Research Article

Received: 7 June 2024 Revised: 7 July 2024 Published online in Wiley Online Library: 31 August 2024

(wileyonlinelibrary.com) DOI 10.1002/jsfa.13851

Microbial biofertilizers and algae-based biostimulant affect fruit yield characteristics of organic processing tomato

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Abstract

BACKGROUND: Microbial biofertilizers and algae-based biostimulants have been recognized for supporting sustainable agriculture. Field experiments were conducted in 2022 and 2023 growing seasons in an organic farm located in Ferrara (Italy) with the aim of evaluating plant growth-promoting microorganisms (PGPMs) and algae-based biostimulants (Biost) in tomato (*Solanum lycopersicum* L.). The experimental treatments were: (i) two microbial biofertilizers (PGPM_1, PGPM_2) and no inoculated plants (No_PGPM); and (ii) two algae-based biostimulant rates (0.5% (Biost_0.5%), 1.0% (Biost_1.0%)) and no application (No_Biost). PGPMs were applied at transplanting, while biostimulants at 15 and 30 days after transplanting. Treatments were replicated three times according to a split-plot experimental design. Plant characteristics were evaluated at 30 days after transplanting in No_Biost treatments. During tomato cultivation, soil plant analysis development (SPAD), nitrogen difference vegetation index (NDVI), leaf area index (LAI) and photosynthetic photon flux density (PPFD) were monitored. Tomato yield was determined.

RESULTS: PGPM_2 showed the highest shoot biomass (132.9 g plant^{-1}), plant height (44.7 cm), leaf number (34.0 plant^{-1}) and root biomass (9.22 g plant^{-1}). Intermediate values were observed in PGPM_1, while all parameters were lower in No_PGPM. Both PGPMs achieved higher values of SPAD, NDVI, PPFD and LAI than No_PGPM. Biost_1.0% increased all measured growth parameters followed by Biost_0.5% and No_Biost, respectively. Tomato yield was the highest for PGPM_2–Biost_1.0% (67.2 t ha^{-1}). PGPMs affected fruit size and sugar content, while biostimulants were associated with color and lycopene.

CONCLUSION: The application of microbial biofertilizers and algae-based biostimulants could be part of environment-friendly practice in organic farming.

Keywords: sustainable production; microbial biofertilizer; foliar application; growth parameters; tomato crop

INTRODUCTION

Beneficial soil microorganisms could replace chemicals and pesticides by enabling the use of sustainable agricultural practices and supporting organic farming.[1] The benefits of using microbial biofertilizers as plant growth-promoting microorganisms (PGPMs) in crop production are well proven; however, their application in agricultural management is still limited.[2] Soil microorganisms including rhizobacteria and fungi play a key role in soil health, biodiversity and productivity of natural and managed ecosystems.[3]

Nowadays, there is a pressing call for an increase in sustainable use of crop nutrients and the application of PGPMs has gained attention as a means for more sustainable agriculture.[4] Soil PGPMs can form a mutualistic plant–microorganism association that can enhance plant performance and tolerance against several stresses, particularly drought stress, leading to successful plant growth and yield enhancement.[5] Indeed, the plant stress

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tolerance increases in terms of various PGPM benefits such as enhanced water and nutrient uptake, amelioration of efficiency of photosynthesis, osmotic adjustment, the production of phytohormones and more efficient antioxidative systems.[6] PGPMs are classified according to their functional activities into: (i) biofertilizers, which facilitate the uptake of specific nutrients from the environment; (ii) phytostimulators, which synthesize compounds or phytohormones for plants; and (iii) bioprotectants or biocontrol, which protect plants from diseases via the production of antifungal metabolites and/or antibiotics.[7] In recent years, several biofertilizer formulations have become commercially available and, when applied directly to seeds or introduced into the plant rhizosphere, can provide nutritional benefits to the host plant.[8] They are a safe alternative to conventional chemical fertilizers and provide sustainable agricultural production worldwide.[9] Biofertilizers improve plant growth in terms of seed germination, shoot and root development, increased biomass and reduced disease incidence.[6]

The development of algae-based biostimulants is emerging as an interesting agronomical strategy to enhance crop performance and develop protection traits against different plant stressors of biotic and abiotic origin.[10] These natural formulations can be easily applied by foliar application and may be a valuable tool in several vegetables,[10] especially under organic farming systems where synthetic agrochemicals are not allowed. In addition, algae-based biostimulants contain a high concentration of growth-promoting components, such as vitamins, phytohormones and amino acids, that may exert a positive effect also on the enhancement of nutraceutical and organoleptic characteristics for vegetables and fruits.[11] All these aspects make the use of algae-based biostimulants a promising strategy for improving the sustainability of the agricultural sector as their foliar application is considered environmentally friendly and a cost-effective alternative compared with agrochemicals obtained by fossil-fuel-consuming processes.[12] Although algae-based biostimulants are applied at low dose, they can exert an efficacious response of plant physiological characteristics determining enhanced crop growth and yield, and improved quality attributes.[13]

This study reported here hypothesized that the application of PGPMs integrated with the foliar application of algae-based biostimulant could represent a suitable strategy for the cultivation of processing tomato in an organic farming system determining an increase of marketable fruit yield and quality. Therefore, the objectives were: (i) to evaluate the effects of PGPMs on plantlet establishment at field conditions, (ii) to evaluate the combined application of PGPMs and algae-based biostimulant on processing tomato growth; and (iii) to investigate the effect of integrated application of PGPMs and algae-based biostimulant on processing tomato yield and fruit quality.

