The Effect of *Trichoderma* spp. on the Composition of Volatile Secondary Metabolites and Biometric Parameters of Coriander (*Coriandrum sativum* L.)

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The aim of this work was to investigate the influence of *Trichoderma* spp. on volatile secondary metabolites and biometric parameters obtained from coriander (*Coriandrum sativum* L.). The fruits of coriander treated with liquid suspension spores of *T. harzianum* strain T22 and of *T. asperellum* strain B35 increased the yield of essential oil (by ~36%); however, it was unaffected in its composition. Moreover, *Trichoderma* spp. influenced the yield and increased the number of seeds of coriander by ~60%. Inoculation seeds with *T. asperellum* strain B35 caused about 2-fold increase in the biomass of the aerial parts of coriander. There was also an increased root colonization by the fungus *Trichoderma* spp., limiting the number of phytopathogenic fungi from genus *Fusarium* observed.

1. Introduction

In recent years, there has been a continuous increase in sales on the pharmaceutical market, or more broadly understood as the market of food, dietary supplements, natural substances, and herbs. Their usefulness in most cases is determined by pharmacopoeial standards (Polish or European Pharmacopoeia). Research shows that the quality of pharmacopoeial raw materials is influenced by many factors, such as (1) the cultivar used in cultivation, (2) type of substrate, (3) time and period of harvesting, or (4) the drying method [1]. The influence of the plant’s condition on the metabolites of secondary herbal raw materials is also known, which can be positively influenced by the use of living microorganisms. Microorganisms that support growth and protect plants from pathogens are a subject of growing interest [2–4]. The possibility of using living microorganisms to increase soil fertility and plant production efficiency has already been used in the middle of the last century [4, 5]. Among soil microorganisms, antagonistic fungi from genus *Trichoderma* are the most commonly studied and used in the biological plant protection and/or integrated pest management (IPM) programs [6]. These fungi are capable not only for stimulation of plant growth but also for induction of its defense mechanisms (i.e., ISR, induced systemic resistance; SAR, systemic acquired resistance) [7–9]. *Trichoderma* fungi produce a number of substances with antibiotic properties and hydrolytic enzymes (cellulases, chitinases, xylanases, pectinases, β-1,3-glucanases, and proteases among others), thanks to which they can quickly colonize plant roots and
compete with phytopathogens for the place of infection or nutrients. Numerous reports show that several strains of *Trichoderma* had a significant reducing effect on plant diseases caused by soilborne and foliar pathogens (such as *Rhizoctonia solani*, *Phytophthora* spp., *Pythium ultimum*, *Fusarium* spp., *Alternaria alternata*, *Sclerotinia* spp., *Gaeumannomyces graminis*, *Thielaviopsis basicola*, *Verticillium dahlia*, and *Botrytis cinerea*) under greenhouse and field conditions [6, 9–13]. The use of *Trichoderma* fungi to protect herbal plants or organic crops can be an alternative to synthetic pesticides. The mechanisms of activity of the *Trichoderma* genus towards pathogens (e.g., antibiosis, micoparasitism, and competition) or stimulation of plant growth or induction of defense mechanisms is indicated widely in the literature [6–11]. However, there are very few papers describing the use of these antagonists in the cultivation of herbs or their impact on the content of aromatic compounds [14, 15].

One of the world’s oldest herbs and species plants is coriander (*Coriandrum sativum* L.). Coriander is an annual, herbal, spicy, and melliferous plant, belonging to the celery family (Apiaceae). The main herbal raw materials are fruits containing large amounts of essential oil (0.2–2.6%) and polyphenolic acids (e.g., linoleic, oleic, palmitic, and stearic acids) as well as protein compounds, cellulose, pectins, mineral salts, and vitamins [14, 16]. Moreover, the coriander essential oils also have antibacterial properties against Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Streptococcus haemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris*) [14, 16].

The data presented in the FEMA report (Flavor and Extract Manufacturers Association) indicate that the global annual production of coriander fruits is about 600,000 tonnes per year (http://www.femaflavor.org). Fruits of coriander are used mainly as a flavoring and aromatic additive in the production of food products (meat, snack, alcohol, and confectionery products). Moreover, coriander oils are included in the group of so-called safe (nontoxic) oils that have produced normal seedlings after 14 days of germination under optimal conditions. The tests were performed on 100 fruits from each combination.

