Check for updates

Received: 5 March 2022 | Revised: 21 May 2022 | Accepted: 27 May 2022

DOI: 10.1111/aab.12794

RESEARCH ARTICLE

Annals of Applied Biology
An international journal of the aab

WILEY

Foliar application of wood distillate boosts plant yield and nutritional parameters of chickpea

Riccardo Fedeli[1] | Andrea Vannini[1] | Silvia Celletti[1] | Viviana Maresca[1] | Silvana Munzi[2,3] | Cristina Cruz[3] | Dmitry Alexandrov[4] | Massimo Guarnieri[1] | Stefano Loppi[1,5]

[1]Department of Life Sciences, University of Siena, Siena, Italy

[2]Centro Interuniversitário de História das Ciências e da Tecnologia Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

[3]Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

[4]Ufa State Aviation Technical University, Ufa, Russia

[5]BAT Center - Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples 'Federico II', Naples, Italy

Correspondence
Stefano Loppi, Department of Life Sciences, University of Siena, Siena 53100, Italy.
Email: stefano.loppi@unisi.it

Abstract

In the quest for eco-friendly products with biostimulant properties, foliar application of wood distillate (WD) was tested on the growth and yield of chickpea (*Cicer arietinum* L.). WD (pyroligneous acid) is a by-product of plant biomass pyrolysis and is rich in biologically active substances like polyphenols, alcohols, acids and esters. In this work, chickpea plants were sprayed weekly with 100 ml 0.25% (v/v) chestnut (*Castanea sativa* Mill.) WD during the whole growing period, and at the end physiological and nutritional analyses were performed both on the whole plant and on seeds. While plant height and weight did not change significantly, seeds showed an increase in diameter (+11.2%) and weight (+33.3%), and in the content of starch (+45.9%), total soluble protein (+12.9%), total polyphenol (+16.4%) and antioxidant power (+28.4%). Overall, the content of essential free amino acids increased, except for lysine (−3.4%), phenylalanine (−10.5%) and methionine (−13.7%). Among all the mineral elements analysed, only potassium and magnesium decreased in WD-treated plants, although values were within the common range for chickpea seeds. These results are a clear demonstration of the effectiveness of the use of WD on increasing the nutritional qualities of the edible parts of crop species, thus representing a possible solution to counteract human malnutrition and famine as well as environmental concerns.

KEYWORDS
amino acids, antioxidant power, proteins, pyroligneous acid, wood vinegar

1 | INTRODUCTION

It is now becoming clear that the massive use of synthetic chemicals commonly used in agriculture is of major concern for the environment and human health, and there is growing interest towards the use of natural compounds that could contribute to mitigate and overcome the negative effects of current agricultural practices (Alewu & Nosiri, 2011; WHO, 1990). As a consequence, there is an increasing number of studies focusing on the evaluation of the potential of bio-based products to replace fertilisers and to improve soil quality and boost crop plant growth (Becagli, Santin, & Cardelli, 2021; Celletti et al., 2021), but also aimed at checking the safety for humans and the

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. *Annals of Applied Biology* published by John Wiley & Sons Ltd on behalf of Association of Applied Biologists.

environment of these products (Celletti et al., 2021; Fačkovcová et al., 2020a, 2020b; Filippelli, Ciccone, Loppi, & Morbidelli, 2021).

One of the most promising bio-based compounds currently being investigated is wood distillate (WD), also known as pyroligneous acid or wood vinegar. WD is a by-product of bio-energy production by pyrolysis of plant biomass (Grewal, Abbey, & Gunupuru, 2018) and is rich in numerous bioactive substances (i.e., polyphenols, alcohols, acids, esters) (Mu, Yu, Wu, & Wu, 2006; Zulkarami, Ashrafuzzaman, Husni, & Ismail, 2011). WD is used as a biostimulant in agriculture owing to its ability to enhance quality, size and weight of edible plant parts (Mungkunkamchao, Kesmala, Pimratch, Toomsan, & Jothityangkoon, 2013; Zulkarami et al., 2011), to increase plant biomass (Zulkarami et al., 2014; Fedeli, Vannini, Guarnieri, Monaci, & Loppi, 2022; Theerakulpisut, Kanawapee, & Panwong, 2016; Vannini, Moratelli, Monaci, & Loppi, 2021), and even to counteract the attack of plant pathogens (Mourant, Yang, Lu, & Roy, 2005; Ratanapisit, Apiraksakul, Rerngnarong, Chungsiriporn, & Bunyakarn, 2009). Furthermore, WD has recently been included in the list of products that can be used in organic farming in Italy (Italian Ministerial Decree 6793, 2018).

