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A Lignin-Rich Extract of Giant Reed (*Arundo donax* L.) as a Possible Tool to Manage Soilborne Pathogens in Horticulture: A Preliminary Study on a Model Pathosystem

Stefania Galletti [1], Stefano Cianchetta [1], Hillary Righini [2,*] and Roberta Roberti [2]

[1] Research Centre for Agriculture and Environment, Council for Agricultural Research and Economics, 40128 Bologna, Italy; stefania.galletti@crea.gov.it (S.G.); stefano.cianchetta@crea.gov.it (S.C.)

[2] Department of Agriculture and Food Sciences, Alma Mater Studiorum, University of Bologna, 40127 Bologna, Italy; roberta.roberti@unibo.it

[*] Correspondence: hillary.righini2@unibo.it; Tel.: +39-051-2096582

Abstract: Finding new sustainable tools for crop protection in horticulture has become mandatory. Giant reed (*Arundo donax* L.) is a tall, perennial, widely diffuse lignocellulosic grass, mainly proposed for bioenergy production due to the fact of its high biomass yield and low agronomic requirements. Some studies have already highlighted antimicrobial and antifungal properties of giant reed-derived compounds. This study aimed at investigating the potential of a lignin-rich giant reed extract for crop protection. The extract, obtained by dry biomass treatment with potassium hydroxide at 120 °C, followed by neutralization, was chemically characterized. A preliminary in vitro screening among several pathogenic strains of fungi and oomycetes showed a high sensitivity by most of the soilborne pathogens to the extract; thus, an experiment was performed with the model pathosystem, *Pythium ultimum*–zucchini in a growth substrate composed of peat or sand. The adsorption by peat and sand of most of the lignin-derived compounds contained in the extract was also observed. The extract proved to be effective in restoring the number of healthy zucchini plantlets in the substrate infected with *P. ultimum* compared to the untreated control. This study highlights the potential of the lignin-rich giant reed extract to sustain crop health in horticulture.

Keywords: giant reed; potassium hydroxide; lignin; *Pythium ultimum*; zucchini; *Cucurbita pepo*; extract; soilborne; polyphenol; crop protection

1. Introduction

Soilborne plant pathogens are disease-causing agents belonging to several species of fungi, oomycetes, bacteria, and nematodes that live in the soil as resting structures on plant residues or also in the absence of the host for brief or extended periods. Once established, soilborne pathogens accumulate in the soil leading to high yield losses, proving difficult to control [1]. The management options of soilborne pathogens are limited [2]. Highly effective synthetic pesticides for soil treatment, such as the fumigant methyl bromide, are no longer allowed, both in European countries and elsewhere, due to their negative impact on the environment. In Europe, there are also restrictions under Reg. (EC) No. 1107/2009 for the use of other soil fumigants: methyl isothiocyanate is not approved, while the status of chloropicrin and 1,3-dichloropropene is currently pending. Moreover, the fungicides for soil treatment, carbendazim and thiophanate methyl, are not approved because of their toxic effect on human and animal health. Currently, soilborne disease management is directed towards sustainable strategies including microbial products based on antagonistic microorganisms, crop rotation, soil solarization, or breeding for resistant plant varieties. However, none of these methods alone can effectively contain soilborne diseases. Among other control means, plant extract and their essential oils have shown

some efficacy against a number of plant pathogens. Indeed, plants contain several bioactive compounds, such as phenols, flavonoid, quinones, and terpenes, have a defensive function against biotic stresses [3]. In vitro studies showed that neem, garlic, and turmeric extracts reduced the colony growth of *Fusarium oxysporum* and *Rhizoctonia solani* [4], while extracts of plants belonging to the *Asteraceae* and *Rubiaceae* families reduced *Fusarium solani* colony growth [5]. On muskmelon, the Fusarium wilt disease was suppressed by soil treatment with extracts from pepper/mustard, cassia, and clove [6], while on cucumber plants, leaf treatment with essential tea tree oil derived from *Melaleuca alternifolia* controlled *Sphaerotheca fuliginea,* the agent of powdery mildew [7]. A combination of both foliar spray and root drenching with a marine plant extract formulation from *Ascophyllum nodosum* effectively reduced the cucumber fungal pathogens *Alternaria cucumerinum*, *Didymella applanata*, *F. oxysporum*, and *Botrytis cinerea* [8]. Among sustainable alternative strategies for the control of soilborne pathogens, Armorex and Fungastop are examples of natural-based products that are commercially available in the USA [4].

Giant reed (*Arundo donax* L.) is a tall, rhizomatous, spontaneous perennial grass that is probably native to the Mediterranean environment but also widely diffused in many subtropical and temperate zones, with an invasive behavior [9]. Its use dates to 5000 BC by Egyptians who utilized the leaves as lining for grain storage and for wrapping mummies. More recently, it was utilized to produce paper/cellulose/viscose, musical instruments, stakes for plants or fishing rods, in addition to being considered an ornamental species [10,11]. For its wide pedo-climatic adaptability and high productivity, even when cultivated with low agronomic inputs [12], over the last decades this lignocellulosic species has gained interest for bioenergy production through direct combustion of the biomass, or, more recently, as feedstock for biorefinery to produce second-generation biofuel, biogas, and other biobased products [13–15]. Notably, the rhizomes, leaves, and stems were being utilized as traditional herbal remedies against several human pathologies; therefore, that giant reed can also be considered a medicinal plant [11]. Interestingly, the scientific literature reports an antimicrobial activity of giant reed extracts: an aqueous extract was able to contrast the biofilm formation of *Staphylococcus aureus* [16], while a methanolic extract showed the maximum effect among other extracts from medicinal plants against *Escherichia coli* and *Pseudomonas aeruginosa* [17].

Few studies have investigated the possible antifungal activity of giant reed lignocellulose-derived compounds: bio-oil obtained from giant reed pyrolysis showed preservative properties when used to treat Scots pine wood against *Basidiomycetes* [18]. In another study, water-soluble compounds recovered from steam-exploded giant reed during the bioethanol production process showed activity against fungal pathogens of horticultural interest [19].

The biological activity of lignin and lignin-derived compounds has been further studied: kraft lignin obtained from the delignification process of the paper industry was able to inhibit the fungal plant pathogen, *Aspergillus niger*, as well as several microbial human pathogens [20]. In addition, alkali-extracted lignin from maize residues of the bioethanol industry showed an antimicrobial effect against Gram+ bacteria and yeasts [21]. A recent review illustrated the possibility to exploit lignin with its polyhydroxyphenol network structure to produce innovative and sustainable agrochemicals, highlighting the ability to promote seedling emergence and plant growth, improve soil quality, and enhance plant resistance against phytopathogens and abiotic stresses [22].

This study aimed at investigating the potential of an innovative extract of giant reed for possible use in horticulture in the context of sustainable plant pathogen management. The extract, obtained after alkali treatment of the dry giant reed biomass, would contain lignin and lignin-derived compounds that could exert biological activity towards microbial pathogens of horticultural interest. This study moved from the chemical characterization of the extract to the evaluation of its effectiveness when applied to a model pathosystem, namely, the soilborne pathogen *Pythium ultimum* and zucchini (*Cucurbita pepo* L.), after a preliminary in vitro screening among several fungi and oomycetes.

2. Materials and Methods

2.1. Isolates of Fungi and Oomycetes

A total of 22 fungal and oomycetes isolates were preliminary tested in vitro for their sensitivity to the giant reed extract. Table 1 reports the list of the species and the origin of the different isolates used in the experiments. They were selected among soilborne and airborne pathogens, wood decay fungi, and pathogenic or nonpathogenic *Trichoderma* species. Most of them were previously isolated from infected tissues or soil samples at the laboratories of the Council for Agricultural Research and Economics (CREA), Research Centre for Agriculture and Environment of Bologna, Italy, or the Department of Agriculture and Food Sciences (DISTAL) of the University of Bologna, and identified based on morphological characteristics or molecular analysis. Wood decay isolates were purchased from public collections or private providers.

Table 1. List of the fungal and *oomycetes* isolates assayed in the preliminary in vitro experiments.

Category	Isolate	Origin
Soilborne pathogenic oomycetes and fungi	*Pythium ultimum* 16	Sugar beet
	Pythium ultimum 22	Sugar beet
	Phytophthora cactorum	Strawberry
	Ceratobasidium sp. RH3	Strawberry
	Rhizoctonia solani DAF3001	Green bean
	Sclerotium rolfsii 1	Sugar beet
	Sclerotium rolfsii 2	Potato
	Sclerotinia sclerotiorum MA3	Alfalfa
	Phoma betae	Sugar beet
	Fusarium oxysporum L1	Tomato
	Fusarium oxysporum 11.22	Tomato
Airborne pathogenic fungi	*Botrytis cinerea* 1	Strawberry
	Botrytis cinerea 2	Green bean
	Alternaria alternata 1	Potato
	Alternaria alternata 2	Tomato
Wood decay fungi	*Phanerochaete chrysosporium* D85242T	VTT, Finland
	Trametes versicolor D83211	VTT, Finland
	Pleurotus ostreatus	Funghi Mara, Italy
Pathogenic *Trichoderma* species	*Trichoderma pleuroticola* 488	*P. ostreatus* substrate
	Trichoderma pleuroti 498	*P. ostreatus* substrate
Nonpathogenic *Trichoderma* species	*Trichoderma gamsii* IMO5	Peach rhizosphere
	Trichoderma afroharzianum B75	Sugar beet rhizosphere

All isolates were maintained on potato dextrose agar (PDA) medium in tubes at 4 °C until use. The PDA medium was prepared as follows: filtered potato broth 1 L (obtained boiling 200 g/L of potatoes in distilled water), supplemented with sucrose 10 g/L, and technical agar nr 3 (Oxoid™, Thermo Fisher, Waltham, MA, USA) 12 g/L. The medium was autoclaved at 120 °C, for 20 min.

