Article

Improvement of Nutraceutical Value of Parsley Leaves (*Petroselinum crispum)* upon Field Applications of Beneficial Microorganisms

Alessia Staropoli [1,2,*], Anastasia Vassetti [1], Maria Michela Salvatore [3], Anna Andolfi [3,4], Maria Isabella Prigigallo [5], Giovanni Bubici [5], Marina Scagliola [6], Pasquale Salerno [6] and Francesco Vinale [2,4,7]

1   Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; an.vassetti@libero.it
2   Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Italy; frvinale@unina.it
3   Department of Chemical Sciences, University of Naples Federico II, 80126 Naples, Italy; mariamichela.salvatore@unina.it (M.M.S.); andolfi@unina.it (A.A.)
4   BAT Center—Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples Federico II, 80055 Portici, Italy
5   Institute for Sustainable Plant Protection, National Research Council, 70126 Bari, Italy; mariaisabella.prigigallo@ipsp.cnr.it (M.I.P.); giovanni.bubici@ipsp.cnr.it (G.B.)
6   Spagro srl, 76121 Barletta, Italy; marina_89@alice.it (M.S.); info@spagro.it (P.S.)
7   Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, 80137 Naples, Italy
*   Correspondence: alessia.staropoli@unina.it

Abstract: Parsley (*Petroselinum crispum*) is an important aromatic herb that has gained importance in food and cosmetic industry, and it is used as medicinal plant due to the presence of compounds with biological activity. Several studies have demonstrated antioxidant, antimicrobial or cancer chemopreventive activity of different parts of parsley plants. We showed that the nutritional value of parsley leaves can be improved by treatments with beneficial microorganisms on the field crop. *Streptomyces fulvissimus* strain AtB-42 and *Trichoderma harzianum* strain T22 were applied, as singly or in combination (microbial consortium), at transplanting and two weeks later. After harvesting, plants were subjected to metabolomic analysis by LC and GC-MS. Spectrometric analysis resulted in the identification of seven polar metabolites. Results showed a significant difference in relative abundance of these metabolites among treatments. The AtB-42 application, alone or in combination with T22, induced the accumulation of petroselinic acid, while T22, alone or in combination, induced the accumulation of xanthotoxol/bergaptol and its derivative xanthotoxin/bergapten. The microbial consortium increased the accumulation of capsanthone compared to single treatments. No statistically relevant differences were found for the volatile fraction. It can be concluded that *S. fulvissimus* and *T. harzianum* significantly induced metabolic profile change of parsley and the accumulation of metabolites with nutraceutical value.

Keywords: microbe-assisted crop production; *Streptomyces*; *Trichoderma*; metabolomics; LC-MS

1. Introduction

Parsley (*Petroselinum crispum* (Mill.) Fuss; family *Apiaceae*) is an aromatic herb which has gained popularity for the food and cosmetic industries due to its antioxidant compounds [1]. The presence of a broad range of active compounds detected in this plant allows the application of parsley as medicinal plant with various proven pharmacological properties including antioxidant, hepatoprotective and neuroprotective [2–4]. Parsley leaf, stem and roots are rich in minerals and bioactive metabolites such as vitamins (vitamin C being the most abundant), essential oils, pigments, polyphenols and fatty acids [5,6].

Liberal et al. (2020) [7] analyzed the phenolic profile and bioactivity of hydroethanolic extracts from parsley leaf samples from 25 cultivars finding that the leaves of all the varieties of *P. crispum* tested were a good source of natural products that confer health benefits. Characterization of these extracts showed the presence of apigenin and kaempferol as the most abundant phenolic compounds, with antioxidant and antimicrobial activity against bacteria and fungi.

Many studies have been conducted in order to evaluate the effect of production factors on yield and on phytochemicals content of different crops [8,9]. Among these, the application of beneficial microorganisms plays an important role in the modern agriculture due to their ability to control phytopathogenic agents through multiple mechanisms, such as antibiosis, mycoparasitism and competition for nutrients and space [10,11]. Biological control involves the use of beneficial organisms, selected strains and/or their metabolites, which can produce positive effects on plants by promoting their growth, increasing resistance levels and promoting the assimilation of nutrients. In the recent years, many microorganisms have been used as Biological Control Agents (BCAs) including strains belonging to bacterial genera and species such as *Agrobacterium*, *Pseudomonas*, *Streptomyces* and *Bacillus*; and fungal genera and species such as *Gliocladium*, *Trichoderma*, *Ampelomyces*, *Candida* and *Coniothyrium* [12]. Streptomycetes are Gram-positive filamentous bacteria in the *Streptomycetaceae* family, widely distributed in soils and rhizospheres. Streptomycetes are one of the major microbial sources of antibiotic molecules used in pharmaceutical and agricultural sector. They produce a wide variety of bioactive secondary metabolites, such as antifungal, antiviral, antitumor, anti-hypertensive and immunosuppressant molecules [13,14]. Moreover, Streptomycetes have an exceptionally large number of hydrolytic enzymes enabling them to interact with other organisms in the environment. All these biological features suggest that Streptomycetes are excellent biological control agents [15–18]. Selected *Trichoderma* species are used as biocontrol agents and bio-stimulants due to their ability to protect plants, compete with pathogens and produce several biologically active compounds including cell wall degrading enzymes and secondary metabolites [19]. The ability of *T. harzianum* strain T22 and *T. atroviride* strain P1 to improve growth was investigated in several experiments on lettuce, tomato and pepper plants under field conditions [16]. It was also tested on parsley plants demonstrating an increase in total fresh and dry mass and root system, superior to the controls when applied on soil under greenhouse conditions [20].

The present study aimed to evaluate the effects of beneficial microorganisms on the metabolic profile of parsley. *Streptomyces fulvissimus* (strain AtB-42) and *T. harzianum* (strain T22) have been selected for the present study due to their well-known characteristic as bio-stimulants and biological control agents [15–21]. In order to investigate the chemical profile of parsley plants (*P. crispum* var. *neapolitanum*) and to detect their changes after treatments, a metabolomic approach based on mass spectrometry was used.

