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Evaluation of the Potential of Agro-Industrial Waste-Based Composts to Control *Botrytis* Gray Mold and Soilborne Fungal Diseases in Lettuce

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Citation: Santos, C.; Monte, J.; Vilaça, N.; Fonseca, J.; Trindade, H.; Cortez, I.; Goufo, P. Evaluation of the Potential of Agro-Industrial Waste-Based Composts to Control *Botrytis* Gray Mold and Soilborne Fungal Diseases in Lettuce. *Processes* **2021**, *9*, 2231. https://doi.org/10.3390/pr9122231

Academic Editors: David Fernández-Calviño, Manuel Arias-Estevez, Ruth Pereira and Joana Lourenço

Received: 5 November 2021
Accepted: 9 December 2021
Published: 10 December 2021

Abstract: Composts are widely used in horticulture as organic amendments to improve the properties of soils. Composts have also been reported to enhance the disease suppressive potential of soils and, therefore, could be used as a strategy for managing plant diseases. The aim of this study was to test the ability of soils amended with four different agro-industrial waste-based composts (chestnut peels and shells, spent coffee grounds, grape marc, and olive leaves) to inhibit the growth and activity of *Botrytis cinerea* and several soilborne pathogens. First, the capacity of aqueous compost extracts to inhibit the growth of *Botrytis cinerea* and five soilborne fungi was evaluated in vitro using a broth macrodilution method. Second, lettuce plants were grown on soils amended with composts and inoculated either with *B. cinerea* or the soilborne fungus *Fusarium oxysporum* Schlechtendahl isolated from lamb's lettuce. The determination of minimal inhibitory concentrations indicated that none of the composts inhibited the mycelium growth of the selected fungal pathogens. However, the pathogens did not cause any damage on plants grown on the chestnut- and olive-based composts. Lettuce yields were also highest for plants grown with composts made from chestnut and olive, irrespective of the amount of compost incorporated into soils (5% or 10%, weight basis). The grape-based compost also exhibited a fertilization effect, although the effect was associated with increased Fusarium wilt severity. Both N immobilization and symbiosis with the compost's microflora were used to explain the pathogenicity of *F. oxysporum* Schlechtendahl in response to amendment with composts made from grape and coffee wastes. The beneficial effects of the chestnut- and olive-based composts reported in this study could be exploited in strategies aimed at reducing reliance on synthetic pesticides for the control of fungi in lettuce cultivation.

Keywords: disease suppressive soils; biological control; lignocellulosic composts; macrodilution assay; soilborne pathogens; agro-industrial wastes; *Botrytis cinerea*; disease severity and incidence

1. Introduction

A decline in soil fertility and health have been reported worldwide during the last decades; changes in soil quality have been largely attributed to an unreasonable application of agrochemicals in farms [1,2]. Several solutions have been proposed to reduce the use of synthetic fertilizers/pesticides, improve soil properties, and ultimately create an eco-sustainable and efficient agriculture. In this regard, the use of composts has been reported to be advantageous for both recycling of wastes and replenishing of soil fertility [3–5]. Moreover, application of composts to soils has been reported by several authors to be a natural tool for managing plant diseases, especially soilborne diseases [2,6,7]. One major aim in composting is to produce end-products with low levels of heavy metals [8]. The final levels of heavy metals in composts, however, vary, depending on the raw material and composting conditions; as such, composts may introduce heavy metals into soils, on the one hand, and remediate soils by changing the mobility and bioavailability of such contaminants, on the other hand [9]. Although composts can cause damage to seeds, phytotoxicity is mainly observed with immature composted materials that are applied at elevated rates to soils [10]. Therefore, most composts are considered safe for application to soils [11–13].

Hoitink et al. [14] first proposed the use of composts for disease control in 1975. Since then, numerous studies have been published on the topic of suppression of plant pathogens with organic amendments. Bonanomi et al. [6] analyzed 250 articles reporting 2423 experiments on the use of several types of organic amendments for disease control and concluded that the suppression of soilborne pathogens can be achieved through the application of compost materials to soils. Diseases caused by soilborne fungi result in significant economic losses and are among the major factors limiting the productivity of agroecosystems [15]. The suppressive effect of composts and compost-derived products on soilborne fungi has been demonstrated with *Verticillium* sp. [7], *Fusarium* sp. [16], *Pythium* sp. [12,17,18], *Rhizoctonia* sp. [18,19], *Alternaria* sp. [20], *Sclerotinia* sp. [18], and *Phytophthora* sp. [11,13]. Composts have also been reported to be useful in the control of plant diseases besides soilborne diseases. Examples are diseases caused by *Botrytis* sp. [7,16]; although most of the damage caused by these pathogens is done by air-borne conidia, several *Botrytis* sp. survive as sclerotia in soil and plant debris, constituting a secondary inoculum on numerous crops [20].

The capacity of composts to suppress pathogens is attributed to an intricate set of mechanisms related to their physical, chemical, and biological properties [21]. Some composts can provide a physical barrier against harmful soilborne pathogens by occupying space around plant roots [22]. Nutrients in composts contribute to the protection of plants against diseases through enhanced nutritional status of the soil, as well as through direct toxicity on pathogens [23]. Biologically active substances in the composts and toxic metabolites released during the decomposition of organic matter can act as pathogenic suppressive compounds [4,12,23,24]. The physical structure and chemical composition of the compost can vary under different abiotic conditions and influence the indigenous microbial population, leading to an enhancement of the disease suppression potential of soils through antagonism [1,22,25], parasitism [26], fungistasis [27], or antibiosis [28,29]. It follows that the viability and distribution of soil microorganisms is highly influenced by the physicochemical properties of the compost [6,30,31]. Thus, the effectiveness of disease control might also depend on factors, such as the physicochemical characteristics of the source material from which the compost is made [32,33], compost maturity, and the mode, timing, and rate of compost application [26,27,34]. The disease suppression ability of a compost material also depends on the pathogen being targeted [16,23,27,32]. This may explain why the inhibitory effect of compost-amended soils on the growth of pathogens is not evident in all cases.

The objective of this work was to test the disease suppression ability of four agroindustrial waste-based composts of different physicochemical properties. The composts were made with chestnut peels + shells, spent coffee grounds, grape marc, and olive leaves and were tested in vitro and in potted lettuce plants against several soilborne fungi and *B. cinerea*. Interesting data were obtained that could be used as support in reducing the use of chemical fungicides in the control of diseases associated with horticultural crops.

