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Combined Biostimulant Applications of *Trichoderma* spp. with Fatty Acid Mixtures Improve Biocontrol Activity, Horticultural Crop Yield and Nutritional Quality

Stefania Lanzuise [1,*,†], Gelsomina Manganiello [1,†], Valentino M. Guastaferro [1], Cono Vincenzo [1], Paola Vitaglione [1], Rosalia Ferracane [1], Alfeo Vecchi [2], Francesco Vinale [3,4], Solomon Kamau [5], Matteo Lorito [1,6] and Sheridan L. Woo [4,6,7]

1 Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; gelsomina.manganiello@unina.it (G.M.); valentinomaria.guastaferro@unina.it (V.M.G.); cono.vince@gmail.com (C.V.); paola.vitaglione@unina.it (P.V.); rosalia.ferracane@unina.it (R.F.); matteo.lorito@unina.it (M.L.)
2 Alpha BioPesticides, St John's Innovation Centre, Cowley Road, Cambridge CB4 0WS, UK; alfeo.vecchi@alphabiocontrol.com
3 Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy; francesco.vinale@unina.it
4 Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Italy; woo@unina.it
5 Department of Land Resource Management and Agricultural Technology (LARMAT), College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 29053, Nairobi 00625, Kenya; solkam08@gmail.com
6 Task Force on Microbiome Studies, University of Naples Federico II, 80055 Portici, Italy
7 Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy
* Correspondence: stefania.lanzuise@unina.it
† These authors contributed equally to this work.

Abstract: The growing demand for safer foods reflects the public perception of the adverse consequences of chemicals used in agriculture. This research aimed at developing new biological formulations based on the combination of small microbial consortia containing two *Trichoderma* spp., with a medium–long chain fatty acids mixture (FAm). The bioactivity of these formulations was investigated on different vegetable crops in terms of biocontrol, growth promotion, yield and quality improvements. FAm application reduced *Botrytis cinerea* necrosis by up to 90% compared to the infected control plants and some of the assayed *Trichoderma* spp. + FAm combinations contained Rhizoctonia disease, reaching more than 90% reduction of tomato and lettuce plant mortality. An increasing yield, ranging to 25 and 90%, was recorded on treated tomato, lettuce and kohlrabi compared to untreated plants. A significant enrichment in carotenoids (+60%) and glucosinolates (+39%) was measured on biotreated plants compared to controls. Untargeted LC-MS analysis revealed a higher content of tomatine and dehydro-phytosphingosine, glycoalkaloids involved in defense responses, in *Trichoderma* spp. + FAm combination-treated plants. The combined biostimulant applications of *Trichoderma* spp. with FAm were able to improve the performances of different horticultural plant species, producing a premium quality marketable vegetable with higher antioxidant content.

Keywords: microbial consortia; bioactive compounds; plant protection; plant growth promotion; *Rhizoctonia solani*; *Botrytis cinerea*; sustainable agriculture

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1. Introduction

The world population is currently 7 billion people, and it is expected to grow to more than 9 billion by 2050 [1], leading to a significant increase in demand for agricultural products, considering human consumption, animal feed, seeds and raw materials for industrial use and biofuel production. The FAO estimates that, in the same period, global food demand will grow around 60%, with peaks of 100% in developing countries [2].

During the past 50 years, increases in food production were achieved mainly by undue application of external inputs and the growth in global consumption of fertilizers, pesticides and herbicides increased considerably [3]. These intensive agricultural practices have caused severe damage to wildlife habitats and negatively affected human health, led to groundwater contamination, reduction in useful microflora and microfauna, increase in greenhouse gas emissions and generalized pollution phenomena [4].

The need to make food production systems more sustainable, allowing significant yield increases, improving crop quality, and reducing the negative impact on the environment and human health, has become imperative [5,6]. To date, sustainable agriculture practices can bring environmental and economic benefits for farmers, communities, and nations, making better use of internal resources and minimizing external input applications. The European Community established a regulatory framework for the sustainable use of plant protection products, promoting integrated pest management and application of beneficial microorganisms and/or their active metabolites as an effective alternative to pesticides [7].

Therefore, the application of beneficial microorganisms is a promising strategy to improve the productivity, reliability, and sustainability of the global food chain. Beneficial plant–microbe associations result in more effective nutrient uptake, improved growth and development, and higher tolerance to biotic and abiotic stresses [1,8–11]. Furthermore, it is well known that the application of beneficial microorganisms can induce plant defense responses in colonized/treated plants, thus producing an increase in crop yield [12–14]. To date, a few hundred products based on bacteria, fungi and yeast are used to enhance crop protection, plant growth and productivity [7,15,16]. However, as reported in the CMC-7 (Component Material Categories, number 7), only beneficial microbes belonging to *Azotobacter* spp., mycorrhizal fungi, *Rhizobium* spp., and *Azospirillum* spp. are registered as biostimulant products, highlighting a deep legislative limitation for other beneficial microorganisms marketing themselves with proven biostimulant activities. In view of these considerations, the European Biostimulant Industry Council (EBIC) is proposing to expand and update the CMV-7 list adding other microbial genera, supported by scientific evidence of their activity and safeness [17]. Among them, different *Trichoderma* spp. are well known for their biostimulant activities. Numerous studies highlighted the growth promotion effect of different *Trichoderma* strains on multiple plant species, such as melon, tomato, cucumber, eggplant, pea, lettuce, rocket, beans, and ornamental plants [13,18–23]. Furthermore, numerous species of *Trichoderma* are used as active ingredients in over 50 bio-fungicides present on the world market for their ability to contain important soil-borne and foliar pathogens, including *Fusarium*, *Sclerotinia*, *Botrytis* and *Pythium* [7]. The effectiveness of *Trichoderma* biocontrol activity is due to various mechanisms of action including competition for space and nutrients, mycoparasitism and the production of lytic enzymes and antimicrobial compounds [24–26]. Furthermore, these fungi are able to interact with the plant by root colonization, increase nutrient bioavailability, produce bioactive compounds useful for the plant metabolism, and induce defense mechanisms [27,28].

The current work was aimed to investigate and define new biological formulations based on small microbial consortia, able to promote plant growth and trigger the host plant defense responses against invading pathogens. The association of multiple compatible microorganisms within biological formulations and their different biocontrol mechanisms may enhance disease suppression [29] Compatible interactions can provide a greater spectrum of action and reliability in different environmental conditions, increasing the possibility of synergistic/additive effects of the present beneficial microbes [30]. For their features, *Trichoderma* spp. represent an important component of these new multifunctional products [31–34]. The effectiveness of bioformulations can be improved combining beneficial microorganisms with other natural substances such as plant or microbial secondary metabolites, which are able to inhibit pathogen development, induce systemic resistance and promote plant growth [6,35]. Several studies reported that free fatty acids from microbial, animal or plant origins represent an important source of bioactive compounds with antibacterial and antifungal activity [36–40]. For instance, [41] highlighted the fungi-

cidal activity of several unsaturated fatty acids such as linolenic, erucic and oleic acids against phytopathogenic fungi *Rhizoctonia solani*, *Pythium ultimum*, *Pyrenophora avenae* and *Crinipellis perniciosa*.

Activity of free fatty acids is mainly associated with their ability to penetrate the phospholipidic layer, increasing the fluidity and permeability of biological membranes, with consequent structural and functional alterations [42,43]. Fatty acids inhibit important enzymatic activities in different cellular compartments, affecting nutrient absorption, generating free radicals from peroxidation and/or lipid autoxidation and inducing direct cell lysis [44–46].

Specifically, the current work aimed at developing innovative formulations containing a medium and long chain (C_{14}–C_{20}) fatty acids mixture (FAm) of vegetable origin in combination with two selected *Trichoderma* strains, *T. harzianum* M10 and *T. virens* GV41, well known as biocontrol agents. In particular, *T. harzianum* M10 is an important producer of harzianic acid, a secondary metabolite able to promote plant growth and to contain the development of multiple plant pathogens such as *Pythium irregulare*, *Sclerotinia sclerotiorum* and *R. solani* [47,48]. On the other hand, biocontrol action of *T. virens* GV41 is mainly associated with its ability to produce antimicrobial compounds, such as gliotoxin and gliovirine, powerful inhibitors of oomycetes [25,49]. In addition, isolates belonging to these *Trichoderma* species are important producers of indolic compounds, which can play an important role in promoting plant growth [50].

The bioactivity of these innovative formulations was investigated on different vegetable crops (tomato, lettuce, kohlrabi) in terms of biocontrol, growth promotion and yield and quality improvements. In addition, an untargeted LC-MS analysis was carried out to investigate their effect in modulating tomato plant secondary metabolite productions.

2. Materials and Methods

2.1. Fatty Acid Mixture Features (FAm)

FAm is the result of two decades' research. It is a formulation based on an active substance containing a specific and unique range of unsaturated carboxylic acids (carbon chain length C_{14}–C_{20}—479.8 g L^{-1}). The raw material is the by-product redundant from the production process for extra virgin olive oil. Olives are obtained from the Mediterranean region and must meet stringent quality standards and specifications to ensure conformity. The extraction and production processes of mixtures are purely physical processes, excluding the addition of chemical substances. Extraction is the result of an ISO Quality Controlled process of multi-stage distillation and fractionation. To ensure solubility in water the unsaturated carboxylic acids are formulated with potassium hydroxide to form potassium salts.

2.2. FAm Biocontrol Activity In Vitro

The biological activity of the Fatty Acid mixture (FAm) against foliar and soil-borne phytopathogens was evaluated in in vitro assays. The FAm efficacy was compared to thiophanate-methyl, a common active ingredient of fungicides, no longer commercially used. The assays were conducted using different concentrations of FAm (0.3%, 0.5%, 1%, 1.5%, 2%, 3%, 4%) in solid PDA, selected on the observed bioactivity in a previous pilot assay (data not shown). Thiophanate-methyl was added to the PDA considering the application doses according to the manufacturer's technical data sheet (0.5%). Plugs of 0.5 cm in diameter from 7-day-old cultures of *Botrytis cinerea* and *Rhizoctonia solani* were placed in the center of the PDA plates. Pathogens growing on non-enriched PDA were used as negative controls. Each treatment consisted of three replicates. The plates were incubated at 25 °C and the radial growth of the phytopathogenic fungi was recorded daily for seven days. The same procedure was applied for the compatibility test between FAm and *Trichoderma* spp.

2.3. FAm Biocontrol Activity In Vivo

The biocontrol activity of FAm against the foliar pathogen *B. cinerea* was evaluated in vivo on tomato (*Solanum lycopersicum* cv. San Marzano), cucumber (*Cucumis sativus* cv. Marketmore) and zucchini (*Cucurbita pepo* cv. San Pasquale) plants. Fifteen-day-old seedlings of each horticultural crop were maintained in 14 cm diameter pots containing sterile soil in the greenhouse at 18/15 °C (day/night), 70% RH with a photoperiod of 12 h day/light, and 12 night/dark. The infection was carried inoculating 10 µL of *B. cinerea* spore suspension (10^6 spores mL^{-1}) on 5 leaves/plant, considering twenty-seven plants per treatment. After pathogen inoculation, the temperature was decreased to 15/10 °C and RH was increased to 90–100%, allowing the pathogen development at the optimum conditions. To evaluate any phytotoxic effect, a pilot test was conducted applying FAm alone at 0.3%, 0.5%, 1%, 1.5%, 2%, 3%, 4% on plant leaves. A further experiment was carried out considering only the best-performing concentrations. In particular, 10 mL of FAm applied at 0.3%, 0.5% and 1% was sprayed on the infected leaves 24 h post-pathogen inoculation. The positive controls were infected applying the same procedure and then were treated with 10 mL of sterile demineralized water; the negative controls consisted of untreated healthy plants. The disease development was monitored for 7 days, recording the number of attacked leaves and the size of the necrotic areas.

