



# Article **Productivity and Nutritional Trait Improvements of Different Tomatoes Cultivated with Effective Microorganisms Technology**

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**Abstract:** The use of ecofriendly strategies, such as the use of Plant Growth Promoting Bacteria, to improve the yield and quality of crops has become necessary to satisfy the growing demand of food and to avoid the use of chemical fertilizers and pesticides. In this study, we report the effects of an innovative microbial inoculation technique, namely Effective Microorganisms (EM), compared with traditional approaches, on productivity and nutritional aspect of four tomato varieties: Brandywine, Corbarino Giallo, S. Marzano Cirio 3, S. Marzano Antico. Results showed an increase of plant productivity as well as an enhanced antioxidant activity mainly in San Marzano Antico and Brandywine varieties treated with EM technology. Moreover, the polyphenol and carotenoid contents also changed, in response to the plant treatments. In conclusion, the application of EM<sup>®</sup> technology in agriculture could represent a very promising strategy in agricultural sustainability.

**Keywords:** tomato; effective microorganisms; agricultural sustainability; antioxidant activity; Plant Growth Promotion Bacteria (PGPB)

# 1. Introduction

According to the data published by the Department of Economic and Social Affairs-Population Division of the United Nations, the human population was 7.7 billion in 2019 and it is expected to reach 9.7 billion in 2050 and 10.9 billion in 2100 [1]. The worldwide increase in world's population has generated a growing demand of food, and consequently the need of developing innovative techniques to enhance agricultural productivity [2]. Chemical fertilizers, manures, and pesticides represent the conventional strategies adopted to increase crop yields and to preserve the plants from pests [3]. However, the indiscriminate use of these chemical products damages the environment by polluting soil, air, and water [4–7].

The unforeseen harmful effects of chemical fertilizers and pesticides, together with the need of providing enough food to the growing world population, led politicians and researchers to focus on sustainability applied to intensive agriculture. The development of new eco-friendly strategies in intensive agriculture is necessary to counteract environmental, ecological, and health risks.

One of the most promising strategies in agricultural sustainability is the use of Plant Growth Promoting Bacteria (PGPB). Plant associated microorganisms can improve plant growth and health through different processes, generally classified as direct or indirect mechanisms [8]. They can directly act as biofertilizers by increasing the availability of resources that normally remain unavailable for the plants [9]. Indirect microbial PGP traits are essentially based on the "biocontrol" of phytopathogens via different systems, such as the production of one or more antibiotics or enzymes (chinatase,  $\beta$ -1,3-glucanase,



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). protease, lipase) or by the control of virulence factors acting on cell-to-cell communication mechanism known as Quorum Sensing (QS) [10].

Tomato is one of the most valuable crops worldwide. Nowadays it is globally produced, mainly thanks to the development of innovative agricultural techniques and the possibility to control growth conditions in the greenhouses.

Tomato perfectly meets industrial and market requirements, not only because of its pleasant organoleptic characteristics (as flavor, appearance and texture), but also as a result of its versatility. It can be directly commercialized as fresh fruit, but it can also be processed as sauce, powder, paste, juice, etc. Moreover, the importance of tomato is due to its high concentration of biologically active molecules, such as polyphenols, carotenoids (lycopene,  $\beta$ -carotene), folate and ascorbate (vitamin C), which makes it highly attractive not only to the agro-food industry, but also in cosmetics and pharmaceuticals [11,12].

Recent studies underlined the association between a diet based on high consumption of fruits and vegetables, with a reduced risk of cardiovascular diseases, cancer, ageing, and other chronic degenerative disorders [13]. Tomato is rich in bioactive phytochemicals whose activities beneficially affect human health because of their antioxidant (free radical scavenging), anti-inflammatory, anticarcinogenic, and antiatherogenic properties [14]. Therefore, new tomato cultivars with an increased bioactive compound level (e.g., lycopene,  $\beta$ -carotene, and polyphenol content), are considered high-quality "functional foods" because of the enhanced nutritional characteristics [15,16].

Two typical tomato varieties of the Campania region (south of Italy), namely San Marzano and Corbarino were selected for our study. San Marzano was selected for its nutritional features and global commercial importance, especially in processing products (peeled, pureed). In particular, both the ancient variety of this cultivar, named "San Marzano Antico" and the hybrid "San Marzano Cirio 3", designed to improve its cultivation parameters (increased plant productivity and better resistance to diseases), were studied. Corbarino, another typical tomato variety of Campania region, specifically in the Corbara area, has also shown interesting nutritional properties. In particular, the yellow variety (Corbarino Giallo) of this cultivar showed a high content of  $\beta$ -carotene associated with low levels of lycopene [17,18]. Brandywine is a tomato variety typical of North America. It was chosen as foreign tomato variety to be compared with typical Italian varieties.

