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Exploring the Potential of Wood Vinegar: Chemical Composition and Biological Effects on Crops and Pests

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Abstract: Wood vinegar is a by-product of the pyrolysis of organic raw materials. In this study, we investigated the chemical composition and biological activity of industrial wood vinegar derived from the pyrolysis of wood pruning waste. The composition of wood vinegar was characterized using liquid chromatography (LC) and gas chromatography–mass spectrometry (GC-MS). Wood vinegar bioactivity was tested against *Bactrocera oleae* under field conditions in an olive grove for two years. Furthermore, wood vinegar was applied in a greenhouse experiment with strawberry plants and in a strawberry field infested with the nematode *Meloidogyne incognita*. Finally, a seed root length bioassay was performed to evaluate the phytotoxicity or biostimulation of wood vinegar on *Eruca sativa*, *Lactuca sativa*, *Lens culinaryis*, *Lolium multiflorum*, and *Solanum lycopersicum*. Our results showed that wood vinegar had a pH of 3.2, with high concentrations of acetic acid (27,840.16 mg L^{-1}) and phenols (54.00 mg L^{-1}). No repellent effect against *B. oleae* was observed when wood vinegar was applied as an aerosol in olive groves. On strawberry plants in greenhouse conditions, wood vinegar showed phytotoxic effects at high concentrations, resulting in a decrease in the total yield of the plants. In the field, at a 1% concentration, wood vinegar led to a significant 15% reduction in the infection caused by *M. incognita* in strawberry plants. Finally, in the in vitro crop bioassay, wood vinegar demonstrated remarkable phytotoxicity effects at high concentrations while promoting root growth when diluted. The efficacy of wood vinegar displayed considerable variability based on concentration and delivery system, emphasizing the need for careful evaluation when considering its application, particularly in diverse crops and production systems.

Keywords: *Meloidogyne incognita*; LC-MS and GC-MS; olive fly; plant growth promotion

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1. Introduction

Pyrolysis is a process wherein waste or plant biomass undergoes degradation through heat in the shortage of oxygen [1,2]. The end products of pyrolysis typically encompass a gaseous fraction known as syngas, a liquid fraction comprising bio-oils, an aqueous fraction, and biochar as a solid fraction [3,4]. Technological applications of pyrolysis products in agroecosystems for these materials have been developed in recent decades [5]. For instance,

researchers have explored the potential of smoke and its derivatives for biotechnological applications concerning plant biostimulation and protection against pathogens and pests [6].

In earlier studies, the smoke generated from various feedstocks, such as wood, straw, and crop residues, was demonstrated to significantly stimulate seed germination and plant growth [7]. Regarding the mechanisms underlying the remarkable effects on seed germination and root biostimulation, Flematti et al. [8] discovered that karrikinolide (KAR1), a compound isolated from the smoke of burned cellulose, promotes the development of many plant species. Smoke also contains a highly active compound called butenolide, extracted from burned plant material and cellulose, which is thermostable and acts at very low concentrations [9]. Butenolide has been shown to stimulate lettuce germination at concentrations as low as 9–10 M [8] and to promote seed germination in numerous other plant species [10].

The bioactive products found in smoke can also be delivered in liquid form. De Lange and Boucher [11] were the first to create smoke water by burning plant tissue in a drum and channeling the smoke through distilled water using a compressor. More recently, Bonanomi et al. [12] demonstrated that smoke water, produced by bubbling smoke generated during biochar production into water, exhibits remarkable biological activities. It has potential applications for promoting plant growth when appropriately diluted and for controlling root-knot nematodes and the olive fruit fly (*Bactrocera oleae*), a significant pest in olive cultivation. However, in addition to the smoke water produced during pyrolysis, another bioactive substance, wood vinegar, can be obtained via condensation and distillation of the smoke [13]. Wood vinegar is a brown or reddish-yellow liquid rich in acetic acid, acetone, phenols, and various other organic compounds with antimicrobial activity [14]. Several studies have reported the ability of wood vinegar to inhibit the growth of pathogenic microbes such as *Salmonella* [15] and *Bacillus subtilis* [16] and plant pathogens such as *Colletotrichum capsici* [17] and *Phytophthora palmivora* [18]. Additionally, some studies have indicated that wood vinegar can control pests such as termites [19], mosquitoes [20], and houseflies [21].

Previous studies have typically investigated the capacity of wood vinegar either to enhance plant growth or to selectively inhibit a limited number of pests, with few reports focusing on simultaneous assessments of diverse agroecosystem organisms [12]. Additionally, prior research employed wood vinegar produced in laboratory settings or artisanal systems, leading to limited quantities and complicating the applicability to agricultural production systems due to the challenges in reproducing the results [6]. In this study, we opted for an industrial-grade wood vinegar obtained from biochar production using woody pruning waste as a feedstock. Initially, the wood vinegar underwent detailed chemical characterization through a combination of liquid chromatography (LC) and gas chromatography–mass spectrometry (GC-MS). Subsequently, we explored the efficacy of wood vinegar, applied as an aerosol, in olive orchards against *Bactrocera oleae* R. Furthermore, wood vinegar was tested on potted strawberry plants in a greenhouse and in open fields on strawberry plants infested with the nematode *Meloidogyne incognita*. Finally, the phytotoxic and biostimulant effects of wood vinegar were investigated in vitro on five plant species, namely, *Eruca sativa* L., *Lactuca sativa* L., *Lens culinaris* L., *Lolium multiflorum* L., and *Solanum lycopersicum* L. The specific hypotheses tested in this study were as follows:

i. Wood vinegar would exhibit acidity, with a chemical composition rich in biologically active compounds, including acetic acid, phenols, and cresol.
ii. The application of wood vinegar would result in a reduction in the infestation levels of *B. oleae* within olive orchards.
iii. Wood vinegar would exhibit concentration-dependent effects on crops, manifesting phytotoxicity at elevated concentrations and biostimulation when significantly diluted.