2.4. Compatibility between FAm and Trichoderma spp.

The compatibility between FAm and the biological control agents was assayed following the procedure reported in Section 2.2. In particular, the radial growth of *Trichoderma harzianum* M10 and *Trichoderma virens* GV41 on PDA enriched with increasing doses of FAm was compared to the radial growth of the same strains in un-amended PDA. Data were collected every 24 h for seven days. The growth inhibition percentage was calculated by using the formula:

$$\text{Growth inhibition } (\%) = 100 - \frac{C - T}{C}$$

where C = *Trichoderma* spp. grown on PDA and T = *Trichoderma* spp. grown on FAm-enriched PDA.

2.5. Biocontrol Assay of FAm + Trichoderma spp. Combinations

The fungal pathogen *Rhizoctonia solani* was grown for 15 days in PDB and the biomass was harvested by filtration through a cheesecloth filter. Five grams of semi-dried fungal biomass were homogenized using an Ultra-Turrax homogenizer (T25, Ika Works Inc., Wilmington, NC, USA) with 100 mL of sterile distilled water. Soil infection was carried out by adding the obtained fungal pathogen suspension kg^{-1} of sterile soil. FAm was applied at 0.3%, 0.5%, and 1%, while combined formulations were obtained by diluting *Trichoderma* spores in the FAm solutions, to obtain a final suspension of 10^6 spores mL^{-1}. Two-week-old lettuce (*Lactuca sativa* cv. Signorella) and tomato (*Solanum lycopersicum* cv. San Marzano) plants were transplanted onto 10 cm diameter pots containing *Rhizoctonia solani*-infested soil. Two days after transplanting, plants were drenched with 10 mL of single (either *Trichoderma* spp. or FAm) and combined components (*Trichoderma* spp. + FAm). Negative controls were obtained by watering soil with 10 mL of sterile distilled water. Each set of treatments consisted of three replicates of 9 plants each, organized in randomized blocks (27 plants per treatment). All the assays were carried out in a greenhouse under controlled temperature and humidity conditions (at 25 °C day/night, 70% RH). Disease development was monitored for 7 days, recording the percentage of plant mortality.

2.6. Growth Promotion Assay of Trichoderma spp. + FAm Combinations

Tomato seeds (cv. San Marzano nano) were transferred to polystyrene boxes (n. 40 holes; Ø 6; 5 mL volume) containing sterile soil. After sowing, each polystyrene box was covered with wet paper and left in the greenhouse under controlled conditions of temperature and humidity (at 25 °C day/night, 70% RH). Ten-day old seedlings were drenched with

5 mL of single strain, FAm solutions or combined formulations, as detailed before. Plants were then transplanted into 12 cm diameter pots containing sterile soil and drenched with 50 mL of single and combined formulations in three replicates of 9 plants each, arranged in randomized blocks. Negative controls were obtained drenching the soil with sterile demineralized water. The formulations were applied weekly for a total of 4 applications over the experimental period. At the end of the fourth week, plants were collected, and stem and root dry weights were recorded.

2.7. Evaluation of Trichoderma spp. + FAm Combinations on Agronomic Parameters

Experiments were carried out in a tunnel greenhouse located at the University of Naples Federico II, Portici (NA), south Italy (40°490 N, 14°150 E; 72 m a.s.l.). Tomato cv. San Marzano (La Semiorto, Italy) lettuce cv. Signorella (La Semiorto, Italy) and kohlrabi cv. di Vienna bianco (La Semiorto, Italy) were used as test crops in field assays. The seeds of the three crops were transferred to polystyrene boxes (n. 40 holes; Ø 6; 5 mL volume) containing double sterilized soil and allowed to germinate in the greenhouse under controlled temperature and humidity conditions (at 25 °C day/night, 70% RH, 12 h photoperiod). One week after emergence, 15 seedlings/treatment were watered with 5 mL of single and combined solutions (*Trichoderma* spp. + FAm). Successive applications were performed weekly, for a period of three weeks. Seedlings were then transplanted in soil under plastic tunnels and drenched with 50 mL of single and combined formulations of *Trichoderma* spp. and FAm (*Trichoderma* spp. + FAm). Subsequent applications were performed every 15 days for a further two months by watering each plant with 150 mL of the different formulations. Each treatment was organized in a completely randomized design. For tomato crop, ripe fruits were harvested three months after seedling transplanting. The harvest was repeated following the scalar fruit ripening from July to September. For lettuce, yield was assessed two months after seedling transplant by measuring head fresh and dry weights, whereas for kohlrabi, the effectiveness of *Trichoderma* spp. + FAm formulations were assessed four months after seedling transplanting by manually measuring (by tape measure) the circumference and fresh weight of the green part of the crop.

2.8. Assessment of Trichoderma spp. + FAm Combination Effects on Nutritional Quality of Crops

Tomato: fresh fruits harvested at the deep red stage were washed and pulverized to a homogenous mixture using a waring blender and an Ultra-Turrax homogenizer (T25, Ika Works Inc., USA). An aliquot of the obtained mixture was lyophilized and passed through a 1 mm sieve. A total of 100 mg was extracted three times using 2.5 mL of ethanol-hexane (4/3 v/v) and filtered through a 0.45 μm polyethersulphone filter (Millex HV13, Millipore, Bedford, MA, USA). The extracts were dried under nitrogen flow in dark tubes and resuspended in 1 mL chloroform with 1% butylated hydroxytoluene (BHT). Twenty microliters of each extract were analyzed using High Pressure Liquid Chromatography (HPLC-LC 10, Shimadzu, Osaka, Japan) with a flow rate of 0.8 mL min^{-1}, diode array detector and a Supelcosil C_{18} column (250 4.6 mm; 5 μm, 100 Å particle size—Supelco, Bellefonte, PA., USA). Carotenoid separations were achieved using the following linear gradient: 82% A, 18% B; 20 min, 76% A, 24% B; 30 min, 58% A, 42% B; 40 min, 39% A, 61% B, 45 min, back to 82% A, 18% B. A and B were acetonitrile and a methanol–hexane–methylene chloride (1:1:1, $v/v/v$) mixture, respectively. Carotenoid quantification was achieved by calibration curves obtained from commercial β-carotene in the linearity range of 1.56–50 μg mL^{-1}, equation y = 76101x, R^2 = 0.999, LOD 0.5 μg mL^{-1} and LOQ 1 μg mL^{-1} and TLC-purified lycopene in the linearity range of 6.25–200 μg mL^{-1}, equation y = 2755.7x, R^2 = 0.975, LOD 2.5 μg mL^{-1} and LOQ 5 μg mL^{-1}, using Class M10-A Shimadzu software. The concentrations of a 1% lycopene solution in hexane and of a 1% β-carotene solution in chloroform were calculated using the extinction coefficients for lycopene (E 1% of 3450 at 471 nm) and for β-carotene (E 1% of 2396 at 465 nm). A sum of the individual carotenoids (Lycopene, β-carotene, fitofluene, fitoene, luteine) are hereafter referred to as total carotenoid content.

Lettuce: About 0.4 g freeze-dried sample of plant material was extracted twice with 8 mL of MeOH/water/formic acid (25/24/3-*v*/*v*/*v*) and filtered through a 0.45 µm polyethersulphone filters (Millex HV13, Millipore, Bedford, MA). An amount of 20 µL of the extract was analyzed using an HPLC system (LC 10, Shimadzu, Osaka, Japan). Separations were done using a Supelcosil C_{18} column (250 4.6 mm; 5 µm, 100 Å particle size—Supelco, Bellefonte, PA, USA). The mobile phases were water with 5% formic acid (A) and methanol (B) with a solvent flow rate of 1 mL min^{-1}. The separation was carried out under a gradient with the following conditions: 5% B in A, reaching 40% B at 25 min, and then remaining isocratic for 5 min. The UV chromatograms were recorded at 330 and 520 nm. Each sample was repeated three times. The identification of phenolic compounds was carried out according to their UV spectra and retention times. Chemical commercially available standards of chlorogenic acid (used for the quantification of caffeic acid derivates as caffeoyl-malic acid, caffeoyl tartaric acid, cynarin) and chicoric acid (Sigma Aldrich, St. Louis, MO, USA) were used to aid the identification of phenolic compounds.

The calibration curve of chicoric acid was constructed in the linearity range 12.5–100 µg mL^{-1}, the equation was y = 97121x, R^2 = 1, LOD 0.2 µg mL^{-1} and LOQ 0.5 µg mL^{-1}. The calibration curve of chlorogenic acid was constructed in the linearity range 12.5–100 µg mL^{-1}, equation was y = 67341, 6819x, R^2 = 0.999, LOD 0.5 µg mL^{-1} and LOQ 1 µg mL^{-1}. Chlorogenic and chicoric acids were quantified using the respective calibration curves. Caffeoyl-tartaric acid, caffeoyl-meso-tartaric acid and cynarin were expressed as chicoric acid equivalents; tartaric acid and malic acid were expressed as chlorogenic acid equivalents [51]. A sum of the individual phenolic compounds is hereafter referred to as total polyphenolic content.

Kohlrabi: glucosinolates were extracted using 70% boiling methanol (1.5 mL) from lyophilized powder (100 mg) in a water bath at 70 °C for 5 min to inactivate endomyrosinase. The mixture was centrifuged at 12,000 g for 10 min at 4 °C, and the supernatant collected. The residue was re-extracted twice following the same procedure, and the supernatants combined. Glucosinolates in the methanol kohlrabi extracts were analyzed using a HPLC-MS system as previously reported by [52]. Mass spectrometry characteristics of analyzed glucosinolates are reported in Table 1.

Table 1. Mass spectrometry characteristics of analyzed glucosinolates.

Compound	$[M-H]^-$ *m*/*z*	Production *m*/*z*
Sinigrin	358	97
Glucoraphanin	436	97
Glucoalyssin	450	97
Glucoiberin	422	97
4-Hydroxyglucobrassicin	463	97
4-Methoxyglucobrassicin	477	97
Glucobrassicin	447	97
Neoglucobrassicin	477	97
Glucoiberverin	406	97
Glucoerucin	420	97
Glucotropaleoin	408	97

Sinigrin and glucotropaeolin standards were purchased from Extrasynthese (Genay, France). The calibration curve of sinigrin was established in the linearity range of 0.1–25 µg mL^{-1}, equation y = 27424x + 18069, R^2 = 0.997, LOD 0.05 µg mL^{-1} and LOQ 0.1 µg mL^{-1}. The calibration curve of glucotropaeolin was constructed in the linearity range 0.1–50 µg mL^{-1}, linear equation was y = 38027x + 31627, R^2 = 0.998, LOD 0.03 µg mL^{-1} and LOQ 0.07 µg mL^{-1}. Glucoraphanin, glucoalyssin, glucoiberin and glucoiberverin were expressed as equiva-

lents of sinigrin; glucobrassicin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, neoglucobrassicin and glucoerucin were expressed as equivalents of glucotropaeolin. Data were reported as the sum of all the glucosinolates identified and quantified in the samples.

2.9. Assessment of Trichoderma spp. + FAm Combination Effects on Tomato Plant Metabolism

The effect of *Trichoderma* spp. + FAm treatments on tomato plant metabolism was investigated analyzing the whole plant organic extracts by mass spectrometry (LC-MS). The untargeted metabolome analysis was carried out as previously reported by [28]. In particular, 10 mg of ground tomato leaves were used for metabolite extraction in 0.8 mL of 20% methanol in water and the chemical profiling was performed in a 6540 UHD Accurate Mass QTOF LC-MS/MS mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The mass spectra were analyzed by submitting them to Agilent MassHunter Profinder and MassProfile Professional Software to compute the annotation and statistical analysis. Only compounds with a minimum absolute abundance of 5000 counts were considered. FC was calculated using the untreated plant (Control) as the reference. Subsequently, the filtered entities were identified using ID browser identification associated with a Metlin Library (Agilent Technologies, Palo Alto, CA, USA).

