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Semi-VOCs of Wood Vinegar Display Strong Antifungal Activities against Oomycete Species *Globisporangium ultimum* and *Pythium aphanidermatum*

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Abstract: Plant disease outbreaks are increasingly exacerbated by climate change and the conditions of stress combinations. They are negatively affecting crop yield and driving threats to food security in many areas of the world. Although synthetic pesticides offer relative success in the control of pests and plant diseases, they are often overused, and this method faces numerous drawbacks, including environmental toxicity, soil degradation, and adverse effects on human health. Therefore, alternatives are being developed and examined, including the biocontrol of pests and pathogens and biomass pyrolysis leading to wood vinegar that has shown great promise in agriculture and organic farming. However, while wood vinegar use is expanding and allows the control of numerous pests and bacterial and fungal diseases, its application to control oomycete diseases is limited. This study aimed to test wood vinegar for the control of oomycete plant pathogens from which six wood vinegars of pistachio, pomegranate, almond, pine, cypress, and walnut were produced. The inhibitory effects of volatile metabolites (semi-VOCs) of different wood vinegars concentrations (100%, 50%, 25%, 12.5%, and 6.25%) were examined against the hyphal growth of *Globisporangium ultimum* and *Pythium aphanidermatum* isolates. An in vitro analysis unambiguously demonstrated that for *Globisporangium ultimum*, the wood vinegar semi-VOCs of almond, pistachio (C 100% and 50%), and walnut (C 100%) totally inhibited mycelial growth. On the other hand, *Pythium aphanidermatum*, pistachio (C 100%, 50%, and 25%), and cypress (C 100%) expressed their abilities to completely inhibit the mycelial growth. Other treatments, including relevant concentrations of pine and pomegranate significantly inhibited the growth of mycelia of both species compared to the control ($p \leq 0.05$). Therefore, wood vinegar could be considered a natural and organic product to use in agriculture to cope not only against pests, bacterial and fungal pests but also against emerging oomycete plant diseases.

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1. Introduction

Plant disease outbreaks are increasing in frequency and are mainly driven by climate change and the conditions of stress combinations [1–5]. The main consequences of emergent disease outbreaks are threats to agroecosystems' stability, compromised food security, and the loss of native biodiversity [6–8]. A relevant example includes *Solanum lycopersicum* Mill. which is among the most consumed crops with increasing production worldwide that is threatened by potential outbreaks of pests, is favored by climate change, and warrants the critical requirement of sustainable approaches to manage emerging diseases [9–12].

The use of synthetic pesticides emerged as one of the most common methods in the management of pests and pathogens [13]. Because of the low efficacy of these synthetic compounds against pathogens, the concentration and pollution of soil and water, and their adverse impact on human health, this method has come under significant scrutiny, and numerous pesticides are at risk of withdrawal [14]. Their effectiveness is more and more compromised by the development of pest and pathogen resistance and their side effects on native biodiversity [15,16]. Synthetic pesticides are considered and documented as "highly hazardous" given their impact on the environment and human health [17]. Alternative methods to synthetic pesticides include mostly biological control approaches [1,18], plant essential oils [19,20], and biomass pyrolysis technologies for the production of wood vinegar [21].

Pyroligneous acid (PA) or wood vinegar is a condensed organic vapor and is a water-soluble liquid produced from the carbonization or slow pyrolysis (thermal degradation at 400 °C and in the absence or near to absence of oxygen) of hardwood materials. The liquor is then separated from sedimentation tar [22–24]. Pyrolysis liquids are referred to in the literature by terms such as pyroligneous acid [25], bio-crude oil [26], wood oil [27], bio-oil [28], pyroligneous tar, pyrolysis oil, wood distillates, bio-fuel oil, liquid smoke [29], and liquid wood [30]. Pyroligneous acid consists of up to 90% H_2O and a mixture of added-value chemical compounds, including syringol, levoglucosan, catechol, acetol, acetic acid, formic acid, propionic acid, valeric acid, pyran, furan, ketones, methanol, formaldehyde, butanol, acetone, furfural, valero-lactone, and phenolic acids (up to 20%) [31,32]. Pyroligneous acid efficiently promotes plant growth, exerts herbicidal and pesticidal activity, and inhibits the growth of microorganisms (e.g., fungi, bacteria, oomycetes, etc.). However, the bio-stimulant action [33,34], pesticidal [35], and herbicidal [36,37] activities depend on the applied concentrations. Pyroligneous acid also has antioxidant, anti-termite, and anti-inflammatory activities [38]. Wood vinegar may enhance the microbial diversity in the soil and improve the quality of the chemical, physical, and biological characteristics of soil and crops as a result. Therefore, PA can be efficiently used as non-chemical fertilizer with pesticidal activities [39–41]. Studies targeting the antimicrobial effect of wood vinegar have not been widely investigated. Nevertheless, the anti-fungal effect of PA was confirmed against causal agents of cucurbits anthracnose, dieback of apple, common root rot of cereals, *Alternaria* blotch of apple, downy mildew of potato, brown and white rot fungi [26,42–44] as well as tumor-inducing and soft rot bacteria [45–47]. The mycelial growth of both oomycetes, *Phytophthora drechsleri* and *Pythium aphanidermatum*, as causal agents of cucumber damping-off, were inhibited with wood vinegar [48]. Pathogens of rice crops, including *Alternaria padwickii*, *Bipolaris oryzae*, and *Cercospora oryzae*, were also adversely inhibited with wood vinegar treatments [49]. Pyroligneous acid has also been efficiently used to cope with *Fusarium graminearum* in wheat [50], *Ovatisporangium* in tomato [21], the *Fusarium* wilt of cucumber [51], *Rhizoctonia solani* (Kuhn), *Verticillum dahliae* (Kleb.), *Fusarium oxysporum* f. sp. *vasinfectum*, *Colletotrichum gleosporoides*, *Pestalotiopsis microspora* and *Ralstonia solanacearum* as documented by Baharom et al. [52].

Oomycetes are eukaryotic and filamentous fungal-like microorganisms and have the ability to attack a broad host range, including crops, forest plants, insects, humans, animals, algae, and other microorganisms [23,24,53]. *Pythium*, *Phytopythium*, *Phytophthora*, and some obligate parasites, including white rusts and downy mildew, can cause severe diseases on many crops with global major ecological and economic effects [25]. Oomycetes are very difficult to control [54,55].

The chemical management of fungal and fungal-like causal agents is not an environmentally friendly approach and can be dangerous to human health. Meanwhile, providing synthetic fungicides and fertilizers in poor and developing countries is expensive for most farmers. On the other hand, PA has several advantages, e.g., a positive impact on soil physicochemical parameters, mitigating the dissemination of antibiotic genes in soils, enhancing the microbial diversity into soils through positive action on soil microbiome, including plant growth promoting bacteria content, plant growth promotion, mitigation of plant stress tolerance, antimicrobial activity against main fungal and bacterial pathogens, and anti-oomycete activity. In order to expand the putative effect of PA on oomycetes in this research, we explore the in vitro inhibition of six samples of wood vinegar volatile components originating from pistachio, pomegranate, almond, pine, cypress, and walnut against *Globisporangium ultimum* and *Pythium aphanidermatum* isolates retrieved from oomycete-infected tomato fields.

2. Materials and Methods

2.1. Pathogen Recovery

In 2022, *G. ultimum* and *P. aphanidermatum* samples were recovered from tomato fields in East-Azarbaijan province, Iran. Diseased parts of *Solanum lycopersicum* consisting of crown and root pieces were surface-sterilized by incubation for 5 min in a solution of 4% sodium hypochlorite. The samples were then incubated for 30 s in a 70% alcohol solution and washed several times in a ddH_2O sterile solution [56]. The samples were subsequently dried for 5 min by placing them on a Whatman paper. A semi-selective oomycete-specific medium was then prepared by adding antibiotics Fluazinam, Nystatin, Ampicillin, and Rifampicin to a Corn Meal Agar (CMA) medium. Samples were incubated for 1–2 days in this medium at 20 °C, and emerging cultures were purified using a single hypha subculture on Water Agar (WA) media. The long-term preservation of cultures was conducted by a subculture of oomycete isolates on slanted vials filled with PCA (Potato Carrot Agar) media and dark incubation until use at 10 °C.

2.2. DNA Extraction and PCR Amplification

Oomycete *Globisporangium ultimum* and *Pythium aphanidermatum* mycelia grown on Potato Carrot Agar (PCA) media were harvested and used as a starting material for DNA extraction according to Möller et al.'s [57] manual procedure. Universal ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAGTCGTAACAAGG) primers [45] were then used for the specific amplification of the ITS-rDNA marker in PCR (Polymerase Chain Reaction) experiments. PCR reactions were performed using 10 ng of genomic DNA and 1.25 U Taq DNA polymerase (Taqara Bio) in a total reaction volume of 50 µL. The reaction mixture consisted of 0.5 µM of ITS4 and ITS5 primers, 5 µL 10 × Ex Taq buffer (20 mM Tris/HCl, pH 8.0, 100 mM KCl), and 4 µL 2.5 mM dNTP supplemented with sterile distilled water to reach the final volume. PerkinElmer 9700 PCR thermal cycler machine (Perkin Elmer, Tabriz, Iran) was used to carry out all amplifications consisting of an initial denaturation step of 95 °C for 5 min followed by 30 Cycles (denaturation at 95 °C for 45–68 °C, annealing at 55 °C for 30 s and extension at 72 °C for 1 min) and a final extension step for 7 min at 72 °C. PCR amplicons were then purified and directly used for DNA sequence determination. Generated DNA sequences were submitted to GenBank [56].

2.3. DNA Sequencing and Phylogeny

A BigDye® terminator v3.1 cycle sequencing kit (Applied Biosystems, Tehran, Iran) was used for purified amplicon sequencing using ITS-rDNA PCR primers according to manufacturer recommendations. Sequencing reactions were run in a 3130xl Genetic Analyzer (Applied Biosystems, Tabriz, Iran) and SeqManII (DNAStar) software, which was used to edit raw sequence files and generate consensus sequences [58]. Neighboring taxa were then identified using generated consensus sequences in a blast search and a comparison against the NCBI nucleotide database. NCBI has retrieved as well as sequences generated during the time course of our study, which was subjected to a multiple sequence alignment using the Mega 6 software [59]. Genetic evolutionary distances were estimated using the Kimura 2-parameter model. Trees were generated based on the maximum likelihood estimation (ML) algorithm [60,61]. For selected tree branches support evaluation, bootstrap analysis by resampling data sets with 1000 replications was performed [62].

