environments

Article

Effects of Wood Distillate (Pyroligneous Acid) on the Yield Parameters and Mineral Composition of Three Leguminous Crops

Pablo Carril [1], Elisabetta Bianchi [1,2,*], Costanza Cicchi [3], Andrea Coppi [2], Marco Dainelli [2], Cristina Gonnelli [2], Stefano Loppi [1,4], Luigia Pazzagli [3] and Ilaria Colzi [2]

1 Department of Life Sciences, University of Siena, Via Mattioli 3, 53100 Siena, Italy; pablo.carril@unisi.it (P.C.); stefano.loppi@unisi.it (S.L.)
2 Department of Biology, University of Florence, Via Micheli 1, 50121 Florence, Italy; andrea.coppi@unifi.it (A.C.); marco.dainelli@unifi.it (M.D.); cristina.gonnelli@unifi.it (C.G.); ilaria.colzi@unifi.it (I.C.)
3 Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Viale Morgagni 50, 50134 Florence, Italy; costanza.cicchi@stud.unifi.it (C.C.); luigia.pazzagli@unifi.it (L.P.)
4 BAT Center—Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples 'Federico II', 80055 Naples, Italy
* Correspondence: e.bianchi@unifi.it

Abstract: The excessive use of chemical fertilizers and pesticides in agriculture is increasing the demand for novel products to improve the quality of crops in a more sustainable way. Wood distillate (WD, pyroligneous acid) is a by-product obtained during the pyrolysis of plant biomass that can be successfully applied in agriculture due to its ability to enhance the growth, size, and weight of edible plant parts. However, there is little information concerning its plant yield-promoting effects on leguminous crops. The present work investigated the effects of WD on the yield, protein content and mineral composition of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* L.) and bean (*Phaseolus vulgaris* L.) plants grown in field conditions. The application of WD showed remarkable yield-promoting effects mostly in lentil plants, which significantly increased plant and shoot biomass, the number and weight of both pods and seeds, as well as the total seed protein content. Furthermore, seeds from WD-treated plants differentially increased the concentration of elements with high nutritional value for human health, including Fe, Ca, Mg and K. These results suggest that the effects of WD among the legumes tested are species-specific and that WD could be an optimal candidate to grow high-yielding legumes with improved seed nutritional quality.

Keywords: wood distillate; chickpea; lentil; bean; seed quality; minerals; proteins; crop yield

check for updates

Citation: Carril, P.; Bianchi, E.; Cicchi, C.; Coppi, A.; Dainelli, M.; Gonnelli, C.; Loppi, S.; Pazzagli, L.; Colzi, I. Effects of Wood Distillate (Pyroligneous Acid) on the Yield Parameters and Mineral Composition of Three Leguminous Crops. *Environments* **2023**, *10*, 126. https://doi.org/10.3390/environments10070126

Academic Editor: Sergio Ulgiati

Received: 28 May 2023
Revised: 14 July 2023
Accepted: 17 July 2023
Published: 19 July 2023

1. Introduction

Breeding crops for 8 billion humans without impacting natural resources is one of the major challenges of current agriculture [1]. To keep pace with population growth, crop production has relied on the application of chemical fertilizers and pesticides. However, their widespread use has led to a rapid degradation of natural resources and the unsustainable use of soil and water [2]. Such critical environmental issues demand alternative measures to improve crop production without jeopardizing human health, farm animals and the environment.

In this context, the valorization of waste plant biomass is becoming a promising approach to find valuable and more eco-friendly products for agricultural application [3]. Recently, pyroligneus acid, also known as wood distillate (WD), was included in the list of products that can be used in organic farming in Italy [4]. WD is a by-product obtained from the distillation of gases produced during the pyrolysis of woody biomass [5]. It is composed of over 200 water-soluble compounds, including phenols, tannins, esters and acetic acids, which are co-related with both its plant growth- and defense-promoting effects [3]. The chemical composition of WD can vary depending on the type of wood (conifer or broadleaf) and plant species used as feedstock [6]. As a consequence, these factors also determine the specific effects of WD on plants [7].

The positive effects of WD have been demonstrated in both horticultural and cereal crops. The foliar application of WD has been shown to protect lettuce (*Lactuca sativa* L.) plants from ozone-induced damage and to positively affect not only biomass accumulation, but also its chlorophyll and sugar content [8–10]. In addition, the beneficial effects of WD have been linked to increased N and P content in basil (*Ocimum basilicum* L.) [11], to an enhanced number of fruits and increased elemental composition in tomato [12], to increased growth, fruit weight and sweetness in rockmelon [13], and to increased shoot growth and grain yield in rice (*Oryza sativa* L.) [14]. To the best of our knowledge, only one study has investigated the effects of WD in legumes [15], and this study showed that chickpea (*Cicer arietinum* L.) plants weekly sprayed with 0.25% (v/v) chestnut-derived WD increased both seed weight and diameter, as well as both seed antioxidant power and seed protein content [15].

Legumes are among the most important crops in agriculture, accounting for 27% of the world's primary food production [16]. They are an excellent source of proteins, carbohydrates, and minerals, such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), and are gaining preference over animal proteins for human consumption [17,18]. In addition, there is a growing recognition of the contribution of legumes to the critical targets under Sustainable Development Goal 2 set out by the FAO, particularly regarding food access, smallholder incomes, as well as sustainable and resilient agriculture [19,20]. However, the unavailability of an adequate number of high-quality seeds and the lack of sustainable growth-boosters are major constraints in legume cultivation, which is often not sufficient to obtain viable production, especially in developing countries [21,22].