2.10. Statistical Analysis

Data obtained from the in vitro/vivo biocontrol activity, growth promotion assay, field experiments and chemical analyses were subjected to the statistical analysis by GraphPad Prism Software. Ordinary one-way ANOVA was applied to test the effects of the *Trichoderma* + FAm combinations on the assessed parameters. In all cases, the statistical analysis of variance (ANOVA) was corrected for multiple comparisons by the Bonferroni hypothesis test, considering a p-value ≤ 0.05.

3. Results

3.1. In Vitro and In Vivo FAm Biocontrol Activity

The radial growth of the foliar pathogen *Botrytis cinerea* on 3% and 4% FAm-containing medium was considerably reduced, compared to the control, 120 h post inoculation (hpi) (Figure 1a), showing a radial growth inhibition of 51% and 87.5%, respectively. No significant differences were detected comparing the effect of 3% and 4% FAm and thiophanate-methyl (TM) and comparing FAm concentration ranging to 0.3% and 2% with the control.

On the other hand, all the assayed FAm concentrations were able to inhibit the growth of the soil-borne pathogen *R. solani*, reaching a 65% pathogen growth reduction at the lowest concentrations (0.3 and 0.5%) (Figure 1b). The bioactivity of FAm applications resulted comparable to those observed for commercial fungicide TM.

The biocontrol activity of FAm was evaluated also in vivo on cucumber (*Cucumis sativus*), zucchini (*Cucurbita pepo*) and tomato (*Solanum lycopersicum*) against the pathogen *B. cinerea* (Figure 2). The product applied at 0.3% and 0.5% showed containment of the disease symptoms for the three tested horticultural crops compared to infected control. However, there were no significant differences between these two treatments (Figure 2a–c). On the other hand, 1% FAm was the most effective treatment in containing *B. cinerea* infection (Figure 2a–c), reducing necrosis by up to 90% compared to the infected control plants.

3.2. Compatibility between FAm and Trichoderma spp.

A reduction in *Trichoderma* radial growth was observed only on 10 and 20% FAm-containing medium and only in the first 48 h post inoculation while, in the following days, the growth resulted comparable to that observed for the control (un-amended PDA). Consistently with these observations, *Trichoderma* spp. was not negatively affected by FAm at all the tested concentrations.

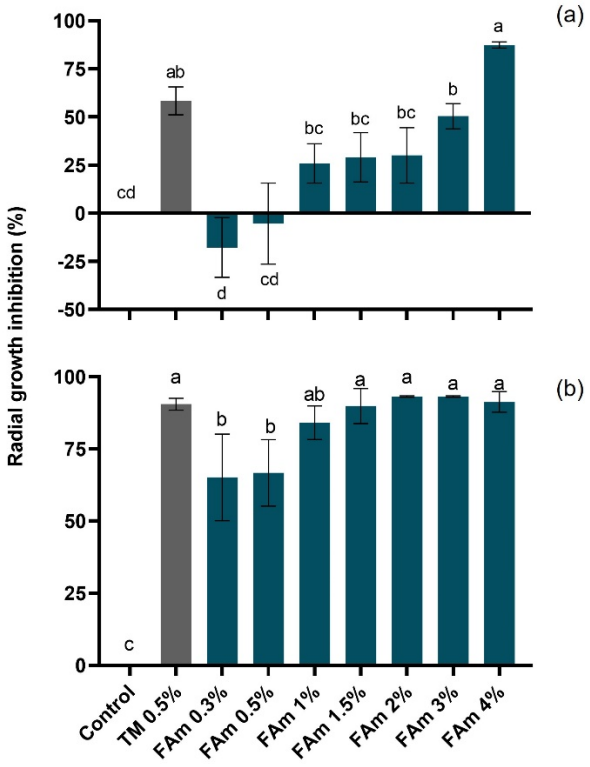


Figure 1. Effect of different FAm concentrations on radial growth of (**a**) *Botrytis cinerea* at 120 h post inoculation (hpi); (**b**) *Rhizoctonia solani* at 96 hpi, expressed as inhibition percentage with respect to the control (pathogen on un-amended PDA). The effect was compared to those obtained with common fungicide thiophanate-methyl (TM). Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and Bonferroni correction test for multiple comparisons.

3.3. Biocontrol Activity of FAm + Trichoderma spp. Combinations

As reported in Figure 3a, all the combinations of *Trichoderma* spp. + FAm were effective in *R. solani* containment, showing a significant reduction in plant mortality. FAm applied individually at concentrations of 0.5% and 1% and the combination GV41 + FAm 0.5% were the most effective treatments in the pathogen control with up to a 96% reduction in tomato plant mortality. However, among the best performing treatments, no significant differences were observed. Similarly, all the treatments reduced the *R. solani* disease severity on lettuce compared to the control and no significant differences were observed between FAm treatments at the three concentrations applied (Figure 3b). The application M10 + FAm 0.5% and GV41 + FAm 1% did not allow the attack and development of the pathogen. A similar result was obtained when the two strains were used in combination with FAm 1% (M10 + GV41 + FAm 1%) as shown in Figure 3b.

3.4. Growth Promotion Effect of Trichoderma spp. +FAm Combinations

The growth promotion effect of *Trichoderma* spp. + FAm combinations was investigated on 40-day-old tomato plants, recording root and stem dry weights. Only the combination of M10 + GV41 + FAm 1% showed a positive effect on both stem and root dry weight. In fact, a 60% increase in root- and 30% increase in stem-dry weight was recorded compared to the untreated control plants (p-value 0.0313 and 0.0306, respectively). (Figure 4a,b). No significant differences were observed when comparing the control and the treatments with FAm at 0.3% and 0.5% concentrations used alone or in combination with *Trichoderma* spp. (data not shown).

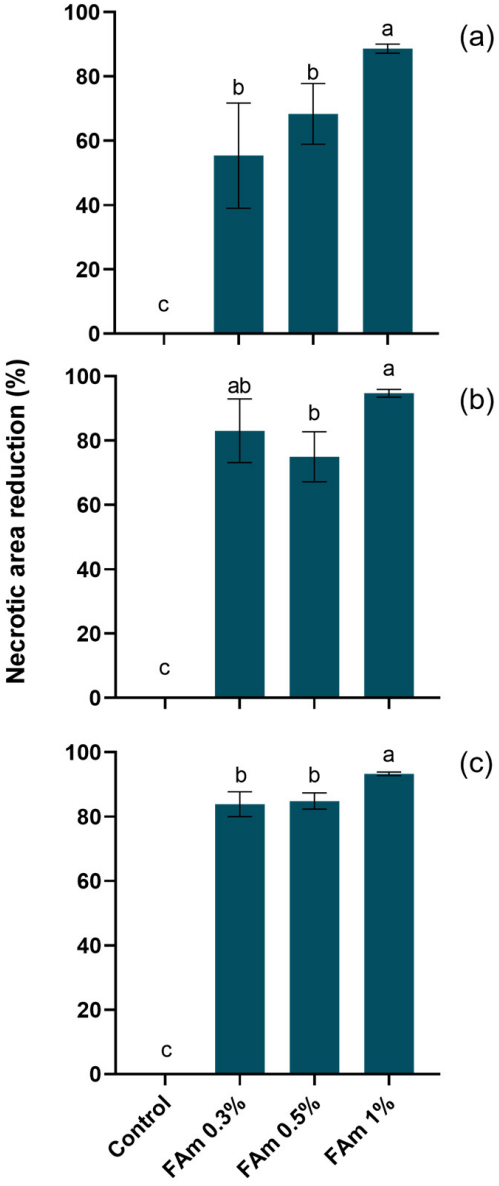


Figure 2. Effect of different concentrations of FAm against *B. cinerea*. Values are expressed as necrotic lesion areas (mm^2) reduction percentage, evaluated 120 h post pathogen inoculation on: (**a**) cucumber (**b**) zucchini, (**c**) tomato. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

3.5. Effectiveness of Trichoderma spp. + FAm Combinations on Agronomic Parameters

The combination of M10 + GV41 + FAm 1% resulted in a significant increase in tomato yield compared to the other treatments (Figure 5). In particular, this treatment resulted in a 70% increase compared to the untreated control plants, 40% compared to FAm 1%, 60% compared to M10 and 45% increase compared to GV41, respectively (Figure 5).

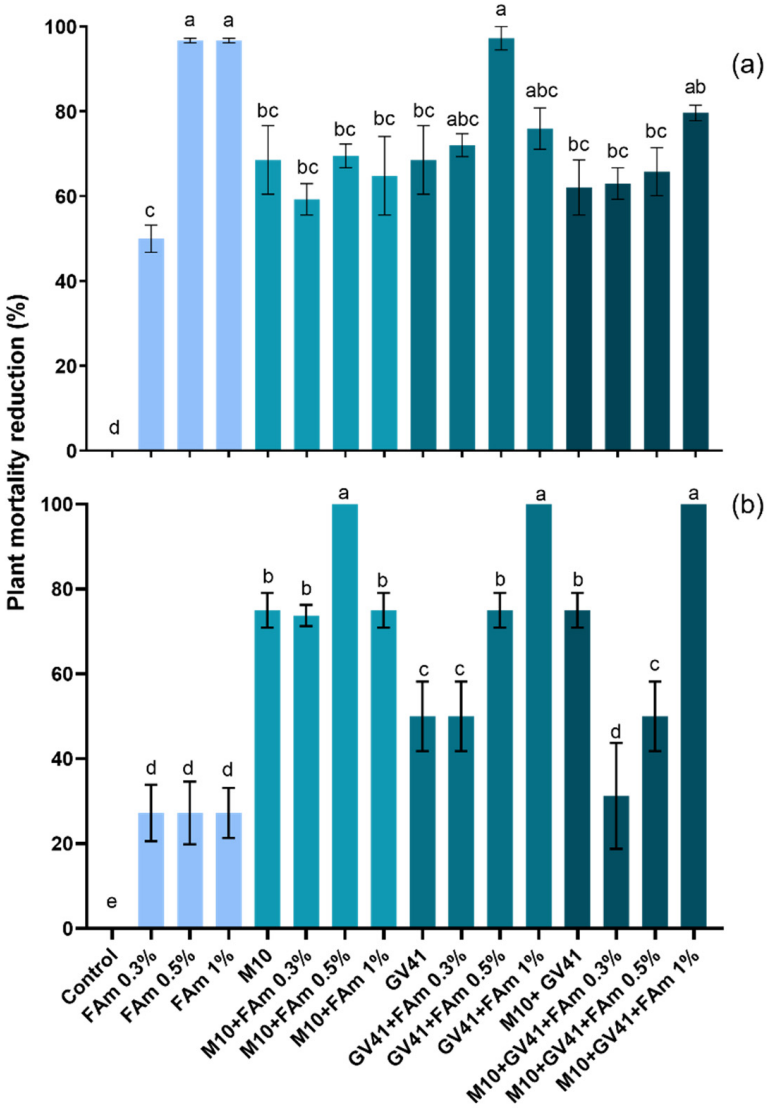


Figure 3. Biocontrol activity of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on *R. solani*-infected (**a**) tomato (**b**) lettuce plants. Values are expressed as a percentage of plant mortality referred to infected and untreated control. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

Furthermore, a 40% increase in tomato yield was also observed for the combination GV41 + FAm 1% compared to the untreated control. No significant differences were observed between *Trichoderma* spp. + FAm combinations and untreated control plants at concentrations lower than 1% (data not shown). In addition, a significant increase in fresh weight of lettuce heads was observed when the combinations M10 + FAm 0.3% and M10 + GV41 + FAm 0.5% were applied, with about a 25% increase compared to the control (Figure 6a).

About a 50% increase in head dry weight was also recorded for GV41 + FAm 0.3% and M10 + FAm 0.3% (Figure 6b).

M10 + FAm 0.5% increased kohlrabi fresh weight by 64%, 97% and 100% compared to M10, untreated control and FAm 0.5%, respectively (Figure 7a). Similarly, M10 + FAm 0.5% application resulted in a significant increase in kohlrabi circumference compared to the untreated control (+30%) and to either FAm or *Trichoderma* spp. treatments (Figure 7b).