2.4. Extraction of Pyroligneous Acid (Wood Vinegar)

Six trees were selected in this study, namely, pistachio, almond, pine, pomegranate, walnut, and cypress. Pyroligneous acid was prepared according to Bridgewater et al. [63,64] and Mohan et al. [39]. Simply, woody biomass materials were burned at 380–430 °C in an environment with reduced oxygen. The produced smoke was finally cooled into a liquid and then refined into wood vinegar. A Karl Fisher titrator (Mettler DL18, Greifensee, Switzerland) was used to determine the amount of water in the wood vinegar samples. The titrator was calibrated with dry methanol. Then, 200 µL of wood vinegar was dropped in a container and titrated with the Karl Fischer reagent until reaching the endpoint [65].

2.5. GC-FID Analysis of Pyroligneous Acid (Wood Vinegar)

Sample preparation was conducted by adding 1.5 mL of concentrated ammonium hydroxide solution to 5 mL aliquots of the aqueous samples in the wood vinegar to increase the pH to around 5. Then, extraction was carried out by adding 3 mL of ethyl acetate (HPLC grade). After liquid–liquid extraction, an organic fraction was used for GC-FID analysis. In previous studies, it has been determined that phenolic compounds are an important group that has been identified in wood vinegar. Therefore, in the current study, the presence of nine phenol derivatives was evaluated by GC/FID using a standard solution [66].

A gas chromatography (Agilent 6850, Santa Clara, CA, USA) equipped with a flame-ionized detector (GC-FID) was used during the quantification of the analytes. The injection port and flame ionization (FID) temperatures were adjusted at 300 °C. The analytes separation was conducted using a temperature programming approach by increasing the column (HP-5, 30 m × 0.25 mm i.d., with a film thickness of 0.25 µm) temperature from 100 °C (maintained for 3 min) to 300 °C at a rate of 12 °C min^{-1}. The column temperature was maintained at 300 °C for 15 min. The ignition of the FID was performed by hydrogen (40 mL min^{-1}) and air (400 mL min^{-1}) gases. One microliter of the extract was injected into the split injector (sampling time of 1 min and split ratio 1:100) [67].

2.6. In Vitro Inhibition of Semi-VOCs of Wood Vinegar against Globisporangium Ultimum and Pythium Aphanidermatum Mycelial Growth

In order to study semi-VOCs of six trees of wood vinegar against the mycelial growth of *G. ultimum* and *P. aphanidermatum* isolates, two-compartment Petri dish plates were used. One side of the plates was filled with 15 mL of potato dextrose agar (PDA), while the other side was filled either with sterile distilled water used as a control or with different concentrations of PA (C 100%, C 50%, C 25%, C 12.5%, and C 6.25%). Agar discs (3 mm) of either *G. ultimum* or *P. aphanidermatum* were placed on the Petri side and filled with a PDA medium. Plates were then sealed with Parafilm M® [45] and incubated for 2–3 days.

Fungal growth was then estimated and used to calculate the percentage of inhibition using the following formula [42]:

$$P = (Gc - Gt)/Gc \times 100$$

P: Percentage of inhibition

Gc: Oomycete growth in control Petri dish

Gt: Oomycete growth in each test Petri dish

2.7. Statistical Analysis

Variance analysis (ANOVA) was used to estimate data statistical analysis. A post hoc Tukey's HSD test was performed whenever a significant effect was detected. For all statistical tests, the level of significance was set at 5% ($p < 0.05$). IBM SPSS Statistics v. 24 program was used for all analyses performed in this study.

3. Results

3.1. Molecular Identification of Globisporangium and Pythium Isolates

Globisporangium and *Pythium* pathogenic isolates recovered from different tomato root rot samples were evaluated using Petri plate growth patterns and morphology. Then, they were subjected to molecular identification. An internal transcribed spacer of ribosomal DNA sequences (ITS-rDNA) of the *G. ultimum* (ON626717, ON626718) and *P. aphanidermatum* (ON626719, ON626720) specimens was similar and nested in a clade with other related species with 99% and 88% bootstrap values, respectively (Figure S1).

3.2. Semi-VOCs Concentrations Activity against Globisporangium Ultimum Mycelial Growth

Wood vinegar samples were prepared with the method of Bridgewater et al. [63,64] and Mohan et al. [39]. Their water content was calculated based on Theapparat et al. [65] and is listed below:

PA of pistachio: 91.5%
PA of almond: 92.4%
PA of walnut: 89.4%
PA of pomegranate: 88.2%
PA of pinus: 86.8%
PA of cypress: 87.4%

Concentration 100%: The best inhibition rates of mycelial growth of *Globisporangium ultimum* isolates were obtained using wood vinegars of almond, walnut, and pistachio. These wood vinegars were then considered to be the most active against *Globisporangium ultimum*. Then, the non-diluted pomegranate, cypress, and pine wood vinegars had lower inhibitory effects compared to pistachio, walnut, and almond non-diluted wood vinegars (Figures 1 and 2).

Concentration 50%: C 50% of all wood vinegars had relative inhibitory effects on the mycelial growth of *G. ultimum* isolate. C 50% of pistachio proved as effective as the non-diluted wood vinegar samples of almond, walnut, and pistachio and completely stopped the growth of the oomycete isolate. C 50% of pistachio was followed closely by almond and cypress wood vinegar treatments that had the same effect (Table 1). Cypress and pomegranate (C 100% and C 50%) treatments gave significantly similar results among each other and were different form the control (Figures 1 and 2). C 50% of pine wood vinegar treatment had significantly the lowest inhibition rate of mycelial growth of *G. ultimum* among all other treatments (Table 1).

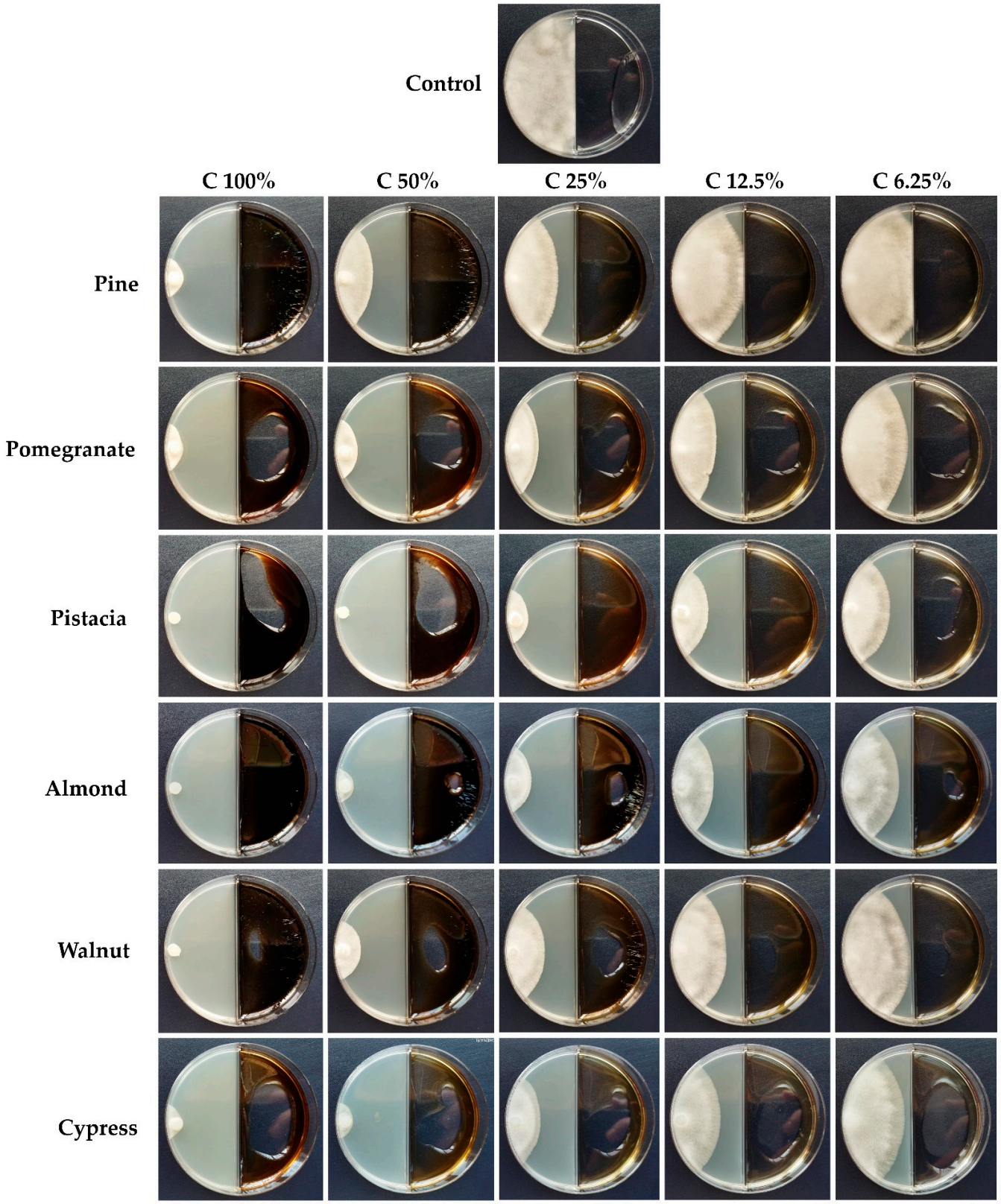

Figure 1. In vitro assay of wood vinegar for six plant species using five concentrations against mycelial growth of *Globisporangium ultimum* (GenBank accession: ON626718) isolate.