2. Materials and Methods

2.1. Preparation of Composts

The composts used in this study were produced at a specialized pilot composting plant under aerobic conditions. Composting was carried out using the following raw materials collected from agri-food industries in the north of Portugal: (i) chestnut peels and shells; (ii) spent coffee grounds (SCG); (iii) grape marc; and (iv) olive leaves. The composting procedure was as described by Santos et al. [35], using wheat straw and silver wattle (*Acacia dealbata*) leaves as bulking agents. Moisture was maintained between 45 and 60% by occasional watering. The composting process was stopped after temperature stabilization which took 156 days for SCG and 147 days for the other raw materials. These composts had good levels of nutrients (P, Ca, Mg, Fe, Cu, Zn, Mn) and phenolic and lignocellulosic compounds (gallic acid, hemicellulose, cellulose, lignin, and lignocellulose), as shown in Tables S1 and S2. The composts also exhibited a high degree of humification and no phytotoxic effect on the germination of seeds from several crop species, including lettuce, tomato, cowpea, parsley, rice, spinach, and faba bean (data not shown). Several physicochemical properties of the composts were characterized through analyses of soluble organic C, soluble organic N, ammonium, nitrate, organic matter, pH, and electrical conductivity (Table S3), as fully described elsewhere [8,35].

2.2. Phytopathogenic Fungi Tested

The ability of the four composts to suppress diseases was tested against some economically relevant phytopathogenic fungi, namely: *Botrytis cinerea* (isolate RW003), *Fusarium oxysporum* (isolate MYA-3072), *Sclerotinia sclerotiorum* (isolate 18015), *Rhizoctonia solani* (isolate 56612), *Alternaria* sp., and *Pythium* sp. Because of confusion over similar names, isolate *F. oxysporum* Schlechtendahl (MYA-3072) from lamb's lettuce (*Valerianella locusta*) was mistakenly used instead of isolate *F. oxysporum* f.sp *lactucae* from lettuce. *F. oxysporum* Schlechtendahl poses a substantial threat to the production of lamb's lettuce in Europe and was first described in Italy in 2003 [36]. The colonization of roots of a wide range of crops by *F. oxysporum* Schlechtendahl has also been reported; however, its ability to cause disease on lettuce cultivars is unknown. *Botrytis*, *Fusarium*, *Sclerotinia*, and *Rhizoctonia* isolates were supplied by the American Type Culture Collection (ATCC; Manassas, VA, USA) and were kept on potato dextrose agar (PDA) in a culture chamber at 25 °C. The *Alternaria* sp. and *Pythium* sp. were isolated from infected lettuce plants grown in the Trás-os-Montes e Alto Douro Region of Portugal. After surface sterilization, root, stem, and leaf sections of the lettuce plants with evident lesions were cut and placed on a PDA medium. Pure fungal cultures of *Alternaria* sp. and *Pythium* sp. were obtained, and the pathogens were maintained on PDA in a culture chamber at 25 °C.

2.3. In Vitro Antifungal Activity of Aqueous Compost Extracts Using a Broth Macrodilution Method

Aqueous compost extracts were prepared by adding 250 g of grounded compost (sieved through pore sizes 2 mm in diameter) with 1 L of sterile distilled water. The mixtures were placed on an orbital shaker (200 rpm) in the dark at 25 °C. After 72 h of incubation, the mixtures were filtered through a Whatman No.1 paper filter [37]; the aqueous compost extracts were collected and stored at 4 °C until used.

The in vitro antifungal activity of the aqueous compost extracts was evaluated against all the phytopathogenic fungi mentioned above using a broth macrodilution method. The broth macrodilution method was carried out following the guidelines of the National Committee for Clinical Laboratory Standards according to protocol M38-A2 [38]. Serial 5-fold dilutions of the aqueous compost extracts were made from 1.6 to 200 $\mu L\ mL^{-1}$ with sterile distilled water. Fungal inoculum suspensions were prepared using mycelium plugs (6 mm diameter) taken from an actively growing culture of each phytopathogenic isolate on PDA [32]. The density of spores in the inoculum was determined by direct microscopic counts and adjusted to ca. 2×10^4 spores mL^{-1}. An aliquot of the inoculum

(80 µL) and 80 µL of aqueous compost extract were pipetted onto a test tube containing the RPMI 1640 medium (ThermoFisher Scientific, Rand Island, New York, NY, USA). Positive and negative control tubes were also prepared. The positive control consisted of 80 µL of fungal inoculum and 80 µLof amphotericin B (XGen Pharmaceuticals, New York, NY, USA); the negative control consisted of 80 µLof fungal inoculum and 80 µL of sterile distilled water. Two tubes were used per aqueous compost extract and dilution. The tubes were incubated in darkness at 30 °C. After 7 d of incubation, the growth of fungal pathogens was visually assessed, and the diameter of the inhibition zone was measured. Minimal inhibitory concentrations (MICs) were determined as the lowest concentrations of extracts at which no fungal growth was observed [39].

2.4. Greenhouse Experiment with Potted Lettuce

The composts were further studied for disease suppressiveness in a greenhouse experiment. A loamy soil was sampled in the topsoil layer (0–30) in the central region of Portugal (latitude: 392.20150 N, longitude: 844.2570 W). The soil was sieved, and its texture (clay 55%, silt 28%, sand 17%; wt wt^{-1}) was determined using standard laboratory procedures. Pots with a volume of 2 L were filled with the soil that was previously steam-sterilized for 45 min in a container.

The disease suppressiveness of the soil-compost mixes was determined in two bioassays: (i) *B. cinerea* (isolate RW003)/lettuce (*Lactuca sativa* 'Maravilha dos Invernos') and (ii) *F. oxysporum* (isolate MYA-3072)/lettuce (*L. sativa* 'Maravilha dos Invernos'). The two fungi tested are responsible for two major devastating and economically important diseases in greenhouse-grown lettuce, namely gray mold [20,40], and Fusarium wilt [23,31]. Fungal inocula were mixed with the potted soil at a density of 10^4 spores g^{-1} soil. Control mixes consisted of soil and 1 mL of water. All the pots were maintained in the greenhouse at 25 °C for 12 days, after which the soils were amended with the coffee-, chestnut-, grape-, and olive-based composts. Amended mixes consisted of compost (5% or 10%, weight basis) and loamy soil (90% or 95%, weight basis), for a total of 27 treatments: [(2 inoculated soils/fungi + 1 non-inoculated soil/control) × 4 composts × 2 proportions of compost) + (2 non-amended and inoculated soils/fungi + 1 non-amended and non-inoculated soil/control)]. The mixes were incubated for 5 d at 25 °C before lettuce sowing in order to allow complete soil infestation.

