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Introducing Autochthonous Bacterium and Fungus Composition to Enhance the Phytopathogen-Suppressive Capacity of Composts against *Clonostachys rosea*, *Penicillium solitum* and *Alternaria alternata* In Vitro

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Abstract: Given their numerous positive characteristics, composts are widely used agriculturally in sustainable development and resource-saving technologies. The management of phytopathogen-suppressive potential and the fertilizing capacity of composts are of great interest. This study examines the impact of introducing the autochthonous compost species *Bacillus subtilis*, *B. amyloliquefaciens*, *Pseudomonas aeruginosa*, and *Aspergillus corrugatus*, both individually and in combination, to composts containing dry matter comprising 36% solid compost and 7% compost suspensions to study their phytopathogen-suppressive and phytostimulation activity. The test phytopathogens were *Clonostachys rosea*, *Penicillium solitum*, and *Alternaria alternata*. This is the first report on compost's potential to biologically control *C. rosea* and *P. solitum*. Classical microbiological and molecular biological methods were used to evaluate the survival rate of microorganisms in compost and validate these results. Test plant (*Raphanus sativus*) germination indexes were determined to evaluate the phytotoxic/phytostimulation effects of the substrates. To assess the effectiveness of biocontrol, mycelial growth inhibition was measured using in vitro tests. The introduction of composition increased the composts' fertilizing properties by up to 35% and improved antagonistic activity by up to 91.7%. Autochthonous bacterial–fungal composition can promote resistance to fungal root and foliar phytopathogens and raise the fertilizing quality of compost.

Keywords: compost; suppressive activity; biocontrol; phytopathogens; phytostimulation; *Clonostachys rosea*; *Alternaria alternata*; *Penicillium solitum*

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1. Introduction

It is widely known that the microbiota of compost is largely responsible for its phytopathogen-suppressive activity. Classical works on the antagonistic activity of compost have revealed that the mechanisms of pathogen suppression in autochthonous microorganisms are complex, and they have been the subject of in-depth study because of the influence of many different factors: differences in compost compositions, the time of antagonist introduction to compost (when composts are additionally enriched) [1], compost age [2], interactions between autochthonous compost species [3], and experimental conditions [4]. This fact has also been mentioned in recent works. Noble et al. (2005) [5] noted that abiotic factors can influence the pathogen-suppressive properties of composts, but the mechanisms are mostly biological. Scheuerell et al. (2005) [6] pointed out that, if a phytopathogen varies even at the species level, the factors influencing the suppressive potential of the compost may differ. St. Martin et al. (2015) [7] described how the complexity and dynamism

inherent to composts, as well as the factors affecting suppressiveness, necessitate carefully selecting optimal parameters for phytopathogen inhibition in specific cases. Diánez et al. (2018) [8] suggested that autochthonous microbiota and diversity in compost communities with different compositions play a key role in varying suppressive activity. Specifically, it is important to select antagonistic microorganisms that are active in a specific range of conditions. It is also reasonable to desire more universal microbial communities for further use as biological controls for a wider range of pathogenic organisms.

The microorganisms that dominate the compost environment play an important role in inhibiting the development of phytopathogens. Regarding bacteria and fungi, our previous studies [9] have shown that the *Bacillus* and *Aspergillus* genera, respectively, are abundant in compost. Other authors have stated that these microbial genera are widespread in compost and note that they are largely responsible for suppressive activity [10].

The contribution of *Bacillus* spp. to the suppressive activity of compost has been widely studied [7,11]. The intensity of *Bacillus* antagonism is caused by the production of a wide range of secondary metabolites belonging to different classes with various mechanisms of action on a wide range of pathogens [12]. Pane and Zaccerdelli (2015) [13] showed that *B. subtilis* and *B. amyloliquefaciens* phylloplane strains can inhibit *A. alternata* development. Ramírez-Cariño et al. (2020) [14] also described bacteria of this genus as an effective biocontrol agent against *A. alternata* and *Fusarium oxysporum*.

Aspergillus species, no less than other common antagonists, have been reported to be responsible for compost's ability to suppress the most important fungal root pathogens. Active antagonism against phytopathogenic organisms such as *Rhizoctonia solani* and *Fusarium oxysporum* [15]; *Sclerotinia sclerotiorum* and *Phytophthora cactorum* [16]; *Phytophthora parasitica* [17]; and *Pythium irregulare* [18] has been reported. *Aspergillus* spp. are the most antagonistic compost fungi [19]. Therefore, this work examines the potential of *Aspergillus corrugatus* as a biocontrol agent, as there is currently no such information on this species.

Members of the genus *Pseudomonas* also play an important role [10,15]. Luo et al. (2019) [20] showed that bacteria of this genus isolated from compost particularly suppressed the growth of *Alternaria* sp. mycelium. According to Al-Ghafri et al. (2020) [21], out of seven strains isolated from compost, two isolates, both belonging to *P. aeruginosa* species, showed the highest efficacy in suppressing phytopathogens. This species was also effective against *Fusarium solani* [22]. Thus, *P. aeruginosa* was considered an important factor in obtaining bacterial–fungal composition in this study.

It should be noted that recent studies have mentioned increased antagonistic activity when bacterial–fungal consortia are used. This is effective for *Pseudomonas* and *Bacillus* genera [3,22–26]. However, there has been no research on *Bacillus*, *Pseudomonas*, and *Aspergillus* used in conjunction to increase disease-suppressiveness of compost. Therefore, autochthonic compost species of these genera were chosen to develop such a phytopathogen-suppressive composition.

The search for combinations of microorganisms is important since synergisms may be quite rare or limited by different conditions, or they may not occur at all. Sometimes the antagonistic combined activity of microorganisms is about the same as when they are used individually [14,27]. Inefficiencies may be due to high microbial competition in the environment [28], as well as high species or particular microbial strains specificity [29]. It has been claimed that *Pseudomonas* bacteria distinctly suppress pathogenic microorganisms exactly in the community with other antagonists. In such communities, the dominant roles of *Bacillus* spp. and micromycetes are often noted [22–26]. For example, Kwok et al. (1987) [3] reported that *Pseudomonas* genus bacteria showed antagonistic activity only in combination with *Trichoderma* genus fungi, and Rajeswari (2019) [25] found that this combination inhibited pathogens best. The efficacy of combining such antagonists can be explained by synergism between the metabolites produced by bacteria and micromycetes [22], thus increasing enzyme complex activity [23].

Interestingly, using bacterial–fungal consortiums can have a prolonged influence, a potential in the environment. González-González et al. (2021) [30] showed that *Aspergillus*

fungi present in compost or in rhizosphere soil where compost was previously applied promoted the spread of plant growth stimulating *Pseudomonas* and *Bacillus* via mycelia. This can have a positive impact on the growth and development of plants and represents a way to improve their protection against pathogens. In [24], the highest suppressive activity against phytopathogens was observed with a combination of *P. fluorescens*, *B. subtilis* and *Trichoderma viride*, which was because they were part of the natural microbial community of the plant rhizosphere. Hammam et al. (2016) [23] showed that the greatest increase in the enzyme activity of peroxidase, polyphenol oxidase, and chitinase was obtained using a mixture of compost and a bacterial–fungal consortium with *B. subtilis* and two species of fungi—*T. harzianum*, *T. viride*. Enhancing the activity of these enzymes, as well as b-1,3-glucanase, when using compost extract improves the level of protection against *Alternaria* spp. [31]. Tao et al. (2020) [26] noted that applying the biocontrol agent *B. amyloliquefaciens* increases the number of soil *Pseudomonas* spp., as well as the synergism between them, and stimulates biofilm formation, which has a positive effect on plant protection. Antoniou et al. (2017) [10] described the beneficial effects of compost microbiota on the rhizosphere community in aspects of plant protection.

The benefits of each antagonist can contribute to biocontrol, thereby increasing the effectiveness of suppression in a range of pathogens. Thus, we used *Clonostachys rosea*, *Penicillium solitum* and *Alternaria alternata* as test phytopathogens that cause root and foliar diseases in plants and fruit spoilage during storage.

