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Article

Microbial Consortium Applications Can Affect Quality and Primary Metabolism of Processing Tomato

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Abstract: Microbial-based biostimulants containing arbuscular mycorrhizal fungi (AMF), *Trichoderma* fungi and plant growth-promoting rhizobacteria (PGPR) have been applied in an open-field tomato cultivation. A two-years field experiment (2020-2021) was performed in Southern Italy on "Heinz 1534" processing tomato hybrid, using three commercial formulations characterized by different microbial consortia (MIC: *Glomus* spp., *Rhizophagus* spp., *Bacillus* spp., *Streptomyces* spp., *Pichia* spp., *Trichoderma* spp.; EKO: *Glomus* spp., *Bacillus* spp., *Streptomyces* spp., *Pseudomonas* spp., *Arthrobotrys* spp., *Monacrosporium* spp., *Paecilomyces* spp., *Myrothecium* spp., *Trichoderma* spp.; FID: *Glomus* spp., *Bacillus* spp., *Trichoderma* spp.) and comparing them to untreated control (CTRL). The effect of growing season and microorganism-based treatments on yield, technological traits and functional quality of tomato fruits was assessed. The year of cultivation (Y) affected yield (with lower fruit weight, higher marketable to total yield ratio and higher % of total defected fruits in 2020), and technological components (higher dry matter, total acidity, total soluble solids in 2020). During the first year of the trial, all evaluated treatments (MIC, EKO and FID) enhanced soluble solids content by 10%, on average, compared to CTRL. Sucrose and lycopene contents were influenced both by the microbial-based treatments and the growing season (greater values found in 2021 with respect to the first year). Y factor also significantly affected all evaluated metabolites contents, except for tyrosine, essential (EAA) and branched-chain (BCAAs) amino acids. Over two years of field trial, FID biostimulant enhanced the contents of proteins (+53.71%), alanine (+16.55%), aspartic acid (+31.13%), γ-aminobutyric acid (+76.51%), glutamine (+55.17%), glycine (+28.13%), monoethanolamine (+19.57%), total amino acids (+33.55), essential amino acids (+32.56%) and branched-chain amino acids (+45.10%) compared to the untreated control. Our findings highlighted the valuable effect of FID microbial inoculant in boosting several primary metabolites (proteins and amino acids) in the fruits of processing tomato crop grown under Southern Italy environmental conditions, although no effect on yield and its components was appreciated.

Keywords: *Solanum lycopersicum*; mycorrhizae; *Trichoderma*; Plant Growth-Promoting Rhizobacteria; biostimulants; fruit quality

1. Introduction

Tomato (*Solanum lycopersicum* L.) ranks 3rd in vegetable crops production after potatoes and cassava, reaching a worldwide yield of around 187 million tons on a cultivated area of almost 5.05 million hectares in 2020 [1,2], and represents an excellent source of nutrients (Ca, K, Mg, organic acids and simple sugars) and health-promoting compounds including vitamins (B, E, C, K), phenolic compounds and carotenoids [3–5].

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Although lycopene [6], polyphenols and ascorbic acid are known as the most important health-related compounds in tomato fruits [3], noteworthy is their content of γ-aminobutyric acid (GABA). The biological role of this non-proteinogenic amino acid is not yet fully clarified, nevertheless GABA is widely recognized as a bioactive and functional compound in humans, as it mainly acts as an inhibitory neurotransmitter and as a restraint for blood pressure in hypertensive patients [7].

Quality of tomato is strongly affected by the genetic background of the variety; however, several external factors affect fruit composition such as agronomic techniques, growing conditions, production methods, harvest time and storage [8–10].

Among agricultural techniques, the application of microbial consortia containing arbuscular mycorrhizal (AMF), Trichoderma fungi, and plant growth-promoting rhizobacteria (PGPR) has been successfully used to improve plant growth, yield, abiotic stress tolerance, nutritional and functional quality of fruits in tomato [11–17]. Their applications are of particular interest in a context of climate change (referring to shifts in air temperature patterns, timing and amount of rainfall, and soil salinization) [18,19] to maintain or increase tomato yield and boost fruit quality attributes [11,20–22].

Considering the growing attention on biostimulants [23,24], several microbial consortia (with different fungi or/and bacterial composition and formulation) are continuously released on the market as beneficial products for tomato crops. Hence, it appears to be necessary to evaluate the effectiveness of different commercial formulations on agronomic and qualitative performance tomato crops in open field trials under different growing conditions.

Microbial inoculants mainly group AMF, fungi and free-living bacteria that act as biofertilizers [29]. These biostimulants have been largely applied to promote plant growth, increase nutrient uptake, stress resistance [29] and tomato fruit quality (soluble solids content, dry matter and metabolites) [14,30,31].

AMF establish a mutualistic symbiosis by creating specialized and highly branched hyphae inside the cell lumen of the roots, called arbuscules, which are efficiently used for nutrient exchange (in particular, P and N) [32]. The relationship is beneficial for both partners, as AMF enhance mineral nutrients exchange between soil and the host, while the plant supplies carbohydrates and represents a safe and necessary home for growth and reproduction of the guest [32].

Trichoderma fungi interact with the host plant colonizing its roots and establishing a symbiotic relationship. A molecular cross-talk is used to activate host defense system and promote plant growth and nutrient uptake [33]. *Trichoderma* is also widely applied for its biocontrol activity against the main root pathogens, which is exerted through mycoparasitism, antibiosis and competition for space and nutrients [34].

PGPR naturally populate the rhizosphere and colonize the root tissues of the host plant [35]. PGPR are endophytes and represent about 2 to 5% of total rhizobacteria and their mechanism of action can be direct or indirect [36]. Direct mode involves the production and the release of several bacterial compounds, such as phytohormones and volatiles, to facilitate nutrients uptake by the plant, lower ethylene level in plant and stimulate induced systemic resistance (ISR). Indirect mechanism is generated by the biocontrol action against diseases through the stimulation of beneficial symbioses or the production of antibiotic compounds.