This study aims to contribute to the discussion on the effectiveness of using PGPB in agriculture. More specifically, the effects of microbial inoculation on four tomato varieties (Brandywine, Corbarino Giallo, S. Marzano Cirio 3, S. Marzano Antico), with a specific EM<sup>®</sup> biofertilizer (EM-SCHWEIZ AG, Switzerland), were evaluated and compared to the same varieties cultivated with traditional methodologies. Carotenoid and polyphenolic contents, together with the antioxidant activity of lipophilic/hydrophilic/polyphenolic extracts, were selected as biomarkers for fruit quality.

#### 2. Materials and Methods

#### 2.1. Tomato Plants and EM Treatment

Tomato plants of Brandywine (BW), Corbarino Giallo (CY), San Marzano Cirio 3 (SMC3) and San Marzano Antico (SMA) were grown in an experimental field named 'Vado Cannata', a field in Accadia (Foggia, Southern Italy) presenting very good exposure to sun and water. Seeds of tomato germinated at the end of March 2018; 45-day-old tomato seedlings were transplanted in the 'Vado Cannata' field. The tomato plants (n.100 for each variety) were divided in three groups according the fertilizing techniques utilized during their growth: CONTROL (only water), BIODIN (common fertilizers), and BOK-EM (Bokashi plus EM-1). Sampling of fruits was performed in August 2018 at the maximum of ripening (red ripe for tomato such as San Marzano Cirio 3, San Marzano Antico, and Brandywine or fully yellow for the variety Corbarino Giallo). All tomato fruits were at the same degree of growth and ripening and without injuries. Fruits were counted and weighed. For each varieties, 40 fruits (randomly sampled) were selected and divided in

two groups that were analyzed separately for chemical-physical parameters and extraction process. Three technical measurements were done for each analysis.

#### 2.2. Biodynamic Agriculture

Biodynamic Agriculture (BIODIN) is one of the most important organic agriculture farming systems. This methodology is based on nitrogen fixation by leguminoses, a crop rotation and the use of organic, mainly composted solid manure [19]. The experimental field dedicated to BIODINtreatment was previously used for leguminose (lens, potato) cultivation for two years. Before the tomato seedlings, the soil was enriched with composted solid manure containing about 9% of minerals (Nitrogen, Phosphorous, Potassium and Magnesium), 38% of organic carbon, and 11% of humic acid. After one month, tomato plants of all varieties were planted. The composted solid manure was used as fertilizer every two weeks.

# 2.3. EM<sup>®</sup> Technology

EM<sup>®</sup> (Effective Microorganisms) technology has been described for the first time in 1970 by Prof. Teruo Higa at Ryukyus University in Okinawa [20]. EM<sup>®</sup> liquid solution included a mix of isolated soil microorganisms, such as lactic (*Lactobacillus plantarum*, *L. casei, Streptococcus lactis*) and photosynthetic bacteria (*Rhodopseudomonas lalustris* and *Rhodobacter speroides*), yeasts (*Saccharomyces cerevisiae* and *Candida utilis*), actinomicetes (*Streptomyces albus* and *S. griseus*) and fungi (*Aspergillus oryzae, Penicillum* sp., *Mucor hiemalis, Trichoderma harzianum*, and *Trichoderma viride*).

The experimental field dedicated to EM<sup>®</sup> technology was treated, at first, with Bokashi product, bran enriched with EM<sup>®</sup> liquid solution (EM-1). The benefit of Bokashi (EM plus organic matter) consists in the ability of the EM to ferment organic matter, thus released nutrients can be used by plants. Two weeks before planting the tomato seedlings, Bokashi was dispersed on the ground, and was mixed in the first 6 cm of soil. The amount of Bokashi used was 200 g mq<sup>-1</sup> of field. Next, EM-1 (liquid solution) was used on tomato plants starting from their transfer to the open field. Before the use, EM-1 was diluted 1:500 (v/v) in water and sprayed on the plants once every 7 days. The fertilizers application scheme was the same for all varieties.