2.2. Giant Reed Meal, Seeds, and Growth Substrate

The giant reed meal was obtained starting from winter-harvested plants collected at the CREA experimental farm located at Anzola dell'Emilia (Bologna, northern Italy) (Figure 1a,b); Table 2 shows the composition of the meal, obtained after milling and sieving (<1.5 mm) the aboveground oven-dried parts, as reported in a previous paper [15].

Seeds of zucchini "Bolognese" (*Cucurbita pepo* L., Blumen Group S.p.A., Milano, Italy) were used for the experiments.

As growth substrates, peat (Terraricca, Cifo srl, S. Giorgio di Piano, Bologna, Italy, 60% humidity, 0.5 g/cm^3 density) and sterilized river sand (1.5 g/cm^3 density) were utilized.
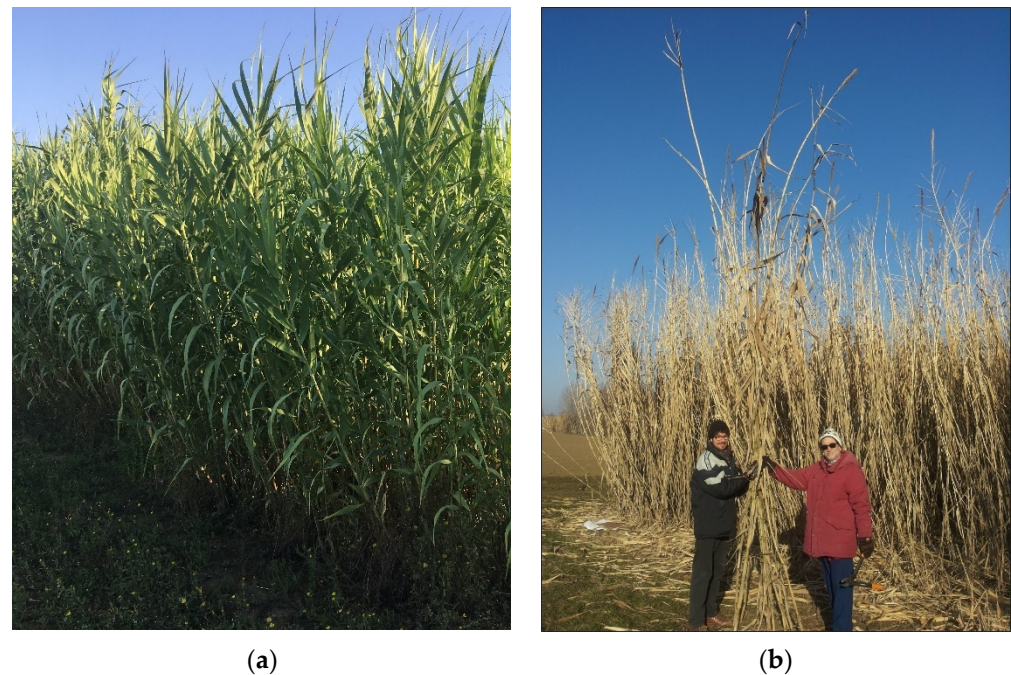
(**a**) (**b**)

Figure 1. (**a**) Giant reed plants at the growing stage; (**b**) plants at the end of the growth cycle. Pictures were taken at the experimental farms of the Council for Agricultural Research and Economics (CREA), Anzola dell'Emilia, Bologna, northern Italy (courtesy of Enrico Ceotto).

Table 2. Composition of the giant reed meal used in the experiments as reported in a previous paper [15] [1]. Standard deviations (sd) are reported in brackets (*n* = 3).

Composition Parameters	Mean Values (sd)
Total solids (%DW) [2]	95.51 (0.02)
Cellulose (%DW)	41.2 (1.5)
Hemicellulose (%DW)	22.5 (0.8)
Lignin (%DW)	10.9 (0.5)
Ash (%DW)	5.19 (0.01)
Total C (%DW)	44.6 (0.60)
Total N (%DW)	0.37 (0.05)
Total P (%DW)	0.05 (0.00)
C/N (mol/mol) [3]	142 (21)
pH in water	5.4 (0.5)

[1] Adapted with permission from Ref. [15]. 2022, Elsevier; [2] Percentage of dry weight at 60 °C; [3] C/N mol/mol can be converted into C/N *w/w* by multiplying the former by 12/14 (C/N molar mass ratio).

2.3. Experimental Design

The workflow can be summarized as follows:

(A) Preparation and chemical characterization of a lignin-rich extract from giant reed dry biomass;

(B) Preliminary tests:
- Toxicity tests of the extract towards fungi and *oomycetes* on poisoned PDA plates;
- Toxicity test of the extract towards *P. ultimum* in peat;
- Sensitivity tests of zucchini to the extract on filter paper, in peat, and in sand;
- Pathogenicity test of *P. ultimum* on zucchini in sand.

(C) Distribution analysis of the lignin-derived polyphenols in peat and sand treated with the extract;

(D) Evaluation of the extract efficacy in the *P. ultimum*–zucchini pathosystem in a growth substrate.

2.4. Giant Reed Extract Preparation and Characterization

The extract was prepared by treating a slurry of the giant reed meal at 10% weight/weight (*w/w*) in water with alkali (KOH 1.6% *w/w* in water) at 120 °C for 20 min; then, the liquid fraction was collected by filtration under vacuum and stored at 4 °C. Just before use, the extract was neutralized (pH 7) with appropriate amounts of H_2SO_4 72% (approximately 0.035 M SO_4^{2-} final) operating under sterile conditions.

To calculate the dry weights of the insoluble and soluble fractions, samples of the alkaline slurry were centrifuged (10 min, 2000 rcf, Allegra X-22 Beckman Coulter); then, the insoluble fraction (three-fold washed) and supernatants (liquid fraction) were dried at 105 °C until constant weight.

Water and total solids were determined according to ISO 12880; ashes and volatile solids according to ISO 12879; pH according to ISO 13037; electrical conductivity according to ISO 13038; total P and K according to Reg CE 2003/2003, met. 3.1.1 and met. 8.1, respectively.

Total organic C was determined according to national regulation DM 21/12/00, Suppl n. 6 [23]. C and N content were determined after elemental analysis by a LECO Truspec® CHN Analyzer (LECO Corporation; Saint Joseph, MI, USA), and the C/N ratio was calculated accordingly.

Free reducing sugars were quantified by the 3,5-dinitrosalicylic acid (DNS) method [24] adapted for 96-well microplates in duplicates [25]. A mix of glucose and xylose (1:1) was included as standards. Briefly, the assay conditions were citrate buffer 50 mM, pH 4.8, 5 min, 95 °C.

The hydrolysable holocellulose content was calculated by dividing by a correction factor of 1.12 of the amount of reducing sugars released in solution after enzymatic saccharification of the giant reed extract to account for the addition of water during hydrolysis. In detail, a sample of 10 mL of extract was saccharified in 15 mL tubes (in triplicate) using the following conditions: citrate buffer 50 mM, pH 4.8, sodium azide (0.2 mg/mL), and enzymes (SAE0020, Sigma-Aldrich, St. Louis, MO, USA) at a cellulase load of 25 FPU/g DW of the substrate. Tubes were continuously mixed in an orbital shaker (200 rpm, 2 mm radius, 50 °C, 120 h). Reducing sugars were quantified by the DNS method described above. Dilutions of the enzyme mix were included as a control.

Lignin-derived polyphenols were quantified by a spectrophotometric analysis as alkali lignin equivalents. In detail, a calibration curve was constructed using dilutions containing known amounts of alkali lignin (Lignin, alkali 370959, Sigma-Aldrich) dissolved in a strongly alkaline solution (KOH 16 g/L). Absorbance values were recorded between 280 and 420 nm, with 2 nm intervals to calculate the area under the curve (AUC). The AUC is directly proportional to the concentration (g/L); thus, the polyphenol concentration in the diluted samples (DPC) could be determined. Immediately before analysis, liquid samples were opportunely diluted with an alkaline solution (KOH 16 g/L), recording the dilution factor (DF), then briefly centrifuged, and analyzed by a spectrophotometer (Infinite 200 PRO series, Tecan) in 96-well microplates (EIA/RIA Clear Flat Bottom Polystyrene Not Treated Microplate, 3591, Corning, Corning, NY, USA) to determine the polyphenol concentration in terms of alkali lignin equivalents. The same KOH solution used to prepare the dilutions was included as a blank. The polyphenol content in undiluted samples (UPC) as g/L was calculated according to the formula:

$$UPC = DPC \times DF \tag{1}$$

Data reported are the mean of 3 experiments.

2.5. Toxicity Tests of the Extract towards Fungi and Oomycetes

A dose-response assay was performed in Petri dishes with five doses (i.e., 0, 10, 20, 40, and 80%, volume/volume, *v/v*) of the giant reed extract towards 22 fungal and oomycetes strains. After neutralization (pH 7) with H_2SO_4, the extract was slowly warmed up to

40 °C and mixed, under sterile conditions, to PDA medium, previously autoclaved and then cooled to 60 °C, to obtain the extract doses of 0, 10, 20, 40% *v/v*. To prepare the 80% dose, a SeaKem LE agarose (Cambrex, East Rutherford, NJ, USA)-based medium, supplemented with an appropriate amount of concentrated sucrose solution, was used instead of PDA to guarantee proper gelling. The final sucrose and agarose concentrations in the 80% dose were 10 and 12 g/L, respectively, as in the PDA. The amended substrates were poured into Petri plates (90 mm in diameter) and centrally inoculated with one agar-mycelium plug taken from the edge of actively growing colonies of the isolates (3 replicates). PDA and PDA supplemented with 5 g/L K_2SO_4 were included as controls, since the extract after neutralization with sulphuric acid contained an equivalent amount of sulphate.

Plates were incubated at room temperature (22 °C). For each plate, two perpendicular colony diameters were measured and averaged after 3–7 days of incubation, depending on the isolate, just before the colony reached the plate edge in the corresponding control (PDA). The growth inhibition percentage was calculated according to the formula:

$$I\ (\%) = \frac{C - T}{C} \times 100 \tag{2}$$

where I (%) is the inhibition percentage of the colony growth, C is the averaged diameter (mm) of the pathogen colony in the control, and T is the averaged diameter (mm) in the amended plate.