Chickpea is the third most widely consumed legume in the world, being cultivated on more than 11.5 million ha of land and in more than 50 countries in South Asia, Australia, the Americas, and the Middle East and Southern Europe (FAOSTAT, 2011). Chickpea seeds are an excellent source of proteins, carbohydrates, and minerals, especially calcium, magnesium, iron and zinc (Cabrera, Lloris, Giménez, Olalla, & López, 2003). In particular, the quality of proteins of these seeds is the highest compared to other types of legume seeds (Jukanti, Gaur, Gowda, & Chibbar, 2012).

The aim of this study was to investigate whether foliar application of WD increases the growth and yield of chickpea (*Cicer arietinum* L.) plants as well as the nutritional parameters of the seeds.

2 | MATERIALS AND METHODS

2.1 | Plant material and growing conditions

Dried chickpea seeds, from a typical variety of Tuscany ('small chickpea from Arezzo') provided by a local farm, were placed in 50-ml tubes, and then stored at 6°C for 3 days for cold stratification (Garcia, Barker, & Journet, 2006) to interrupt seed dormancy and promote germination. Subsequently, seeds were sterilised with 3% (v/v) sodium hypochlorite (NaClO) for 2 min and then washed thoroughly with deionised water. Finally, seeds were allowed to germinate for 1 week at 15°C in the dark in Petri dishes. All material for seed germination (i.e., Petri dishes, pipette tips, deionised water, tweezers and filter papers) was sterilised using UV lamps for 1 h to inhibit the growth of pathogens such as fungi and bacteria. The resulting seedlings were placed in plastic pots (10 cm × 10 cm × 12 cm) filled with soil for 2 weeks. Afterwards, 20 plants were planted in the ground at the Botanical Garden of the University of Siena, Italy. Ten plants, randomly selected, were treated weekly with foliar applications of 0.25% (v/v) chestnut (*Castanea sativa* Mill.) WD (BioDea® WD, Arezzo, Italy) and the remaining 10 plants (control) with water. The experiment lasted 4 months (March–July 2021), that is, until all the plants were mostly (>50%) dry. Analysis of WD provided by the producer indicates 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}. After 4 months of the growing period, whole plants were harvested and carried to the laboratory for subsequent analyses.

2.2 | Plant features

2.2.1 | Plant height, plant dry weight and seeds measurements

Prior to harvest, plant height was measured considering the maximal distance between the ground level and its apex. After harvest, plants were dried at 40°C for 1 week and then their total dry weight was measured. The number of pods and seeds, as well as seed diameter and weight were also determined.

2.3 | Nutritional parameters

2.3.1 | Sample preparation

Prior to analyses, chickpea seeds from each plant were finely ground with mortar and pestle.

2.3.2 | Soluble sugars and starch

For the determination of the content of soluble sugars, ground samples (100 mg) were homogenised in 2 ml of deionised water and then centrifuged at 15,000 rpm for 5 min. The supernatant was filtered at 0.45 μm using a syringe filter and then directly analysed by HPLC (Waters 600 system) equipped with a Waters 2410 refractive index detector. Sugar separation was allowed using deionised water as mobile phase, eluted at 0.5 ml min^{-1}, and a Waters Sugar-Pak I ion-exchange column (6.5 mm × 300 mm) kept at 90°C using an external temperature controller (Waters Column Heater Module). Quantification was obtained through calibration curves prepared by dissolving analytical sugars (Sigma) in deionised water at concentrations of 0.1–20 mg ml^{-1}.

The starch content was determined following the method described by Loppi et al. (2021). Briefly, ground samples (50 mg) were homogenised in 2 ml dimethyl sulfoxide (DMSO). Then 0.5 ml of HCl 8 M was added and samples were placed in a ventilated oven for 30 mins at 60°C. After cooling, 0.5 ml of NaOH 8 M and 7 ml of deionised water were added. Samples were then centrifuged at 4,000 rpm for 5 mins, and 0.5 ml of supernatant was added to 2.5 ml of Lugol's solution (HCl 0.05 M, 0.03% I_2, and 0.06% KI). After 15 min, samples were read at 605 nm with a UV-VIS spectrophotometer (Agilent

8453). Quantification was run using a calibration curve (10–400 μg ml^{-1}) prepared with pure starch (Merck).