Although WD may be a promising alternative to sustainably increase crop production, its effect across different legume plants has never been investigated. In light of the ecological and economic benefits of legumes, this study aims to investigate whether WD has positive effects on the yield parameters and the nutritional content of bean (*Phaseolus vulgaris* L.), chickpea (*C. arietinum* L.), and lentil (*Lens culinaris* L.), three of the most important leguminous crops for human consumption worldwide [23,24]. The present work expands scientific knowledge on the possible phytostimulatory effects of WD and its potential role in the sustainable improvement of legume production.

2. Materials and Methods

2.1. Plant Material, Growth Conditions and Yield Parameters

Bean (*Phaseolus vulgaris* L.), chickpea (*C. arietinum* L.), and lentil (*Lens culinaris* L.) seeds were kindly provided by Del Colle Srl. Chestnut (*Castanea sativa* Mill.). Wood distillate (WD, BioDea®, Arezzo, Italy) was chosen owing to its previously reported beneficial effects on chickpea plants [15]. The WD provided by the producer possessed the following characteristics: pH 3.5–4.5; acetic acid 2–2.3% (v/v); density 1.05 kg L^{-1}; and polyphenol content in the range of 22–25 g L^{-1}. The element concentration in pure WD was determined as described in Section 2.3. and it was as follows: Fe 3.2 ± 0.05 mg L^{-1}, Na 4.9 ± 0.4 mg L^{-1}, K 32.9 ± 0.6 mg L^{-1}, Ca 944.2 ± 5.3 mg L^{-1}, Zn 3.6 ± 0.1 mg L^{-1}, and Mg 16.0 ± 1.0 mg L^{-1}. Thus, approximately the following contents of nutritional elements were sprayed onto the leaves (mg): Fe 0.0006, Na < 0.001, K 0.006, Ca 0.018, Zn 0.0007 and Mg 0.0003; or were used for the fertigation (mg): Fe 0.001, Na > 0.001, K 0.009, Ca 0.02, Zn 0.001 and Mg 0.0004. Seeds of each legume species were sown in separate plots in a crop field of Del Colle Srl. located at Bientina (Pisa, Tuscany, Italy). Plants were grown in a soil characterized by 26% clay, 37% sand, 3.3% organic matter, 17 cmol Kg^{-1} of cation-exchange capacity and a pH of 7.9. No mineral fertilizers were added. Each plot (2.5 m × 4 m) consisted of six rows at 50 cm intervals, and twenty seeds per row were sown at 20 cm intervals (120 plants per each legume species; sowing density: 12 seeds/m^2). Approximately 100 mL of WD was applied for both foliar and fertigation treatments, previously diluting the WD in tap water to a 0.2% (*v*/*v*) concentration for foliar treatments and to 0.3% (*v*/*v*) for fertigation treatments, according to the producer's instructions. Plants from the first three rows had their leaves sprayed weekly and were fertigated every two weeks with WD. The other three rows were treated in the same way but using tap water only (control). Plants were grown for four months (April–July 2022), until they were dry. Both whole plants and shoots were individually weighed, and the number, mean weight and total weight of both pods and seeds were recorded.

2.2. Protein Quantification

For protein quantification, ten seeds from each plant were randomly selected and pooled. To extract and quantify proteins, dry seeds were crushed with a mortar and pestle and then ground with an Ultra Turrax (T 25 Stirrer ULTRA-TURRAX®, IKA-Werke GmbH & Co. KG, Staufen, Germany)) at 220 V and 170 W for 20 s, with 5 cycles. Then, 0.1 g of then fine powder obtained was weighed out and added to 1 mL of a glacial extracting solution 10% TCA/acetone, according to [25]. The proteins were left to precipitate overnight at −20 °C and then the samples were centrifuged at 13,200× *g* for 15 min at 4 °C. The pellet was washed twice with cold acetone and centrifuged again, then the supernatant was discarded, and the pellet was dried to remove residual acetone. The proteins extracted from the samples were resuspended in PBS (phosphate saline buffer, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 8 mM, Na_2HPO_4, 2 mM KH_2PO_4) and finally they were quantified using the Bradford assay [26]. The results were reported as mg of protein in 100 mg of seeds.

2.3. Mineral Determination in the Seeds and in WD

For element quantification, ten seeds from each plant were randomly selected and pooled. Seeds were carefully washed with demineralized water, dried at 70 °C for two days, ground with a mortar and pestle, and then dried again. About 0.1 g of seed powder was mineralized with 10 mL of 69% HNO_3 in a microwave digestion system (Mars 6, CEM Matthews, NC, USA) with maximum temperature of 200 °C for 10 min [27]. In the case of WD, 20 mL of pure WD was mineralized with 1.5 mL of 69% HNO_3 and 1 mL 37% HCl, according to [28]. Samples were diluted at a 1/20 dilution ratio with 0.5% $LaCl_3$ in 1% HNO_3. The concentration of micro- and macro-elements (K, Ca, Mg, Fe, Zn, and Na) was measured by means of atomic absorption spectroscopy (PinAAcle 500, Perkin Elmer) and expressed on a dry weight basis in the case of seeds, and in mg/L in the case of WD. To verify the method's reliability and accuracy, certified reference materials (grade BCR, Fluka Analytical, Sigma-Aldrich) were used. Recoveries were within ±10% and the precision was >95%.