For selected isolates, the half-maximal inhibitory concentration (IC_{50}) was calculated after probit linearization of the growth inhibition values I (%). Data were obtained from 2 independent experiments, with 3 replicates for each dose.

The plugs of the isolates showing 100% growth inhibition were transferred onto PDA plates and further incubated for up to 15 days to verify fungistatic/fungicidal effects.

For *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, after 20 days of incubation, the number of sclerotia produced in the amended and control plates (3 replicates) as well as their sensitivity were also recorded. To test the sensitivity, the sclerotia were collected, dried onto sterilized filter paper, and rehydrated again on filter paper imbibed with 2 mL of extract at the doses of 0 (distilled water, control), 20, 40, and 80% for 24 h. The imbibed sclerotia were transferred into 96-well microplates on PDA supplemented with streptomycin 500 μg/mL (1 sclerotium per well, 28 sclerotia per each dose and control). After 48 h incubation at room temperature, the percentage of germinated sclerotia was counted by visual observations under a stereomicroscope. The test was repeated.

2.6. Toxicity Test of the Extract towards Pythium ultimum 22 in Peat

The experiment was conducted in a greenhouse at DISTAL, in plastic trays (16 × 14 × 10 cm) filled with 500 g of peat (60% humidity), at temperatures of 21–24 °C, and under artificial lightning (16/8 h light/dark). The pathogen inoculum was prepared by finely mincing 72 h old colonies grown on a PDA medium with sterile distilled water [26]. Peat in each tray was evenly inoculated with 100 mL of the pathogen suspension at 2% *w/w* (4 replicates) and covered with a plastic bag. Three days after incubation in the dark, the peat was treated with the extract by irrigation (250 mL per tray, undiluted dose), then covered with plastic bags and further incubated for 48 h in the dark. Nontreated trays (control) received the same volumes of distilled water. In order to check the fungitoxic effect of the extract against *P. ultimum*, portions of treated-inoculated and untreated-inoculated (control) peat were sampled (3 g per tray, 12 g per treatment pooled together). For each treatment, 10 g of peat were utilized to determine the dry weight at 105 °C, while the remaining 2 g were stirred in sterile distilled water. Then, 1 mL of the dilutions 1:100 and 1:1000 (*w/w*) was plated on a semi-selective medium (PARP) [27] in Petri dishes. The characteristic pathogen colonies were visually counted after 48 h of incubation at 19 °C to determine the number of colony-forming units per gram of peat on a dry weight basis (CFU/g DW) (3 replicates).

2.7. Sensitivity Test of Zucchini to the Extract

Considering that the effectiveness of the extract would be evaluated in the pathosystem *P. ultimum*–zucchini, it was first examined if the extract could have any effects on zucchini seed germination and seedling growth. The sensitivity of zucchini to the extract was tested on filter paper, peat, and sand.

Test on filter paper. The test was conducted on Petri plates (90 mm in diameter). Ten seeds per plate were positioned between two disks of sterile filter paper, each imbibed with 5 mL of distilled water (dose 0%, control) or the extract at the doses of 20, 40, 60, and 80% *v/v*, 4 replicates. Plates were incubated at 25 °C for 7 days in the dark/light (8/16 h), when the number of normally germinated seeds according to standard procedures [28] and the rootlet length were recorded. The normal seedlings were oven-dried (105 °C, 48 h) up to constant weight to determine the dry weight.

Test in peat. This test was conducted in aluminum trays (10.5 × 8 × 4.5 cm) with holes in the bottom, filled with 50 g of peat (100 mL final volume), soaked with 25 mL of undiluted extract (prepared as reported in Section 2.4) or water (control), and covered with plastic film, 3 replicates. The 25 mL volume represented the highest dose that could be absorbed by 50 g of peat. After 48 h incubation at room temperature (22 °C), the trays were watered once with 25 mL of distilled water with the aim of partially removing the extract to reduce its potential phytotoxicity. Then, ten seeds per tray were sown, the trays were covered with transparent plastic film and incubated at 25 °C for 11 days in the dark/light (8/16 h). Finally, the percentage of normal seedlings according to standard procedures [29] was recorded, and the root apparatus development was visually compared to the untreated control.

Test in sand. This assay was performed in trays as above, filled with 300 g of sterile dry river sand moistened with 50 mL of water (200 mL final volume). The trays were treated with 40 mL of undiluted extract (prepared as reported in Section 2.4) or water (control), 3 replicates, and covered with plastic film. The 40 mL volume represented the highest dose that could be absorbed by 300 g of sand. After 48 h incubation at room temperature (22 °C), the trays were watered 4 times with 25 mL of water, aimed at partially removing the extract as specified above for peat. Ten seeds were sown in each tray, incubated at 20 °C for 14 days in the dark/light (8/16 h), and covered with transparent plastic film. Finally, the percentage of normal seedlings was recorded according to standard procedures [29], and the root apparatus development was visually compared to the untreated control.

For both the tests in peat and in sand, the substrate and the liquid percolated from the trays after watering were sampled for lignin-derived polyphenol analysis as described below (Section 2.9).

2.8. Pathogenicity Test of P. ultimum 22 towards Zucchini

The isolate *P. ultimum* 22 was checked for pathogenicity towards zucchini "Bolognese". The inoculum was prepared as described above (Section 2.6). Aluminum trays (10 × 8 × 4.5 cm) filled with autoclaved river sand (300 g) were inoculated with the pathogen (0.7% *w/w*) suspended in 50 mL of water, 3 replicates. Non-inoculated trays were included as the control. Each tray was sown with ten seeds and incubated at 20 °C in a growth chamber (16/8 h light/dark) for 11 days. After incubation, damping-off symptoms were checked, and the percentage of non-symptomatic plantlets was recorded.

2.9. Determination of the Lignin-Derived Polyphenols in Treated Peat and Sand

To determine the fate of water-soluble polyphenols in peat and sand treated with the giant reed extract (Section 2.7) a spectrophotometric analysis was performed (Section 2.4), quantifying the input amount (i.e., endogenously present in the substrate (END) plus exogenously added with the extract (EXO)) and its distribution after watering (i.e., percolated

out of the tray (PER), free in solution (FREE), and adsorbed to the substrate (ADS)). This latter was calculated by difference according to Equation (2):

$$ADS = END + EXO - PER - FREE. \tag{3}$$

Calculations were performed on a fresh weight (FW) basis, accounting for each fraction's total weight per tray. Then, the data obtained were converted and presented per liter of substrate (accounting for density). This choice allowed for a better comparison between peat and sand substrates characterized by very different density values.

Sampling and analysis. A total of 5 g FW of each substrate (peat or sand) treated with the extract or untreated (Section 2.7) was randomly collected soon after tray watering, then diluted 1:5 in water and centrifuged. Supernatants were filtered through 1 μm syringe filter and stored at 5 °C before analysis. The extract and the liquid percolated from treated and untreated trays after tray watering were carefully collected and weighted. Then, convenient samples were centrifuged, filtered, and stored at 5 °C before analysis. Samples were opportunely diluted with an alkaline solution (KOH 16 g/L), recording the dilution factor (DF), briefly centrifuged, and analyzed for the polyphenol content (DPC) as described in Section 2.4. The lignin-derived polyphenol content (grams of alkali lignin equivalents/grams of sample) in undiluted sampled materials (UPCMs) was thus calculated according to the Formula (4):

$$UPCM = \text{Undiluted sample specific volume} \times DPC \times DF, \tag{4}$$

where the undiluted sample specific volume corresponds to the reciprocal of its density in g/L (previously measured).

2.10. Assessment of the Extract Efficacy in the P. ultimum 22–Zucchini Pathosystem

The experiment was performed in a growth chamber at CREA using river sand as a model growth substrate, because in this substrate, the partial removal of the extract resulted in more efficiency. The sand was distributed in aluminum trays, inoculated with the pathogen (0.7% w/w), and then treated with the extract utilizing the protocols described above (Section 2.6 for inoculum preparation and Section 2.7 for the extract treatment). Four treatments were compared according to a completely randomized block design with 4 replicates (trays): (1) inoculated with the pathogen and treated with the extract; (2) non-inoculated and non-treated control; (3) non-inoculated and treated control; (4) inoculated and nontreated control. Three days after the treatment, the trays were watered with the aim of partially removing the extract and then sown with ten seeds each. All trays received the same amount of liquids.

Eleven days after sowing, samples of 40 g of sand per thesis were collected (10 g per replicate, then pooled) to determine the extract's fungitoxic effect on the pathogen CFU. For each thesis, the dry weight at 105 °C was determined on 38 g of sand, while the remaining 2 g were suspended in distilled water and utilized for serial dilutions on PARP medium in Petri dishes (3 replicates) to determine the number of CFU/g of the pathogen, as already described (Section 2.6).

Thirteen days after sowing, the number of healthy plantlets was recorded, the root apparatus was checked, and the total dry weight was determined after oven-drying at 105 °C. The experiment was repeated.

2.11. Statistical Analysis

Depending on the experimental design, data were submitted to one-way or two-way analysis of variance, after arcsine transformation of percentage data. Means were separated by Tukey's test at $p \leq 0.05$ significance level. All analyses were performed with PAST 4.09 program.

3. Results

3.1. Extract Characterization

The results of the neutralized extract of giant reed were rich in lignin-derived polyphenols and potassium but low in nitrogen. It was also characterized by a C/N ratio of 63, the presence of phosphorus, and small amounts of holocellulose and free reducing sugars. (Table 3).

Table 3. Characterization of the giant reed extract obtained by treating a slurry of dry meal (10% weight/weight in water) with alkali (KOH 1.6% weight/weight in water) at 120 °C for 20 min after filtering and neutralization with H_2SO_4. The standard deviation (sd) of the mean is reported in brackets (*n* = 3 experiments).