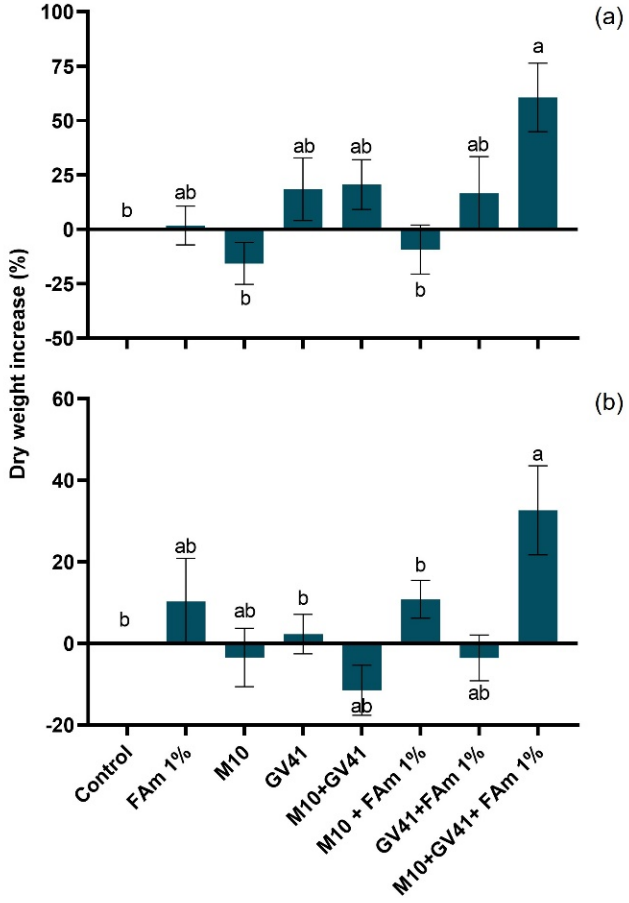


Figure 4. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on tomato plant dry weight of (**a**) root (**b**) stem. Data are expressed as dry weight increase percentage compared to the control. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and Bonferroni correction test for multiple comparisons.

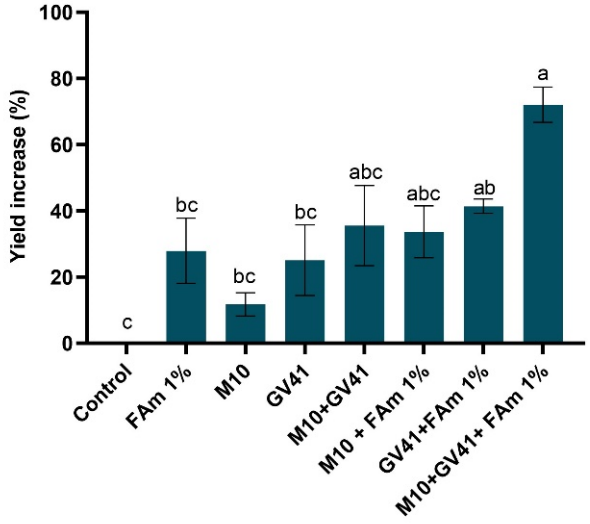


Figure 5. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on tomato yield. Data are expressed as yield increasing percentage compared to the control. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

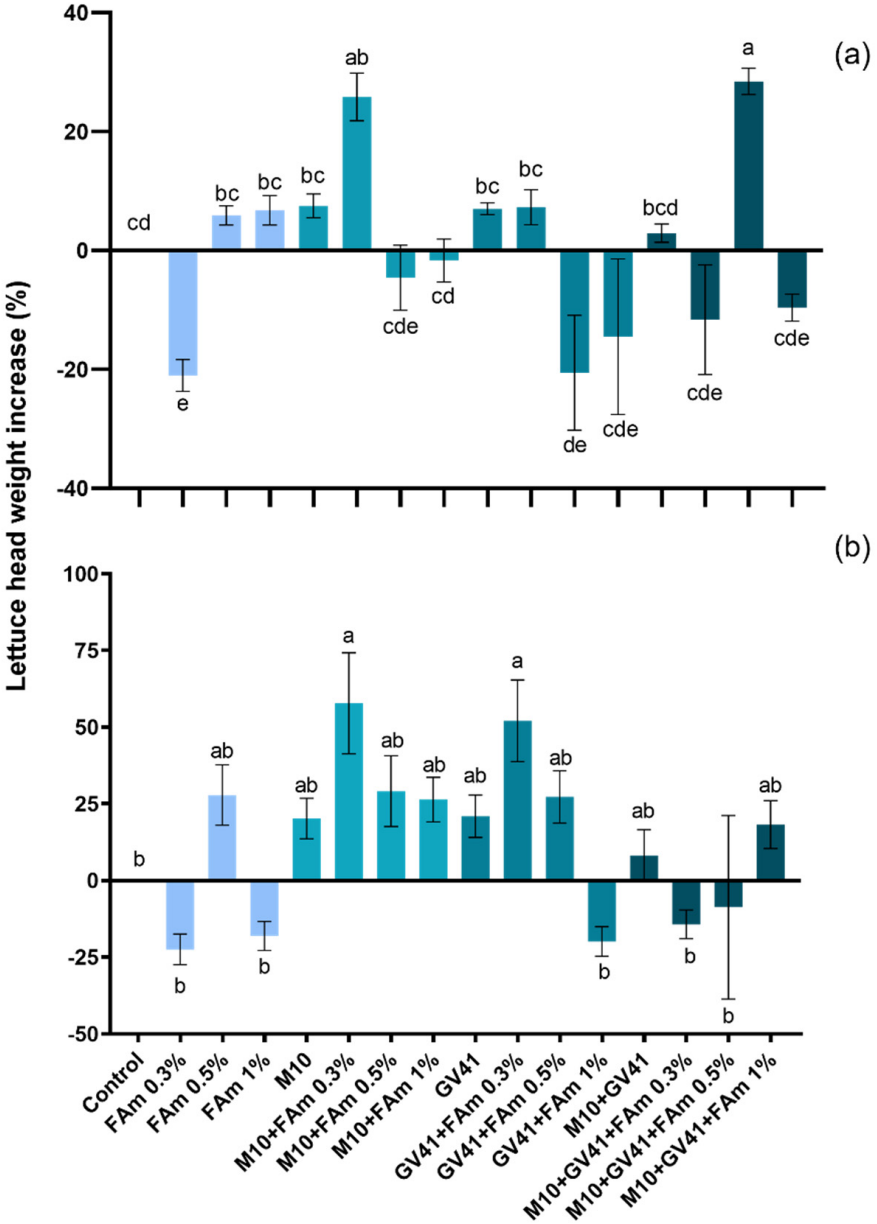


Figure 6. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on lettuce head (**a**) fresh weight (**b**) dry weight. Data are expressed as weight increase percentage compared to the control. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

3.6. Effect of Trichoderma spp. + FAm Combinations on Nutritional Quality of Crops

As shown in Figure 8, the application of M10 + GV41 + FAm 1% led to a significant increase in total carotenoid content by 60% compared to the untreated control. However, a significant carotenoid content reduction was observed in tomato fruits treated with M10 + FAm 1%. No significant differences were observed when FAm was applied at concentrations of 0.3% and 0.5% or in combination with the *Trichoderma* strains (data not shown).

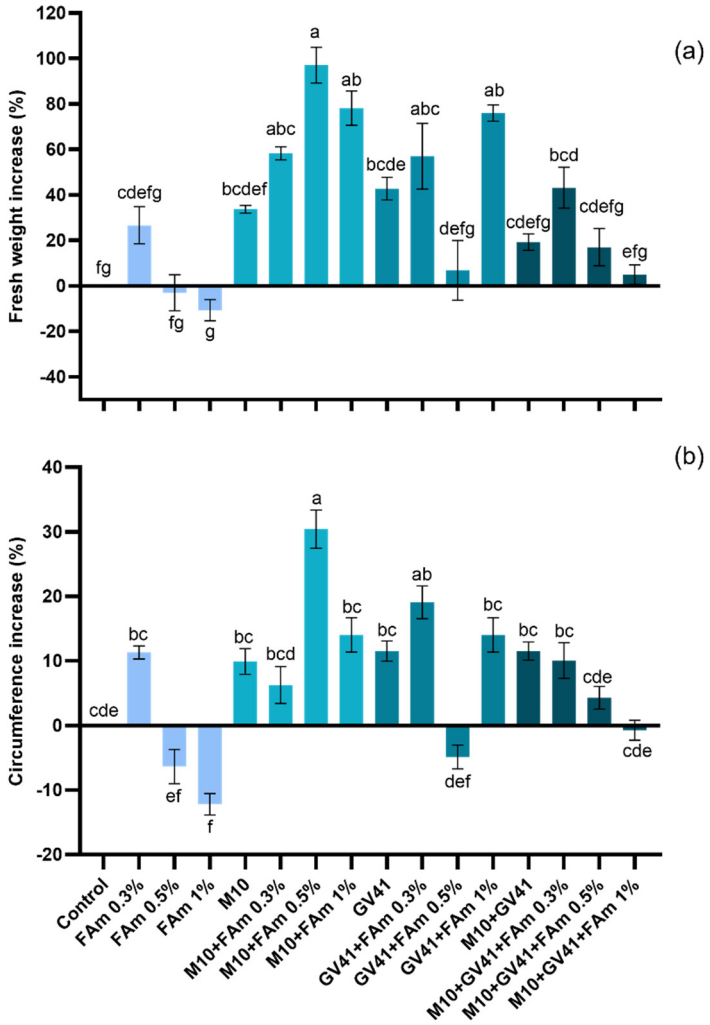


Figure 7. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on kohlrabi growth. (**a**) cabbage fresh weight (**b**) cabbage circumference. Data are expressed as a percentage of increasing compared to the control. Bars with different letters are significantly different (p-value $\leq$ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

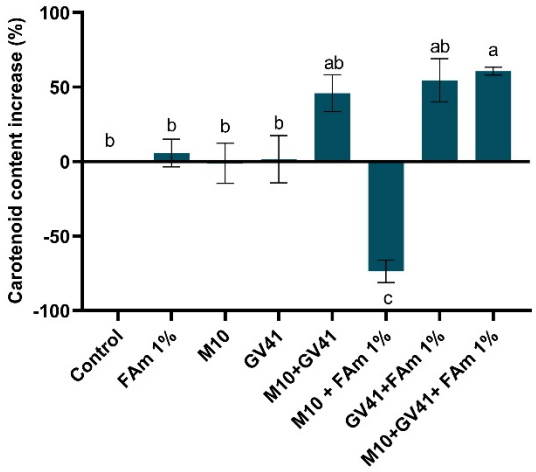


Figure 8. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on tomato fruit carotenoid content expressed as carotenoid content increases (%). Bars with different letters are significantly different (p-value $\leq$ 0.05) according to ANOVA and Bonferroni correction test for multiple comparisons.

M10 was the most effective treatment on lettuce, resulting in a significant increase in total polyphenols compared to the control (+110%) (Figure 9). The polyphenol content detected in plants treated with the single components or their combination resulted comparable to the control.

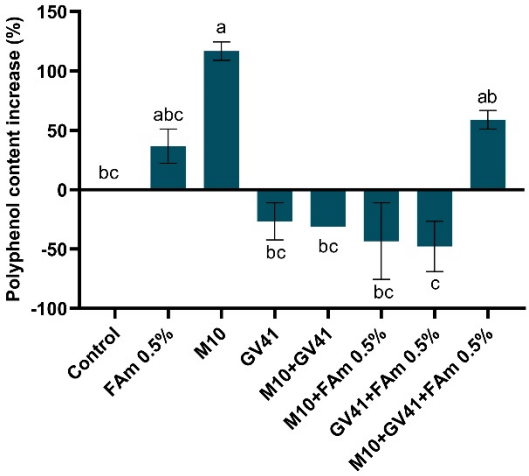


Figure 9. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on lettuce polyphenol content. Values are expressed as polyphenols percentage increases compared to the control. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

Kohlrabi glucosinolate content was significantly higher in plants treated with M10 + FAm 0.5% compared to untreated plants (+39%) and to those treated with single components (+55% compared to M10 and +60% compared to FAm 0.5%) (Figure 10). On the contrary, there was a decrease in glucosinolate content in plants treated with the combinations M10 + FAm 1%, GV41 + FAm at 0.5 and 1%, M10 + GV41 + FAm at 0.3 and 0.5%.