MATERIALS AND METHODS

Site description and experimental design and treatments

A field trial was carried out at the F.lli Baretta farm located in Ferrara, Italy (44°72′70 N, 12°08;12 E, altitude 2 m) in 2022 and 2023 growing seasons of processing tomato in two nearby fields previously cultivated with wheat (*Triticum aestivum* L.). The experimental area was set up in an organic farm characterized by an attenuate thermos-Mediterranean climate (UNESCO-FAO classification) with annual precipitation of 617 mm (average of the last 30-year period). Annual air temperature is 14.2 °C, the minimum temperature dropped below 0 °C in December to February, while the maximum air temperature is observed in July and August (37 °C). Average soil characteristics in the 0–30 cm soil profile were: 34.4% sand, 49.0% silt and 16.6% clay; pH 7.8 (water, 1:2.5); 1.13% organic matter (Lotti); 1.29% total nitrogen (Kjeldahl). The experimental treatments were the following. (a) Three applications of PGPMs as microbial biofertilizers: two commercial formulates (MICOSAT F® (produced by CCS Centro Colture Sperimentali, Aosta, Italy) containing a mixture of selected mycorrhizal fungi, bacteria and *Streptomyces* derived from rhizosphere (PGPM_1) and MYCOUP (produced by BIOGARD Division, Bergamo, Italy) composed of a mycorrhizae-forming fungus and bacteria (PGPM_2)) and no application of microbial biofertilizer (No_PGPM). (b) Two rates of algae-based biostimulant (0.5% (Biost_0.5%), 1% (Biost_1.0%)), and no treated tomato plants with algae-based biostimulant (No_Biost). In both tomato growing seasons, the experimental design was a split plot with three replications of randomized blocks, where the main plots were the microbial biofertilizers and the sub-plots were the rate of algae-based biostimulant. The size of the main experimental plot was 54 m^2 (4.5 m × 12 m) and the sub-plot size was 18 m^2 (4.5 m × 4 m). The commercial formulates of microbial biofertilizers contain the following. (i) PGPM_1: symbiont fungi as 40 g of crude inoculum containing species of the genus *Glomus* (*Glomus* spp. GB 67, *G. mosseae* GP 11, *G. viscosum* GC 41), *Trichoderma* spp. content 3×10^5 CFU g^{-1}, *Agrobacterium radiobacter* AR 39, *Bacillus amyloliquefaciens* BA 41; *Pochonia chlamydosporia* PC 50, *Trichoderma harzianum* TH 01, *Streptomyces* spp. SB 14, *Pichia pastoris* PP 59; (ii) PGPM_2: arbuscular mycorrhizal fungi (AMF) part by *Glomus iranicum* var. *tenuihypharum* 1% (120 spreads g^{-1}) and rhizosphere bacteria content 103 UFC g^{-1}. The algae-based biostimulant was produced in the botanical laboratory of the University of Ferrara and consisted of the exhausted low-salinity BG11 medium deriving from the autotrophic cultivation of the green microalga *Neochloris oleoabundans* UTEX 1195, as described in Baldisserotto *et al.*[14] The exhausted medium was harvested by centrifugation (8000 × *g*, 10 min) at the beginning of the stationary phase of growth (*ca* 28 days).

Farming practices

Farming practices were carried out according to the EC Regulation concerning organic production and the labeling of organic products. In both tomato growing seasons, soil tillage was performed in September by plowing at the maximum tillage depth of 30 cm, then the soil was left bare during the winter season, emerging weeds being removed mechanically by means of disk harrowing. In March, 56 kg ha^{-1} of K_2O, 68 kg ha^{-1} of CaO, 24 kg ha^{-1} of MgO and 192 kg ha^{-1} of SO_3 were applied by means of a commercial product allowed for organic farming uniformly distributed on the soil surface and incorporated into the soil by means of disk harrowing to prepare the tomato transplanting bed. Tomato (*Solanum lycopersicum* L.) seeds of a commercial variety cv. Heinz 1301 F1, characterized by a determinate growth habit with good vigor and yield, and medium oval fruit, were sown and grown in a nursery (Bronte Garden, Venice, Italy). One-month-old tomato seedlings were manually transplanted under field conditions on 28 April 2022 and 3 May 2023. Tomato seedlings were transplanted in paired rows at 40 cm from one another and 140 cm between the paired rows at a density of 4 plants m^{-2}. Both commercial microbial biofertilizers were applied at tomato transplanting according to the suggested protocol. PGPM_1 was applied at a rate of 10 kg ha^{-1} being placed in the transplanting furrow just before the tomato seedling

transplanting, while PGPM_2 was administered at a rate of 3 kg ha^{-1} applied with drip irrigation 5 days after tomato transplanting. In addition, no inoculated tomato plants were cultivated (hereafter called No_PGPM). Moreover, at 15 and 30 days after tomato transplanting, algae-based biostimulant was applied as foliar spraying at concentrations of 0.5 and 1%, respectively. The application of a concentration of 0% of the biostimulant (No_Biost) was by spraying tap water. For each application, both rates of algae-based biostimulant were applied early in the morning (between 09:00 and 10:00), with an average air temperature of 20–24 °C and 30–50% relative humidity, by using a hand sprayer. In addition, no treated tomato plants with algae-based biostimulant were cultivated. Drip irrigation was adopted for the tomato crop with the aim of reintegrating the 90% of the water lost through evapotranspiration estimated by means of a digital weather station placed 500 m from the experimental field. Fertirrigation was adopted for supplying nutrients during the tomato growing season, a total of 120 kg ha^{-1} of N being applied in five applications. In both growing seasons, there was no biotic adversity revealed to justify foliar treatment on tomato plants; therefore, control means were not used in both tomato growing seasons. Tomato plants were manually harvested one time on 8 August 2022 and 9 August 2023.

Sampling and measurements

Tomato seedling establishment

Thirty days after tomato transplanting, 10 tomato seedlings of each biofertilizer grown in field conditions under No_Biost treatment (no application of algae-based biostimulant) were carefully collected from the soil taking care to remove the whole plant (shoots + roots). Collected plants were carefully cleaned and subjected to determination of shoot and root characteristics. Average shoot and root length, number of leaves, length and width of leaves, soil plant analysis development (SPAD) readings, shoot diameter and branches of leaves were noted using a measuring tape (cm) for each treated tomato plant. As well, the root and shoot average dry and fresh weight were determined separately.

Tomato plant development and fruit yield

During the entire tomato growing seasons, the nutrient status of the tomato plants was estimated by means of a chlorophyll concentration meter (MC-100) of the 4th fully grown leaf from the top of the plant and a RapidScan CS-45 canopy sensor. Ten measurements per treatment were taken in each replication and averaged. Furthermore, an Accupar LP-80 ceptometer was used for measuring the leaf area index (LAI) of tomato plants subjected to biofertilizer and biostimulant treatments. The photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) transmitted by the tomato canopy was measured by placing the Accupar LP-80 ceptometer five times in the middle of tomato paired rows at ground level during clear days between 11:00 and 13:00. The fraction of PPFD was determined by applying the following formula:

$$\text{Fraction of PPFD intercepted} = [1-(I_o/I_t)]$$

where I_o is the average PPFD measured five times on the ground and it is the same measurement performed at the top of the tomato canopy. The intercepted PPFD index could be equal to 1 or 0 indicating all no PPFD intercepted, respectively. The readings of all monitored parameters (SPAD, nitrogen difference vegetation index (NDVI), LAI and PPFD) were performed every 12 days throughout both growing seasons starting at the 12th day after tomato transplanting (T1).