2. Materials and Methods

2.1. Wood Vinegar Production

The woody pruning residues, following collection in the orchards, were crushed into pieces no longer than 10 cm, and underwent a pyro-gasification process, reaching temperatures of 800 °C. The resulting biosyngases were cooled in order to condense a pyrolytic liquid, commonly referred to as wood vinegar (WV), which was collected and preserved in plastic containers.

2.2. Chemical and Metabolomic Analysis

Chemical analyses were carried out for the following parameters: total organic carbon (method: Dlgs 7276 of 31 May 2016 Suppl. 13 n. 2), acetic acid (method: IS 08.03/161 2017), propionic acid (method: IS 08.03/161 2017), total phenols (method: EPA 3510C 1996 + EPA 8270E 2018), pH [22], electrical conductivity [23], total suspended solids (method: APAT CNR IRSA 2090 B Man 29 2003), ammonia nitrogen (method: UNI 11669:2017), sulfates (method: APAT CNR IRSA 4020 Man 29 2003), sulfites (method: APAT CNR IRSA 4150 Man 29 2003), sulfides (method: APHA Standard Methods 4500), chlorides (method: APAT CNR IRSA 4020 Man 29 2003), total phosphorus (method: M.U. 2252:08), nitrates (method: APAT CNR IRSA 4020 Man 29 2003), aluminum (method: EPA 3015A 2007 + EPA 6020B 2014), arsenic (method: EPA 3015A 2007 + EPA 6020B 2014), iron (method: EPA 3015A 2007 + EPA 6020B 2014), manganese (method: EPA 3015A 2007 + EPA 6020B 2014), hydrocarbons (method: UNI EN 14039:2005), and acetone (method: EPA 5021A 2014 + EPA 8260D 2018).

For the extraction of nonpolar metabolites in wood vinegar, the method outlined by Flematti et al. [24] was employed. In brief, 1 L of wood vinegar underwent extraction three times with 200 mL of dichloromethane (DCM), followed by drying with anhydrous sodium sulfate (Na_2SO_4) and subsequent evaporation under vacuum at 40 °C in a rotary evaporator. The resulting extract underwent characterization through mass spectrometric analysis. DCM was reconstituted in methanol (MeOH) to achieve a final concentration of 1 mg mL^{-1}. A 7 µL volume was injected into an Agilent HP 1260 Infinity Series liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA), coupled with a quadrupole time-of-flight mass spectrometer (Q-TOF, Agilent Technologies, Santa Clara, CA, USA), and a diode array detector (DAD, Agilent Technologies, Santa Clara, CA, USA). Sample characterization adhered to the protocol detailed by Staropoli et al. [25].

The DCM extract was also analyzed using gas chromatography–mass spectrometry. Prior to analysis, an aliquot of the extract was resuspended in ethyl acetate to obtain a final concentration of 100 ppm, and the extract was derivatized with N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (Fluka, Buchs, Switzerland). The reaction was carried out in an ultrasonic bath (Sonorex, Bandelin Electronic GmbH & Co. K, Berlin, Germany) for 30 min. The sample was then injected into an Agilent 8890 GC instrument (Agilent Technologies) coupled to an Agilent 5977B MSD system (Agilent Technologies). The temperature gradient and other chromatographic and spectrometric parameters were adjusted according to the method described by Staropoli et al. [26].

2.3. Effect of Wood Vinegar on Bactrocera oleae under Field Conditions

During the summers of 2022 and 2023, field trials were carried out to assess the efficacy of wood vinegar as a repellent for *B. oleae* in an olive orchard. The orchard, featuring the Frantoio cultivar, was situated in Cicerale (southern Italy, 40°19′ N 15°07′ E, 186 m a.s.l.). Undiluted wood vinegar (100%) was utilized in falcon tubes (120 mm × 27 mm ø), each containing 30 mL of the solution. These tubes were strategically positioned in the canopy, with five tubes per canopy, affixed to branches using plastic wire (Supplementary Figure S1). The treatment application occurred in mid-June, organized in sets of three adjacent trees, totaling 15 falcon tubes. Each tube was covered with parafilm and sealed with a modified screw cap, featuring a single hole at the center to facilitate evaporation of wood vinegar. Two control treatments were implemented: the application of zeolite sprayed over the canopy, a product commonly used in organic agriculture to repel *B. oleae* [27], and spraying

olives with water. Zeolite was used to assess whether wood vinegar could serve as a replacement for zeolite as a repellent product. In mid-October, during the ripening stage, the frequency of *B. oleae* infestation was further assessed. In detail, the drupes from each tree were individually harvested using an olive harvester on a plastic net placed beneath the olive trees. For each plant, 300 olives were randomly selected from the net, and infestation was visually assessed by observing the entrance and exit holes of the pest. The entire experiment was repeated in 2023 using the same experimental design and methodology.