The aim of the present research was to determine the effect of coriander fruits treatment with *Trichoderma* fungi on the composition and content of volatile secondary metabolites. This study also investigated the influence of *Trichoderma* fungi on the biometric features of coriander and the degree of root colonization with these fungi. The degree of colonization of roots by toxicogenic fungi of the *Fusarium* genus was also examined.

### 2. Materials and Methods

In the study, coriander fruits (*Coriandrum sativum* var. *microcarpum*) of the Pallas variety belonging to the plants from the oil group (Legutko, Poland) were used. The study examined two strains of antagonistic fungi: *T. harzianum* strain T22 (commercial biological preparation Trianum, Koppert B.V., the Netherlands) and the *T. asperellum* strain B35.

The *T. asperellum* B35 strain comes from the collection of the Agricultural Microbiology Lab, Department of Plant Protection, Wroclaw University of Environmental and Life Sciences, Poland. This strain stimulates the growth of plants and effectively limits a number of diseases of the root system of onions, cabbage, cucumbers, peppers, tomatoes, leek, and celery [20–22].

#### 2.1. Field Experiment and Biometric Analyses

Single-factor field experiment was carried out from 8th of May up to 27th August 2015 in Pawłowice Research Station (51°09′N, 17°06′E) belonging to Wroclaw University of Environmental and Life Sciences. The experiment was arranged with four replicates, on 10 m² elementary plots. The forecrop of experiment in 2014 was oats. In 2015, prior to sowing, mineral fertilization was applied uniformly throughout the field at a dose of 40.0 kg N: 30.0 kg P: 50.0 kg K per hectare.

The following treatments were applied: (1) control (dry fruit); (2) soaking fruits in sterile water for 10 min; and soaking fruits for 10 min in a conidia spores suspension of (3) *T. harzianum* T22 and (4) *T. asperellum* B35. The suspension of *Trichoderma* fungi spores was prepared by mixing the preparation with distilled water in an amount to obtain $1 \times 10^7$ spores 1 mL⁻¹. Conidial densities in the suspension were determined by use of a hemocytometer under a light microscope. The coriander fruits were poured into a suspension and soaked for 10 min, mixing it constantly; then, the seeds were dried at room temperature and sown.

Germination energy and capacity were determined before sowing. According to ISTA [23] regulations, germination energy was determined as the percentage of seeds that have been produced by seedlings classified as normal after 7 days of germination under optimal conditions. The germination capacity was expressed as a percentage of seeds that have produced normal seedlings after 14 days of germination under optimal conditions. The tests were performed on 100 fruits from each combination.

After the growing season, 25 randomly selected plants were collected from each plot and biometric analyses were carried out. The number of fruits, the mass of about 1000 pieces (MTS), and the weight of fruits were calculated. The biomass increase of the aboveground parts and roots was also measured.

#### 2.2. Root Colonization

Root colonization with *Trichoderma* fungi was examined in the period of full vegetation—before the harvest of the raw material for analyses on the content of active compounds. Roots were harvested from representative plants. The roots after mechanical soil removal were thoroughly washed with running tap water to remove adhering soil particles and then were rinsed with a sterile solution of 0.1 M MgSO₄ $\times$ 7H₂O. The roots were aseptically cut into ~5-mm fragments and transferred on a PDA medium (Potato Dextrose Agar, Emapol, Poland) with
30 μg mL⁻¹ streptomycin (to inhibit growth of bacteria colony). Eight fragments were placed per plate and incubated at 28°C. Growing colonies were observed daily. After incubation, the percent degree of roots colonization with *Trichoderma* and *Fusarium* fungi was determined [21, 24].