At harvesting, four tomato plants per plot were harvested to determine yield and fruit characteristics. Tomato fruits were collected based on marketable tomato yield (number and weight) considering red and disease-free fruits, and unmarketable tomato fruits were divided into green fruits (number) and rotten fruits (number). The marketable tomato fruits collected in the field were taken to the Laboratory of Food Science and Technology of the University of Ferrara for analytical measurements. Tomato fruit samples were washed to remove dirt and dried, and then their longitudinal and equatorial dimensions were measured as indicators of fruit size.[15] Also, the skin firmness was measured with a penetrometer (FT-327, TR Turoni & Co., Forlì, Italy) equipped with an 8 mm diameter tip and the results were expressed as the maximum force (N) required to penetrate the probe into the tomato pulp. Flesh color measurements were carried out with a colorimeter (PCE-RGB2, PCE Deutschland GmbH, Germany) on five randomly selected areas of each selected tomato fruit. For quality parameter quantification, the soluble solids content (SSC, °Brix) was measured in the fruit juice using a refractometer (PCE-018, PCE Deutschland GmbH, Germany). The pH was measured following the official method 'pH Measurement of Water' (AOAC 973.41) with a pH meter (Mettler Toledo, Milan, Italy). For the determination of lycopene and β-carotene content in marketable tomato fruits, 5 g of homogenate suspension of tomato was weighed and added to 25 mL of a 2:1:1 hexane–methanol–acetone mixture with butylated hydroxytoluene (0.5%, w/v), shaking for 30 min with a magnetic stirrer, according to the method described by Fish *et al.*[16] The flasks were immediately wrapped with an aluminium foil to limit the light degradation of carotenoids. After the separation of the organic phase, the nonpolar layer containing lycopene and β-carotene was finally collected and spectrophotometrically analyzed (UV–visible spectrophotometer, Beckman, USA) measured in a 1 cm pathlengh quartz cuvette at different wavelengths of 453, 505, 645 and 663 nm. Chl a, Chl b, β-carotene and lycopene content was calculated[17] from the following equations:

$$\text{Lycopene}\left(\text{mg}\,(100\,\text{mL})^{-1}\right) = -0.0485A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

$$\beta\text{–Carotene}\left(\text{mg}\,(100\,\text{mL})^{-1}\right) = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

where A is absorbance.

The titratable acidity (TA) was measured by means of supernatant obtained from frozen tomato pieces ground and homogenized. TA, expressed as malic acid, was determined by titrating 10 g of tomato homogenized into 50 mL of water with 0.1 mol L^{-1} NaOH to an end point of pH = 8.1.[18] The acidity was calculated as % (g (100 g)$^{-1}$) of malic acid equivalents. For all chemical parameters analyzed, five measurements were taken per replication and then the averages of the readings were considered.

Statistical analysis

Analysis of variance was performed on all data collected during both growing seasons of tomato crop by adopting the JMP statistical software package 4.0,[19] considering the growing season (year) as a repeated measure across time.[20] Before the analysis,

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

all data were subjected to Shapiro–Wilk and Leven tests to verify their normality and heteroscedasticity. A split-plot experimental design was adopted for tomato yield and yield characteristics, where microbial biofertilizer was considered the main factor and algae-based biostimulant as a split factor. Means were compared according to Fisher's protected least significant difference (LSD) test at the 5% level of significance ($P \leq 0.05$). Data for SPAD, NDVI, LAI and fraction of PPFD intercepted were presented in this study as means during the growing seasons associated with their respective standard error (± SE). Tomato fruit characteristics were subjected to canonical discriminant analysis (CDA) to evaluate their association with the microbial biofertilizers and algal-based biostimulant. A vector diagram based on the total canonical coefficient of each qualitative characteristic of tomato fruit from the canonical function was combined into the same plot.

RESULTS

Tomato seedling establishment

At 30 days after transplanting, tomato plants subjected to both PGPM_1 and PGPM_2 showed greater values of plant characteristics compared to No_PGPM tomato plantlets (Table 1). Shoot fresh biomass was the highest in PGPM_2 followed by PGPM_1, while it was lowest in No_PGPM (132.9, 85.9 and 71.3 g plant^{-1}, respectively). A similar trend was observed in the shoot dry biomass, which ranged from 18.9 to 10.3 g plant^{-1} (Table 1). Tomato plants subjected to PGPM_1 exhibited the highest plant height followed by PGPM_2 and No_PGPM (44.7, 42.8 and 40.2 cm, respectively). Stem diameter, leaf number and SPAD readings were greater in PGPM_1 and PGPM_2 compared with No_PGPM (Table 1). Furthermore, plants subjected to PGPM_1 and PGPM_2 showed a longer and denser root system compared with the No_PGPM plants (Fig. 1). Indeed, root length was greater in PGPM_1 and PGPM_2 compared with No_PGPM (on average 33.9 *versus* 21.7 cm), while root dry biomass was the highest in PGPM_2, intermediate in PGPM_1 and low in No_PGPM (9.22, 8.68 and 6.35 g plant^{-1}, respectively; Table 1).

Table 1. Main effects of microbial biofertilizer on tomato plant characteristics at 30 days after transplanting (mean ± SE; $n = 10$). Mean values belonging to the same factor without common letters are statistically different according to LSD ($P < 0.05$)

Treatment	Shoot fresh biomass (g plant^{-1})	Shoot dry biomass (g plant^{-1})	Plant height (cm)	Stem diameter (mm)	Leaves (plant^{-1})	Leaf length (cm)	Leaf width (cm)	SPAD	Root dry biomass (g plant^{-1})	Root length (cm)
No_PGPM	71.3 ± 7.23 ^{c}	10.3 ± 1.29 ^{c}	40.2 ± 0.44 ^{c}	7.3 ± 0.33 ^{b}	28.0 ± 2.65 ^{b}	4.54 ± 0.07 ^{b}	2.50 ± 0.10 ^{a}	46.5 ± 1.00 ^{b}	6.35 ± 0.70 ^{b}	21.7 ± 3.87 ^{b}
PGPM_1	85.9 ± 4.83 ^{b}	14.3 ± 1.48 ^{b}	44.7 ± 0.60 ^{a}	8.7 ± 0.88 ^{a}	33.7 ± 0.90 ^{a}	4.91 ± 0.05 ^{ab}	2.75 ± 0.02 ^{a}	49.8 ± 0.65 ^{a}	8.68 ± 0.14 ^{ab}	32.4 ± 2.69 ^{a}
PGPM_2	132.9 ± 4.74 ^{a}	18.9 ± 1.27 ^{a}	42.8 ± 0.44 ^{b}	8.3 ± 0.33 ^{a}	34.0 ± 1.01^{a}	5.35 ± 0.05 ^{a}	2.89 ± 0.11 ^{a}	51.1 ± 0.90 ^{a}	9.22 ± 0.37 ^{a}	35.4 ± 3.52 ^{a}

Tomato plant development and fruit yield

Tomato plant development

Although during the whole tomato cultivation period no differences were detected in phenological phases among the treatments, tomato plants subjected to PGPM inoculation tended to show significantly higher values of SPAD and NDVI throughout the entire growing season with respect to No_PGPM (Fig. 2). In general, SPAD readings of tomato plants followed a positive trend from T1 until T6 and then slowly decreased. NDVI readings showed a similar trend, even if shorter in time, and values showed an increase until T3 then decreased. SPAD and NDVI (under the different PGPM applications) were significantly correlated ($R^2 = 0.404$, $P < 0.001$), increasing as the biostimulant rate increased (Fig. 2). Specifically, for all the biostimulant doses, results showed that plants under PGPM_1 and PGPM_2 had higher levels of SPAD and NDVI compared to No_PGPM plants.