#### 2.4. Analysis of Chemical-Physical Parameters

The total soluble solids (TSS) content of samples, expressed as °Brix, was estimated by using refractometer and sucrose (Sigma-Aldrich, Italy) as standard. The total titratable acidity (TA) of samples was evaluated by means of 0.1 N NaOH titration up to achieve a pH of 8.1 value. TA was expressed as grams of acid citric equivalent for 100 g of fresh product (g CA/100 g FW) [21].

#### 2.5. Extraction Process

For the bioactive components evaluation, each sample (100–250 g) was homogenized in a blender and then centrifuged at  $13,848 \times g$  for 20 min. The supernatants, consisting of the hydrophilic fraction, were collected and used in the antioxidant assay while pellets were extracted with diethyl ether (1:2 w/v; 20 min under stirring in the dark, repeated 3 times with fresh solvent) and methanol (1:2 w/v; 30 min under shaking in the dark, repeated 3 times with fresh solvent) with the aim to recover lipophilic and polyphenolic fractions, respectively [11].

#### 2.6. Antioxidant Activity

The antioxidant activity was evaluated by using DMPD, DPPH, and ABTS spectrophotometricmethods, which are based on the capacity of different components to inhibit the DMPD and ABTS radical cations (DMPD+ and ABTS+, respectively) and DPPH• radical [22–24]. The antioxidant capacity was expressed as mg eq Trolox 100g<sup>-1</sup> of fresh product.

# 2.7. Bioactive Compound Contents

# 2.7.1. Polyphenols

The total polyphenol content was estimated by using the Folin-Ciocalteau method. Briefly, 50  $\mu$ L of Folin-Ciocalteau's phenol reagent, a volume of samples ranging from 10 to 50  $\mu$ L and 800  $\mu$ L of deionized water were accurately mixed. After 1 min, 100  $\mu$ L of 20% sodium carbonate solution was added and further mixed. A final volume of 1 mL was reached by adding deionized water. Quercetin was used as standard. Samples were kept at room temperature for 2 h and then the total phenol content was estimated by reading at  $\lambda$ 765 nm (DU-Beckman, 5350 Lakeview Parkway South Drive, Indianapolis, IN 46268, USA).

#### 2.7.2. Lycopene and β-Carotene

Diethyl ether extracts from each sample were analyzed in order to estimate their lycopene and  $\beta$ -carotene contents by reverse-phase HPLC. The system was a Shimadzu LC-6A (Columbia, MD, USA) with a Kromasil 100A C18 column (5 µm, 250 mm × 10 mm; Phenomenex, Bologna, Italy), SPD 10A VP UV-visible detector, CR 3A recorder, SCL 10A VP system controller and Chemstation Class-VP 5.0 integration software. Immediately before injection, the diethyl ether extracts were dissolved in 2 mL of HPLC-grade dichloromethane and filtered with a 0.22 µm PTFE syringe filter. HPLC analysis was performed using the following chromatographic conditions: Gradient elution with water (A) and acetone (B), 25%/75% (v/v) A/B for 15 min, 5%/95% A/B for 12 min, 100% B for 5 min, and then return to starting condition in 5 min before next injection; flow rate, 3 mL min<sup>-1</sup>; wave length of UV detector, 450 nm, sensitivity adjusted to 0.04 AUFS (absorbance units full scale); room temperature. Solutions of standards (Lycopene and  $\beta$ -carotene by Sigma-Aldrich, Via Monte Rosa, 93,20149 Milano, Italy) were prepared at concentration of 1 mg mL<sup>-1</sup>. The lycopene and  $\beta$ -carotene in each sample were identified by comparison of the retention times, and by co-injection with standards.

#### 2.8. Statistical Analysis

All measurements were carried out in triplicate and results were statistically analyzed using GraphPad Prism 5.0 software to determine the mean  $\pm$  standard error of the mean (SEM) of at least three technical measurements. Statistical significance was assessed by one-way analysis of variance (ANOVA), and Tukey's multiple comparison test was used to obtain *p* values. Differences were considered significant at *p* < 0.05.

# 3. Results

The EM treatment (Bokashi plus EM-1), which was applied to the soil and then during the growth of tomato plants significantly affected the productivity of San Marzano Antico and Brandywine varieties, including the number and weight of the fruits (Table 1). No marked differences were detected for Corbarino Giallo and S. Marzano Cirio 3 between BIODIN and BOK+EM treatments (Tables S1 and S2).

The total yield expressed as Kg of fruits was improved by BIODIN and BOK+EM treatments in comparison with control. However, the use of EM technologies enhanced the yield of about 20%, more than BIODIN treatment. In particular, the improvement with BOK+EM was observed in the total number of fruits and in their weight.