A. alternata is one of the world's most common phytopathogenic fungi, causing both leaf spot and brown spot diseases [32]. Its injuriousness is very high, and it is a causal agent of early blight in tomatoes [13,14]. Recently, the possibility of root rot and fruit rot during storage caused by *A. alternata* was reported in [33,34]. *P. solitum* is an important causative agent in pomaceous and citrus fruit spoilage during storage [35,36]. Its resistance to some fungicides has also been reported, which makes the issue of biocontrol especially important [37]. Furthermore, Zhang et al. (2020) [38] first reported on the possibility of root rot in saffron plants specifically caused by *P. solitum*. *C. rosea* was previously used as a biocontrol agent because of its active mycoparasitism [39,40]. However, in recent years, researchers identified root rot in grain legumes and medicinal crops caused by *C. rosea* [41–44]. In addition, Coyotl-Pérez et al. (2022) [45] described this species as a phytopathogen that causes soft rot in avocado fruit.

The potential use of composts to suppress *P. solitum* and *C. rosea* has not yet been evaluated, so our work is the first to research this possibility.

Compost is regarded as a universal biological control agent, and we suggest increasing its suppressive properties against phytopathogens by introducing antagonist microorganism composition, for which compost would be a natural environment. At the same time, the phytostimulation activity of the compost is expected to be improved.

The aim of the study was to determine the effectiveness of a new bacterial–fungal composition based on autochthonous phytopathogen-antagonistic microorganisms (*B. subtilis*, *B. amyloliquefaciens*, *P. aeruginosa* and *A. corrugatus*) in increasing the suppressive potential of compost and compost suspension against the phytopathogens *C. rosea*, *P. solitum*, and *A. alternata*, as well as the effect of such introduction on the fertilizing properties of substrates.

The objectives of the study were as follows: (1) to evaluate the survival rate of microorganisms introduced to composts with different dry matter contents; (2) to determine the effect of their introduction on the phytotoxity/phytostimulation capacity of the compost; and (3) to establish changes in the suppressive activity of compost following the introduction of microorganisms, both individually and in composition, against three phytopathogens, *C. rosea*, *P. solitum*, and *A. alternata.*

Sterilizing composts and compost suspensions usually decreases their phytopathogen suppressive activity [46]. Thus, non-sterile stable compost was used in this study, and microbial inoculates were used to supplement the autochthonous microbial community of this compost.

2. Materials and Methods

2.1. Compost Mixture

Mature compost from a mixture of food and agro-waste was obtained in our previous studies [9], where its physicochemical and biological properties are described. The composting and full maturity periods were 98 days and ~10 months, respectively. The sample comprised mature compost, satisfying the quality criteria, and had high agrochemical values (parameters).

The initial water content of the compost was 20%. In addition to the classic version of solid compost, the use of its liquid form has increased in recent years, particularly for foliar disease control [47]. Before introducing microorganisms, the compost was moistened for optimum water content of 60% and for a compost suspension with water content of 92%. The next step was introducing microorganisms to these composts. In total, 10 mL of inoculum (or water and LB medium in control variants) was added per 100 g of solid compost and per 90 mL of compost suspension (the variants are described in more detail below). Final water contents in all variants were 64% and 93% for the solid compost and compost suspension, respectively. Thus, there were two compost variations used in this work, with 36% and 7% dry-mass matter content (hereafter, C36 and C7, respectively).

We did not filter the liquid form of the compost. Ismael and St. Martin (2017) [48] showed that using filtered liquid compost (without microbial cells) with the same fertilizing capacity as unfiltered compost leads to a significant reduction in plant growth. Thus, using an unfiltered compost suspension (C7) is suggested to be perspective.

2.2. Obtaining Microbial Inoculums

Microbial inoculums were obtained to supplement the existing microbial community of the compost with biocontrol organisms. Among these bacteria, we used pure cultures of *B. subtilis* (Genbank accession number: PRJNA979896), *B. amyloliquefaciens* (Genbank accession number: PRJNA979896) and *P. aeruginosa* (GenBank accession number: PRJNA979896), previously isolated by us from compost. The bacteria were cultivated in rich liquid LB medium (Luria–Bertani) in flasks at 28 °C with constant shaking at 130 rpm for 2–3 days. Composition of LB medium (g L^{-1}): tripton—10.0, yeast extract—5.0, sodium chloride—5.0. Subsequently, 2–3-day-old bacterial cultures were introduced to the compost.

Bacterial suspension titers were determined using a photoelectric concentration colorimeter, CFC-2 (ZOMZ, Zagorsk, Russia). To determine the bacteria concentration, optical density values were used based on international standards of turbidity according to McFarland at a wavelength of 600 nm. A graduation chart was plotted to find the necessary values. Thus, the titers in bacterial suspensions were as follows: *B. subtilis*—4.5×10^9 colony-forming units (CFUs) mL^{-1}; *B. amyloliquefaciens*—3.5×10^9 CFU mL^{-1}; *P. aeruginosa*—6.0×10^9 CFU mL^{-1}.

A culture of the *Aspergillus corrugatus* micromycete (Genbank accession number: OR244202), isolated from compost [9], was also used. To obtain a spore inoculum, the fungus biomass was grown on Petri dishes using potato dextrose agar (PDA). Cultivation was performed at 28 °C for 7 days. Following this, a spore suspension was prepared in water. To determine the number of spores in the suspension, the serial dilution technique was chosen, and a PDA medium was used. The number of CFUs for the prepared suspension was 5.5×10^6 mL^{-1}.

Composition of the PDA medium (per liter): potato extract—230 mL; glucose—20.0 g; agar—20.0 g.

Before preparing the microorganism composition, the possibility of antagonism between the organisms was evaluated. The method of perpendicular strokes on solid media was used for this purpose, considering the possibility of growth inhibition zones where the microorganisms could be antagonists (Figure S6). Antagonistic activity was previously tested for pure cultures (see Figures S9–S11).

Pure cultures of root and foliar plant pathogens *C. rosea* (Genbank accession number: OR244207); *P. solitum* (Genbank accession number: OR244208); and *A. alternata* (Gen-

bank accession number: OR259104) from the Research Group of Microbial Processes of Organic Waste Conversion (Research Center of Biotechnology RAS, Moscow, Russian), collection were used. The required micromycete biomass was grown on Petri dishes on PDA medium. Cultivation was carried out at 28 °C for 7 days. For the use of this material, see Section 2.6 below.

2.3. Introduction of Microorganisms to the Compost

The inoculums were added to the compost in 10 mL volumes per 100 g of solid compost and per 90 mL of compost suspension. Thus, the titers in the composts for the bacterial agents were as follows: *B. subtilis*—4.5 × 10^8, *B. amyloliquefaciens*—3.5 × 10^8, *P. aeruginosa*—6.0 × 10^8 CFU per g and ml of C36 and C7, respectively. Then, 10 mL of the *A. corrugatus* spore suspension was added to the composts. Its titer was 5.5 × 10^5 CFU per g or mL of C36 or C7, respectively. The microorganism composition was formed from the four species described above, and their titers in the composts were about 10^8 CFU g^{-1} (mL^{-1}) for each bacterial agent and ~10^5 CFU g^{-1} (mL^{-1}) for fungal agent. The final CFU ratio of fungi and bacteria in the composition was 1:1000.

The C7 variants were incubated at 28 °C with continuous shaking (130 rpm). The C36 variants were incubated at 28 °C without mixing under conditions meant to prevent moisture loss through evaporation. The incubation time was 14 days. A list of all variants is in Table 1.

Table 1. Variant list of obtained composts: control composts with water and LB medium (instead of inoculate) and composts after introducing phytopathogen antagonistic microorganisms (individually and in composition).

Compost Variant Abbreviation	Inoculum	Variant Name
C36	*B. subtilis*	C36B1
	B. amyloliquefaciens	C36B2
	P. aeruginosa	C36P
	A. corrugatus	C36A
	composition	C36C
C7	*B. subtilis*	C7B
	B. amyloliquefaciens	C7B2
	P. aeruginosa	C7P
	A. corrugatus	C7A
	composition	C7C
Control variants		
C36	water	C36W
	LB medium	C36L
C7	water	C7W
	LB medium	C7L

Despite the complexity of measuring phytopathogen-suppressive capacity when using non-sterile composts, we consider this to be an optimal variant because this work focuses on practical issues, such as increasing suppressive activity in general, and only indirectly considers antagonism mechanisms, so data obtained this way are sufficient. Moreover, the use of stable compost is closer to industrial conditions, where the best method is to apply a biopreparation, which improves the suppressive activity of the substrate already in cooled compost in the aging stage or just before it is applied to the soil [49].