2.4. Statistical Analysis

The data approached a normal distribution (Shapiro–Wilk test, $p < 0.05$), and hence a Student's *t*-test for independent samples was used to check for statistically significant ($p < 0.05$) differences between plants treated with water (control) and those treated with WD.

3. Results

3.1. Yield Parameters

The application of WD positively affected all investigated yield parameters in *L. culinaris*. Both total and above-ground plant biomass in this species increased significantly (3.5 g in WD-treated vs. 2.15 g in control plants) (Figure 1a,b). In addition, WD-treated *L. culinaris* showed an increased number of both pods (37.4 in WD-treated vs. 23.5 in control) and seeds (50.6 in WD-treated vs. 29.6 in control) per plant (Figure 1c,f), as well as an increase in the total and mean weight of both pods (2.4 g in WD-treated vs. 0.9 g in control) and in the mean weight of pods (0.05 g in WD-treated vs. 0.03 g in control plants) (Figure 1d,e). Moreover, an increase in the total weight of seeds (2.11 g in WD-treated vs.

0.9 g in control) and the mean weight of seeds (0.04 g in WD-treated vs. 0.03 g in control) (Figure 1g,h) per plant was also recorded in *L. culinaris*. Regarding the other two species, only *P. vulgaris* showed a significant increase in the number of pods (3.6 in WD-treated vs. 2.5 in control).

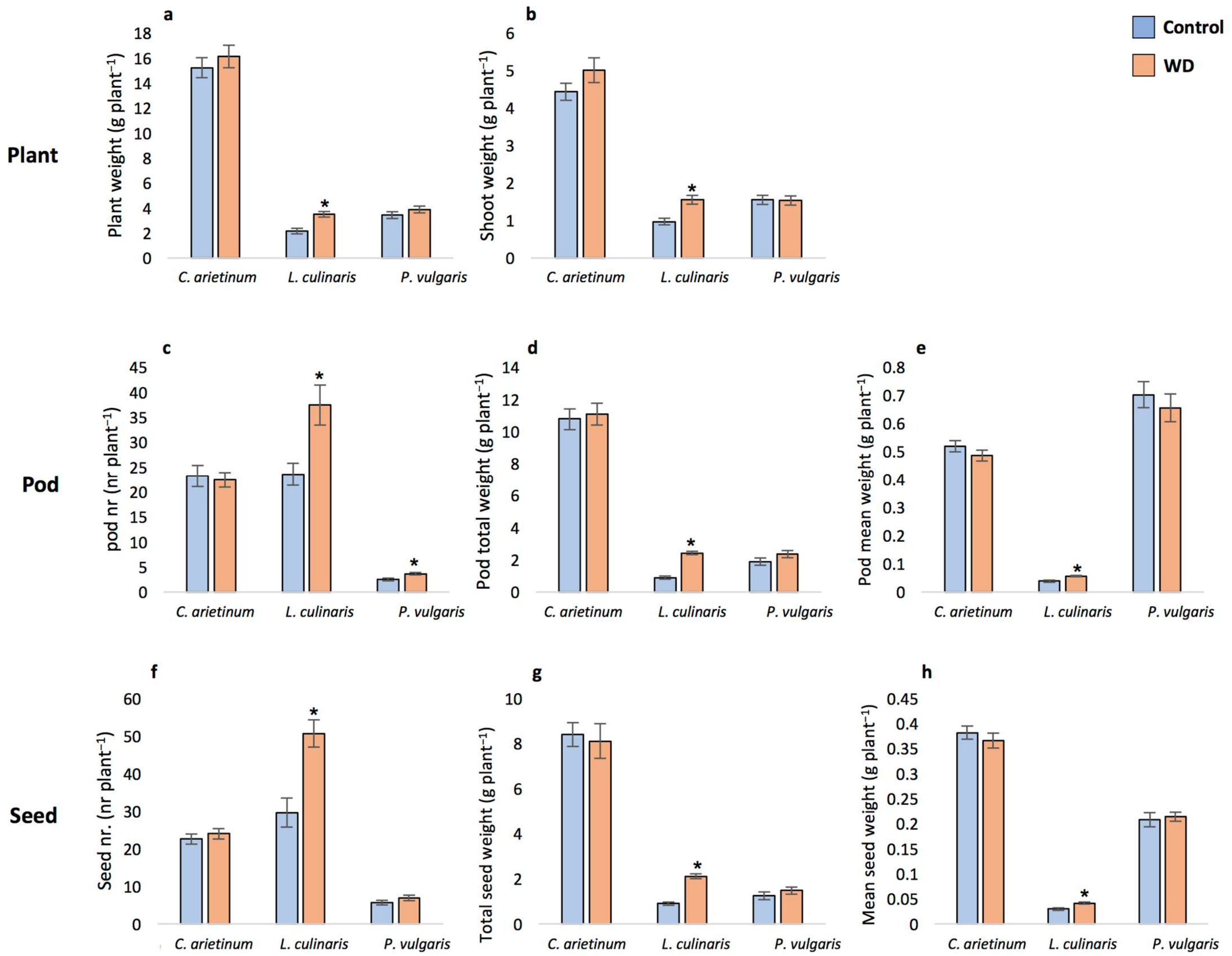

Figure 1. Yield parameters (mean ± standard error) of *C. arietinum*, *L. culinaris* and *P. vulgaris* plants treated with water (Control) or with 0.2% wood distillate (WD). (**a**) Plant fresh weight; (**b**) shoot fresh weight; (**c**) pod number; (**d**) total pod weight; (**e**) mean pod weight; (**f**) seed number; (**g**) total seed weight; (**h**) mean seed weight. * = Significant difference between control and WD-treated plants.