Fruits of all varieties were further investigated for their chemical-physical parameters [21]. For all varieties, no relevant differences were detected between the treatments CONTR, BIODIN, and BOK+EM (Table 2 and Table S3).

TREATMENT -	Total Yield (kg)		Total Number of Fruits		Average Yield for Plant (Kg)		Average Weight of Fruit (g)		Average Number of Fruits per Plant	
	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine
CONTR	44.23 (a) *	53.07 (a)	$774\pm8.0$ (a)	$929\pm10$ (a)	$0.79\pm0.48$ (a)	$0.95 \pm 0.58$ (a)	$51.6\pm18.7$ (a)	$61.9\pm22.5$ (a)	$14.4\pm7.2$ (a)	$17.3 \pm 8.6$ (a)
BIODIN	101.67 (b) *	122.00 (b)	$1607\pm12$ (b)	$1928\pm14$ (b)	$1.85\pm0.71$ (b)	$2.22\pm0.85$ (b)	$64.4\pm9.4$ (b)	$77.3 \pm 11.4$ (b)	$27.3\pm7.9$ (b)	$32.8\pm9.5~\text{(b)}$
BOK+EM	123.80 (c) *	148.56 (c)	$1897\pm11$ (c)	$2276\pm13~\mathrm{(c)}$	$1.96\pm1.14$ (b)	$2.35\pm1.37$ (b)	$74.5\pm15.3$ (b)	$89.5\pm18.4$ (b)	$25.3\pm12.1~\text{(b)}$	$30.4\pm14.5~\text{(b)}$

Table 1. Production data of San Marzano Antico and	Brandywine tomato varieties.
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Results are expressed as mean  $\pm$  SEM. \* Letters in brackets indicate the significance for *p* < 0.05 (different letters indicate average values statistically different).

# Table 2. Total soluble solids (TSS) content, pH, and total titratable acidity (TA) of San Marzano Antico and Brandywine tomato varieties.

TREATMENT	рН		TSS (°BRIX)		TA (g CA/100 gFW)		Maturity Index (TSS/TA)	
	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine	San Marzano Antico	Brandywine
CONTR	$4.40\pm0.14$	$4.37\pm0.15$	$5.05\pm0.76$ (a) *	$5.03 \pm 0.72$ (a)	$0.32\pm0.05$ (a)	$0.32\pm0.05$ (a)	$15.66 \pm 2.02$ (a)	$15.63 \pm 2.00$ (a)
BIODIN	$4.36\pm0.08$	$4.32\pm0.06$	$4.36\pm0.45$ (b)	$4.32\pm0.43$ (b)	$0.29\pm0.04$ (b)	$0.27\pm0.04$ (b)	$15.31 \pm 2.36$ (a)	$15.27 \pm 2.35$ (a)
BOK+EM	$4.43\pm0.11$	$4.40\pm0.14$	$5.24 \pm 0.28$ (a)	$5.23 \pm 0.27$ (a)	$0.27\pm0.02$ (b)	$0.25\pm0.02$ (b)	$19.69 \pm 2.08$ (b)	$19.64\pm2.06$ (b)

Results are expressed as mean  $\pm$  SEM. \* Letters in brackets indicate the significance for p < 0.05 (different letters indicate average values statistically different). TSS, Total soluble solids; TA, total titratable acidity; CA, citric acid.

All samples were extracted with different solvents with the aim to evaluate the antioxidant activity of different fractions: hydrophilic, lipophilic, and methanolic. The amount of lipophilic extracts ranged between 34.4 mg100 g<sup>-1</sup> fresh product (San Marzano Cirio 3+EM) and 77.9 mg100 g<sup>-1</sup> fresh product (Corbarino Giallo), while the amount of methanolic extracts ranged between 489.9 mg100 g<sup>-1</sup> fresh product (San Marzano Antico+EM) and 1161,7 mg100 g<sup>-1</sup> fresh product (Corbarino Giallo). No significant differences were detected in hydrophilic fraction and pH, and none correlation between different treatments and yield of extracts was found (Table S4).

The total antioxidant activity related to hydrophilic, methanolic, and lipophilic fractions was evaluated by using DMPD, DPPH, and ABTS methods, respectively.

The antioxidant activity, related to different extracts of samples and expressed as mg Trolox100  $g^{-1}$  of fresh product, is reported in Figure 1A–C.