### 2.3. Analysis of the Content of Essential Oils

Determination of the composition and content of volatile metabolites was carried out by the method of hydrodistillation in the Deryng apparatus [25]. In a 250 mL round-bottom flask, 30 g of fresh coriander fruits was placed, 100 mL of redistilled water was added, and hydrodistillation was carried out for 3 hours. The collected distillate (extracted into 1 mL of cyclohexane and dried with Na₂SO₄) was stored at a temperature of −15°C until the GC-MS analysis was performed. The determination of the composition of volatile fractions was performed according to the chromatographic method developed by Szumny et al. [26]. About 50 μL of the previously prepared solution of the essential oils was diluted ten times in cyclohexane and subjected to the GC-MS analysis (injection of 1 μL).

A gas chromatograph of the PerkinElmer Clarus 680 coupled to a mass spectrometer was used to identify volatile compounds. The separation was performed using an Elite-5MS column (30 m × 0.25 mm × 0.25 μm film thickness). The identification of the compounds was carried out by (1) comparing the EI-MS spectrum of the compound with the spectrum in the NIST14 data library; (2) comparison of Kovats retention index of the identified molecules with data included in the NIST14, NIST Webbook database, and (3) comparison of retention times with commercially available standards isolated from plants.

In addition, chromatographic analysis (GC-MS) of the main components of the obtained essential oils was confirmed by performing nuclear magnetic resonance spectra. The structures of compounds were confirmed on the basis of the comparison of signals obtained from NMR analysis with literature data. The spectra ¹H NMR, ¹³C NMR, and HSQC were performed on the Bruker Avance DRX-500 apparatus, using solutions in CDCl₃.

Specific rotation was measured on the Autopol IV Automatic Polarimeter (Rudolph) equipped with a thermostatic system. The analysis was performed in ethanol at a concentration expressed in g 100 mL⁻¹.

The enantiomeric excess of linalool was determined based on the GC analysis using a chiral column, separation on cyclodextrin associated with dimethyl polysiloxane (CP-Chirasil-DEX CB), with the 25 m, 0.25 mm, 0.25 μm film thickness.

### 2.4. Statistical Analyses

The data from field experiment, biometric analyses, and root colonization were subjected to the analysis of variance using Tukey’s test (p < 0.05) using STATISTICA 13.3 software for Windows.

### 3. Results and Discussion

In the first stage of the study, the influence of *Trichoderma* fungi on the germination energy and capacity of the coriander seeds was evaluated (Table 1). The germination energy of fruits depended on the used treatment. Soaking coriander fruits in water and spore suspension of *Trichoderma* spp. increased the germination energy in the range from 18% to 47%. Treatment of fruits with *T. harzianum* strain B35 increased the germination energy by 20% in comparison with water and Trianum treatment. The experimental combinations used did not affect the germination capacity of the coriander fruits.

The development of coriander during the growing season depended on the method of seed treatment before sowing (Table 2). Coriander growing on control objects had a slower development during the entire growing season, but it was fruiting earlier and had a slightly shorter growing season. A similar rate of coriander development was observed on objects with fruits soaked in water before sowing. In turn, biological treatment of coriander fruit with T22 and B35 strains accelerated the development of plants from 4 days during emergence to 6 days during flowering. It also extended the flowering period, fruit settling, and the growing season. *Trichoderma* species also had a positive influence on the increase in yield and fruit number in the range from 55% to 64% (Table 3). Newman et al. [13] had obtained similar effects in their study, while applying *T. harzianum* spores during the tomato cultivation. In our research, after inoculation fruits with *T. asperellum* strain B35, an ∼2-fold increase in plant biomass was obtained compared with the remaining experimental combinations. At the same time, an increased colonization of the roots by *Trichoderma* species was found in the treatment of fruits with T22 and B35 strains in comparison with objects a and b. Sobolewski et al. [27] investigating carrots had also observed significant increase in height and mass of the plants after applying *Trichoderma* spp. preparation instead of standard T 75 DS/WS (2012) preparation.

Moreover, from the coriander roots, phytopathogenic fungi from the *Fusarium* genus (*F. culmorum*, *F. oxysporum*, and *F.avenaceum*) were isolated. The roots were colonized by these fungi most numerous, whose fruits before sowing was not biologically treated. This may be related to the faster colonization of these specific micromycetes by antagonistic fungi *Trichoderma* and their parasitic activity against these phytopathogens [12, 20–22].

