improved fruit metabolic activities leading to the synthesis of more metabolites, glucose, and acids through biostimulant treatments.

P.p. extract, rich in nutrient compounds [29,30], can stimulate plant growth and promote carbohydrate synthesis, resulting in an increase in soluble solids. Similarly, Bagyaraj et al. [43] demonstrated that mycorrhizae can enhance nutrient uptake by plant roots, increasing sugar and soluble solids content.

3.3. Antioxidant Parameters

The later planting time led to significantly higher values of all the antioxidant parameters measured (Table 3).

Table 3. Effects of planting time and biostimulant type on antioxidant activities and compounds of tomato fruits.

Treatment	HAA Ascorbic Acid eq. 100 g ⁻¹ f.w.	LAA Trolox eq. 100 g ⁻¹ f.w.	Polyphenols mg Gallic Acid g ⁻¹ d.w.	TAA mg g ⁻¹ f.w.
Planting time				
4 May	2.74 ± 0.15	10.5 ± 0.5	2.46 ± 0.12	161.9 ± 8.0
1 June	5.39 ± 0.25 *	13.8 ± 0.7 *	2.92 ± 0.14 *	242.7 ± 12.0 *
Biostimulant				
Control	3.53 ± 0.18 d	9.5 ± 0.5 d	2.40 ± 0.12 c	177.8 ± 8.0 c
C.t.	4.02 ± 0.20 c	13.1 ± 0.7 ab	2.72 ± 0.14 ab	199.6 ± 10.0 b
F.v.	5.28 ± 0.25 b	10.9 ± 0.6 c	2.55 ± 0.13 bc	198.1 ± 9.0 b
P.p.	5.78 ± 0.28 a	13.0 ± 0.7 ab	2.96 ± 0.15 a	226.6 ± 12.0 a
AMF	4.81 ± 0.24 b	10.6 ± 0.5 c	2.73 ± 0.14 ab	210.8 ± 10.0 ab
AMF + C.t.	4.86 ± 0.24 b	12.5 ± 0.6 b	2.71 ± 0.14 ab	200.2 ± 10.0 b
AMF + F.v.	5.27 ± 0.25 b	13.6 ± 0.7 ab	2.60 ± 0.13 bc	204.6 ± 10.0 b
AMF + P.p.	5.95 ± 0.29 a	14.1 ± 0.7 a	2.93 ± 0.15 a	220.7 ± 11.0 ab
Source of variance				
Planting time (P)	*	*	*	*
Biostimulant (B)	*	*	*	*
P × B	n.s.	n.s.	n.s.	n.s.

n.s.: not significant; *: significant at $p < 0.05$. Within each column, values with the same letters do not significantly differ according to Duncan's multiple range test at $p < 0.05$. Values followed by $\pm$ sign are the standard errors of the mean.

The application of the P.p. extract and the combination of P.p. and AMF resulted in the overall best results of antioxidant activities and compounds. Particularly, they averagely increased HAA, LAA, polyphenols, and TAA by 66%, 42%, 23%, and 26%, respectively, compared to the untreated control (Table 3).

Late-ripened fruits had considerably greater total carotenoid and lycopene concentrations as well as hydrophilic and lipophilic antioxidant activities, according to a study regarding high-lycopene tomato cultivars at different ripening stages, usually linked to late-transplanted crops [44].

The algae *Cystoseira* spp., *Fucus* spp., and *Padina* spp. contain polysaccharides, phenolic compounds, and fatty acids that could enhance both hydrophilic and lipophilic antioxidant activities through various pathways. Fucoidans, which are polysaccharides present in all three types of algae, contribute to hydrophilic antioxidant activity by counteracting reactive oxygen species (ROS) in water-based settings, thus helping safeguard plant cells from oxidative stress, as demonstrated by Sousa et al. [45].

Phenolic chemicals, as phlorotannins, show potent antioxidant activities by effectively neutralizing reactive oxygen species (ROS) and offering substantial hydrophilic antioxidant activity, which enhances the plant's ability to withstand environmental challenges, as demonstrated by Obluchinskaya et al. [46].