2. Materials and Methods

2.1. Field Trial

The trial was established in an open field located at 41°13′22.0″ N, 16°20′00.2″ E, Andria, Apulia, Italy. In January, 30 day old parsley seedlings were transplanted in single rows at 10 cm distance on the row and 20 cm between rows. The trial consisted of four treatments: control (C), *T. harzianum* strain T22 (T), *S. fulvissimus* strain AtB-42 (S) and a combination of the two microorganisms (S + T). The treatments were carried out at transplanting by dipping roots for 15 min in conidia/spore suspensions (1×10^7 conidia or spores per milliliter) and one month later by soil drenching 25 mL per plant of conidia/spore suspensions (1×10^7 conidia or spores per milliliter).

Starting from a glycerol stock collection tube (Corning® cryogenic vials, Merck KGaA, Darmstadt, Germany), AtB-42 inoculum was prepared in 1 L bottles with 300 mL potato dextrose broth (PDB, Condalab, Madrid, Spain), incubated at 25 °C and 200 rpm for 7 days. Then, the culture was centrifuged at $8000 \times g$ for 10 min to remove the supernatant and resuspend the pellet in sterile distilled water. Spore concentration was estimated by using

a Thoma chamber. *S. fulvissimus* strain AtB-42 was previously selected from a consortium of ca. 300 rhizosphere-competent isolates [15]. *Trichoderma harzianum* strain T22 was purchased from Koppert (Trianum WG, Koppert Biological Systems, Piracicaba, SP, Brazil). Inoculum of *T. harzianum* strain T22 was prepared by suspending the commercial product in water to achieve the desired conidia concentration (final concentration for treatments: 10^7 spore mL^{-1}).

A randomized complete block design with three blocks was adopted. Blocks were separated by untreated plants (extra plants) to avoid any contamination during the applications of microorganisms. Thirty plants per treatment in each block were used for a total of 360 plants in the entire trial.

Plants were cultivated according to the agronomic practices commonly used in the farm. At the end of crop cycle (three months), plant height and shoot fresh weight were measured on 10 plants per treatment. Twenty plants (five biological replicates per treatment) were stored at −80 °C until the metabolomics analysis was completed. Biometric index data were analyzed by one-way ANOVA using Minitab statistical software (Minitab, LLC, State College, PA, USA). Significant differences among treatments were estimated according to Tukey's test with a 0.05 significance level.

2.2. Metabolite Extraction

Lyophilized powder obtained from leaves was subjected to extraction in organic solvents. In particular, 100 mg of powder for each biological replicate was suspended in 2 mL of methanol (MeOH; LC-MS purity) and vortexed for 30 s. Samples were then centrifuged for 10 min at 14,000 rpm at 4 °C. Each supernatant was diluted 1:10 in MeOH and injected into the LC-MS qTOF (liquid chromatography-mass spectrometry-quadrupole-time of flight) system. All reagents used were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

2.3. Isolation of Volatile Fraction

Leaf samples (50 g) stored at −80 °C were hydrodistilled with 600 mL of distilled water in Clevenger apparatus for 2 h according to the European Pharmacopoeia [22]. The water containing dissolved oils was extracted three times with dichloromethane (50 mL), and the extracts were collected and dried over anhydrous sodium sulphate. The samples were stored in sealed glass vials at −20 °C before the analysis. The extraction was carried out by triplicate.

2.4. LC-MS Analysis

Analyses were conducted on an Agilent HP 1260 Infinity Series liquid chromatograph coupled to a Q-TOF mass spectrometer and equipped with a DAD system (Agilent Technologies, Santa Clara, CA, USA). A Zorbax Extend C-18 column (4.6 × 50 mm, 3.5 μm, Agilent Technologies) was used for chromatographic separation, held at a constant temperature of 37 °C. The analyses were performed at a 0.6 mL min^{-1} flow rate, using a linear gradient system composed of 0.1% (*v/v*) formic acid in water (eluent A) and 0.1% (*v/v*) formic acid in acetonitrile (eluent B). The gradient program was as follows: from 5% to 70% eluent B in 4 min, isocratic at 70% of eluent B from 4 to 5 min; from 70% to 80% of eluent B from 5 to 8 min and from 80% to 100% eluent B from 8 to 10 min; and, finally, lowering to starting conditions (5% eluent B) from 10 to 15 min. After returning to the initial conditions, equilibration was achieved after 1 min. The injection volume was 10 μL. UV spectra were collected by DAD every 0.4 s from 190 to 750 nm, with a resolution of 2 nm. The MS system was equipped with a Dual Electrospray Ionization (ESI) source and operated both in positive and negative mode. The capillary was maintained at 2000 V, fragmentor voltage at 180 V, cone 1 (skimmer 1) at 45 V and Oct RFV at 750 V. Gas flow rate was set at 11 L min^{-1}, at 350 °C, and the nebulizer was set at 45 psig. Mass spectra were recorded within the *m/z* range 100–1700 as centroid spectra, with three scans per second. In order to perform real time mass-lock correction, a solution consisted of purine ($C_5H_4N_4$,

m/z 121.050873, 10 μmol L^{-1}), and hexakis (1H,1H,3H-tetrafluoropentoxy)-phosphazene (C$_{18}$H$_{18}$O$_6$N$_3$P$_3$F$_{24}$, m/z 922.009798, 2 μmol L^{-1}) was constantly infused by an Isocratic pump (1260 Infinity Series, Agilent Technologies) with a flow rate of 0.06 mL min^{-1}. All MS and HPLC parameters were set with Agilent MassHunter Data Acquisition Software, rev. B.05.01.

2.5. GC-MS Analysis

The analyses of the essential oils were performed by using an Agilent 6850 GC (Agilent Technologies) coupled to an Agilent 5973 Inert MS. The amount of 2 μL of each sample was injected in splitless mode into an HP-5MS capillary column (5% phenyl methyl poly siloxane stationary phase). The injection temperature was 250 °C, and the temperature ramp raised the column temperature from 70 °C to 280 °C: 70 °C for 1 min; 10 °C·min^{-1} until reaching 170 °C; and 30 °C·min^{-1} until reaching 280 °C. Subsequently, it was held at 280 °C for 5 min. Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The solvent delay was set to 4 min. Measurements were performed under electron impact (EI) ionization (70 eV) in full scan mode (m/z 29–550) at a frequency of 3.9 Hz. The EI ion source and quadrupole mass filter temperatures were kept, respectively, at 200 °C and 250 °C.

The identification of compounds was based on comparison of their mass spectra with those recorded in the NIST 14 mass spectral library (https://www.nist.gov/srd/nist-standard-reference-database-1a, accessed on 9 July 2021). Furthermore, the identification was supported by Kovats retention index (RI) calculated for each analyte by the Kovats equation, using the standard *n*-alkane mixture in the range C7-C40 (Sigma-Aldrich, Saint Louis, MO, USA).