2.4. Microbial Survival Rate in Compost

The number of fungi in the compost was evaluated using the serial ten-fold dilution technique, which has been used in other studies [50]. In total, 100 mcL of each prepared dilution was spread on the surface of the PDA medium, and Petri dishes were incubated for

7 days at 28 °C. Then, the colonies were counted, and a CFU value per g (mL) of compost was calculated.

For bacteria, a similar survival rate evaluation was performed using LB medium, and the CFU values were obtained after 3 days of incubation in Petri dishes at 28 °C.

2.5. Influence of Pure Cultures of Antagonistic Microorganisms and Composts after the Introduction of Microorganisms on the Seed Germination Index of the Test Plant (Raphanus sativus)

The effect of the composts on the germination index values was tested according to the method described in the work of Luo et al. (2017) [51]. The variants C36B1, C36B2, C36P, C36A, C36C, C7B1, C7B2, C7P, 174 C7A and C7C, as well as C36W, C7W, C36L, and C7L (without introducing microorganisms), were examined. Aqueous compost suspensions (with tap water, 1:30 ratio) were centrifuged at 7000 rpm for 15 min using a microcentrifuge: Mini-15K (Allsheng, Hangzhou, China). Thirty seeds from the test plant (*Raphanus sativus*) were placed on filter paper in Petri dishes. In total, 5 mL of supernatant was added to each one. For the control, 5 mL of tap water was added. The dishes were covered and incubated at 25 °C for 72 h in a dark room [51]. Then, the root length of the seedlings and the number of germinated seeds were measured. The germination index was calculated according to [52]:

$$\mathrm{GI}\ (\%) = \frac{G1 \times L1}{G2 \times L2} \times 100 \quad (1)$$

where $G1$ is the number of seeds germinated in contact with the test solution;

$L1$ is the average root length of the seedlings grown in contact with the test solution, mm;

$G2$ is the number of seeds germinated in contact with tap water;

$L2$ is the average root length of the seedlings grown in contact with tap water, mm.

Pure cultures of the antagonists were examined using the analogous method. Cell-free supernatant was used after centrifuging (7000 rpm, 15 min) the cell suspensions in nutrient medium. Bacteria were cultured in LB medium. Fungi were cultured in Czapek medium (composition (g L^{-1}): sucrose—30.0; 184 sodium nitrate—2.0; dipotassium phosphate—1.0; magnesium sulfate—0.5; potassium chloride—0.5; iron sulfate—0.01). In addition, supernatants were diluted to 1:1000 (with sterile distilled water). Tap water and undiluted media were used as controls.

The experiments were conducted with three replicates.

2.6. In Vitro Suppressiveness of Composts

Agar disks with germinated pathogen mycelium (0.5 cm) were placed in the center of a Petri dish (9 cm) with PDA medium. Around the disk, lumps (1.5 cm in diameter) of solid compost (C36) were placed on the surface of the nutrient medium on the edge of the Petri dish around 3 cm from the pathogen disk. For clarity, this method is presented below (Figure 1).

For the compost suspension (C7), we used a variation of the well-cut diffusion technique described by Pane et al. (2012) [50]. Four wells (1.5 cm in diameter) were made in a solid agar medium around 3 cm from the pathogen agar disk (0.5 cm). The wells were "sealed" underneath with a few drops of water agar (15 g per L), and then 100 mcL of each compost variant was introduced to each well.

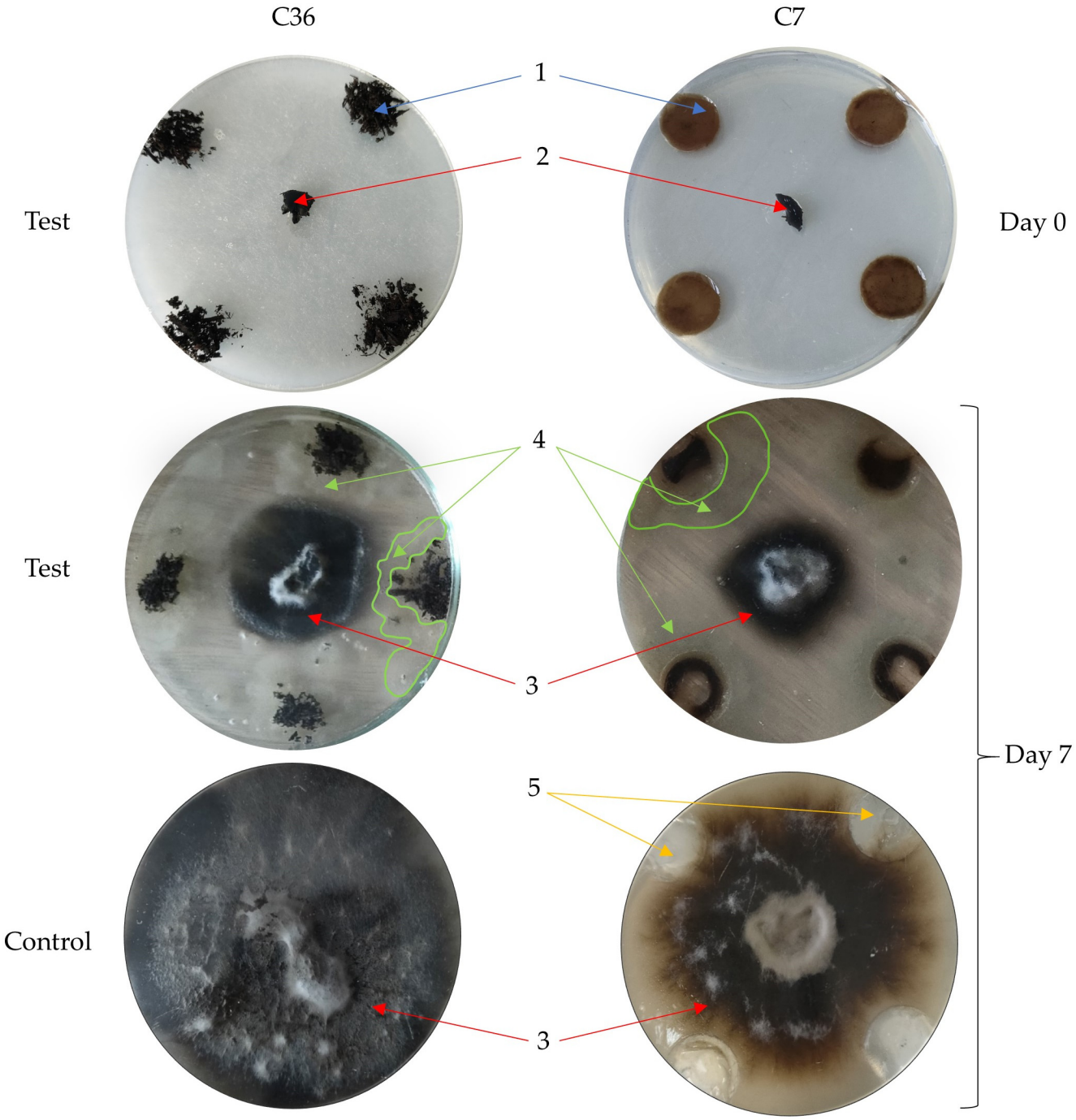

Figure 1. Analysis of the phytopathogen-suppressive potential of composts C36 and C7: 1—variants of composts C36 and C7; 2—initial agar disk with phytopathogen mycelium; 3—germinated phytopathogen after incubation for 7 days at 28 °C; 4—antagonists from composts grown on agarized nutrient media; 5—sterile distilled water in the wells; Test, Control—compost variants with and without the introduction of antagonists, respectively; Day 0, Day 7—start and end of incubation, respectively.

