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Effect of Seed Dressing and Soil Chemical Properties on Communities of Microorganisms Associated with Pre-Emergence Damping-Off of Broad Bean Seedlings

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Abstract: Combating soil pathogens that disable plant emergence is among the most difficult challenges of global agriculture. Legumes, preferred in sustainable cultivation systems, are particularly sensitive to pre-emergence damping-off of seedlings. Seed dressing is therefore a very important element in the cultivation technology. The aim of this study was to compare the impact of biological (*Pythium oligandrum*) and chemical (carboxin + thiuram) seed dressing on the quantitative and qualitative composition of microorganisms participating in the epidemiology of this disease, under specific hydrothermal conditions and chemical properties of the soil (pH, humus, macro-, and micronutrient). Microorganism identification was done using the MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) technique. Species were assigned to frequency groups, and populations of pathogens, saprophytes, and antagonists were identified. The biodiversity of these communities was expressed with Simpson's Reciprocal, Shannon–Wiener, and Evenness (Shannon) indices. In individual variants of seed pre-treatment, the correlations between individual edaphic factors and the suppression of pre-emergence damping-off, the number of isolates obtained from infected seedlings, and the share of individual trophic groups of fungi were assessed. The main causes of pre-emergence damping-off of broad bean seedlings are *Ilyonectria destructans*, *Globisporangium irregulare*, *Fusarium equiseti*, *Rhizoctonia solani*, and *Fusarium solani*. Eliminating seed treatment results in a seedling mortality rate of 33.5–42.5%. The effectiveness of the chemical protection product is 44.2% and 25.9%. Carboxin and thiuram reduce the diversity of microorganisms involved in the pathogenesis of pre-emergence damping-off and limit the presence of antagonistic fungi. Under the influence of *P. oligandrum*, there was a five-fold increase in the population of antagonists. An increase in humus in the soil reduces the percentage of diseased broad bean seedlings.

Keywords: soil-borne and non-pathogenic fungi; carboxin + thiuram; soil chemical properties; biodiversity; pre-emergence damping-off; sustainable development

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1. Introduction

The broad bean (*Vicia fabae* L. var. *major* L.) belongs to the *Fabaceae* family and is grown throughout the world. This species plays an important role in the sustainable development of agriculture. It derives nitrogen through the biological binding of atmospheric nitrogen (N_2) and its conversion to NH_3 [1,2]. Thus, it is possible to minimize or completely eliminate the use of industrial nitrogen fertilizers in broad bean cultivation. The low C:N ratio in

organic residues contributes to the integrated management of soil fertility, which has a beneficial effect on successive crops [3]. Therefore, it is a very important element in crop rotation, reduces production costs, protects the environment, and serves food security [4,5].

Broad bean seeds are rich in lysine protein, oligosaccharides, macronutrients, choline, folic acid, lecithin, B vitamins, fluorine, and mineral salts [6–9]. In addition, the broad bean is a source of bioactive compounds: Phytoestrogens, phytohemogluteins, saponins, and polyphenolic compounds that protect the human body against inflammation, metabolic disorders (diabetes), coronary heart disease, and high blood pressure [10–12].

The global increase in the area of broad bean cultivation is associated primarily with the global demand for high-quality protein and functional food ingredients in the human diet [4,13]. China, with its longest recorded tradition of broad bean consumption, is a leader in broad bean cultivation [14,15]. According to Turco et al. [9], broad beans have an important place in the Indian, Mediterranean, English, African, Middle Eastern, and South American diets.

In all climatic zones, damping-off diseases are an important factor limiting the yield and quality of legumes, including broad beans, peas, beans, lentils, soybeans, chickpeas, lupine, and lupins [16–20]. According to many authors, the damping-off of seedlings (which is caused mainly by soil fungi) is the most troublesome problem in the global production of agricultural and forestry plants [21–24]. In addition, the shredded mycelium or fruiting bodies of fungi of the genera *Fusarium*, *Phoma*, and *Alternaria* wintering in the infected seeds may be an important source of infection [25–27]. The critical period of disease development is in the first weeks after sowing and is determined by environmental factors. At low temperatures, which delay emergence, soil fungi can colonize seeds faster. As a result of their activity, the seeds and sprouts break down—they die. This kind of damping-off, which is very difficult to diagnose, is referred to as pre-emergence damping-off.

Numerous epidemiological reports indicate that fungi-like organisms (oomycetes) represented by the genus *Pythium*, *Phytophthora*, and *Aphanomyces euteiches* participate in the pathogenesis of pre-emergence damping-off [28–30]. In the etiology of legume seedling damping-off, *P. ultimum* and *P. irregulare* have been identified most frequently [31]. In legume cultivation, yield losses caused only by *Pythium* spp. are estimated at 50% [21]. Under high soil moisture content, species classified in oomycetes develop rapidly, and their oospores move in the soil easily, infecting germinating roots and the hypocotyl.

The species most frequently determined at various study sites were *Fusarium solani* (Mart.) Sacc. and *F. avenaceum*. The pathogenic complex causing pre-emergence damping-off of broad bean and field bean also includes *F. oxysporum*, *F. acuminatum*. *F. equiseti*, *F. graminearum*, *F. semitectum*, *F. tricinctum*, and *F. culmorum* [19]. Furthermore, in the case of bean and soy cultivation, significant contributions of *Macrophomina paseolina* and *Sclerotinia rolfsii* have been reported [22,32]. *M. paseolina* has many host plants, and it proves burdensome on plantations of not only legumes, but also vegetables and fruit trees [33]. The complex etiology of pre-emergence damping-off, the polyphagous nature of the majority of its pathogens, and their survival in the soil in the form of robust structures make the disease very difficult to fight; whereas, within Oomycetes, pathogenic species belonging to *Pythium* and *Phytophthora*, live intercellularly and penetrate the plant cells through the suction cups [34]. In the conventional cultivation system, synthetic fungicides (seed treatment) and soil fumigants are frequently used, but their efficiency is not always satisfactory [17,35]. This is because frequent fungicide application creates favorable conditions for the immunization of pathogens [36,37]. The use of multiple fungicides during the growing season causes severe consequences to soil and aquatic ecosystems. Unfortunately, apart from target organisms, they also eliminate useful microorganisms, macrophytes, invertebrates, and vertebrates from these environments. The loss of biological diversity of the soil environment is a global problem [38–41]. Its direct effect is the reduction of soil biological activity as well as deterioration of plant, animal, and human health.

The restoration of biodiversity constitutes the basis for ecological programs for integrated disease management implemented for the development of sustainable agriculture.

Organic fertilization plays a key role in stimulating soil biological activity and creating ecosystem balance [42–45]. The relative balance in the soil between groups of parasitic, saprophytic, and antagonistic microorganisms ensures better plant health.

Replacing chemical protection with biological agents enables the avoidance of negative consequences for the environment. To deal with pathogens, *Bacillus subtilis* strain QST 713, *Pseudomonas clororaphis* strain MA342, *Streptomyces* K61 bacteria as well as *Trichoderma*, *Gliocladium*, *Fusarium*, and *Oomycetes–Pythium* fungi are used [46–49]. Many researchers have expressly recommended *Pythium oligandrum* Drechsler for agricultural use [48,50–52]. The phytosanitary benefits for plants primarily stem from the rapid colonization of roots by *P. oligandrum* and the synergistic effect of its mechanisms: Antagonism towards soil pathogens, plant immunity induction, and stimulation of the production of precursors for auxins responsible for plant growth. With the latest advances in molecular technology, these mechanisms are very well understood; nevertheless, little is known about the interactions between *P. oligandrum* and the ecosystem. The effects of the chemicals used are also very rarely assessed at the ecosystem level [53]. The impact of agricultural management systems (crop rotation, cultivation, fertilization, and conservation measures) on soil microbial diversity is relatively well understood [54]. However, we still lack a broader ecosystem approach in which microbial populations are assessed in a non-soil environment [55]. Thus far, it has not been examined whether (and if so, to what degree) practices of biological and chemical seed treatment translate into diversity in microorganism communities that contribute to pre-emergence seedling death. Partially, this gap is being addressed by our 3-year study. Considering the above aspects, we selected the two most popular cultivars of broad beans in Poland and one two-component fungicide (carboxin + thiuram; among the two recommended agents for seed treatment) and the biological agent *P. oligandrum*.

The objective of the study was to determine the effectiveness of broad bean seed treatment with *Pythium oligandrum* as well as carboxin + thiuram in the protection against pre-emergence damping-off of seedlings. Learning about microorganisms involved in the epidemiology of this disease was another objective that in the future would enable the formulation of an optimal strategy for the management of pre-emergence damping-off of seedlings. That is why in our study we analyzed whether the selected seed treatments influence the changes in communities (total population) of these microorganisms: (a) Their belonging to the group of eudominants, dominants, subdominants, recedents, or subrecedents; (b) trophic structure: Percentage of pathogenic, saprobiontic, and antagonistic microorganisms; and (c) biodiversity. The significant impact of edaphic factors on the studied features of microorganism populations as well as the occurrence of pre-emergence damping-off of broad bean seedlings were expressed by the correlation coefficient.

2. Materials and Methods

2.1. Field Experiments

A 3-year field experiment was established at the Experimental Station of the University of Agriculture in Krakow located in Prusy (20°4′21.007″ E, 50°6′48.403″ south-eastern Poland). A two-factorial experiment with the most widely grown broad bean cultivars in Poland—Windsor Biały and Hangdown Biały—and seed treatments with Polyversum WP and Vitavax 200 FS (fluid suspension concentrate) SL was carried out in three replications using the random sub-block method. The size of the plot for observation and harvest was 20 m^2, and the paths between the plots were 1 m wide. Cereals (winter wheat in 2014, oat in 2015, spring barley in 2016) were used each year as a forecrop for broad beans. Before sowing, broad bean seeds were treated with the following preparations:

Biological: Polyversum WP (powder for suspension in water), which contains 106 oospores of P. oligandrum fungus in 1 g of the preparation. According to the labels, 10 g biopreparation per kg of seeds was used for seed soaking.

Chemical: Vitavax 200 FS (carboxin, a compound of the carboxyanilide group, 200 g; thiuram, a compound of the dithiocarbamate group, 200 g in 1 L of the preparation), in 4 $mL \cdot kg^{-1}$ of seeds.