3.2. Total Proteins and Mineral Composition

Lentil seeds from WD-treated plants showed a significantly higher concentration of total proteins (17% in WD-treated vs. 15% in control), while no significant changes were found in *C. arietinum* and *P. vulgaris* (Figure 2a).

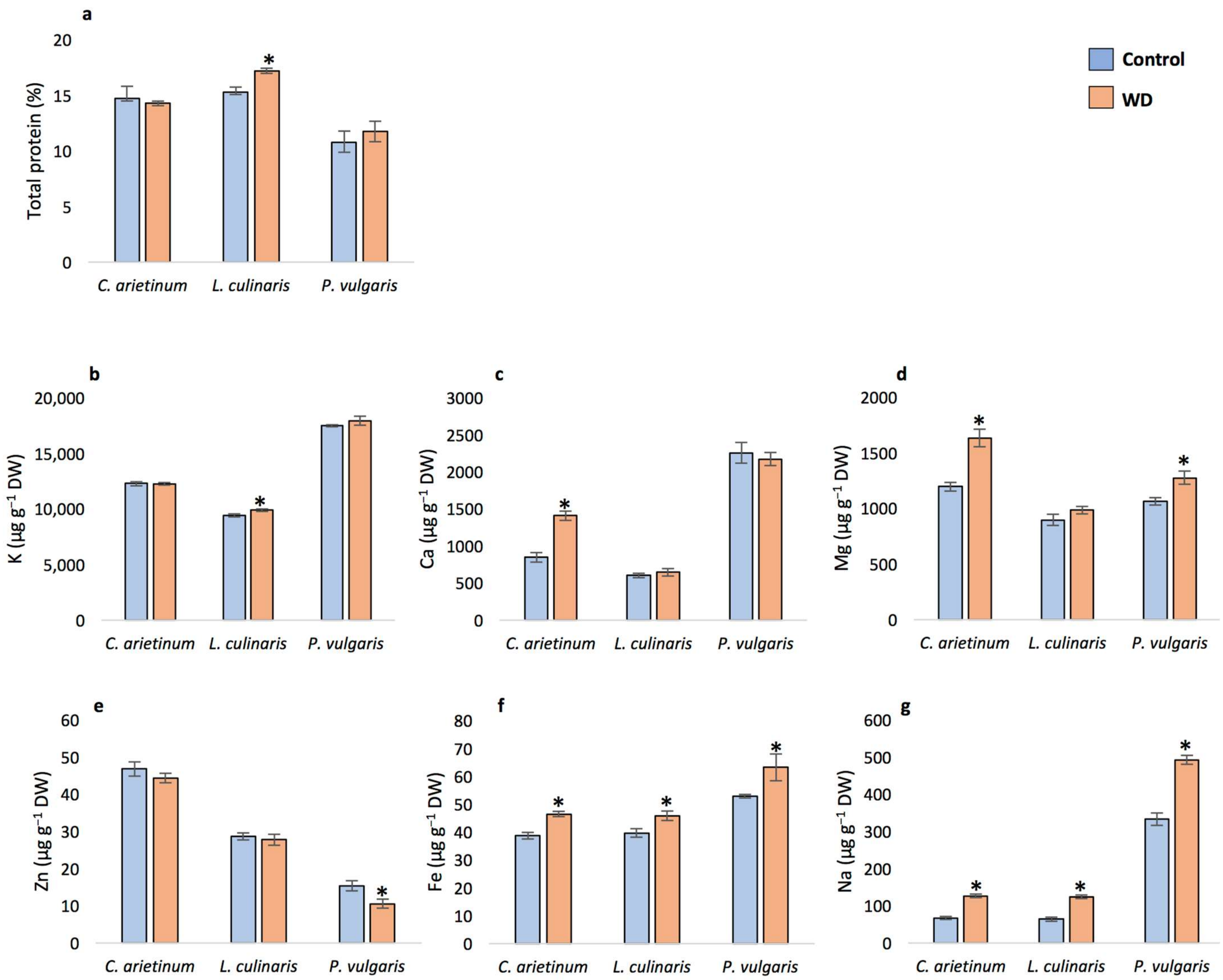

Figure 2. Total protein and mineral content (mean ± standard error) in *C. arietinum*, *L. culinaris* and *P. vulgaris* treated with water (Control) or with 0.2% wood distillate (WD). (**a**) Total protein concentration; (**b**–**g**) mineral concentration of (**b**) K, (**c**) Ca, (**d**) Mg, (**e**) Zn, (**f**) Fe, and (**g**) Na. * = significant difference between control and WD-treated plants.