Composition Parameters [2]	Mean Values (sd)
Water (%FM [1])	94.7 (0.2)
Total solids (TS [2]) (%FM)	5.3 (0.2)
Volatile solids (%FM)	2.6 (0.1)
Ashes (%FM)	2.7 (0.1)
pH in water	6.5–7.2
Electrical conductivity (S/m)	0.30 (0.01)
Total P (%FM)	0.009 (0.002)
Total K (%FM)	0.84 (0.02)
Total organic C (%FM)	2.0 (0.1)
C (% TS)	36 (0.5)
N (% TS)	0.57 (0.1)
C:N ratio (weight/weight)	63 (10)
Hydrolysable holocellulose (%FM)	0.42 (0.02)
Free reducing sugars (%FM)	0.14 (0.04)
Lignin-derived polyphenols (%FM) [3]	1.7 (0.15)

[1] FM: fresh matter; [2] TS: total solids; [3] obtained by dividing the measured concentration (g/L) of alkali lignin equivalents by 10.

Moreover, the presence of sulphate equivalent to 5 g/L K_2SO_4 was estimated based on the amount of sulphuric acid used for the extract neutralization.

The electrical conductivity value (0.3 S/m) was largely determined by K and sulphate ions.

3.2. Toxicity of the Extract towards Fungi and Oomycetes

The fungal and oomycetes isolates tested in vitro showed a differential response towards the doses of the giant reed extract, variable from sensitivity to stimulation, depending on the species and sometimes also on the isolate (Figure 2a). No significant differences in colony growth were observed between PDA and PDA amended with K_2SO_4 (Figure 2a); thus, all the data are expressed as the percentage of the control on PDA. Figure 2b shows a test of growth-recovery for different isolates performed transferring on unamended medium mycelium plugs that appeared completely inhibited on substrate amended with the giant reed extract.

Among the soilborne pathogens (Figure 3a,c), *Phoma betae* and *Fusarium oxysporum* L1 and 11.22 grew well, even in the presence of the highest extract dose, and the former was also highly stimulated compared to the control, up to a 40% dose (Figure 3c). Apart from these three isolates, the growth of the other soilborne isolates was gradually inhibited, partially or totally, with the increasing extract doses (Figure 3a). At an 80% dose, *Rhizoctonia solani*, *Ceratobasidium* sp., and *S. sclerotiorum* still showed growth values of 29, 29, and 13% of the control, respectively (Figure 3a,c). *Phytophthora cactorum* was the unique isolate showing a complete growth reduction from a 20% dose. Both *P. ultimum* isolates were highly inhibited at a 40% dose and did not grow at all at 80%, with the isolate 22 appearing more sensitive than 16 at the intermediate doses (Figure 3a). A strong effect was observed with *S. rolfsii* since the colony growth of both isolates was highly inhibited from the 20% and stopped from the 40% dose (Figure 3a). Two weeks later, sclerotia production by these two isolates was also significantly reduced at a 40% dose; differently, *S. sclerotiorum* continued

to produce sclerotia up to the maximum tested dose, even with a significant reduction with respect to the other doses (Figure 3b). However, all the sclerotia produced by *S. rolfsii* 1 and 2 and *S. sclerotiorum* grown on extract-amended PDA were able to germinate when transferred on the unamended substrate.
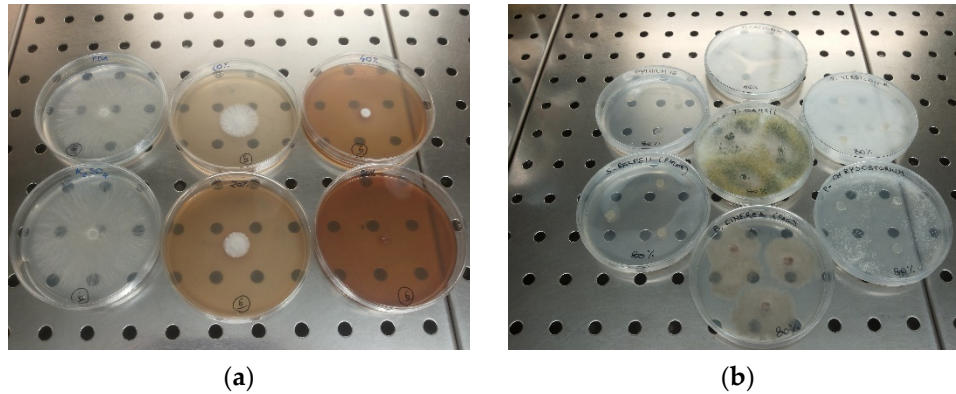

(a)   (b)

Figure 2. (**a**) Effect of the increasing doses of the giant reed extract on the colony growth of a representative isolate (*Sclerotium rolfsii*); (**b**) test of the growth-recovery of different isolates on unamended medium after the occurrence of total inhibition on substrate amended with the giant reed extract.

Among the airborne pathogens, the two isolates of *Botrytis cinerea* behaved differently: the growth of the isolate 1 was gradually inhibited, while the isolate 2 was highly stimulated at the 20 and 40% doses, compared to control; nevertheless, both were almost completely inhibited at the highest dose (Figure 3d). Only one of the two isolates of *Alternaria alternata* (2) showed sensitivity to the extract, with a gradual growth inhibition up to 58% at the 80% dose. On the contrary, isolate 1 of *A. alternata* did not show any inhibition, even at the maximum dose, compared to the control, and it was also highly stimulated at the intermediate doses (Figure 3d).

The two nonpathogenic *Trichoderma* species (i.e., *T. gamsii* and *T. afroharzianum*) were gradually inhibited up to a complete stopping at the 80% dose (Figure 3e). Among the two pathogenic *Trichoderma* species, *T. pleuroti* behaved similarly to the nonpathogenic ones, while *T. pleuroticola* showed a growth reduction at the 40% dose, but it was never completely inhibited even at the maximum dose, still showing a growth of 31% compared to control (Figure 3e).

The white-rot fungi *Trametes versicolor* and *Phanerochaete chrysosporium* showed high sensitivity to the extract since their growth was dramatically reduced already at the 20% dose and stopped at 40% (Figure 3f). By contrast, the other wood decay agent, *P. ostreatus*, showed a moderate inhibition at this dose (33%), but it was completely inhibited at the dose of 80% (Figure 3f).

To further determine the extract's effect, the mycelium plugs that did not show any growth were collected and transferred on unamended PDA as shown in Figure 2b. Interestingly, almost all of the mycelium plugs were able to regrow, except those of *S. rolfsii* 2, *P. ultimum* 16, and *P. ultimum* 22, meaning that the inhibitory effect was irreversible for these isolates. Thus, *P. ultimum* 22 was selected for further experiments, also due to the higher sensitivity to the intermediate doses of the extract compared to isolate 16 (Figure 3a). This was also confirmed by the calculated IC_{50} values, which corresponded to a dose of 17.2% ± 0.1 and 23.5% ± 1.3% *v/v*, respectively.

The sensitivity of *P. ultimum* 22 to the extract was also confirmed in peat: 48 h after the treatment, the number of CFU/g peat DW was significantly reduced by 62% in infected + treated trays, while it did not vary significantly in the untreated trays (only infected) (Table 4). This is in accordance with the statistically significant interaction time × treatment found after two-way ANOVA.
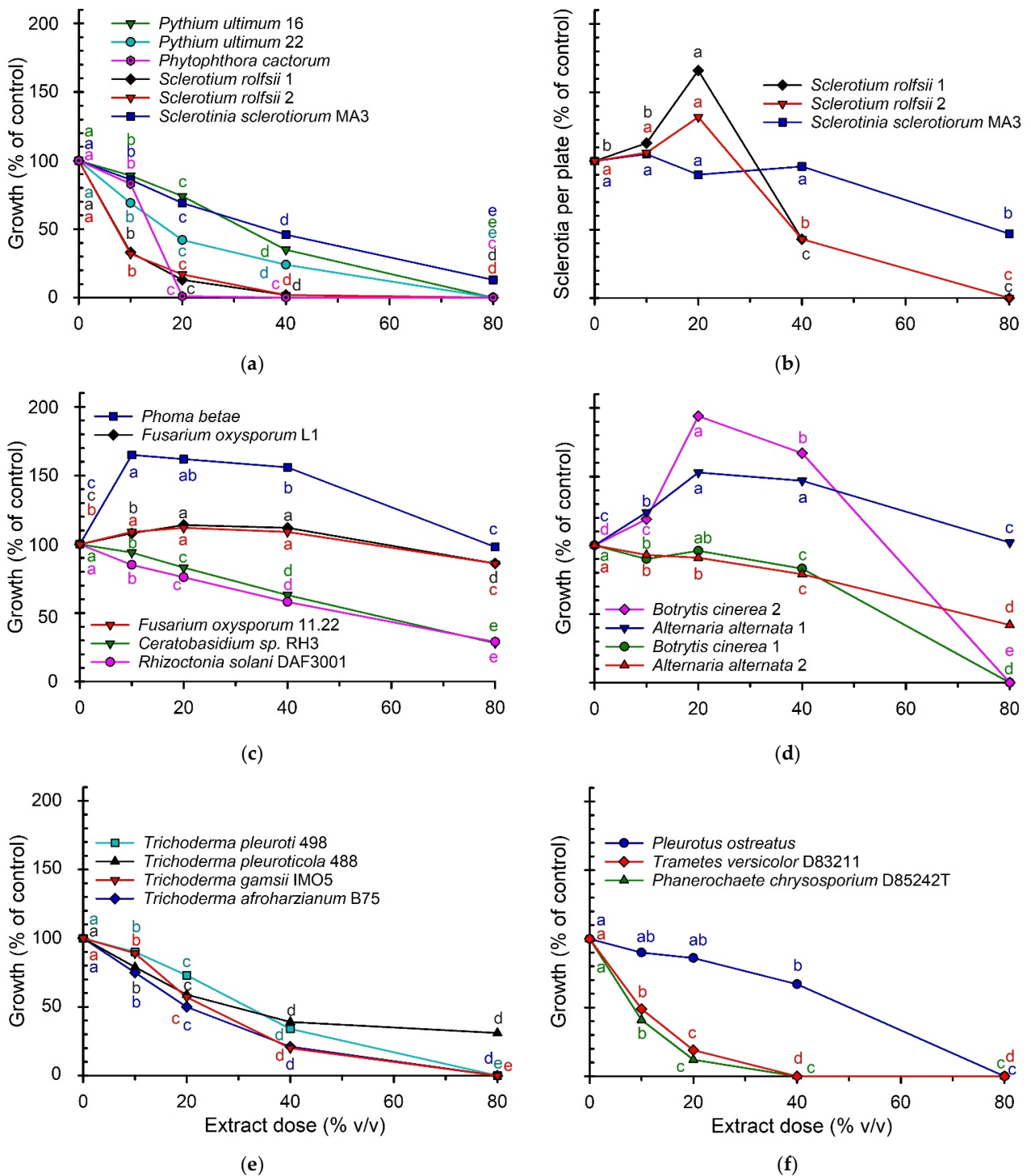
Figure 3. Screening of a collection of fungi and oomycetes for sensitivity to the giant reed extract in PDA plates amended with increasing doses of the extract (% volume/volume, *v/v*). Colony growth of (**a**,**c**) soilborne pathogens; (**d**) airborne fungi; (**e**) pathogenic and nonpathogenic *Trichoderma* spp.; (**f**) wood decay fungi. Panel (**b**) shows the sclerotia production by *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. Both the parameters (i.e., colony growth and number of sclerotia per plate) are expressed as a percentage of the control (0% dose). For each isolate, mean values sharing the same letter were not different at $p < 0.05$ according to Tukey's test after one-way ANOVA (*n* = 3). See also Tables S1 and S2 in the Supplementary Materials reporting mean and standard deviation values.