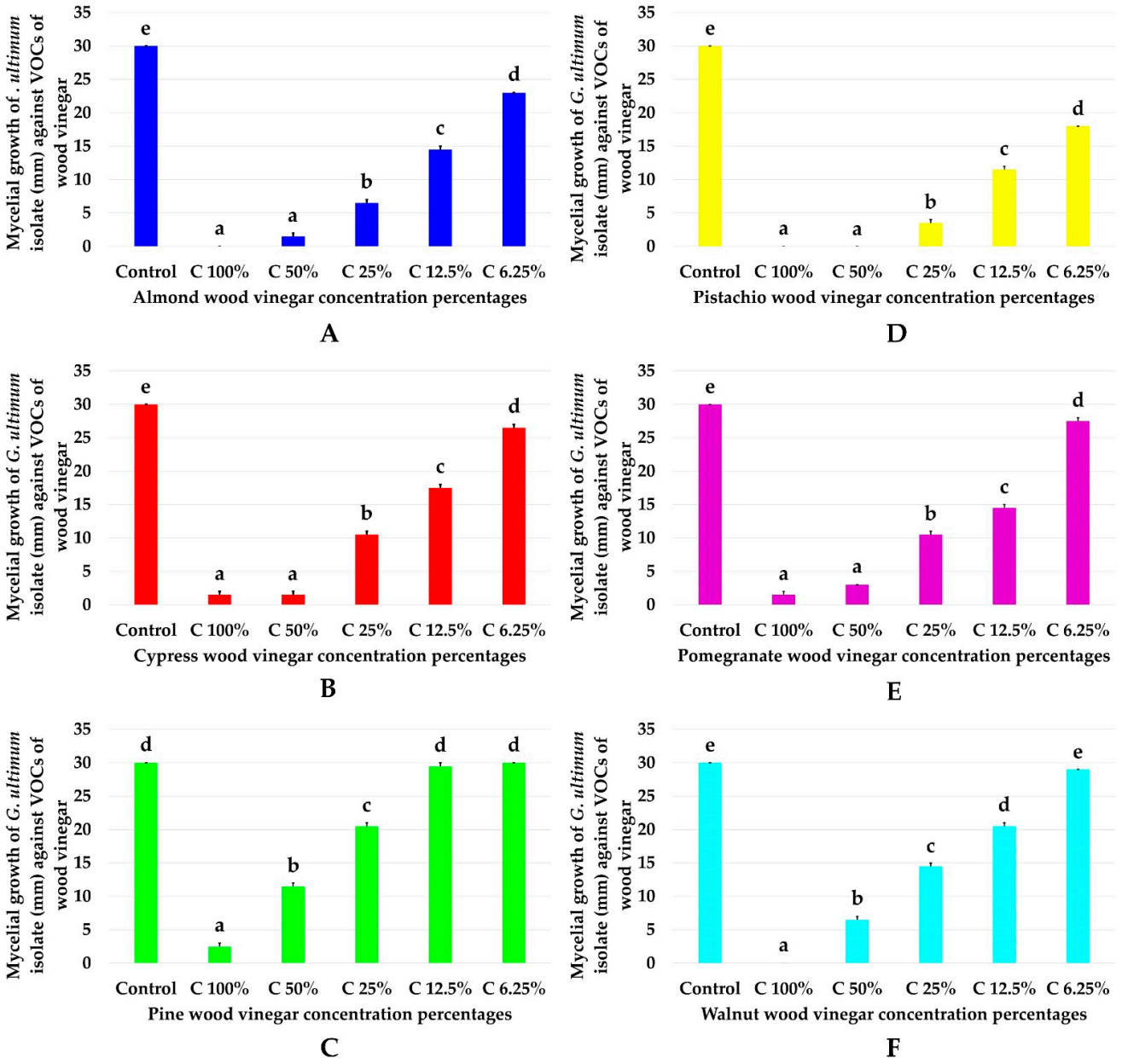

Figure 2. Bar-charts of different wood vinegars (**A**): Almond, (**B**): Cypress, (**C**): Pine, (**D**): Pistachio, (**E**): Pomegranate, (**F**): Walnut wood vinegars) and their effectiveness against *Globisporangium ultimum* (GenBank accession: ON626718). Data presents the mean ± standard error. Letters present on the different bars indicate significant differences across treatments ($p < 0.05$) estimated using Tukey's HSD test.

Table 1. Effectiveness evaluation of semi-VOCs of wood vinegars against *Globisporangium ultimum* in different concentrations with Tukey multi-comparison analysis. Data presents mean ± standard error. Rows labelled with different letters are significantly different among the treatments at $p < 0.05$ using Tukey's HSD test.

Treatment-Concentration	Mycelial Growth (mm)	Tukey Multi-Comparison Analysis
Almond wood vinegar + C 100%	0	a
Cypress wood vinegar + C 100%	1.5 ± 0.5	ab
Pine wood vinegar + C 100%	6.5 ± 0.5	c

Table 1. *Cont.*

Treatment-Concentration	Mycelial Growth (mm)	Tukey Multi-Comparison Analysis
Pistachio wood vinegar + C 100%	14.5 ± 0.5	e
Pomegranate wood vinegar + C 100%	23 ± 0.0	h
Walnut wood vinegar + C 100%	1.5 ± 0.5	ab
Almond wood vinegar + C 50%	1.5 ± 0.5	ab
Cypress wood vinegar + C 50%	10.5 ± 0.5	d
Pine wood vinegar + C 50%	17.5 ± 0.5	f
Pistachio wood vinegar + C 50%	26.5 ± 0.5	i
Pomegranate wood vinegar + C 50%	2.5 ± 0.5	b
Walnut wood vinegar + C 50%	11.5 ± 0.5	d
Almond wood vinegar + C 25%	20.5 ± 0.5	g
Cypress wood vinegar + C 25%	29.5 ± 0.5	jk
Pine wood vinegar + C 25%	30 ± 0.0	k
Pistachio wood vinegar + C 25%	0	a
Pomegranate wood vinegar + C 25%	0	a
Walnut wood vinegar + C 25%	3.5 ± 0.5	b
Almond wood vinegar + C 12.5%	11.5 ± 0.5	d
Cypress wood vinegar + C 12.5%	18 ± 0.0	f
Pine wood vinegar + C 12.5%	1.5 ± 0.5	ab
Pistachio wood vinegar + C 12.5%	3 ± 0.0	b
Pomegranate wood vinegar + C 12.5%	10.5 ± 0.5	d
Walnut wood vinegar + C 12.5%	14.5 ± 0.5	e
Almond wood vinegar + C 6.25%	27.5 ± 0.5	ij
Cypress wood vinegar + C 6.25%	0	a
Pine wood vinegar + C 6.25%	6.5 ± 0.5	c
Pistachio wood vinegar + C 6.25%	14.5 ± 0.5	e
Pomegranate wood vinegar + C 6.25%	20.5 ± 0.5	g
Walnut wood vinegar + C 6.25%	29 ± 0.0	jk

Concentration 25%: For treatments with pistachio, almond, pomegranate, and cypress (both with the same effect), walnut and pine had the strongest to lowest effect on mycelial growth, respectively (Table 1).

Concentration 12.5%: For treatments using pistachio, almond, and pomegranate (both with the same effect), cypress, walnut, and pine wood vinegars proved effective in inhibiting *G. ultimum* mycelial growth and had from the strongest to the lowest inhibition rates, respectively (Table 1).

Concentration 6.25%: We found a decreasing inhibition rate using pistachio, almond, cypress, pomegranate, walnut, and pine wood vinegar treatments against *G. ultimum*, respectively (Table 1). A comparison of inhibition rate percentages of wood vinegar treatments toward *G. ultimum* allowed us to presume that a significant decrease in *G. ultimum* mycelial growth was achieved using all PA semi-VOCs (Table 1).

Generally, treatments (e.g., almond wood vinegar + C 100%, pistachio wood vinegar + C 25%, pomegranate wood vinegar + C 25%, cypress wood vinegar + C 6.25%) have the best inhibition against mycelial growth of *G. ultimium*. The treatment (pine wood vinegar + C 25%) had low efficacy against the oomycete.

3.3. Efficacy of Semi-VOCs Concentrations against Pythium Aphanidermatum Mycelial Growth

Concentration 100%: Cypress and pistachio wood vinegars had the highest inhibition rates of *P. aphanidermatum* mycelial growth among all the other wood vinegars investigated in our study. The concentration was 100% for both pistachio, cypress, and pomegranate vinegars, while almond wood vinegars were significantly similar to each other, different from other treatments, and performed with the highest inhibition rates (Figures 3 and 4).