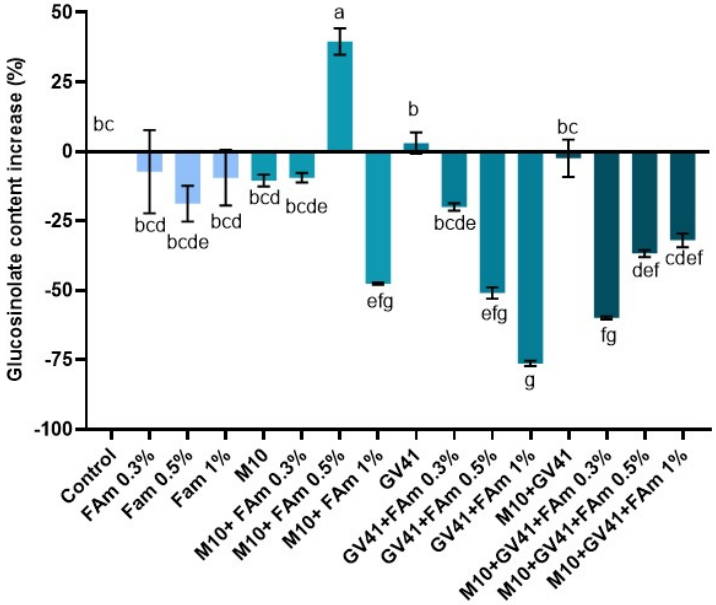
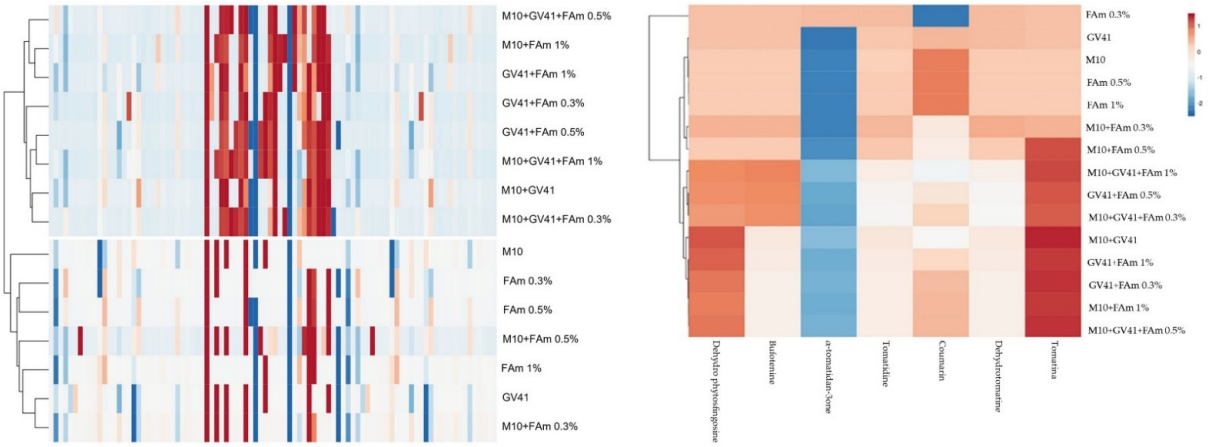


Figure 10. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulations on kohlrabi glucosinolate content. Values are expressed as the increase percentage compared to the control. Bars with different letters are significantly different (p-value ≤ 0.05) according to ANOVA and the Bonferroni correction test for multiple comparisons.

3.7. Effect of Trichoderma spp. + FAm Combinations on Tomato Plant Metabolism

As reported in Figure 11, the results showed a clear separation between metabolic profiles obtained from the plants treated with the individual components (M10, FAm, GV41) and those obtained from the plants treated with the different *Trichoderma* spp. + FAm formulations. M10 + FAm 0.3% and M10 + FAm 0.5% treatments showed a greater similarity with those obtained using the individual components. Moreover, in the *Trichoderma* spp. + FAm cluster, about 50% of the detected compounds were more abundant than those recorded in untreated control plant extracts. In general, 21 out of 60 compounds were identified but only those with an identification score >75% were used in further analysis (Table 2). Many of these compounds belong to the class of steroidal glycoalkaloids (SGAs) such as alpha-tomatine (10,335,497 Da), tomatidine (415.346 Da), dehydrotomatine (1031.53 Da) and 5-α-tomatidan-3-one (413.33 Da). The abundance of tomatine and dehydro-phytosphingosine was higher in all the *Trichoderma* spp. + FAm combinations, except for M10 + FAm 0.3%. On the contrary, the content of dehydro-tomatine and tomatidine was higher in plants treated with individual components (FAm or *Trichoderma* isolates) compared to those treated with the *Trichoderma* spp. + FAm combinations. On the other hand, cumarin and bufotenine did not show any cluster specificity.



(**a**) (**b**)

Figure 11. Effect of *Trichoderma* spp., FAm and *Trichoderma* + FAm formulation treatments on metabolic profiles of tomato plants. Hierarchical clustering of (**a**) all the differentially abundant metabolites and (**b**) selected metabolites detected in the organic extracts. Values are expressed in Log FC (normalized by quantile). Rows were centered and unit variance scaling was applied. Columns were clustered using correlation distance and average linkage. Analysis was performed by ClustVis software.

Table 2. List of putatively identified selected metabolites (identification score >80%) detected by LC-MS of tomato-treated organic extracts.

	Dehydro Phytosfingosine	Bufotenine	A−Tomatidan−3−One	Tomatidine	Coumarin	Dehydrotomatine	Tomatine
FAm 0.3 %	−0.1456	−0.1456	−0.12479	−0.11483	−4.82014	−0.1238	−0.1456
FAm 0.5 %	−0.34555	−0.34555	−20.1518	−0.32685	4.396446	−0.23038	−0.34555
FAm 1 %	−0.22871	−0.22871	−20.035	−0.19447	4.629767	−0.21834	−0.22871
M10	−0.22533	−0.22533	−20.0316	−0.45842	4.568698	−0.20868	−0.22533
GV41	−0.28736	−0.28736	−20.0936	−0.6035	0.218449	0.19646	0.28736
M10 + GV41	16.81207	−0.45301	−20.2593	−0.12306	−0.12754	−0.61138	20.77753
M10 + FAm 0.3%	0.232552	0.232552	−19.5737	−0.10955	−4.44199	0.413509	0.232552
M10 + FAm 0.5%	−0.48115	−0.48115	−20.2874	−0.29364	−5.15569	−0.48943	6.805254
M10 + FAm 1%	15.85772	−1.03429	−20.8406	−0.42998	9.630247	−1.09503	21.37197
GV41 + FAm 0.3%	16.29603	−0.64539	−20.4517	−0.52558	9.144241	0.72401	21.7276
GV41 + FAm 0.5%	16.271	17.01381	−20.4952	−0.50401	4.304544	0.74871	21.61315
GV41 + FAm 1%	16.02612	−0.68987	−20.4961	−0.70976	4.336587	−0.7407	19.50915
M10 + GV41 + FAm 0.3%	16.17436	17.29442	−20.5374	−0.30001	8.920802	−0.87419	21.44018
M10 + GV41 + FAm 0.5%	16.20062	−0.72027	−0.5265	−0.3068	9.610603	−0.88789	21.98139
M10 + GV41 + FAm 1%	15.98161	16.83258	−20.6264	−0.6515	−5.49467	0.84643	21.90122

4. Discussion

Fungi belonging to the *Trichoderma* genus are able to colonize roots and interact with the host plants, increasing the bioavailability of nutrients, producing bioactive compounds useful for the plant metabolism and inducing defense mechanisms. The beneficial effects of *Trichoderma* spp. on plant growth include the enhancement of nutrient uptake and photosynthetic activity and the stimulation of different metabolic processes that positively affect yields and the quality of the treated crops [8,27,53,54]. These beneficial microbes produce secondary metabolites, natural bioactive substances which are currently being evaluated as potential biopesticides [55,56]. Natural compounds from microbial or other sources, able to function as plant stimulants or protectors, can be applied in combination with beneficial microbes for more efficient and wide-ranging agricultural formulations [6]. Among them, free fatty acids derived from vegetable matrices can represent an important reservoir of compounds with proven antimicrobial activity [36,37]. This research aimed at investigating and developing new biological formulations based on the combination of microbial consortia, containing two different *Trichoderma* spp., with a medium and long chain (C_{14}–C_{20}) fatty acids mixture (FAm), applicable in a perspective of sustainable agriculture. The effectiveness of FAm in terms of pathogen growth inhibition was compared to one of the most applied commercial ingredients (thiophanate-methyl), previously used in conventional agriculture and active against Botrytis disease and root rot. The results showed that a FAm mixture at concentrations of 2 and 4% inhibited the growth of the tested phytopathogens, with an effect comparable to commercial fungicides. There are several fatty acids already known for their antifungal activity [41,57,58]. As reported by [57], some of them, such as butyric, caproic, caprylic, capric, lauric, myristic, palmitic, oleic, and linoleic acids, are able to inhibit the growth and development of phytopathogenic fungi as *Alternaria solani*, *Colletotrichum lagenarium*, *Fusarium oxysporum* f. sp. *cucumerinum* and *Fusarium oxysporum* f. sp. *lycopersici*, highlighting a greater efficacy of saturated fatty acids compared to unsaturated ones. FAm used in this study showed a different efficacy in containing the development of the tested phytopathogens. This effect could be associated with the different sterol content in the cell membrane of the target fungi. Higher activity of fatty acids has been reported towards fungi characterized by a membrane with low sterol content [59]. The close correlation between the fungicidal activity and the composition of cellular structures was also demonstrated by [60]. The authors showed that a silent mutant for the biosynthesis of ergosterol of *Saccharomyces cerevisiae* (ERG4-) was unable to grow in the presence of medium and long chain fatty acids. Similarly, [58] reported an increased activity of cis-9-heptadecenoic acid against *Phytophthora infestans*, *P. aphanidermatum* and *B. cinerea*, pathogens characterized by a low or zero membrane sterol content.

The FAm efficacy in containing *B. cinerea* disease symptoms at all the tested concentrations could be associated with the formation of a protective film that acts as a physical barrier, preventing the penetration of the pathogen in the plant tissue. Disease control could be determined by the induction of plant resistance mechanisms since fatty acids can act as elicitors of defense responses [61]. In addition, [62] showed that spray treatment of hexanoic acid was able to deter the growth of *B. cinerea* on tomato, thus reducing necrosis by up to 40% compared to the untreated control plants. In the current study, a 90% reduction in necrosis was observed after applying 1% FAm. This significant reduction in terms of disease severity could be related to the different chemical nature of the fatty acids contained in the mixture.

Multiple studies reported that different fatty acids have a beneficial interaction with the plant by increasing not only defense responses but also plant growth vigor [63–67]. In the current study, an early tomato seeds germination and a significant increase in the seed germination percentage were observed when the seeds were treated with 0.3% FAm. Only the application of the pure FAm solution resulted in total inhibition of germination. Seeds contain a considerable amount of reserve lipids, which are used in the early stages of germination. Application of FAm at low concentrations (0.3%) in our case could have enhanced the availability of nutrients that hastened the germination process. The early

germination positively impacted stem and root length and plant weight, since these parameters were higher in all FAm concentrations (0.3%, 0.5 % and 1%) tested. This concurs with other studies which have shown beneficial effects of fatty acid mixtures on different crops as reported by [63,66,67]. In particular, [63] showed an increase in the root dry weight of cucumber and tomato plants applying a mixture of palmitic and oleic acids.