Antagonism was assessed based on the presence of fungal growth suppression, expressed via mycelial growth inhibition—MGI (%)—which was measured according to the formula described in [53,54]:

$$\mathrm{MGI} = \frac{\mathrm{S(control)} - \mathrm{S(test)}}{\mathrm{S(control)}} \times 100 \quad (2)$$

where, S(control) is the radial growth area of the phytopathogen in the control variants (without introducing biocontrol microorganisms), mm^2, and S(test) is the radial growth area of phytopathogen in test variants (with biocontrol microorganisms), mm^2.

As a control for the compost suspensions (C7), sterile distilled water was added to the wells around the pathogen disk; for C36, lumps of compost were not placed around the disk. Thus, the results were fixed after the phytopathogen mycelium reached the wells with water (C7) or the edge of the Petri dish (C36) in the control variants. Three parallel experiments were conducted [54].

2.7. Analysis of Bacterial Survival Rate in Compost Using Molecular Biological Methods

This step of the work evaluated the survival rate of bacterial agents introduced to the composts and was necessary to confirm the results of the analysis conducted with classical microbiological methods.

The total number of prokaryotes in composts with different final dry matter contents (C36 and C7) was evaluated. One phytopathogen antagonist culture—*B. amyloliquefaciens*—was chosen as an example to verify the survival of the introduced microorganisms (also for the C36 and C7 variants). We used the samples without incubation (0 days, on the same day when introduction was performed) and samples after 14 days of incubation at 28 °C. Samples with the compost suspension (C7) were centrifuged (4000 rpm, 30 min). For further analysis used the obtained sediments. C36 samples were used in their initial form without pretreatment. The C36L and C7L composts—without introduced microorganisms but with added LB nutrient medium—were used as control variants in order to consider possible impact of LB medium on the autochthonous microbiota. Compost samples with introduced antagonistic microorganisms were used as experimental variants (a detailed description is presented in the previous sections of the methodology). A summary list of variants is presented in Table 2.

Table 2. List of variants used to analyze the survival rate of introduced microorganisms.

Variant Abbreviation	Description
C36L0	Control variant: compost with 36% dry matter content with added LB nutrient medium and without introduced microorganisms, 0 days (without incubation).
C7L0	Control variant: compost with 7% dry matter content with added LB nutrient medium and without introduced microorganisms, 0 days (without incubation).
C36C0	Test variant: compost with 36% dry matter content with introduced antagonist microorganism composition, 0 days (without incubation).
C7C0	Test variant: compost with 7% dry matter content, with the introduced antagonist microorganism composition, 0 days (without incubation).
C36L14	Control variant: compost with 36% dry matter content with added LB nutrient medium and without introduced microorganisms, 14 days of incubation.
C7L14	Control variant: compost with 7% dry matter content with added LB nutrient medium and without introduced microorganisms, 14 days of incubation.
C36C14	Test variant: compost with 36% dry matter content with introduced antagonist microorganism composition, 14 days of incubation.
C7C14	Test variant: compost with 36% dry matter content with introduced antagonist microorganism composition, 14 days of incubation.

The genomic DNA of *B. amyloliquefaciens* was isolated from freshly grown cells using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's instructions. A shotgun WGS library preparation and sequencing were performed by BioSpark Ltd. (Moscow, Russia) using the KAPA HyperPlus Library Preparation Kit (KAPA Biosystems, London, UK) according to the manufacturer's protocol and

the NovaSeq 6000 system (Illumina, San Diego, CA, USA) with a reagent kit that could read 100 nucleotides from each end. Raw reads were processed with Trimmomatic [55] for adapter removal and quality filtering. Read assembly was performed with Unicycler v0.4.8 [56]. Thus, 181,379,615 reads were obtained, which were then assembled into 44 contigs (N50—288,378; L50—5), with a total length of 3,966,887 bp. The genome was annotated using PGAP [57]. In total, 3971 genes were identified: 3778 protein-coding genes, 76 tRNA-coding genes, 3 rRNA-coding genes (1 5S, 1 16S, 1 23S), 5 ncRNAs, and 109 pseudogenes.

Highly specific primers were designed for *B. amyloliquefaciens*, suitable for detecting this strain even in the presence of closely related strains.

The target gene for *B. amyloliquefaciens* was holA encoding DNA polymerase III subunit delta. Primer sequences: Bam-holA-F 5′-CATAATCCATCACTGCAAGT-3′ and Bam-holA-R 5′-235GCCAACCAATTCCGACTG-3′. Amplicon length—185 bp. Program: (95–5′); (95–15″; 56–15″; 72–15″)—35 cycles.

DNA preparations were isolated for the described variants (Table 2) using the FastDNA™ SPIN Kit for Soil. For each of the eight preparations, real-time PCR was performed with universal primers for all prokaryotes for the V4 region of the 16S rRNA gene [58] and with a system of highly specific primers to detect the target microorganism (*B. amyloliquefaciens*, holA). Each measurement was performed with three replicates.

2.8. Statistical Analysis

Statistical analyses were conducted using analyses of variance (ANOVA); mean values were compared using Duncan's test with a 95% confidence interval ($p \leq 0.05$). Statistica version 8.0 (StatSoft, Inc., Tulsa, OK, USA) was used. All experiments were carried out with threefold repetitions, and the results are presented as means with standard deviations.

3. Results and Discussion

3.1. Microbial Survival Rate in Compost

The method of counting we used can obtain approximate data. This is particularly relevant for bacteria. For micromycetes, it is easier to obtain a more accurate result using this technique due to the visibility. In this work, it was sufficient to know that, after introducing a concrete microorganism, there was generally no titer decrease. However, some confirmation that this occurs due to the survival of the introduced microorganisms was shown by using molecular biological methods on *B. amyloliquefaciens* example.

The initial titers of *B. subtilis*, *B. amyloliquefaciens*, and *P. aeruginosa* introduced to the compost were 4.5×10^8, $3.5 \times 10^{8,}$ and 6.0×10^8 CFU g^{-1} (mL^{-1}), respectively. After 14 days of incubating the C7 variants at 28 °C, the bacterial counts were 1.6×10^9, 1.2×10^{10} and 8.0×10^9 CFU g^{-1} (mL^{-1}) for those with introduced *B. subtilis* (C7B1), *B. amyloliquefaciens* (C7B2), and *P. aeruginosa* (C7P), respectively. We found that after 14 days of incubating C36 variants at 28 °C, the bacterial titer decrease was not significant: 1.7×10^7, 1.8×10^7 and 2.1×10^8 CFU g^{-1} for variants with *B. subtilis* (C36B1), *B. amyloliquefaciens* (C36B2), and *P. aeruginosa* (C36P), respectively (Figure S1). *A. corrugatus* showed a slight decrease in the titer after incubation for 14 days at 28 °C, from 5.5×10^5 to 3.0×10^4 CFU g^{-1} and 1.0×10^4 CFU ml^{-1} for C36A and C7A, respectively (Figure S2). Therefore, all inoculated microorganisms survived in composts with 36% dry matter content. In the C7 compost, *A. corrugatus* also survived, and meanwhile, the bacterial titer increased. The results are shown in Table 3.

Table 3. Bacterial and fungal titers after introducing antagonistic microorganisms to C36 and C7 composts.

Culture	Initial Titer, CFU per g (mL) of C36 and C7	Bacterial And Fungal Titers on Day 14 of Incubation, CFU per g of C36	Bacterial and Fungal Titers on Day 14 of Incubation, CFU per mL of C7
B. subtilis	4.5×10^8	1.7×10^7	1.6×10^9
B. amyloliquefaciens	3.5×10^8	1.8×10^7	1.2×10^{10}
P. aeruginosa	6.0×10^8	2.1×10^8	8.0×10^8
A. corrugatus	5.5×10^5	3.0×10^4	1.0×10^4

The survival rate of the *B. amyloliquefaciens* introduced to the compost was evaluated via molecular biology methods using qPCR (Table 4).

Table 4. Total number of prokaryotes and survival rate of *B. amyloliquefaciens* (number of 16S rRNA gene copies ± standard deviation; number of holA gene copies ± standard deviation).