2.6. Statistical Analysis of Metabolomics Data

Statistical analysis was carried out by using Mass Profile Professional software, version 13.1.1 (Agilent Technologies). Raw data of leaf extracts were grouped by the treatment applied in the field (i.e., single strains or microbial consortium), and these groups were subjected to one-way ANOVA (p-value < 0.05) and to fold change ≥2.0. Thus, the results obtained were subjected to principal components analysis (PCA) and hierarchical clustering in order to compare metabolic profiles of plants and to detect differences induced by different treatments. Statistically relevant compounds were identified using a plant database, FooDB Version 1.0 (www.foodb.ca, accessed on 10 March 2021; freely available), and by comparison with data available in the literature. Among the detected molecules, only those with a mass error below 10 ppm and a sufficient score were reported.

3. Results

3.1. Plant Growth

Beneficial microbial treatments positively affected the growth and development of parsley. At the end of crop cycle (3 months), plant height increased in plants treated with single cultures (T22 or AtB42) compared to the control, and the fresh weight increased in all treatments compared to the control (Figure 1).

3.2. Metabolomic Analysis

An extraction protocol, starting from lyophilized leaf powder, was carried out. The extracts were subjected to LC-MS analysis. The resulting total ion chromatograms (TIC) and mass spectra were analyzed, and putatively identified compounds were obtained by comparison with data reported in literature and a freely available electronic food database (FooDB). Figure 2 shows the chromatogram of the extract of T22-treated plants (recorded in positive mode). Putatively identified metabolites are reported in Table 1, while Figures S1 and S2 show the comparisons between the recorded chromatograms for the different conditions in positive and negative mode, respectively.
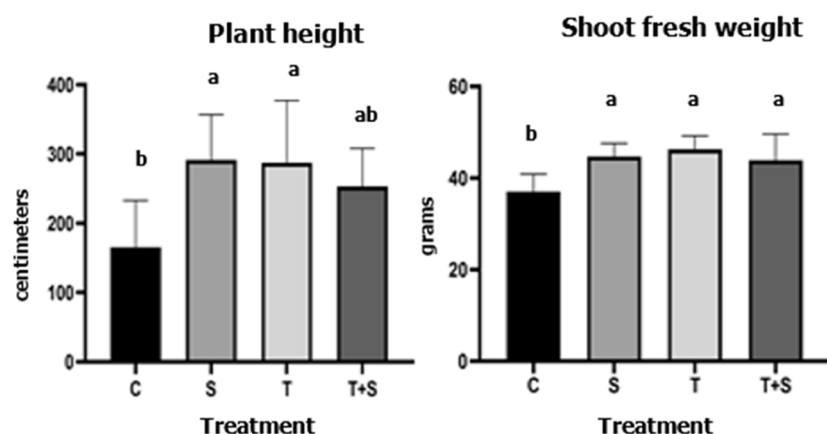
Figure 1. Variation of plant height (**left**) and shoot fresh weight (**right**) of parsley plants treated with *Streptomyces fulvissimus* AtB-42 (S), *Trichoderma harzianum* T22 (T), microbial consortium (S + T) and control group (C). Treatments with the same letter are not significantly different according to Tukey's test (*p*-value < 0.05). Error bars represent standard deviation.
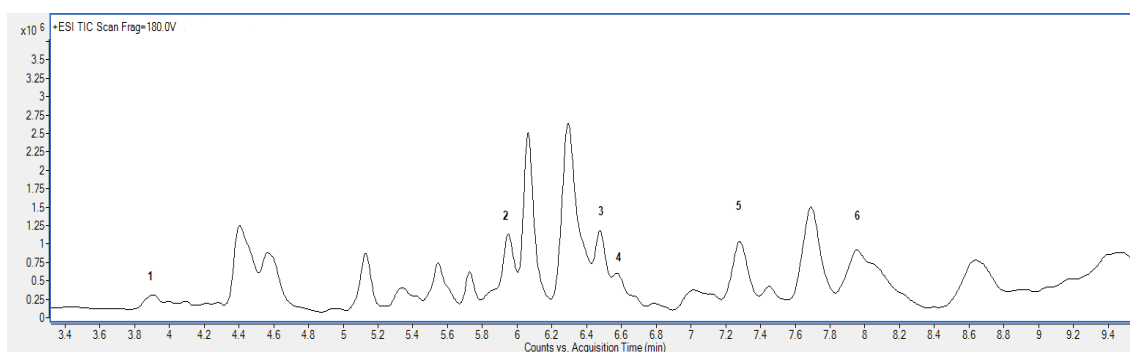

Figure 2. Magnified total ion chromatogram of the extract of T22-treated plants recorded in positive mode. Putatively identified metabolites and the corresponding peak numbers in the figure are reported in Table 1.

Table 1. Putatively identified metabolites in parsley extract obtained by LC-MS analysis. Data include peak number as reported in Figure 2, retention time, experimental and theoretical mono-isotopic mass and molecular formula.

N.	Compound	RT (min)	Experimental Mass (Da)	Theoretical Mass (Da)	Molecular Formula
1	Isorhamnetin 3,7-di-*O*-beta-glucopyranoside	3.848667	640.1663	640.1639	$C_{28}H_{32}O_{17}$
2	Xanthotoxin/Bergapten *	6.000033	216.0427	216.0422	$C_{12}H_8O_4$
3	Capsanthone	6.425001	582.4098	582.4072	$C_{40}H_{54}O_3$
4	Petroselinic acid	6.530999	282.257	282.2558	$C_{18}H_{34}O_2$
5	Bergaptol/Xanthotoxol *	7.286808	202.0272	202.0266	$C_{11}H_6O_4$
6	Piperochromanoic acid	7.955463	356.198	356.1988	$C_{22}H_{28}O_4$
-	Quinic acid **	0.9503336	192.0642	192.0633	$C_7H_{12}O_6$

* Stereoisomers for which it was not possible to make a distinction in the chromatogram. ** Putatively identified molecule from LC-MS analysis performed in negative mode.