The obtained results unambiguously indicate the effect of coriander fruits treatment with *Trichoderma* strains tested on the content of essential oils (Table 4). The amount of volatile fraction for fruits treated with the Trianum preparation increased by ∼36%. A similar effect was obtained by Ratnakumari et al. (2014) [15], observing the increase in the content of the mint essential oil (*Mentha arvensis*) grown on the substrate inoculated with the *T. harzianum* strain NFCCI 2241 and *T. ovosporum* strain NFCCI 2689. The authors isolated 40 to 50% more volatile fractions from plants compared with controls. Singh et al. (2002) [14] observed an increase in the content of a dozen or so percent
of the essential oil contained in Pogostemon cablin, in the soil inoculated with T. harzianum strain ATCC PTA-3701.

Studies carried out with the GC-MS technique allowed to identify 26 compounds (Table 4). In plants from the control objects a and b, monoterpenoid alcohol, linalool (77%), was the component with the highest content. In contrast, plants from the objects c and d, received 81.8% on average. Other characteristic components were \( \gamma \)-terpinene, \( \alpha \)-pinene, \( p \)-cymene, camphor, geranyl acetate, and geraniol. The highest amount of linalool was found in the oil isolated from Jantar coriander growing in Estonia. Furthermore, in obtained oil, higher contents of linalool and geranyl acetate were observed, than in oils obtained from coriander fruits in Netherlands, Russia, and France. In general, the fruits of European coriander in comparison with Asian ones are characterized by a higher

| Table 1: Germination energy and capacity of the coriander fruits (%) |
|---------------------------------------------------------------|
| No. | Experimental combinations | Germination energy (%) | Germination capacity (%) |
| 1.  | Control | 38<sup>a</sup> | 80<sup>a</sup> |
| 2.  | Fruits soaked in water | 48<sup>b</sup> | 95<sup>a</sup> |
| 3.  | Fruits treated with T. harzianum T22 | 45<sup>b</sup> | 98<sup>a</sup> |
| 4.  | Fruits treated with T. harzianum B35 | 56<sup>c</sup> | 98<sup>a</sup> |

The values in individual columns marked with the same letters differ significantly from the control objects (p = 0.05).

| Table 2: Length of phenological phases of coriander in the growing season. |
|---------------------------------------------------------------|
| Phenological phase | Control<sup>*</sup> | Fruits soaked in water<sup>*</sup> | Fruits treated with T. harzianum T22<sup>*</sup> | Fruits treated with T. harzianum B35<sup>*</sup> |
| Sowing | 08.05 | | | |
| Beginning of emergence | 15 | 12 | 12 | 12 |
| Full emergence | 4 | 4 | 3 | 3 |
| Leafing | 7 | 7 | 7 | 7 |
| Formation of shoots | 15 | 14 | 13 | 13 |
| Beginning of budding | 16 | 21 | 17 | 17 |
| Beginning of flowering | 11 | 11 | 9 | 9 |
| Full flowering | 6 | 6 | 4 | 4 |
| Formation of fruit | 10 | 14 | 16 | 16 |
| Plant drying | 20 | 18 | 23 | 23 |
| Harvest | 27.08 | | | |

<sup>*</sup>Number of days of individual development phases.