Sample	Number of 16S rRNA Prokaryote Gene Copies per g of Compost	Number of holA Gene Copies per g of Compost
C36L0	$1.12 (\pm 0.13) \times 10^8$	n.p.p. [1]
C7L0	$3.51 (\pm 0.67) \times 10^8$	n.p.p.
C36C0	$1.23 (\pm 0.19) \times 10^8$	$2.40 (\pm 0.35) \times 10^5$
C7C0	$4.24 (\pm 1.22) \times 10^7$	$2.45 (\pm 0.63) \times 10^4$
C36L14	$1.84 (\pm 0.53) \times 10^5$	n.p.p.
C7L14	$1.28 (\pm 0.30) \times 10^7$	n.p.p
C36C14	$1.89 (\pm 0.42) \times 10^8$	$8.54 (\pm 1.43) \times 10^3$
C7C14	$3.94 (\pm 0.50) \times 10^7$	$5.24 (\pm 0.76) \times 10^2$

[1] n.p.p.—no PCR product.

The total number of prokaryotes in the control variants (without introducing microorganisms) decreased after 14 days of incubation; in the case of variant C36L14, this was more intensive than for C7L14: three orders of magnitude vs. one order of magnitude (Table 4), respectively. This shares a common trend with the results obtained using classical microbiological analysis methods (Table 3). Adding LB nutrient media likely had a stimulating effect on the autochthonous microbiota of these composts, after which, competition for nutrients increased. At the same time, the variants with the compost suspension (C7) initially contained more easily available soluble compounds than the variants with solid compost (C36). Thus, in the latter case, the competition between microorganisms was more intense, which caused a greater decrease in the number of prokaryotes.

Because of the introduction, the number of microorganisms in all types of compost generally did not decrease after 14 days of incubation, which agrees with the results of microbial abundance analysis based on the seeding on nutrient media.

The results of the analysis carried out with primers specific to *B. amyloliquefaciens* show that the microorganism survived after introducing to both 36% and 7% dry matter composts. At the same time, in both the C36C14 and C7C14 variants, a drop in the number of holA gene copies by three orders of magnitude was observed after 14 days of incubation (Table 4). The differences in the results obtained using molecular biological and classical microbiological methods are because the former were more specific to a concrete microorganism, while the latter were characterized by some overestimation, which was influenced by the diversity of the compost microbiota, in particular, initially including the autochthonous species *B. amyloliquefaciens*.

3.2. Effects of Pure Microbial Cultures and Composts with Those Cultures on the Seed Germination Index of Raphanus sativus

This stage of the research allowed us to establish the possibility of antagonistic microorganisms and compost after introducing these microorganisms to cause the phytotoxic

effects. The effects of *B. subtilis*, *B. amyloliquefaciens*, *P. aeruginosa*, and *A. corrugatus* pure cultures as well as the C36 and C7 with these microorganisms on the garden radish (*R. sativus*) seed germination index were examined to identify phytotoxic and phytostimulatory effects and their possible causes. These results also provide an estimate of the potential fertilizing properties of the substrates; see Tables 5 and 6.

Table 5. Effect of antagonist microorganism culture supernatants without dilution (CS) and with 1:1000 ratio dilution (CS 1:1000) on the germination index (GI, mean value ± standard deviation, %) of the test plant (*R. sativus*) seeds.

Culture	GI [1], %	
	CS	CS 1:1000
B. subtilis	0.0 ± 0.0 [a]	63.9 ± 6.9 [c]
B. amyloliquefaciens	0.0 ± 0.0 [a]	82.9 ± 12.1 [d]
P. aeruginosa	0.0 ± 0.0 [a]	38.1 ± 1.3 [b]
A. corrugatus	0.0 ± 0.0 [a]	48.2 ± 1.6 [b]

[1] Interpretation of GI values according to [52]: 0–40%—sensible inhibition; 40–80%—slight inhibition; 80–120%—no effect; >120%—stimulation. [a–d] Different letters indicate that the values are statistically significantly different ($p < 0.05$, Duncan's test).

Table 6. Effects of C36 and C7 with and without antagonists on the seed germination index (GI, mean value ± standard deviation, %) of the test plant (*R. sativus*) after 14 days of incubation.

Variant Name	GI [1], %
C36B2	80.6 ± 8.7 [a]
C36W	84.9 ± 15.4 [ab]
C36B1	87.8 ± 2.0 [abcd]
C7B1	89.7 ± 4.7 [abcd]
C7B	90.3 ± 4.1 [abcd]
C7B2	91.8 ± 1.0 [abcd]
C36B	96.9 ± 0.5 [abcde]
C36B	98.5 ± 15.2 [abcde]
C7W	103.8 ± 4.0 [cdef]
C7B	108.5 ± 7.4 [def]
C36L	109.4 ± 3.7 [def]
C7L	110.6 ± 11.2 [def]
C36C	114.8 ± 1.9 [ef]
C7C	120.9 ± 9.4 [f]

[1] Interpretation of GI values according to [52]: 0–40%—sensible inhibition; 40–80%—slight inhibition; 80–120%—no effect; >120%—stimulation. [a–f] Different letters indicate that the values are statistically significantly different ($p < 0.05$, Duncan's test).

3.2.1. Effect of Pure Cultures on Germination Index Values

We found that significant plant growth retardation was mainly caused by the influence of the liquid nutrient medium used to cultivate the antagonistic microorganisms (Figure S3). The data show that the concentrated LB medium suppressed *R. sativus* seed germination. The GI value was 0.0%, which can be interpreted as total inhibition. The Czapek medium used for *A. corrugatus* in this assay provided a higher ($p < 0.05$) value (28.5 ± 1.9%), but it also had a phytotoxic effect (from 0 to 40%) due to Cesaro grading (2015) [52]. By diluting culture supernatants (at a ratio of 1:1000), the negative effect of the nutrient medium was neutralized (Table 5, Figure S5).

The results reflect the possibility of growth inhibition, possibly due to the influence of *P. aeruginosa* and *A. corrugatus* metabolites. The values of the germination indexes obtained from the effect of the non-diluted supernatants of these cultures show complete growth suppression (Table 5, Figure S4). After dilution, the GI values increased ($p < 0.05$) but still showed slight inhibition of plant growth for *P. aeruginosa* and *A. corrugatus*, statistically at

the same level ($p > 0.05$, one group of results). For the *B. subtilis* and *B. amyloliquefaciens* cultures no growth inhibition was observed when diluting the culture supernatants, which may indicate that the negative effect in the in the case of initial variants (without dilution) was induced specifically by the nutrient medium (Table 5). For *B. amyloliquefaciens*, the GI was significantly higher ($p < 0.05$) than for *B. subtilis*.

In another study [17], results differing from ours were obtained. It was shown that culture extracts of *Aspergillus* species used as biocontrol agents did not cause a phytotoxic effect. Conversely, Khattak et al. (2014) [59] obtained results similar to our findings. Their work showed that extracts of *Aspergillus* sp. isolated from the rhizosphere of peppermint (*Mentha piperita*) had herbicidal activities against the test plants, which was reflected in the reduction in common duckweed (*Lemna minor*) growth and the complete suppression of milk thistle (*Silybum marianum* L.) seed germination.

The reduced GI values for the variants in which plants contacted with the *P. aeruginosa* cultural supernatant were likely induced by hydrogen cyanide produced by these bacteria. Blom et al. (2010) [60] showed that this compound promotes oxidative stress in plants. It has also been reported that the cyanogenesis inherent to rhizosphere bacteria of the genus *Pseudomonas* can inhibit plant growth, which is particularly relevant to weed plants [61].

3.2.2. Impact of Composts with Antagonists on Germination Index Values

Based on the values of the germination indexes we obtained, the C36 and C7 compost variants did not suppress plant development after introducing antagonistic microorganisms after 14 days of incubation under the conditions described above (Table 6). In this part of the assay, the C36L and C7L variants were also tested. The values for these variants represent the potential impact of the LB nutrient medium components on the phytostimulation capacity of these composts.