Table 4. Number of colony-forming units (CFUs) of *Pythium ultimum* 22 per gram of peat, on a dry weight basis, retrieved from the trays infected and treated with the undiluted giant reed extract, before and after the treatment (at 0 and 48 h) in comparison with the infected and nontreated trays.

	Treatment (CFU $\times 10^5$/g Peat Dry Weight) [1]	
Time from Treatment (h)	**Infected**	**Infected + Treated**
0	0.91 ab [2]	1.22 a
48	1.09 a	0.47 b

[1] Two-way ANOVA results: time ($p < 0.05$); treatment (not significant); time $\times$ treatment ($p < 0.05$); [2] Means in rows or columns followed by the same letter did not differ significantly according to Tukey's test ($p < 0.05$).

3.3. Sensitivity Test of Zucchini to the Giant Reed Extract

Test on filter paper. The extract negatively affected the germination of zucchini seeds on filter paper (Figure 4). The percentage of normal seedlings gradually decreased from 90% in the control down to 42% at the 40% dose. Complete inhibition was observed at the 60% dose. The root length and the dry weight were significantly reduced, as well, with the increasing doses (Figure 4).
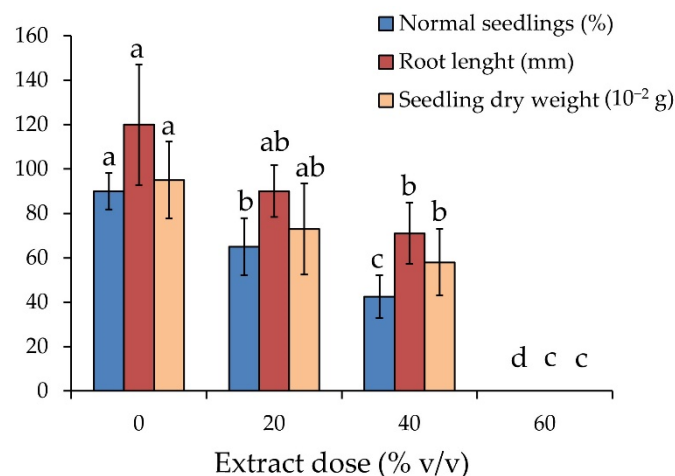

Figure 4. Effects of increasing doses (% volume/volume, *v/v*) of the giant reed extract on the germination of zucchini seeds, on filter paper, measured as a percentage of normal seedling, rootlet length, and total dry weight. Means labelled with the same letter within the same parameter did not differ for $p \leq 0.05$ (Tukey's test after one-way ANOVA). Bars represent standard deviation values.

Test in peat and sand. The use of the undiluted extract in peat and sand, followed by watering, did not reduce the emergence percentage of zucchini compared to untreated control, even if a slight but not statistically significant ($p \geq 0.05$) decrease was observed from 87 to 70% and from 97 to 83% in peat and in sand, respectively (Table S3). Thus, the watering step in peat and sand before sowing was effective in minimizing the inhibitory effect of the extract on the germinating seeds previously observed on filter paper.

3.4. Lignin-Derived Polyphenol Distribution in Peat and Sand

Figure 5 shows the distribution of lignin-derived polyphenols in peat compared to sand, both treated with the giant reed extract in tray experiments.

The analysis showed the presence of endogenous water-soluble polyphenols in the peat (shaded orange bar), while endogenous polyphenols were not detected in sand, as expected. The extract provided 3.3–3.6 g polyphenols per L substrate (exogenous). Watering did not remove completely the extract, but unexpectedly, both in the case of peat and sand, most of the polyphenol amount remained adsorbed to the substrate and only a relatively small amount remained free in solution. However, watering determined a relevant removal of polyphenols in sand only (percolated), corresponding to 33% of the added extract (exogenous) (Figure 5).
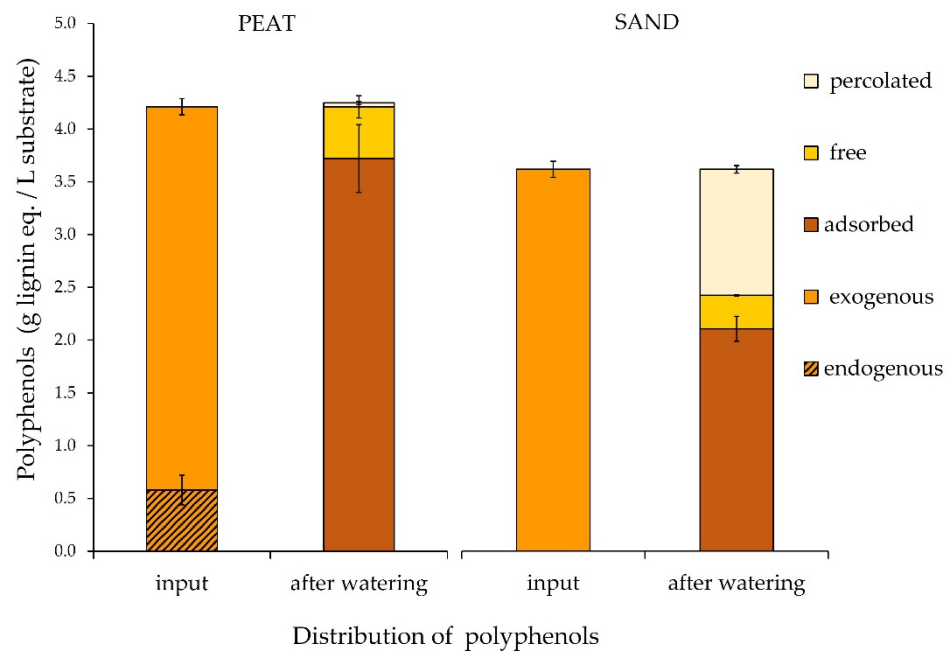
Figure 5. Distribution of lignin-derived polyphenols in peat and sand treated with undiluted giant reed extract in tray experiments. Columns represent the quantity of polyphenols in input (endogenously present in the substrate or exogenously added with the extract) and after watering (adsorbed into the substrate, free in solution, or percolated out of the tray). Bars represent standard deviation values (*n* = 3).

3.5. Extract Efficacy in the P. ultimum 22–Zucchini Patho-System

In a preliminary pathogenicity test, *P. ultimum* 22 was particularly aggressive towards zucchini, being able to attack the germinating seeds and reduce the emergence by 63.4% compared to the control. In addition, the development of the root apparatus in the inoculated trays appeared markedly reduced (Figure 6).

Figure 6. Pathogenicity test of *Pythium ultimum* 22 towards zucchini plantlets. Effect of development reduction of the root apparatus in plantlets from the inoculated trays (right) compared to the non-inoculated control (left).

In the experiment with the pathosystem, the treatment with the extract did not reduce the percentage of healthy plantlets compared to the control in the non-inoculated trays (Figure 7a). The pathogen infection instead halved the percentage of healthy plantlets (from 85 to 39%), while the treatment with the extract restored the percentage of healthy plants up to a value of 73%, which was not statistically different from the noninfected controls (Figure 7a). Moreover, the treatment with the extract significantly reduced the pathogen CFU/g by 90% compared to the control, measured 14 days after the treatment (Figure 7b).