2.3.3 | Free amino acids and total soluble proteins

The content of free amino acids (FAAs) was determined by means of a Waters LC1 HPLC system, equipped with an Agilent column C18 (250 mm × 4.6 mm with 5 μm of particle size), thermostated at 20°C, and a Waters 470 scanning fluorescence detector (excitation at 250 nm, detection at 395 nm). The solvents used were: (A) 22.9% (w/v) sodium acetate/water, 7.7% (v/v) phosphoric acid/water, and 4.1% (v/v) triethylamine/water; (B) 60% (v/v) acetonitrile/water. According to the AccQtag protocol, 10 μl of each reconstituted sample was derivatized with amino acids (Cohen & Michaud, 1993), using the AQC fluorescent reagent and 0.02 M borate buffer (pH 8.6). The concentration of each amino acid was estimated by matching the area under the chromatogram peak to the standard (WAT088122, Waters), using the Clarity software (DataApex).

For the total soluble proteins, approximately 50 mg of seed powder was homogenised in 5 ml of deionised water and centrifuged at 4,000 rpm for 5 min. Then, 0.4 ml of the supernatant was added to 1.6 ml of Bradford solution (Sigma-Aldrich). The content was determined using a UV-Vis spectrophotometer (Agilent 8453), by reading the absorbance of the samples at 595 nm. Quantification was done using a calibration curve, prepared with concentrations in the range 20–80 μg ml^{-1}, of bovine serum albumin (BSA) (Sigma-Aldrich) as standard. Results were expressed as mg of BSA equivalent on a dry weight basis (mg BSA eq g^{-1} dw).

2.3.4 | Vitamin E

The concentration of Vitamin E was determined using 1.0 g of ground seeds; the samples were homogenised in 1 ml of pure ethanol and centrifuged at 4,000 rpm for 10 min. The supernatant (50 μl) was taken and injected in a Waters 600 HPLC system on Agilent C18 column (250 mm × 4.6 mm with 5 μm of particle size) at a constant flux of 0.5 ml min^{-1}. The running time of analysis was about 30 min, the wavelength was 295 nm using a Waters 996 Photodiode Array Detector. The mobile phase used was methanol/acetonitrile (90:10, v/v) with 9 mM triethanolamine (1.29 ml L^{-1}). A standard curve of pure tocopherol (Merck KGaA), ranging from 10 to 300 μg ml^{-1}, was used for determining the concentration of vitamin E in the samples.

2.3.5 | Total antioxidant power and polyphenols

For the determination of the total antioxidant power, 100 mg of ground seeds was homogenised in 2 ml of 80% (v/v) ethanol and then centrifuged at 15,000 rpm for 5 min. An aliquot of supernatant (200 μl) was added to 1 ml of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution, previously prepared by dissolving 1.85 mg of this compound in 50 ml of 80% methanol (v/v). To compare the antioxidant power of the samples, a blank and a control were prepared by adding 200 μl of 80% (v/v) ethanol in 1 ml of 80% (v/v) methanol and in 1 ml of DPPH solution, respectively. The reaction for all preparations was conducted in the dark for 1 h and then the absorbance was read at 517 nm with a UV-Vis spectrophotometer (Agilent 8453). Results were expressed as a percentage of antiradical activity (ARA%), according to the following formula:

$$\mathrm{ARA}\% = 100 \times [1 - (\text{sample absorbance}/\text{control absorbance})].$$

The total polyphenol content (TPC) was measured following the method described by Henríquez et al. (2010). Briefly, 100 mg of ground seeds was homogenised in 4 ml of 70% (v/v) acetone and then centrifuged at 4,000 rpm for 5 min. The supernatant (0.5 ml) was added to 3 ml of deionised water, 0.125 ml of Folin-Denis' reagent (Sigma-Aldrich), 0.750 ml of saturated Na_2CO_3, and finally 0.950 ml of deionised water. Samples were placed in an oven at 37°C for 30 min. Afterwards, they were centrifuged at 4,000 rpm for 5 min and their absorbance was read at 765 nm by using a UV-Vis spectrophotometer (Agilent 8453). For the quantification, the absorbance of the samples was referred to a calibration curve (5–20 μg ml^{-1}) of gallic acid (Sigma-Aldrich) used as standard. Results were expressed as mg of gallic acid equivalent on a dry weight basis (mg GAE g^{-1} dw).