Regarding the concentration of the investigated elements (Figure 2b–g), treatment with WD consistently increased the levels of Fe in the seeds of all species (46.5 μg g^{-1} DW in WD-treated vs. 38.7 μg g^{-1} DW in the control for *C. arietinum*, 45.8 μg g^{-1} DW in WD-treated vs. 39.7 μg g^{-1} in the control for *L. culinaris*; 63.2 μg g^{-1} DW in WD-treated vs. 52.8 μg g^{-1} DW in the control for *P. vulgaris*). The amount of Na also increased in the three species (126.8 μg g^{-1} DW in WD-treated vs. 67.4 μg g^{-1} DW in the control for *C. arietinum*; 124.4 μg g^{-1} DW in WD-treated vs. 64 μg g^{-1} in the control for *L. culinaris;* 493 μg g^{-1} DW in WD-treated vs. 333.2 μg g^{-1} DW in control for *P. vulgaris*). Furthermore, WD significantly increased the concentration in *C. arietinum* seeds (1634 μg g^{-1} in WD-treated vs. 1192 μg g^{-1} in the control for Mg and 1405.3 μg g^{-1} in WD-treated vs. 845.8 μg g^{-1} in the control for Ca). An increase in Mg levels was also observed in *P. vulgaris* (1276.2 μg g^{-1} in WD-treated vs. 1071.4 μg g^{-1} in control). Finally, the levels of K increased significantly only in *L. culinaris* (9866 μg g^{-1} in WD-treated vs. 9399.5 μg g^{-1} in control), while the concentration of Zn was reduced in WD-treated *P. vulgaris* (10.6 μg g^{-1} in WD-treated vs. 15.4 μg g^{-1} in control).

4. Discussion

Few studies have focused on the effects of WD application on legume crops grown in field conditions [29]. On the contrary, field experiments involving WD effects in the field have been performed with other plant species. For example, rice plants sprayed with 1/100 diluted WD both onto their leaves and onto the soil demonstrated an increased plant height and tiller production [30]. In addition, lettuce, cabbage, and cucumber plants that were leaf-sprayed with 500-fold diluted WD accumulated more vitamin C and demonstrated improved productivity [31]. More recently, Ref. [32] showed that rapeseed plants foliar sprayed with 1/400-fold diluted WD displayed a significantly increased seed yield, leaf area index, and number of pods. To the best of our knowledge, this is the second set of evidence reported for *C. arietinum* and the first set of evidence reported for both *L. culinaris* and *P. vulgaris* regarding the positive effects of WD on plant growth and yield. The yield-promoting effects of WD were mostly observed in *L. culinaris*—in addition to increasing total plant and shoot biomass, WD treatment almost doubled the number of seeds per plant and more than doubled their mean weight. As for *P. vulgaris*, the number of pods significantly increased in WD-treated plants, and a similar trend was observed for both seed number and weight, although this trend was not statistically significant. The observed WD-mediated increase in plant yield is consistent with previous reports focused on tobacco [33] as well as on both horticultural [9,10,34] and cereal [14] crops. In contrast, all yield parameters of *C. arietinum* were similar to the control, consistent with [15], although these authors observed a significant increase in the weight of WD-treated seeds. This might be due to the lower concentration of WD used in this study (0.2% vs. 0.25%), as well as its lower application frequency (every two weeks vs. every week). The different yield effects found between these three legumes indicate that the effects of WD are likely species-specific and that the concentrations tested are optimal to improve *L. culinaris* productivity. In this regard, higher WD concentrations may be tested for *P. vulgaris* and *C. arietinum* in future studies. In addition, the evaluation of both physiological and biochemical changes (e.g., chlorophyll content, proline content or shifts in the plant oxidative metabolism) could help in determining eventual stress-related responses to WD in these two crops.

Similarly to the yield parameters, WD treatment increased the concentration of total soluble proteins only in *L. culinaris* seeds. A higher protein amount after WD application has also been observed in rapeseed plants [33]. The present results are particularly relevant from a nutritional point of view, as leguminous crops are a central part of the human diet and could significantly compensate for the forecasted shortage of animal proteins [18]. However, it remains to be further investigated why *C. arietinum* and *P. vulgaris* did not respond as well. Also, in this case, the present results are at variance with those of [15], which found an increased protein content after WD foliar treatment, but without any fertigation.

The application of WD also enhanced the concentration of elements with high nutritional value, including Fe, Mg, Ca, and K. Deficiencies of these nutrients are highly prevalent among populations and could be alleviated via food fortification approaches. In this context, the production of seeds with a higher Fe content could critically contribute to reducing the number of subjects affected by iron deficiency, which is the micronutrient treated with the most concern worldwide regarding human nutrition [34,35]. Likewise, improved lentil seeds with higher K or chickpea seeds with higher Mg and Ca could contribute to reducing nutritional deficiencies related to diabetes, hypokalemia or osteoporosis [36,37]. Seeds from WD-treated *P. vulgaris* showed a decrease in Zn concentration, while this element was not affected in *C. arietinum* or in *L. culinaris*. Although Zn concentration in *P. vulgaris* was slightly lower compared to the ranges previously reported (10 $\mu g\ g^{-1}$ vs. 13 to 33.3 $\mu g\ g^{-1}$ [38,39]), the causes of this decrease in response to WD remain to be further investigated. It is worth noting that an increase in Na levels was observed in seeds from WD-treated plants. The concentration of this element both in control and WD-treated plants was below the values reported in the literature in the case of chickpea seeds (67–127 $\mu g\ g^{-1}$ vs. 188–285 $\mu g\ g^{-1}$ DW [40–42]) and lentil (64–124 $\mu g\ g^{-1}$ vs. 300–790 $\mu g\ g^{-1}$ DW [43,44]), although it was above that reported for