2.4. Effect of Wood Vinegar on Strawberry Plants

The objective of the first experiment with strawberry plants was to evaluate the effect of wood vinegar at different concentrations and identify the best ones to use in field trials. The experiment was carried out at the Department of Agriculture of the Federico II University in Naples, Italy (40°48′40.3″ N, 14°20′33.8″ E; 75 m a.s.l.). The area features a typical Mediterranean climate, characterized by an annual mean temperature of 16.2 °C, with monthly mean temperatures ranging from 25.9 °C in August to 9.1 °C in January. The experiment involved the application of four different percentages of wood vinegar, namely, 1%, 5%, and 10%, plus the control, where plants were treated with water, resulting in a total of 32 pots of 1.5 L (8 pots per treatment). A refrigerated strawberry plant was placed in each pot that received 200 mL of the respective wood vinegar percentage once a week. The pots were placed in a greenhouse from March to June 2022; after the four-month production cycle, the biometric data of the plants were quantified by measuring the dry weights of the roots, leaves, stem, and fruits.

2.5. Effect of Wood Vinegar on Meloidogyne incognita in the Strawberry Field

The experiment was conducted in Ferrara, Italy, with the experimental field situated in Lagosanto (44°46′ N 12°08′ E), on a farm specializing in the nursery production of strawberry plants. The regional climate is generally temperate, featuring an average annual temperature of 13.2 °C and an annual precipitation of 652 mm. The soil composition of the experimental field includes 70% sand, 21.6% silt, and 8.3% clay, with a pH of 7.9. Other soil attributes comprise 2.3% organic matter, 6% limestone, 1% active limestone, a conductivity of 275 μS cm^{-1}, and nutrient levels of 63.6% nitrogen, 145.6% phosphorus, and 137.2% potassium, with a C/O ratio of 10.3 (method: D.M. n.179 of 11 May 1992, D.M. n.185 of 13 September 1999). The experiment was conducted in large plots (2000 m^2) with three replications arranged in randomized blocks. Before initiating the experiment, the soil underwent fumigation treatment with chloropicrin, injected approximately 30 cm below the soil surface. Subsequently, the experimental field received applications of wood vinegar at two percentages: 1% and 5% at a rate of 2500 L ha^{-1}. Wood vinegar application occurred once every two weeks. Additionally, a standard NPK fertilizer was applied at rates of 150 kg ha^{-1} of nitrogen (N), 80 kg ha^{-1} of phosphorus (P), and 120 kg ha^{-1} of potassium (K). The production cycle for the production of strawberry plants began with the transplanting of mother plants in May and ended with the harvesting of the plants the following winter in January. At the end of the cycle in January 2023, we conducted an assessment of the plant sizes, categorizing them into three commercial categories based on their dimensions: large, medium, and small. Moreover, nematode infestation was evaluated following the methods of Hallman et al. [28].

2.6. Crop Phytotoxicity Bioassay

The experiment involved five plant species: *E. sativa*, *L. sativa*, *L. culinaris*, *L. multiflorum*, and *S. lycopersicum*. The in vitro experiment was conducted in 90 mm Petri dishes with seeds positioned on sterile filter paper (grade 5 Whatman). Each Petri dish was moistened with 10 mL of wood vinegar at one of five concentrations: 0.01%, 0.1%, 1%, 10%, and 100% (undiluted), along with a control group receiving distilled sterile water only. For each species, a total of 20 seeds were distributed across each Petri dish, resulting in 100 seeds per species (5 concentrations of wood vinegar × 20 seeds per Petri dish, including the control

with water only). The Petri dishes were then placed in a growth chamber set at 22 ± 2 °C in darkness. Seedling root length was measured after 72 h for *E. sativa* and *L. sativa* and after 168 h for *L. culinaris* and *L. multiflorum*, and *S. lycopersicum.*

2.7. Statistical Analysis

The statistical analysis of the experimental data was conducted using the analysis of variance (ANOVA) test to assess the overall differences among the treatment groups for each variable measured. Following the ANOVA, a pairwise Duncan post hoc test was performed to identify specific differences between pairs of treatment groups. All statistical analyses were carried out using STATISTICA 13 software (TIBCO Software, New York, NY, USA). The significance level for all statistical analyses was set at $p < 0.05$.