The aims of this work were to *i)* assess the effect of three commercial products on yield components, technological traits and functional quality of processing tomato fruits; and to *ii)* identify the biostimulant better enhancing tomato fruit quality on a conventionally-managed processing tomato in the most important area of production in Southern Italy (Apulia region).

2. Results

2.1. Meteorological data

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The mean maximum and minimum air temperatures and total rainfall during the cropping cycles (April-May to August-September) were 29.5 °C and 17.0 °C and 89 mm for the year 2020, and 31.3 °C and 17.5°C and 120.2 mm for the year 2021, respectively (Figure 2).

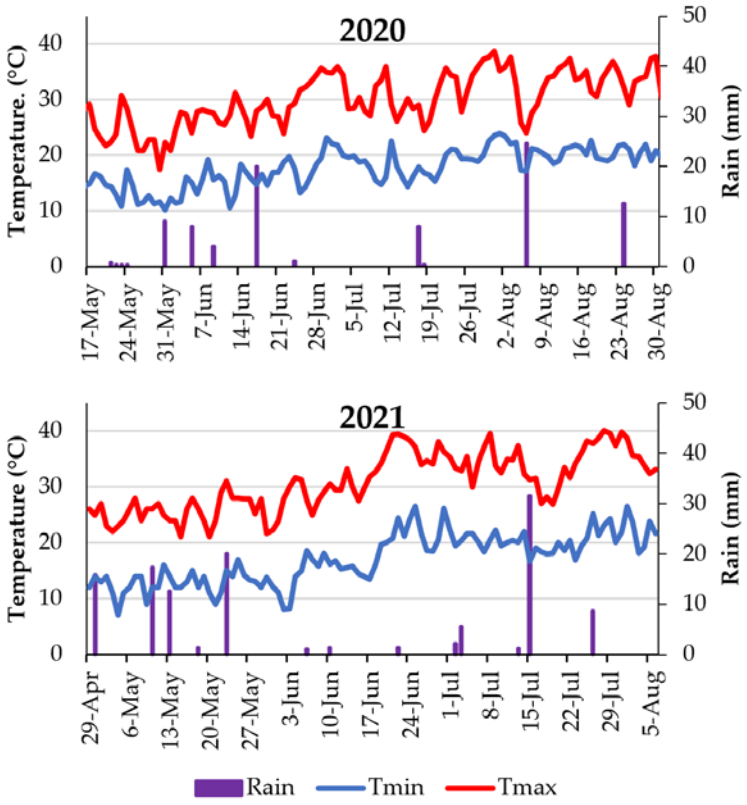

Figure 1. The mean maximum and minimum air temperatures and total rainfall during 2020 and 2021 growing seasons.

2.2. Yield and its components

As reported in Table 1, green yield (GY) was higher in 2021 (25.31 t ha^{-1}) with respect to 2020 (13.55 t ha^{-1}). Conversely, RY was lower in 2021 (3.73 t ha^{-1}) compared to the previous year (6.13 t ha^{-1}). No significant variations were found for MY (80.89 t ha^{-1} in 2020 and 79.52 t ha^{-1} in 2021) and TY (100.58 t ha^{-1} in 2020 and 108.56 t ha^{-1} in 2021), while MY to TY ratio was significantly higher in 2020 than 2021 year (80.89% and 73.74%, respectively). FW (69.91 g, as mean) was greater in 2021 than in 2020 (71.63 g *vs* 68.19 g), although no effect of this yield component was appreciable on MY across two years of field experiment.

Regarding fruit defects, microbial biostimulants had not significant effects in comparison with the untreated CTRL (4.42% of SsF, 14.67% of VrF, accounting to 19.08% of TDF, on average). However, valuable differences between the two years were appreciated. Indeed, in the first year the percentages of SsF and VrF were higher (5.83% and 26.50%, respectively) than in 2021 (3.00% and 2.83% for SsF and VrF, respectively).

Finally, no significant effect of T x Y interaction was found for the all yield-related traits reported in Table 1.

2.3. Chemical and technological traits

Fructose, total sugars (as sum of Glc, Fru and Suc), starch and lycopene contents were increased in 2021 with respect to 2020 (+31,2%, +15.8%, +67,3% and 32.1% for Fru, Tsu, Sta and Lyc, respectively) (Table 2). Conversely, a higher content of sucrose was detected in

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2020 compared to 2021 (0.82 μg g^{-1} and 0.23 μg g^{-1}, respectively). No significant variation of glucose and polyphenols contents was found in tomato fruits produced in both years.

MIC and FID induced an higher sucrose level with respect to EKO biostimulant (0.73, 0.64 and 0.27 μg g^{-1}, respectively), although MIC, EKO and FID treatments did not significantly differ from CTRL. EKO biostimulant also increased lycopene content in comparison with the CTRL (7.99 and 6.56 mg g^{-1}, respectively) (Table 2).

Regarding another relevant antioxidant compounds of tomato fruits, polyphenols were not affected by different biostimulant treatments in both years of field trial.

The applications of microbial consortia significantly affected sucrose content compared to CTRL in 2020. In particular, MIC increased Suc by 83.1%, while EKO inoculant worsened this trait (-63.4%) with respect to CTRL. (Table 2).

With respect to the technological quality of tomato fruits, TtA, SSC and DM were negatively affected by 2021 growing season (0.41 g%, 5.21 °Brix and 6.03 g%, respectively) compared to 2020 (0.51 g%, 5.41 °Brix and 6.53 g%, respectively), while pH was higher in 2021 (4.56) with respect to 2020 (4.41). No statistical difference was recorded among the three treatments and CTRL for all technological parameters. Conversely, a significant effect of T x Y interaction was found for SSC which was improved by biostimulants (+8.75%, +9.94%, +11.33% for MIC, EKO, and FID) with respect to untreated CTRL in the first year of experiment.