The control consisted of untreated seeds.

The sowing of broad bean seeds took place in the first or second decade week of April, with 50-cm spacing between rows of 10 cm each. The seeds were placed in the soil at a depth of 6 cm. During the growing season, weeds were controlled mechanically in all combinations and no additional protection against pathogens and pests was applied.

2.2. Characteristics of Soil and Climatic Conditions

The soil at the site of the experiment is degraded chernozem, formed from loess, with a granulometric composition of silt loam (SiL) [56,57]. Prior to broad beans sowing, the pH was determined in soil samples by the potentiometric method; the nitrate nitrogen content was determined by the colorimetric method using a spectrophotometer (or by ion-selective electrode); the humus content by the Tiurin method; and the content of absorbable macroelements (P, K, Ca, and Mg) by methods commonly accepted at Chemical-Agricultural Stations. Furthermore, the content of microelements was determined in the soil by commonly used methods.

The soil was slightly acidic (pH_{H2O} = 6.56) (Table S1). The arable layer of the soil was rich in macroelements; it contained, on average, 15.67 mg N-NO_3, 73 mg P, 115.33 mg K, 596 mg Ca, and 65.33 mg Mg in 100 g. The humus content was 2.28%.

In the analyzed growing seasons, the period from sowing to emergence (April–May) was characterized by variable hydrothermal conditions (Table S2). In general, temperatures and rainfall in April and May in all seasons exceeded the multiannual means recorded for them. The exception was 2015 when, in relation to the multi-annual period, lower precipitation in April and a 1.1 °C temperature fall in May were recorded, accompanied by more than four times higher precipitation (and the Sielianiov coefficient K = 7.5 indicated extreme humidity). In turn, May 2017 was very dry (K = 0.5) and warm (15.0 °C). The best hydrothermal conditions for broad bean emergence were in 2016. During this season, both April and May recorded the highest temperatures (10.2 °C and 18.7 °C, respectively), and precipitation of 138 mm provided optimal moisture (Table S2).

2.3. Evaluation of Pre-Emergence Damping-Off of Broad Bean Seedlings

After the emergence of broad bean in the BBCH (scales used in EU countries to identify phytophenological phases of crops, the abbreviation BBCH comes from German: Biologische Bundesanstalt, Bundessortenamt und CHemische) 11–13 phase (BBCH 11—first leaf unfolded; BBCH 13—the 3rd leaf phase), evaluation of seedling infection by pathogenic fungi that contributes to their complete death was carried out. The missing plants (empty places) were counted in the rows of each plot, which was the evidence for pre-emergence blight (foot-rot) of broad bean seedlings. The severity of this disease was evaluated using the following formula:

$$\text{Disease incidence} = \text{Number of diseased seedlings}/\text{Total number of seedlings} \times 100 \quad (1)$$

In turn, the effectiveness of the applied seed treatments was calculated according to Abbott's formula [58].

$$E = (A - B)/A \times 100\% \quad (2)$$

where E is the effectiveness, A is the infection in the control combination, and B is the infection in the protected combination.

2.4. Isolation and Identification of Fungi Colonizing Pre-Emergence-Infested Seedlings

The material for mycological analysis was obtained immediately after the field health status evaluation. Infected non-germinated or poorly germinated seedlings (lack of formed organs above the soil surface) were dug from the central rows of the plots. In accordance with the adopted phytopathological procedure, 40 seedlings were collected from each combination and surface cleaned under running water. With a sterile scalpel, they were cut into 10 pieces at the site of pathological changes. From the border between the healthy

tissue and the diseased tissue, 2–3 mm fragments were cut out and disinfected for 30 s in 0.5% sodium hypochlorite (Javel, Warszawa, Polska). They were rinsed three times in sterile distilled water and dried on sterile gauze. Sets of 10 pieces were placed onto PDA (Potato Dextrose Agar) solidified in Petri dishes (150 mm in diameter), with 0.05 $g \cdot L^{-1}$ chloramphenicol. Four hundred fragments were taken from each combination. The dishes were incubated at a temperature of 23 °C for 5–10 days with a 12 h light cycle. The appearing cultures of fungi were subcultivated on PDA (potato peptone 4 $g \cdot L^{-1}$; glucose 20 $g \cdot L^{-1}$; agar $g \cdot L^{-1}$) and WA (water agar). The number of isolates obtained was counted in each combination.

Species identification in pure fungal cultures was done by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique, defining the protein profiles of ribosomes for which the reference spectral database is available in the Bruker Daltonics GmbH library [59]. Specimens of filamentous fungi were prepared for identification according to the standard procedure developed by Bruker Daltonics GmbH &Co. KG, Bremen, Germany. A small amount of 3-day fungal hyphae was transferred to test tubes with an 8 mL modified liquid medium (cat. no. 221014; Becton Dickinson). They were tightly closed with a cap and placed in the SB2 rotator (cat. no. Y552; Carl Roth GmbH & Co. KG, Bremen, Germany). These were incubated at the top with mixing until a sufficient amount of biological material was found.

The solution of alpha-cyano-4-hydroxycinnamic acid was prepared—HCCA matrix (cat. no. 8255344). To the tube containing HCCA, 250 µL of standard solvent was admeasured (50% acetonitrile, 47.5% water, 2.5% trifluoroacetic acid; cat. no. 19182, Sigma-Aldrich). The contents of the tubes were shaken on the vortex at room temperature until the solution became clear. The tubes with fungal culture were pulled out of the rotator, placed on a table, and left for approximately 10 min until the precipitate settled to the bottom. Then, 1.5 mL of the precipitate was transferred to the Eppendorf tube, which was centrifuged for 2 min at 13,000 rpm (table centrifuge, Biofuge fresco; cat. no. 75005510, Thermo Scientific). The resulting supernatant was removed, and the mixture in the tube was supplemented with 1 mL deionized water and centrifuged again. Thereafter, 300 µL water and 900 µL absolute ethanol (EtOH) were added to the washed precipitate lump. The whole formula was shaken on the vortex and then centrifuged at maximum speed (13,000 rpm). The supernatant was removed very carefully with the pipette, centrifuged for several seconds, and the remaining ethanol was then completely removed. The precipitate lump was left to dry for a few minutes. Then, depending on the amount of precipitate, 10–20 µL 70% formic acid was added (in the case of a large lump, a maximum of 100 µL was used). The tube was supplemented with the same amount of acetonitrile and carefully mixed, then centrifuged for 2 min at approximately 13,000 rpm.

In the next step, 1 µL crude protein extract (supernatant) was pipetted onto a MALDI steel plate and allowed to dry at room temperature. Then, 1 µL HCCA solution was applied, and it was also allowed to dry at room temperature.

The plate was placed in a MALDI Biotyper microflex spectrometer, computer-linked to the database of reference spectra for fungi contained in the Filamentous Fungi Library 1.0 (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The Maldi biotyper miroflex software, which allows the identification of fungi, is MBT Compass Ruo, version 4.1. (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The identification of each isolate was carried out twice, and results were expressed in logarithmic values. Regarding the values of identification indices, 2.3–3.00 indicates a reliable diagnosis of the fungal species; 2.0–2.29 indicates reliable identification to the level of the genus and probable identification to the species level; 1.7–1.99 indicates the probable result to the level of the genus; and 0.00–1.69 indicates an uncertain result of identification.

Correct identification at the species level was obtained for 17 cases, the genus was correctly determined in eight cases, and the result was unreliable (or complete lack of identification) in seven cases. All fungal cultures with an index lower than 2.3 were identified using a standard phytopathological method. Pure fungal cultures were identified on

the basis of morphological characteristics, determined Macroscopically (color, structure, height, density, aerial mycelium and reverse, and colony shape). The Nikon Eclipse E-200 MV optical microscope (Tokyo, Japan) with a magnification of 200x with computer image analysis was used to observe and evaluate the vegetative and conidia-forming hyphae and spores (asexual and sexual characteristics of color, shape, and size). The following species were identified using mycological keys as well as numerous monographic studies [60–63]: *Ascochyta fabae*, *Clonostachys rosea*, *Fusarium avenaceum*, *F. poae*, *F. sporotrichioides*, *Globisporangium irregulare*, *Ilyonectria destructans*, *Juxtiphoma eupyrena*, *Mortierella alpina*, *Ulocladium consortiale*, *Sclerotinia sclerotiorum*, *Trichoderma harzianum*, and *Verticillium albo-atrum*.

2.5. Statistical Analysis

The results concerning the occurrence of seedling blight were subjected to three-way ANOVA, and the significance of differences was verified by the Student's t-test at a significance level of $\alpha = 0.05$ using the STATISTICA version 10.0 package (StatSoft, Krakow, Poland). For the analysis of linear correlation, Pearson (rxy) was used to assess the relationship between:

(1) The number of isolates obtained from infected seedlings, independent variable (x), and the percentage of broad bean seedlings damping-off was adopted as a dependent variable (s).

(2) Content of particular macroelements, microelements, pH, and humus in the soil (independent variables, x), and dependent variables (y):

The number of seedlings that died due to pre-emergence damping-off, the number of isolates obtained from seedlings dead due to pre-emergence damping-off, the share of pathogens, and saprophytic and antagonistic fungi in communities colonizing the infected seedlings—in particular, variants of pre-sowing treatments of broad bean seeds.

2.6. Descriptive Statistics

Fungal communities colonizing dead broad bean seedlings in specific combinations of the experiment were characterized using the following population parameters:

The incidence of individual species $C = a/b \times 100\%$, where:

a is the number of occurrences of a given species and b is the number of all isolates (100%).

On this basis, fungi were assigned to attendance groups: Eudominants, more than 10.0% of total isolated fungi; dominants, 5.1–10.0%; subdominants, 2.1–5.0%; recedents, 1.1–2.0%; and sub-recedents, less than 1.0%.