(**a**)

(**b**)
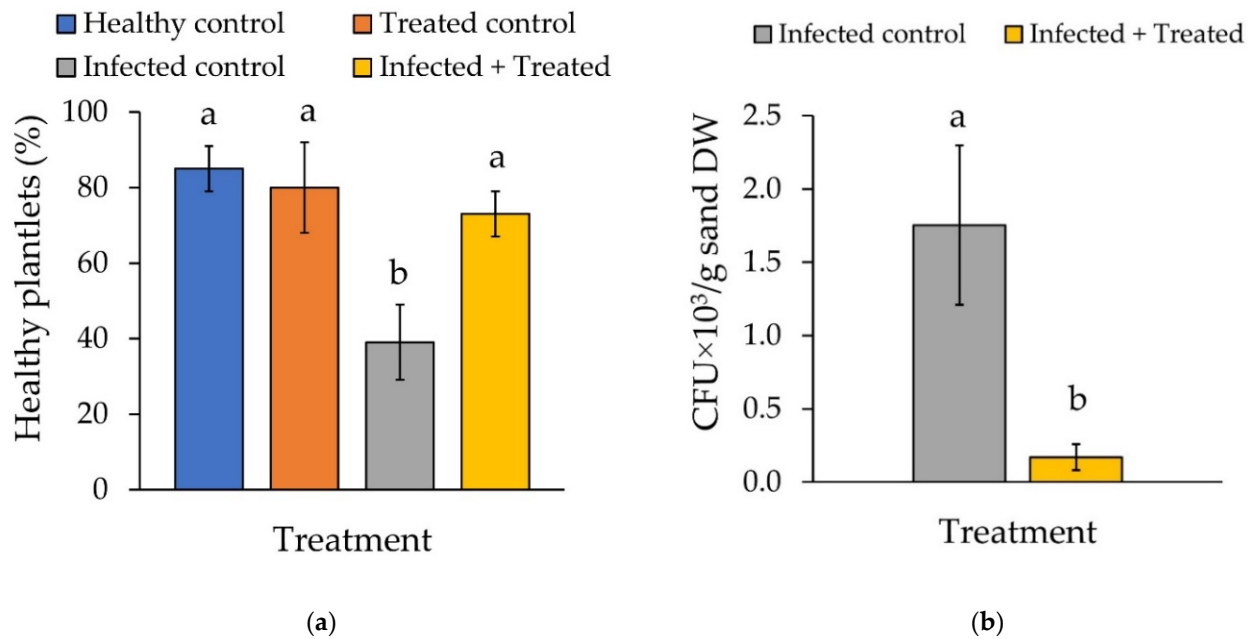
Figure 7. Effects of the treatment with the undiluted giant reed extract on the *Pythium ultimum* 22–zucchini pathosystem in sand: (**a**) percentage of healthy plantlets of zucchini in the infected and treated trays (yellow column) compared to the different controls (i.e., healthy, infected, or treated) 16 days after the treatment; (**b**) number of colony-forming units (CFUs) of *P. ultimum* 22 per g of sand dry weight (DW) in the infected and treated trays (yellow column) in comparison with the infected control, 14 days after the treatment. Bars represent the standard error of the mean. Columns labelled with the same letter did not differ for $p \leq 0.05$ (Tukey's test after one-way ANOVA).

Finally, the root apparatus of plants collected from the treated trays showed a development similar to that of the control, while it appeared reduced in the infected trays (Figure 8). Notably, the infected and treated plantlets showed a well-developed root apparatus if compared to that of the controls (Figure 8).
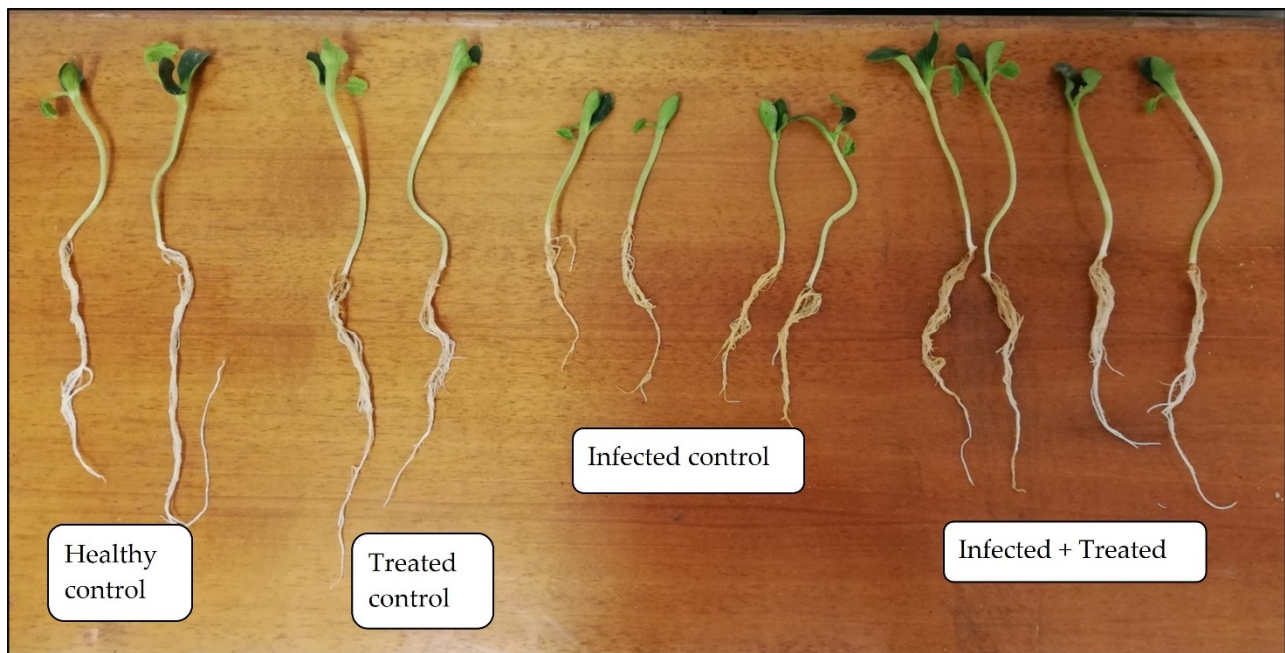
Figure 8. Effects of the treatment with the undiluted giant reed extract on the *Pythium ultimum* 22–zucchini pathosystem in sand. Plantlet development in the infected and treated trays (on the right) compared to the different controls (i.e., healthy, treated, or infected) 16 days after the treatment.

4. Discussion

4.1. Extract Characterization

The giant reed extract obtained by treating the giant reed dry biomass with potassium hydroxide can be considered a liquid organic amendment with possible ameliorative effects on the physicochemical characteristics of the soil.

Due to the high content of soluble lignin-derived compounds, the extract's application to the soil could improve soil physical characteristics while increasing C sequestration. Application of lignin provided a film-forming liquid mulch with the effect of improving soil aggregates, warming, preserving soil moisture, and protecting seedlings. [22]. In addition, soil pH, organic matter, cation exchange capacity, and available P and K can be increased by lignin amendment. Lignin application to arable soils also reduced the accumulation of heavy metals Cu, Zn, Cd, Pb, Cr, and Ni in wheat plants [30].

As a soil amendment, the use of an extract with low N content like this would not cause N leaching in areas where mandatory limits exist [31]; such a low N level was due to the plant harvesting at the end of the growth cycle when most of the nitrogen contained in the aboveground biomass had been translocated to the sink (rhizomes) for regrowth in the following season [32]. If needed, the N content of the extract can be easily increased by harvesting younger giant reed plants, which contain a high N level [12]. Thus, it is possible to modulate the fertilizing properties of the extract by utilizing plants harvested at different growth stages.

The electrical conductivity value of the extract (0.3 S/m) was far below the values of other organic fluids used as a soil amendment, for instance, the olive mill wastewaters (1 S/m) [33]. This confirms the possible use of this extract as a soil amendment.

The use of potassium hydroxide for the alkaline extraction, instead of another alkali (i.e., NaOH), enriched the extract with potassium, providing the extract with additional potential fertilizing properties. The use of KOH to pretreat the lignocellulosic biomass before anaerobic digestion for biogas production has been already proposed in the literature aiming to obtain a coproduct (digestate) with a high fertilization value [13,34].

Since nutrients can also affect disease resistance, K and P provided with the extract may also exert additional beneficial activities; K may promote the development of thicker

outer walls in epidermal cells, thus preventing disease attack, while P may promote a vigorous root development permitting seedlings to escape the disease [35].

4.2. Toxicity of the Extract towards Fungi and Oomycetes

The giant reed extract contains biologically active compounds as shown by the broad fungitoxic activity observed in the screening of different species of fungi and oomycetes of agricultural interest. This biological activity could be related to the presence of lignin or soluble lignin-derived phenolic compounds, generated by the biomass treatment with potassium hydroxide. Indeed, the alkali used to obtain the extract removes the cross-links among xylan, lignin, and other hemicelluloses by solvation and saponification of the ester bonds and also further degrades lignin into different phenolic compounds [36,37].

The antimicrobial activity of lignin obtained from alkali-treated lignocellulosic feedstocks has already been reported in the literature: kraft lignin obtained from the paper industry by alkali treatment of spruce and eucalyptus showed strong activity against a range of human microbial pathogens and against the fungal plant pathogen *Aspergillus niger* [20]. Antimicrobial effects on Gram+ bacteria and yeasts have also been reported for alkali-extracted lignin from lignocellulosic maize biomass [21]. The antifungal activity of lignin-related phenolic compounds was investigated by a large in vitro screening that highlighted a higher toxicity towards true fungi (excluding *Zygomycetes*) than mitosporic ones [38].

In addition to lignin and derived compounds, the giant reed extract utilized in this work may also contain other bioactive molecules with antifungal properties. Indeed, high-temperature treatment of giant reed can generate a mixture of water-soluble compounds (such as furfural, 5-hydroxymethylfurfural, acetic, and formic acid) that have shown inhibitory activity towards pathogenic fungi of agricultural interest such as *A. alternata*, *B. cinerea*, *Colletotrichum acutatum*, *Cladosporium fulvum*, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *melonis*, *F. solani* f. sp. *pisi*, and *V. dahliae* [19].

Surprisingly, the wood pathogens tested in our study (i.e., *P. chrysosporium*, *T. versicolor*, and *P. ostreatus*) resulted among the most sensitive isolates tested, while a good tolerance to lignin-derived compounds was expected since these strains can utilize lignin as a carbon source [39]. This finding suggests the possible presence of different kinds of inhibitory compounds in the giant reed extract.

Soilborne pathogens also appeared highly sensitive to the extract in the in vitro experiments; thus, we decided to verify, firstly, the giant reed extract's toxicity against *P. ultimum* 22 inoculated in a peat substrate and then its efficacy in the pathosystem model, *P. ultimum*–zucchini, in view of possible use in horticulture.