The results related to the effects of PGPM and algae-based biostimulant on LAI and on the fraction of PPFD intercepted by tomato plants during the growing cycle showed that values for Biost_1.0% of all treatments are higher compared with No_Biost (Fig. 3). For LAI, the trend is the same in all treatments, even if with differences in PGPM_2 with biostimulant application. Conversely, for the fraction of PPFD intercepted, the trend is similar in all treatments with differences in PGPM treatment with application of

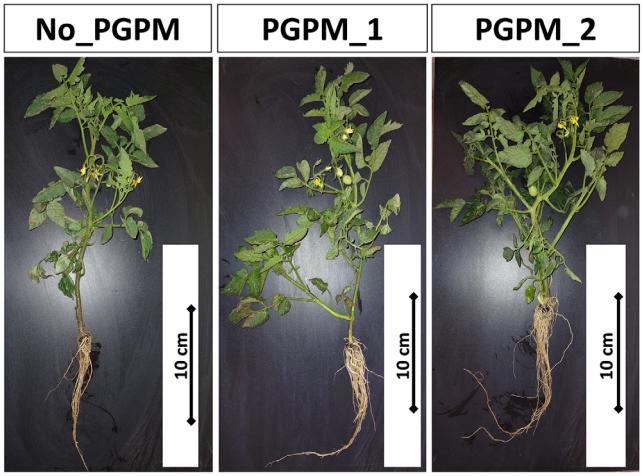

Figure 1. Tomato plants collected 30 days after tomato transplanting in No_Biost treatments.

biostimulant at 0% and 0.5%. Results have generally shown that LAI and fraction of PPFD intercepted increased when both PGPMs were applied regardless of the biostimulant dose compared to No_PGPM (Fig. 3). As the dose of biostimulant increased, the response of plants in terms of LAI increased notably, but only increased slightly for the fraction of PPFD intercepted.

Tomato fruit yield and quality

Tomato fruit yield was the highest fo PGPM_2–Biost_1.0% (67.2 t ha^{-1} FW; Table 2). On the other hand, the application of Biost_0.5% in the treatment with PGPM_1 and PGPM_2 was more effective on tomato yield and straw value than without PGPM (on average 44.3 *versus* 33.8 t ha^{-1} FW and 1.47 *versus* 1.30 t ha^{-1} DW, respectively). Under No_Biost, tomato fruit yield was high in PGPM_2 followed by PGPM_1 and No_PGPM (30.3, 27.0 and 26.0 t ha^{-1} FW, respectively). Similarly, the highest number of marketable tomato fruits (165 fruits m^{-2}) was observed under PGPM_2–Biost_1.0% (Fig. 4), while the lowest number of

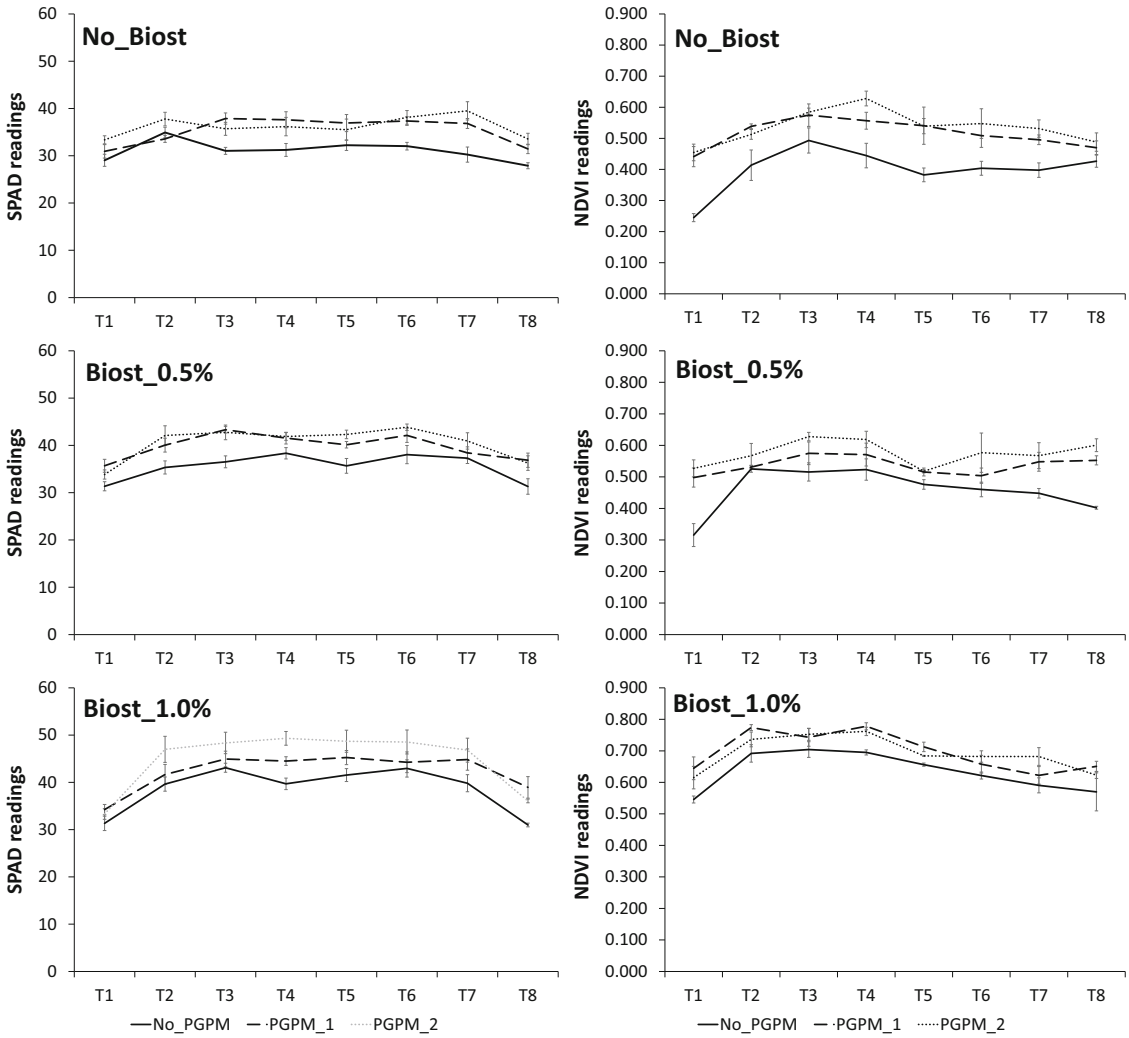

Figure 2. Effects of microbial biofertilizer and algae-based biostimulant on SPAD and NDVI readings of tomato leaves during the growing cycle of the crop. Data correspond to the 2022 and 2023 growing seasons. Error bars represent ± standard error from mean (n = 6). T1 to T8 indicate the dates of measurements after processing tomato transplanting with an interval of 12 days. T1 = 12 days after tomato transplanting.