The percentage share of pathogens and potentially pathogenic, saprotrophic, and antagonistic species was isolated in the fungal community structure. The classification was preceded by pathogenicity tests. In terms of diversity indices, species richness (S) is the simplest and most basic criterion for biodiversity assessment. This factor assigns equal significance to all species, thus characterizing biodiversity in an insufficient manner. The Evenness (Shannon) Index ($J_{H'}$) constitutes a supplement, the values of which range from 0 to 1 and inform of the evenness of distribution of a species in a given population (1, complete evenness). The relationship between evenness and species richness is expressed by the Simpson's Reciprocal (1/D), the minimum value of which is 1, meaning that a species represents a community. The Shannon–Wiener index (H′) is the most useful one for comparative assessment, and its values increase with the increase of species contribution uniformity. Indexes of the biodiversity of microorganism communities were calculated according to the patterns in Gardi and Jeffery [64].

Simpson's Reciprocal Index(1/D) as $D = (\sum_{i=1}^{S} [n_i (n_i-1)])/(N(N-1))$,

where S is the number of species, n is the number of individuals of the given species, and N is the total number of individuals of all analyzed species.

Shannon–Wiener index (H′) as $H' = -\sum_{i=1}^{S} p_i \ln f_0 [p_i]$

pi is the relative abundance of each species. This is the calculated proportion of individuals in a species relative to the total number of individuals in the community: ni/N.

Evenness (Shannon) ($J_{H'}$) as $J_{H'} = H'/H\_max$ where $H\_max = \ln f_0 S$

3. Results and Discussion

3.1. Pre-Emergence Damping-Off of Seedlings Depending on the Examined Factors

When assessing the biodiversity of microorganism communities colonizing pre-emergence infected seedlings, we focus our attention on the entire ecosystem: The plant, soil environment, hydrothermal conditions, and seed-treatment procedure. A strongly infected seedling is treated as a microhabitat, which, in a given growing season, transforms into a non-being that is manifested by seedling death. However, these remain in the soil, and may constitute an infectious inoculum for plant roots, or negatively increase the natural biocontrol potential. This is determined by the percentage of seedlings covered by pre-emergence damping-off as well as the biodiversity of microorganisms that colonize them, and the direct interactions between them. Our study is pioneering in this respect, and the absence of literature in this field constitutes strong proof of this fact.

Despite the fact that our discussion concentrates on the pre-emergence damping-off of broad bean seedlings, we do notice similarities between the epidemiology of this disease in other more frequently studied legume species. The complexity of etiological factors, and their varying environmental preferences, means the disease is capable of developing under any conditions. However, in cultivation areas with different climatic conditions, disease severity is observed in areas with very high humidity and lower temperatures [21,28,29,31]. This has been corroborated by our study, where an extremely humid and cool emergence period in 2015 led to the highest mean seedling reduction of 30.6% (DI) (Figure.1a). In the growing seasons 2016 and 2017, characterized by similar hydrothermal conditions, almost the same intensity of broad bean pre-emergence damping-off was found. The applied seed treatments significantly and strongly limited the development of pre-emergence damping-off. However, a nearly twice lower share of dead seedlings was recorded after seed treatment with carboxin + thiuram. The tested broad bean cultivars showed similar resistance to this disease. At the same time, seed-treatment procedures showed significantly higher effectiveness with respect to the Hangdown Biały variety (Figure 1b). In turn, their 13.5% lowest effectiveness was found in the first year of the study and was almost four times higher in subsequent years. Irrespective of the growing season and broad bean cultivar, seeds treated with carboxin + thiuram, compared to *P. oligandrum*, gave approximately 20% better protective effects.

A significant interaction between the incidence of seedling pre-emergence damping-off and the effectiveness of seed treatment was found between the type of applied preparations, the growing season, and broad bean variety (Table 1). The highest rate of seedling infection (42.7%) was recorded in the last year of the study for an unprotected (controlled) Hangdown Biały variety. The treatment of the Windsor Biały cultivar seeds with *P. oligandrum* in 2016 and 2017 compared to the second variety resulted in a significant reduction in the share of seedlings with the symptoms of pre-emergence damping-off. However, significantly higher effectiveness (45.4%) was demonstrated only for 2016. An impressive protective effect of 78.4% by carboxin and thiram was obtained for this cultivar in 2017, which was characterized by the most favorable hydrothermal conditions.

3.2. Qualitative Analysis of Fungi and Oomycota Colonizing the Seedlings That Died Due to Pre-Emergence Damping-Off

Irrespective of the broad bean variety and the type of preparations used for seed treatment, 32 species of fungi and *Globisporangium irregulare* (syn. *P. irregulare*), an oomycete representative, participated in the process of pre-emergence damping-off of the seedlings (Table 2).

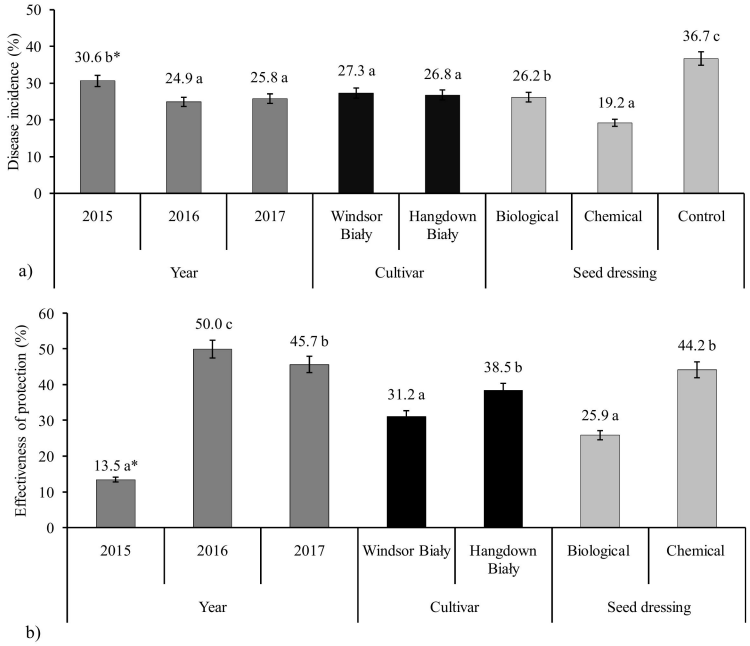

Figure 1. (**a**) Pre-emergence damping-off of broad bean seedlings depending on the examined factors. (**b**) Protective efficacy of the applied seed treatments. * Means within a category followed by the same letter are not significantly different (LSD, $\alpha = 0.05$).

Table 1. Increase in pre-emergence damping-off of the Broadleaf seedlings (DI—disease incidence) and effectiveness of seed treatment.

Treatments	Cultivars	DI—Disease Incidence %			E-effectiveness of Protection in %		
		2015	2016	2017	2015	2016	2017
Pythium oligandrum	Windsor Biały	30.1 gh *	21.3 c	22.5 cd	10.1 b	45.4 f	34.6 e
	Hangdown Biały	32.1 hi	32.1 hi	27.3 fg	6.0 a	33.9 e	36.1 e
Carboxin + thiuram	Windsor Biały	28.4 g	16.7 b	23.4 cde	15.2 c	57.2 g	32.5 e
	Hangdown Biały	25.3 ef	14.9 b	9.2 a	25.8 d	59.5 g	78.4 h
Control	Windsor Biały	33.5 i	37.9 k	34.8 ijk			
	Hangdown Biały	34.2 ij	37.1 jk	l			

* Means in each column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$).

The only eudominant in the population of isolated fungi was *Ilyonectria destructans* (syn. *Cylindrocarpon destructans*), whose share constituted 10.2%. In turn, the group of dominants with a total share of 29.6% included pathogens such as *G. irregulare* (syn. *P. irregulare*), *F. equiseti*, *R. solani*, and *F. solani*. Moreover, subdominants constituted quite an abundant group in the pathogen community (22.5%), and were most abundantly represented by *Botrytis cinerea*, *Ascochyta fabae*, *F. proliferatum*, *F. culmorum*, *Fusarium oxysporum*, and *F. avenaceum*. Parasitic recedents included *Alternaria alternata*, *Didymella glomerata* (*Phoma glomerata*), *Juxtiphoma eupyrena* (syn. *Phoma eupyrena*), and *F. sporotrichoides*. Of the remaining six species included in the sub-recedents, a steadiness of occurrence was demonstrated for *Sclerotinia sclerotiorum*, *F. poae*, and *Verticillium albo-atrum*. The saprobiont community was most abundantly represented by *Epicoccum nigrum* (4.2%), *Mortierella alpina* (3.8%), *Cladosporium cladosporioides* (3.6%), and *Aspergillus niger* (2.4%), whereas the group of antagonistic fungi was represented by two species belonging to *Trichoderma* and *Clonostachys rosea* (syn. *Gliocladium roseum*), which constituted 6.9% in total.

Table 2. Frequency of fungi isolation from dead seedlings due to pre-emergence damping-off. Trophic structure and biodiversity of microbial populations isolated.