The two nonpathogenic *Trichoderma* isolates, representing the beneficial soil microflora, showed tolerance at low-extract doses. Notably, the ability of *Trichoderma* to tolerate and/or recover after soil treatment with fungitoxic compounds is well supported [40,41]. Therefore, it is possible that a soil treatment with the giant reed extract should not have a detrimental effect on soil *Trichoderma* species, in principle, but dedicated experiments in bulk soil should be performed to verify this effect.

4.3. Sensitivity of Zucchini to the Giant Reed Extract

The high sensitivity shown by zucchini in the filter paper test suggested the presence of anti-germinative compounds in the extract, as several potential allelochemicals have been characterized in giant reed including indoles, ketones, esters, and alcohols [42]. An anti-germinative effect of an aqueous extract of giant reed leaves reported for lentil growing on filter paper was attributed to a delay in the germination process and a reduction in the seedling vigor index [43]. The same extract applied to peat caused growth inhibition on lentils with a higher sensitivity of the rootlets compared to the shoots. These authors speculated that the phytotoxic effect could be attributed to a direct and more intensive contact between roots and allelochemicals and to a higher permeability of roots compared to shoot tissues [43]. Consequently, in our study on zucchini, the treatment of peat and

sand with the extract was followed by a watering step to remove possible allelochemicals before sowing, and this actually allowed to obtain good emergence values. Lignin or lignin-derived compounds in the extract may cause anti-germinative effects or even turn to be biostimulants. Accordingly, ammonium lignosulfonate, derived from the paper industry, exerted an anti-germinative effect on beans, while a biostimulant effect was observed at low doses [44]. Moreover, in maize, very low doses (10 ppm) of water-soluble lignin isolated from miscanthus and giant reed biomass stimulated emergence and early growth [45]. A positive effect on tomato emergence and growth after field application of alkali-extracted lignin from flax was also reported [46].

4.4. Lignin-Derived Polyphenol Distribution in Peat and Sand

As already reported, among the possible allelochemicals contained in the giant reed extract, there are lignin-derived polyphenols generated from the lignocellulosic biomass treatment with alkali [37]. For this reason, we studied the distribution of polyphenols in peat and sand after treatment with the extract and watering to detect possible interactions between the substrates and the extract. The results showed that the watering step did not completely remove the polyphenols, which instead remained largely adsorbed by the substrates. This phenomenon was less marked in sand than in peat, also because leaching from sand is easier than from peat. However, the amounts of free polyphenols in the solution and available for seed imbibition and rootlet uptake resulted sufficiently diluted for good zucchini growth, both in peat and in sand, as observed in the sensitivity tests. The peat's capability to adsorb environmental pollutants, including polyphenols, is well recognized [47,48]. Recently, low-cost filters based on peat have been proposed to remove wood-derived polyphenols from water [49]. Various mechanisms are thought to be involved in the biosorption process; however, it is now recognized that ion exchange is the most prevalent mechanism [50]. Similarly, the capability to adsorb anionic, cationic, and nonionic compounds has been reported in the literature for various types of sand; thus, sand has been proposed as a low-cost adsorbing material to be used to remove coloring substances from industrial effluents [51].

4.5. Extract Efficacy in the P. ultimum–Zucchini Pathosystem

The experiment with the pathosystem *P. ultimum*–zucchini in sand confirmed the activity of the extract observed in the preliminary experiments. The giant reed extract applied at presowing in the infected substrate proved to be effective both in restoring the number of healthy plantlets, compared to nontreated control, and in reducing the pathogen CFU, as two weeks after the treatment a significant CFU reduction was still observed. Thus, the watering step after the treatment did not negatively interfere with the extract's activity. This was probably due to the adsorption phenomenon described above [51] that allowed to keep a reservoir of bioactive compounds in the system and maybe also allowed the treatment's efficacy to last over time.

While the literature reports some examples of in vitro bioactivity of lignin and lignin-related compounds towards microorganisms [20,21,38], few studies have been concerned with in vivo evaluations of these compounds for crop protection purposes. The present research reports for the first time the use of an extract of alkali-treated giant reed in a pathosystem model. De Corato et al. [19] highlighted the antifungal activity in a greenhouse of liquid wastes (SELWs) recovered during the bioethanol production process from different steam-exploded lignocellulosic biomass (*Miscanthus sinensis*, wheat straw and giant reed), even if giant reed SELW was the least effective. This harsh and highly energy-demanding technology is very effective in delignifying plant biomass but also partially degrades the holocellulose-generating toxic residues, such as furfurals and acetic and formic acids, which are responsible for the antifungal activity. Zschiegner [52] invented a plant growth regulator based on lignosulfonate that can enhance the natural resistance of plants against viruses, fungi, and bacterial pests. It cannot be excluded that our giant reed extract could also have the similar ability; thus, dedicated experiments could help to investigate possible

priming or induced resistance mechanisms such as the activation of specific plant metabolic pathways and related marker genes [53].

This study reports preliminary findings on the bioactivity of the giant reed extract on a model pathosystem in tray experiments. With regard to possible application in the field, the extract dose used corresponded to 3–4.8 L m^{-2}, which is technically feasible. In addition, dose optimization or/and localized application in the field could also be considered with a reduction in the volume applied per surface unit. The technology necessary to obtain the extract is simple; moreover, the alkaline extraction could be carried out at room temperature, with obvious economic advantages [13].

Overall, the results of this study highlight an interesting potential for the use of the giant reed extract as a liquid soil improver with biological activity against soilborne pathogens such as *P. ultimum*. This pathogen affects the first crop phases and causes a pre- and post-emergence damping-off that is particularly insidious in horticulture because few chemical products are available for soil application. In addition, the repeated use of the same chemical product can increase the risk of developing resistance in the pathogen population. The phasing out of many chemicals for soil disinfestation, such as methyl bromide, has increased the demand for research regarding alternatives to controlling soilborne diseases and their recrudescence under climate change [54,55].

5. Conclusions

The awareness of the need to safeguard human and environmental health suggests the setting up of bio-based strategies in crop protection. Thus, this lignin-rich extract of alkali-treated giant reed could represent an innovative product to sustain crop health, with possible adjunctive fertilizing and amending properties. The results obtained with the model pathosystem, *P. ultimum*–zucchini, suggest both testing the extract's efficacy at a larger scale and enlarging the study to other soilborne pathogens that are particularly sensitive to the extract, such as *S. rolfsii*, or towards wood decay fungi. Further studies could regard the definition of the appropriate extract doses for practical use, the durability of the effectiveness of the extract's application in preventing disease, as well as a complete chemical characterization of the extract to highlight other possible bioactive compounds that may have a role in sustaining plant health.

Giant reed requires low pedo-climatic and agronomic inputs to produce huge biomass amounts; thus, the availability of biomass to produce the extract would not represent a limiting factor. The solvent-free process utilized for the extraction would also meet the demand for the adoption of environmentally friendly technologies. Finally, the utilization of potassium hydroxide for the alkali extraction would present an advantage to enrich the extract with K and would thus confer fertilizing properties.

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References

1. Haas, D.; Défago, G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* **2005**, *3*, 307–319. [CrossRef]
2. Chellemi, D.O.; Gamliel, A.; Katan, J.; Subbarao, K.V. Development and deployment of systems-based approaches for the management of soilborne plant pathogens. *Phytopathology* **2016**, *106*, 216–225. [CrossRef] [PubMed]
3. Gwinn, K.D. Bioactive natural products in plant disease control. In *Studies in Natural Products Chemistry*; Rahman, A., Ed.; Elsevier: Amsterdam, The Netherlands, 2018; Volume 56, pp. 229–246. [CrossRef]
4. Zaker, M. Natural plant products as eco-friendly fungicides for plant diseases control- a review. *Agriculturists* **2016**, *14*, 134–141. [CrossRef]
5. Niño, J.; Narváez, D.M.; Mosquera, O.M.; Correa, Y.M. Antibacterial, antifungal and cytotoxic activities of eight *Asteraceae* and two *Rubiaceae* plants from Colombian biodiversity. *Braz. J. Microbiol.* **2006**, *37*, 566–570. [CrossRef]
6. Bowers, J.H.; Locke, J.C. Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of Fusarium wilt in the greenhouse. *Plant Dis.* **2000**, *84*, 300–305. [CrossRef] [PubMed]
7. Reuveni, M.; Sanches, E.; Barbier, M. Curative and suppressive activities of essential tea tree oil against fungal plant pathogens. *Agronomy* **2020**, *10*, 609. [CrossRef]
8. Jayaraman, J.; Norrie, J.; Punja, K.Z. Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. *J. Appl. Phycol.* **2011**, *23*, 353–361. [CrossRef]
9. Lewandowski, I.; Scurlock, J.M.; Lindvall, E.; Christou, M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenerg.* **2003**, *25*, 335–361. [CrossRef]
10. Antal, G. Giant reed (*Arundo donax* L.) from ornamental plant to dedicated bioenergy species: Review of economic prospects of biomass production and utilization. *Int. J. Hortic. Sci.* **2018**, *24*, 39–46. [CrossRef]
11. Al-Snafi, E.A. The constituents and biological effects of *Arundo donax*—A review. *Int. J. Phytopharm. Res.* **2015**, *6*, 34–40. Available online: https://api.semanticscholar.org/CorpusID:202637327 (accessed on 1 April 2022).
12. Ceotto, E.; Vasmara, C.; Marchetti, R.; Cianchetta, S.; Galletti, S. Biomass and methane yield of giant reed (*Arundo donax* L.) as affected by single and double annual harvest. *Glob. Change Biol. Bioenerg.* **2021**, *13*, 393–407. [CrossRef]
13. Vasmara, C.; Cianchetta, S.; Marchetti, R.; Ceotto, E.; Galletti, S. Potassium hydroxyde pre-treatment enhances methane yield from giant reed (*Arundo donax* L.). *Energies* **2021**, *14*, 630. [CrossRef]
14. Cianchetta, S.; Nota, M.; Polidori, N.; Galletti, S. Alkali pre-treatment and enzymatic hydrolysis of *Arundo donax* for single cell oil production. *Environ. Eng. Manag. J.* **2019**, *18*, 1693–1701.
15. Cianchetta, S.; Polidori, N.; Vasmara, C.; Ceotto, E.; Marchetti, R.; Galletti, S. Single cell oil production from hydrolysates of alkali pre-treated giant reed: High biomass-to-lipid yields with selected yeasts. *Ind. Crops Prod.* **2022**, *178*, 114596. [CrossRef]
16. Quave, L.C.; Plano, R.W.L.; Pantuso, T.; Bennet, C.B. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol.* **2008**, *118*, 418–428. [CrossRef]
17. Shirkani, A.; Mozaffari, M.; Zarei, M. Antimicrobial effects of 14 medicinal plant species of Dashti in Bushehr province. *Iran. S. Med. J.* **2014**, *17*, 49–57. Available online: http://ismj.bpums.ac.ir/article-1-504-en.html (accessed on 1 April 2022).
18. Temiz, A.; Akbas, S.; Panov, D.; Terziev, N.; Alma, M.H.; Parlak, S.; Kose, G. Chemical composition and efficiency of bio-oil obtained from giant cane (*Arundo donax* L.) as a wood preservative. *Bioresources* **2013**, *8*, 2084–2098. [CrossRef]
19. De Corato, U.; Viola, E.; Arcieri, G.; Valerio, V.; Cancellara, A.F.; Zimbardi, F. Antifungal activity of liquid waste obtained from the detoxification of steam-exploded plant biomass against plant pathogenic fungi. *Crop Prot.* **2014**, *55*, 109–111. [CrossRef]
20. Gordobil, O.; Herrera, R.; Yahyaoui, M.; Ilk, S.; Kaya, M.; Labidi, J. Potential use of kraft and organosolv lignins as a natural additive for healthcare of products. *RSC Adv.* **2018**, *8*, 24525–24533. [CrossRef]
21. Dong, X.; Dong, M.; Lu, Y.; Turley, A.; Jin, T.; Wu, C. Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production. *Ind. Crops Prod.* **2011**, *34*, 1630–1631. [CrossRef]
22. Ahmad, U.M.; Ji, N.; Li, H.; Wu, Q.; Song, C.; Liu, Q.; Ma, D.; Lu, X. Can lignin be transformed into agrochemicals? Recent advances in the agricultural applications of lignin. *Ind. Crops Prod.* **2021**, *170*, 113646. [CrossRef]