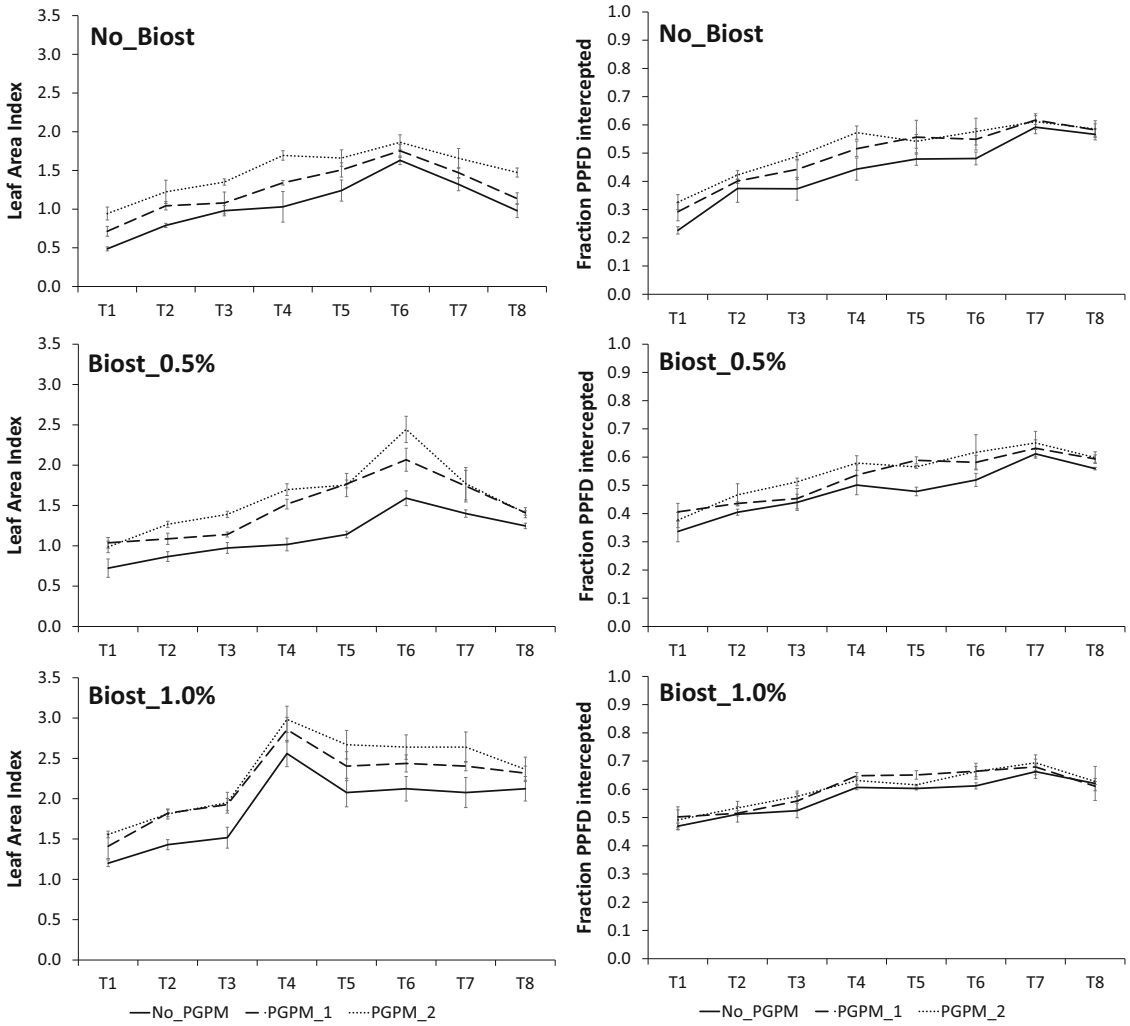

Figure 3. Effects of microbial biofertilizer and algae-based biostimulant on LAI and fraction of PPFD intercepted of tomato plants during the growing cycle of the crop. Data correspond to the 2022 and 2023 growing seasons. Error bars represent ± standard error from mean ($n = 6$). T1 to T8 indicate the dates of measurements after processing tomato transplanting with an interval of 12 days. T1 = 12 days after tomato transplanting.

Table 2. Interaction effects of microbial biofertilizer and algae-based biostimulant on marketable fruit yield and straw of tomato (mean ± SE; $n = 6$)

	Tomato yield (t ha^{-1} FW)		
	No_Biost	Biost_0.5%	Biost_1.0%
No_PGPM	26.0 ± 1.12 ^{bB}	33.8 ± 2.30 ^{bAB}	40.4 ± 5.07 ^{bA}
PGPM_1	27.0 ± 2.52 ^{abC}	42.3 ± 4.57 ^{aB}	63.3 ± 6.26 ^{aA}
PGPM_2	30.3 ± 3.79 ^{aC}	46.3 ± 7.09 ^{aB}	67.2 ± 6.26 ^{aA}
	Tomato straw (t ha $^{-1}$ DW)		
	No_Biost	Biost_0.5%	Biost_1.0%
No_PGPM	1.05 ± 0.14 ^{aB}	1.30 ± 0.11 ^{bAB}	1.50 ± 0.20 ^{bA}
PGPM_1	1.12 ± 0.12 ^{aB}	1.35 ± 0.09 ^{abB}	1.75 ± 0.23 ^{abA}
PGPM_2	1.18 ± 0.17 ^{aC}	1.59 ± 0.23 ^{aB}	1.96 ± 0.22 ^{aA}

Values belonging to the same characteristic and treatment with different letters in rows for algae-based biostimulant rate (upper-case letter), and in columns for microbial biofertilizer (lower-case letter) are statistically different according to LSD (0.05).FW, fresh weight; DW, dry weight.