3. Results

3.1. Wood Vinegar Chemistry and Metabolomics

The wood vinegar exhibited acidic properties with a pH of 3.2 and an electrical conductivity of 1389.10 μS cm^{-1}. The analysis revealed a concentration of 54.00 mg L^{-1} of phenol and a 27,840.16 mg L^{-1} (2.78%) concentration of acetic acid. The composition included 26.00 mg L^{-1} of total suspended solids; 8.30 mg L^{-1} of ammonium nitrogen; 72,447.06 mg L^{-1} of propionic acid; and sulfates, sulfites, and sulfides at levels of 53.70, 0.32, and 0.12 mg L^{-1}, respectively. Chlorides were measured at 29.20 mg L^{-1}, with 0.97 mg L^{-1} of total phosphorus, 5.20 mg L^{-1} of nitrates, 0.92 mg L^{-1} of aluminum, 0.02 mg L^{-1} of arsenic, 183.00 mg L^{-1} of iron, 1.11 mg L^{-1} of manganese, 15,600.00 mg L^{-1} of total organic carbon, 77.10 mg L^{-1} of hydrocarbons, and 320.00 mg L^{-1} of acetone (Table 1). Further analysis of the organic extract using LC-MS identified 54 compounds, with 2 compounds putatively identified through comparison with an internal database for plant secondary metabolites. These compounds were nicotinic acid (also known as niacin or vitamin B3) and malvalic acid (Table 2). The DCM extract of the wood vinegar underwent GC-MS analysis, revealing 133 chromatographic peaks, freom which 23 compounds were identified by comparing their mass spectra with the NIST library and their retention index (Table 3). The identified compounds belonged to various classes, including phenolic derivatives (guaiacol, syringol, eugenol, catechol, etc.), fatty acids (oleic, stearic, and palmitic acids), polyalcohols (glycerol), and pyrimidine derivatives (2,5-dimethyl-4-pyrimidinamine).

Table 1. Chemical and biochemical parameters of wood vinegar.

Parameter	Unit	Result
Acetic acid	mg L^{-1}	27,840.1
Propionic acid	mg L^{-1}	72,447.0
Phenols	mg L^{-1}	54.1
pH	-	3.2
Electrical conductivity	μS cm^{-1}	1389.1
Total suspended solids	mg L^{-1}	26.0
Ammonia nitrogen	mg L^{-1}	8.3
Sulfates	mg L^{-1}	53.7
Sulfites	mg L^{-1}	0.32
Sulfides	mg L^{-1}	0.12
Chlorides	mg L^{-1}	29.2
Total phosphorus	mg L^{-1}	0.97
Nitrates	mg L^{-1}	5.2
Aluminum	mg L^{-1}	0.92
Arsenic	mg L^{-1}	0.02
Iron	mg L^{-1}	183
Manganese	mg L^{-1}	1.11
Total organic carbon	mg L^{-1}	15,600.4
Hydrocarbons	mg L^{-1}	77.1
Acetone	mg L^{-1}	320.0

Table 2. Putatively identified wood vinegar compounds by LC-MS analysis; RT = retention time.

Name	Formula	Monoisotopic Mass (Da)	RT (min)
Nicotinic acid	$C_6H_5NO_2$	123.0318	1.475
Malvalic acid	$C_{18}H_{32}O_2$	280.2424	6.697

Table 3. Identified wood vinegar compounds by GC-MS analysis (RT = retention time).

Name	RT (min)
Phenol, TMS derivative	5.617
o-Cresol, TMS derivative	6.673
m-Cresol, TMS derivative	6.8
p-Cresol, TMS derivative	6.938
Guaiacol, TMS derivative	8
Diethylene glycol, 2TMS derivative	8.231
Glycerol, 3TMS derivative	8.699
2-Methyl-4-oxo-3,4-dihydro-2H-pyran-3-yl isobutyrate	8.834
Catechol, 2TMS derivative	9.279
2,5-Dimethyl-4-pyrimidinamine, TMS derivative	9.575
4-Methylcatechol, 2TMS derivative	10.242
Vanillin, TMS derivative	10.271
Syringol, TMS derivative	10.374
Protocatechuic aldehyde, 2TMS derivative	11.141
4-Ethylsyingol, TMS derivative	12.035
Eugenol, TMS derivative	12.161
m-Ethoxycarbonylaniline	12.301
2-Methoxymandelic acid, ethyl ester, TMS derivative	13.429
Syringaldehyde, TMS derivative	13.785
Vanillic acid, 2TMS derivative	13.838
Propyl vanillate, TMS derivative	14.586
Palmitic acid, TMS derivative	15.24
Oleic acid, (Z)-, TMS derivative	15.301
Stearic acid, TMS derivative	16.085

3.2. Effects of Wood Vinegar on Bactrocera oleae

Overall, the results indicated a statistically significant difference among the tested treatments concerning *B. oleae* infestation (Supplementary Table S1). Notably, significant distinctions were observed between trees treated with zeolite and the control trees. In contrast, wood vinegar exhibited no repellent effect on the flies under field conditions when compared to untreated control trees. These findings remained consistent during the second year of data collection (Figure 1).