Lettuce seeds were sown by hand in plastic trays (41 L × 41 l × 5 H cm) filled with peat and allowed to germinate for 24 h in a growth chamber at 28 °C in dark conditions. Germinated seeds were transferred to the greenhouse (25 °C). After the plants had reached the second true-leaf stage (ca. 12 d after transfer to the greenhouse), one seedling was transplanted in each pot. Leaves of seedlings grown on the soil/compost mixes inoculated with *B. cinerea* were gently covered with the mixes in order to promote infection. A randomized complete block design with four replicates for each potting mix was used for the experiment. Each replicate consisted of a tray containing two pots placed on a mat. Each pot was watered to 80% of field capacity by addition of water to the prescribed weight every other day. The plants were not treated with fungicides, nor fertilized.

Disease development was assessed regularly by observing the appearance of symptoms. The experiment was ended when plants in one treatment showed complete wilting or died, i.e., 30 days after transplantation. Disease severity was estimated by assigning symptom indexes/grades using the following scale: 1 = no symptoms; 2 = leaf yellowing; 3 = stem wilting; and 4 = plant dead [31,34]. At harvest time, shoots were cut from each plant at the soil level, and roots were washed free of soil. The number of infected plants was recorded (i.e., plants with visible leaf yellowing or stem wilting, or plants with blackened stems or roots). The number of healthy plants was also counted, and disease incidence was calculated as the percentage of infected plants. Shoots and roots were dried for 72 h at 80 °C to determine the dry weights.

2.5. Statistical Analyses

The in vitro experiment was carried out with two replicates for qualitative purposes. The greenhouse experiment was carried out with four (n = 4), replicates, which allowed results to be expressed as mean ± standard deviation. In the case of the greenhouse experiment, each replicate comprised two plants; for each parameter, the average value for the two plants was used in statistical analyses. Before statistical analyses, disease incidence and disease severity data were arcsine square root (x/100)- and ln-transformed, respectively. One-way, two-way, and three-way analysis of variance (ANOVA) were carried out using Statistix 10.0 (Analytical Software, Tallahassee, FL, USA). In most cases, it was not possible to estimate the interaction effects among the three studied factors (compost source, proportion of compost in soil, and fungus isolate) because of plant death or missing values. Thus, only one-way ANOVA data are discussed. The Tukey's test was used to assess the significance ($p < 0.05$) of differences between the means. A Pearson correlation analysis was also performed to identify the effects of the different physicochemical variables measured in the composts in previous experiments [8,35] on disease incidence and severity assessed in the present study.

3. Results and Discussion

3.1. In Vitro Antifungal Activity of Composts

In the present work, the antifungal activity of four composts was tested in vitro against six phytopathogenic fungi. All the fungi isolates exhibited noticeable mycelial growth over the period between 3 and 4 days (Figure 1), an indication that all the aqueous compost extracts were inactive against the fungi irrespective of the concentrations. All fungi showed growth inhibition to a variable degree when tested with Amphotericin B (positive control) with MIC values between 2.5 and 56 μL mL^{-1}. These MIC values were within the ranges of those reported by other authors using the broth macrodilution method [38,39]. *R. solani* was the most sensitive fungus (MIC = 2.5 μL mL^{-1}). The antifungal properties of plant-derived products are attributed to the presence of bioactive chemical compounds. Bioactive compounds in several cases exhibit their activity via direct interaction with the pathogens: for example, by facilitating perforation of microbial cell walls, altering the activity of an enzyme or the expression of a gene [12]. The absence of inhibitory effects with the composts in this study indicates no direct action on the fungi through fungistasis [25,27].

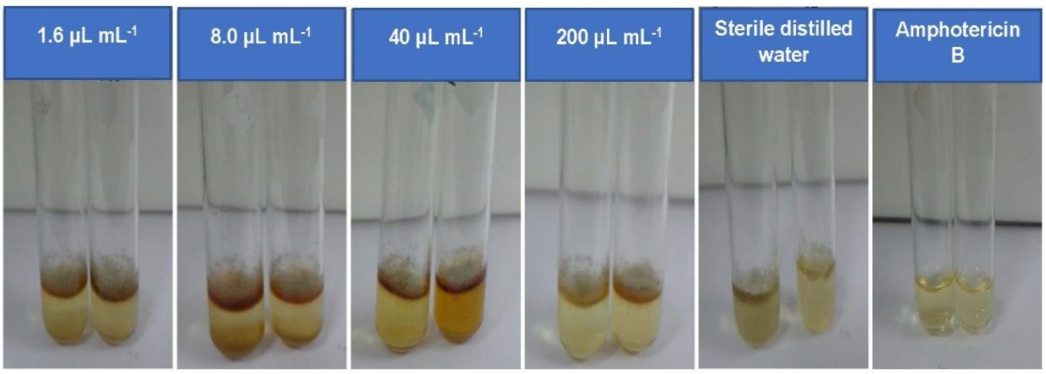

Figure 1. Example of broth macrodilution assay of aqueous extract from the grape-based compost at concentrations (from left to right) of 1.6 μL mL^{-1}, 8 μL mL^{-1}, 40 μL mL^{-1}, and 200 μL mL^{-1}, against 2 × 10^{4} spores mL^{-1} suspension of *S. sclrerotiorum* (isolate 18015) 7 days after inoculation, when compared with sterile distilled water (negative control) and Amphotericin B (positive control).

3.2. Disease Suppressiveness of Composts

Lettuce plants were grown in soil-compost mixes inoculated either with *B. cinerea* or *F. oxysporum*. Disease severity data in Figure 2a show that plants grown on non-inoculated mixes were free of diseases. Hence, the ability of composts to suppress lettuce diseases was assessed in comparison with the non-amended and inoculated loamy soils. The composts differed in their ability to suppress gray mold caused by *B. cinerea* and Fusarium wilt caused by *F. oxysporum*.