Species of Fungi	Cultivar		Year			Seed Dressing			Mean	Group of Attendance
	Windsor Biały	Hangdown Biały	2015	2016	2017	Control	Biological	Chemical		
Pathogenic fungi										
Ilyonectria destructans	9.4	11.2	9.3	9.1	12.0	10.2	10.6	9.6	10.2	Eudominants (10.2)
Globisporangium irregulare	8.1	9.5	16.3	7.1	5.7	8.3	8.3	13.4	9.6	
Fusarium equiseti (2.54) *	8.4	8.4	7.7	10.2	7.7	8.6	8.4	8.8	8.5	Dominants (29.6)
Rhizoctonia solani (2.80)	6.2	5.2	6.8	6.9	3.7	2.5	6.3	10.7	6.0	
Fusarium solani (2.47)	6.0	4.5	6.1	5.9	5.0	7.3	4.7	4.4	5.5	
Botrytis cinerea (2.92)	4.5	5.9	4.0	4.1	6.5	5.6	4.8	3.9	5.0	
Ascochyta fabae	4.9	4.0	3.1	5.2	4.5	4.4	4.3	4.1	4.3	
Fusarium proliferatum (2.72)	3.9	3.8	4.5	4.1	3.2	4.2	3.2	4.4	3.9	Subdominants (22.5)
Fusarium culmorum (2.51)	4.7	2.6	2.4	3.7	4.9	3.3	3.5	4.7	3.7	
Fusarium oxysporum (2.83)	2.6	3.5	4.2	2.9	2.3	3.6	2.6	3.0	3.1	
Fusarium avenaceum	3.4	1.8	3.0	3.4	1.7	3.6	2.3	1.5	2.5	
Alternaria alternata (2.96)	1.7	1.7	1.2	1.6	2.2	3.1	0.1	1.4	1.6	
Didymella glomerata (2.39)	1.7	1.6	2.6	1.6	1.0	1.9	1.9	1.3	1.7	Recedents (5.6)
Juxtiphoma eupyrena	1.0	1.4	1.0	1.2	1.4	1.5	1.0	1.1	1.2	
F. sporotrichioides	1.0	1.3	1.7	0.6	1.0	0.4	2.4	0.6	1.1	
Phoma herbarum (2.77)	1.0	1.1	0.9	1.6	0.6	0.9	1.2	0.9	1.0	
Sclerotinia sclerotiorum	1.2	0.7	1.0	0.6	1.2	1.4	0.6	0.7	0.9	
Fusarium poae	1.0	0.9	0.7	0.9	1.0	1.0	0.6	0.8	0.9	Subrecedents (4.3)
Ulocladium consortiale	1.0	0.8	-	1.1	1.5	1.6	0.2	0.5	1.0	
Verticillium albo-atrum	0.6	0.5	0.3	0.5	0.7	0.3	1.0	0.2	0.5	
Total	72.4	70.7	77.2	72.5	67.8	73.6	67.9	76.2		
Nonpathogenic fungi										
Saprotrophic fungi										
Epicoccum nigrum (2.93)	1.5	7.2	2.1	3.7	6.6	3.7	5.7	3.1	4.2	
Mortierella alpina	5.7	2.7	3.3	4.2	3.7	4.0	3.7	3.4	3.8	
Cladosporium cladosporioides (2.61)	3.8	4.2	2.6	4.7	3.6	4.6	3.9	1.7	3.6	Subdominants (16.1)
Aspergillus niger (2.93)	2.3	2.2	1.9	1.7	3.2	1.3	0.9	5.9	2.4	
Rhizopus stolonifera (2.58)	31	1.2	2.1	2.4	2.1	3.5	1.1	1.5	2.1	
Penicillium expansum (2.49)	1.3	2.0	1.7	1.1	2.0	2.1	0.4	2.4	1.6	
Mucor spp.	1.4	1.4	1.4	1.4	1.5	0.7	2.2	1.5	1.4	Recedents (4.1)
Sarocladium strictum (2.74)	1.5	0.5	0.9	1.4	0.9	1.3	0.1	1.8	1.1	

Table 2. *Cont.*

Species of Fungi	Cultivar		Year			Seed Dressing			Mean	Group of Attendance
	Windsor Biały	**Hangdown Biały**	**2015**	**2016**	**2017**	**Control**	**Biological**	**Chemical**		
Penicillium spp.	0.5	0.4	-	0.9	0.4	0.3	0.9	-	0.4	Subrecedents (0.43)
Acremonium rutilum	0.1	0.0	-	0.1	-	-	0.1	-	0.03	
Total saprotrophic fungi	21.2	21.8	16.0	21.6	24.0	21.5	19.0	21.3		
Antagonistic fungi										
Trichoderma koningii (2.69)	3.2	2.8	3.7	2.9	2.8	1.5	7.0	0.7	3.1	Subdominants (5.3)
Trichoderma harzianum	1.9	2.8	1.7	1.5	3.4	2.4	2.8	1.3	2.2	
Clonostachys rosea	1.3	1.9	1.4	1.5	2.0	1.0	3.3	0.5	1.6	Recedents (1.6)
Total antagonistic fungi	6.4	7.5	6.8	5.9	8.2	4.9	13.1	2.5		
Number of isolates	1341	1255	919	1285	1308	1702	1150	1041		
The species richness (S)	33	32	30	33		32	32	32	31	
Simpson's Reciprocal Index (1/D)	18.43	16.95	14.82	19.43		18.82	19.25	18.93	14.90	
Shannon–Wiener index (H′)	1.28	1.30	1.11	1.39		1.37	1.39	1.34	1.14	
Evenness (Shannon) ($J_{H'}$)	0.39	0.40	0.35	0.41		0.42	0.41	0.42	0.35	

* Score value MALDI-TOF MS—reliable fungi species identification (2.30–3.00)

The infected seedlings of the Hangdown Biały variety were more often colonized by pathogens such as *I. destructans*, *G. irregulare*, *Botrytis cinerea*, and *F. oxysporum*, and this occurred four times more often by the saprophytic species *Epicoccum nigrum* (Table 2). In turn, the seedlings of the Windsor Biały variety were colonized by *R. solani*, *F. solani*, and *Ascochyta fabae*, and among the non-pathogenic species, by *Mortierella alpina* and *Rhizopus stolonifer*.

The pre-sowing treatment of the seeds with both biological and chemical preparations resulted in a reduction of most species isolation frequencies. However, a four-times-higher share of *R. solani* and 5.1% of *G. irregulare* was found in a batch of infected seedlings originating from the carboxin + thiuram-protected variants as compared to the control. In addition, this type of seed treatment was most favorable for the colonization of infected seedlings by *A. niger*. Similarly, after applying the biological protection of broad bean seedlings, the *R. solani* pathogen was noted twice as often, and *F. sporotrichioides* infested the plants five times more often. Furthermore, the share of *T. koningii* was ten times higher than in the case of chemical protection (Table 2).

Furthermore, variability in the frequency of individual species occurrence was noted in the analyzed growing seasons. Markedly high soil moisture content does not favor legume seed germination, as rapid water uptake results in the loss of membrane integrity and cotyledon damage [29,65]. This facilitates seed and germ infection by soil pathogens. *P. ultimum* and P. irregulare have been the most common pathogens found on legume seedlings [28,66,67]. However, in the present study, it was only *G. irregulare* (syn. *P. irregulare*; Table 3). In the combination with chemical seed treatment, we observed both the lowest (4.9%) incidence of the species in the dry period as well as the highest (27.4%) in the period with the highest rainfall. In the control, and with biological seed treatment, the stress associated with excess water content in soil increased the frequency of *G. irregulare* determinations from infected seedlings two-fold, on average. We have determined that, at the lowest air temperature (10.9 °C) and highest (6.98) soil pH, *G. irregulare* is the predominant culprit in broad bean pre-emergence damping-off. This is confirmed in the study of Alcala et al. [31]. On the other hand, in 2017, *I. destructans* fungus was the sole dominant, probably due to higher temperature (15 °C) and lower rainfall levels (Table 3). Within all combinations, *Botrytis cinerea* and antagonistic *T. harzianum* were isolated at a higher frequency. The saprophytic *Epicoccum nigrum* species was ranked as dominant. Thus, in various areas of legume cultivation, the paradigm for determining the preference of the disease via hydrothermal requirements of pathogens transferred via soil is valid [16,19,68]. Moreover, we draw attention to the fact that, independently of the used treatments, the contribution of *I. destructans*, compared to *G. irregulare*, was more stable within the years, and it was determined as dominant by as many as five times. The literature lacks information on the share of this species in a complex causing pre-emergence damping-off of legume seedlings. There are reports indicating the high pathogenicity of *C. destructans* toward underground parts of numerous plant species [24,69–72]. We predict that climate warming may lead to an increase in the soil density of *G. irregulare*.

Garbeva et al. [54] emphasize that the plant species (cultivar) and protective treatments constitute a significant factor that determines the composition of soil microorganisms. With the statistically non-differentiated intensity of pre-emergence damping-off of seedlings between cultivars (Figure 1a), a smaller number of isolates were obtained from infected broad bean seedlings of the Hangdown Biały cultivar (Table 2); nevertheless, the frequency of the major pathogens, G. irregulare and I. destructans, was higher than for the Windsor Biały.

In our study, fungi represented by 32 species constituted 72.6% to 95.1% of total isolates obtained from seedlings infected by pre-emergence damping-off (Table 4). Their more intensive occurrence in the 2016 and 2017 seasons may have stemmed from acidic soil pH (pH 6.01 and 6.25, respectively). Among the pathogens, *F. equiseti* and *R. solani* (Teleomorph: *Thanatephorus cucumeris*) exceeded the threshold of 10% contribution on two occasions. Various *Fusarium* species constitute a constant component of the pre-emergence damping-off complex of legume seedlings [19]. On the other hand, *R. solani* is the most dangerous bean pathogen [73,74] of 15 other alimentary plant species [75]. It is surprising that, in

2016, these species colonized infected broad bean seedlings originating from a combination of chemical seed treatment at the highest frequency (Table 3). This indicates the lack of efficiency of the carboxin + thiuram mixture against these pathogens. Exceptional immunity to synthetic fungicides is demonstrated by *R. solani* [22,75]. In such a situation, the use of bioagents constitutes an alternative method of reducing the frequency of occurrence of pathogens and disease severity, without a negative impact on the environment.

Table 3. Impact of seed treatment and growing season on frequency, biodiversity, and the trophic structure of microorganisms colonizing infected broad bean seedlings.