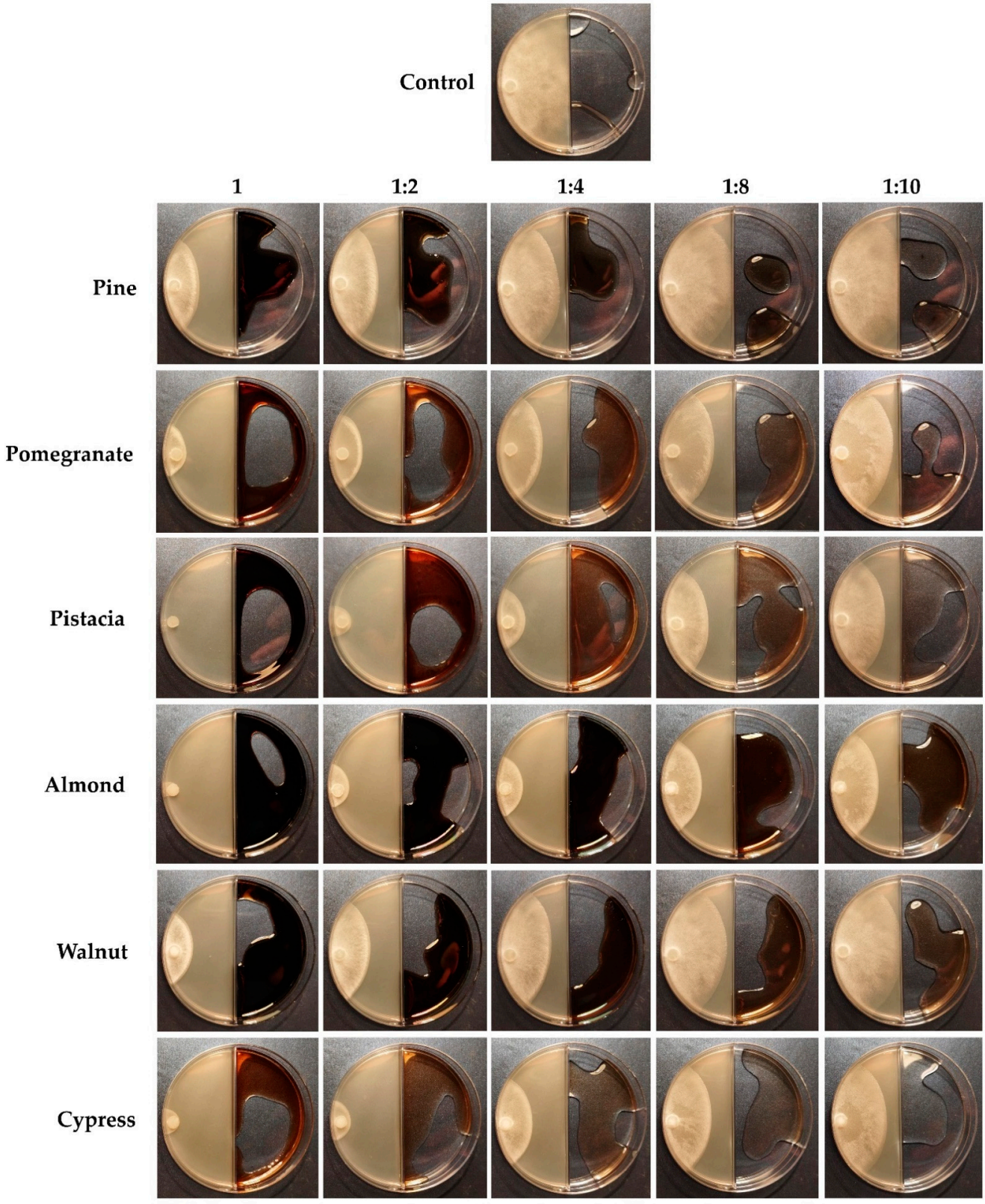

Figure 3. Concentrations of the six plant species wood vinegar in vitro assays for the inhibition of mycelial growth for the *Pythium aphanidermatum* (ON626719) isolate.

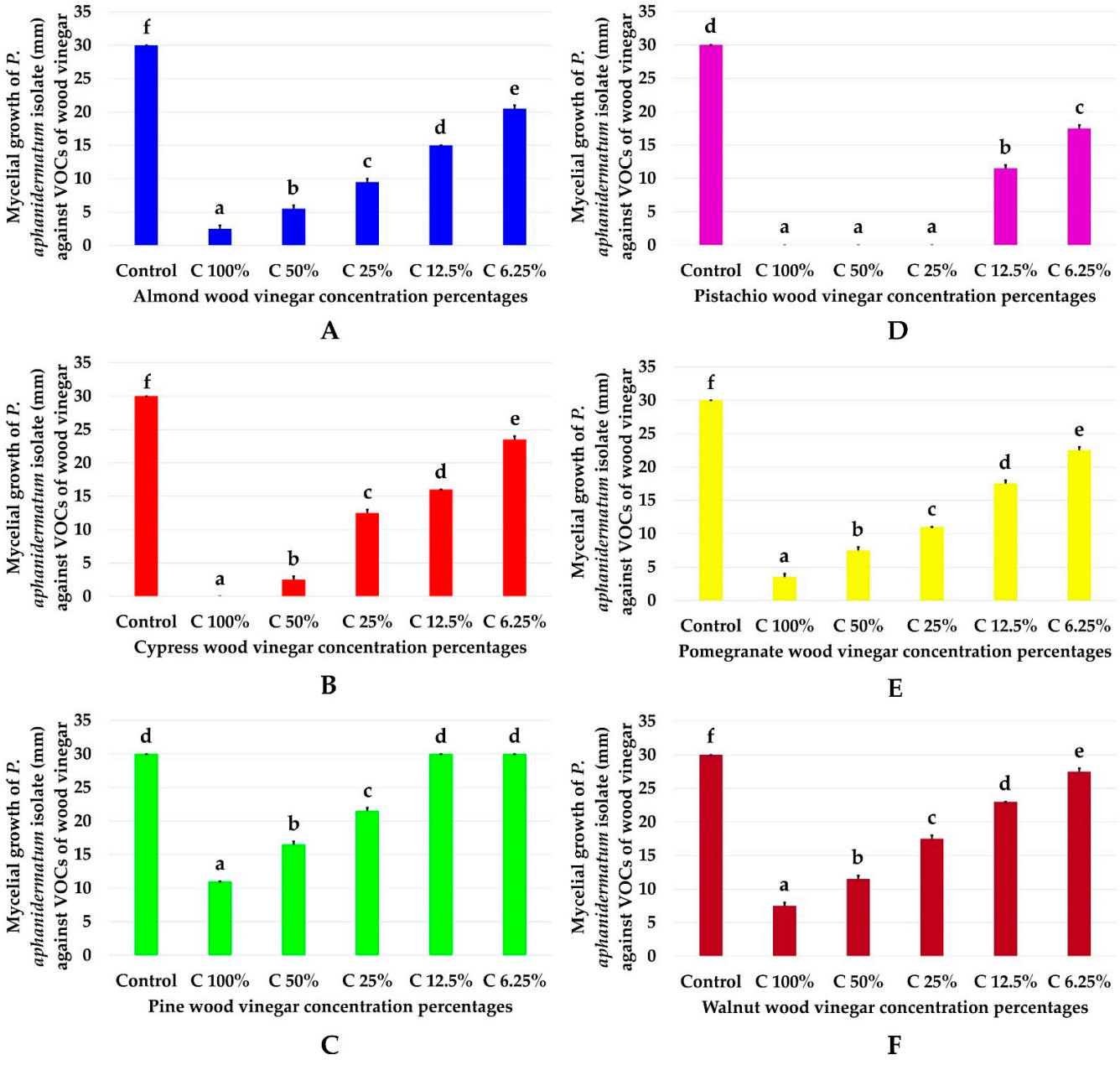

Figure 4. Performance of plant Pyroligneous acid semi-VOCs (**A**): Almond, (**B**): Cypress, (**C**): Pine, (**D**): Pistachio, (**E**): Pomegranate, (**F**): Walnut wood vinegars) for the inhibition of *Pythium aphanidermatum* (ON626719). Data presents the mean ± standard error. Letters present on the different bars indicate significant differences across treatments ($p < 0.05$) estimated using Tukey's HSD test.

Concentration 50%: The wood vinegars of pistachio and cypress (both similar to each other), pomegranate and almond (both similar to each other), and walnut and pine had from the highest to lowest inhibition of *P. aphanidermatum* mycelial growth, respectively.

Concentration 25%: Treatments with almond, pomegranate, pistachio, cypress, walnut, and pine had, respectively, the strongest to lowest mycelial growth inhibition (Table 2). Concentrations of 100%, 50%, and 25% of pistachio wood vinegar had the highest inhibition rates of the mycelial growth of *P. aphanidermatum*. Concentrations of 100%, 50%, and 25% of pistachio were similar to a concentration of 100% for the cypress treatment, which completely stopped the growth of *P. aphanidermatum* (Figures 3 and 4). Then, walnut and pine had lower inhibitory effects, respectively. Significantly, pine treatment had the lowest

inhibition rate against the mycelial growth of *P. aphanidermatum* in comparison to all other treatments (Table 2).

Table 2. Effectiveness evaluation of semi-VOCs of wood vinegars against *Pythium aphanidermatum* in different concentrations with Tukey multi-comparison analysis. Data presents mean ± standard error. Rows labelled with different letters are significantly different among the treatments at $p < 0.05$ using Tukey's HSD test.

Treatment-Concentration	Mycelial Growth (mm)	Tukey Multi-Comparison Analysis
Almond wood vinegar + C 100%	0	a
Cypress wood vinegar + C 100%	3.5 ± 0.5	bc
Pine wood vinegar + C 100%	7.5 ± 0.5	de
Pistachio wood vinegar + C 100%	0	a
Pomegranate wood vinegar + C 100%	11 ± 0.0	fg
Walnut wood vinegar + C 100%	2.5 ± 0.5	b
Almond wood vinegar + C 50%	0	a
Cypress wood vinegar + C 50%	7.5 ± 0.5	de
Pine wood vinegar + C 50%	11.5 ± 0.5	fg
Pistachio wood vinegar + C 50%	2.5 ± 0.5	b
Pomegranate wood vinegar + C 50%	16.5 ± 0.5	hi
Walnut wood vinegar + C 50%	5.5 ± 0.5	cd
Almond wood vinegar + C 25%	0	a
Cypress wood vinegar + C 25%	11 ± 0.5	fg
Pine wood vinegar + C 25%	17.5 ± 0.5	i
Pistachio wood vinegar + C 25%	12.5 ± 0.5	g
Pomegranate wood vinegar + C 25%	21.5 ± 0.5	jk
Walnut wood vinegar + C 25%	9.5 ± 0.5	ef
Almond wood vinegar + C 12.5%	11.5 ± 0.5	fg
Cypress wood vinegar + C 12.5%	17.5 ± 0.5	i
Pine wood vinegar + C 12.5%	23 ± 0.0	k
Pistachio wood vinegar + C 12.5%	16 ± 0.0	hi
Pomegranate wood vinegar + C 12.5%	30 ± 0.0	m
Walnut wood vinegar + C 12.5%	15 ± 0.0	h
Almond wood vinegar + C 6.25%	17.5 ± 0.5	i
Cypress wood vinegar + C 6.25%	22.5 ± 0.5	jk
Pine wood vinegar + C 6.25%	27.5 ± 0.5	l
Pistachio wood vinegar + C 6.25%	23.5 ± 0.5	k
Pomegranate wood vinegar + C 6.25%	30 ± 0.0	m
Walnut wood vinegar + C 6.25%	20.5 ± 0.5	j

Concentration 12.5%: Treatments using pistachio, almond, cypress, pomegranate, walnut, and pine wood vinegar proved to be the highest to lowest in effectiveness for the inhibition of the *P. aphanidermatum* mycelium growth, respectively (Table 2).

Concentration 6.25%: We found a decreasing inhibition rate using pistachio, almond, pomegranate, cypress, walnut, and pine (both had the same effect) wood vinegar treatments of *P. aphanidermatum* mycelial growth, respectively. The comparison of the inhibition rate percentages of wood vinegar treatments of *P. aphanidermatum* empowered us to prove that all semi-VOCs significantly decreased *P. aphanidermatum* mycelial growth with significantly the same effect on it (Table 2).