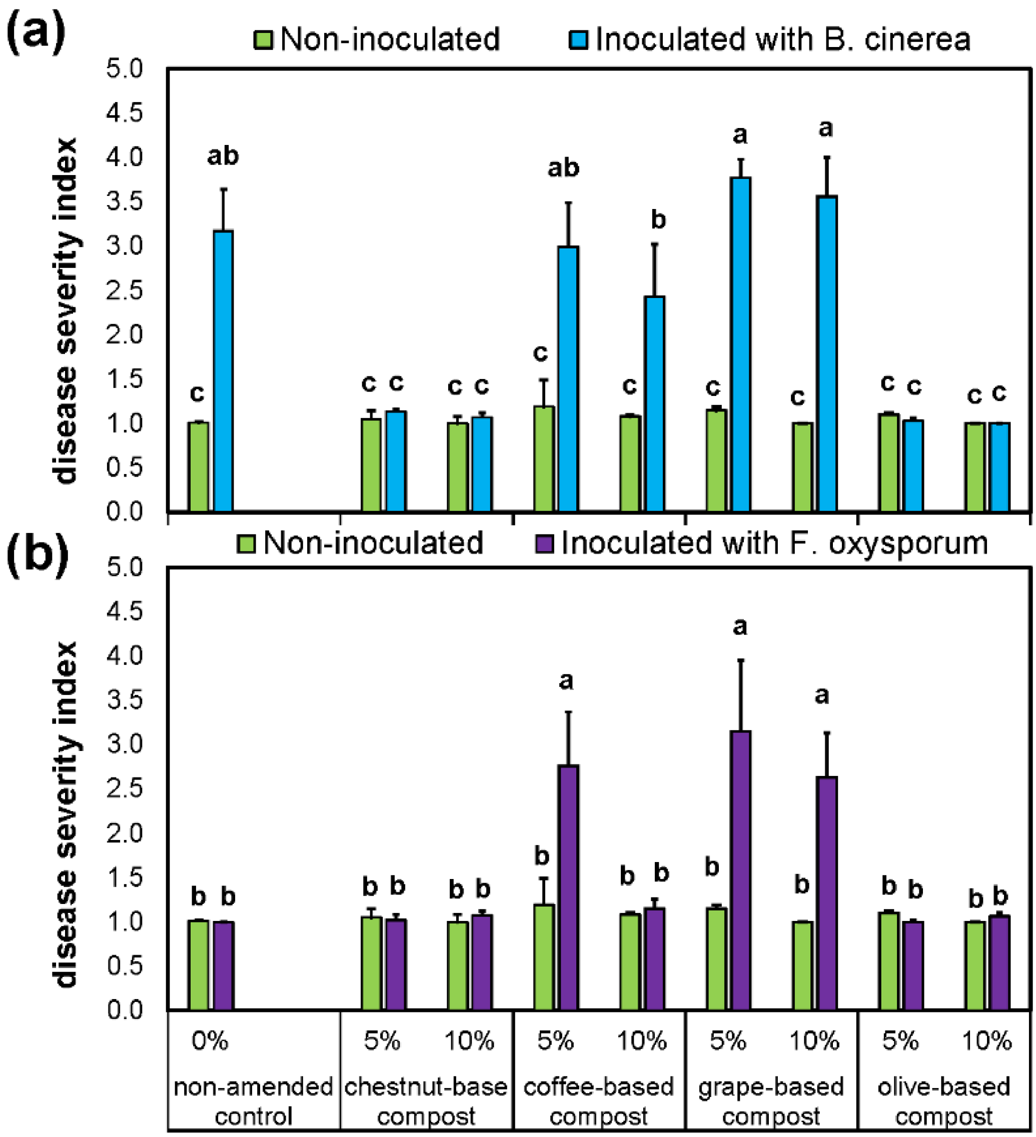

Figure 2. Disease severity of (**a**) gray mold and (**b**) Fusarium wilt in lettuce plants grown on soil/compost mixes inoculated with *B. cinerea* and *F. oxysporum*. The composts were incorporated into soils at proportions of 5% and 10% (weight basis). Control treatments consisted of non-amended soils. A semi-quantitative scale from 1 to 4 was used to rate the severity of the disease, where: 1 = no symptoms; 2 = leaf yellowing; 3 = stem wilting; and 4 = plant dead. Different letters above the bars indicate significant differences among means ($p < 0.05$; ANOVA/Tukey's test).

Control plants grown on soil inoculated with *B. cinerea* exhibited typical symptoms of gray mold (disease severity index = 3.17), including soft brown rot on the stems and at the base of older leaves and wilting of leaves; a fuzzy grey to brown discoloration or tan mold could be seen on some young leaves [40]. As compared to the non-amended and inoculated medium, two composts (i.e., chestnut- and olive-based composts) clearly induced the suppression of gray mold disease; no disease symptom was observed on plants grown on the two mixes irrespective of the proportion of composts incorporated into the soil (Figure 2a). Since none of the aqueous compost extracts from the chestnut- and olive-based composts inhibited the mycelial growth of *B. cinerea*, it can be inferred that the two composts indirectly induced a systemic resistance in the plants [28,29]. The aggressiveness of *B. cinerea* is linked to an oxidative burst-suppressing agent that suppresses the hypersensitive response, a form of cell death often associated with plant resistance to pathogen infection [40]. It is possible that the composts induced oxidative burst in host plants which suppressed the hyphal growth of *B. cinerea* and, consequently, inhibited fungal infection [41,42]. Plants grown on the coffee- and grape-based composts were severely affected by gray mold. Composts made from grape wastes showed similar behavior to the inoculated control and obtained disease severity indexes of 3.56 to 3.77 depending on the proportion of compost incorporated into soils (Figure 2a).

With respect to Fusarium wilt, control plants inoculated with *F. oxysporum* did not exhibit the typical symptoms of the disease, such as seedling wilt and leaf yellowing [31]. Therefore, infected control plants remained healthy; similarly, plants grown on the composts made from chestnut and olive did not exhibit any symptoms of Fusarium wilt (Figure 2b). The most likely reason for such an absence of symptoms is that isolate *F. oxysporum* Schlechtendahl from lamb's lettuce was not pathogenic on lettuce. The severity of Fusarium wilt is affected by inoculum source and density in soil, which is expected to decline when a non-susceptible crop is grown [36,43]. Although the isolate from lamb's lettuce has genetic sequences and other characteristics that are very close to *F. oxysporum* f. sp. *lactucae* [44], the levels of aggressiveness of the two isolates are different. The objective posed in this work was to accomplish a severe enough inoculation to achieve plant death in the control group inoculated with *F. oxysporum* Schlechtendahl. Unfortunately, the objective was not achieved. Surprisingly, however, symptoms of Fusarium wilt were clearly observed on lettuce plants grown in soil amended with the coffee-based compost at 5% and the grape-based compost at 5% and 10%, indicating that the pathogen could colonize and produce inoculum on a non-host (Figure 2b). It is now well known that a non-pathogenic strain can be converted to a pathogenic strain by transferring lineage-specific genes or even chromosomes under certain circumstances [36]. Factors, such as symbiosis with bacteria, can also explain the pathogenicity or non-pathogenicity of some strains of *F. oxysporum*. Ectosymbiotic bacteria, for example, can silence the expression of genes involved in fungal pathogenesis, thereby changing the characteristics of the hyphae [44]. The aggressiveness of *F. oxysporum* Schlechtendahl observed in this study may be due to a direct response to bacterial substances liberated by the compost's microflora. The presence of different races of *F. oxysporum* displaying varying degrees of virulence complicates the effective use of control measures. More than 80 putative host-specific formae speciales members of the *F. oxysporum species* complex have been described [43], which indicates a huge number of potential hosts. The results of this study could have a direct relationship on the production of horticultural crops in settings where some putatively nonpathogenic isolates on some crops may become pathogenic on other crop species. It would be important to recover *F. oxysporum* Schlechtendahl from the vascular stele of lettuce grown on the two coffee and grape substrates in future studies and determine using pathogenicity tests and molecular assays the differences between the recovered pathogens and the initial isolate MYA-3072.