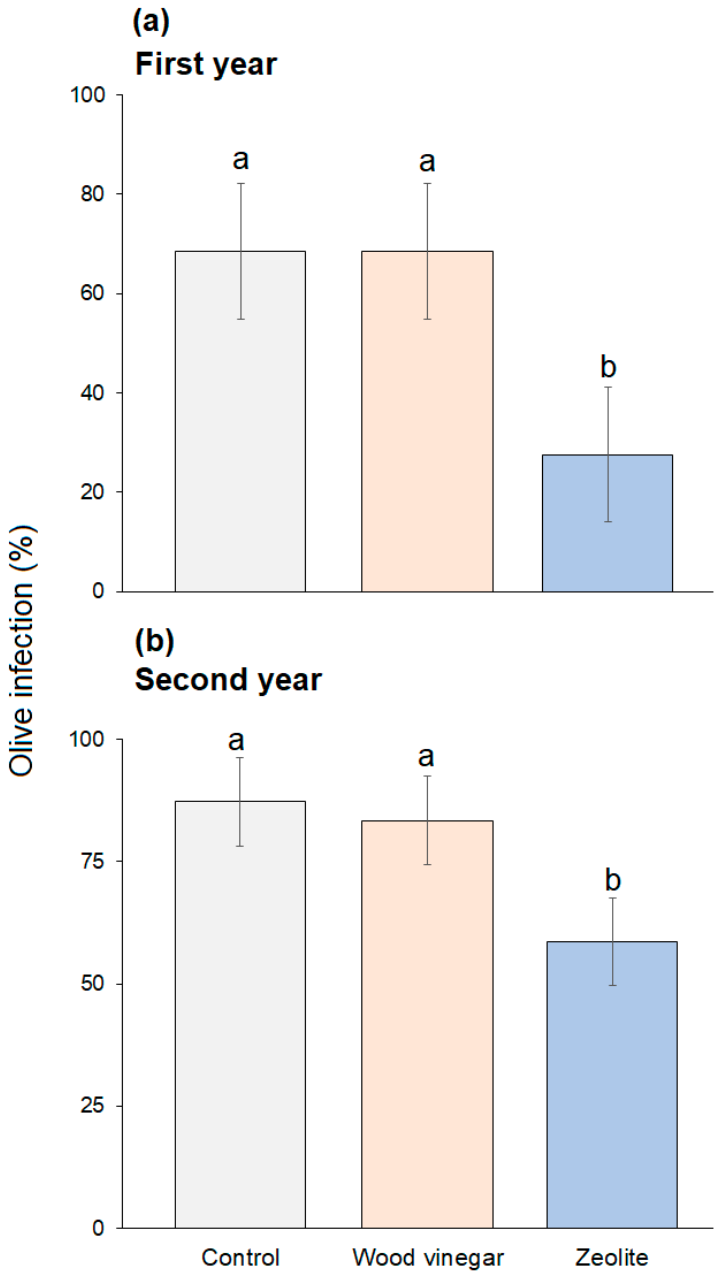

Figure 1. Percentage of olive drupe infection with *B. oleae* following three different treatments: wood vinegar, zeolite, and control without treatment, after the end of the first (**a**) and second year (**b**) of the experiment. Data are expressed as a percentage of fly-infested olives. Different letters indicate statistically significant differences (Duncan's test, $p < 0.05$).

3.3. Effects of Wood Vinegar on Strawberry Plants

The findings revealed a statistically significant difference among the tested treatments with respect to the total yield of strawberry plants. Notably, significant distinctions were evident between the strawberry plants treated with 5% and 10% as compared to the control. In particular, a notable decrease in the yield of strawberry plants treated with higher percentages of wood vinegar was observed. This reduction was observed in different components of the plant, including the leaves, fruits, stem, and roots (Figure 2).