Our study also shows the compatibility between FAm and two different *Trichoderma* strains in enhancing crop performances. For instance, none of the tested FAm concentrations inhibited the development of the two beneficials, although a slowdown in growth was observed in the first hours on medium enriched with FAm 10 and 20%. This slowdown could be related to an adaptation of *Trichoderma* spp. to the substrate and to the activation of detoxification mechanisms, which include β-oxidation, esterification or decarboxylation reactions as suggested by [68]. Fungi belonging to the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Cladosporium* and *Fusarium* can carry out these bioconversions to remove toxic metabolites [68]. *T. harzianum* resulted able to grow in the presence of pelargonic acid (nonanoic acid, C9:0) at the same concentrations, which inhibited the growth of two cocoa pathogens (*Crinipellis perniciosa* Stahel and *Moniliophthora roreri*) [69]. Similarly, it has been shown that the use of Fungicover®, a product based on fatty acids, improves the effectiveness of *Candida sake* and *Pantoea agglomerans* in the biocontrol of *B. cinerea* [70,71]. The effectiveness of the *Trichoderma* + FAm combination in containing *B. cinerea* and *R. solani* could be related to a positive interaction between the organic and microbial components. In addition, the formulation can stimulate defense mechanisms in plants. For example, [28] reported that *T. harzianum* M10 can activate defense responses in tomato plants infected with *R. solani*, through the overexpression of genes involved in the signaling of ethylene/jasmonic acid (ET/JA) and acid salicylic (SA) pathways. Similarly, fatty acids are known to act as elicitors of defense responses. In particular, it has been shown that lipoxygenases (LOX) can use fatty acids as substrate, with the consequent formation of hydroperoxides, precursors of oxylipins, molecules similar to phytoalexins [72], and regulatory molecules as derivatives of JA [73–75]. Therefore, the increased biocontrol effects observed in our experiments could be associated with a direct action on the pathogen of both components, and with stimulation of plant defense responses.

Trichoderma spp. + FAm treatments effectively promoted growth and improved yield for all the horticultural crops tested. However, there was no single *Trichoderma* + FAm formulation that was able to produce similar effects across the vegetable crops assayed. This could be associated with the differences arising from the specific physiology of the crops. Several studies have shown that the beneficial interaction of *Trichoderma* with plants can be strictly linked to specific plant species [21,76,77].

In the past, the biostimulant effect was evaluated considering the improvement of tolerance mechanisms under biotic stresses or focusing on plant (pigment content, photosynthesis) or fruit physiological parameters (size, shape, weight yield and color) [78]. To date, only few studies reported the effect of biostimulants on the improvement of nutraceutical features of treated plants, meaning this field has still been little explored [79–81]. In this work, the content of the principal nutritional factors with antioxidant activity was determined, specifically evaluating carotenoids, polyphenols and glucosinolates for tomato, lettuce, and kohlrabi, respectively. Our results showed that plants treated with different *Trichoderma* spp. + FAm combinations were significantly enriched in beneficial compounds (carotenoids, polyphenols and glucosinolates) compared to plants treated with FAm or *Trichoderma* spp. These compounds are known to positively contribute to human health, by protecting key biological constituents such as lipoproteins, membranes, and DNA from oxidative processes [82]. Multiple studies reported an inverse association between vegetable consumption containing glucosinolates and the risk of chronic degenerative diseases [83]. The same correlation has been highlighted for tomato consumption and the risk of some types of cancer, cardiovascular diseases and altered macular degeneration [1,84–87]. This protective effect has been attributed mainly to pro-vitamin A and other carotenoids, precursors of essential vitamins and antioxidants [88]. It is well

documented that several *Trichoderma* spp. can increase the content of secondary metabolites with antioxidant activity in different plant species. For example, [89] found that treatments with *T. harzianum* T22 on grapevines increased yield and polyphenols content in grapes. In a more recent study [12], reported a significant increase in ascorbic acid and anthocyanin content in strawberries treated with *Trichoderma* strain GV41 compared to the untreated controls. Other studies have shown greater yield and content of antioxidant compounds (ascorbic acid, β-carotene and lycopene) in plants fertilized with compost enriched with *Trichoderma* spp. [90,91]. Furthermore, the biosynthesis of these compounds has been associated strictly with the activation of the plant defense pathways [92,93]. In this work, the observed increase in antioxidants could be attributed to the ability of *Trichoderma* spp. to make nutrients more bioavailable, to increase soil fertility and to positively modulate the plant secondary metabolism, increasing the production of phytochemicals, and trigger plant defense responses.

The abundance of different metabolites was also observed in plants treated with individual components compared to those treated with different *Trichoderma* + FAm formulations, resulting in the formation of two distinct clusters. Among the compounds putatively identified, some of them belong to the class of steroidal glyco-alkaloids (SGAs) such as tomatine, tomatidine, dehydrotomatin and 5-α-tomatidan-3-one. The SGAs are specialized anti-nutritional metabolites constitutively produced in plants and frequently reported as determinants of resistance to fungal attack. For example, tomatine is involved in defense mechanisms against a broad spectrum of phytopathogenic agents, acting specifically on membrane sterols [94]. Coumarin and bufotenine were also detected among the identified compounds. Coumarins are metabolites derived from the phenylpropanoid pathway, involved in plant structural and chemical defense strategies [95]. On the other hand, bufotenine belongs to the phyto-serotonin class, molecules implicated in multiple processes such as flowering, ion permeability, signaling, detoxification processes and cellular protection, acting as antioxidants [96]. The significant abundance of these compounds in *Trichoderma* spp. + Fam-treated plants indicated that the combined formulation applications can trigger plant defense responses.

5. Conclusions

Our study demonstrated that the combined biostimulant applications of *Trichoderma* spp. with fatty acid mixtures improved biocontrol activity, growth promotion and yield of treated plant species. Interestingly, these innovative bio-formulations were more effective than the treatment with the individual components (microbial or organic) not only in improving yield but also in producing a premium quality marketable vegetable with higher antioxidant content. Further research will allow a better understanding of molecular and physiological mechanisms underlying the ability of the formulation components to increase phytochemicals, which could help to improve the nutraceutical properties of crops. Therefore, the combination of specifically selected *Trichoderma* strains with natural bioactive substances represents a promising, efficient, and sustainable strategy for improving disease management and increasing yields in the context of eco-sustainable agriculture.

Author Contributions: Conceptualization, S.L., A.V. and M.L.; methodology, S.L.; software, G.M.; validation, S.L. and G.M., formal analysis, S.L. and G.M.; investigation, S.L., G.M., V.M.G., C.V., R.F. and P.V.; resources, F.V., M.L. and S.L.W.; data curation, S.L. and G.M.; writing—original draft preparation, S.L. and G.M; writing—review and editing, G.M. and S.K.; visualization, S.L. and G.M.; supervision, S.L.W., A.V. and M.L.; project administration, M.L.; funding acquisition, S.L.W. and M.L. All authors have read and agreed to the published version of the manuscript.

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References

1. Carillo, P.; Woo, S.L.; Comite, E.; El-Nakhel, C.; Rouphael, Y.; Fusco, G.M.; Borzacchiello, A.; Lanzuise, S.; Vinale, F. Application of *Trichoderma harzianum*, 6-Pentyl-α-Pyrone and Plant Biopolymer Formulations Modulate Plant Metabolism and Fruit Quality of Plum Tomatoes. *Plants* **2020**, *9*, 771. [CrossRef]
2. Hertel, T.W. The Global Supply and Demand for Agricultural Land in 2050: A Perfect Storm in the Making. *Am. J. Agric. Econ.* **2011**, *93*, 259–275. [CrossRef]
3. Santos, M.S.; Rodrigues, T.F.; Nogueira, M.A.; Hungria, M. The Challenge of Combining High Yields with Environmentally Friendly Bioproducts: A Review on the Compatibility of Pesticides with Microbial Inoculants. *Agronomy* **2021**, *11*, 870. [CrossRef]
4. Tudi, M.; Daniel Ruan, H.; Wang, L.; Lyu, J.; Sadler, R.; Connell, D.; Chu, C.; Phung, D.T. Agriculture Development, Pesticide Application and Its Impact on the Environment. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1112. [CrossRef]
5. Guzmán-Guzmán, P.; Porras-Troncoso, M.D.; Olmedo-Monfil, V.; Herrera-Estrella, A. *Trichoderma* Species: Versatile Plant Symbionts. *Phytopathology* **2019**, *109*, 6–16. [CrossRef]
6. Comite, E.; El-Nakhel, C.; Rouphael, Y.; Ventorino, V.; Pepe, O.; Borzacchiello, A.; Vinale, F.; Rigano, D.; Staropoli, A.; Lorito, M.; et al. Bioformulations with Beneficial Microbial Consortia, a Bioactive Compound and Plant Biopolymers Modulate Sweet Basil Productivity, Photosynthetic Activity and Metabolites. *Pathogens* **2021**, *10*, 870. [CrossRef]
7. Woo, S.L.; Ruocco, M.; Vinale, F.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, A.; Lanzuise, S.; Manganiello, G.; Lorito, M. *Trichoderma*-Based Products and Their Widespread Use in Agriculture. *Open Mycol. J.* **2014**, *8*, 71–126. [CrossRef]
8. Fiorentino, N.; Ventorino, V.; Woo, S.L.; Pepe, O.; de Rosa, A.; Gioia, L.; Romano, I.; Lombardi, N.; Napolitano, M.; Colla, G.; et al. *Trichoderma*-Based Biostimulants Modulate Rhizosphere Microbial Populations and Improve N Uptake Efficiency, Yield, and Nutritional Quality of Leafy Vegetables. *Front. Plant Sci.* **2018**, *9*, 743. [CrossRef]
9. Schirawski, J.; Perlin, M. Plant—Microbe Interaction 2017—The Good, the Bad and the Diverse. *Int. J. Mol. Sci.* **2018**, *19*, 1374. [CrossRef]
10. Stringlis, I.A.; Yu, K.; Feussner, K.; de Jonge, R.; van Bentum, S.; van Verk, M.C.; Berendsen, R.L.; Bakker, P.A.H.M.; Feussner, I.; Pieterse, C.M.J. MYB72-Dependent Coumarin Exudation Shapes Root Microbiome Assembly to Promote Plant Health. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E5213–E5222. [CrossRef] [PubMed]
11. Silletti, S.; di Stasio, E.; van Oosten, M.J.; Ventorino, V.; Pepe, O.; Napolitano, M.; Marra, R.; Woo, S.L.; Cirillo, V.; Maggio, A. Biostimulant Activity of Azotobacter Chroococcum and *Trichoderma harzianum* in Durum Wheat under Water and Nitrogen Deficiency. *Agronomy* **2021**, *11*, 380. [CrossRef]
12. Lombardi, N.; Caira, S.; Troise, A.D.; Scaloni, A.; Vitaglione, P.; Vinale, F.; Marra, R.; Salzano, A.M.; Lorito, M.; Woo, S.L. *Trichoderma* Applications on Strawberry Plants Modulate the Physiological Processes Positively Affecting Fruit Production and Quality. *Front. Microbiol.* **2020**, *11*, 1364. [CrossRef]
13. Rouphael, Y.; Carillo, P.; Colla, G.; Fiorentino, N.; Sabatino, L.; El-Nakhel, C.; Giordano, M.; Pannico, A.; Cirillo, V.; Shabani, E.; et al. Appraisal of Combined Applications of *Trichoderma* Virens and a Biopolymer-Based Biostimulant on Lettuce Agronomical, Physiological, and Qualitative Properties under Variable N Regimes. *Agronomy* **2020**, *10*, 196. [CrossRef]
14. Manganiello, G.; Nicastro, N.; Caputo, M.; Zaccardelli, M.; Cardi, T.; Pane, C. Functional Hyperspectral Imaging by High-Related Vegetation Indices to Track the Wide-Spectrum *Trichoderma* Biocontrol Activity Against Soil-Borne Diseases of Baby-Leaf Vegetables. *Front. Plant Sci.* **2021**, *12*, 630059. [CrossRef]
15. Mishra, J.; Arora, N.K. Bioformulations for Plant Growth Promotion and Combating Phytopathogens: A Sustainable Approach. In *Bioformulations: For Sustainable Agriculture*; Springer: New Delhi, India, 2016; pp. 3–33.
16. Aamir, M.; Rai, K.K.; Zehra, A.; Dubey, M.K.; Kumar, S.; Shukla, V.; Upadhyay, R.S. Microbial Bioformulation-Based Plant Biostimulants: A Plausible Approach toward next Generation of Sustainable Agriculture. In *Microbial Endophytes*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 195–225.
17. Castiglione, A.M.; Mannino, G.; Contartese, V.; Bertea, C.M.; Ertani, A. Microbial Biostimulants as Response to Modern Agriculture Needs: Composition, Role and Application of These Innovative Products. *Plants* **2021**, *10*, 1533. [CrossRef]
18. Martínez-Medina, A.; del Mar Alguacil, M.; Pascual, J.A.; van Wees, S.C.M. Phytohormone Profiles Induced by *Trichoderma* Isolates Correspond with Their Biocontrol and Plant Growth-Promoting Activity on Melon Plants. *J. Chem. Ecol.* **2014**, *40*, 804–815. [CrossRef] [PubMed]