Generally, treatments (e.g., Almond wood vinegar + C 100%, C 50%, and C 25%, pistachio wood vinegar + C 100%) had the best inhibition against the mycelial growth of *P. aphanidermatum*. The treatment (pomegranate wood vinegar + C 12.5% and C 6.25%) had the lowest efficacy against the species (Table 2).

3.4. GC-FID Analysis of Wood Vinegar Samples

GC-MS analysis of the wood vinegar samples described in this study revealed the presence of numerous organic compounds (Table 3). Detected phenolic compounds included 2,6-dimethoxy phenol (21.4 to 32.3%), 2-methoxyphenol (10.2 to 19.3%), 4-methoxy-1,2-benzenediole (4.1 to 5.6%), catechol (3.2 to 8.3%), 3,4-dimethoxyphenol (2.4 to 5.3), 4-ethyl-2-metoxyphenol (2.3 to 5.6%), 2-methylphenol (1.9 to 2.9%), phenol (0.6 to 1.9%), and cresol (0.13 to 1.6%). The results show that the profile of the chromatograms was similar, but the amounts of phenolic compounds were different. 2,6-dimethoxy phenol and 2-methoxyphenol were found in higher concentrations, respectively. Additionally, the results indicated that the highest and lowest concentration of 2,6-dimethoxy phenol was in the wood vinegar of pine and pomegranate, respectively. The phenolic compound with the lowest concentration was related to the phenol in the wood vinegar of pine (Table 3 and Figure 5). The antimicrobial activity of wood vinegar compounds is described in Table 1.

Table 3. GC-FID analysis results of wood vinegar samples and their biological activity. Numbers are percentages of each compound.

Compound Name	Almond	Walnut	Pinus	Pomegranate	Pistachio	Cypress	Biological Activity	References
Phenol	1.9	1.3	0.6	1.2	0.9	0.6	Antimicrobial activity	[68]
2-methylphenol	2.9	2.7	2.4	2.2	2.3	1.9	Cyclooxygenase (COX)-2 inhibitor	[69]
2-methoxyphenol	10.2	11.1	19.3	11.4	17.2	17.3	Antibacterial activity	[27]
Cresol	1.6	2.3	0.97	0.21	0.21	0.13	Inhibition of biofilm formation and neutralization of bacterial toxins	[70]
Catechol	3.2	4.6	4.2	5.9	8.3	3.2	Antimicrobial activities on three bacteria (*Pseudomonas putida*, *Pseudomonas pyocyanea*, *Corynebacterium xerosis*) and two fungi (*Fusarium oxysporum*, *Penicillium italicum*)	[71]
4-ethyl-2-metoxyphenol	2.6	2.9	3.9	3.6	5.6	2.3	Inhibitory effects against mycelial growth, conidial formation, and germination, and deoxynivalenol (DON) biosynthesis in *Fusarium graminearum*	[72]
4-methoxy-1,2-benzenediole	4.9	4.9	5.6	4.9	4.8	4.1	antimicrobial activities against food-borne bacteria	[73]
2,6-dimethoxy phenol	26.4	27.6	32.3	21.4	22.1	25.9	Inhibitory effect against *Escherichia coli* (NBRC 3301) and *Bacillus subtilis* (NBRC 3134).	[74]
3,4-dimethoxy-phenol	2.6	4.9	2.4	5.3	3.4	3.6		

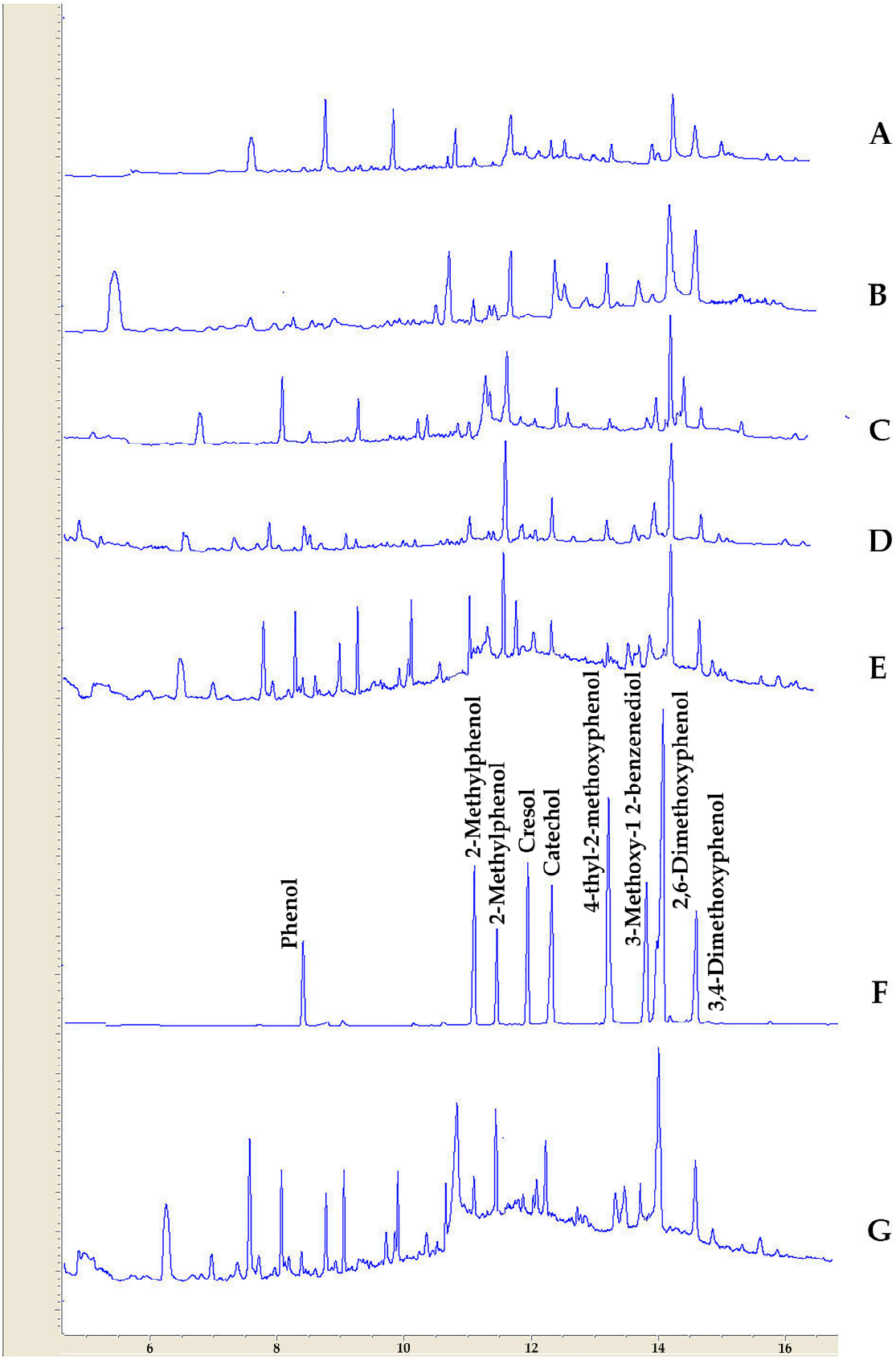

Figure 5. GC-FID chromatograms of wood vinegars along with standard solutions of phenol derivatives of wood vinegars of (**A**) almond, (**B**) walnut, (**C**) pine, (**D**) pomegranate, (**E**) pistachio, (**F**) standard solutions of phenol derivatives, and (**G**) cypress.

4. Discussion

Oomycete diseases are considered nowadays to be an abiding threat to agriculture and forestry [6,53,58]. Huge economic losses are more and more documented, as well as environmental damage [75,76]. The management of oomycete diseases proves difficult and is a significant challenge for agriculture. Most chemical treatments are ineffective due to pathogen resistance to these fungicides [14]. Chemical control has also caused huge environmental damage, including the loss of biodiversity in treated areas and contamination of soil [77], drinking source waters [78], and human health [79]. Alternatives to chemical control are more and more warranted by the general public and policymakers [1]. Two main emerging trends are biological control [5,80,81] and the use of non-chemical compounds, e.g., wood vinegar [21].

All the above arguments in favor of using wood vinegar in agriculture and the absence of studies documenting its activity against oomycete plant pathogens prompted us to study wood vinegar semi-VOCs extracted from different woody tree materials on two oomycete pathogens: *G. ultimum* and *P. aphanidermatum*.

Our results clearly documented that the semi-VOCs of all wood vinegar compounds originating from woody material of pistachio, pomegranate, almond, pine, cypress, and walnut, which were used in the current study, had an inhibitory impact on *G. ultimum* and *P. aphanidermatum* isolates. While non-diluted pistachio, walnut, and almond wood vinegars proved effective in stopping the mycelial growth of *G. ultimum,* all other wood vinegars proved effective in decreasing the pathogen mycelial growth with different inhibition rates. Interestingly, the pistachio concentration at 50% also proved effective in completely blocking *G. ultimum* mycelial growth. Concerning *P. aphanidermatum,* while pistachio and cypress proved effective in stopping the mycelial growth of *G. ultimum,* all other wood vinegars proved effective in inhibiting this pathogen growth with different inhibition rates. Concentrations of 50% and 25% pistachio were able to block *P. aphanidermatum* mycelial growth. These results confirm the results reported by Chenari Bouket et al. [21], who also documented the inhibition of *Ovatisporangium* sp. by the non-diluted wood vinegar of pistachio, cypress, and almond. In this study, none of the diluted wood vinegar proved effective in blocking the growth of *Ovatisporangium* sp. [21]. We speculate that wood vinegar pistachio could be regarded as a promising source of volatile compounds against oomycete plant pathogens.