The disease suppressiveness of the composts was also graded with the disease incidence parameter, as shown in Table 1. Disease incidence results were generally in agreement with disease severity results, e.g., disease incidence of 0% for plants grown on non-inoculated mixes, and 0% for plants grown on the chestnut- and olive-based composts inoculated with *B. cinerea* and *F. oxysporum*. Lettuce plants grown on the coffee- and grape-based composts were highly susceptible to both diseases, with more than 25% of disease incidence; some plants even scored ca. 100% disease incidence of Fusarium wilt (Table 1). Related to the composts made from grape marc and coffee grounds, the data show that a compost proportion of 10% was more effective in limiting the spread of Fusarium wilt compared to 5%, although, in some cases, the effect was not significant ($p > 0.05$) (Figure 2; Table 1). A dose-dependent increase in the antimicrobial activity of composts has been demonstrated in several studies [45,46].

Table 1. Disease incidence of (a) gray mold and (b) Fusarium wilt in lettuce plants grown on soil/compost mixes inoculated with *B. cinerea* and *F. oxysporum*. The composts were incorporated into soils at proportions of 5% and 10% (weight basis). Control treatments consisted of non-amended Scheme 100. Different letters in a column or a row indicate significant differences among means ($p < 0.05$; ANOVA/Tukey's test).

Treatments	Proportion of Compost in Soil	Non-Inoculated Mixes	*Botrytis cinerea*-Inoculated Mixes	*Fusarium oxysporum*-Inoculated Mixes
Control (non-amended)	0%	0.00 ± 0.00 e	82.36 ± 2.10 d	0.00 ± 0.00 e
Chestnut peels+sheels	5%	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 e
	10%	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 e
Coffee grounds	5%	0.00 ± 0.00 e	48.82 ± 7.13 c	55.62 ± 9.79 c
	10%	0.00 ± 0.00 e	26.44 ± 8.11 d	0.00 ± 0.000 e
Grape marc	5%	0.00 ± 0.00 e	50.02 ± 6.92 c	96.10 ± 5.31 a
	10%	0.00 ± 0.00 e	75.15 ± 6.21 b	39.28 ± 1.57 cd
Olive leaves	5%	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 e
	10%	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 e

Globally, the results from this study could be explained by the capacity of composts to enhance or diminish the disease suppressive potential of soils. A great diversity of beneficial microorganisms naturally colonizes composts, and this may explain the suppression characteristics of the chestnut- and olive-based composts. Several composts obtained from chestnut residues have been characterized by the presence of putative antagonistic soil microorganisms and plant growth promoting rhizobacteria belonging to free-living (N_2)-fixing aerobic bacteria and *Pseudomonas* spp., with suppressive effects on *S. minor* and *R. solani* in tomato [47,48]. Several bacterial and fungal genera recovered in the grape marc-based compost, such as *Bacillus*, *Fusarium*, and *Penicillium*, have also been identified as antagonists of several pathogens [49,50]. Bacteria, in particular, produce a wide range of different antibiotics that exert a suppressive effect with regards to soil-borne phytopathogens [49]. SCG is rich in *Trichoderma* species that were shown to strongly inhibit mycelial growth of *S. sclerotiorum* and *P. nicotianae* [51].

3.3. Lettuce Growth on Composts

The whole plant yield of lettuce grown on the different mixes ranged from 1.78 to 10.41 g plant^{-1} (Figures 3 and 4). There was no difference ($p < 0.05$) in yield among the non-amended control treatments whether the soils were non-inoculated or inoculated with *B. cinerea* and *F. oxysporum*. On average, the chestnut- and olive-based composts increased lettuce head yields by 302 to 619% when compared to the control. The above findings were irrespective of the proportion of compost and the presence of fungi in soils. However, for plants grown on 10% compost, non-inoculated and Botrytis-inoculated soils produced more biomass than Fusarium-inoculated soils. These results corroborated the findings reported by Santos et al. [3] using the same composts and the lettuce varieties Maravilha dos Invernos and Quatro Estações. In addition, composts made from grape marc also exhibited a fertilization effect (Figures 3 and 4). This showed that, despite aggravating the disease (Figure 2; Table 1), the grape-based compost had a beneficial effect on plant growth, attesting to the fact that the incorporation of organic amendments into soil can have a direct impact on plant health and crop productivity [52]. The whole plant yield was not affected for mixes that received the coffee-based compost incorporated into soils at 5% (Figure 4).