19. Gravel, V.; Antoun, H.; Tweddell, R.J. Growth Stimulation and Fruit Yield Improvement of Greenhouse Tomato Plants by Inoculation with *Pseudomonas* Putida or *Trichoderma* Atroviride: Possible Role of Indole Acetic Acid (IAA). *Soil Biol. Biochem.* **2007**, *39*, 1968–1977. [CrossRef]
20. MacKenzie, A.J.; Starman, T.W.; Windham, M.T. Enhanced Root and Shoot Growth of *Chrysanthemum* Cuttings Propagated with the Fungus *Trichoderma harzianum*. *HortScience* **1995**, *30*, 496–498. [CrossRef]
21. Ousley, M.A.; Lynch, J.M.; Whipps, J.M. Potential of *Trichoderma* Spp. as Consistent Plant Growth Stimulators. *Biol. Fertil. Soils* **1994**, *17*, 85–90. [CrossRef]
22. Yedidia, I.; Benhamou, N.; Chet, I. Induction of Defense Responses in Cucumber Plants (*Cucumis sativus* L.) by the Biocontrol Agent *Trichoderma harzianum*. *Appl. Environ. Microbiol.* **1999**, *65*, 1061–1070. [CrossRef] [PubMed]
23. Caruso, G.; El-Nakhel, C.; Rouphael, Y.; Comite, E.; Lombardi, N.; Cuciniello, A.; Woo, S.L. *Diplotaxis tenuifolia* (L.) DC. Yield and Quality as Influenced by Cropping Season, Protein Hydrolysates, and *Trichoderma* Applications. *Plants* **2020**, *9*, 697. [CrossRef] [PubMed]
24. Elad, Y. Biological Control of Foliar Pathogens by Means of *Trichoderma harzianum* and Potential Modes of Action. *Crop Prot.* **2000**, *19*, 709–714. [CrossRef]
25. Howell, C.R. Mechanisms Employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. *Plant Dis.* **2003**, *87*, 4–10. [CrossRef]
26. Markovich, N.A.; Kononova, G.L. Lytic Enzymes of *Trichoderma* and Their Role in Plant Defense from Fungal Diseases: A Review. *Appl. Biochem. Microbiol.* **2003**, *39*, 341–351. [CrossRef]
27. Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I.; Lorito, M. *Trichoderma* Species—Opportunistic, Avirulent Plant Symbionts. *Nat. Rev. Microbiol.* **2004**, *2*, 43–56. [CrossRef]
28. Manganiello, G.; Sacco, A.; Ercolano, M.R.; Vinale, F.; Lanzuise, S.; Pascale, A.; Napolitano, M.; Lombardi, N.; Lorito, M.; Woo, S.L. Modulation of Tomato Response to *Rhizoctonia solani* by *Trichoderma harzianum* and Its Secondary Metabolite Harzianic Acid. *Front. Microbiol.* **2018**, *9*, 1966. [CrossRef]
29. Sarma, B.K.; Yadav, S.K.; Singh, S.; Singh, H.B. Microbial Consortium-Mediated Plant Defense against Phytopathogens: Readdressing for Enhancing Efficacy. *Soil Biol. Biochem.* **2015**, *87*, 25–33. [CrossRef]
30. Stockwell, V.O.; Johnson, K.B.; Sugar, D.; Loper, J.E. Mechanistically Compatible Mixtures of Bacterial Antagonists Improve Biological Control of Fire Blight of Pear. *Phytopathology* **2011**, *101*, 113–123. [CrossRef]
31. Duffy, B.K.; Weller, D.M. Biological Control of Take-All of Wheat in the Pacific Northwest of the USA Using Hypovirulent *Gaeumannomyces graminis* Var. tritici and Fluorescent Pseudomonads. *J. Phytopathol.* **1996**, *144*, 585–590. [CrossRef]
32. Jetiyanon, K. Defensive-Related Enzyme Response in Plants Treated with a Mixture of *Bacillus* Strains (IN937a and IN937b) against Different Pathogens. *Biol. Control* **2007**, *42*, 178–185. [CrossRef]
33. Kannan, V.; Sureendar, R. Synergistic Effect of Beneficial Rhizosphere Microflora in Biocontrol and Plant Growth Promotion. *J. Basic Microbiol.* **2009**, *49*, 158–164. [CrossRef] [PubMed]
34. Srivastava, R.; Khalid, A.; Singh, U.S.; Sharma, A.K. Evaluation of Arbuscular Mycorrhizal Fungus, Fluorescent *Pseudomonas* and *Trichoderma harzianum* Formulation against *Fusarium oxysporum* f. sp. lycopersici for the Management of Tomato Wilt. *Biol. Control* **2010**, *53*, 24–31. [CrossRef]
35. Singh, P.K.; Vijay, K. Biological Control of *Fusarium* Wilt of *Chrysanthemum* with *Trichoderma* and Botanicals. *J. Agric. Technol.* **2011**, *7*, 1603–1613.
36. Řiháková, Z.; Plocková, M.; Filip, V.; Šmidrkal, J. Antifungal Activity of Lauric Acid Derivatives against *Aspergillus niger*. *Eur. Food Res. Technol.* **2001**, *213*, 488–490. [CrossRef]
37. Dawson, T.E.; Mambelli, S.; Plamboeck, A.H.; Templer, P.H.; Tu, K.P. Stable Isotopes in Plant Ecology. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 507–559. [CrossRef]
38. Yff, B.T.S.; Lindsey, K.L.; Taylor, M.B.; Erasmus, D.G.; Jäger, A.K. The Pharmacological Screening of Pentanisia Prunelloides and the Isolation of the Antibacterial Compound Palmitic Acid. *J. Ethnopharmacol.* **2002**, *79*, 101–107. [CrossRef]
39. Desbois, A.P.; Lebl, T.; Yan, L.; Smith, V.J. Isolation and Structural Characterisation of Two Antibacterial Free Fatty Acids from the Marine Diatom, *Phaeodactylum tricornutum*. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 755–764. [CrossRef]
40. Dilika, F.; Bremner, P.D.; Meyer, J.J.M. Antibacterial Activity of Linoleic and Oleic Acids Isolated from Helichrysum Pedunculatum: A Plant Used during Circumcision Rites. *Fitoterapia* **2000**, *71*, 450–452. [CrossRef]
41. Walters, D.R.; Walker, R.L.; Walker, K.C. Lauric Acid Exhibits Antifungal Activity Against Plant Pathogenic Fungi. *J. Phytopathol.* **2003**, *151*, 228–230. [CrossRef]
42. Kabara, J.J.; Swieczkowski, D.M.; Conley, A.J.; Truant, J.P. Fatty Acids and Derivatives as Antimicrobial Agents. *Antimicrob. Agents Chemother.* **1972**, *2*, 23–28. [CrossRef]
43. Cerdeiras, M.P.; Fernández, J.; Soubes, M.; Vero, S.; Ferreira, F.; Moyna, P.; Olano, I.; Vázquez, A. A New Antibacterial Compound from Ibicella Lutea. *J. Ethnopharmacol.* **2000**, *73*, 521–525. [CrossRef]
44. Wang, L.L.; Johnson, E.A. Inhibition of Listeria Monocytogenes by Fatty Acids and Monoglycerides. *Appl. Environ. Microbiol.* **1992**, *58*, 624–629. [CrossRef]
45. Thompson, P.A.; Calvert, S.E. Carbon-Isotope Fractionation by a Marine Diatom: The Influence of Irradiance, Daylength, PH, and Nitrogen Source. *Limnol. Oceanogr.* **1994**, *39*, 1835–1844. [CrossRef]