	Biological Seed Dressing			Chemical Seed Dressing			Control		
	2015	**2016**	**2017**	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**
Pathogenic Fungi									
Ilyonectria destructans	11.8	8.2	11.7	7.0	10.5	11.4	8.9	9.0	12.6
Globisporangium irregulare	12.4	6.0	6.4	27.4	8.0	4.9	11.8	7.5	5.5
Fusarium equiseti	8.4	8.2	8.5	5.7	13.0	7.6	8.4	10.2	7.2
Rhizoctonia solani	6.7	7.1	5.0	11.5	12.0	8.6	3.8	3.6	
Fusarium solani	4.5	4.9	4.6	3.2	4.5	5.4	9.3	7.5	5.2
Botrytis cinerea	4.5	3.4	6.4	1.9	4.0	5.9	5.1	4.8	6.9
Ascochyta fabae	3.4	6.3	3.2	2.5	5.5	4.3	3.4	4.2	5.7
Fusarium proliferatum	2.8	3.0	3.9	5.1	5.0	3.2	5.5	4.5	2.6
Fusarium culmorum	1.7	4.5	4.3	4.5	2.5	7.0	1.7	3.9	4.3
Fusarium oxysporum	3.4	2.2	2.1	4.5	3.0	1.6	4.6	3.3	2.9
Fusarium avenaceum	2.2	4.1	0.7	2.5	2.0		3.8	3.6	3.4
Alternaria alternata			0.4	0.6	2.5	1.1	2.5	2.4	4.3
Didymella glomerata	3.9	1.5	0.4	1.3	2.0	0.5	2.5	1.5	1.7
Juxtiphoma eupyrena	1.1	0.7	1.1	0.6	1.0	1.6	1.3	1.8	1.4
Fusarium sporotrichioides	4.5	0.7	2.1	1.3	0.5			0.6	0.6
Phoma herbarum	1.1	2.2	0.4	0.6	2.0		0.8	0.9	1.1
Sclerotinia sclerotiorum		0.7	1.1	0.6	1.0	0.5	2.1	0.3	1.7
Fusarium poae		1.1	0.7	1.3		1.1	0.8	1.2	1.1
Ulocladium consortiale		0.7			0.5	1.1		1.8	2.9
Verticillium albo-atrum		1.5	1.4	0.6			0.4		0.6
Including the type of Fusarium	27.5	28.7	26.9	28.1	30.5	25.9	34.1	34.8	27.3
Total	76.6	67.0	64.4	82.9	79.5	65.9	76.7	72.4	71.7
Saprotrophic Fungi									
Epicoccum nigrum	3.9	4.9	8.2	1.3	1.5	6.5	1.3	4.2	5.5
Mortierella alpina	2.8	3.7	4.6	1.9	4.0	4.3	4.6	4.8	2.6
Cladosporium cladosporioides	2.2	5.6	3.9		2.5	2.7	4.6	5.4	3.7
Aspergillus niger		1.5	1.1	5.1	4.0	8.6	1.3	0.6	2.0
Rhizopus stolonifer	1.1	0.7	1.4	1.3	1.0	2.2	3.4	4.5	2.6
Penicillium expansum		0.4	0.7	2.5	2.5	2.2	2.5	0.9	2.9
Mucor spp.	2.2	1.9	2.5	2.5	1.0	1.1		1.2	0.9
Sarocladium strictum		0.4		1.3	2.0	2.2	1.3	1.8	0.9
Penicillium spp.		1.9	0.7					0.6	0.3
Acremonium rutilum		0.4							
Total	12.2	21.4	23.1	15.9	18.5	29.8	19.1	24.0	21.5
Antagonistic Fungi									
Trichoderma koningii	9.0	6.0	6.0	0.6	1.0	0.5	1.7	1.5	1.4
Trichoderma harzianum	1.7	3.0	3.6	0.6	0.5	2.7	2.5	0.9	3.7
Clonostachys rosea	4.5	2.6	2.8		0.5	1.1		1.2	1.7
Total	15.2	11.6	12.5	1.2	2.0	4.3	4.2	3.6	6.8
Number of isolates	1175	1250	1025	1025	900	1200	1700	1380	2025
Indicators of the Biodiversity of Fungal Communities									
The species richness (S)	23	31	29	28	29	27	27	31	31
Simpson's Reciprocal Index (1/D)	16.24	22.27	18.26	9.66	16.40	18.62	18.55	19.62	19.56
Shannon-Wiener (H′)	1.05	1.62	1.35	1.01	1.21	1.21	1.28	1.33	1.56
Evenness (Shannon) ($J_{H'}$)	0.33	0.47	0.40	0.30	0.36	0.37	0.39	0.39	0.45
Group of Attendance	>10%	-Eudominants		5.1–10%	-Dominants		2.1–5.0%	-Subdominants	
	1.1–2.0%	-Recedents		<1.0%	-Subrecedents				

Table 4. Relationship between the structure of a microorganism community colonizing infected seedlings, biodiversity indices, assimilable macro- and micronutrient forms, and the incidence of pre-emergence damping-off and its associated microorganisms (correlation coefficients).

Independent Variables (*x*)	Dependent Variables (*y*)														
	Disease Incidence			Number of Isolates			Share								
							Pathogens			Saprobiontic			Antagonists		
	B*	CH	C	B	CH	C	B	CH	C	B	CH	C	B	CH	C
pH H_2O	0.86	0.97	−0.88	−0.05	0.15	0.23	0.84	0.45	0.93	−0.92	−0.44	−0.99	0.99	−0.50	−0.10
Humus content	−0.99	−0.93	−0.99	−0.50	0.42	0.33	−0.99	−0.86	−0.98	0.98	0.86	0.64	−0.84	0.89	0.63
N	0.46	0.15	−0.42	1.00	−0.99	−0.98	0.48	0.87	0.31	−0.33	−0.87	0.34	−0.05	−0.83	−0.98
P	−0.45	−0.15	0.42	−1.00	0.99	0.98	−0.48	−0.86	−0.31	0.33	0.87	−0.34	0.05	0.83	0.98
K	−0.35	−0.03	0.32	−0.99	0.99	0.99	−0.38	−0.80	−0.20	0.22	0.81	−0.44	0.17	0.76	0.96
Ca	0.99	0.97	−0.99	0.37	−0.28	−0.19	0.99	0.78	0.99	−0.99	−0.77	−0.75	0.91	−0.82	−0.51
Mg	0.75	0.92	−0.77	−0.25	0.34	0.42	0.73	0.27	0.84	−0.83	−0.25	−0.99	0.98	−0.33	0.09
Cu	−0.57	−0.28	0.54	−0.99	0.97	0.95	−0.59	−0.92	−0.44	0.45	0.93	−0.21	−0.08	0.90	0.99
Zn	−0.56	−0.26	0.52	−0.99	0.98	0.95	−0.58	−0.91	−0.42	0.43	0.92	−0.23	−0.06	0.89	0.99
Mn	−0.88	−0.69	0.87	−0.82	0.76	0.70	−0.89	−0.99	−0.80	0.81	0.99	0.26	−0.53	0.99	0.89
Fe	−0.91	−0.99	0.92	−0.04	−0.05	−0.14	−0.89	−0.53	−0.96	0.93	0.52	0.93	−0.99	0.67	0.30
Participation:															
Pathogens	0.99	0.62	−0.99	Explanations:			$r_{xy} > 0.9$			Very strong dependence					
Saprobiontic	−0.99	−0.61	0.70				$0.7 < r_{xy} < 0.9$			Quite strong dependence					
Antagonists	0.86	−0.67	0.56				$0.4 < r_{xy} < 0.7$			Moderate dependence					
The species richness (S)	−0.86	−0.04	0.97				$0.2 < r_{xy} < 0.4$			Weak dependence					
Simpson's Reciprocal Index (1/D)	−0.54	−0.96	0.95				$r_{xy} < 0.2$			No dependency					
Shannon-Wiener (H′)	−0.71	−0.99	0.81												
Evenness (Shannon) ($J_{H'}$)	−0.68	−0.98	0.70												

* **B**—Biological seed dressing; **CH**—Chemical seed dressing; **C**—Control.

3.3. Biodiversity and Trophic Structure of Microbial Populations Isolated from Pre-Emergence-Infested Broad Bean Seedlings

Among the analyzed factors, environmental conditions in individual growing seasons determined this character to the greatest degree, altering the counts (richness) of species within the range of 30 to 33 (Table 2). In our study, its value ranged from 0.35 to 0.42. The lowest value indicated that the total number of microorganisms is least evenly distributed between species isolated from seedlings infected in 2015, and in the subjects of chemical seed treatment. The highest uniformity (0.42) was observed with the same species richness of 32 characterized communities of microorganisms colonizing infected seedlings from biologically protected seeds, as well as from the growing season of 2017.

The vegetative season of 2016 was most favorable for the diversity of microorganism communities colonizing broad bean seedlings (19.43) (Table 2). On the other hand, carboxin + thiuram treatment of seeds and environmental conditions in 2015 restricted biodiversity to an equal extent. Adequately, the highest diversity was characterized by microorganism assemblages with the highest evenness and richness. Variable hydrothermal conditions and chemical properties of soils in the studied growing seasons clearly modified the trophic structure of these microorganisms. The population of pathogenic organisms increased with the increase of the Sielianinow coefficient (K). Its highest value (7.5) noted in May 2015 (extreme wetness, accompanied by low temperatures) corresponded to a 77.2% share of pathogens (Table S2 and Table 2), whereas the shift of this index (1.4) in the direction optimal for plant development resulted in a 4.7% increase in non-pathogenic fungi share, with a concurrent reduction in the pathogen population. In turn, very dry conditions (K = 0.4) in 2017 moved this limit by another 4.7%, which was reflected in the reduction of the population of pathogens to 67.8%.

Under the influence of broad bean seed treatment with *P. oligandrum* oospores, the pre-emergence infected seedlings with the highest frequency (13.1%) were colonized by antagonistic fungi (*T. koningii*, *T. harzianum*, and *Clonostachys rosea* syn. *Gliocladium roseum*), which were more than five-fold less abundant in the chemically protected plot (2.5%); (Table 2). This also reduced the contribution of *R. solani* (Table 3). Moreover, Brožová [76] states that not only does *P. oligandrum* reduce the density of *R. solani*, *Fusarium* spp., and many other important pathogens in the soil, but it also increases the antagonist population. The antagonism of *Trichoderma* fungi against *R. solani* has been determined in Mayo et al.'s [73] studies. Other mechanisms, such as food competition and immunity gene induction, have also been demonstrated [51,73,77]. *C. rosea* has a high potential of combating plant disease and, in particular, the mycoparasitism against *S. sclerotiorum* that was demonstrated in a study by Sun et al. [78]. Our studies confirm this fact because, at the highest concentration of 4.5%, *C. roseum* and *S. sclerotiorum* were completely absent from the community of fungi colonizing infected seedlings (Table 3). According to Koutb and Ali [79], *Epicoccum purpurascens* (syn. *E. nigrum*), which colonizes legume seedlings, is an antagonist to *P. irregulare* and is capable of efficient reduction of its pathogenic activity. The increased frequency of *E. nigrum* determinations was associated with the reduction of *G. irregulare* population, which was particularly well marked in the object without the use of seed treatments (Table 3). In the preceding study, saprophytic fungi—*E. nigrum*, *C. cladosporioides*, *Penicillium* spp., *Mucor*, and antagonistic *Trichoderma* and *Gliocladium*—restricted the infection of potato tubers by a pathogenic fungi complex [44]. Furthermore, in the present study, independently of seed treatment, the elevated total share of saprophytes and antagonists was reflected by the improved health of broad bean seedlings.