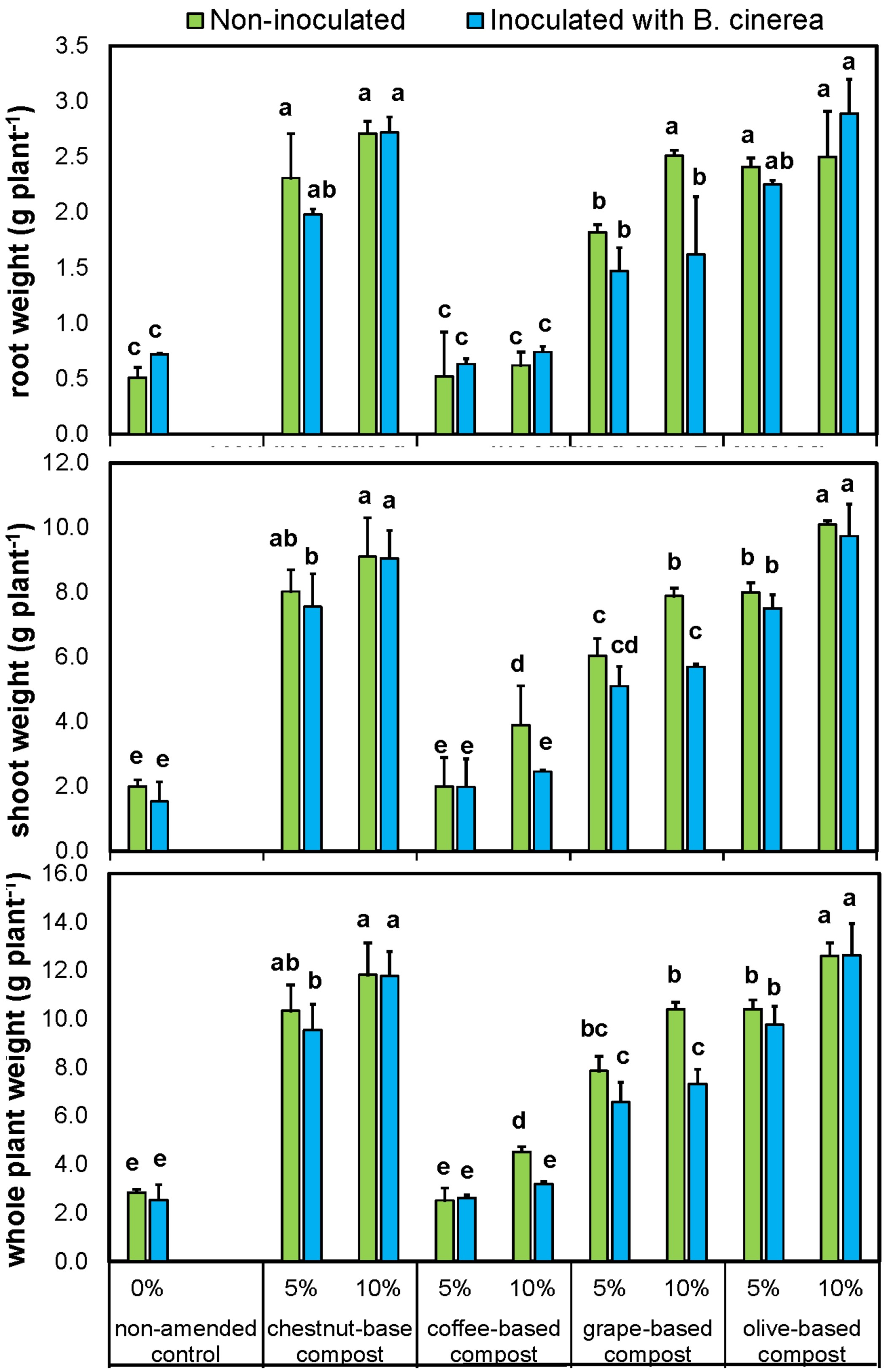

Figure 3. Shoot and root yields (dry weight basis) of lettuce plants grown on soil/compost mixes inoculated with *B. cinerea*. The composts were incorporated into soils at proportions of 5% and 10% (weight basis). Control treatments consisted of non-amended soils. Different letters above the bars indicate significant differences among means ($p < 0.05$; ANOVA/Tukey's test).

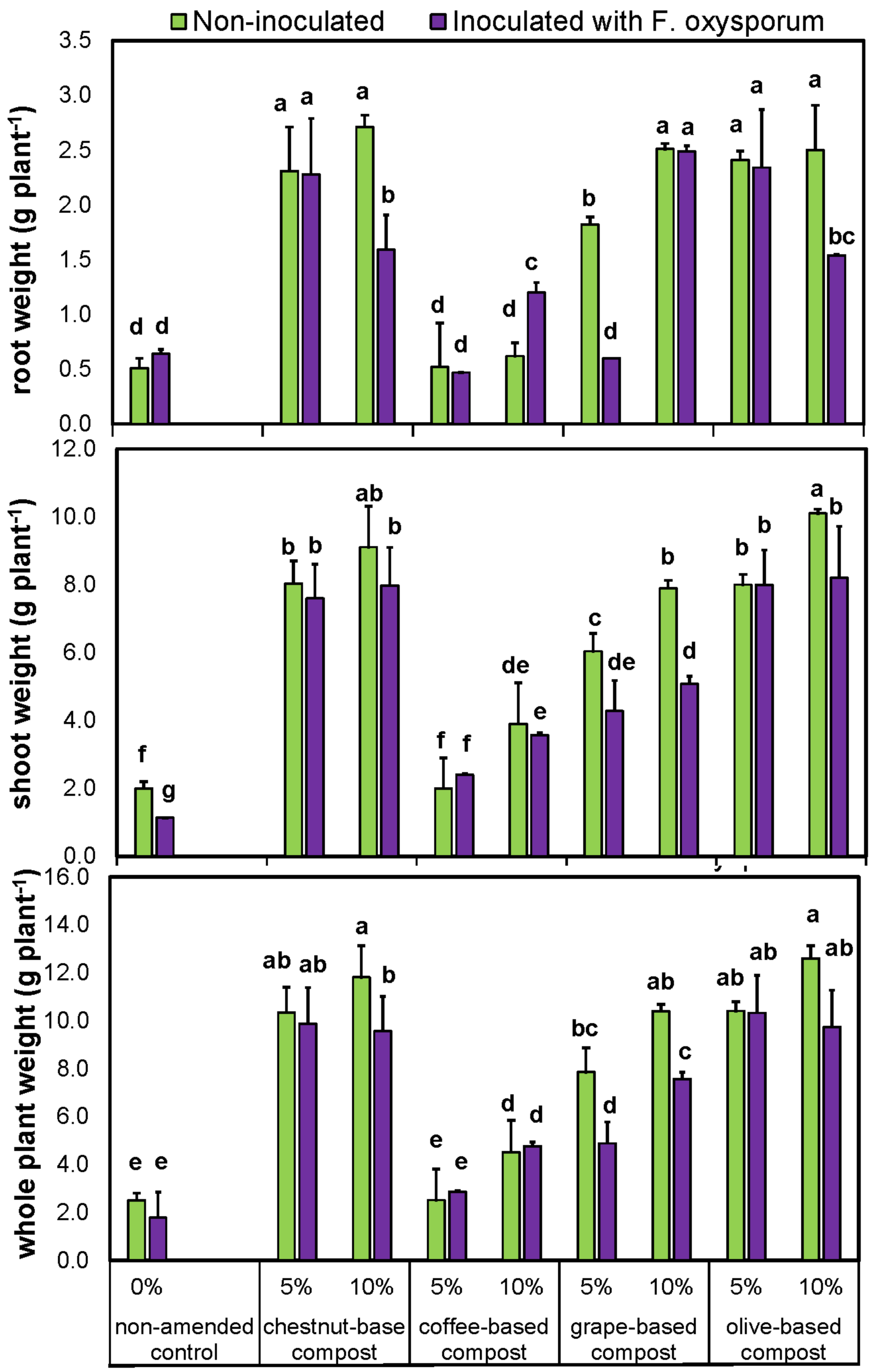

Figure 4. Shoot and root yields (dry weight basis) of lettuce plants grown on soil/compost mixes inoculated with *F. oxysporum*. The composts were incorporated into soils at proportions of 5% and 10% (weight basis). Control treatments consisted of non-amended soils. Different letters above the bars indicate significant differences among means ($p < 0.05$; ANOVA/Tukey's test).