| Table 3: Coriander’s biometrics and roots colonization. |
|---------------------------------------------------------------|
| Specification | Control | Fruits soaked in water | Fruits treated with T. harzianum T22 | Fruits treated with T. harzianum B35 |
| Plant height (cm) | 40<sup>a</sup> | 39<sup>a</sup> | 51<sup>b</sup> | 52<sup>b</sup> |
| Mass of the superpowers from 1 plant (g) | 29.4<sup>a</sup> | 32.0<sup>a</sup> | 37.5<sup>b</sup> | 64.0<sup>c</sup> |
| The weight of roots from 1 plant (g) | 3.4<sup>a</sup> | 4.0<sup>a</sup> | 4.3<sup>b</sup> | 5.6<sup>b</sup> |
| Number of fruits from one plant (pcs) | 112<sup>a</sup> | 157<sup>b</sup> | 174<sup>b</sup> | 185<sup>b</sup> |
| Weight of fruit from 1 plant (g) | 2.0<sup>a</sup> | 2.6<sup>b</sup> | 3.1<sup>b</sup> | 3.2<sup>b</sup> |
| Mass of 1000 seeds (g) | 9.3<sup>a</sup> | 9.2<sup>a</sup> | 10.7<sup>b</sup> | 11.1<sup>b</sup> |
| Colonization of roots of Trichoderma spp. (%) | 29<sup>a</sup> | 34<sup>a</sup> | 71<sup>b</sup> | 68<sup>b</sup> |
| Colonization of roots of Fusarium (%) | 50<sup>a</sup> | 51<sup>a</sup> | 23<sup>b</sup> | 26<sup>b</sup> |
| Colonization of roots of F. oxysporum (%) | 32<sup>a</sup> | 34<sup>a</sup> | 16<sup>b</sup> | 18<sup>b</sup> |
| Colonization of roots of F. culmorum (%) | 9<sup>ab</sup> | 13<sup>a</sup> | 6<sup>b</sup> | 6<sup>b</sup> |
| Colonization of roots of F. avenaceum (%) | 9<sup>a</sup> | 4<sup>b</sup> | — | 2<sup>b</sup> |

Values in individual lines marked with the same letters differ significantly from the control objects according to Tukey’s test (p = 0.05).
To improve the quantitative chemical composition of the coriander essential oils, the fruits were soaked in the culture broth of selected biological control agents based on Trichoderma asperellum, T. harzianum, Pseudomonas spp. and T. harzianum. Particularly in the case of the fruits treated with T. harzianum, there was an increase of 28.8% in the percentage of linalool (78%), 25% in α-terpinene (5%), 11% in camphor (2%), and 7% in linalool oxide (1%).

Table 4: Quality and quantity composition of coriander oil and its efficiency.

| No. | Name               | Control* Share % | Soaked fruits* Share % | Fruits treated with T. harzianum T22* Share % | Fruits treated with T. asperellum B35* Share % |
|-----|--------------------|-------------------|------------------------|-----------------------------------------------|-----------------------------------------------|
| 1   | Hexanal            | 0.04              | 0.02                   | 0.03                                           | 0.02                                           |
| 2   | Heptanal           | 0.03              | 0.01                   | 0.03                                           | 0.06                                           |
| 3   | Tricycylene        | 0.01              | 0.03                   | 0.01                                           | 0.01                                           |
| 4   | α-Thujene          | 0.03              | 0.04                   | 0.02                                           | 0.02                                           |
| 5   | α-Pineene          | 2.16              | 2.19                   | 1.31                                           | 1.67                                           |
| 6   | Camphone           | 0.38              | 1.11                   | 0.42                                           | 0.39                                           |
| 7   | Sabineine          | 0.21              | 0.2                    | 0.14                                           | 0.17                                           |
| 8   | β-Pineene          | 0.35              | 0.59                   | 0.2                                            | 0.58                                           |
| 9   | Myrcene            | 0.45              | 0.42                   | 0.29                                           | 0.31                                           |
| 10  | α-Terpineene       | 0.04              | 0.05                   | 0.02                                           | 0.03                                           |
| 11  | p-Cymene           | 0.54              | 0.02                   | 0.4                                            | 0.62                                           |
| 12  | Limonene           | 1.18              | 0.45                   | 0.96                                           | 0.97                                           |
| 13  | 1,8-Cineole        | 0.05              | 0.33                   | 0.23                                           | 0.12                                           |
| 14  | γ-Terpineene       | 3.72              | 3.47                   | 2.75                                           | 3.28                                           |
| 15  | trans-Linalool oxide | 0.20           | 0.15                   | 0.14                                           | 0.09                                           |
| 16  | Terpinolene        | 0.26              | 0.25                   | 0.21                                           | 0.27                                           |
| 17  | Linalool           | 0.18              | 0.45                   | 0.96                                           | 0.97                                           |
| 18  | Camphor            | 0.21              | 0.77                   | 0.21                                           | 0.04                                           |
| 19  | Bornolene          | 0.22              | 0.08                   | 0.16                                           | 0.12                                           |
| 20  | Terpinen-4-ol      | 0.60              | 0.17                   | 0.39                                           | 0.31                                           |
| 21  | α-Terpineole       | 0.19              | 0.04                   | 0.37                                           | 2.55                                           |
| 22  | Decanal            | 0.10              | 0.04                   | 0.05                                           | 0.04                                           |
| 23  | Citronellol        | 4.43              | 1.75                   | 2.31                                           | 1.92                                           |
| 24  | Geraniol           | 0.03              | 0.3                    | 0.02                                           | 0.08                                           |
| 25  | Thymol             | 0.69              | 0.44                   | 0.5                                            | 0.36                                           |
| 26  | Geranyl acetate    | 0.55              | 0.64                   | 0.75                                           | 0.68                                           |