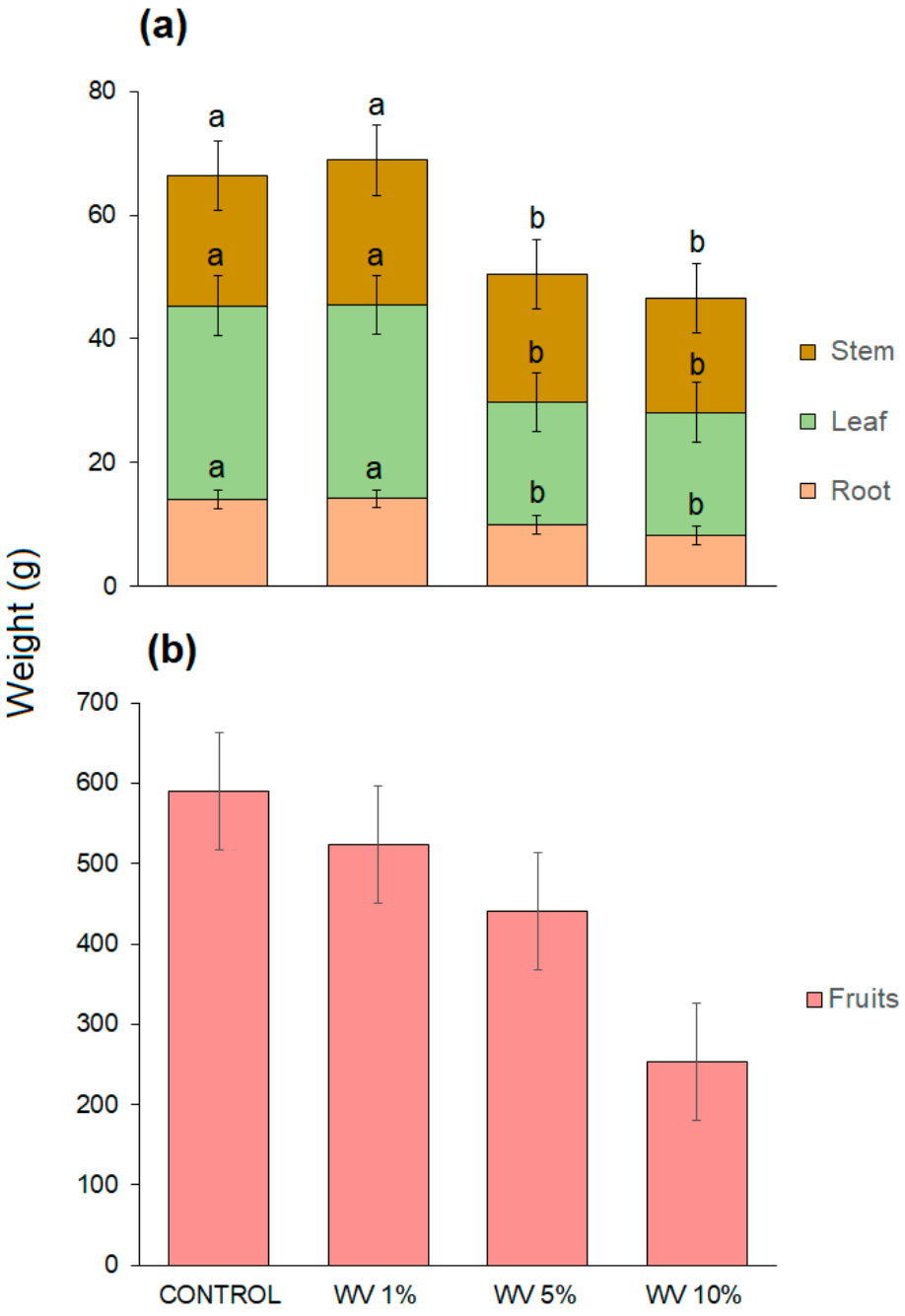

Figure 2. Total fresh weight of stem, leaves, and roots, following three different treatments: 1%, 5% or 10% wood vinegar (**a**) and total fruit weight (**b**). The values are the average of eight replicates ± the standard deviation. Different letters indicate statistically significant differences (Duncan's test, $p < 0.05$).

3.4. Effects of Wood Vinegar on Meloidogyne incognita

In the field experiment, the outcomes revealed a statistically significant difference concerning strawberry plants infected with the nematode *M. incognita*. Notably, significant distinctions were observed in the strawberry field treated with 1% wood vinegar, which resulted in a notable decrease in infection by 15% compared to that in the control. Additionally, in terms of plant size, a general increase was observed in the strawberry fields where treatments of 1% and 5% wood vinegar were applied (Figure 3).

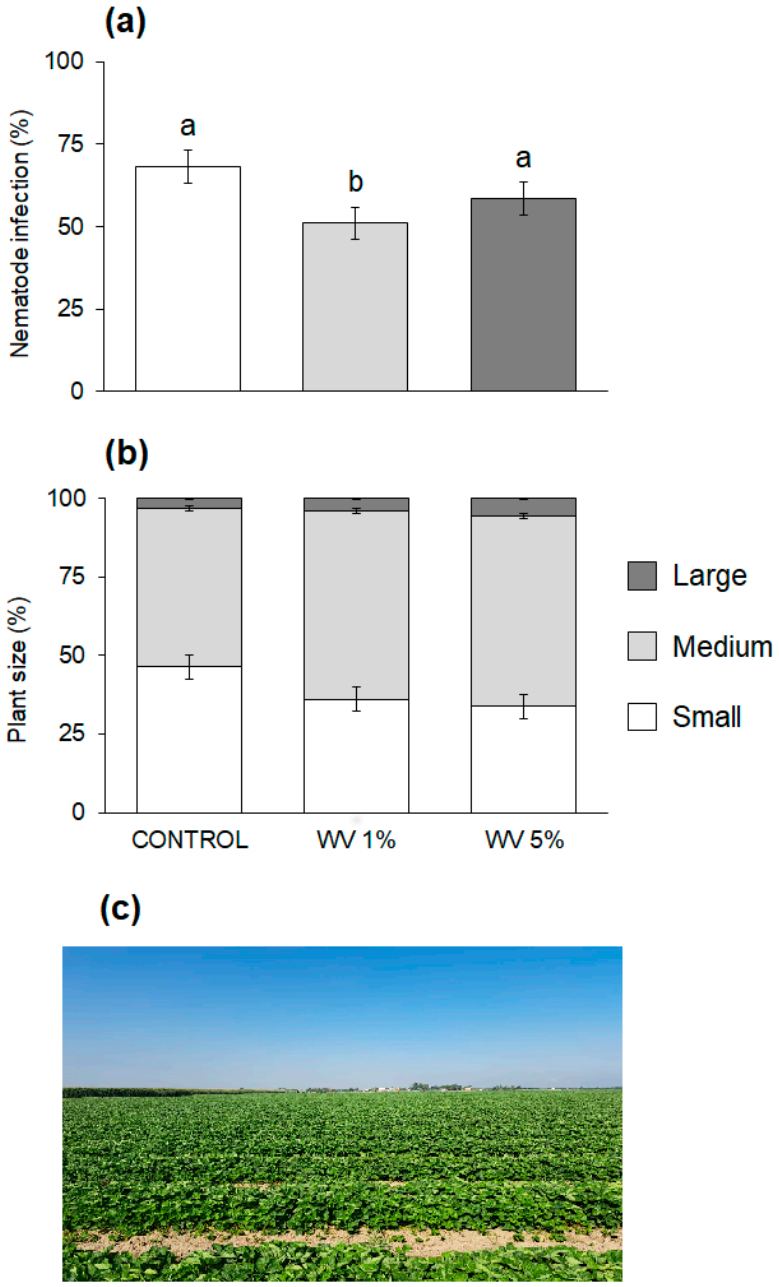

Figure 3. Infection rate of strawberry plants with *M. incognita* following two different treatments: 1% or 5% of wood vinegar, and control without treatment (**a**); distribution of plants in commercial categories following the treatments (**b**); and picture of the experimental strawberry field (**c**). Data are expressed as the percentage of strawberry plants infected by the nematode. Different letters indicate statistically significant differences (Duncan's test, $p < 0.05$).