P. vulgaris (333–492 μg g^{-1} vs. 30–180 μg g^{-1}; [38,45]). Nevertheless, the concentration of Na in the diluted WD employed in this study (~15 μg L^{-1}) was far below the 0.5 g L^{-1} 'no risk' limit set by the FAO concerning salt concentrations in irrigation water [46]. To the best of our knowledge, how the application of WD enhances the concentration of elements such as Fe, Mg, Ca or Na in the seed remains unknown. A higher expression of auxin-responsive genes in WD-treated tomato plants was recently observed by [47]. In relation to this, WD could contain auxin-like substances that induce ATPase pumps in root tissues, thus favoring the entrance of cations, as previously shown in humic substances [48]. However, there is no experimental basis that has yet proven this hypothesis. Therefore, while initial results are encouraging, further studies are needed to dig into the processes influenced by WD that generate such differences in the mineral composition across the species tested, such as their absorption into the roots or their allocation in the seed. In parallel, future studies should also focus on the effects of WD on the plant-associated microbiota, as microorganisms critically contribute to maintaining plant growth and health in agricultural systems.

5. Conclusions

The present study showed the positive effect of WD treatment on the yield and nutritional composition of three legume species. Lentil plants showed an increased yield in terms of both pod and seed weight/number, as well as an improved concentration of total soluble proteins. Moreover, the treatment with WD differentially enhanced the content of mineral elements of high nutritional value in the seeds, such as Fe, K, Ca, and Mg. Overall, these results suggest that WD is an optimal candidate to sustainably boost sources of proteins and micronutrients in the human diet by generating high-yielding legume seeds with improved nutritional quality. Given the widespread impact of nutritional deficiencies on general health, the correction of these deficiencies through WD-based food fortification approaches should be considered. Lastly, and in line with the Farm to Fork strategy of the European Union, this study suggests that WD holds huge potential as a novel "green" alternative in the replacement of chemical fertilizers.