2.4. Primary metabolites content

As for most of the previously analyzed traits, the year of cultivation significantly affected all evaluated metabolites contents, except for Tyr, EAA and BCAAs. In fact, the contents of proline, histidine (included in the essential amino acids), asparagine, alanine, serine, ornithine, and glycine in tomato fruits in 2020 were of +108.5%, +86.5%, +72.5%, +69.3%, +51.0%, +50.0% and +21,4% higher than those of 2021. On the contrary, soluble proteins, GABA, aspartic acid, MEA, glutamine and glutamic acid contents were lower in the first year of field trial (-63.6%, -33.1%, -30.9%, -30.3%, 29.9% and 21.9%, respectively).

In 2021, FID biostimulant increased Prot (8.47 mg g^{-1}) and TAA contents (98.04 μmol g^{-1}) in comparison with untreated CTRL (5.05 mg g^{-1} and 57.19 μmol g^{-1}, respectively) (Table 3). Moreover, in the same year, EAA (including isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine) and BCAAs (including isoleucine, leucine and valine) contents were improved by FID biostimulant, which significantly differed from EKO and MIC biostimulants and the untreated CTRL (+80% and +115% compared to CTRL, respectively) (Table 3).

In Figure 2, a heat map analysis summarizing statistically significant tomato fruits' responses to different commercial biostimulant treatments (MIC, EKO and FID) compared to the control is represented. Based on this analysis, significant decreases in alanine and glycine content were found for MIC treatment with respect to the untreated CTRL in 2020 (-46,4% and -46,6%, respectively). A great effect of FID was noticed in 2021; indeed, this microbial consortium boosted the content of GABA, glutamine, glycine, essential amino acids (in particular that of arginine), aspartic acid and MEA (+136.7%, +110.1%, +95.6%, +80%, +53.5% and 39.2%) in comparison with respective controls (Figure 3).

Table 1. Effects of treatments (MIC = Micosat F Tab Plus, EKO = EKOprop NX, FID = Fidelius, CTRL = control), year and treatment x year interaction on yield components and fruit defects (cv "Heinz 1534").

	RY (t ha^{-1})	GY (t ha^{-1})	MY (t ha^{-1})	TY (t ha^{-1})	RY/TY (%)	GY/TY (%)	MY/TY (%)	FW (g)	SsF (%)	VrF (%)	TDF (%)
Treatment											
MIC	4.61	19.68	75.80	100.10	4.67	19.35	75.98	71.45	3.00	17.17	20.17
EKO	4.70	20.94	79.80	105.43	4.65	19.19	76.16	69.06	4.67	14.00	18.67

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FID	5.45	24.18	82.05	111.67	4.90	21.34	73.75	71.17	4.17	15.00	19.17
CTRL	4.97	12.93	83.18	101.08	4.93	12.49	82.58	67.96	5.83	12.50	18.33
Year											
2020	6.13 a	13.55 b	80.89	100.58	6.09 a	13.02 b	80.89 a	68.19 b	5.83 a	26.50 a	32.33 a
2021	3.73 b	25.31 a	79.52	108.56	3.48 b	23.17 a	73.34 b	71.63 a	3.00 b	2.83 b	5.83 b
Treatment x Year											
MIC 2020	6.26	10.85	79.42	96.54	6.45	11.15	82.40	69.54	3.00	32.33	35.33
EKO 2020	6.23	19.25	78.14	103.62	6.21	17.31	76.48	67.82	7.00	26.00	33.00
FID 2020	6.29	15.39	82.02	103.70	6.01	14.90	79.09	70.55	5.67	25.00	30.67
CTRL 2020	5.74	8.72	83.98	98.45	5.70	8.70	85.60	64.86	7.67	22.67	30.33
MIC 2021	2.96	28.52	72.18	103.65	2.89	27.56	69.56	73.36	3.00	2.00	5.00
EKO 2021	3.16	22.62	81.46	107.24	3.09	21.07	75.84	70.29	2.33	2.00	4.33
FID 2021	4.60	32.96	82.08	119.64	3.80	27.78	68.42	71.79	2.67	5.00	7.67
CTRL 2021	4.19	17.15	82.37	103.70	4.15	16.29	79.56	71.06	4.00	2.33	6.33
Significance											
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year	*	**	ns	ns	**	**	*	*	*	***	***
Treatment x Year	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mean	4.93	19.43	80.21	104.57	4.79	18.10	77.12	69.91	4.42	14.67	19.08

RY = rotten yield, GY = green yield, MY = marketable yield, TY = total yield, RY/TY = RY to TY ratio, GY/TY = GY to TY ratio, MY/TY = MY to TY ratio, FW = average fruit weight, SsF = sunscald fruits, VrF = fruits with viral symptoms, TDF = total defected fruits. ns, *, **, *** = non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences between treatments, year and treatment x year according to Tuckey's range test ($p \leq 0.05$).

Table 2. Effects of treatments (MIC = Micosat F Tab Plus, EKO = EKOprop NX, FID = Fidelius, CTRL = control), year and treatment x year interaction on chemical and technological characteristics of tomato fruits (cv "Heinz 1534").