To date, more than 200 chemical constituents of pyroligneous acid have been identified from different resources [82]. Many studies have shown that pyroligneous acid contains high levels of phenolic compounds, which are pyrolytic products of lignin [83–85]. Lignin is a stable biopolymer that is present in plants and forms the main structure of plant cell walls, especially in wood and bark. This biopolymer is composed mainly of hydroxyphenylpropane (C6–C3) units such as coniferyl alcohol, sinapyl alcohol, and 4-hydroxycinnamyl alcohol [86]. The cleavage of ether and carbon–carbon bonds in lignin leads to the formation of phenolic compounds. In accordance with lignin units of wood vinegar, the phenol compounds identified from the wood vinegars could be classified as syringol-type, including 2,6-dimethoxy phenol, guaiacol-type, including 2-methoxyphenol and 4-ethyl-2-metoxyphenol and benzenediol-type, and Catechol. According to Table 3, sinapyl alcohol and coniferyl alcohol were the most abundant lignin units from the wood vinegars of six tree species pistachio, pomegranate, almond, pine, cypress, and walnut, which is consistent with the earlier findings of Yang et al. [82] on *Litchi chinensis* wood vinegar.

A study conducted on the wood vinegar of *Litchi chinensis* [86,87] showed that more than 70% of all wood vinegar compositions were identified as phenolic compounds, with three major components: 2,6-dimethoxyphenol (Syringol, 29.54%), 2-methoxyphenol (guaiacol, 12.36%), and 3,5-dimethoxy-4 hydroxytoluene (11.07%), which is consistent with our results obtained from the GC-FID characterization of PA volatiles of six tree species, including pistachio, pomegranate, almond, pine, cypress, and walnut. The GC-FID characterization of PA volatiles for the six tree species revealed a similar composition for the different wood vinegars. Detected phenolic compounds, including 2,6-dimethoxy phenol,

2-methoxyphenol, 4-methoxy-1,2-benzenediole, catechol, 3,4-dimethoxyphenol, 4-ethyl-2-metoxyphenol, phenol, and cresol were detected in earlier studies [63,64,84,88,89], where 2,6-dimethoxy phenol (21.4 to 32.3%) and 2-methoxyphenol (10.2 to 19.3%) were found in higher concentrations, respectively.

These compounds have been described as excellent antimicrobial agents [90,91], similar to our findings (Table 1). The precise composition of our wood vinegar samples is now available after this study and will allow us to test wood vinegar's individual compounds and their antimicrobial activity. In line with this fact is how several authors have indicated that it is impossible to determine the exact mode of action for wood vinegars to inhibit pathogens, given the huge diversity of wood vinegars' volatile compounds [63,64,84,88,89]. Several studies were attributed, similarly to our work, on the antimicrobial activity of wood vinegar and its individual components [92,93]. Given that the PA composition of the different woody materials is similar, we tried to link the most active wood vinegars (pistachio, almond, and cypress), but we failed to find a direct link between a major compound and the activity of specific wood vinegar. Mederios et al. [93] rejected the implication of compounds present in pyroligneous acid (guaiacol, phenols, and furfural) in the observed antimicrobial activity and rather suggested that the low pH due to acetic acid that was present in pyroligneous acid was the real cause for the activity. Our study confirms the hypothesis of Mederios et al. [93]. A possible confirmation of our observations is provided by the fact that several fertilization regimes aiming at lowering soil pH have been used to manage oomycete diseases since oomycete species do not tolerate acidic pH in soil [94,95]. More work is therefore needed to highlight the origin of the observed antimicrobial activity of pyroligneous acid and to estimate the part of the antimicrobial activity that could be attributed to compounds present in pyroligneous acid (guaiacol, phenols, and furfural) and to the low pH due to the acetic acid present in pyroligneous acid. Ongoing experiments are being conducted by our group and aim to characterize precisely the compounds present in our pistachio wood vinegar and purify them and test them for antimicrobial activity, which will help to enrich the debate and to advance the field.

5. Conclusions

Wood vinegar is a basic solution for dealing with forest biomass or other woody plant material. Wood vinegar, in addition to being effective in its dealings with biomass wastes, has tremendous added value extending from the amelioration of soil parameters and plant growth promotion to the management of plant pests and diseases. Following the main findings of our manuscript, which documents its efficiency against oomycete plant diseases that are recalcitrant to classical highly environmental and health-damaging synthetic pesticides, we highly recommend the use of wood vinegar to deal with emerging oomycetes and other disease outbreaks; the use of local biomass materials according to the general cleaner agricultural production principle that stands for proactive and preventive approaches is needed to fulfill human needs and develop processes that are ecologically and economically efficient.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres14010029/s1, Figure S1: Phylogenetic positions of *Globisporangium ultimum* areeo_ach17 and areeo_ach46 isolates (GenBank accession numbers: ON626717, ON626718) and *Pythium aphanidermatum* areeo_ach07 and areeo_ach12 (GenBank accession numbers: ON626719, ON626720) among other oomycete spp. based on ITS-rDNA sequences neighbor-joining examination. Only bootstrap values higher than 50% are indicated on corresponding branches. MH107069 *Phytopythium litorale* PL 80 was used as an outgroup taxon.

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References

1. Belbahri, L.; Chenari Bouket, A.; Rekik, I.; Alenezi, F.N.; Vallat, A.; Luptakova, L.; Petrovova, E.; Oszako, T.; Cherrad, S.; Vacher, S.; et al. Comparative Genomics of *Bacillus amyloliquefaciens* Strains Reveals a Core Genome with Traits for Habitat Adaptation and a Secondary Metabolites Rich Accessory Genome. *Front. Microbiol.* **2017**, *8*, 1438. [CrossRef]
2. Ristaino, J.B.; Anderson, P.; Brauman, K.A.; Cunniffe, N.J.; Federoff, N.; Garrett, K.A.; Gilligan, C.; Holmes, T.; Martin, M.; MacDonald, G.K.; et al. The Persistent Threat of Emerging Plant Diseases to Global Food Security. *Proc. Nat. Acad. Sci. USA* **2021**, *118*, e2022239118. [CrossRef]
3. Malhi, G.S.; Kaur, M.; Kaushik, P. Impact of Climate Change on Agriculture and Its Mitigation Strategies: A Review. *Sustainability* **2021**, *13*, 1318. [CrossRef]
4. Desaint, H.; Aoun, N.; Deslandes, L.; Vailleau, F.; Roux, F.; Berthome, R. Fight hard or die trying: When plants face pathogens under heat stress. *New Phytol.* **2021**, *229*, 712–734. [CrossRef]
5. Balla, A.; Silini, A.; Cherif-Silini, H.; Chenari Bouket, A.; Moser, W.K.; Nowakowska, J.A.; Oszako, T.; Benia, F.; Belbahri, L. The Threat of Pests and Pathogens and the Potential for Biological Control in Forest Ecosystems. *Forests* **2021**, *12*, 1579. [CrossRef]
6. Prospero, S.; Vercauteren, A.; Heungens, K.; Belbahri, L.; Rigling, D. *Phytophthora* diversity and the population structure of *Phytophthora ramorum* in Swiss ornamental nurseries. *Plant Pathol.* **2013**, *62*, 1063–1071. [CrossRef]
7. Corredor-Moreno, P.; Saunders, D.G.O. Expecting the unexpected: Factors influencing the emergence of fungal and oomycete plant pathogens. *New Phytol.* **2020**, *225*, 118–125. [CrossRef]
8. Shahzad, A.; Ullah, S.; Dar, A.A.; Sardar, M.F.; Mehmood, T.; Tufail, M.A.; Shakoor, A.; Haris, M. Nexus on climate change: Agriculture and possible solution to cope future climate change stresses. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 14211–14232. [CrossRef]
9. FAO. FAO Database. Food and Agriculture Organization, United Nations. 2016. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 1 February 2023).
10. Du, Y.D.; Cao, H.X.; Liu, S.Q.; Gu, X.B.; Cao, Y.X. Response of yield, quality, water and nitrogen use efficacy of tomato to different levels of water and nitrogen under drip irrigation in Northwestern China. *J. Integr. Agric.* **2017**, *16*, 1153–1161. [CrossRef]
11. Gadekallu, T.R.; Rajput, D.S.; Reddy, M.P.K.; Lakshmanna, K.; Bhattacharya, S.; Singh, S.; Jolfaei, A.; Alazab, M. A novel PCA–whale optimization-based deep neural network model for classification of tomato plant diseases using GPU. *J. Real-Time Image Process.* **2021**, *18*, 1383–1396. [CrossRef]
12. Nankishore, A.; Farrell, A.D. The response of contrasting tomato genotypes to combined heat and drought stress. *J. Plant Physiol.* **2016**, *202*, 75–82. [CrossRef]
13. Umetsu, N.; Shirai, Y. Development of novel pesticides in the 21st century. *J. Pest. Sci.* **2020**, *45*, 54–74. [CrossRef]
14. Cherrad, S.; Charnay, A.; Hernandez, C.; Steva, H.; Belbahri, L.; Vacher, S. Emergence of boscalid-resistant strains of *Erysiphe necator* in French vineyards. *Microbiol. Res.* **2018**, *216*, 79–84. [CrossRef]
15. Zhu, Q.; Yang, Y.; Zhong, Y.; Lao, Z.; O'Neill, P.; Hong, D.; Zhang, K.; Zhao, S. Synthesis, insecticidal activity, resistance, photodegradation and toxicity of pyrethroids (A review). *Chemosphere* **2020**, *254*, 126779. [CrossRef]
16. Solla, A.; Moreno, G.; Malewski, T.; Jung, T.; Klisz, M.; Tkaczyk, M.; Siebyla, M.; Pérez, A.; Cubera, E.; Hrynyk, H.; et al. Phosphite Spray for the Control of Oak Decline Induced by *Phytophthora* in Europe. *For. Ecol. Manag.* **2021**, *485*, 118938. [CrossRef]
17. Tkaczyk, A.; Mitrowska, K.; Posyniak, A. Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: A review. *Sci. Total Environ.* **2020**, *717*, 137222. [CrossRef]
18. Naamala, J.; Smith, D.L. Relevance of Plant Growth Promoting Microorganisms and Their Derived Compounds, in the Face of Climate Change. *Agronomy* **2020**, *10*, 1179. [CrossRef]
19. Raveau, R.; Fontaine, J.; Lounès-Hadj Sahraoui, A. Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review. *Foods.* **2020**, *9*, 365. [CrossRef]
20. Tilocca, B.; Cao, A.; Migheli, Q. Scent of a Killer: Microbial Volatilome and Its Role in the Biological Control of Plant Pathogens. *Front. Microbiol.* **2020**, *11*, 41. [CrossRef]