Author Contributions: Conceptualization: E.B., I.C., A.C., C.G. and S.L.; Methodology: E.B., P.C., I.C., C.G. and S.L.; Formal analysis and investigation: E.B., P.C., C.C., A.C., M.D. and L.P.; Writing—original draft preparation: P.C.; Writing—review and editing: E.B., P.C., I.C., C.C., A.C., M.D., C.G., S.L. and L.P.; Funding acquisition: S.L.; Resources: I.C., C.G. and L.P.; Supervision: I.C. and S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the University of Florence (Fondi di Ateneo 2021) and by the University of Siena (project CLASS "Ceci, distillato di legno, biochar e intelligenza artificiale per un sistema agrifood smart e sostenibile" funded by Regione Toscana and Esperia Srl.).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors want to thank the University of Florence (Fondi di Ateneo 2021) and the University of Siena (Project CLASS "Ceci, distillato di legno, biochar e intelligenza artificiale per un sistema agrifood smart e sostenibile" funded by Regione Toscana and Esperia Srl.) for the funding. The authors are also grateful to Del Colle Srl. enterprise for having kindly provided the legume seeds and the crop fields used for the experiment, as well as to Francesco Barbagli (BioDea and BioEsperia Srl.) for kindly providing the wood distillate. The authors are also grateful to Matteo De Filippis and Prandvera Guri for their contribution in the experimental work. The publication was made with the contribution of the researcher M.D., with a research contract co-funded by the European Union—PON Research and Innovation 2014–2020 in accordance with Article 24, paragraph 3a), of Law No. 240 of 30 December 2010, as amended, and Ministerial Decree No. 1062 of 10 August 2021.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. *The State of Food Security and Nutrition in the World 2020: Transforming Food Systems for Affordable Healthy Diets*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2020; Volume 2020.
2. FAO Statistical Yearbook 2022. 2022. Available online: http://www.fao.org (accessed on 15 January 2023).
3. 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]
4. Italian Ministerial Decree 6793. 18 July 2018. Available online: https://www.gazzettaufficiale.it/eli/id/2018/09/05/18A05693/sg (accessed on 20 June 2022).
5. Mathew, S.; Zakaria, Z.A. Pyroligneous acid—The smoky acidic liquid from plant biomass. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 611–622. [CrossRef] [PubMed]
6. Terzopoulou, P.; Kamperidou, V. Chemical characterization of Wood and Bark biomass of the invasive species of Tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle), focusing on its chemical composition horizontal variability assessment. *Wood Mater. Sci. Eng.* **2022**, *17*, 469–477. [CrossRef]
7. Agoncillo, E.S.; Alcate, V.; Philippines, O.M. Vegetable Seed Germination Enhancement Using Different Levels of Pyroligneous Acid (PA). *Glob. J. Biol. Agric.* **2018**, *2*, 14–18.
8. Vannini, A.; Moratelli, F.; Monaci, F.; Loppi, S. Effects of wood distillate and soy lecithin on the photosynthetic performance and growth of lettuce (*Lactuca sativa* L.). *SN Appl. Sci.* **2021**, *3*, 113. [CrossRef]
9. Vannini, A.; Fedeli, R.; Guarnieri, M.; Loppi, S. Foliar application of wood distillate alleviates ozone-induced damage in lettuce (*Lactuca sativa* L.). *Toxics* **2022**, *10*, 178. [CrossRef]
10. Fedeli, R.; Vannini, A.; Guarnieri, M.; Monaci, F.; Loppi, S. Bio-baseds 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]
11. Becagli, M.; Santin, M.; Cardelli, R. Co-application of wood distillate and biochar improves soil quality and plant growth in basil (*Ocimum basilicum*). *J. Plant Nutr. Soil Sci.* **2022**, *185*, 120–131. [CrossRef]
12. Ofoe, R.; Qin, D.; Gunupuru, L.R.; Thomas, R.H.; Abbey, L. Effect of pyroligneous acid on the productivity and nutritional quality of greenhouse tomato. *Plants* **2022**, *11*, 1650. [CrossRef]
13. Zulkarami, B.; Ashrafuzzaman, M.; Husni, M.O.; Ismail, M.R. Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. *Aust. J. Crop Sci.* **2011**, *5*, 1508–1514.
14. Simma, B.; Polthanee, A.; Goggi, A.S.; Siri, B.; Promkhambut, A.; Caragea, P.C. Wood vinegar seed priming improves yield and suppresses weeds in dryland direct-seeding rice under rainfed production. *Agron. Sustain. Dev.* **2017**, *37*, 1–9. [CrossRef]
15. 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]
16. Del Borghi, A.; Strazza, C.; Magrassi, F.; Taramasso, A.C.; Gallo, M. Life Cycle Assessment for eco-design of product–package systems in the food industry—The case of legumes. *Sustain. Prod. Consum.* **2018**, *13*, 24–36. [CrossRef]
17. Venter, C.S.; Van, E.E. More legumes for better overall health. *S. Afr. J. Clin. Nutr.* **2001**, *172*, 280.
18. Śmiglak-Krajewska, M.; Wojciechowska-Solis, J. Consumption preferences of pulses in the diet of polish people: Motives and barriers to replace animal protein with vegetable protein. *Nutrients* **2021**, *13*, 454. [CrossRef]
19. Considine, M.J.; Siddique, K.H.; Foyer, C.H. Nature's pulse power: Legumes, food security and climate change. *J. Exp. Bot.* **2017**, *68*, 1815–1818. [CrossRef]
20. Calles, T. The international year of pulses: What are they and why are they important. *Agric. Dev.* **2016**, *26*, 40–42.
21. Suter, M.; Connolly, J.; Finn, J.A.; Loges, R.; Kirwan, L.; Sebastià, M.T.; Lüscher, A. Nitrogen yield advantage from grass–legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Glob. Chang. Biol.* **2015**, *21*, 2424–2438. [CrossRef]
22. Van Loon, M.P.; Deng, N.; Grassini, P.; Edreira, J.I.R.; Wolde-Meskel, E.; Baijukya, F.; Marou, H.; van Ittersum, M.K. Prospect for increasing grain legume crop production in East Africa. *Eur. J. Agron.* **2018**, *101*, 140–148. [CrossRef]
23. Sidhu, J.S.; Zafar, T.; Benyathiar, P.; Nasir, M. Production, processing, and nutritional profile of chickpeas and lentils. In *Dry Beans and Pulses: Production, Processing, and Nutrition*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2022; pp. 383–407. [CrossRef]
24. Shrestha, S.; van't Hag, L.; Haritos, V.S.; Dhital, S. Lentil and Mungbean protein isolates: Processing, functional properties, and potential food applications. *Food Hydrocoll.* **2022**, *135*, 108142. [CrossRef]
25. Branlard, G.; Bancel, E. Protein extraction from cereal seeds. In *Plant Proteomics: Methods and Protocols*; Humana Press Inc.: Totowa, NJ, USA, 2007; pp. 15–25. [CrossRef]
26. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
27. Bettarini, I.; Colzi, I.; Coppi, A.; Falsini, S.; Echevarria, G.; Pazzagli, L.; Selvi, F.; Gonnelli, C. Unravelling soil and plant metal relationships in Albanian nickel hyperaccumulators in the genus *Odontarrhena* (syn. *Alyssum* sect. *Odontarrhena*, Brassicaceae). *Plant Soil* **2019**, *440*, 135–149. [CrossRef]
28. Bianchi, E.; Coppi, A.; Nucci, S.; Antal, A.; Berardi, C.; Coppini, E.; Fibbi, D.; Del Bubba, M.; Gonnelli, C.; Colzi, I. Closing the loop in a constructed wetland for the improvement of metal removal: The use of *Phragmites australis* biomass harvested from the system as biosorbent. *ESPR* **2021**, *28*, 11444–11453. [CrossRef] [PubMed]