Data obtained by LC-MS analysis, i.e., molecular weights, retention times and intensity values, were analyzed statistically. Results were then subjected to Principal Component Analysis (Figure 3) and depicted as hierarchical cluster (Figures S3 and S4). The two graphs indicate that there is a difference in metabolic profiles depending on the treatment applied on parsley plants. A distinct separation among samples differently treated is evident in principal components (PC1–PC2) of the variance in the LC-MS dataset, particularly for data obtained in negative mode.
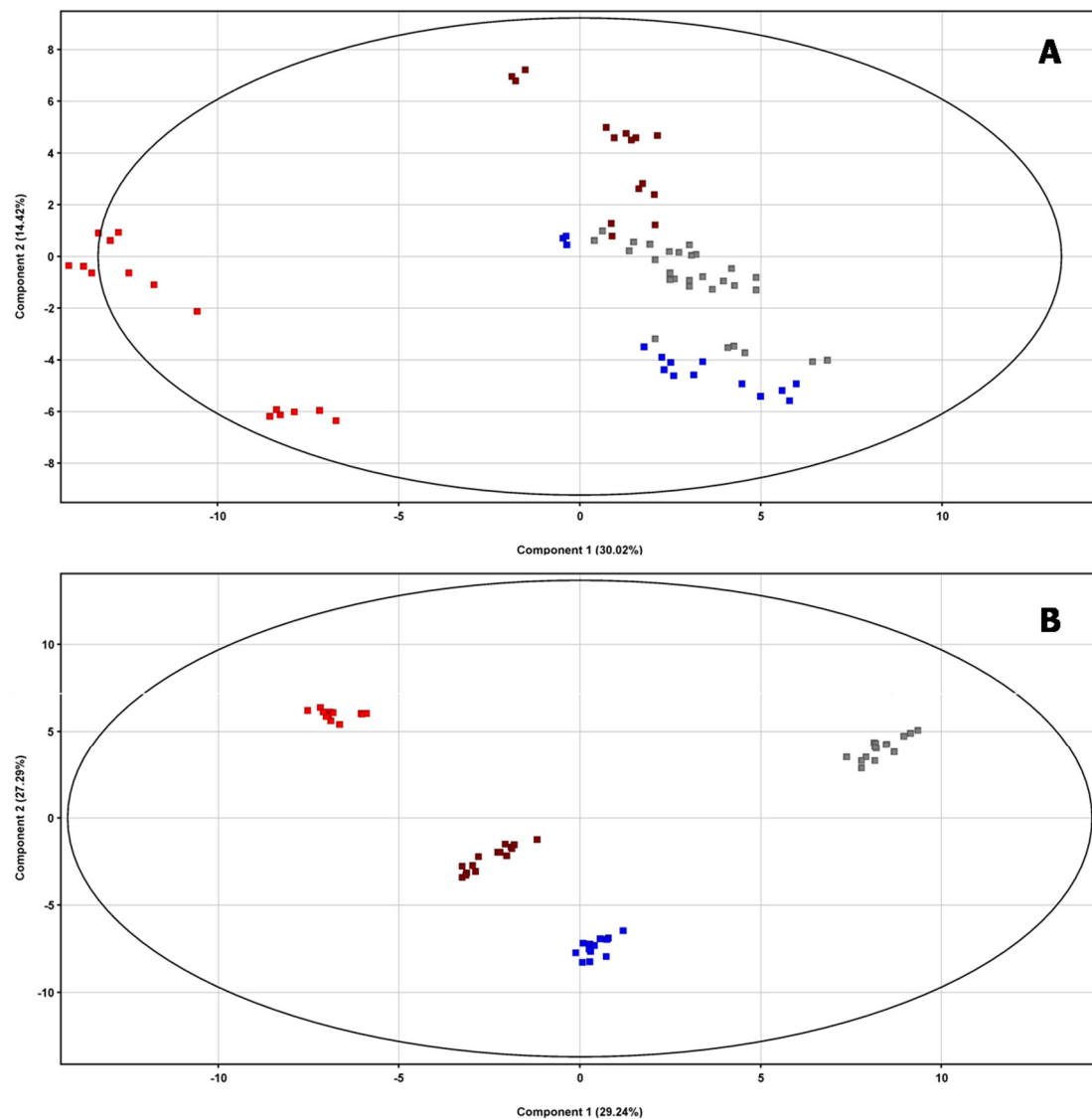
Figure 3. Principal components analysis (PCA) score plots of the LC-MS data acquired for positive (**A**) and negative (**B**) mode. Each group of replicates subjected to different treatments is depicted with a different color: control group (C) is red; *Streptomyces* group (S) is blue; *Trichoderma* group (T) is grey; microbial consortium (S + T) is brown.

Metabolomic analysis highlighted several differentially accumulated metabolites (Tables S1 and S2) for which their abundance is dependent on the treatment applied, and it is related to the function performed in plant. Among those metabolites, six were identified in positive mode analysis and one in negative mode and reported in Table 2 together with regulation against control group and single cultures.

With the aim to reveal potential differences in volatile compounds produced by *P. crispum* treated with different beneficial microorganisms, GC-MS analyses of the hydrodistilled oils of leaves were conducted. Many investigators have studied the chemical composition of the volatile fraction of parsley, and it has been found to be variable [23–26]. The essential oils of samples were essentially constituted by apiol, α-pinene, 1,3,8-p-mentha-triene, myristicin, β-phellandrene and myrcene. Apiol is the main constituent in the examined parsley cultivars. The results of our investigation show no significant effect of treatments on the volatile fraction of the parsley leaves.

Table 2. Putatively identified metabolites that are differentially accumulated in plants treated with *Streptomyces* (S), *Trichoderma* (T) or a mix of those two (S + T) compared to control group (C) plants and the microbial consortium (S + T) compared to *Streptomyces* (S) and *Trichoderma* (T) single culture.

Compound	Regulation				
	S vs. C	T vs. C	S + T vs. C	S + T vs. S	S + T vs. T
Xanthotoxin/Bergapten *	↓	↑	↓	↓	↓
Piperochromanoic acid	↑	↑	↓	↓	↓
Xanthotoxol/Bergaptol *	↓	↓	↓	↑	↓
Isorhamnetin 3,7-di-*O*-beta-glucopyranoside	↓	↓	↓	↓	↓
Petroselinic acid	↑	↓	↓	↓	↑
Capsanthone	↓	↓	↓	↑	↑
Quinic acid	↑	↑	↑	↑	↑

* Stereoisomers for which it was not possible to make a distinction in the chromatogram. ↑ Upregulated against C, S or T group. ↓ Downregulated against C, S or T group.