No substrate with antagonistic microorganisms manifested phytotoxic effects. At the same time, the indexes were higher ($p < 0.05$) than the control (C36W) variant on average, by 3%, 14% and 16% for C36 composts inoculated with *B. subtilis*, *P. aeruginosa*, and *A. corrugatus*, respectively. For the C7 compost, there was no excess compared with the control (C7W) for the variants with individual cultures. After adding *A. corrugatus*, no statistically significant difference was observed ($p > 0.05$, one group of results), despite a 4.5% increase in the average GI.

According to Cesaro grading (2015) [52] introducing an antagonist composition to the C7 compost had a phytostimulation effect (higher than 120%) on the *R. sativus* growth. Introducing this composition to the C36 compost had no phytotoxic or phytostimulation effect (as the GI was between 80 and 120%) on the test plant; instead, it was closer to the "stimulates plant growth" range. Therefore, the values of the germination index significantly exceeded ($p < 0.05$) the control values on average by 35 and 16% for C36C and C7C, respectively. The findings may indirectly indicate an improvement in the fertilizing properties of composts when *B. subtilis*, *B. amyloliquefaciens*, *P. aeruginosa*, and *A. corrugatus* are used in combination. Martínez-Gallardo et al. (2020) [62] reported that using a fungal consortium including the *Aspergillus* genus for the bioaugmentation of wastewater sludge reduces phytotoxic effects.

For the control variants with added LB nutrient medium, instead of water, there was an increase ($p < 0.05$) in the GI values, on average by 29% (significant increase) and 7% (slight increase; the results are in the close statistical group) for C36L and C7L, respectively. This may be achieved by stimulating the autochthonous microbiota of substrates caused by nutrient medium components influence [63].

In general, the GI values for the compost variants with 7% dry matter were higher than those for the variants with 36% dry matter. This is likely due to differences in the initial dissolved nutrient compound contents in these substrates. The C7 variants contained more water and more dissolved compounds than the C36 variants. This may be manifested as better assimilation of nutrient elements in the underdeveloped root systems of the

seedlings and an increase in GI values compared with C36 variants, where the assimilation of elements was slower.

It is important to note that the compost with higher seed germination index values is promising not just in terms of fertilizing qualities. High results according to this assessment criterion show that this substrate can reduce phytotoxic effects and, consequently, be of great benefit to crop disease control. Gur et al. (1998) [64] showed that apple tree diseases that occur during replanting can be reduced by absorbing phytotoxic compounds produced by soil phytopathogens when composts are used.

3.3. Changes in the Suppressive Capacity of Composts after Introducing Antagonistic Microorganisms

Among the three phytopathogenic organisms, the most expressive suppressive activity in the compost was against *C. rosea*. The best variant was the compost with 36% dry matter and an introduced antagonist composition (C36C), with an MGI value of 91.7% (Figure 2a, Table 7). We assume that the fungus *A. corrugatus* made a significant contribution to the suppression of *C. rosea* because its individual introduction was also highly effective in C36A (MGI value, 91.4%), which was not significantly different from variant C36C ($p > 0.05$).

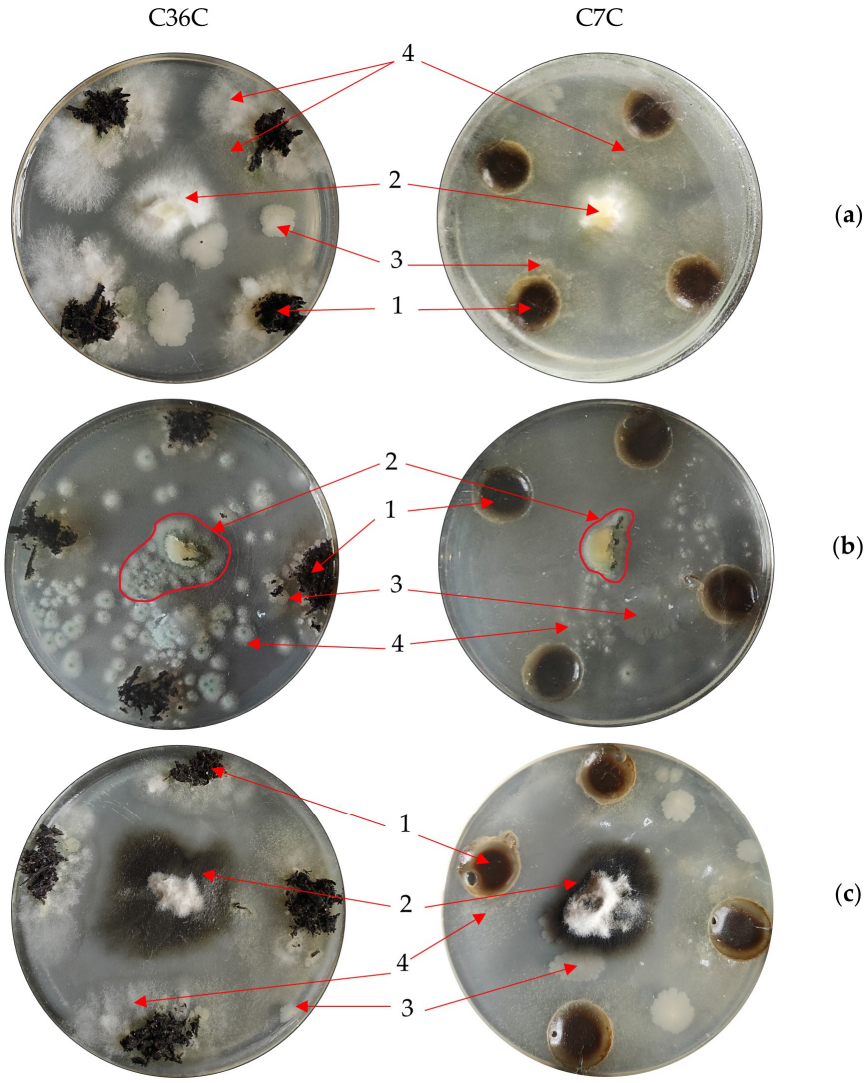

Figure 2. Suppressive activity of composts C36 and C7 inoculated with phytopathogen antagonist composition against (**a**) *C. rosea*; (**b**) *P. solitum*; (**c**) *A. alternata*. 1—compost (C36) lump and compost suspension (C7) in a well; 2—phytopathogens; 3—bacterial agents from composts; 4—fungal agents from composts.

Table 7. Suppressive activity of C36 and C7 variants against *C. rosea*, *P. solitum* and *A. alternata*, indicated by a mycelial growth inhibition value % (mean ± standard deviation).

Variant Name	MGI (%)		
	C. rosea	***P. solitum***	***A. alternata***
C36W	86.3 ± 1.0 ^{bc}	11.1 ± 0.0 ^{a}	76.6 ± 3.7 ^{c}
C36L	86.0 ± 1.5 ^{bc}	26.0 ± 6.7 ^{b}	73.1 ± 2.4 ^{c}
C36B1	84.0 ± 4.8 ^{bc}	83.4 ± 3.2 ^{fg}	71.1 ± 4.9 ^{c}
C36B2	89.5 ± 3.5 ^{bc}	70.6 ± 0.6 ^{de}	74.9 ± 4.5 ^{c}
C36P	87.1 ± 1.8 ^{bc}	59.0 ± 12.7 ^{cd}	77.0 ± 1.5 ^{c}
C36A	91.4 ± 1.7 ^{c}	77.2 ± 8.9 ^{ef}	76.7 ± 4.1 ^{c}
C36C	91.7 ± 0.7 ^{c}	84.0 ± 3.5 ^{fg}	81.9 ± 1.1 ^{c}
C7W	58.4 ± 5.8 ^{a}	0.0 ± 0.0^{a}	41.4 ± 15.3 ^{b}
C7L	54.4 ± 2.7 ^{a}	0.0 ± 0.0 ^{a}	26.5 ± 13.9 ^{a}
C7B1	56.6 ± 10.6 ^{a}	0.0 ± 0.0 ^{a}	75.1 ± 5.4 ^{c}
C7B2	90.5 ± 4.7 ^{c}	58.3 ± 4.8 ^{c}	78.1 ± 2.5 ^{c}
C7P	53.3 ± 4.7 ^{a}	0.0 ± 0.0 ^{a}	77.6 ± 5.9 ^{c}
C7A	79.9 ± 3.1 ^{a}	67.1 ± 5.2 ^{cde}	81.0 ± 5.5 ^{c}
C7C	81.9 ± 1.0 ^{bc}	89.9 ± 2.5 ^{g}	81.9 ± 6.8 ^{c}

$^{a–g}$ Different letters in the same column indicate that the values are statistically significantly different ($p < 0.05$, Duncan's test).