Assemblages of microorganisms inhabiting pre-emergence damping-off of infected broad bean seedlings from objects with chemical seed treatment in the 2015 and 2016 seasons had the highest share of pathogens (82.9% and 79.5%); in 2015, a record frequency of saprophytes of 29.8% with dominant *A. niger* (8.6%) and *E. nigrum* (6.5) was observed (Table 3). According to Anil Kumar and Rajkumar [80], in an in vitro test, *A. niger* inhibited the surface growth of *Fusarium oxysporum* by as much as 80.3%. We believe that the lowest frequency of occurrence of *F. oxysporum* (1.6%) recorded, as well as the elimination of *F.*

avenaceum, could have stemmed from the activity of *A. niger*, among other things. The lack of adequate studies in the literature suggests careful conclusions and to abandon the assessment of individual interactions between microorganisms for the sake of a holistic approach. The study demonstrated the interaction of weather factors and seed treatment in shaping the community of microorganisms contributing to the pre-emergence death of broad bean seedlings (Table 3). It is worth noting that the most stable system between pathogens and non-pathogenic fungi was created by fungal communities that colonized the infected seedlings of the control object. The share of pathogens was in the range of 71.7–76.7% and non-pathogenic fungi were in the range 28.3–23.3%. A slightly wider (8% for pathogenic organisms and 8.2% for non-pathogenic organisms) range of changes between the seasons was recorded in a batch of infected seedlings from plots where the seeds had been treated with biopreparation. In addition, this type of treatment in each year of the study generated the largest proportion of antagonistic fungi, with the largest share (15.2%) recorded in 2015 (Table 3). In this case, *T. koningii* was in the dominant rank, accounting for as much as 9% of the total number of isolated microorganisms. At the same time, the smallest and least diverse population of saprotrophic fungi was found (12.2%), as well as the eudomination of pathogens such as *G. irregulare* and *I. destructans*. In subsequent growing seasons, *T. koningii* also dominated with a constant 6% share. Overall, the proportion of antagonistic fungi decreased to 11.6% and 12.5%, respectively, but almost double the proportion of saprotrophs was recorded compared to 2015.

It was observed that the greatest changes in the communities of fungi isolated from diseased seedlings occurred under the influence of chemical treatments. Carboxin + thiram eliminated antagonistic fungi from them. Contrary to biological protection, their strongest exclusion was recorded in 2015 (a very wet and cold May favored the development of pre-emergence damping-off of seedlings). The total share of subrecedents *T. koningii* and *T harzianum* was only 1.2%. The combination had the highest proportion of pathogens, as high as 82.9%, and among them, a record 27.4% proportion of *G. irregulare*. On the other hand, in the extremely dry, yet warm May 2017, 29.8% of the saprotrophic fungi were recorded. Among them, *A. niger* (8.6%) and *E. nigrum* (6.5%) were dominant.

3.4. Assessment of the Relationship between Fungal Communities Colonizing Infected Broad Bean Seedlings, Soil Chemical Properties and Disease Severity

We are the first to assess the relationship between the share of individual groups of fungi colonizing infected seedlings in determined edaphic conditions with seed treatment and DI. Our hypothesis that seed treatment with a mixture of carboxin and thiram reduces, to a greater extent than *P. oligandrum*, the biodiversity of microorganism communities colonizing infected broad bean seedlings, has been confirmed (Table 3). However, the results of correlation between indices of this biodiversity, trophic groups of the microorganisms, and DI were surprising. In general, with the exception of two cases, values of these correlations in objects with seed treatment were of a negative nature and were exactly opposite to those observed in the control (Table 4).

Within biological seed treatment, the increase in pathogen contribution was accompanied by the intensification of seedling pre-emergence damping-off, and this relationship was very strong ($r = 0.99$). In the control, this relationship had an exactly opposite character of a negative variable ($r = -0.99$). The increased share of saprophytes corresponded to a reduced amount of DI; a very strong relationship was found in biological seed treatment ($r = -99$), moderate in the chemical ($r = -0.61$), and a positive variable ($r = 0.70$) in the control. The literature provides convincing information on the positive correlation between microorganism diversity and soil capacity to inhibit plant diseases [54]. By transferring this to our study, this is confirmed only in combinations with seed treatment; lower DI values correspond to increased biodiversity indices.

In the 3-year experiment with regard to the majority of distinguished edaphic factors, no increased variability was observed (Table S1). However, independent variables assumed in the study had variable effects on the tested parameters (Table 4). Values of correlation coefficients indicate that not all factors are significant, and the type of seed treatment

used may additionally modify those interactions. With the increase of humus content in the soil, the percentage of seedlings infected by pre-emergence damping-off decreased, and it remained stable in all seed-treatment variants. Moreover, better health could have stemmed from the reduced share of pathogenic organisms, particularly with the lack of seed treatment or use of biological treatment, the use of which additionally increases saprophyte population. An increase in humus content in soil (for instance, via organic matter supplementation) constitutes a significant factor in the restoration of the microbial balance and the alleviation of the influence of soil-transferred pathogens [44,45,54].

With increased soil pH (range 6.01–6.98) and the available Ca and Mg forms, together with the lack of seed treatment, seedling damping-off suppression, an increased share of pathogens, and reduced contribution of saprophytes were observed. However, a very strong positive variable was observed after seed treatment. These relationships fully reflect the environmental preferences of the previously presented microorganism communities.

A poor relationship between macronutrients such as nitrogen, phosphorus, potassium, and the number of seedlings dying from infection (DI) has been demonstrated. However, their relationships with the number of isolates obtained from infected seedlings were found to be strong, yet variable. Increased soil phosphorus and potassium content contributed to increased total microorganism populations colonizing infected seedlings that originated from control fields and from chemical protection. On the other hand, a very strong negative correlation between these variables was found in the presence of biological seed treatment (Table 4).

Based on our study, we conclude that the biodiversity of microbial communities associated with pre-emergence damping-off of broad bean seedlings is moderately dependent on soil pH, humus content, the availability of different forms of Ca and Mg, and microelements such as Fe, B, and Mn (Table S3). On the other hand, it does not depend on the content of macroelements N, P, and K, nor microelements Cu and Zn. The correlation coefficients indicate that any treatment aimed at increasing soil pH including calcium and magnesium fertilization may result in a reduction in the biodiversity of endophytic microorganisms of diseased seedlings. Similar effects can also be expected in soils rich in boron or fertilized with it. In our experiment, a positive moderate relationship was found between the listed biodiversity indices and the humus content of the soil. Our previous publications and those of other authors indicate that this soil component plays a key role in shaping soil biodiversity [42–45,54]. In light of the above, ensuring an adequate level of humus in the soil may be one of the more effective methods for improving plant health.

4. Conclusions

Each vegetative season, between 33.5% and 42.7% broad bean seedlings die due to pre-emergence damping-off. On average, seed treatments with *P. oligandrum* reduce this index to 26.2%, and with the carboxin + thiuram mixture, it is further reduced to 19.2%. Hydrothermal conditions determine the efficiency of biological protection to a greater extent than that of chemical protection. Moreover, edaphic factors such as pH, humus content, and available macro- and micronutrient forms determine the structure of soil microorganisms, which in turn influence the degree of seedling infection (DI). The quantitative and qualitative composition of microorganisms that infect and colonize seedlings is the reflection of the phytosanitary state of the soil and the suppression of individual microorganisms under the influence of seed treatments. We would like to emphasize that the total CFU count isolated from 1 g of infected seedlings moderately correlates with the DI. With the same values of macronutrients (N, P, and K) and micronutrients (Cu and Zn) available in the soil, the type of applied treatment considerably changes the nature of interactions with the amount of isolated CFU. In the case of carboxin + thiram mixtures, the correlation was positive and, with regard to *P. oligandrum*, it was negative.

Nineteen pathogenic fungi species, as well as *G. irregulare*, an Oomycota representative that was isolated at the frequency of 9.6%, participated in the process of pre-emergence damping-off. The stability of occurrence and highest frequency were found in *I. destructans*

(10.2%), *F. equiseti* (8.5%), *R. solani* (6.0%), and *F. solani* (5.5%). Infected broad bean seedlings further constitute a habitat for ten saprophytic and three antagonist fungi species. *P. oligandrum*, contrary to carboxin + thiuram, does not reduce the biodiversity of microorganism communities colonizing on—and in—the infected seedlings. Indeed, chemical treatment protects seedlings against pre-emergence damping-off over 18.3% more efficiently, on average; nevertheless, it favors their colonization by pathogens, with concomitant reduction of antagonistic fungi population. Dead seedlings with dominant pathogen communities remaining in the soil in the given vegetative season, without environmental resistance, constitute an infectious inoculum for the root system of the cultivated plants as well as the successive crops. On the other hand, under the influence of *P. oligandrum*, there was a five-fold increase in the population of antagonist fungi: *T. koningii*, *T. harzianum*, and *C. rosea* may contribute to the reduction of the infectious potential of soil and improved plant health.

These study results can be treated as a predictive basis for the determination of both the soil infectious potential and biocontrol.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11091889/s1, Table S1: Chemical analysis of arable soils during the studied growing seasons, Table S2: Weather conditions in the region of experiment in the years 2015–2017, Table S3: Relationship between the assimilable macro- and micronutrient forms, and biodiversity indices of microorganism community colonizing infected seedlings (correlation coefficients).