All the structures of the molecules putatively identified by LC and/or GC-MS analyses are reported in Figure 4.
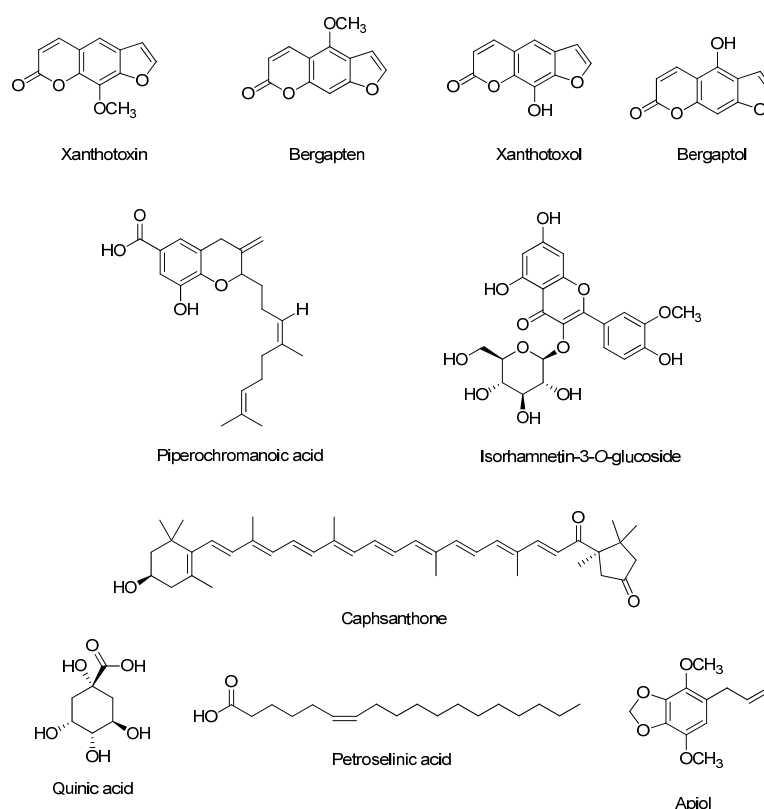

Figure 4. Structures of the molecules putatively identified from LC-MS and GC-MS analysis. Apiol is the metabolite identified from GC-MS analysis, and it is the most abundant compound in parsley extract.

4. Discussion

In the present study we investigated the effects of *S. fulvissimus* strain AtB-42, *T. harzianum* strain T22 and their mixture on a parsley crop in the field, together with the alteration of the plant metabolic profile after treatments. T22 and AtB42 were selected because they are well-known BCAs and bioactive metabolite-producers. Our data (Figure 1) show that single inoculants (T22 or AtB-42) affected the growth by increasing plant height after two treatments, while the microbial consortium (T22 + AtB-42) positively affected the shoot

fresh weight. These data are consistent with other studies which have shown the effect of different fungal and bacterial strains on different crops [20,27–30].

Previous research has documented the biological activity (antioxidant, hepatoprotective, antimicrobial) of several classes of phytochemicals that can be found in different parts of parsley, such as flavonoids, coumarins or lipids [31–35]. However, to our knowledge, this is the first study to investigate the change in parsley metabolome induced by treatments with biocontrol agents. It is evident from Table 2 that the application of different treatments based on beneficial microbes affected the metabolic profile of parsley both in terms of different molecules detected and in terms of relative abundance. Among the differentially accumulated molecules, we putatively identified xanthotoxin/bergapten, piperochromanoic acid, xanthotoxol/bergaptol, isorhamnetin 3,7-di-O-beta-glucopyranoside, petroselinic acid, capsanthone and quinic acid. These metabolites belong to different classes of natural products and are the main active components already isolated and detected in parsley [5].

Coumarin and its derivatives (bergapten, isopimpinellin and xanthotoxin, etc.) are a class of compounds widely distributed in *Apiaceae* family, which is one of the most prominent food sources of coumarins. These molecules are reported to have curative, preventive or nutritive value and also showed antimicrobial activity [36]. Pathogen-infected plants of several species demonstrated an increased level in coumarin derivatives [37]. Cultured parsley cells treated with fungal elicitors increased the production of furanocoumarins xanthotoxol and bergaptol, and it was also demonstrated that xanthotoxin and bergapten were the most abundant coumarin derivatives accumulated in the culture fluid of elicitor-treated parsley cells [38]. Our findings show that bergaptol/xanthotoxol was less abundant in treated plants compared to control. Furthermore, from the comparison of plants treated with AtB-42 + T22 consortium (S + T) and plants treated with AtB-42 (S) alone, bergaptol/xanthotoxol was more abundant in the first group. A similar trend applies to their product xanthoxin/bergapten that resulted in less abundance in treated plants than in control, except for plants treated with T22 (T) in which a greater accumulation of this compound was evident. We may suppose that the application of beneficial microorganisms such as *Trichoderma* and *Streptomyces* explicated an effect as biocontrol agents preventing any pathogen infection and inducing a minor production of coumarins and their derivatives.

The compound isorhamnetin 3,7-di-*O*-beta-glucopyranoside belongs to flavonoids, which are a class of compounds dominant in parsley. Flavonoids are plant natural products with a wide range of activity: They contribute to plant color, have roles in plant growth and development and exhibit a wide range of biological properties including anti-microbial, insecticidal and estrogenic activities [39]. Flavonoids extracted from parsley leaves demonstrated antioxidant, hepato-protective and antidiabetic properties in vivo [5]. As reported in Table 2, this compound is always downregulated in treated plants compared to the control. However, plants treated with *Streptomyces* (S) or *Trichoderma* (T) alone showed a greater accumulation of isorhamnetin 3,7-di-*O*-beta-glucopyranoside compared to plants treated with both microorganisms (S + T).

Petroselinic acid is a fatty acid that was isolated for the first time from *P. crispum* seeds oil in 1909 and is present as a major component in parsley seeds. Petroselinic acid is an important oleochemical active principle for food, cosmetic, chemical and pharmaceutical industry due to its interesting properties such as anti-aging and anti-inflammatory. A considerable amount of antimicrobial activity against several bacteria, yeast and mold species was observed [40,41]. In the present study, we detected a greater accumulation of this compound in plants treated with *Streptomyces* (S) against control and in plants treated with microbial consortium (S + T) vs. *Trichoderma* (T) treated plants. Yeganehpoor et al. in 2017 [42] estimated the effects of different conditions, including bio-fertilizer-based treatments, on chemical profile of coriander. A higher level of petroselinic acid was detected in plants treated with bio-fertilizer and in plants subjected to water stress.