The presence of fatty acids, such as palmitic and oleic acids, in the mentioned algae contributes to the lipophilic antioxidant activity which is achieved by safeguarding cellular membranes from lipid peroxidation, preserving membrane integrity, and diminishing oxidative damage [47].

Our hypothesis is that the combined action of the mentioned chemicals amplifies the overall antioxidant activity, offering extensive defense against oxidative stress and enhancing the overall health and development of plants.

Rouphael et al. [28] demonstrated that the production and accumulation of bioactive compounds, such as ascorbic acid, may be linked to the direct or indirect effects of biostimulant application on antioxidant development in plant tissues.

Di Stasio et al. [48] reported that *A. nodosum*-based algal extracts significantly augmented the ascorbic acid content of fruits, even under salt stress conditions.

3.4. Biochemical Activities

CAT activity was significantly more intense in the later tomato crop cycle, whereas GPOX was not affected by planting time (Table 4).

Table 4. Effects of planting time and biostimulant type on CAT (mmol min^{−1} mg^{−1} Prot) and GPOX (mmol min^{−1} mg^{−1} Prot) in tomato fruits.

Treatment	CAT (mmol min ^{−1} mg ^{−1} Prot)	GPOX (mmol min ^{−1} mg ^{−1} Prot)
Planting time		
4 May	1.83 ± 0.09	16.6 ± 0.8
1 June	8.12 ± 0.40 *	16.1 ± 0.8 n.s.
Biostimulant		
Control	1.17 ± 0.06 d	14.47 ± 0.7 c
C.t.	1.76 ± 0.09 bc	19.87 ± 1.0 b
F.v.	1.61 ± 0.08 c	18.48 ± 0.9 b
P.p.	2.55 ± 0.13 a	24.97 ± 1.2 a
AMF	1.67 ± 0.08 c	18.28 ± 0.9 b
AMF + C.t.	1.77 ± 0.09 bc	18.86 ± 0.9 b
AMF + F.v.	1.93 ± 0.10 b	18.04 ± 0.9 b
AMF + P.p.	2.45 ± 0.12 a	23.71 ± 1.2 a
Source of variance		
Planting time (P)	*	n.s.
Biostimulant (B)	*	*
P × B	n.s.	n.s.

n.s.: not significant; *: significant at $p < 0.05$. Within each column, values with the same letters do not significantly differ according to Duncan's multiple range test at $p < 0.05$. Values followed by ± sign are the standard errors of the mean.

The P.p. extract and the combined AMF + P.p. treatment led on average to 114% and 68% higher values of CAT and GPOX, respectively, compared to the untreated control (Table 4).

According to a study on tomato genotypes subjected to high temperatures, the later transplant resulted in increased CAT and GPOX activity, because to lessen oxidative damage, the late transplanted plants had to cope with more intense heat stress, which increased the activity of antioxidant enzymes [49].

Wozniak et al. [50] investigated over 50 biostimulants, including algae extracts and humic compounds, and found that one of the main effects was related to controlling the overproduction of reactive oxygen species (ROS) and protecting cell membranes. The

heightened antioxidant enzymatic activity and secondary metabolite production in plants demonstrate these protective mechanisms [51].

In addition to the mentioned benefits associated with biostimulant use, careful monitoring is essential to prevent any issues connected with biostimulant application and, in this respect, the implementation of regulation is needed to guarantee their safe management [52] while calling for a compromise in the balance between large-scale production and premium quality targets.

However, further research is needed to assess the efficiency of algae use in agriculture in connection with the evolution of their production processes [53].

4. Conclusions

From this research, it arose that the early-May planting time elicited a higher tomato fruit yield than the early-June one, but the later transplant resulted in overall better quality.

This study also highlighted the positive effects of biostimulants, singly or as a combination of different types, such as brown algae extracts and arbuscular mycorrhizal fungi, on the yield, plant growth, and fruit quality and antioxidants of yellow tomato. Particularly, the extract of the algae species *Padina pavonica*, singly or in combination with AMF, resulted in the overall highest values of fruit yield and quality attributes, as well as reduced oxidative stress in tomato plants, proving their important role as environmentally friendly tools in sustainable crop management.

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