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References

1. Biswas, B.; Gresshoff, P.M. The role of symbiotic nitrogen fixation in sustainable production of biofuels. *Int. J. Mol. Sci.* **2014**, *15*, 7380–7397. [CrossRef]
2. Mus, F.; Crook, M.B.; Garcia, K.; Garcia Costas, A.; Geddes, B.A.; Kouri, E.D.; Paramasivan, P.; Ryu, M.H.; Oldroyd, G.E.; Poole, P.S.; et al. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl. Environ. Microbiol.* **2016**, *82*, 3698–3710. [CrossRef] [PubMed]
3. Franke, A.C.; van den Brand, G.J.; Vanlauwe, B.; Giller, K.E. Sustainable intensification through rotations with grain legumes in Sub-Saharan Africa: A review. *Agric. Ecosyst. Environ.* **2018**, *261*, 172–185. [CrossRef]
4. Multari, S.; Stewart, D.; Russell, W.R. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 511–522. [CrossRef]
5. Wahbi, S.; Prin, Y.; Thioulouse, J.; Sanguin, H.; Baudoin, E.; Maghraoui, T.; Oufdou, K.; Le Roux, C.; Galiana, A.; Hafidi, M.; et al. Impact of wheat/faba bean mixed cropping or rotation systems on soil microbial functionalities. *Front. Plant Sci.* **2016**, *7*, 1364. [CrossRef]
6. Giménez, M.A.; Drag, S.R.; De Greef, D.; Gonzalez, R.J.; Lobo, M.O.; Samman, N.C. Rheological, functional and nutritional properties of wheat/broad bean (*Vicia faba*) flour blends for pasta formulation. *Food Chem.* **2012**, *134*, 200–206. [CrossRef]
7. Łabuda, H.; Buczkowska, H. Biologically active substances in the broad bean green seeds after storage in the pods. *Acta Sci. Pol.-Hortorum Cultus* **2014**, *13*, 83–93.
8. Revilla, I. Impact of thermal processing on faba bean (*Vicia faba*) composition. In *Processing and Impact on Active Components in Food*; Academic Press: Cambridge, MA, USA, 2015; Volume 40, pp. 337–343. [CrossRef]
9. Turco, I.; Ferretti, G.; Bacchetti, T. Review of the health benefits of Faba bean (*Vicia faba* L.) polyphenols. *J. Food Nutr. Res.* **2016**, *55*, 283–293.

10. Chaieb, N.; González, J.L.; Lópezesas, M.; Bouslama, M.; Valiente, M. Polyphenols content and antioxidant capacity of thirteen faba bean (*Vicia faba* L.) genotypes cultivated in Tunisia. *Food Res. Int.* **2011**, *44*, 970–977. [CrossRef]
11. Bouchenak, M.; Lamri-Senhadji, M. Nutritional quality of legumes, and their role in cardiometabolic risk prevention: A review. *J. Med. Food* **2013**, *16*, 185–198. [CrossRef] [PubMed]
12. Luo, Y.; Xie, W.; Jin, X.; Tao, B.; Chen, Q.; Zhu, W. Impact of sprouting pretreatment on phytic acid and polyphenol level of faba bean (*Vicia fabae* L.) flour. *Int. Food Res. J.* **2013**, *20*, 1133–1137.
13. Gasim, S.; Hamad, S.A.A.; Abdelmula, A.; Ahmed, I.A.M. Yield and quality attributes of faba bean inbred lines grown under marginal environmental conditions of Sudan. *Food Sci. Nutr.* **2015**, *3*, 539–547. [CrossRef] [PubMed]
14. Alemu, G.Y.; Tadele, Y.A. Management of faba bean gall disease through the use of host resistance and fungicide foliar spray in Northwestern Ethiopia. *Adv. Crop Sci. Tech.* **2017**, *5*, 254. [CrossRef]
15. Li, L.; Yang, T.; Liu, R.; Redden, B.; Maalouf, F.; Zong, X. Food legume production in China. *Crop J.* **2017**, *5*, 115–126. [CrossRef]
16. Abdel-Monaim, M.F. Improvement of biocontrol of damping-off and root rot/wilt of fabae bean by salicylic acid and hydrogen peroxide. *Mycobiology* **2013**, *41*, 47–55. [CrossRef]
17. Abdel-Monaim, M.F. Integrated management of damping-off, root and/or stem rot diseases of chickpea and efficacy of the suggested formula. *Not. Sci. Biol.* **2011**, *3*, 80–88.
18. Chang, K.F.; Hwang, S.F.; Gossen, B.D.; Strelkov, S.E.; Turnbull, G.D.; Bing, D.J. Effect of seeding practices, temperature and seed treatments on fusarium seedling blight of narrow-leaved lupin. *Can. J. Plant Sci.* **2011**, *91*, 859–872. [CrossRef]
19. Chang, K.F.; Conner, R.L.; Hwang, S.F.; Ahmed, H.U.; McLaren, D.L.; Gossen, B.D.; Turnbull, G.D. Effects of seed treatments and inoculum density of *Fusarium avenaceum* and *Rhizoctonia solani* on seedling blight and root rot of faba bean. *Can. J. Plant Sci.* **2014**, *94*, 693–700. [CrossRef]
20. Mitiku, M. Management of root rot diseases of cool season food legumes crops in Ethiopia. *J. Plant Sci.* **2017**, *5*, 104–109. [CrossRef]
21. Hwang, S.F.; Ahmed, H.U.; Turnbull, G.D.; Gossen, B.D.; Strelkov, S. Effect of seeding date and depth, seed size and fungicide treatment on *Fusarium* and *Pythium* seedling blight of canola. *Can. J. Plant Sci.* **2015**, *95*, 293–301. [CrossRef]
22. Reznikov, S.; Vellicce, G.R.; González, V.; de Lisi, V.; Castagnaro, A.P.; Ploper, L.D. Evaluation of chemical and biological seed treatments to control charcoal rot of soybean. *J. Gen. Plant Pathol.* **2016**, *82*, 273. [CrossRef]
23. Lamichhane, J.R.; Dürr, C.; Schwanck, A.A.; Robin, M.H.; Sarthou, J.P.; Cellier, V.; Messéan, A.; Aubertot, J.-N. Integrated management of damping-off diseases. *Agron. Sustain. Dev.* **2017**, *37*, 10. [CrossRef]
24. Farh, M.E.-A.; Kim, Y.-J.; Kim, Y.-J.; Yang, D.-C. *Cylindrocarpon destructans/Ilyonectria radicicola*-species complex: Causative agent of ginseng root-rot disease and rusty symptoms. *J. Ginseng Res.* **2018**, *42*, 9–15. [CrossRef] [PubMed]
25. El-Samawaty, A.M.; Omar, M.; El-Naggar, M.; Yassin, O. Amer. Pathological assessment of seed borne fungi involved in cotton seedlings damping-off. *J. Plant Sci.* **2012**, *7*, 85–95. [CrossRef]
26. Gleń, K.; Boligłowa, E.; Gospodarek, J. Fungi colonising broad bean seeds depending on protection method. *Pol. J. Agron.* **2013**, *12*, 9–16. (In Polish) [CrossRef]
27. Gleń-Karolczyk, K.; Boligłowa, E.; Gospodarek, J. Mycological purity of broad bean (*Vicia faba* L.) seeds in the conditions of companion planting and differentiated protection. *J. Res. Appl. Agric. Eng.* **2016**, *61*, 126–131.
28. You, M.P.; Rensing, K.; Renton, M.; Barbetti, M.J. Modeling effects of temperature, soil, moisture, nutrition and variety as determinants of severity of *Pythium* damping-off and root disease in subterranean clover. *Front. Microbiol.* **2017**, *8*, 2223. [CrossRef]
29. Kato, M.; Minamida, K.; Tojo, M.; Kokur, T.; Hamaguchi, H.; Shimada, S. Association of *Pythium* and *Phytophthora* with pre-emergence seedling damping-off of soybean grown in a field converted from a paddy field in Japan. *Plant Prod. Sci.* **2013**, *16*, 95–104. [CrossRef]
30. Carmona, M.A.; Sautua, F.J.; Grijalba, P.E.; Cassina, M.; Pérez-Hernández, O. Effect of potassium and manganese phosphites in the control of *Pythium* damping-off in soybean: A feasible alternative to fungicide seed treatments. *Pest Manag. Sci.* **2018**, *74*, 66–374. [CrossRef]
31. Alcala, A.V.C.; Paulitz, T.C.; Schroeder, K.L.; Porter, L.D.; Derie, M.L.; du Toit, L.J. *Pythium* species associated with damping-off of pea in certified organic fields in the Columbia Basin of Central Washington. *Plant Dis.* **2016**, *100*, 916–925. [CrossRef]
32. Valenciano, J.B.; Casquero, P.A.; Boto, J.A.; Marcelo, V. Evaluation of the occurrence of root rots on bean plants (*Phaseolus vulgaris*) using different sowing methods and with different techniques of pesticide application. *N. Zeal. J. Crop. Hortic. Sci.* **2006**, *34*, 291–298. [CrossRef]
33. Luna, M.P.R.; Mueller, D.; Mengistu, A.; Singh, A.K.; Hartman, G.L.; Wise, K.A. Advancing our understanding of charcoal rot in soybeans. *J. Integr. Pest Manag.* **2017**, *8*, 1–8. [CrossRef]
34. Judelson, H.S.; Ah-Fong, A.M.V. Exchanges at the Plant-Oomycete Interface That Influence Disease. *Plant Physiol.* **2019**, *179*, 1198–1211. [CrossRef] [PubMed]
35. Howell, C.R. Effect of seed quality and combination fungicide–*Trichoderma* spp. seed treatments on pre- and postemergence damping-off in cotton. *Phytopathology* **2007**, *97*, 66–71. [CrossRef]
36. Baćmaga, M.; Wyszkowska, J.; Kucharski, J. The effect of the Falcon 460 EC fungicide on soil microbial communities, enzyme activities and plant growth. *Ecotoxicology* **2016**, *25*, 1575–1587. [CrossRef]
37. Singh, Z.; Kaur, J.; Kaur, R.; Hundal, S.S. Toxic effects of organochlorine pesticides: A Review. *Am. J. BioSci.* **2016**, *4*, 11–18. [CrossRef]