23. Gazzettaufficiale.it. Available online: https://www.gazzettaufficiale.it/eli/gu/2001/01/26/21/sg/pdf (accessed on 24 November 2021).

24. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]

25. Cianchetta, S.; Galletti, S.; Burzi, P.L.; Cerato, C. A novel microplate-based screening strategy to assess the cellulolytic potential of *Trichoderma* strains. *Biotechnol. Bioeng.* **2010**, *107*, 461–468. [CrossRef]

26. Righini, H.; Francioso, O.; Di Foggia, M.; Prodi, A.; Martel Quintana, A.; Roberti, R. Tomato seed biopriming with water extracts from *Anabaena minutissima*, *Ecklonia maxima* and *Jania adhaerens* as a new agro-ecological option against *Rhizoctonia solani*. *Sci. Hortic.* **2021**, *281*, 109921. [CrossRef]

27. Kannwischer, M.E.; Mitchell, D.J. The influence of a fungicide on the epidemiology of black shank of tobacco. *Phytopathology* **1978**, *68*, 1760–1765. [CrossRef]

28. ISTA. *International Rules for Seed Testing*; International Seed Testing Association: Zürich, Switzerland, 2010.

29. Don, R. *ISTA Handbook on Seedling Evaluation*; International Seed Testing Association: Zürich, Switzerland, 2006.

30. Zhang, S.; Wang, S.; Shan, X.-Q.; Mu, H. Influences of lignin from paper mill sludge on soil properties and metal accumulation in wheat. *Biol. Fertil. Soils* **2004**, *40*, 237–242. [CrossRef]

31. Wang, Z.H.; Li, S.X. Nitrate N loss by leaching and surface runoff in agricultural land: A global issue (a review). *Adv. Agron.* **2019**, *156*, 159–217. [CrossRef]

32. Nassi o Di Nasso, N.; Roncucci, N.; Triana, F.; Tozzini, C.; Bonari, E. Seasonal nutrient dynamics and biomass quality of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) as energy crops. *Ital. J. Agron.* **2011**, *6*, 152–158. [CrossRef]

33. Dakhli, R.; Khatteli, H.; Ridha, L.; Taamallah, H. Agronomic application of olive mill waste water: Short-term effect on soil chemical properties and barley performance under semiarid Mediterranean conditions. *Int. J. Environ. Qual.* **2018**, *27*, 1–17. [CrossRef]

34. Jaffar, M.; Pang, Y.; Yuan, H.; Zou, D.; Liu, Y.; Zhu, B.; Korai, R.M.; Li, X. Wheat straw pretreatment with KOH for enhancing biomethane production and fertilizer value in anaerobic digestion. *Chin. J. Chem. Eng.* **2016**, *24*, 404–409. [CrossRef]

35. Dordas, C. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agron. Sustain. Dev.* **2008**, *28*, 33–46. [CrossRef]

36. Sun, Y.; Cheng, J.J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour. Technol.* **2002**, *83*, 1–11. [CrossRef]

37. McIntosh, S.; Vancov, T. Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw. *Biomass Bioenerg.* **2011**, *35*, 3094–3103. [CrossRef]

38. Guiraud, P.; Steiman, R.; Seiglemurandi, F.; Benoitguyod, J.L. Comparison of the toxicity of various lignin-related phenolic compounds toward selected fungi perfecti and fungi imperfecti. *Ecotoxicol. Environ. Saf.* **1995**, *32*, 29–33. [CrossRef]

39. Cianchetta, S.; Di Maggio, B.; Burzi, P.L.; Galletti, S. Evaluation of selected white-rot fungal isolates for improving the sugar yield from wheat straw. *Appl. Biochem. Biotechnol.* **2014**, *173*, 609–623. [CrossRef]

40. Galletti, S.; Sala, E.; Leoni, O.; Burzi, P.L.; Cerato, C. *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biol. Control* **2008**, *45*, 319–327. [CrossRef]

41. Madhavi, G.B.; Devi, G.U. Effect of combined application of biofumigant, *Trichoderma harzianum* and *Pseudomonas fluorescens* on *Rhizoctonia solani* f. sp. sasakii. *Indian Phytopathol.* **2018**, *71*, 257–263. [CrossRef]

42. Hong, Y.; Hu, H.Y.; Sakoda, A.; Sagehashi, M. Isolation and characterization of antialgal allelochemicals from *Arundo donax* L. *Allelopath. J.* **2010**, *25*, 357–368.

43. Abu-Romann, S. Allelopathic effect of *Arundo donax*, a mediterranean invasive grass. *Plant Omics J.* **2015**, *8*, 287–288. Available online: https://www.pomics.com/aburomman_8_4_2015_287_291.pdf (accessed on 1 April 2022).

44. Popa, V.I.; Dumitru, M.; Volf, I.; Anghel, N. Lignin and polyphenols as allelochemicals. *Ind. Crops Prod.* **2008**, *27*, 144–149. [CrossRef]

45. Savy, D.; Cozzolino, V.; Nebbioso, A.; Drosos, M.; Nuzzo, A.; Mazzei, P.; Piccolo, A. Humic-like bioactivity on emergence and early growth of maize (*Zea mays*, L.) of water-soluble lignins isolated from biomass for energy. *Plant Soil* **2016**, *402*, 221–233. [CrossRef]

46. Balas, A.; Popa, V.I. The influence of natural aromatic compounds on the development of *Lycopersicon esculentum* plantlets. *Bioresources* **2007**, *2*, 363–370. [CrossRef]

47. Mckay, G. Peat for environmental applications: A review. *Dev. Chem. Eng. Miner. Process.* **1996**, *4*, 127–155. [CrossRef]

48. Ahmaruzzaman, M. Adsorption of phenolic compounds on low-cost adsorbents: A review. *Adv. Colloid Interface Sci.* **2008**, *143*, 48–67. [CrossRef]

49. Svensson, H.; Marques, M.; Svensson, B.M.; Mårtensson, L.; Bhatnagar, A.; Hogland, W. Treatment of wood leachate with high polyphenols content by peat and carbon-containing fly ash filters. *Desalin. Water Treat.* **2015**, *53*, 2041–2048. [CrossRef]

50. Crini, G. Non-conventional low-cost adsorbents for dye removal: A review. *Bioresour. Technol.* **2006**, *97*, 1061–1085. [CrossRef]

51. Bello, O.S.; Bello, I.A.; Adegoke, K.A. Adsorption of dyes using different types of sand: A review. *S. Afr. J. Chem.* **2013**, *66*, 117–129. Available online: https://journals.co.za/doi/pdf/10.10520/EJC134491 (accessed on 1 April 2022).

52. Zschiegner, H. Plant Tonic for Induction and Improvement of Resistance. DE4404860A1, 31 August 1995.

53. Mauch-Mani, B.; Baccelli, I.; Luna, E.; Flors, V. Defense priming: An adaptive part of induced resistance. *Annu. Rev. Plant Biol.* **2017**, *68*, 485–512. [CrossRef]
54. Martin, F.N. Development of alternative strategies for management of soilborne pathogens currently controlled with methyl bromide. *Annu. Rev. Phytopathol.* **2003**, *41*, 325–350. [CrossRef]
55. Panth, M.; Hassler, S.C.; Baysal-Gurel, F. Methods for management of soilborne diseases in crop production. *Agriculture* **2020**, *10*, 16. [CrossRef]