	Glc (μg g^{-1})	Fru (μg g^{-1})	Suc (μg g^{-1})	Tsu (μg g^{-1})	Sta (μg g^{-1})	PP (μg mg^{-1})	Lyc (mg g^{-1})	pH	TtA (g% citric acid)	SSC (°Brix)	DM (g)
Treatment											
MIC	12.63	9.06	0.73 a	22.42	6.27	0.29	6.77 ab	4.44	0.48	5.35	6.28
EKO	12.11	9.52	0.27 b	21.90	6.52	0.31	7.99 a	4.52	0.44	5.33	6.33
FID	12.05	9.47	0.64 a	22.15	5.98	0.30	7.04 ab	4.48	0.47	5.38	6.32
CTRL	12.08	9.88	0.48 ab	22.44	6.31	0.30	6.56 b	4.50	0.45	5.17	6.19
Year											
2020	11.58	8.20 b	0.82 a	20.60 b	4.69 b	0.29	6.11 b	4.41 b	0.51 a	5.41 a	6.53 a
2021	12.86	10.76 a	0.23 b	23.86 a	7.85 a	0.31	8.07 a	4.56 a	0.41 b	5.21 b	6.03 b
Treat. X Year											
MIC 2020	12.24	7.80	1.30 a	21.34	4.60	0.26	5.40	4.39	0.54	5.47 a-c	6.58
EKO 2020	12.07	8.24	0.26 c	20.57	5.06	0.30	7.67	4.42	0.47	5.53 ab	6.74
FID 2020	10.61	7.87	1.03 ab	19.50	4.41	0.30	5.59	4.38	0.52	5.60 a	6.56
CTRL 2020	11.39	8.89	0.71 b	20.99	4.71	0.30	5.80	4.44	0.49	5.03 d	6.24
MIC 2021	13.03	10.33	0.16 c	23.51	7.95	0.31	8.15	4.49	0.42	5.23 a-d	5.98
EKO 2021	12.16	10.80	0.27 c	23.23	7.99	0.33	8.30	4.61	0.40	5.13 cd	5.92
FID 2021	13.50	11.07	0.24 c	24.81	7.55	0.30	8.50	4.58	0.41	5.17 b-d	6.07
CTRL 2021	12.77	10.87	0.24 c	23.88	7.91	0.30	7.32	4.56	0.41	5.30 a-d	6.14

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Significance											
Treatment	ns	ns	***	ns	ns	ns	*	ns	ns	ns	ns
Year	ns	***	***	*	***	ns	***	**	***	*	***
Treat. X Year	ns	ns	***	ns	ns	ns	ns	ns	ns	*	ns
Mean	12.22	9.48	0.53	22.23	6.27	0.30	7.09	4.48	0.46	5.31	6.28

Glc = glucose, Fru = fructose, Suc = sucrose, Tsu = total sugars, Sta = starch, PP = polyphenols, Lyc = lycopene, pH, TtA = titratable acidity, SSC = soluble solids content, DM = fruit dry matter, pH, TtA = titratable acidity, SSC = soluble solids content, DM = fruit dry matter. ns, *, **, *** = non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences between treatments, year and treatment x year according to Tukey's range test ($p \leq 0.05$).

Table 3. Effects of treatments (MIC = Micosat F Tab Plus, EKO = EKOprop NX, FID = Fidelius, CTRL = control), year and treatment x year interaction on primary metabolites of tomato fruits (cv "Heinz 1534").

	Prot (mg g^{-1})	**Ala (µmol g^{-1})**	**Asn (µmol g^{-1})**	**Asp (µmol g^{-1})**	**GABA (µmol g^{-1})**	**Gln (µmol g^{-1})**	**Glu (µmol g^{-1})**	**Gly (µmol g^{-1})**	**MEA (µmol g^{-1})**	**Orn (µmol g^{-1})**	**Pro (µmol g^{-1})**	**Ser (µmol g^{-1})**	**Tyr (µmol g^{-1})**	**TAA (µmol g^{-1})**	**EAA (µmol g^{-1})**	**BCAAs (µmol g^{-1})**
Treatment																
MIC	3.60 b	1.82 b	4.36 b	5.08 b	11.16 b	14.33 b	14.14	0.23 c	0.45 b	0.16	0.80	1.06	0.71 b	58.28 b	2.29 b	0.94 b
EKO	3.69 b	2.52 ab	4.97 ab	4.92 b	11.15 b	14.99 b	13.63	0.29 bc	0.44 b	0.14	0.66	1.26	0.72 b	59.70 b	2.59 b	1.09 b
FID	5.30 a	3.06 a	5.50 ab	7.24 a	18.31 a	22.65 a	16.18	0.41 a	0.55 a	0.15	0.71	1.47	1.02 a	82.71 a	3.42 a	1.48 a
CTRL	3.45 b	2.63 ab	6.20 a	5.52 b	10.37 b	14.60 b	14.78	0.32 b	0.46 ab	0.15	0.73	1.23	0.81 ab	61.93 b	2.58 b	1.02 b
Year																
2020	2.14 b	3.15 a	6.66 a	4.65 b	10.22 b	13.71 b	12.87 b	0.34 a	0.39 b	0.18 a	0.98 a	1.51 a	0.78	59.63 a	2.72	1.12
2021	5.88 a	1.86 b	3.86 b	6.73 a	15.28 a	19.57 a	16.49 a	0.28 b	0.56 a	0.12 b	0.47 b	1.00 b	0.86	71.67 b	2.72	1.14
Treat. X Year																
MIC 2020	2.30 c	2.10 b-d	4.99	4.16 b	8.90 b	10.89 b	11.05	0.22 b	0.41 bc	0.19 a	1.17 a	1.16	0.64	49.60 b	2.12 b	0.88 b
EKO 2020	2.30 c	3.38 ab	6.50	4.28 b	9.66 b	13.10 b	10.27	0.34 ab	0.34 c	0.15 bc	0.87 b	1.50	0.75	54.90 b	2.51 b	1.07 b
FID 2020	2.13 c	3.23 a-c	7.14	5.18 b	11.98 b	15.73 b	15.01	0.39 a	0.39 bc	0.18 ab	0.90 b	1.72	0.88	67.38 b	3.14 ab	1.28 ab
CTRL 2020	1.85 c	3.92 a	8.00	4.98 b	10.33 b	15.12 b	15.17	0.42 a	0.41 bc	0.19 a	0.99 ab	1.68	0.83	66.67 b	3.11 ab	1.27 ab
MIC 2021	4.90 b	1.54 d	3.72	6.01 b	13.43 b	17.77 b	17.24	0.23 b	0.49 bc	0.12 c	0.43 c	0.95	0.78	66.95 b	2.45 b	1.01 b