21. Chenari Bouket, A.; Narmani, A.; Tavasolee, A.; Elyasi, G.; Abdi, A.; Naeimi, S.; Sharifi, K.; Oszako, T.; Alenezi, F.N.; Belbahri, L. In Vitro Evaluation of Wood Vinegar (Pyroligneous Acid) Semi-VOCs Inhibitory Effect against a Fungus-like Microorganism *Ovatisporangium* (*Phytopythium*) Isolate Recovered from Tomato Fields in Iran. *Agronomy* **2022**, *12*, 1609. [CrossRef]
22. Arshad, U.; Naveed, M.; Javed, N.; Gogi, M.D.; Ali, M.A. Biochar Application from Different Feedstocks Enhances Plant Growth and Resistance against *Meloidogyne incognita* in Tomato. *Int. J. Agric. Biol.* **2020**, *24*, 961–968.
23. Thines, M. Phylogeny and evolution of plant pathogenic oomycetes—A global overview. *Eur. J. Plant Pathol.* **2014**, *138*, 431–447. [CrossRef]
24. Van West, P.; Beakes, G.W. Animal pathogenic Oomycetes. *Fungal Biol.* **2014**, *118*, 525–526. [CrossRef]
25. Larousse, M.; Rancurel, C.; Syska, C.; Palero, F.; Etienne, C.; Industri, B.; Nesme, X.; Bardin, M.; Galiana, E. Tomato root microbiota and *Phytophthora parasitica*-associated disease. *Microbiome* **2017**, *5*, 56. [CrossRef]
26. Tiilikkala, K.; Fagernas, L.; Tiilikkala, J. History and use of wood pyrolysis liquids as biocide and plant protection product. *Open Agric. J.* **2010**, *4*, 111–118. [CrossRef]
27. Grewal, A.; Abbey, L.; Gunupuru, L.R. Production, Prospects and Potential Application of Pyroligneous Acid in Agriculture. *J. Anal. Appl. Pyrolysis.* **2018**, *135*, 152–159. [CrossRef]
28. Mathew, S.; Zakaria, Z.A. Pyroligneous acid—The smoky acidic liquid from plant biomass. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 611–622. [CrossRef]
29. Souza, J.B.G.; Ré-Poppi, N.; Raposo, J.L. Characterization of pyroligneous acid used in agriculture by gas chromatography-mass spectrometry. *J. Braz. Chem. Soc.* **2012**, *23*, 610–617. [CrossRef]
30. Balat, M.; Balat, M.; Kırtay, E.; Balat, H. Main routes for the thermo-conversion of biomass into fuels and chemicals. Part 1: Pyrolysis systems. *Energy Convers. Manag.* **2009**, *50*, 3147–3157. [CrossRef]
31. Mansur, D.; Yoshikawa, T.; Norinaga, K.; Hayashi, J.; Tago, T.; Masuda, T. Production of ketones from pyroligneous acid of woody biomass pyrolysis over an iron-oxide catalyst. *Fuel* **2013**, *103*, 130–134. [CrossRef]
32. Araujo, E.S.; Pimenta, A.S.; Feiji, F.M.C.; Castro, R.V.O.; Fasciotti, M.; Monteiro, T.V.C.; Lima, K.M.G. Antibacterial and antifungal activities of pyroligneous acid from wood of *Eucalyptus urograndis* and *Mimosa tenuiflora*. *J. Appl. Microbiol.* **2017**, *124*, 85–96. [CrossRef]
33. Fanfarillo, E.; Fedeli, R.; Fiaschi, T.; de Simone, L.; Vannini, A.; Angiolini, C.; Loppi, S.; Maccherini, S. Effects of Wood Distillate on Seedling Emergence and First-Stage Growth in Five Threatened Arable Plants. *Diversity* **2022**, *14*, 669. [CrossRef]
34. Fedeli, R.; Vannini, A.; Celletti, S.; Maresca, V.; Munzi, S.; Cruz, C.; Alexandrov, D.; Guarnieri, M.; Loppi, S. Foliar application of wood distillate boosts plant yield and nutritional parameters of chickpea. *Ann. Appl. Biol.* **2023**, *182*, 57–64. [CrossRef]
35. Fedeli, R.; Vannini, A.; Guarnieri, M.; Monaci, F.; Loppi, S. Bio-Based Solutions for Agriculture: Foliar Application of Wood Distillate Alone and in Combination with Other Plant-Derived Corroborants Results in Different Effects on Lettuce (*Lactuca sativa* L.). *Biology* **2022**, *11*, 404. [CrossRef]
36. Liu, X.; Zhan, Y.; Li, X.; Li, Y.; Feng, X.; Baghavathiannan, M.; Zhang, C.; Qu, M.; Yu, J. The use of wood vinegar as a non-synthetic herbicide for control of broadleaf weeds. *Ind. Crops Prod.* **2021**, *73*, 114105. [CrossRef]
37. Chu, L.; Liu, H.; Zhang, Z.; Zhan, Y.; Wang, K.; Yang, D.; Liu, Z.; Yu, J. Evaluation of Wood Vinegar as an Herbicide for Weed Control. *Agronomy* **2022**, *12*, 3120. [CrossRef]
38. Wititsiri, S. Production of wood vinegars from coconut shells and additional materials for control of termite workers, *Odontotermes* sp. and striped mealy bugs, *Ferrisia virgata*. *Songklanakarin J. Sci. Technol.* **2011**, *33*, 349–354.
39. Mohan, D.; Pittman, C.U.; Steele, P.H. Pyrolysis of wood/biomass for bio-oil: A critical review. *Energy Fuels* **2006**, *20*, 848–889. [CrossRef]
40. Steiner, C.; Das, K.C.; Garcia, M.; Förster, B.; Zech, W. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia* **2008**, *51*, 359–366. [CrossRef]
41. Wei, Q.; Ma, X.; Dong, J. Preparation, chemical constituents and antimicrobial activity of pyroligneous acids from walnut tree branches. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 24–28. [CrossRef]
42. Jung, K.-H. Growth inhibition effect of pyroligneous acid on pathogenic fungus, *Alternaria mali*, the agent of *Alternaria* blotch of apple. *Biotechnol. Bioprocess Eng.* **2007**, *12*, 318–322. [CrossRef]
43. Chen, S.; Feng, Y.; Li, S.; Mu, J. Research on Inhibition Effect of MDF Pyrolysis Condensate Liquids against Two Kinds of Fungi. In Proceedings of the 55th International Convention of Society of Wood Science and Technology, Beijing, China, 27–31 August 2012; pp. 1–7.
44. Mmojieje, J.; Hornung, A. The potential application of pyroligneous acid in the UK Agricultural Industry. *J. Crop. Improv.* **2015**, *29*, 228–246. [CrossRef]
45. Chalermsan, Y.; Peerapan, S. Wood-vinegar: By-product from rural charcoal kiln and its roles in plant protection. *Asian J. Food. Agro-Indust.* **2009**, *2*, 189–195.
46. Mahmud, K.N.; Yahayu, M.; Sarip, S.H.M.; Rizan, N.H.; Min, C.B.; Mustafa, N.F.; Ngadiran, S.; Ujang, S.; Zakaria, Z.A. Evaluation on Efficiency of Pyroligneous Acid from Palm Kernel Shell as Antifungal and Solid Pineapple Biomass as Antibacterial and Plant Growth Promoter. *Sains Malays.* **2016**, *45*, 1423–1434.
47. Saberi, M.; Sarpeleh, A.; Askary, H. Management of damping-off and increasing of dome growth traits of cucumber in greenhouse culture using citrus wood vinegar. *Appl. Res. Plant Protec.* **2015**, *4*, 99–111.