3.4. Relationships among Lettuce Growth, Disease Incidence/Severity, and Physicochemical Characterisics of the Composts

In this work, a correlation analysis was carried out aiming to assess the relationships among the physicochemical characteristics of the composts (Tables S1–S3) and disease incidence/severity (Figures 2–4; Table 1).

The most striking observations in Table 2 are the positive correlations between lettuce growth and C content and between lettuce growth and the lignin:holocellulose ratio. The C:N ratio of the compost made from coffee was the lowest (C:N = 8.68). The chestnut- and olive-based compost has a C:N of more than 20 (Table S3), which is greater than most horticultural soils [53]. The four composts showed no variance in the total phenolic content. The grape- and chestnut-based composts generally had cellulose and hemicellulose contents higher than the other composts (Table S2). However, the only parameter that seemed to favor plant growth and limit the severity of gray mold was the lignin:holocellulose ratio (Table 2). The lignin:holocellulose ratio is a common predictor of litter decomposition rate; as the ratio increases, the decay rate also increases [54]. These correlations suggest that the degradation of complex and resistant polymeric substrates, such as cellulose, hemicellulose, pectin, and lignin, proceeded faster in the chestnut- and olive-based substrates, providing readily available nutrients to plants. Increasing nutrient supply surely stimulated plant growth, while depressing gray mold infection.

Table 2. Correlations among disease prevalence of gray mold and Fusarium wily on lettuce, and physicochemical parameters. All data from Tables S1–S4 were submitted to a Pearson correlation analysis. Green color indicates $p < 0.10$; light green color indicates $p < 0.05$; light green color with bold numbers indicates $p < 0.01$.

	Yield Gray Mold 5%	**Yield Gray Mold 10%**	**Yield Fusarium Wilt 5%**	**Yield Fusarium Wilt 10%**	**Incidence Gray Mold 5%**	**Incidence Gray Mold 10%**	**Incidence Fusarium Wilt 5%**	**Incidence Fusarium Wilt 10%**	**Severity Fusarium Wilt 5%**	**Severity Fusarium Wilt 10%**	**Severity Gray Mold 5%**	**Severity Gray Mold 10%**
Total phenolics	−0.52	−0.58	−0.60	−0.55	0.60	0.59	0.23	0.21	0.22	0.20	0.58	0.59
Gallic acid	0.46	0.46	0.40	0.66	−0.47	−0.30	0.57	0.52	0.54	0.56	−0.06	−0.13
Hemicellulose	0.01	0.04	0.01	0.26	−0.13	−0.14	0.63	0.36	0.68	0.42	0.19	0.08
Cellulose	−0.06	−0.13	−0.18	−0.27	0.34	0.51	−0.18	0.18	−0.30	0.12	0.21	0.33
Lignin	0.75	0.68	0.62	0.68	−0.51	−0.16	0.05	0.45	−0.10	0.43	−0.29	−0.22
Lignocellulose	0.45	0.36	0.28	0.33	−0.13	0.23	0.11	0.54	−0.06	0.51	0.02	0.13
Lignin/holocellulose	0.89	0.91	0.91	**0.97**	−0.94	−0.88	−0.06	0.04	−0.09	0.06	−0.71	−0.73
Soluble organic C	0.25	0.19	0.05	0.25	0.08	0.44	0.58	0.87	0.42	0.85	0.37	0.44
Soluble organic N	0.21	0.13	0.00	0.06	0.22	0.60	0.24	0.71	0.04	0.66	0.32	0.46
NH^{4+}	−0.04	−0.08	−0.07	−0.27	0.19	0.23	−0.50	−0.23	−0.55	−0.28	−0.07	0.03
NO^{3}-	0.05	−0.03	−0.15	0.16	0.20	0.51	0.86	**1.00**	0.84	**1.00**	0.60	0.62
Organic matter	0.08	0.84	0.80	0.74	−0.67	−0.37	−0.31	0.13	−0.45	0.10	−0.60	−0.50
C	**0.98**	**0.97**	0.94	**0.97**	−0.89	−0.61	−0.11	0.19	−0.21	0.19	−0.67	−0.63
N	−0.58	−0.53	−0.47	−0.32	0.23	−0.09	0.40	−0.16	0.58	−0.10	0.32	0.16
C/N	0.69	0.65	0.60	0.45	−0.39	−0.09	−0.48	0.07	−0.64	0.01	−0.48	−0.32
pH	0.92	0.89	0.83	0.87	−0.67	−0.33	−0.28	0.21	−0.43	0.17	−0.58	−0.47
EC	0.05	0.00	−0.04	−0.25	0.26	0.42	−0.49	−0.03	−0.61	−0.11	−0.01	0.15
P	−0.32	−0.32	−0.36	−0.03	0.22	0.21	0.91	0.57	0.95	0.62	0.59	0.47
Ca	−0.02	0.01	0.01	−0.25	0.14	0.13	−0.58	−0.36	−0.62	−0.41	−0.20	−0.09
Mg	−0.43	−0.38	−0.33	−0.13	0.09	−0.18	0.49	−0.06	0.65	0.01	0.26	0.09
Fe	−0.09	0.00	0.08	0.11	−0.27	−0.55	0.03	−0.41	0.18	−0.35	−0.24	−0.38
Cu	0.46	0.48	0.44	0.68	−0.53	−0.41	0.51	0.40	0.50	0.45	−0.14	−0.23
Zn	0.54	0.52	0.44	0.67	−0.44	−0.18	0.52	0.61	0.44	0.63	−0.05	−0.07
Mn	−0.08	−0.14	−0.25	0.10	0.24	0.46	**0.97**	0.94	0.90	**0.96**	0.67	0.63

A positive correlation between the compost pH and lettuce yield was also found in the present study (Table 2). Unfortunately, the physicochemical characteristics of the soil:compost mixes were not determined. However, soil organic amendments are known to improve plant growth by directly improving key soil physical, chemical, and biological properties [55]. In a SCG-amendment pot study [53], soil pH increased slightly over time such that the amended soils were eventually less acidic than the control; at the end of the experiment, plant biomass growth of tomato was related to the pH, N content, and net mineralization of the composts. A similar phenomenon might have happened in the pot study reported here. The absence of correlations among C, pH, disease incidence, and severity suggests that the physicochemical properties of the composts affected plant growth more than the presence of the pathogens in soils. Horticultural plants with different C and pH preferences are likely to respond differently to composts. For instance, the population of Actinomycetes which are antagonistic to *F. oxysporum* increases with increasing pH [56]. A negative correlation was found between compost pH and *F. oxysporum* severity on muskmelon seedlings [5].