3.5. Plant Bioassay

The application of wood vinegar exerted a significant impact on root length across all tested plant species (Supplementary Table S2). Our findings indicate that lower concentrations of wood vinegar, such as 1%, 0.1%, and 0.01%, fostered growth in all the plant species under examination. Specifically, *S. lycopersicum*, *L. multiflorum*, *L. sativa*, and *L. culinaris* exhibited significantly enhanced root growth, peaking at 0.1%, and subsequently declining with an increase in wood vinegar concentration, ultimately leading to complete inhibition at 100%. Conversely, for *E. sativa*, no concentration-dependent effect was observed among 1%, 0.1%, and 0.01%, but complete inhibition of root growth was noted at 10% (Figure 4).

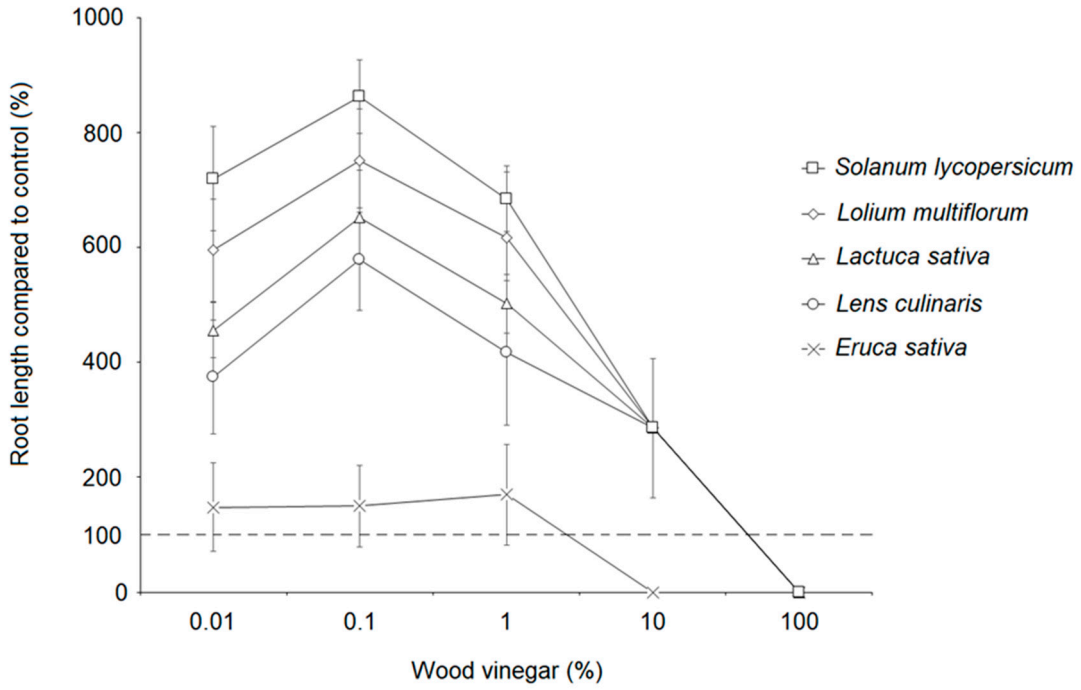

Figure 4. Effects of wood vinegar at five concentrations on the root length of five seed types. Data are expressed as percentages compared to the control without treatment (=100%), associated statistics are reported in Supplementary Table S2.

4. Discussion

Regarding *B. oleae* infestation on olives, our study revealed that wood vinegar did not reduce the incidence of this pest, contrary to expectations based on prior research. Specifically, Bonanomi et al. [12] reported a repellent effect of smoked water on *B. oleae*, reversing the attraction of adult flies to fresh olives. Smoking has historically been employed to protect food from insect attacks, although the underlying mechanisms remain insufficiently understood. Scarpati et al. [29] and Liscia et al. [30] identified various volatile compounds released by olive trees, influencing the behavior of *B. oleae*. The chemical composition of wood vinegar, distinct from smoked water, may explain the disparate responses observed. Jesu et al. [31] demonstrated a significant reduction in *B. oleae* infestation through the aerosol application of smoked water in olive groves, suggesting its potential as a control agent. In our study, the lack of control effectiveness with wood vinegar might be attributed to differences in the chemical profiles of wood vinegar and smoked water, both produced through pyrolysis. Analyzing the GC-MS results highlighted compounds unique to wood vinegar (e.g., cresols, glycerol, catechol, syringol, and eugenol) and others exclusive to smoked water (e.g., cyclopentanone, maltol, and furfural). Additionally, the concentration used (100%) might have saturated olfactory receptors, potentially diminishing the repellent effect. For example, in the *Aedes aegypti* mosquito, continuous exposure to the repellent N,N-diethyl-m-toluamide (DEET), one of the most-used mosquito repellents, resulted in a decreased response in mosquitoes, which showed behavioral insensitivity. The authors suggested that this insensitivity be caused by a reduction in the volume of glomeruli and the corresponding synapse loss over long-term exposure to the chemical, as well as to a nonassociative learning process, such as habituation [32]. Further investigations incorporating various concentrations and delivery systems, such as aerosol or direct canopy application, are imperative to comprehensively assess wood vinegar's potential to control *B. oleae* under field conditions, taking into consideration that the direct application of wood vinegar, due to the presence of compounds considered toxic, could be phytotoxic, unlike aerosol application.