Piperochromanoic acid belongs to a class of compounds known as prenol lipids. Prenol lipids (PR) are synthesized from the 5-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate. The simple isoprenoids (linear alcohols, diphosphates and

so on) are formed by the successive addition of C5 units and are classified according to the number of these terpene units. This class includes the carotenoids, which are precursors of vitamin A and possess antioxidant effects [43].

Lipids are one of the most important biomolecules found in all plant tissues: they are a major component of bio-membranes and platform for lipid signaling. Abiotic stress such as water deficiency and high temperature triggers lipid-dependent signaling cascades, which control the expression of specific gene clusters and activate plant adaptation processes [44]. The application of single inoculants of *Trichoderma* (T) and *Streptomyces* (S) enhanced the production of piperochromanoic acid compared to the control.

Capsanthone is a member of the chemical class of xanthophylls, which are carotenoids containing an oxygenated carotene backbone. Carotenoids are pigments found in photosynthetic bacteria, some species of archaea and fungi, plants and animals. These compounds also act as photo-protectors, antioxidants, color attractants and precursors of plant hormones. [45]. The abundance of capsanthone is increased in plants treated with the mix (S + T) compared to plants treated with single culture of *Streptomyces* (S) and *Trichoderma* (T).

Quinic acid is a dominant acid in green coffee beans and is also a product of the degradation of chlorogenic acids, which are one of the most abundant polyphenols present in green coffee beans and certain fruits and vegetables. Chlorogenic acids have been shown to contribute to the antioxidant, anti-inflammatory, antipyretic and antineoplastic properties of green coffee bean extracts [46]. A recent study demonstrated that methanol extract of *P. crispum* has an important level of quinic acid. Furthermore, this extract showed antioxidant activity, anti-adhesion and anti-proliferative proprieties against human glioblastoma cells [47]. Quinic acid detected through LC-MS analysis was upregulated in treated plants. This result is in agreement with previous studies that evaluated the effects of single specie or microbial consortia on pea plants [48] and of a combination of *T. harzianum* and compost on maize leaves [49].

The results of our investigation show no significant effect of treatments on the volatile fraction of parsley leaves. Dini et al. and Gębarowska et al. reported changes in the volatolome of olive tree and coriander after treatments with *T. harzianum* strain T22 [8,50]. Moreover, Coppola et al. demonstrated that T22 enhanced tomato defense responses against aphids by inducing a higher production of volatile organic compounds (VOCs) [51].

In this investigation, the experimental design, based on interaction of parsley and beneficial microbes in field conditions, has been used as model to improve sustainable agricultural products. *S. fulvissimus* strain AtB-42 and *T. harzianum* strain T22, when either applied as single inoculants or in consortium, produce significant changes in the chemical profile of parsley leaves. The enhanced production of metabolites with antioxidant, anti-inflammatory or also anti-aging activity improve the commercial value of parsley.

Some compounds, such as isorhamnetin 3,7-di-*O*-beta-glucopyranoside, piperochromanoic acid and capsanthone, have been detected for the first time in parsley, and their abundance in plant tissue is still unknown. Further research is needed to gain deeper understanding of the biosynthetic pathway that results in the production of these molecules.

mode and subjected to statistical analysis (one-way ANOVA $p < 0.05$ and fold change > 2.0), Table S1: Metabolites obtained from LC-MS data (positive mode) that are differentially accumulated in plants treated with *Streptomyces* (S), *Trichoderma* (T) or a mix of those two (S + T) compared to control group (C) plants and the microbial consortium (S + T) compared to *Streptomyces* (S) and *Trichoderma* (T) single culture, Table S2: Metabolites obtained from LC-MS data (negative mode) that are differentially accumulated in plants treated with *Streptomyces* (S), *Trichoderma* (T) or a mix of those two (S + T) compared to control group (C) plants and the microbial consortium (S + T) compared to *Streptomyces* (S) and *Trichoderma* (T) single culture.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mahmood, S.; Hussain, S.; Malik, F. Critique of medicinal conspicuousness of Parsley (*Petroselinum crispum*): A culinary herb of Mediterranean region. *Pak. J. Pharm. Sci.* **2014**, *27*, 193–202.
2. Farzaei, M.H.; Abbasabadi, Z.; Ardekani, M.R.S.; Rahimi, R.; Farzaei, F. Parsley: A review of ethnopharmacology, phytochemistry and biological activities. *J. Tradit. Chin. Med.* **2013**, *33*, 815–826. [CrossRef]
3. Dini, I. Chapter 14—Spices and Herbs as Therapeutic Foods. In *Handbook of Food Bioengineering, Food Quality: Balancing Health and Disease*; Holban, A.M., Mihai Grumezescu, A., Eds.; Academic Press: Cambridge, MA, USA, 2018; pp. 433–469.
4. Dini, I.; Laneri, S. Nutricosmetics: A brief overview. *Phytother. Res.* **2019**, *33*, 3054–3063. [CrossRef] [PubMed]
5. Dobricev, N.; Zabkur, J.; Voca, S.; Pliestic, S.; Galic, A.; Delic, A.; Fabek Uher, S. Bioactive compounds content and nutritional potential of different parsley parts (*Petroselinum crispum* Mill.). *J. Cent. Eur. Agric.* **2019**, *20*, 900–910. [CrossRef]
6. Agyare, C.; Appiah, T.; Boakye, Y.D.; Apenteng, J.A. Petroselinum crispum: A review. In *Medicinal Spices and Vegetables from Africa, Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases*, 1st ed.; Kuete, V., Ed.; Academic Press: Cambridge, MA, USA, 2017; pp. 527–547.
7. Liberal, A.; Fernandes, A.; Polyzos, N.; Petropoulos, S.A.; Dias, M.I.; Pinela, J.; Petrović, J.; Soković, M.; Ferreira, I.C.F.R.; Barros, L. Bioactive properties and phenolic compound profiles of turnip-rooted, plain-leafed and curly-leafed parsley cultivars. *Molecules* **2020**, *25*, 5606. [CrossRef] [PubMed]
8. Dini, I.; Marra, R.; Cavallo, P.; Pironti, A.; Sepe, I.; Troisi, J.; Scala, G.; Lombari, P.; Vinale, F. *Trichoderma* Strains and Metabolites selectively increase the production of volatile organic compounds (VOCs) in Olive trees. *Metabolites* **2021**, *11*, 213. [CrossRef]
9. Dini, I.; Graziani, G.; Gaspari, A.; Fedele, F.L.; Sicari, A.; Vinale, F.; Cavallo, P.; Lorito, M.; Ritieni, A. New strategies in the cultivation of Olive trees and repercussions on the nutritional value of the Extra Virgin Olive oil. *Molecules* **2020**, *25*, 2345. [CrossRef]
10. Vejan, P.; Abdullah, R.; Khadiran, T.; Ismail, S.; Nasrulhaq Boyce, A. Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. *Molecules* **2016**, *21*, 573. [CrossRef]
11. Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Marra, R.; Woo, S.L.; Lorito, M. *Trichoderma*–plant–pathogen interactions. *Soil Biol. Biochem.* **2008**, *40*, 1–10. [CrossRef]
12. Bubici, G. *Streptomyces* spp. as biocontrol agents against *Fusarium* species. *CAB Rev.* **2018**, *13*, 50. [CrossRef]
13. Sivalingam, P.; Hong, K.; Pote, J.; Prabakar, K. Extreme environment *Streptomyces*: Potential sources for new antibacterial and anticancer drug leads? *Int. J. Microbiol.* **2019**, *2019*, 20. [CrossRef] [PubMed]
14. Manteca, Á.; Yagüe, P. *Streptomyces* Differentiation in liquid cultures as a trigger of secondary metabolism. *Antibiotics* **2018**, *7*, 41. [CrossRef] [PubMed]
15. Bubici, G.; Marsico, A.D.; D'Amico, M.; Amenduni, M.; Cirulli, M. Evaluation of *Streptomyces* spp. for the biological control of corky root of tomato and *Verticillium* wilt of eggplant. *Appl. Soil Ecol.* **2013**, *72*, 128–134. [CrossRef]
16. Vinale, F.; Sivasithamparam, K. Beneficial effects of *Trichoderma* secondary metabolites on crops. *Phytother. Res.* **2020**, *34*, 2835–2842. [CrossRef] [PubMed]