Among the bacterial agents, *B. amyloliquefaciens* is of interest, and its introduction was effective in both C36 and C7 (MGIs of 89.5 and 90.5%, respectively). In C36, these bacteria have slightly less influence than the best two variants described above; based on our statistical analysis, it was in the intermediate group between the lowest and highest results in all ranges of the C7 and C36 variants. C7 was even slightly more effective than variant C7C with the introduction of a microbial composition and it statistically differed from all variants with individual culture inoculants ($p < 0.05$).

B. subtilis and *P. aeruginosa* showed no similar effects. The MGIs for C36B1 and C7B1 were a few percentages lower than controls C36W and C7W, respectively, but were not significant ($p > 0.05$, one group with controls). There was a slight increase for C36P (87.1% inhibition), while C7P had a smaller effect than the control (53.3%). However, as with *B. subtilis* variants, in terms of statistics, it was in one group of results with the controls.

The compost without introducing of microorganisms was effective against this phytopathogen by itself, and the addition of LB nutrient medium had no influence on the suppressive potential (no significant difference compared with the control, $p > 0.05$) while diluting the compost (suspension variants, C7) caused a significant reduction ($p < 0.05$) in the inhibition efficiency (MGIs for C36W, C36L, C7W, and C7L: 86.3, 86.0, 58.4, and 54.4%, respectively).

Thus, *C. rosea* supersession likely depends largely on the antagonistic activity of specific compost fungal autochthonous species and their abundance in the environment. Given the results in Figure 2a (4) and Table 7, we can see the role of fungus in inhibiting *C. rosea* mycelial growth. *B. amyloliquefaciens* is also an effective biocontrol agent.

Antagonist inoculation increased the suppressive potential of the initial composts best (i.e., compared with the control) against *P. solitum*, despite its generally low suppression efficiency compared with the other two phytopathogens (Table 7). Among the C36 variants, the microbial composition (MGI, 84.0%; control MGI, 11.1%) and *B. subtilis* (83.4%) were the best inoculants (statistically significant differences compared with other C36 variants, $p < 0.05$, and were included in the same group of results). The fungal contribution was also recorded (C36A—MGI, 77.2%, a statistically close group of results with the best two variants). Treatment with *P. aeruginosa* provided suppression of fungal mycelial growth by 59.0%.

Using LB nutrient medium instead of water in the C7L variant did not enhance the suppression of the phytopathogen (the MGIs were at the same levels for C7W and C7L,

$p > 0.05$). However, the LB components in C36 had some impact in terms of suppressive capacity (a significant difference between the C36W and C36L variants, $p < 0.05$). This may be directly or indirectly due to stimulating the autochthonous microbiota of the compost and specific changes in synthesis of some metabolites effective against *P. solitum*. Given the values showing the influence of individual culture introductions, *B. subtilis* actively inhibits the mycelium growth of *P. solitum*, as described above. The nutrient medium components (when LB was introduced to the C36L variant instead of water) likely stimulated these autochthonous bacteria, as they are often dominant in compost communities. Thus, this is an effective way to repress this fungus.

The best mycelial growth inhibition (MGI 89.9%) was obtained by introducing a microbial composition to compost suspension C7C (significantly significant differences compared with all ranges of the C7 and C36 variants, $p < 0.05$, but statistically close to the results for the C36C and C36B1; Figure 2b).

Individually introducing *B. subtilis* and *P. aeruginosa* cultures to the compost suspension was ineffective against *P. solitum* (MGIs for C7B1 and C7P, 0.0%), and there was no statistically significant difference compared with controls C7W and C7L ($p > 0.05$). Using *B. amyloliquefaciens* (C7B2) and *A. corrugatus* (C7A) caused the suppression of the phytopathogen by 58.3% and 67.1%, respectively, and significantly differed from all C7 variants ($p < 0.05$).

Generalizing these results, *P. solitum* is sufficiently resistant to the influence of compost, and its mycelial growth is more difficult to inhibit, its reduction mostly depends on the presence and concentration of antagonists in the medium. The *Bacillus* genus made a significant contribution when introduced to both C36 and C7, and *A. corrugatus* made a significant contribution when introduced to the compost suspension.

In evaluating the biocontrol potential of the analyzed variants against *Alternaria alternata*, it should be noted that the suppression increases caused by introducing microorganisms to C36 (compared with the control) were less intense than for the other phytopathogens. However, inoculating the compost suspensions (C7 variants) with microorganisms was more effective than inoculating the C36 variants given the increases in suppression (Table 7, Figure 2b).

The LB nutrient medium components did not improve the antagonistic activity, and in the C7L variant, the result was lower (MGI 26.5%) than that of the C7W control variant (41.4%) ($p < 0.05$).

For *A. alternata*, the most successful inoculum was the composition of all four microorganisms, which, when introduced to both C36 and C7, achieved a mycelial growth inhibition rate of 81.9% (Figure 2c). At the same time, variant C7A was also highly effective; the estimated rate of mycelial inhibition was 81.0%. However, statistically, the MGI values for all C36 variants were at approximately the same level, in some cases slightly higher (C36P) or slightly lower (C36B1, C36B2) than the control (in a range of 71.1–76.7%) (Figure S8), but this was not significant ($p > 0.05$, one group of results). As noted for the compost suspension variants, the differences in variants with introduced microorganisms compared with controls C7W and C7L were greater; however, among all the biocontrol agents (including microbial composition), the MGI was in about the same range of values (75.1–77.0%, $p > 0.05$, one group).

In general, when using the antagonist composition, the mycelium growth inhibition values of the three phytopathogens were in a relatively narrow range of 81.9–91.7% for both types of compost (C36 and C7). The standard deviation did not exceed 8%, which may reflect a lower degree of scatter in the data. All of this may indicate more stable results in comparison with the use of single cultures.

Considering all the analysis results, the values obtained after applying composts with 36% dry matter were more consistent and higher. To some extent, this may be due to the way microorganisms grow when introduced to a solid or liquid medium. In the latter case, the microorganism takes longer to adapt to conditions; in the former case, the environment

is closer to its native one, which leads it to rapidly achieve the maximum rate of biomass accumulation [65,66].

Scheuerell and Mahaffee (2004) [67] and Deepthi and Reddy (2013) [68] reported that the suppressive activity of composts may be more dependent on their various characteristics than on the number of antagonists in the medium. However, it is worth noting that variants C36W and C36L, without microbial inoculation, showed minimal suppressive activity against *P. solitum*, while C7W and C7L did not inhibit the fungus (Figure S7). Suppressing this phytopathogen likely depends greatly on the number of antagonists in the compost. Since there are known cases of resistance to some chemical fungicides in this species [37] and based on the results of the analysis—which show that the growth of *P. solitum* mycelium is less affected by inhibition than *C. rosea* and *A. alternata*, a larger amount of microbial fungicide compounds is probably needed to provide the proper level of biocontrol. Interestingly, a similar trend associated with the concentration of biocontrol agents in the medium was also shown for *C. rosea*.

It is worth noting that no strong differences were found when inoculants were used to inhibit the growth of *A. alternata*. Both in individual cultures and in microbial combination cases, suppression was observed at approximately the same level, with a nonsignificant increase for the combination. The introduction enhanced the suppressive potential of the initial compost suspensions (C7 variants), a feature that is difficult to observe in solid compost (C36 variants). Pane et al. (2012) [50] reported significant variability in suppressing *A. alternata* with compost tea, and this was independent of the type of extractant used to prepare the teas (in other words, it did not depend on the abiotic factor or certain additives). Deepthi and Reddy (2013) [68] noted that introducing microorganisms in combination can even reduce suppressive activity against *A. alternata*. However, conversely, the authors note that sometimes individual isolates and composts exposed under alternative conditions can be more effective in suppressing this phytopathogen. This can have a more significant effect than increasing the number of antagonistic microorganisms in the substrate. Ramírez-Cariño et al. (2020) [14] noted that individual treatments with antagonists from the *Bacillus* and *Trichoderma* genera provided the same *A. alternata* biocontrol effect as a combined application. Thus, suppressing *A. alternata* may be greatly influenced by abiotic factors that affect the autochthonous microbiota of composts.