Earlier investigations [11,12] have noted that the effects of smoke water, derived from burned plant tissue, were concentration-dependent, leading to phytotoxic effects at higher concentrations and neutral or stimulatory effects at lower concentrations. Similarly, our findings with wood vinegar demonstrated a comparable response in plant species, where undiluted solutions resulted in complete inhibition, while highly diluted solutions stimulated root growth. This dual response was evident in both the crop bioassay and the greenhouse experiment. The phenomenon of hormesis, wherein organic chemical inhibitors can promote root growth even at low concentrations, likely explains the concentration-dependent responses observed, consistent with previous findings on biochar [33] and wood vinegar. The potential role of karrikins, specifically butenolide 3-methyl-2H-furo [2,3-c] pyran-2-one (KAR1), known plant growth regulators found in smoke from burned plant tissue, was considered. Karrikins have been linked to stimulatory effects on seed germination and root and shoot growth in various plant taxa, including non-fire-adapted species like *Arabidopsis thaliana* [34]. While karrikins were detected in smoked water, their absence in wood vinegar suggests the involvement of other substances in the observed stimulation of plant growth at low concentrations. Further studies are necessary to identify these substances contributing to plant development at low concentrations while potentially being phytotoxic at higher concentrations. Understanding these mechanisms will enhance our knowledge of wood vinegar's impact on plant growth and aid in its judicious application in agricultural practices.

As indicated by our chemical analyses, wood vinegar exhibits an exceptionally low pH, suggesting potential direct phytotoxic effects on root growth [35]. The acidity of wood vinegar is known to influence the solubility of allelochemical compounds, such as organic acids and phenols, thereby amplifying their phytotoxicity under acidic conditions [36]. Additionally, the phytotoxicity of heavy metals, including aluminum and manganese, is reported to increase at low pH [37]. Furthermore, our GC-MS analyses unveiled the presence of numerous phenolic compounds. Considering the pronounced phytotoxicity observed with undiluted wood vinegar, recent studies have explored its potential use as a herbicide [38,39]. For instance, wood vinegar derived from the pyrolysis of apple branches has demonstrated efficacy as a nonselective herbicide in dormant turfs and for general weed control [39,40]. These findings suggest a promising new application of wood vinegar as an effective herbicide, particularly when applied to bare soil before seeding. Further research in this direction may unveil practical strategies for harnessing the herbicidal properties of wood vinegar in various agricultural contexts.

Plant parasitic nematodes pose a significant threat to annual crop yields, representing a major challenge to global food security given their widespread association with various crops. Surprisingly, the impact of wood vinegar on plant-parasitic nematodes has not been explored in previous studies. Recently, Marra et al. [41] demonstrated the nematocidal effect of water extracts from biochar produced from olive mill waste on juveniles of *M. incognita* under laboratory conditions. Building upon these findings, our study suggests that wood vinegar may have the potential to reduce the population of plant-parasitic nematodes in the soil. The inhibitory effects observed may be attributed to the phenolics and other phytochemical compounds present in wood vinegar. While these results are promising, further in vivo investigations are essential to comprehensively understand the role and impact of wood vinegar on soil nematode populations. Specifically, additional research is warranted to assess the effectiveness of wood vinegar against different nematode species, such as *Meloidogyne*, and to explore the underlying mechanisms contributing to its nematocidal properties. This knowledge could pave the way for innovative strategies in nematode management within agricultural systems.

5. Conclusions

In conclusion, the current body of research on the effects of wood vinegar on various pests is limited, necessitating new trials tailored to each cropping system. To unlock the full potential of wood vinegar, it is crucial to determine the optimal concentration and

the most effective delivery system for specific agricultural applications. For instance, the inherent phytotoxicity of wood vinegar could be harnessed for herbicidal purposes using the undiluted product, while the stimulation of plant growth might be achieved through the application of the same material at diluted concentrations. Our study underscores the notable biological activity of wood vinegar against both pests and crops. However, it emphasizes that the concentration and mode of administration are pivotal factors that significantly influence the effectiveness of this product across diverse cropping systems. Future research actions should explore and refine these variables to ensure the judicious and efficient application of wood vinegar in diverse agricultural contexts. Through this ongoing exploration, wood vinegar holds promise as a versatile and eco-friendly tool for pest management and crop enhancement in agricultural practices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14010114/s1, Figure S1: Photos of field trials (A) to test the effect of wood vinegar delivered from 50 mL plastic tubes fixed under tree canopy (B, arrows) to evaluate the repellent effect towards B. oleae in olive groves in Cicerale (Southern Italy); Table S1: Summary of the Generalized Linear Modelling (GLM) testing (significant values in bold at $p < 0.05$) for main effects of wood vinegar treatments on *Bactrocera oleae*; Table S2: Summary of the Generalized Linear Modelling (GLM) testing (significant values in bold at $p < 0.05$) for main and interactive effects of plant species and tested concentrations.

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