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EKO 2021	5.09 b	1.67 cd	3.43	5.56 b	12.64 b	16.88 b	16.98	0.23 b	0.53 b	0.13 c	0.45 c	1.03	0.70	64.51 b	2.66 b	1.11 b				
FID 2021	8.47 a	2.89 a-d	3.86	9.30 a	24.64 a	29.56 a	17.35	0.43 a	0.71 a	0.12 c	0.53 c	1.22	1.16	98.04 a	3.69 a	1.68 a				
CTRL 2021	5.05 b	1.34 d	4.41	6.06 b	10.41 b	14.07 b	14.39	0.22 b	0.51 b	0.12 c	0.46 c	0.79	0.80	57.19 b	2.05 b	0.78 b				
Significance																				
Treatment	***	*	*	***	***	***	ns	***	*	ns	ns	ns	**	**	*	*				
Year	***	***	***	***	***	***	**	*	***	***	***	***	ns	**	ns	ns				
Treat. X Year	***	*	ns	*	***	**	ns	**	**	*	*	ns	ns	**	*	*				
Mean	4.01	2.51	5.26	5.69	12.75	16.64	14.68	0.31	0.47	0.15	0.72	1.26	0.82	65.65	2.72	1.13				

Prot = proteins, Ala = alanine, Asn = asparagine, Asp = aspartic acid, GABA = γ-aminobutyric acid, Gln = glutamine, Glu = glutamic acid,Gly = glycine, MEA = monoethanolamine, Orn = ornithine, Pro = proline, Ser = serine, Tyr = tyrosine, TAA = total amino acids, EAA = essential amino acids and BCAAs = branched-chain amino acids. ns, *, **, *** = non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences between treatments, year and treatment x year according to Tuckey's range test ($p \leq 0.05$).

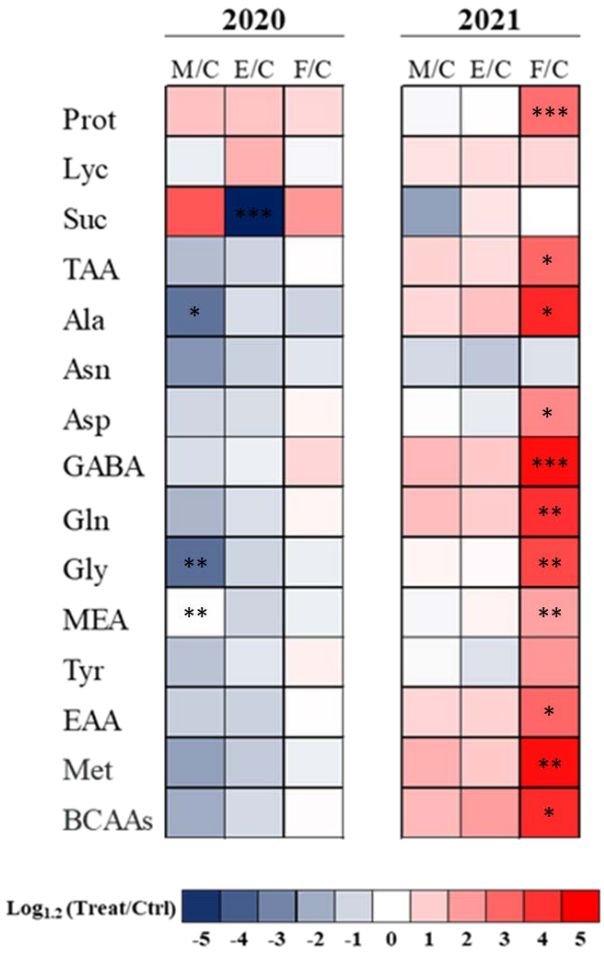

Figure 2. Heat map analysis summarizing statistically significant tomato fruits' responses to different commercial biostimulant treatments (M = Micosat F Tab Plus, E = EKOprop NX, F = Fidelius)

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compared to C = control. Results were calculated as Logarithm base 1.2 (Log1.2) of treatments/control in the two years of agronomic cultivation (2020-2021) and visualized using a false color scale with red indicating an increase and blue a decrease. No differences were visualized by blank squares. *, **, *** = significant at $p \leq 0.05$, 0.01, and 0.001, respectively and indicate significant differences between treatment x year according to Tuckey's range test ($p \leq 0.05$).

3. Discussion

Microbial-based biostimulants are often applied by tomato growers to improve yield and boost fruit quality under different environmental conditions. Moreover, these biostimulants have great effects under abiotic stress conditions (reduced water availability, soil salinity, and low endowment of nutrients), representing a valuable resource to mitigate the threatening consequences of changing climate on processing tomato crops [18,19,23,37].

In the present work, the effect of three commercial microbial-based biostimulants containing AMF, Trichoderma fungi and PGPR (Micosat F Tab Plus, EKOprop NX and Fidelius) on yield, technological traits and functional quality of tomato fruits was assessed.

Most of the evaluated attributes were significantly affected by the growing season. No variations in total and marketable yield were found between 2020 and 2021, although significant differences in terms of phytopathological problems were found in the two experimental years. In fact, during the crop cycle of 2020, valuable infections by *Fusarium* spp. (a fungi causing tracheomycotic disease) and *Tomato Spotted Wilt Orthotospovirus* (TSWV) were detected on the tomato plants. TSWV was measured at the harvest as number of fruits with viral symptoms (VrF), accounting up to 26.5% in the first year of field-trial. Infections by TSWV and *Fusarium* soil-born fungi accelerated the tomato crop cycle causing earlier plant ageing and fruit ripening (higher MY and RY to TY ratios in 2020). Furthermore, plants decay and leaves fall, as effect of both diseases, resulted in an over-exposition of the fruits to the sunlight and therefore in higher percentage of SsF (sunscald fruits) in 2020 in respect to 2021 field-trial. A higher percentage of GY on TY in 2021 than the previous year was related to an early harvesting as well as a good phytosanitary status of the plants and a great availability of water by rainfall and irrigation supplies. According to our findings, Colla et al., 1999 [38] reported a positive effect of increases in irrigation volumes on fruit coverage and fruit weight, as well as on reduction of sun burning on the fruits. The same relationship between FW and water regime was also reported by Di Cesare et al., 2012 and Patanè and Saita, 2015 [39,40]. Variations in DM, pH, TtA, and SSC could be related to a different irrigation volume between the year of experiment. Indeed, different works highlighted worsening in DM, SSC and TtA attributes under increasing in water supplies in processing tomato crops [38–41].