29. Pangnakorn, U.; Watanasorn, S.; Kuntha, C.; Chuenchooklin, S. Application of wood vinegar to fermented liquid bio-fertilizer for organic agriculture on soybean. *Asian J. Food Agro-Ind.* **2009**, *2*, S189–S196.
30. Rogelio, R.M. Alternative growth ehancers for rice production: Usefulness of wood vinegar (PA) in irrigated rice (PSB rc18). *J. Biol. Agric. Healthc.* **2018**, *8*, 82–98.
31. Mu, J.; Yu, Z.M.; Wu, W.Q.; Wu, Q.L. Preliminary study of application effect of bamboo vinegar on vegetable growth. *For. Ecosyst.* **2006**, *8*, 43–47. [CrossRef]
32. Zhu, K.; Gu, S.; Liu, J.; Luo, T.; Khan, Z.; Zhang, K.; Hu, L. Wood vinegar as a complex growth regulator promotes the growth, yield, and quality of rapeseed. *Agronomy* **2021**, *11*, 510. [CrossRef]
33. Mao, K.; Li, S.; Li, B.; Wu, W.; Wei, C.; Yuan, S.; Niu, Y.; Du, H.; Zhang, L. Effect of wood vinegar on growth, yield and quality of upper leaves of flue-cured tobacco of Nanzheng. *Southwest China J. Agric. Sci.* **2019**, *32*, 645–652.
34. Mungkunkamchao, T.; Kesmala, T.; Pimratch, S.; Toomsan, B.; Jothityangkoon, D. Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (*Solanum lycopersicum* L.). *Sci. Hortic.* **2013**, *154*, 66–72. [CrossRef]
35. Guzmán-Maldonado, S.H.; Acosta-Gallegos, J.; Paredes-López, O. Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L). *J. Sci. Food Agric.* **2000**, *80*, 1874–1881. [CrossRef]
36. Venn, B.J.; Mann, J.I. Cereal grains, legumes and diabetes. *Eur. J. Clin. Nutr.* **2004**, *58*, 1443–1461. [CrossRef]
37. Rebello, C.J.; Greenway, F.L.; Finley, J.W. A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. *Obes. Rev.* **2014**, *15*, 392–407. [CrossRef]
38. Petropoulos, S.A.; Fernandes, Â.; Plexida, S.; Chrysargyris, A.; Tzortzakis, N.; Barreira, J.C.; Barros, L.; Ferreira, I.C. Biostimulants application alleviates water stress effects on yield and chemical composition of greenhouse green bean (*Phaseolus vulgaris* L.). *Agronomy* **2020**, *10*, 181. [CrossRef]
39. Gelin, J.R.; Forster, S.; Grafton, K.F.; McClean, P.E.; Rojas-Cifuentes, G.A. Analysis of seed zinc and other minerals in a recombinant inbred population of navy bean (*Phaseolus vugaris* L.). *Crop Sci.* **2007**, *47*, 1361–1366. [CrossRef]
40. Ray, H.; Bett, K.; Tar'an, B.; Vandenberg, A.; Thavarajah, D.; Warkentin, T. Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. *Crop Sci.* **2014**, *54*, 1698–1708. [CrossRef]
41. Ereifej, K.I.; Al-Karaki, G.N.; Hammouri, M.K. Seed chemical composition of improved chickpea cultivars grown under semiarid Mediterranean conditions. *Int. J. Food Prop.* **2001**, *4*, 239–246. [CrossRef]
42. Daur, I.; Khan, I.A.; Jahangir, M. Nutritional quality of roasted and pressure-cooked chickpea compared to raw (*Cicer arietinum* L.) seeds. *Sarhad J. Agric.* **2008**, *24*, 117.
43. Zia-Ul-Haq, M.; Ahmad, S.; Shad, M.A.; Iqbal, S.; Qayum, M.; Ahmad, A.; Luthria, D.L.; Amarowicz, R. Compositional studies of lentil (*Lens culinaris* Medik.) cultivars commonly grown in Pakistan. *Pak. J. Bot.* **2011**, *43*, 1563–1567.
44. Sahi, S.T.; Ghazanfar, M.U.; Afzal, M.; Wakil, W.; Habib, A. Influence of inoculation with Ascochyta lentis on mineral contents (Na, Ca, Mg, Zn, Cu and Fe) of susceptible and resistant lines of lentil (*Lens culinaris* Medik.). *Pak. J. Bot.* **2010**, *42*, 375–382.
45. Paredes, M.; Becerra, V.; Tay, J. Inorganic nutritional composition of common bean (*Phaseolus vulgaris* L.) genotypes race Chile. *Chil. J. Agric. Res.* **2009**, *69*, 486–495. [CrossRef]
46. Brouwer, C.; Goffeau, A.; Heibloem, M. Chapter 7: Salty soils. In *Irrigation Water Management: Training Manual No. 1-Introduction to Irrigation*; FAO—Food and Agriculture Organization of the United Nations: Rome, Italy, 1985.
47. Ofoe, R.; Gunupuru, L.R.; Wang-Pruski, G.; Fofana, B.; Thomas, R.H.; Abbey, L. Seed priming with pyroligneous acid mitigates aluminum stress, and promotes tomato seed germination and seedling growth. *Plant Stress* **2022**, *4*, 100083. [CrossRef]
48. Canellas, L.P.; Teixeira Junior, L.R.L.; Dobbss, L.B.; Silva, C.A.; Medici, L.O.; Zandonadi, D.B.; Façanha, A.R. Humic acids crossinteractions with root and organic acids. *Ann. Appl. Biol.* **2008**, *153*, 157–166. [CrossRef]