17.　Ebrahimi-Zarandi, M.; Bonjar, G.H.S.; Riseh, R.S.; El-Shetehy, M.; Saadoun, I.; Barka, E.A. Exploring two *Streptomyces* species to control *Rhizoctonia solani* in tomato. *Agronomy* **2021**, *11*, 1384. [CrossRef]

18.　Bonaldi, M.; Chen, X.; Kunova, A.; Pizzatti, C.; Saracchi, M.; Cortesi, P. Colonization of lettuce rhizosphere and roots by tagged *Streptomyces*. *Front. Microbiol.* **2015**, *6*, 25. [CrossRef]

19.　Dini, I.; Graziani, G.; Fedele, F.L.; Sicari, A.; Vinale, F.; Castaldo, L.; Ritieni, A. Effects of *Trichoderma* biostimulation on the phenolic profile of Extra-Virgin Olive oil and Olive oil by-products. *Antioxidants* **2020**, *9*, 284. [CrossRef] [PubMed]

20.　Barroso, F.M.; Pereira Costa Muniz, P.H.; Milan, M.D.; Silva dos Santos, W.; Cássia de Faria Ferreira, N.; Rodriguez, F.; Carvalho, D.D.C. Growth promotion of Parsley (*Petroselinum crispum* L.) using commercial strains of *Trichoderma* spp. *J. Agric. Sci.* **2019**, *11*, 493–499. [CrossRef]

21.　Saraylou, M.; Nadian Ghomsheh, H.; Enayatizamir, N.; Rangzan, N.; St. Clair Senn, S. Some plant growth promoting traits of *Streptomyces* species isolated from various crop rhizospheres with high root colonization ability of spinach (*Spinacia oleracea* L.). *Appl. Ecol. Environ. Sci.* **2021**, *19*, 3069–3081. [CrossRef]

22.　European Pharmacopoeia Commission. *European Pharmacopoeia*; Maisonneuve: Sainte-Ruffine, France, 1975; pp. 68–80.

23.　Gruszecki, R. The effect of growth stage on essential oil composition of 'Hamburg' parsley. *Acta Hort.* **2007**, *826*, 391–396. [CrossRef]

24.　Lamarti, A.; Badoc, A.; Bouriquet, R. A chemotaxonomic evaluation of *Petroselinum crispum* (Mill.) AW Hill (parsley) marketed in France. *J. Essent. Oil Res.* **1991**, *3*, 425–433. [CrossRef]

25.　Snoussi, M.; Dehmani, A.; Noumi, E.; Flamini, G.; Papetti, A. Chemical composition and antibiofilm activity of *Petroselinum crispum* and *Ocimum basilicum* essential oils against *Vibrio* spp. strains. *Microb. Pathog.* **2016**, *90*, 13–21. [CrossRef] [PubMed]

26.　Petropoulos, S.A.; Daferera, D.; Akoumianakis, C.A.; Passam, H.C.; Polissiou, M.G. The effect of sowing date and growth stage on the essential oil composition of three types of parsley (*Petroselinum crispum*). *J. Sci. Food Agric.* **2004**, *84*, 1606–1610. [CrossRef]

27.　John, R.P.; Tyagi, R.D.; Prevost, D.; Brar, S.K.; Pouleur, S.; Surampalli, R.Y. Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. *Spadzuki* and *Pythium arrhenomanes* as a growth promoter of soybean. *Crop. Prot.* **2010**, *29*, 1452–1459. [CrossRef]

28.　Doni, F.; Anizan, I.; Che Radziah, C.M.Z.; Salman, A.H.; Rodzihan, M.H.; Wan Mohtar, W.Y. Enhancement of rice seed germination and vigour by *Trichoderma* spp. *Res. J. App. Sci. Eng. Technol.* **2014**, *7*, 4547–4552. [CrossRef]

29.　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]

30.　Suárez-Moreno, Z.R.; Vinchira-Villarraga, D.M.; Vergara-Morales, D.I.; Castellanos, L.; Ramos, F.A.; Guarnaccia, C.; Degrassi, G.; Venturi, V.; Moreno-Sarmiento, N. Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. *Front. Microbiol.* **2019**, *10*, 290. [CrossRef] [PubMed]