In the first year of experiment, all microbial-based biostimulants positively affected SSC in comparison with the CTRL, in fully accordance with previous works [42,43]. SSC represents a crucial parameter in quality evaluation of processing tomato fruits and thus for its profitability, as the optimization of industrial processes (e.g., production of tomato paste) is highly dependent on soluble solids content of the incoming fruits [44].

EKO treatment over the two years of experiment significantly increased lycopene content, which is the main antioxidant molecule of tomato fruit, responsible for the bright red color, the quality and the shelf-life of tomato and tomato-derived products [21]. This carotenoid is able to detoxify ROS, particularly hydroxyl radicals, and stimulate antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase in plant cells, providing an increased resistance to oxidative stresses (e.g., salinity, drought, high light, etc.) and a shelf-life extension. Moreover, it can exert the same action in animal cells, thus improving the nutritional and nutraceutical properties of tomatoes [45], in particular preventing oxidative stress mediated carcinogenesis [46–48]. In the year 2020, EKO also resulted in decreasing in sucrose content, which was probably used as source of carbon skeletons and/or energy for the synthesis of lycopene, since very high

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costs for the synthesis of organic compounds for oxidative stress or osmotic protection (50–70 moles ATP for mole) are required [49]. Whereas sucrose underwent a non-statistically significant but effective accumulation in fruits from plants under Fidelius and Micosat treatments compared to control ones, thus probably exerting a function of osmolyte and stabilizer of macromolecules, as previously shown in leaves and roots of rice plant under water shortage [50]. In these plants, the environmental stress induced an increase of the expression of sucrose synthase (*OsSuSy*) and sucrose transporter (*SUC2*) thus enhancing sucrose synthesis and transport under water stress conditions [50].

FID in 2021 was able to induce a strong increase of GABA in tomato fruits. GABA in its zwitterionic form may act as an osmolyte balancing the water potential during cellular dehydration, and as an antioxidant stabilizing and protecting the structure and function of macromolecules [51]. Moreover, GABA is widely recognized as a bioactive and functional compound in humans, as it, in addition to its role of hypotensive, can increase immune functions under stress, prevent diabetes and cancer and control blood cholesterol levels [52–54]. Over two years, FID treatment was also able to produce an increase of tyrosine content, which was independent from protein degradation, since a higher Prot content was induced by the same biostimulant in comparison with the control. Tyrosine could function as a precursor in the synthesis of tocopherols and other lipophilic antioxidants [55][55], but it could itself function as an antioxidant as suggested by Shahidi and Zhong, 2007 [56]. FID increased only in 2021 the content of monoethanolamine (MEA), a metabolite deriving from the decarboxylation of the photorespiratory serine and fundamental for the synthesis or regeneration of membrane phospholipids, functions that allow the plant to have a greater resistance to ROS. Therefore, the application of FID allowed tomato plants to reuse the photorespiratory amino acids and ammonium to synthesize this useful metabolite and decreasing the pressure of photorespiration on stressed plants [57].

An increase in the content of total AA, alanine (Ala), glutamine (Gln) and essential amino acids (EAA), in particular methionine (Met) and branched-chain amino acids (BCAAs; leucine, isoleucine and valine together) were detected under FID treatment over two years, particularly in 2021. Alanine is a precursor of CoA and is also implicated in membrane phospholipid synthesis, fatty acid synthesis and degradation, and plays an active role in secondary metabolism and plant response to biotic and abiotic stresses [58]. The increase of glutamine under abiotic stress has been reported in wheat [59,60], as well as its possible role in osmotic adjustment and macromolecule protection [61,62]. Glutamate content that can affect tomato flavor and fruit palatability since it can elicit intense umami taste, was not affected by biostimulant treatments [57]. Essential amino acids (EAA) content was also enhanced by FID treatment, particularly in the second year of experiment. Their improvement and in particular that of BCAAs can be useful both as compatible compounds and as alternative electron donor for the mitochondrial electron transport chain [63–65]. Moreover, BCAAs have a high nutraceutical value since recent findings demonstrated that they are able to decrease oxidative stress in mice and rats by an unknown mechanism [66,67]. The accumulation of methionine can be correlated to its role as precursor of BCAAS, in particular of isoleucine [64].

4. Materials and Methods

4.1. Location

An agronomic trial was carried out in an open field at Foggia (41°32’45.4”N; 15°36’18.4”E) (Southern Italy) during a two-year period (2020-2021) in a typical Haploxerepts soil (Soil Taxonomy; USDA, 2014) [68]. Physical and chemical soil properties were as follows: sand 41%, silt 20%, clay 39%, limestone 19.3 g kg^{-1}, pH 7.52, organic matter 22.9 g kg^{-1}, total nitrogen 1.66‰, P_2O_5 10.6 mg kg^{-1}, K_2O 268 mg kg^{-1}, C/N ratio 7.99, and Cation Exchange Capacity (CEC) 25.1 meq 100 g^{-1}. The climate of this region was typically Mediterranean.