The nutrient status of the soil has also been reported to affect the microbial community and disease outcomes [31,57–59]. In this study, an effect of the NO^{3-} content of the composts on the disease severity and incidence of Fusarium wilt was indicated (Table 2). The grape and coffee-based composts tented to have higher NO^{3-} contents than the other composts (Table S3). The four composts had similar organic matter contents that ranged from 927.10–959.37 g C kg^{-1} (Table S3). As organic matter decomposes in the soil, N is made available through microbial mineralization processes that yield ammonium (NH^{4+}) and nitrate (NO^{3-}). NH^{4+} and NO^{3-} can be taken up by plants or accumulate in the soil. NH^{4+} and NO^{3-} can also be taken up by microbes to support their own metabolic needs, a process known as microbial immobilization [53]. Differences in compost NO^{3-} contents observed in this study demonstrate the occurrence of microbial N immobilization processes in substrates inoculated with *F. oxysporum* Schlechtendahl, and this might be one of the causes of transition from a non-aggressive status to an aggressive one.

Apart from C and N, the composts were found to be valuable sources of several other elements (Table S1) acting as micronutrients that could promote plant growth. However, no correlation was found between disease severity and elemental composition, except for Mn. Indeed, Mn was positively correlated with both measured disease-related variables of Fusarium wilt, with similar correlation indexes ($R^2 > 0.9$). Mn has received most attention in the scientific literature as a toxic element. However, Mn is known as an activator of a crop's internal defense responses, especially when it comes to root diseases caused by soil pathogens, such as *Verticillium* and *Fusarium* [60]. How Mn levels in the composts stimulate virulence of a *F. oxysporum* isolate on a non-host is a key research question for further investigations.

Differential benefits and risks according to crop species are common for most organic amendments [61]. In this study, the trend of plant growth response was chestnut $\geq$ olive > grape > coffee-based composts. Data in Figures 2–4 and Tables 1 and 2 indicate that the composts made from chestnut and olive are capable to provide both disease suppression and fertilizing effects and, therefore, represent optimal substrates to support lettuce growth. In Mediterranean countries, chestnut represents an important resource for an economy that generates tons of wastes [47]. The anticipated benefits of composts made from chestnut wastes could be harnessed for seed germination, seedling development, and horticultural production. A good number of studies have been made on the use of olive mill wastes as soil amendments; suppressive effects of olive mill waste-based composts on different phytopathogens have also been demonstrated [62]. Using olive leaves in composting is a new concept [63], and the results from this study fully endorse the potential of leaves-based composts as suppressive amendments against plant pathogens. A large number of microbes appeared in microbiological analyses of grape marc-based composts produced by Santos et al. [49]. These microbes were extremely effective antagonists against Pythium damping-off in cucumber but not against Phytophthora root rot in tomato, *Fusarium oxysporum* f. sp. *Radiciscucumerinum* in melon and *R. solani* in radish [49]. These data demonstrated that composts, in general, must be evaluated on a case-by-case basis. The use of the grape-based compost used in this study could be particularly advantageous in those nursery industries where other biocontrol agents are already available for managing soil-borne pathogens [7], exploiting their potential fertilizing effects. The compost from SCG presented several positive physicochemical properties, such as a low C:N. A low C:N is usually an indication of increase soil fertility [55]. Despite that feature, however, the composts made from SCG negatively affected plant growth and tolerance to pathogens. The most frequently cited issue with using fresh SCG as a growing medium is their high caffeine levels [51,53]. Caffeine is an alkaloid that has a toxic effect on a broad range of organisms at variable concentrations. Caffeine reduces N availability to plants and inhibits germination and plant growth [64]. Addition of SCG to soil has been found to reduce the growth of several crop species [53,64]. In order to avoid these drawbacks, SCG must be composted before their use in agriculture. The data from this study suggest that composting of SCG was insufficient to eliminate caffeine toxicity. In addition, even though plant growth was increased, phytotoxic effects of SGC seemed to be more dominant than the changes in N availability, which exacerbated the susceptibility of plants to diseases, as compared with non-amended soil. Thus, is not recommended that the coffee-based compost studied here be applied as a soil amendment in horticultural production systems.

4. Conclusions

In this study, the disease suppressive capacity of four compost materials were evaluated in vitro against several phytopathogenic fungi using the broth microdilution assay, and in potted lettuces grown in a greenhouse. It was found that the chestnut- and olive-based composts were effective in controlling gray mold and Fusarium wilt diseases caused by *B. cinerea* and *F. oxysporum*, respectively, resulting in improved lettuce growth. With respect to Fusarium wilt, however, the relationship between the compost and disease suppression in lettuce could not be ascertained, since the virulence of the lamb's lettuce isolate used (*F. oxysporum* Schlechtendahl) was lower than the one expected from *F. oxysporum* f.sp *lactucae*. The results would possibly have been different if a lettuce isolate has been tested. Nevertheless, this study yielded an important information about the ability of *F. oxysporum* to colonize and incite symptoms on crops on which it does not usually cause disease. In most cases, the proportion of composts incorporated into the soil (5% or 10% weight basis) did not affect the results, denoting the possibility of saving production costs by using low amounts of the composts. Application of a grape-based compost to soil increased yield but also the percentage of necrotic plants. The application of compost made from coffee grounds did not have a beneficial effect on plant growth and health. Further studies are required to (i) define the best application mode for the composts, (ii) identify appropriate doses for better disease control and optimum lettuce yield, and (iii) analyze the long-term impacts of the tested composts on soil properties. The knowledge gained from these studies will facilitate sustainable disease management in agriculture by harnessing the potential of agricultural wastes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9122231/s1, Table S1: Mineral composition of the composts, Table S2: Phenolic composition of the composts, Table S3: Physicochemical characteristics of the composts, Table S4: Lettuce head yield.

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

Funding: This work was supported by European Investment Funds (FEDER/COMPETE/POCI-Operational Competitiveness and Internationalization Program) under the project POCI-0145-FEDER-006958 and by national funds provided by FCT—Portuguese Foundation for Science and Technology, under the projects PTDC/AGR-AAM/102006/2008, SFRH/BD/81473/2011 and UIDB/04033/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available within the article and in supplementary materials.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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