Our results were varied for the three species of fungal pathogens. Indeed, heterogeneity in the effect of different antagonists on phytopathogens has been found by other researchers. Daami-Remadi et al. (2012) [69] showed that *Aspergillus, Penicillium*, and *Trichoderma* genera isolated from low-dry-matter compost inhibited two species of *Pythium* in different ways. All fungi achieved 100% suppression of *P. aphanidermatum*, but for *P. ultimum*, the results ranged from 15 to 96%. De Corato et al. (2018) [15] observed significant differences in the inhibition of pathogens by composts ranging from 25 to 80% overall when examining a wide range of phytopathogens.

Pane et al. (2012) [50] suggested that the antagonism may experience an antibiotic-like effect without physical antagonist–pathogen interactions, which was indicated by the presence of distinct growth inhibition zones. Zouari et al. (2020) [27] focused on the idea that the specificity of cell wall degradation enzyme synthesis (proteolytic, lipolytic, chitinolytic potential) of antagonists isolated from compost extracts greatly influences and inhibits fungal pathogens. Therefore, concrete fungal suppression can be caused by differences in the native autochthonous microbiota of composts and the suppressive potential of one or more communities. Considering the above aspects, variation in some environmental conditions may result in the succession of the microbial community and, accordingly, changes in the suppressive properties of the substrate. This can also include changes in the production of metabolites by compost-native microbiota after further inoculation with individual microorganisms. In our case, as shown in Figure 2, there were variants with both a distinct zone of inhibition (an antibiotic-like effect), and other interactions between antagonists and pathogens were observed (Figure S8). This may indicate the presence of several suppression mechanisms at once. The major contribution of certain mechanisms and

the specifics producing highly effective metabolites in relation to concrete phytopathogens should be examined in detail. We studied the possibility of generally enhancing the suppressive properties of composts by introducing various additional inoculants. These issues are of interest and need to be investigated in more depth.

Therefore, forming communities approximating natural ones is a promising process, as well as deeper studies on changes in the mechanisms of production highly effective metabolites against phytopathogens using new composition. However, as can be seen from the above description, a large number of reports on bacterial–fungal interactions are based on the *Bacillus*, *Pseudomonas*, and *Trichoderma* genera; therefore, studying these aspects regarding the role of *Aspergillus* fungi is of interest, as they are commonly found in compost and the rhizosphere and (as shown in our study) significantly contribute to phytopathogen suppression. The data obtained in this research have the potential for further in-depth study because they only indirectly show the effect of introducing *A. corrugatus*, in association with *Pseudomonas* and *Bacillus* bacteria, to compost without a deeper investigation of the antagonism mechanism.

4. Conclusions

The additional enrichment of the autochthonous microbiota of composts with biological control agents is beneficial. This is relevant not only for standard solid compost but also for compost suspensions, extending the range of compost uses because of the possibility of applying different types of treatments in specific cases. Applying composition comprising compost-native bacteria and fungi such as *B. subtilis*, *B. amyloliquefaciens*, *P. aeruginosa*, and *A. corrogatus*, as opposed to single cultures, can provide an integrated effect. This will lead to an increase in the fertilizing capacity of substrates and phytostimulation, which may indirectly improve plant protection efficiency. In our study, introducing composition stabilized manifestations of suppressive activity; i.e., the results became more predictable. All of this enhanced the controllability of the process of phytopathogen biological control using compost. This is extremely important for this kind of substrate given the complexity and variability of the microbiota, on which the potential of suppression greatly depends. This is the first report on the disease-suppressive potential of composts regarding *C. rosea* and *P. solitum*. Thus, the results represent a leading trend in the biological control of the described phytopathogens. In the future, it will be of interest to investigate the best variants in vivo and broaden our understanding of the mechanisms of suppression.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy13112841/s1, Figure S1: Survival rate of bacteria introduced to compost (C7) after introducing bacteria: (a) *Bacillus amyloliquefaciens*; (b) *Bacillus subtilis*; (c) *Pseudomonas aeruginosa*. Colonies formed on agarized LB medium after surface seeding from serial ten-fold dilutions (variants of three dilutions in two-fold replications are presented). Figure S2: *Aspergillus corrugatus*: (a) colonies of the fungus formed on PDA medium after surface seeding from one of the spore suspension serial dilutions; (b) colony formed on PDA medium via surface seeding from the last serial dilution of the compost suspension (C7) (analysis of the survival rate in compost after introducing fungus). Figure S3: Effect of undiluted liquid nutrient LB (Luria–Bertani) and Czapek media on test plant (*Raphanus sativus*) seed growth: (a) control (water)—no plant growth inhibition; (b) LB medium—complete inhibition of plant growth; (c) Czapek medium—lower but significant inhibition of plant growth. Figure S4: Inhibition of seed germination induced by culture supernatants without dilution: (a) *Bacillus amyloliquefaciens*; (b) *Bacillus subtilis*; (c) *Pseudomonas aeruginosa*; (d) *Aspergillus corrugatus*. Figure S5: Analysis results for diluted (1:1000) culture supernatants: (a) *Bacillus subtilis;* (b) *Bacillus amyloliquefaciens;* (c) *Pseudomonas aeruginosa*; (d) *Aspergillus corrugatu*s. *B. subtilis* and *B. amyloliquefaciens*—no seed germination inhibition observed; *P. aeruginosa*—normal plant growth was noted; *A. corrugatus*—less active growth. Figure S6: Evaluation of the antagonist microorganism's compatibility before forming a composition: normal development of all four cultures. No zones of growth inhibition were noted, and compatibility was confirmed. At the top horizontally, the fungus *A. corrugatus*; bacteria are perpendicular: from left to right—*B. amyloliquefaciens*, *B. subtilis*, *P. aeruginosa*. Figure S7: Examples of no suppression of *Penicillium solitum*: (a) C7B1—phytopathogen

spread over the entire surface of the Petri dish and grows in the wells with the compost suspension; (b) C7L—*P. solitum* grows in the wells with the compost suspension; (c) C7W—extensive spread of the pathogen and abundant growth in the wells; (d) C36W—phytopathogen grows on one of the compost lumps. Figure S8: Examples of the suppression effect (less mycelial growth inhibition of *Alternaria alternata* than in the best variants, with introduced microorganism composition): left—the effect of variant C36B2; right—C36L. C36L physical interaction with compost micromycetes and phytopathogen is observed; for C36B2, both zones of inhibition and physical interaction are visible (different antagonism mechanisms). Figure S9—Examples of the suppression effect (pure culture of antagonistic bacteria *B. subtilis* against phytopathogens): (a) bacteria spread over the entire surface of the Petri dish, no growth of *A. alternata* is observed; (b) bacteria spread over the entire surface of the Petri dish, no growth of *C. rosea* is observed; (c) bacteria spread over the entire surface of the Petri dish, no growth of *P. solitum* is observed. Three repetitions of each experiment. Figure S10—Examples of the suppression effect (pure culture of antagonistic bacteria *B. amyloliquefaciens* against phytopathogens): left—bacteria spread over the entire surface of the Petri dish, no growth of *C. rosea* is observed; right—bacteria spread over the entire surface of the Petri dish, no growth of *A. alternata* is observed. Figure S11—Examples of the suppression effect (pure culture of antagonistic fungus *A. corrugatus* against phytopathogens): (a) the fungus takes up from 47 to 94% of the Petri dish area, *P. solitum* takes up from 6 to 29% of the Petri dish area; (b) the fungus takes up from 86 to 94% of the Petri dish area, *C. rosea* takes up from 6 to 14% of the Petri dish area. Three repetitions of each experiment.

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