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4.2. Experimental design and microbial-based treatments

The processing tomato hybrid "Heinz 1534" (Furia Seed, Monticelli Terme (PR), Italy), showing determinate habitus and blocky/round fruits, was adopted in this experiment. Seedlings were transplanted on 19th May 2020 and 29th April 2021 in paired rows, with a 0.38 m spacing along the rows and 0.40 m among the rows. The distance between pairs was 1.40 m, with a plant density of 2.24 plants m^{-2}.

N, K and P requirements were calculated on the basis of soil analysis and the expected fruit yield (under these environmental conditions), and were supplied before transplanting and during the crop cycle according to the phenological growth stages (125 kg ha^{-1} of N, 76 kg ha^{-1} of K_2O, and 109 kg ha^{-1} of P_2O_5 in 2020; 80 kg ha^{-1} of N, 140 kg ha^{-1} of K_2O, and 107 kg ha^{-1} of P_2O_5 in 2021).

Irrigation scheduling was based on evapotranspiration of the crop (Etc) and calculated as Etc = Eto × Kc. Eto (reference evapotranspiration) and tomato crop coefficient (Kc) were determined according to Hargreaves and Samani [69] and Allen et al. [70]. Water supplying (equal to 100% Etc) occurred when 40% of total available water was depleted, according to the evapotranspiration method reported by Doorenbos and Pruitt [71]. Finally, a total irrigation volume equal to 3694 m^3 ha^{-1} of H_2O were applied in 2020 through 19 supplies, while 23 irrigations were done in 2021 throughout crop cycle, amounting to 4500 m^3 ha^{-1} of H_2O.

Three commercial consortia of microorganisms were evaluated: Glomus aggregatum, G. intraradices, G. mossae, G. etunicatum, Bacillus amyloliquefaciens, B. licheniformis, B. subtilis, B. laterosporus, B. mojavensis, Trichoderma harzanium, T. koningii (FID) [Fidelius, Intertec, Bibbiena (AR), Italy]; Glomus spp., Bacillus spp., Streptomyces spp., Pseudomonas spp., Arthrobotrys spp., Monacrosporium spp., Paecilomyces spp., Myrothecium spp., Trichoderma spp. (EKO) [EKOprop NX (Green Ravenna srl, Ravenna (RA), Italy]; Glomus coronatum, G. caledonium, G. mosseae, G. viscosum, Rhizophagus irregularis, B. subtilis, Streptomyces spp., Pichia pastoris, Trichoderma harzanium, T. viride (MIC) [Micosat F Tab Plus (CCS, Quart (AO), Italy] (Table S1, Supplementary Materials).

These products, thereafter referred as FID, MIC and EKO, were compared with untreated control (CTRL). Treatments were carried out at two times: 48 hours before transplant and at 10 days after transplanting (DAT). The first application was carried out in plant nursery by dipping the seedlings (raised in cellular containers) up to the collar in an aqueous solution for each of the three commercial blends at these concentrations: 0.2 g L^{-1} for FID or MIC and 0.1 g L^{-1} for EKO. Ten days after transplanting a second inoculum was done supplying the commercial microorganism consortia through drip irrigation (without fertilizers dissolved in water) at doses of 1 kg ha^{-1} for FID and EKO, and 2 kg ha^{-1} for MIC.

Weed control and plant protection were performed according to the cultivation protocols of the Apulia region (Italy).

A randomized block design with three replication was realized and each plot, containing 20 plants, measured 3.8 m × 1.8 m (6.84 m^2).

4.3. Yield and Merceological Assessment

Manual harvestings were carried out on 2 September in 2020 (106 DAT) and on 6 August in 2021 (99 DAT), when about 90% of fruits were ripe. Total yield (TY) was assessed sorting and weighting the fruits in three commercial categories: MY = marketable yield, GY = green yield (unripe fruits) and rotten yield (RY). RY to TY (RY/TY), GY to TY (GY/TY) and MY to TY (MY/TY) ratios were also reported in percentage. Mean fruit weight (FW) was evaluated on 100 red-ripe fruits randomly chosen from each plot. The same sample was used to determine the incidence (%) of sunscald (SsF) and fruits infected by TSWV (*Tomato Spotted Wilt Orthotospovirus*) (VrF) showing typical chlorotic blotches and ringspots as symptoms.

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4.4. Technological Characteristics

Dry matter content (DM) was determined drying 20 g of homogenized tomato sample in a stove at 72 °C until constant weight. The soluble solids content (SSC) was assessed using a digital refractometer (Refracto 30PX, Mettler-Toledo, Novate Milanese, IT), and results were expressed as °Brix. pH and titratable acidity (TtA) were evaluated using the pH-Matic 23® titroprocessor, equipped with a pH electrode (model 5011T) (Crison Instruments, Barcelona, Spain). TtA was expressed as g of citric acid 100 g^{-1} juice [72,73].

4.5. Fruit Metabolic Profiling

Thirty well-ripened fruits per plot were washed and dried, and then sliced and homogenized in a Waring blender (2 L capacity, Model HGB140, PartsTown, Addison, IL, USA) for 1 min, then shock frozen in liquid nitrogen and transported on dry ice to the laboratory of Plant Crop Physiology of University of Campania "Luigi Vanvitelli," where they were ground to a fine powder in liquid nitrogen and either used immediately for assays or stored at –80 °C.