*Exp. = experimental; Lit. = literature. *KI = Kovac retention index; MS = comparison of the EI-MS spectrum obtained with the NIST11 library; S = comparison of retention time with a commercial or isolated pattern (chromatographic standard); NMR = comparison with literature shifts 13C and 1H NMR. **Average of the three replicates; *percentage share acc. to the GC-MS analysis; ⋆ mean ± SD (standard deviation); ** weight-volume percentage, determined according to the Deryng apparatus scale.

content of linalool (∼30%) [30]. Results similar to ours were received by Sriti et al. [31] and Figueiredo et al. [32], who identified ∼40 compounds, of which the main components were also linalool, α-pinene, γ-terpinene, camphor, and geraniol. The quantitative analysis carried out by Msaada et al. [28] and Ravi et al. [33] showed the presence of 0.35 and 0.39% of the essential oils in coriander fruits.

In previous studies, the presence of (S)-(+)−linalool (also called coriandrol) was found in the coriander essential oils. In fact, linalool is not a pure (S) isomer and contains from 12 to 15% of the (R) isomer [34, 35]. Oliver [36] in his work studied the samples of coriander essential oils for the presence of isomeric (S)-(−)-linalool. By means of gas chromatography (GC) and the use of a chiral column, he found the presence of 88% of (S)-(+)−linalool and 12% of (R)-(−)-linalool. The GC analysis we conducted on a column with a chiral filling and a proper rotation test confirmed the presence of (S)-(+)−linalool in the tested material. The presence of (S)-(−)-linalool in the essential oils was also found by Gaydou et al. [37] and Sadowski et al. [38]. Our research yielded very similar contents of (S)-(−)-linalool −82%.

According to the European Pharmacopoeia, the fruits of coriander should contain no less than 0.3% of essential oils. The percentage content of components was also characterized: limonene (1.5–5%), geraniol (0.5–3%), geranyl acetate (0.5–4%), camphor (3–6%), linalool (65–78%), p-cymene (0.5–4%), γ-terpinene (1.5–8%), α-terpinene (0.1–1.5 %), α-pinene (3–7%). Comparing the data in the Pharmacopoeia with the data obtained in our work, it was found that the contents of camphor, α-pinene, and limonene slightly deviate from the acceptable standards.

The conducted research is becoming particularly interesting in the context of recent reports that indicate the use of coriander in animal nutrition, which improves their well-being. These results were demonstrated by Abou-Elkhair et al. [39] in the broiler studies and Mohammed et al. [40] in research on the use of Awassi sheep and rams. In addition, as emphasized by Bahat et al. [41], Sriti et al. [32], Rezaei et al., [42] and Prachayasittkul et al. [43], the interest in coriander is constantly growing. Therefore, it is a good premise for efforts such as those undertaken in our research to intensify its production and improve the quantitative chemical composition.

### 4. Summary

The obtained results indicate the purposefulness of using biological control agents based on Trichoderma species in...
the cultivation of coriander as a source of essential oils. The comparison of efficiency of the distillates obtained with steam proves the positive effect of \textit{T. asperellum} B35 on coriander. And most importantly, increasing the yield of essential oils obtained does not mean a change in composition. This oil is in the upper ranges of pharmacopeial standards. At the same time, the use of antagonistic fungi affects the improvement of biometric parameters of the plant. An increased yield and the number of coriander fruits were found to be at the level of \~60\%. In the experimental combination with \textit{T. asperellum} B35, the biomass of the aerial parts of the plant was twice as large.

**Data Availability**

The NMR and GC-MS data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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