46. Shin, S.Y.; Bajpai, V.K.; Kim, H.R.; Kang, S.C. Antibacterial Activity of Bioconverted Eicosapentaenoic (EPA) and Docosahexaenoic Acid (DHA) against Foodborne Pathogenic Bacteria. *Int. J. Food Microbiol.* **2007**, *113*, 233–236. [CrossRef]
47. Vinale, F.; Flematti, G.; Sivasithamparam, K.; Lorito, M.; Marra, R.; Skelton, B.W.; Ghisalberti, E.L. Harzianic Acid, an Antifungal and Plant Growth Promoting Metabolite from *Trichoderma harzianum*. *J. Nat. Prod.* **2009**, *72*, 2032–2035. [CrossRef] [PubMed]
48. Vinale, F.; Nigro, M.; Sivasithamparam, K.; Flematti, G.; Ghisalberti, E.L.; Ruocco, M.; Varlese, R.; Marra, R.; Lanzuise, S.; Eid, A.; et al. Harzianic Acid: A Novel Siderophore from *Trichoderma harzianum*. *FEMS Microbiol. Lett.* **2013**, *347*, 123–129. [CrossRef]
49. Viterbo, A.; Wiest, A.; Brotman, Y.; Chet, I.; Kenerley, C. The 18mer Peptaibols from *Trichoderma* Virens Elicit Plant Defence Responses. *Mol. Plant Pathol.* **2007**, *8*, 737–746. [CrossRef] [PubMed]
50. Contreras-Cornejo, H.A.; Macías-Rodríguez, L.; Cortés-Penagos, C.; López-Bucio, J. *Trichoderma Virens*, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis. *Plant Physiol.* **2009**, *149*, 1579–1592. [CrossRef] [PubMed]
51. Rouphael, Y.; Petropoulos, S.A.; El-Nakhel, C.; Pannico, A.; Kyriacou, M.C.; Giordano, M.; Troise, A.D.; Vitaglione, P.; de Pascale, S. Reducing Energy Requirements in Future Bioregenerative Life Support Systems (BLSSs): Performance and Bioactive Composition of Diverse Lettuce Genotypes Grown Under Optimal and Suboptimal Light Conditions. *Front. Plant Sci.* **2019**, *10*, 1305. [CrossRef] [PubMed]
52. Caporale, A.G.; Vitaglione, P.; Troise, A.D.; Pigna, M.; Ruocco, M. Influence of Three Different Soil Types on the Interaction of Two Strains of *Trichoderma harzianum* with *Brassica rapa* subsp. *Sylvestris* cv. *Esculenta*, under Soil Mineral Fertilization. *Geoderma* **2019**, *350*, 11–18. [CrossRef]
53. Lorito, M.; Woo, S.L.; Harman, G.E.; Monte, E. Translational Research on *Trichoderma*: From 'Omics to the Field. *Annu. Rev. Phytopathol.* **2010**, *48*, 395–417. [CrossRef] [PubMed]
54. Mukherjee, M.; Mukherjee, P.K.; Horwitz, B.A.; Zachow, C.; Berg, G.; Zeilinger, S. *Trichoderma*–Plant–Pathogen Interactions: Advances in Genetics of Biological Control. *Indian J. Microbiol.* **2012**, *52*, 522–529. [CrossRef] [PubMed]
55. Kumari, I.; Hussain, R.; Sharma, S.; Geetika; Ahmed, M. Microbial Biopesticides for Sustainable Agricultural Practices. In *Biopesticides*; Woodhead Publishing: Cambridge, UK, 2022; pp. 301–317.
56. Das, T.; Tudu, C.K.; Nandy, S.; Pandey, D.K.; Dey, A. Role of Fungal Metabolites as Biopesticides: An Emerging Trend in Sustainable Agriculture. In *Volatiles and Metabolites of Microbes*; Academic Press: San Diego, CA, USA, 2021; pp. 385–407.
57. Altieri, C.; Cardillo, D.; Bevilacqua, A.; Sinigallia, M. Inhibition of *Aspergillus* spp. and *Penicillium* spp. by Fatty Acids and Their Monoglycerides. *J. Food Prot.* **2007**, *70*, 1206–1212. [CrossRef] [PubMed]
58. Avis, T.J.; Anguenot, R.; Neveu, B.; Bolduc, S.; Zhao, Y.; Cheng, Y.; Labbè, C.; Belzile, F.; Belanger, R.R. Usefulness of Heterologous Promoters in the *Pseudozyma Flocculosa* Gene Expression System. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 456–462. [CrossRef] [PubMed]
59. Liu, S.; Ruan, W.; Li, J.; Xu, H.; Wang, J.; Gao, Y.; Wang, J. Biological Control of Phytopathogenic Fungi by Fatty Acids. *Mycopathologia* **2008**, *166*, 93–102. [CrossRef]
60. Benyagoub, M.; Bel Rhlid, R.; Bélanger, R.R. Purification and Characterization of New Fatty Acids with Antibiotic Activity Produced BySporothrix Flocculosa. *J. Chem. Ecol.* **1996**, *22*, 405–413. [CrossRef]
61. McDonough, V.; Stukey, J.; Cavanagh, T. Mutations in Erg4 Affect the Sensitivity of Saccharomyces Cerevisiae to Medium-Chain Fatty Acids. *Biochim. Biophys. Acta (BBA)—Mol. Cell Biol. Lipids* **2002**, *1581*, 109–118. [CrossRef]
62. Aranega-Bou, P.; de la O Leyva, M.; Finiti, I.; García-Agustín, P.; González-Bosch, C. Priming of Plant Resistance by Natural Compounds. Hexanoic Acid as a Model. *Front. Plant Sci.* **2014**, *5*, 488. [CrossRef]
63. Leyva, M.O.; Vicedo, B.; Finiti, I.; Flors, V.; del Amo, G.; Real, M.D.; García-Agustín, P.; González-Bosch, C. Preventive and Post-Infection Control of *Botrytis Cinerea* in Tomato Plants by Hexanoic Acid. *Plant Pathol.* **2008**, *57*, 1038–1046. [CrossRef]
64. Preisig, C.L.; Kuć, J.A. Metabolism by Potato Tuber of Arachidonic Acid, an Elicitor of Hypersensitive Resistance. *Physiol. Mol. Plant Pathol.* **1988**, *32*, 77–88. [CrossRef]
65. Bostock, R.M.; Quatrano, R.S. Regulation of *Em* Gene Expression in Rice. *Plant Physiol.* **1992**, *98*, 1356–1363. [CrossRef]
66. Ruan, W.; Wang, J.; Pan, J.; Li, H.; Wang, J.; Zhang, F.; Gao, Y. Effects of Sesame Seed Cake Allelochemicals on the Growth Cucumber (*Cucumis sativus* L. cv. Jinchun 4). *Allelopath. J.* **2005**, *16*, 217–225.
67. Weibin, R.; Mohan, L.; Jie, P.; Jing, W.; Wenlong, L.; Chengcang, M.; Jingguo, W.; Yubao, G. Effect of Oil Cakes on the Growth of Cucumber (*Cucumis sativus* L.) under Continuous Cropping System and Its Mechanism. *Sci. Agric. Sinica.* **2003**, *36*, 1519–1524.
68. Kinderlerer, J.L. Fungal Strategies for Detoxification of Medium Chain Fatty Acids. *Int. Biodeterior. Biodegrad.* **1993**, *32*, 213–224. [CrossRef]
69. Aneja, M.; Gianfagna, T.J.; Hebbar, P.K. *Trichoderma harzianum* Produces Nonanoic Acid, an Inhibitor of Spore Germination and Mycelial Growth of Two Cacao Pathogens. *Physiol. Mol. Plant Pathol.* **2005**, *67*, 304–307. [CrossRef]
70. Calvo-Garrido, C.; Elmer, P.A.G.; Viñas, I.; Usall, J.; Bartra, E.; Teixidó, N. Biological Control of Botrytis Bunch Rot in Organic Wine Grapes with the Yeast Antagonist *Candida Sake* CPA-1. *Plant Pathol.* **2013**, *62*, 510–519. [CrossRef]
71. Cañamás, T.P.; Viñas, I.; Torres, R.; Usall, J.; Solsona, C.; Teixidó, N. Field Applications of Improved Formulations of Candida Sake CPA-1 for Control of Botrytis Cinerea in Grapes. *Biol. Control* **2011**, *56*, 150–158. [CrossRef]
72. Weber, H.; Chételat, A.; Caldelari, D.; Farmer, E.E. Divinyl Ether Fatty Acid Synthesis in Late Blight–Diseased Potato Leaves. *Plant Cell* **1999**, *11*, 485–493. [CrossRef]

73. Creelman, R.A.; Mullet, J.E. Biosynthesis and Action of Jasmonates in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 355–381. [CrossRef]
74. Farmer, E.E.; Weber, H.; Vollenweider, S. Fatty Acid Signaling in Arabidopsis. *Planta* **1998**, *206*, 167–174. [CrossRef]
75. Weber, H. Fatty Acid-Derived Signals in Plants. *Trends Plant Sci.* **2002**, *7*, 217–224. [CrossRef]
76. Baker, R. *Trzchoderma* spp. as Plant-Growth Stimulants. *Crit. Rev. Biotechnol.* **1988**, *7*, 97–106. [CrossRef]
77. Tucci, M.; Ruocco, M.; de Masi, L.; de Palma, M.; Lorito, M. The Beneficial Effect of *Trichoderma* spp. on Tomato Is Modulated by the Plant Genotype. *Mol. Plant Pathol.* **2011**, *12*, 341–354. [CrossRef]
78. Mannino, G.; Gentile, C.; Ertani, A.; Serio, G.; Bertea, C.M. Anthocyanins: Biosynthesis, Distribution, Ecological Role, and Use of Biostimulants to Increase Their Content in Plant Foods—A Review. *Agriculture* **2021**, *11*, 212. [CrossRef]
79. Mannino, G.; Campobenedetto, C.; Vigliante, I.; Contartese, V.; Gentile, C.; Bertea, C.M. The Application of a Plant Biostimulant Based on Seaweed and Yeast Extract Improved Tomato Fruit Development and Quality. *Biomolecules* **2020**, *10*, 1662. [CrossRef]
80. Kocira, S. Effect of Amino Acid Biostimulant on the Yield and Nutraceutical Potential of Soybean. *Chil. J. Agric. Res.* **2019**, *79*, 17–25. [CrossRef]
81. Graziani, G.; Ritieni, A.; Cirillo, A.; Cice, D.; di Vaio, C. Effects of Biostimulants on Annurca Fruit Quality and Potential Nutraceutical Compounds at Harvest and during Storage. *Plants* **2020**, *9*, 775. [CrossRef] [PubMed]
82. Szeto, Y.T.; Kwok, T.C.Y.; Benzie, I.F.F. Effects of a Long-Term Vegetarian Diet on Biomarkers of Antioxidant Status and Cardiovascular Disease Risk. *Nutrition* **2004**, *20*, 863–866. [CrossRef]
83. Higdon, J.; Delage, B.; Williams, D.; Dashwood, R. Cruciferous Vegetables and Human Cancer Risk: Epidemiologic Evidence and Mechanistic Basis. *Pharmacol. Res.* **2007**, *55*, 224–236. [CrossRef]
84. Sesso, H.D.; Liu, S.; Gaziano, J.M.; Buring, J.E. Dietary Lycopene, Tomato-Based Food Products and Cardiovascular Disease in Women. *J. Nutr.* **2003**, *133*, 2336–2341. [CrossRef]
85. Omoni, A.O.; Aluko, R.E. The Anti-Carcinogenic and Anti-Atherogenic Effects of Lycopene: A Review. *Trends Food Sci. Technol.* **2005**, *16*, 344–350. [CrossRef]
86. Stahl, W.; Sies, H. Bioactivity and Protective Effects of Natural Carotenoids. *Biochim. Biophys. Acta (BBA)-Mol. Basis Dis.* **2005**, *1740*, 101–107. [CrossRef]
87. Burton-Freeman, B.; Reimers, K. Tomato Consumption and Health: Emerging Benefits. *Am. J. Lifestyle Med.* **2011**, *5*, 182–191. [CrossRef]
88. Mayne, S.T. Beta-carotene, Carotenoids, and Disease Prevention in Humans. *FASEB J.* **1996**, *10*, 690–701. [CrossRef]
89. Pascale, A.; Vinale, F.; Manganiello, G.; Nigro, M.; Lanzuise, S.; Ruocco, M.; Marra, R.; Lombardi, N.; Woo, S.L.; Lorito, M. *Trichoderma* and Its Secondary Metabolites Improve Yield and Quality of Grapes. *Crop Prot.* **2017**, *92*, 176–181. [CrossRef]
90. Khan, M.Y.; Haque, M.M.; Molla, A.H.; Rahman, M.M.; Alam, M.Z. Antioxidant Compounds and Minerals in Tomatoes by *Trichoderma*-Enriched Biofertilizer and Their Relationship with the Soil Environments. *J. Integr. Agric.* **2017**, *16*, 691–703. [CrossRef]
91. Molla, A.H.; Manjurul Haque, M.; Amdadul Haque, M.; Ilias, G.N.M. *Trichoderma*-Enriched Biofertilizer Enhances Production and Nutritional Quality of Tomato (*Lycopersicon esculentum* Mill.) and Minimizes NPK Fertilizer Use. *Agric. Res.* **2012**, *1*, 265–272. [CrossRef]
92. Halkier, B.A.; Gershenzon, J. Biology and Biochemistry of Glucosinolates. *Annu. Rev. Plant Biol.* **2006**, *57*, 303–333. [CrossRef] [PubMed]
93. Njoroge, S.M.C.; Vallad, G.E.; Park, S.-Y.; Kang, S.; Koike, S.T.; Bolda, M.; Burman, P.; Polonik, W.; Subbarao, K.V. Phenological and Phytochemical Changes Correlate with Differential Interactions of *Verticillium Dahliae* with Broccoli and Cauliflower. *Phytopathology* **2011**, *101*, 523–534. [CrossRef]
94. Sandrock, R.W.; VanEtten, H.D. Fungal Sensitivity to and Enzymatic Degradation of the Phytoanticipin α-Tomatine. *Phytopathology* **1998**, *88*, 137–143. [CrossRef]
95. Stringlis, I.A.; de Jonge, R.; Pieterse, C.M.J. The Age of Coumarins in Plant–Microbe Interactions. *Plant Cell Physiol.* **2019**, *60*, 1405–1419. [CrossRef] [PubMed]
96. Wojtaszek, P.; Roshchina, V.V. Neurotransmitters in Plant Life. *Ann. Bot.* **2003**, *92*, 166. [CrossRef]