4.6. Starch and soluble sugars analysis

Starch and soluble sugars were extracted according to Dell'Aversana et al. 2021 [74] with some modifications. Fresh tomato fruits (20 mg) were submitted to two subsequent extractions with 250 ml ethanol 80% (v:v) and a final extraction with 150 ml ethanol 50% (v:v) at 80 °C for 20 min. The tubes were cooled in ice and centrifuged at 14,000 g for 10 min at 4 °C. The clear supernatants of the three following extractions were pooled together and stored at -20 °C until analysis. The pellets of the ethanolic extraction were heated at 90 °C for 2 h in 500 µL of 0.1 M KOH (Salbitani et al. 2022 [75]). After cooling on ice, the samples were acidified to pH 4.5, mixed 1:1 with a hydrolysis buffer containing sodium acetate 50 mM pH 4.8, α-amylase 2 U/ml and amyloglucosidase 20 U/ml, and incubated at 37 °C for 18 h. The samples were centrifuged at 14,000 g for 10 min at 4 °C, and the supernatant containing the glucose derived from starch hydrolysis was used for measurement. The content of glucose, fructose, and sucrose in the ethanolic extracts, and the glucose derived by starch were determined by an enzymatic assay coupled with reduction of pyridine nucleotides and the increase in absorbance at 340 nm was recorded using a Synergy HT spectrophotometer (BioTEK Instruments, Bad Friedrichshall, Germany) as described in Carillo et al. 2019 [45]. The content of sugars was expressed as mg g^{-1} DW.

4.7. Polyphenols and lycopene analysis

The total polyphenol content was determined by using the Singleton Folin–Ciocalteu [76] method with some modifications. An aliquot (50 mg) of tomato samples were suspended in 700 µL of 60% methanol (v:v), then centrifuged at 25 °C for 10 minutes at 13,000 g. Aliquots of the clear supernatant (35 µl) were added to 125 µl of the Folin–Ciocalteu reagent diluted with H_2O milli Q (1:1 v:v) and 650 µL of 3% (w:v) sodium carbonate. After 90 minutes of reaction at room temperature, the absorbance at 760 nm was determined by a Synergy HT spectrophotometer (BioTEK Instruments, Bad Friedrichshall, Germany). The total polyphenol content in the samples was evaluated with a standard curve obtained using known concentrations of gallic acid (GAE) as a standard. Total phenols were expressed as mg GAE g^{-1} DW. Lycopene concentration (mg g^{-1} DW) was evaluated according to Sadler et al. 1990 [77], with the modifications described in Carillo et al. 2019 [45]. An aliquot of tomato samples (50 mg) was suspended in 380 µL of hexane:acetone:methanol (2:1:1 v/v), containing 0.05% (w/v) BHT (butylated hydroxytoluene) to minimize oxidation. Blanks were prepared without tomato extracts. The suspensions were mixed and continuously agitated on an orbital shaker for 30 min. Samples were then centrifuged at 4 °C for 10 min at 14 000 g, and an aliquot of 100 µL of the organic phase of orange color was transferred in an Eppendorf tube and 1.4 mL hexane was added. The absorbance at

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472 nm was measured by a microplate reader. Lycopene concentration was estimated for comparison with standard curves of pure lycopene and expressed as mg g^{-1} DW.

4.8. Soluble proteins and free amino acid analysis

Soluble proteins were extracted by mixing 20 mg of fresh tomato fruit with a buffer containing 200 mM TRIS-HCl pH 7.5 and 500 mM $MgCl_2$ at 4 °C for 24 h. The clear supernatants (10 µl) were added to 190 µl of Protein Assay Dye Reagent Concentrate (Bio-Rad, Milan, Italy) diluted with H_2O milli Q (1:5 v:v). The soluble protein content in the samples was calculated by comparison with standard curves obtained using known concentrations of bovine serum albumin (BSA) as the reference standard (Carillo et al. 2019). Proteins were expressed as mg g^{-1} DW. Free amino acids were extracted from 40 mg of tomato samples in 1 ml ethanol:water (40:60 v:v) overnight at 4 °C and estimated by HPLC after pre-column derivatization with *o*-phthaldialdehyde (OPA) according to Dell'Aversana et al. 2021 [74]. Proline was determined in the same ethanolic extract according to Carillo et al. 2019 [45] and expressed as µmol g^{-1} DW.

4.9. Data Analysis

All data were submitted to Analysis of variance (one-way ANOVA) by using GENSTAT 17th software package (VSN International, Hemel Hempstead, UK). Tukey test was used to separate means, when the F test of ANOVA for treatment was significant at $p < 0.05$. MY/TY, RY/TY and GY/TY ratios, expressed as percentage, were submitted to Arcsin transformation before ANOVA analysis.

A heat map was generated in Excel, summarizing the responses of the two years cultivation and the three biostimulant treatments. Results were calculated as the logarithm base 1.2 ($Log_{1.2}$) ratio of biostimulant treatments to control plants. Results were visualized using a false color scale with red indicating an increase and blue a decrease, whereas white squares indicated no differences (Giordano et al. 2022 [78][78]).

5. Conclusions

Microbial-based biostimulants represent promising means to improve yield and quality in processing tomato crop. Several commercial products based on AMF, *Trichoderma* and PGPR consortia are continuously released on the market, making necessary to evaluate their effectiveness under different environments and growing conditions. Our findings revealed no significant effect of MIC, EKO and FID treatments on yield, its components and most of the technological traits. Despite the significant influence of the year of cultivation, which was mainly related to phytopathological problems affecting the crop in 2020, over two years FID biostimulant enhanced several evaluated parameters (proteins content, alanine, aspartic acid, γ-aminobutyric acid, glutamine, glycine, monoethanolamine, total amino acids, essential amino acids and branched-chain amino acids) of tomato fruits with respect to the control. Furthermore, all biostimulant products enhanced soluble solids content in 2020. Based our evidence, FID appears as the best microbial inoculant to enhance primary metabolites (proteins and amino acids) in processing tomato fruit (cv "Heinz 1534") under Southern Italy growing conditions.

The results of this study contribute to obtaining more applied insights into the use of microbial-based biostimulants capable of improving the nutritional and functional quality of processing tomato crop. However, due to the complexity of the applied microbial consortia, it remains to be clarified whether it is useful to apply formulates containing more than 3-4 microorganisms (including fungi, bacteria and other). In fact, microorganisms live in dynamic equilibrium into the soil and compete for space and resources. This aspect should therefore be investigated and clarified.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: "Detailed composition of commercial formulations containing different microbial consortia".

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