31.　Novak, A.F.; Clark, G.C.; Dupuy, H.P. Antimicrobial activity of some ricinoleic acid derivatives. *J. Am. Oil Chem. Soc.* **1960**, *38*, 321–324. [CrossRef]

32.　Abdellatief, S.A.; Galal, A.A.A.; Farouk, S.M.; Abdel-Daim, M.M. Ameliorative effect of parsley oil on cisplatin-induced hepato-cardiotoxicity: A biochemical, histopathological, and immunochemical study. *Biomed. Pharmacother.* **2017**, *86*, 482–491. [CrossRef] [PubMed]

33.　El-Sayed, M.M.; Metwally, N.H.; Ibrahim, I.A.; Abdel-Hady, H.; Abdel-Wahab, B.S.A. Antioxidant activity, total phenolic and flavonoid contents of *Petroselinum crispum* Mill. *J. Appl. Life Sci.* **2018**, *19*, 1–7. [CrossRef]

34.　Caboni, P.; Saba, M.; Oplos, C.; Aissani, N.; Maxia, A.; Menkissoglu-Spiroudi, U.; Casu, L.; Ntalli, N. Nematicidal activity of furanocoumarins from parsley against *Meloidogyne* spp. *Pest. Magan. Sci.* **2015**, *71*, 1099–1105. [CrossRef]

35.　de Menezes Epifanio, N.M.; Cavalcanti, L.R.I.; dos Santos, K.F.; Duarte, P.S.C.; Kachlicki, P.; Ozarowski, M.; Riger, C.J.; de Almeida Chaves, D.S. Chemical characterization and in vivo antioxidant activity of parsley (*Petroselinum crispum*) aqueous extract. *Food Funct.* **2020**, *11*, 5346–5356. [CrossRef] [PubMed]

36.　Matos, M.J.; Santana, L.; Uriarte, E.; Abreu, O.A.; Molina, E.; Yordi, E.G. Coumarins—An important class of phytochemicals. In *Phytochemicals—Isolation, Characterization and Role in Human Health*, 1st ed.; Venket Rao, A.V.A., Rao, L.G., Eds.; IntechOpen: London, UK, 2015.

37.　Murray, R.D.H.; Mendez, J.; Brown, S.A. *The Natural Coumarins. Occurance, Chemistry and Biochemistry*, 1st ed.; John Wiley & Sons Ltd.: New York, NY, USA, 1982.

38.　Hauffe, K.D.; Hahlbrock, K.; Scheel, D. Elicitor-stimulated furanocoumarin biosynthesis in cultured parsley cells: S-adenosyl-L-methionine: BergaptoI and S-adenosyl-L-methionine: Xanthotoxol O-methyltransferases. *Z. Naturforsch C* **1986**, *41*, 228–239. [CrossRef]

39.　Harborne, J.B. Flavonol O-glycosides. In *The Handbook of Natural Flavonoids*, 1st ed.; Harborne, J.B., Baxter, H., Eds.; John Wiley & Sons Ltd.: New York, NY, USA, 1999; Volume 1, pp. 297–548.

40.　Uitterhaegen, E.; Sampaio, K.A.; Delbeke, E.I.P.; De Greyt, W.; Cerny, M.; Evon, P.; Merah, O.; Talou, T.; Stevens, C.V. Characterization of French coriander oil as source of petroselinic acid. *Molecules.* **2016**, *21*, 1202. [CrossRef] [PubMed]

41.　Placek, L.L. Review on petroselinic acid and its derivatives. *J. Am. Oil Chem. Soc.* **1963**, *40*, 319–329. [CrossRef]

42.　Yeganehpoor, F.; Zehtab-Salmasi, S.; Ghassemi-Golezani, K.; Shafagh-Kolvanagh, J.; Dastborhan, S. The effect of water stress, salicylic acid and bio-fertilizer on quality of leaf and seed essential oil, and oil components of coriander. *Net. J. Agric. Sci.* **2017**, *5*, 38–47. [CrossRef]

43. Donato, P.; Dugo, P.; Mondello, L. Chapter 8: Separation of Lipids. In *Liquid Cromathography*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2017.
44. Hou, Q.; Ufer, G.; Bartels, D. Lipid signaling in plant responses to abiotic stress. *Plant Cell Environ.* **2016**, *39*, 1029–1048. [CrossRef] [PubMed]
45. Maoka, T. Carotenoids as natural functional pigments. *J. Nat. Med.* **2020**, *74*, 1–16. [CrossRef]
46. Narita, Y.; Inouye, K. Chlorogenic acids from coffee. In *Coffee in Health and Disease Prevention*, 1st ed.; Preedy, V.R., Ed.; Elsevier Science & Technology: Amsterdam, The Netherlands, 2014; Volume 1, pp. 189–197.
47. Aissani, N.; Albouchi, F.; Sebai, H. Anticancer Effect in human glioblastoma and antioxidant activity of *Petroselinum crispum L.* methanol extract. *Nutr. Cancer* **2020**, *29*, 1–9. [CrossRef]
48. Jain, A.; Singh, A.; Chaudhary, A.; Singh, S.; Singh, H.B. Modulation of nutritional and antioxidant potential of seeds and pericarp of pea pods treated with microbial consortium. *Food Res. Int.* **2014**, *64*, 275–282. [CrossRef]
49. Vinci, G.; Cozzolino, V.; Mazzei, P.; Monda, H.; Spaccini, R.; Piccolo, A. An alternative to mineral phosphorus fertilizers: The combined effects of *Trichoderma harzianum* and compost on *Zea mays*, as revealed by ^{1}H NMR and GC-MS metabolomics. *PLoS ONE.* **2018**, *13*, e0209664. [CrossRef] [PubMed]
50. Gębarowska, E.; Pytlarz-Kozicka, M.; Nöfer, J.; Łyczko, J.; Adamski, M.; Szumny, A. The effect of *Trichoderma* spp. on the composition of volatile secondary metabolites and biometric parameters of coriander (*Coriandrum sativum* L.). *J. Food Qual.* **2019**, *2019*, 5687032. [CrossRef]
51. Coppola, M.; Cascone, P.; Chiusano, M.L.; Colantuono, C.; Lorito, M.; Pennacchio, F.; Rao, R.; Woo, S.L.; Guerrieri, E.; Digilio, M.C. *Trichoderma harzianum* enhances tomato indirect defense against aphids. *Insect Sci.* **2017**, *24*, 1025–1033. [CrossRef] [PubMed]