2.3.6 | Mineral elements

About 200 mg of ground seeds was mineralised with a mixture of 3 ml of 70% (v/v) HNO_3, 0.2 ml of 50% (v/v) HF, and 0.5 ml of 30% (v/v) H_2O_2, using a microwave digestion system (Milestone Ethos 900, Metrohm) at 280°C and 55 bar. The content of Ca, Cu, Fe, K, Mg, Na, P, S and Zn were quantified with an Inductively Coupled Plasma – Mass Spectrometry (ICP-MS, Perkin Elmer NexION 350). The analytical quality was measured using the NCS certified standard reference material DC 73350 '*Poplar leaves*'; recoveries were in the range 94–112%. The precision of analyses was estimated by the coefficient of variation of five replicates and was always >97%. the results are expressed on a dry weight basis (mg kg^{-1}_{DW}).

2.4 | Statistical analysis

To disentangle the effect of WD treatment on the diameter and weight of chickpea seeds, a linear mixed-effect model was fitted for each variable, with treatment as fixed effect plant as random effect (Brauer & Curtin, 2018). For model validation, scatterplots of the residual and fitted values were used to check for homoscedasticity, and normal probability (qqnorm) plots as well as the Shapiro–Wilk test to check for normality. Models were fitted using the restricted maximum likelihood estimation, and significance of the models was checked with type III ANOVA using the Satterthwaite method (Luke, 2017). For all the other parameters analysed, a permutation

TABLE 1 Plant height and biomass, number of pods, and number, weight, and diameter of seeds in chickpea plants after foliar application of water (CTRL) or 0.25% (v/v) wood distillate (WD)

Physiological parameter	CTRL	WD
Plant height (cm)	66.8 ± 4.4 a	71.2 ± 4.4 a
Plant biomass (g)	36.4 ± 7.5 a	49.9 ± 17.2 a
Number of pods	85.5 ± 18.8 a	76.0 ± 13.3 a
Number of seeds	74.0 ± 20.3 a	80.3 ± 20.5 a
Seed diameter (mm)	6.05 ± 0.22 b	6.73 ± 0.19 a
Seed weight (mg)	0.18 ± 0.02 b	0.24 ± 0.02 a

Note: All parameters are expressed as mean ± standard error. Different letters indicate statistically significant differences ($p < 0.05$).

TABLE 2 Total soluble sugars, starch content, total soluble proteins, vitamin E, total polyphenol content (TPC) and total antioxidant power (ARA%) in seeds of chickpea plants after foliar application of water (CTRL) or 0.25% (v/v) wood distillate (WD)

Nutritional parameter	CTRL	WD
Total soluble sugars (g 100 g^{-1})	1.7 ± 0.1 a	1.8 ± 0.1 a
Starch content (mg g^{-1})	18.3 ± 1.10 b	26.6 ± 1.5 a
Total soluble proteins (mg g^{-1})	14.4 ± 0.4 b	16.3 ± 0.6 a
Vitamin E (mg 100 g^{-1})	6.8 ± 0.66 a	6.7 ± 0.5 a
TPC (mg g^{-1})	0.55 ± 0.03 b	0.64 ± 0.02 a
ARA%	39.6 ± 3.3 b	50.9 ± 3.2 a

Note: All parameters are expressed as mean ± standard error. Different letters indicate statistically significant differences ($p < 0.05$).

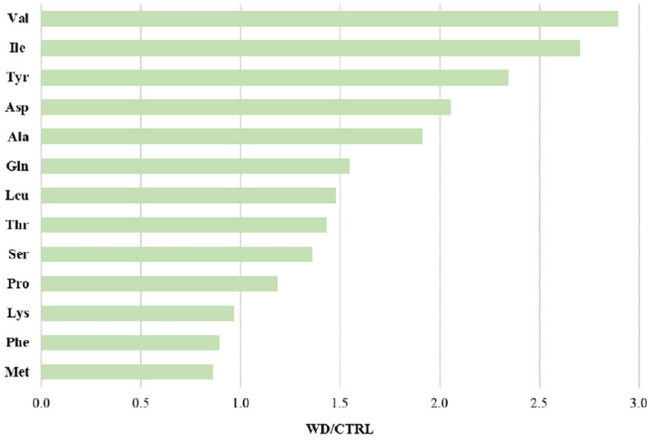

FIGURE 1 Content of free amino acids in chickpea seeds. Data are reported as ratios between wood distillate-treated and control plants. Ala, alanine; Asp, aspartic acid; Gln, glutamic acid; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine

t-test was used to check for differences between WD-treated and control samples. All calculations were run using the free R software (R Core Team, 2021).