38. Wagg, C.; Bender, S.F.; Widmer, F.; van der Heijden, M.G.A. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5266–5270. [CrossRef]
39. Bender, S.F.; Wagg, C.; Van der Heijden, M.G.A. An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability crossref. *Trends Ecol. Evol.* **2016**, *31*, 440–452. [CrossRef]
40. Lori, M.; Symnaczik, S.; Mäder, P.; De Deyn, G.; Gattinger, A. Organic farming enhances soil microbial abundance and activity—a meta-analysis and meta-regression. *PLoS ONE* **2017**, *12*, e0180442. [CrossRef]
41. Rillig, M.C.; Lehmann, A.; Lehmann, J.; Camenzind, T.; Rauh, C. Soil biodiversity effects from field to fork. *Trends Plant Sci.* **2018**, *23*, 17–24. [CrossRef] [PubMed]
42. Meng, J.; Li, L.; Liu, H.; Li, Y.; Li, C.; Wu, G.; Yu, X.; Guo, L.; Cheng, D.; Muminov, M.A.; et al. Biodiversity management of organic orchard enhances both ecological and economic profitability. *PeerJ* **2016**, *4*, e2137. [CrossRef]
43. Das, S.; Jeong, S.T.; Das, S.; Kim, P.J. Composted cattle manure increases microbial activity and soil fertility more than composted swine manure in a submerged rice paddy. *Front. Microbiol.* **2017**, *8*, 1702. [CrossRef] [PubMed]
44. Gleń-Karolczyk, K.; Boligłowa, E.; Antonkiewicz, J. Organic fertilization shapes the biodiversity of fungal communities associated with potato dry rot. *Appl. Soil Ecol.* **2018**, *129*, 43–51. [CrossRef]
45. Mierzwa-Hersztek, M.; Gleń-Karolczyk, K.; Gondek, K. Fungistatic activity of composts with the addition of polymers obtained from thermoplastic corn starch and polyethylene—An innovative cleaner production alternative. *Sci. Total Environ.* **2018**, *635*, 1063–1075. [CrossRef] [PubMed]
46. Habtegebriel, B.; Boydom, A. Biocontrol of faba bean black root rot caused by *Fusarium solani* using seed dressing and soil application of *Trichoderma harzianum*. *J. Biol. Control* **2016**, *30*, 169–176. [CrossRef]
47. El-Shennawy, R.Z. Biological control of root-rot and wilt disease of faba bean using some bioagents. *J. Plant Prot. Pathol.* **2011**, *2*, 195–202.
48. Benhamou, N.; Le Floch, G.; Vallance, J.; Gerbore, J.; Grizard, D.; Rey, P. *Pythium oligandrum*: An example of opportunistic success. *Microbiology* **2012**, *158*, 2679–2694. [CrossRef]
49. Khabbaz, S.E.; Abbasi, P.A. Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. *Can. J. Microbiol.* **2014**, *60*, 25–33. [CrossRef]
50. Takenaka, S.; Sekiguchi, H.; Nakaho, K.; Tojo, M.; Masunaka, A.; Takahashi, H. Colonization of *Pythium oligandrum* in the tomato rhizosphere for biological control of bacterial wilt disease analyzed by real-time PCR and confocal laser-scanning microscopy. *Phytopathology* **2008**, *98*, 187–195. [CrossRef]
51. Heydari, A.; Pessarakli, M. A review on biological control of fungal plant pathogens using microbial antagonists. *J. Biol. Sci.* **2010**, *10*, 273–290. [CrossRef]
52. Takenaka, S.; Ishikawa, S. Biocontrol of sugar beet seedling and taproot diseases caused by *Aphanomyces cochlioides* by *Pythium oligandrum* treatments before transplanting. *Jpn. Agric. Res. Q.* **2013**, *47*, 75–83. [CrossRef]
53. Tanentzap, A.J.; Lamb, A.; Walker, S.; Farmer, A. Resolving conflicts between agriculture and the natural environment. *PLoS Biol.* **2015**, *13*, e1002242. [CrossRef] [PubMed]
54. Garbeva, P.J.A.; van Veen, J.D.; van Elsas, J.D. Microbial diversity in soil:Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu. Rev. Phytopathol.* **2004**, *42*, 243–270. [CrossRef] [PubMed]
55. Morrison-Whittle, P.; Lee, S.A.; Goddard, M.R. Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems. *Agric. Ecosyst. Environ.* **2017**, *246*, 306–313. [CrossRef]
56. Soil Survey Staff. *Keys to Soil Taxonomy*, 12th ed.; USDA-Natural Resources Conservation Service: Washington, DC, USA, 2014.
57. Świtoniak, M.; Kabała, C.; Charzyński, P. Proposal of English equivalents for the soil taxa names in the Polish Soils Classification. *Soil Sci. Annu.* **2016**, *67*, 103–116. [CrossRef]
58. Cwalina-Ambroziak, B. The efficiency of biological and chemical protection of potato plants against late blight (*Phytophthora infestans* /Mont./de Bary) and early blight (*Alternaria* spp.). *Pol. J. Agron.* **2012**, *11*, 3–9.
59. De Carolis, E.; Posteraro, B.P.; Lass-Flörl, C.; Vella, A.; Florio, A.R.; Torelli, R.; Girmenia, C.; Colozza, C.; Tortorano, A.M.; Sanguinetti, M.; et al. Species identification of *Aspergillus*, *Fusarium* and *Mucorales* with direct surface analysis by matrix-assisted laser desorption ionization timeof-flightmass spectrometry. *Clin. Microbiol. Infect.* **2012**, *18*, 475–484. [CrossRef] [PubMed]
60. Barnet, H.L.; Hunter, B.B. *Illustrated Genera of Imperfect Fungi*, 4th ed.; APS Press: St. Paul, MN, USA, 1998; p. 218.
61. Dugan, F. *The Identification of Fungi: An Illustrated Introduction with Keys, Glossary, and Guide to Literature*; The American Phytopathological Society: St. Paul, MN, USA, 2006; p. 176.
62. Leslie, J.F.; Summerell, B.A. *The Fusarium Laboratory Manual*, 1st ed.; Blackwell Publishing Ltd.: Oxford, UK; London, UK, 2006; p. 388.
63. Klaus, H.; Domsch, W.; Gams, W.; Anderson, T. Compendium of Soil Fungi, 2nd ed.Wiley Subscription Services, Inc.: St. Paul, MN, USA, 2008; p. 672.
64. Gardi, C.; Jeffery, S. *Soil Biodiversity*; European Commission, Institute for Environmental and Sustainability: Luxemburg, 2009; Volume 10, pp. 2788–7831.
65. Finch-Savage, W.E.; Bassel, G.W. Seed vigour and crop establishment: Extending performance beyond adaptation. *J. Exp. Bot.* **2016**, *67*, 567–591. [CrossRef]
66. Urrea, K.; Rupe, J.C.; Rothrock, C.S. Effect of fungicide seed treatments, cultivars, and soils on soybean stand establishment. *Plant Dis.* **2013**, *97*, 807–812. [CrossRef]

67. Li, Y.P.; You, M.P.; Colmer, T.D.; Barbetti, M.J. Effect of timing and duration of soil saturation on soilborne *Pythium* diseases of common bean (*Phaseolus vulgaris*). *Plant Dis.* **2015**, *99*, 112–118. [CrossRef]
68. Macedo, R.; Sales, L.P.; Yoshida, F.; Silva-Abud, L.L.; Lobo, M. Potential worldwide distribution of *Fusarium* dry root rot in common beans based on the optimal environment for disease occurrence. *PLoS ONE* **2017**, *12*, e0187770. [CrossRef] [PubMed]
69. Rego, C.; Farropas, L.; Nascimento, T.; Cabral, A.; Oliveira, T. Black foot of grapevine: Sensitivity of *Cylindrocarpon destructans* to fungicides. *Phytopathol. Mediterr.* **2006**, *45*, 93–100.
70. Kurzawińska, H. Fungi inhabiting the rhizosphere of persian cyclamen and their impact. *Phytopathologia* **2010**, *57*, 5–10.
71. Pastucha, A.; Kołodziej, B. The effect of irrigation and foliar fertilization on the colonization of american ginseng (*Panax quinquefolium* L.) Diseased parts by different micro-organisms. *Acta Agrobot.* **2010**, *63*, 179–188. [CrossRef]
72. Jamali, S.; Nasimi, Z. First report of black-foot disease, caused by *Cylindrocarpon destructans*, on ornamental marigold (*Tagetes minuta*) in Iran. *J. Plant Prot. Res.* **2014**, *54*, 139–143. [CrossRef]
73. Mayo, S.; Gutiérrez, S.; Malmierca, M.G.; Lorenzana, A.; Campelo, M.P.; Hermosa, R.; Casquero, P.A. Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. *Front. Plant Sci.* **2015**, *6*, 685. [CrossRef]
74. Torres, S.V.; Vargas, M.M.; Godoy-Lutz, G.; Porch, T.P.; Beaver, J.S. Isolates of *Rhizoctonia solani* can produce both web blight and root rot symptoms in common bean (*Phaseolus vulgaris* L.). *Plant Dis.* **2016**, *100*, 1351–1357. [CrossRef] [PubMed]
75. Anderson, J.P.; Sperschneider, J.; Win, J.; Kidd, B.; Yoshida, K.; Hane, J.; Saunders, D.G.; Singh, K.B. Comparative secretome analysis of *Rhizoctonia solani* isolates with different host ranges reveals unique secretomes and cell death inducing effectors. *Sci. Rep.* **2017**, *7*, 10410. [CrossRef]
76. Brožová, J. Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant protection. *Plant Protect. Sci.* **2002**, *38*, 29–35.
77. Marroni, I.V.; Moura, A.B.; Ueno, B. Chemical and biological treatments of castor bean seeds: Effect on germination, emergence and associated microorganismus. *Rev. Bras. Sementes* **2012**, *34*, 21–28. [CrossRef]
78. Sun, Z.-B.; Sun, M.-H.; Zhou, M.; Li, S.-D. Transformation of the endochitinase gene *Chi67-1* in *Clonostachys rosea* 67-1 increases its biocontrol activity against *Sclerotinia sclerotiorum*. *AMB Express* **2017**, *7*, 1–9. [CrossRef]
79. Koutb, M.; Ali, E.H. Potential of *Epicoccum purpurascens* Strain 5615 AUMC as a biocontrol agent of *Pythium irregulare* root rot in three leguminous plants. *Mycobiology* **2010**, *38*, 286–294. [CrossRef] [PubMed]
80. Anil Kumar, R.; Rajkumar, H.G. Antagonistic effect of rhizospheric Aspergillus species against *Fusarium oxysporum f. sp. lycopersici*. *Int. J. Chem. Anal. Sci.* **2014**, *5*, 39–42.