marketable tomato fruits was registered when biostimulant and PGPM were not applied (70 fruits m^{-2}). No significant differences were noticed in the number of marketable tomato fruits between plants that were under the highest dose of biostimulant (Biost_1.0%) and both PGPM_1 and PGPM_2. Moreover, results have shown that increasing the biostimulant dose had a positive effect on the number of marketable tomato fruits; in fact, under Biost_1.0% the number of marketable tomato fruits significantly increased compared with No_Biost application. Generally, equatorial diameter of tomato fruits was higher in PGPM_2 than PGPM_1 and No_PGPM (on average 4.0 *versus* 3.8 cm, respectively). The longitudinal diameter was greater for microbial biofertilizer than No_PGPM (on average 5.5 *versus* 5.0 cm, respectively; Table 3). No differences were detected concerning the growing season and algae-based biostimulant on marketable tomato fruit size. Color characteristics were generally not significant for all factors tested in the study, except for the R color that tended to be higher in 2023 than 2022 growing season (Table 3). The firmness of tomato fruits was higher in 2022 compared with 2023 growing season (5.84 *versus* 5.37 N, respectively), while among the microbial biofertilizer treatments it was greater in No_PGPM, intermediate in PGPM_2 and low in PGPM_1 (6.01, 5.56 and 5.24 N, respectively). The SSC was affected only by growing season and was higher in 2022 than 2023 (5.24 *versus*. 5.66 °Brix, respectively), while TA and pH were not significantly affected by the experimental treatments (Table 4). The sugar to acidity ratio (SAR) was the highest in PGPM_1 followed by PGPM_2 and No_PGPM (18.16, 18.23 and 17.48, respectively), while among the algae-based biostimulant treatments, Biost_1.0% showed the greatest SAR (18.76). Regarding the β-carotene and lycopene contents, these were both the highest in PGPM_1 (7.68 and 59.58 mg (100 g)$^{-1}$ FW, respectively) and in Biost_1.0% (7.34 and 56.87 mg (100 g)$^{-1}$ FW, respectively; Table 4). Quality parameters of marketable tomato fruits based on the microbial biofertilizer and algae-based biostimulant treatments were evaluated by means of CDA (Fig. 5). Regarding the microbial biofertilizers, the first two canonical variables generally accounted for 60.9% of the total variance. CDA showed a tendency towards differentiation among marketable tomato fruit characteristics. Fruit size, in terms of equatorial and longitudinal dimension (F_Weight, F_Width and F_Length), seemed to be associated with PGPM_1, while SSC was associated with both PGPM_1 and PGPM_2 treatments. Conversely, Tot_Ac, B_color, G_color and Penetrometer seemed to be associated with the No_PGPM tomato plants. Other tomato fruit characteristics, such as lycopene (Lyc), carotenoids (Car) and β-carotene (B_Car) did not look to be associated with any microbial biofertilizer treatment (Fig. 5(A)). Regarding the algae-based biostimulant treatments, CDA showed that fruit size characteristics seemed to be associated with the highest biostimulant rate (Biost_1.0%), while Lyc and Car vectors are

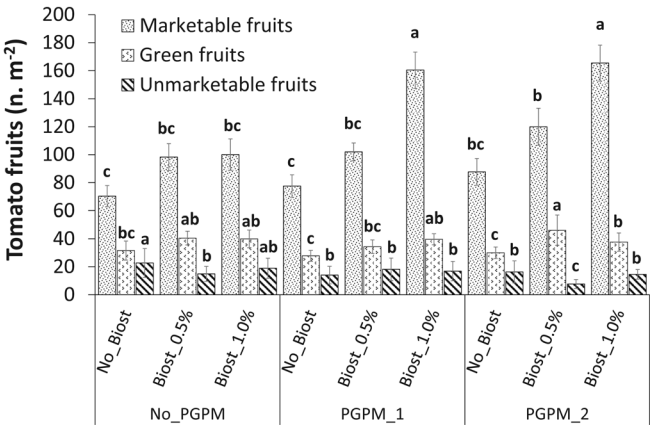

Figure 4. Interaction effect of microbial biofertilizer and algae-based biostimulant on marketable, green and unmarketable tomato fruits. Values belonging to the same characteristic followed by the same letter are not significantly different according to LSD (0.05). Error bars represent ± standard error from mean ($n = 6$).

Table 3. Main effects of growing season, microbial biofertilizer and algae-based biostimulant on physical quality parameters of marketable tomato fruits (mean ± SE; $n = 27$, 18 and 18 for growing season, microbial fertilizer and algae-based biostimulant, respectively)

	Equatorial diameter (cm)	Longitudinal diameter (cm)	R color	G color	B color	Firmness (N)
2022	3.9 ± 0.03 ^{a}	5.4 ± 0.05 ^{a}	207.3 ± 2.09 ^{b}	94.0 ± 1.55 ^{a}	69.2 ± 1.18 ^{a}	5.84 ± 0.15 ^{a}
2023	3.8 ± 0.04 ^{a}	5.3 ± 0.05 ^{a}	219.4 ± 2.29 ^{a}	97.4 ± 1.35 ^{a}	74.2 ± 1.36 ^{a}	5.37 ± 0.14 ^{b}
No_PGPM	3.8 ± 0.03 ^{b}	5.0 ± 0.06 ^{b}	213.7 ± 3.02 ^{a}	96.0 ± 2.22 ^{a}	72.9 ± 1.87 ^{a}	6.01 ± 0.18 ^{a}
PGPM_1	3.8 ± 0.05 ^{b}	5.4 ± 0.06 ^{a}	213.3 ± 2.21 ^{a}	95.7 ± 1.25 ^{a}	71.5 ± 1.10 ^{a}	5.24 ± 0.15 ^{b}
PGPM_2	4.0 ± 0.05 ^{a}	5.6 ± 0.07 ^{a}	213.0 ± 3.30 ^{a}	95.5 ± 1.84 ^{a}	70.8 ± 1.64 ^{a}	5.56 ± 0.20 ^{ab}
No_Biost	3.8 ± 0.04 ^{a}	5.3 ± 0.04 ^{a}	212.5 ± 3.57 ^{a}	96.3 ± 1.93 ^{a}	70.9 ± 1.87 ^{a}	5.69 ± 0.21 ^{a}
Biost_0.5%	3.8 ± 0.06 ^{a}	5.3 ± 0.08 ^{a}	216.4 ± 2.73 ^{a}	95.2 ± 1.84 ^{a}	71.3 ± 1.50 ^{a}	5.52 ± 0.16 ^{a}
Biost_1.0%	3.9 ± 0.04 ^{a}	5.4 ± 0.06 ^{a}	211.1 ± 2.71 ^{a}	95.7 ± 1.75 ^{a}	72.9 ± 1.62 ^{a}	5.60 ± 0.19 ^{a}
Microbial biofertilizer (A)	*	*	ns	ns	ns	*
Algae-based biostimulant (B)	ns	ns	ns	ns	ns	ns
A × B	ns	ns	*	ns	ns	ns
Growing season (C)	ns	ns	**	ns	ns	*
C × A	ns	ns	ns	ns	ns	*
C × B	*	*	ns	ns	ns	ns
C × A × B	ns	ns	ns	ns	ns	ns

Mean values belonging to the same factor without common letters are statistically different according to LSD ($P \leq 0.05$).ns, no significant differences; R color, red color; G color, green color; B color, blue color.