**Figure 1.** Antioxidant activity of lipophilic (**A**), methanolic (**B**), and hydrophilic (**C**) extracts evaluated by means of ABTS, DPPH, and DMPD methods, respectively. Polyphenol content (**D**) was evaluated by using the Folin–Ciocalteau method. BW (Brandywine), CY (Corbarino Giallo), SMC3 (San Marzano Cirio 3), SMA (San Marzano Antico). Results are shown as mean of three different measurements  $\pm$  SD. Statistical analysis was performed by subjecting data from the two different treatments BIODIN and BOK+EM versus CONTROL to Student's *t*-test. Statistically significant differences are indicated with: \* significant (*p* < 0.05) and \*\* very significant (*p* < 0.01).

Data showed that among all varieties, Brandywine and San Marzano Antico treated with BOK+EM revealed an increase of antioxidant capacity in the three fractions (lipophilic A, methanolic B, and hydrophilic C) compared with CONTROL and BIODIN treatments. The increase was significantly marked in Brandywine variety both for lipophilic extract (an increment of more than 70%) and methanolic fraction (an increment of more than 40%). Results of the Folin Ciocalteau assay (Figure 1D) performed on all samples, indicated a major polyphenol contents in Brandywine and San Marzano Antico varieties treated with BOK+EM (9.83 and 4.92 mg eq. quercetin100 g<sup>-1</sup> fresh product, respectively) compared with the same varieties treated with CONTROL and traditional method BIODIN (7.34 and 2.98 mg eq. quercetin100 g<sup>-1</sup> fresh product for BW and SMA, respectively). The enhancement in bioactive compound content was also confirmed by HPLC analysis, which was performed on the lipophilic extracts of each sample (Table 3). In particular, especially

in Brandywine variety but also in San Marzano Antico, an increase in lycopene and  $\beta$ carotene amounts was registered. These results are in agreement with the best antioxidant potential observed in lipophilic extracts of Brandywine and San Marzano Antico varieties.

**Table 3.** Lycopene and  $\beta$ -carotene contents in lipophilic extracts of tomatoes obtained by HPLC analysis.

Samples	Lycopene (mg 100 g <sup>-1</sup> Fresh product)	β-Carotene (mg 100 g <sup>-1</sup> Fresh Product)		
Brandywine CONTR	$20.16 \pm 1.5$ (a) *	$8.32 \pm 0.7$ (a)		
Brandywine BIODIN	$30.79 \pm 1.8$ (b)	$13.68 \pm 1.3$ (b)		
Brandywine + EM-1	$35.25 \pm 1.6$ (b)	$32.90 \pm 1.9$ (c)		
Corbarino giallo CONTR	n.d.	$15.62 \pm 1.1$ (a)		
Corbarinogiallo BIODIN	$3.89 \pm 0.4$ (a)	$46.76 \pm 2.3$ (b)		
Corbarinogiallo + EM-1	$3.34 \pm 0.4$ (a)	$26.70 \pm 1.8$ (c)		
San Marzano Cirio 3 CONTR	$9.87 \pm 0.8$ (a)	$3.25 \pm 1.1$ (a)		
San Marzano Cirio 3 BIODIN	$23.15 \pm 1.6$ (b)	$13.89 \pm 1.2$ (b)		
San Marzano Cirio 3 + EM-1	$14.47 \pm 1.2$ (c)	$7.23 \pm 0.8$ (c)		
San Marzano Antico CONTR	$10.26 \pm 1.3$ (a)	$4.04 \pm 0.6$ (a)		
San Marzano Antico BIODIN	$18.33 \pm 1.5$ (b)	$7.33 \pm 0.6$ (b)		
San Marzano Antico + EM-1	$18.40\pm1.4$ (b)	$8.22\pm0.9$ (b)		

Results are expressed as mean  $\pm$  SEM. \* Letters in brackets indicate the significance for p < 0.05 (different letters indicate average values statistically different between different treatments for each tomato variety). n.d. not detected.

# 4. Discussion

The optimization of tomato cultivation through a reduction of chemical fertilizers and pesticides is a topic of growing interest. A recent study reported that bacterial strains residing in the rhizosphere and endophytes of different tomato cultivars showed plant growth-promoting (PGP) abilities in vitro, which make them potentially applicable to eco-friendly fertilizing systems based on microbial inoculation [25].

The beneficial effects of microbial inoculation on the growth, yield, and nutritional parameters of tomato have been discussed by Berger et al. (2017) [26]. They reported an increased plant yield production and also taste-affecting compounds during the ripening process in tomato plants inoculated with the plant growth-promoting bacterium (PGPB) *Kosakonia radicincitans*.