48. Chuaboon, W.; Ponghirantanachoke, N.; Athinuwat, D. Application of wood vinegar for fungal disease controls in paddy rice. *Appl. Environ. Res.* **2016**, *38*, 77–85. [CrossRef]
49. Hwang, Y.H.; Matsushita, Y.I.; Sugamoto, K.; Matsui, T. Antimicrobial effect of the wood vinegar from *Cryptomeria japonica* sapwood on plant pathogenic microorganisms. *J. Microbiol. Biotechnol.* **2005**, *15*, 1106–1109.
50. Gao, T.; Bian, R.; Joseph, S.; Taherymoosavi, S.; Mitchell, D.R.G.; Munroe, P.; Xu, J.; Shi, J. Wheat straw vinegar: A more cost-effective solution than chemical fungicides for sustainable wheat plant protection. *Sci. Total Environ.* **2020**, *725*, 138359. [CrossRef] [PubMed]
51. Maliang, H.D.; Wang, P.W.; Chen, A.; Liu, H.; Lin, H.; Ma, J.Y. Bamboo Tar as a Novel Fungicide: Its Chemical Components, Laboratory Evaluation, and Field Efficacy against False Smut and Sheath Blight of Rice and Powdery Mildew and Fusarium Wilt of Cucumber. *Plant Dis.* **2021**, *105*, 331–338. [CrossRef]
52. Bahoram, N.A.; Rahman, M.H.A.; Shahrun, M.S.; Suherman, F.H.S.; Masdar, S.N.H. Chemical composition and antimicrobial activities of wood vinegars fromcarambola, coconut shells and mango against selectedplant pathogenic microorganisms. *Malaysian J. Microbiol.* **2020**, *16*, 438–445. [CrossRef]
53. Lara, E.; Belbahri, L. SSU rRNA reveals major trends in oomycete evolution. *Fungal Divers.* **2011**, *49*, 93. [CrossRef]
54. Fisher, M.C.; Gurr, S.J.; Cuomo, C.A.; Blehert, D.S.; Jin, H.; Stukenbrock, E.H.; Stajich, J.E.; Kahmann, R.; Boone, C.; Denning, D.W.; et al. Threats posed by the fungal kingdom to humans, wildlife, and agriculture. *mBio* **2020**, *11*, e00449-20. [CrossRef]
55. Chepsergon, J.; Motaung, T.E.; Bellieny-Rabelo, D.; Moleleki, L.N. Organize, Don't Agonize: Strategic Success of *Phytophthora* Species. *Microorganisms* **2020**, *8*, 917. [CrossRef] [PubMed]
56. Chenari Bouket, A.; Arzanlou, M.; Tojo, M.; Babai-Ahari, A. *Pythium kandovanense* sp. nov., a fungus-like eukaryotic microorganism (Stramenopila, Pythiales) isolated from snow-covered ryegrass leaves. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 2500–2506. [CrossRef] [PubMed]
57. Möller, E.M.; Bahnweg, G.; Sandermann, H.; Geiger, H.H. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res.* **1992**, *20*, 6115–6116. [CrossRef] [PubMed]
58. Belbahri, L.; Calmin, G.; Sanchez-Hernandez, E.; Oszako, T.; Lefort, F. *Pythium sterilum* sp. nov. isolated from Poland, Spain and France: Its morphology and molecular phylogenetic position. *FEMS Microbiol. Lett.* **2006**, *255*, 209–214. [CrossRef]
59. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. Mega6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef]
60. Saitou, N.; Nei, M. The neighbor-joining method: A new method for re-constructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
61. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef]
62. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols, a Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.
63. Bridgewater, A.V. Renewable fuels and chemicals by thermal processing of biomass. *Chem. Eng. J.* **2003**, *91*, 87–102. [CrossRef]
64. Bridgewater, A.V. Biomass fast pyrolysis. *Thermal Sci.* **2004**, *8*, 21–50. [CrossRef]
65. Theapparat, Y.; Chandumpai, A.; Leelasuphakul, W.; Laemask, N.; Ponglimanont, C. Physicochemical Characteristics of Wood Vinegars from Carbonization of *Leucaena leucocephala*, *Azadirachta indica*, *Eucalyptus camaldulensis*, *Hevea brasiliensis* and *Dendrocalamus asper*. *Kasetsart. J. (Nat. Sci.)* **2014**, *48*, 916–928.
66. Pimenta, A.; Fasciotti, M.; Monteiro, T.V.C.; Lima, K.M.G. Chemical Composition of Pyroligneous Acid Obtained from *Eucalyptus* GG100 Clone. *Molecules* **2018**, *23*, 426. [CrossRef]
67. Miyardan, F.N.; Mogaddam, M.R.A.; Farajzadeh, M.A.; Nemati, M. Combining modified graphene oxide-based dispersive micro solid phase extraction with dispersive liquid–liquid microextraction in the extraction of some pesticides from zucchini samples. *Microchem. J.* **2022**, *182*, 107884. [CrossRef]
68. Alves, M.J.; Ferrerira, I.C.F.R.; Forufe, H.J.C.; Abreu, R.M.V.; Martins, A.; Pintado, M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *J. Appl. Microbiol.* **2013**, *115*, 346–357. [CrossRef]
69. Fujisawa, S.; Ishihara, M.; Murakami, Y.; Atsumi, T.; Kadoma, Y.; Yokoe, I. Predicting the Biological Activities of 2-Methoxyphenol Antioxidants: Effects of Dimers. *In Vivo* **2007**, *21*, 181–188.
70. Zaborowska, M.; Kucharski, J.; Wyszkowska, J. Biochemical and microbiological activity of soil contaminated with o-cresol and biostimulated with *Perna canaliculus* mussel meal. *Environ. Monit. Assess.* **2018**, *190*, 602. [CrossRef]
71. Kocaçalışkan, I.; Talan, I.; Terzi, I. Antimicrobial Activity of Catechol and Pyrogallol as Allelochemicals. *Z. Naturforsch.* **2006**, *61*, 639–642. [CrossRef]
72. Gao, T.; Zhang, Y.; Shi, J.; Mohamed, S.R.; Xu, J.; Liu, X. The Antioxidant Guaiacol Exerts Fungicidal Activity Against Fungal Growth and Deoxynivalenol Production in *Fusarium graminearum*. *Front. Microbiol.* **2021**, *12*, 762844. [CrossRef]
73. Kim, M.-G.; Lee, H.-S. 1,2-Benzendiol Isolated from Persimmon Roots and Its Structural Analogues Show Antimicrobial Activities against Food-borne Bacteria. *J. Korean Soc. Appl. Biol. Chem.* **2014**, *57*, 429–433. [CrossRef]
74. Oka, S.; Kuniba, R.; Tsuboi, N.; Tsuchida, S.; Ushida, K.; Tomoshige, S.; Kuramochi, K. Isolation, synthesis, and biological activities of a bibenzyl from *Empetrum nigrum* var. japonicum. *Biosci. Biotechnol. Biochem.* **2020**, *84*, 31–36. [CrossRef]

75. Paul, B.; Bala, K.; Lassaad, B.; Calmin, G.; Sanchez-Hernandez, E.; Lefort, F. A new species of *Pythium* with ornamented oogonia: Morphology, taxonomy, internal transcribed spacer region of its ribosomal RNA, and its comparison with related species. *FEMS Microbiol. Lett.* **2006**, *254*, 317–323. [CrossRef]
76. Moralejo, E.; Clemente, A.; Descals, E.; Belbahri, L.; Calmin, G.; Lefort, F.; Spies, C.F.; McLeod, A. *Pythium recalcitrans* sp. nov. revealed by multigene phylogenetic analysis. *Mycologia* **2008**, *100*, 310–319. [CrossRef] [PubMed]
77. Meftaul, I.M.; Venkateswarlu, K.; Dharmarjan, R.; Annamali, P.; Megharaj, M. Pesticides in the urban environment: A potential threat that knocks at the door. *Sci. Total Environ.* **2020**, *711*, 134612. [CrossRef]
78. Dong, W.W.; Zhang, Y.; Quan, X. Health risk assessment of heavy metals and pesticides: A case study in the main drinking water source in Dalian, China. *Chemosphere* **2020**, *242*, 125113. [CrossRef]
79. Maggi, F.; la Cecilia, D.; Tang, F.H.M.; McBratney, A. The global environmental hazard of glyphosate use. *Sci. Total Environ.* **2020**, *717*, 137167. [CrossRef]
80. Cheffi, M.; Chenari Bouket, A.; Alenezi, F.N.; Luptakova, L.; Belka, M.; Vallat, A.; Rateb, M.E.; Tounsi, S.; Triki, M.A.; Belbahri, L. *Olea europaea* L. Root Endophyte *Bacillus velezensis* OEE1 Counteracts Oomycete and Fungal Harmful Pathogens and Harbours a Large Repertoire of Secreted and Volatile Metabolites and Beneficial Functional Genes. *Microorganisms* **2019**, *7*, 314. [CrossRef]
81. Alenezi, F.N.; Slama, H.B.; Bouket, A.C.; Cherif-Silini, H.; Silini, A.; Luptakova, L.; Nowakowska, J.A.; Oszako, T.; Belbahri, L. *Bacillus velezensis*: A Treasure House of Bioactive Compounds of Medicinal, Biocontrol and Environmental Importance. *Forests* **2021**, *12*, 1714. [CrossRef]
82. Yang, J.F.; Yang, C.H.; Liang, M.T.; Gao, Z.J.; Wu, Y.W.; Chuang, L.Y. Chemical composition, antioxidant, and antibacterial activity of wood vinegar from Litchi chinensis. *Molecules* **2016**, *21*, 1150. [CrossRef]
83. Velmurugan, N.; Han, S.S.; Lee, Y.S. Antifungal activity of neutralized wood vinegar with water extracts of *Pinus densiflora* and *Quercus serrata* saw dusts. *Int. J. Environ. Res.* **2009**, *3*, 167–176.
84. Baimark, Y.; Niamsa, N. Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. *Biomass Bioenergy* **2009**, *33*, 994–998. [CrossRef]
85. Ma, X.H.; Wei, Q.; Zhang, S.S.; Shi, L.; Zhao, Z. Isolation and bioactivities of organic acids and phenols from walnut shell pyroligneous acid. *J. Anal. Appl. Pyrolysis* **2011**, *91*, 338–343. [CrossRef]
86. Amen-Chen, C.; Pakdel, H.; Roy, C. Production of monomeric phenols by thermochemical conversion of biomass: A review. *Bioresour. Technol.* **2001**, *79*, 277–299. [CrossRef]
87. Aguirre, J.L.; Baena, J.; Martín, M.T.; Nozal, L.; González, S.; Manjón, J.L.; Peinado, M. Composition, Ageing and Herbicidal Properties of Wood Vinegar Obtained through Fast Biomass Pyrolysis. *Energies* **2020**, *13*, 2418. [CrossRef]
88. Baimark, Y.; Threeprom, J.; Dumrongchai, N. Utilization of wood vinegars as sustainable coagulating and antifungal agents in the production of natural rubber sheets. *J. Environ. Sci. Technol.* **2008**, *1*, 157–163. [CrossRef]
89. Kadota, M.; Niimi, Y. Effects of charcoal with pyroligneous acid and barnyard manure on bedding plants. *Sci. Hort.* **2004**, *101*, 327–332. [CrossRef]
90. Orlo, E.; Russo, C.; Nugnes, R.; Lavorgna, M.; Isidori, M. Natural Methoxyphenol Compounds: Antimicrobial Activity against Foodborne Pathogens and Food Spoilage Bacteria, and Role in Antioxidant Processes. *Foods* **2021**, *10*, 1807. [CrossRef]
91. Li, N.; Jing, S.; Wang, H.; Cavaco-Paulo, A. Production of antimicrobial powders of guaiacol oligomers by a laccase-catalyzed synthesis reaction. *Process. Biochem.* **2021**, *111*, 213–220. [CrossRef]
92. Congguang, Z.; Shihai, L.; Fuqing, X.; Quan, B.; Ling, Q. Chemical Characteristics and Antimicrobial Performance of Wood Vinegar Produced from Pyrolysis of Polyploidy Mulberry Branches. *J. Biobased Mater. Bioenergy* **2019**, *13*, 812–819.
93. Mederios, L.C.D.; Gasparotto, L.H.S. Pyroligneous acid and antibacterial activity: Criticism of a paper by Araújo et al. (2018). *J. Appl. Microbiol.* **2022**, *132*, 1768–1770. [CrossRef]
94. Hoitink, H.A.J.; Powell, C.C. Fighting *Phytophthora*—A guide to combating *Phytophthora* root rot and dieback in ericaceous crops. *Am. Nurserym.* **1990**, *171*, 67–73.
95. Hoitink, H.A.J.; Inbar, Y.; Boehm, M.J. Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. *Plant Dis.* **1991**, *75*, 869–873. [CrossRef]