Table 4. Main effects of growing season, microbial biofertilizer and algae-based biostimulant on chemical quality parameters of marketable tomato fruits (mean ± SE; n = 27, 18 and 18 for growing season, microbial fertilizer and algae-based biostimulant, respectively)

	SSC (°Brix)	TA (%)	SAR	pH	β-Carotene (mg (100 g)$^{-1}$ FW)	Lycopene (mg (100 g)$^{-1}$ FW)
2022	5.24 ± 0.01 ^{b}	0.29 ± 0.006 ^{a}	18.06 ± 0.40 ^{a}	4.27 ± 0.03 ^{a}	6.76 ± 0.59 ^{a}	52.91 ± 2.17 ^{b}
2023	5.66 ± 0.07 ^{a}	0.32 ± 0.007 ^{a}	17.68 ± 0.56 ^{a}	4.23 ± 0.02 ^{a}	7.12 ± 0.40 ^{a}	57.15 ± 1.02 ^{a}
No_PGPM	5.42 ± 0.08 ^{a}	0.31 ± 0.010 ^{a}	17.48 ± 0.69 ^{b}	4.27 ± 0.03 ^{a}	6.11 ± 0.57 ^{b}	51.33 ± 1.47 ^{b}
PGPM_1	5.45 ± 0.07 ^{a}	0.30 ± 0.013 ^{a}	18.16 ± 0.52 ^{a}	4.25 ± 0.03 ^{a}	7.68 ± 0.53 ^{a}	59.58 ± 1.25 ^{a}
PGPM_2	5.47 ± 0.09 ^{a}	0.30 ± 0.011 ^{a}	18.23 ± 0.52 ^{ab}	4.24 ± 0.03 ^{a}	6.90 ± 0.70 ^{b}	53.35 ± 1.04 ^{b}
No_Biost	5.44 ± 0.07 ^{a}	0.31 ± 0.010 ^{a}	17.54 ± 0.50 ^{b}	4.26 ± 0.02 ^{a}	6.62 ± 0.58 ^{b}	53.79 ± 0.95 ^{b}
Biost_0.5%	5.46 ± 0.09 ^{a}	0.31 ± 0.013 ^{a}	17.61 ± 0.68 ^{b}	4.21 ± 0.03 ^{a}	6.86 ± 0.69 ^{b}	54.44 ± 1.48 ^{ab}
Biost_1.0%	5.44 ± 0.08 ^{a}	0.29 ± 0.010 ^{a}	18.76 ± 0.56 ^{a}	4.29 ± 0.03 ^{a}	7.34 ± 0.43 ^{a}	56.87 ± 1.32 ^{a}
Microbial biofertilizer (A)	ns	ns	**	ns	*	*
Algae-based biostimulant (B)	ns	ns	*	ns	*	*
A × B	ns	ns	ns	ns	ns	ns
Growing season (C)	**	ns	*	ns	ns	***
C × A	ns	ns	ns	ns	ns	*
C × B	ns	ns	ns	ns	ns	ns
C × A × B	ns	ns	ns	ns	ns	ns

Mean values belonging to the same factor without common letters are statistically different according to LSD ($P \leq 0.05$).ns, no significant differences; SSC, soluble solid content; TA, titratable acidity; SAR, sugar/acidity ratio.

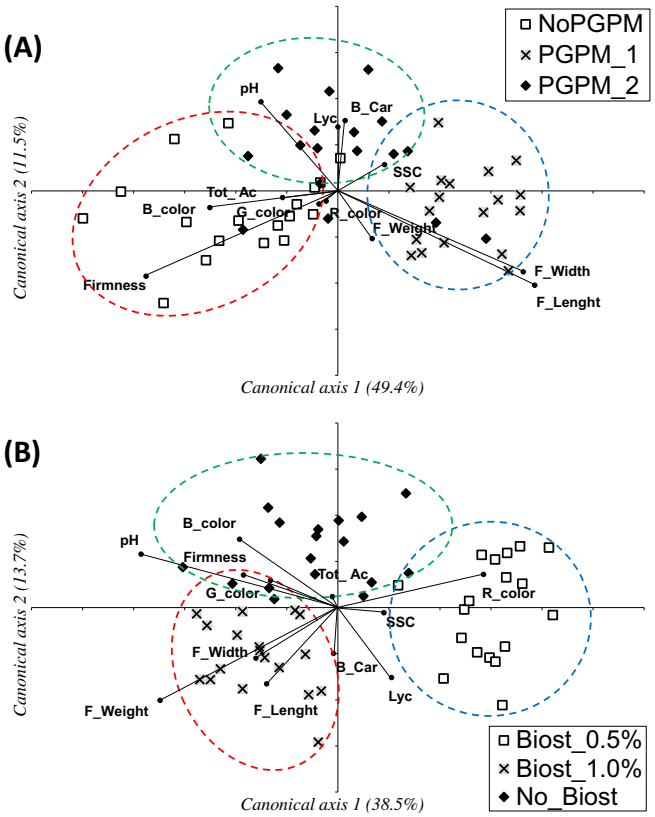

Figure 5. Biplot from CDA of the quality characteristics of tomato fruits collected at tomato harvesting grouped by microbial biofertilizer (A) and algae-based biostimulant (B). Data are combined for 2022 and 2023 growing seasons.

placed in the middle of both algae-based biostimulants and in the opposite position of the No_Biost. Penetrometer and Tot_Ac seemed to be associated with the No_Biost treatment (Fig. 5(B)).

DISCUSSION

Nowadays, it is well known that PGPMs and biostimulants facilitate plant growth.[21] Similarly, in this study, tomato plants after 30 days of transplanting showed enhanced morphological parameters, such as shoot and root biomass, number of leaves and plant height, when subjected to microbial biofertilizers in accordance with the findings of Roussis *et al.*[22] The same authors revealed that PGPMs determined more evident root systems implying that microbial biofertilizers by extending the root absorbing area increase the absorption surface and improve plant access to nutrients. This improves seedling rooting from the earliest stages after transplanting, reducing the problems associated with transplanting stress.[23] The enhancement of leaf number, size and chlorophyll content observed in tomato plants subjected to both microbial biofertilizers is indicative of less difficulty for seedling establishment and a rapid development of the tomato plants compared with No_PGPM. These morphological characteristics associated with a greater root system, especially in PGPM_2, may mitigate the negative effect of drought.[9] An increase in root growth parameters could have a positive effect on the nutritional status of the inoculated plants, since increasing the volume of soil explored by the roots.[24] Backer *et al.*[25] reported that PGPMs stimulate the production of plant hormones, plant defense-related traits and cell-wall-related genes, determining the development of longer roots.