One of the most ancient eco-friendly strategy in agriculture is the Biodynamic Agriculture. This methodology is based on nitrogen fixation by leguminoses, a crop rotation, and the use of organic, mostly composted, solid manure. The use of biodynamic agriculture can bring multiple benefits to tomato crops, both in terms of biometric parameters of the plant (plant height, fruit yield, etc.), and in the improvement of fruit quality [19]. Results reported in the present paper confirmed the efficiency of biodynamic treatment in tomato cultivation. Indeed, the productivity and nutritional qualities of all investigated tomato varieties grown with BIODIN treatment were improved compared to CONTROL treatment.

In previous papers, Higa described, for the first time, different pools of beneficial microorganisms, containing more than 80 species (photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, etc.), isolated from the soil and named effective microorganisms (EM), presenting their use in agriculture [20,27].

According to the data reported in literature, the present study, conducted on tomato plants, confirm the positive effects of EM inoculation on the growth of plants and nutritional aspect of fruits, in term of antioxidant activity. The benefits of EM technology have been assigned to several factors, such as the release of nutrients from Bokashi (EM plus organic matter), an improved photosynthesis, a major production of bioactive substances (such as hormones and enzymes), controlling soil diseases and accelerating the decomposition of lignin materials in the soil [28].

These factors could affect the biosynthesis of secondary metabolites, including polyphenols and carotenoids; then, an enhancement of antioxidant activity as well as bioactive metabolite contents were observed in some of the investigated tomato varieties. Previous papers revealed the role of EM compost as soil supplements, to obtain several benefits to tomato crop, both in terms of plant biometric parameters (plant height, fruit yield, etc.), and also increasing the fruit quality in terms of lycopene and Vitamin C contents, antioxidant activity, and defense enzyme activities [29,30]. The present study also suggested that the application of EM technology could improve the content of bioactive compounds in tomato fruits, in particular of polyphenols as resulted by Folin–Ciocalteau and DPPH methods. However, lipophilic and hydrophilic compounds also changed in their content or composition in light of an increased antioxidant activity estimated by ABTS and DMPD methods.

Since the first report [20], the scientific interest towards the use of EM in crop production has increased and its beneficial effects has been reported. In particular, a very interesting paper described the findings of a long-term field experiment for soil fertility and crop yield improvement by using effective microorganisms on wheat, one of the most important food crops in China [31]. The wheat straw biomass as well as grain yields and straw and grain nutrition parameters significantly increased when treated with EM technology compared with untreated samples. Similar results were achieved on apple, pea, rice, bean, soybean, and cotton plants [32–37]. Our results also confirm that the use of EM technologies enhanced the yield of crop production. In particular, an improvement of tomato fruits yield of about 20%, more than BIODIN treatment, was observed.

However, the use of EM technology did not have similar effects on investigated tomato varieties. A previous paper reported a study performed on bacterial strains isolated from different tomato cultivars. Among the total of 23 isolates, 11 were rhizospheric strains, residing in the rhizosphere (soil) or phyllosphere (the aerial habitat influenced by plants), and 12 were endophytic strains that reside in specific tissues of the plant (such as root cortex or xylem) and develop a close association with the plant, with exchange of nutrients, enzymes, functional agents and also "signals" [25]. Therefore, given that the soil and aerial condition were the same for investigated tomato varieties, their different behavior to EM technology could be linked to different endophytic microbial population that could act synergistically or not with EM.

Other beneficial effects of EM technology reported in literature were not limited to plant growth but also to composting process, waste treatment (water and solid), and phenol degradation [38–42].

# 5. Conclusions

The research of novel bio-based technology in agriculture is necessary both for ensuring soil quality and harvest protection, and for eco-sustainable production system by the reduction of consumption of chemical fertilizers and synthetic pesticides.

In conclusion, this study showed that the application of EM technology in agriculture represent a very promising eco-friendly strategy for increase crop production and for the enhancement in yield and healthy quality of crops.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2077-0 472/11/2/112/s1, Table S1. Production data of San Marzano Cirio 3 tomato variety. Table S2. Production data of Corbarino Giallo tomato variety. Table S3. Total soluble solids (TSS) content, pH and total titratable acidity (TA) of San Marzano Cirio 3 and Corbarino Giallo tomato varieties. Table S4. Overview table of tomato extraction steps.

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