Although during the whole tomato cultivation period in both growing seasons, no differences were detected in phenological phases among the treatments, tomato plants subjected to PGPM inoculation tended to show significantly higher values of SPAD and NDVI throughout the entire growing season with respect to No_PGPM. The increased plant growth conditions observed in inoculated plants determined an enhancement of plant architecture by producing a high number of leaves that improved their efficacy in terms of LAI and fraction of PPFD intercepted. Similarly, Mohanty *et al.*[26] observed that microbial biofertilizers improved

shoot growth, resulting in enhanced crop productivity. Similarly, foliar application of the algae-based biostimulant resulted in an increased tomato plant response in terms of LAI and fraction of PPFD intercepted. Cai *et al.*[27] showed that algal extracts may represent a source of substances associated with plant growth regulation and foliar spraying can facilitate their absorption in plants via stomata faster than root absorption. Indeed, the metabolite content in the algae-based biostimulants could pass during the opening and closing of the stomatal pores of tomato leaves and be transported in the whole plant. Cozzolino *et al.*[28] showed that the application of biostimulants may affect plant metabolism determining an enhanced resilience of treated plants against biotic and abiotic stresses. In this study, LAI and fraction of PPFD intercepted were enhanced when subjected to algae-based biostimulant, especially at the highest rate (Biost_1.0%), probably being associated with healthy plants with reduced impacts due to environmental stressors. In addition, tomato plants subjected to algae-based biostimulants showed high values of SPAD and NDVI, which are closely related to higher N content in the leaves, throughout the whole growing seasons of tomato crop. This behavior is in line with the findings of Battacharyya *et al.*[29] who observed that algal extract may integrate with fertilization strategy to improve nutrient uptake, especially nitrogen that resulted in increased root and shoot tissues. Shaaban[30] showed that algal extracts contain also other substances such as phytohormones, enzymes and vitamins that may play an important role in the assimilation of plant nutrients and their translocation, leading to significant increases in crop yield. Carillo *et al.*[31] demonstrated that inoculated plants were more vigorous, with larger fruits and a higher level of production compared with non-inoculated plants. In tomato crop, Wang *et al.*[32] reported that PGPMs can promote fruit and flower production, prolong the total duration of flowering and increase fruit yield. Furthermore, it is important to emphasize the greater number of marketable fruits and the lower number of rotten and green fruits in the treated plants compared to the untreated ones. Luna *et al.*[33] studied the influence of the application of different PGPMs on the yield, the number of fruits and the weight of fruits in tomato plants. The application of PGPMs by improving the nutritional status of plants induces changes in secondary metabolism by increasing the formation of nutraceutical compounds.[34] Decreased fruit number, increased fruit weight and diameter plus reduced fruit yields have been reported in tomatoes treated with different concentrations of biostimulants.[35-37] A study reported by Lakshmi *et al.*[38] states that biostimulant concentration may influence SSC and lycopene; in fact, the substances in the biostimulant are able to stimulate the latter's metabolic pathway. Sutharsan *et al.*[36] explained that fruit quality increases as biostimulant/biofertilizer does, probably because there is a greater availability of compounds, which contributes to plant growth regulator synthesis. Aguilera *et al.*[39] demonstrated that PGPMs can improve tomato yield and lycopene concentration compared with controls. PGPM application on tomato plants can affect quality characteristics, such as lycopene, sweetness index, β-carotene and lutein in greenhouse conditions[40] and field conditions.[41] Ordookhani *et al.*[42] found that the application of *Pseudomonas* + *Azotobacter* + *Azosprillum* + AMF treatment had the most effect on lycopene, antioxidant activity and potassium contents in tomato. According to Kaushal *et al.*,[43] the application of PGPMs could improve crop productivity in a sustainable and safer way compared to chemical inputs and therefore can be a solution for increasing and maintaining sustainable agricultural production with less environmental impact. Indeed, the adoption of plant growth-promoting rhizobacteria has become an important strategy in sustainable agriculture due to the possibility of reducing synthetic fertilizers, promoting plant growth and health and enhancing soil quality.[44] In addition, plants treated with PGPMs and/or algae-based biostimulants have a higher yield than untreated plants and confirmed the findings of Nasuelli *et al.*[45] who indicated that the effects of a mixed inoculation approach have been described as being greater than those of a single-kingdom inoculum. For example, increasing experimental data suggest that a close interaction between microalgae and bacteria synergistically affects their respective physiological and metabolic processes.[46] Moreover, co-cultivation/treatment of microorganisms (microalgae, bacteria, fungi) is increasingly considered an approach with promising implications for environmental biotechnology, also focused on the production of vegetables with improved quality.[47] Based on the result of this study, it is possible to state that plant colonization by PGPMs and application of algae-based biostimulant are an eco-friendly agricultural method to improve plant growth and productivity confirming the current increased interest of growers in biofertilizers and biostimulants, especially under organic farming systems where the application of synthetic products is avoided.

CONCLUSION

This study showed that the application of PGPMs combined with the foliar application of algae-based biostimulants can support the growth and fruit yield of processing tomatoes under organic cropping systems. The inoculation of tomato plants with PGPMs improved plant morphological parameters determining well-established plants mitigating transplanting stress of tomato seedlings and showing more resilient tomato plants compared to the non-inoculated plants for the whole growing season. At tomato harvesting, the improved fruit yield and the high value of marketable tomatoes showed that the combined application of PGPMs and algae-based biostimulants represents a promising strategy for improving sustainable vegetable production in organic farming. The adoption of innovative solutions in agriculture such as microbial biofertilizer and algae-based biostimulants can represent valid tools for regenerative agriculture able to match natural processes with sustainable agricultural productivity.

ACKNOWLEDGEMENTS

This research is a part of Valentina Quintarelli's PhD thesis entitled 'Impact of sustainable agronomic practices for the improvement of agricultural production', funded by the University of Ferrara. The paper is funded under the National Recovery and Resilience Plan (NRRP), Mission 04 Component 2 Investment 1.5 – NextGenerationEU, call for tender no. 3277 dated 30/12/2021. Open access publishing facilitated by Universita degli Studi di Ferrara, as part of the Wiley - CRUI-CARE agreement.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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