TABLE 3 Content of mineral elements in seeds of chickpea plants after foliar application of water (CTRL) or 0.25% (v/v) wood distillate (WD)

Mineral element (mg kg^{-1} dw)	CTRL	WD
K	14,859 ± 139 a	13,182 ± 300 b
P	5,216 ± 133 a	4,976 ± 113 a
S	3,606 ± 166 a	3,413 ± 126 a
Mg	1,748 ± 29 a	1,502 ± 42 b
Ca	1,096 ± 64 a	1,092 ± 109 a
Na	128 ± 8 a	138 ± 10 a
Fe	75 ± 2 a	72 ± 2 a
Zn	50 ± 2 a	46 ± 3 a
Cu	11.7 ± 0.3 a	10.8 ± 0.6 a

Note: All parameters are expressed as mean ± standard error. Different letters indicate statistically significant differences ($p < 0.05$).

3 | RESULTS

Foliar application of 0.25% WD significantly increased both the diameter (+11.2%) and weight (+33.3%) of seeds, but differences in plant height and dry weight, as well as in the number of pods and seeds, were not statistically significant (Table 1).

Plants treated with WD showed a significantly higher content of starch (+45.9%), total soluble proteins (+12.9%), total polyphenols (+16.4%) and total antioxidant power (+28.4%), while the content of soluble sugars and vitamin E did not differ (Table 2).

The response of FAAs was complex. After foliar treatment with WD, a group of amino acids, that is, valine (+187.5%), isoleucine (+168.8%), tyrosine (+136.8%), aspartic acid (+105.8%), alanine (+96%), showed a marked increase, while another group, that is, glutamic acid (+55.2%), leucine (+46.2%), threonine (+40%), serine (+35%) and proline (+18.2%) showed a much lesser pronounced increase. In contrast, another group of amino acids, that is, lysine (−3.4%), phenylalanine (−10.5%), and methionine (−13.7%), was slightly decreased (Figure 1).

The content of seven out of nine mineral elements was similar in seeds from WD-treated and control plants, while a significant reduction was observed for K (−11.3%) and Mg (−14.1%) in seeds from WD-treated plants (Table 3).

4 | DISCUSSION

Our results showed a clear positive effect of WD on the yield and nutritional properties of chickpea seeds. Consistently, a *c*. 10% weight increase has been reported for seeds of rapeseed (*Brassica napus* L.) after application of 0.25% WD (Zhu et al., 2021).

Starch is the main energy reserve in plants and one of the most important carbohydrates in chickpea seeds, being also the major source of dietary energy for humans (Mahadevamma, Shamala, & Tharanathan, 2004; Salimath et al., 2007). Plants release starch under

adverse events and accumulate it when the conditions become favourable (Fincher, 1989; Thalmann & Santelia, 2017). In our study, the remarkable increase in starch (+45.9%) in WD-treated plants suggests a very beneficial effect of WD on chickpea. This result is consistent with the doubled starch content found in 0.25% and 0.50% WD-treated lettuce plants (Fedeli et al., 2022). Similarly, also Jee and Cho (2005) reported that WD at concentrations within 0.5% increased the starch content in the roots of the orchid *Neofinetia falcata* Thunb. Nevertheless, studies on the effects of WD on starch accumulation are still limited and this topic deserves further investigation.

A high starch content in seeds is usually associated with a higher concentration of FAAs since during seed development sugars are mobilised from the leaves to the seeds, where sugars will be converted into starch which, in turn, will be converted to FAAs, essential for protein synthesis (Fait et al., 2006; Weber, Borisjuk, & Wobus, 2005), thus likely indicating an overall plant well-being. To the best of our knowledge, this study is the first one documenting an effect of WD in modulating the content of FAAs. Our results showed that the content of some FAAs was markedly increased by the treatment with WD, while the same effect on others was less intense. At the same time, some FAAs were slightly reduced. These different trends likely depend on the specific role of each amino acid in the physiological activities of plants. For example, methionine is the first amino acid for the protein synthesis (Amir, Cohen, & Hacham, 2019) and acts as a precursor for the synthesis of other amino acids, such as isoleucine, leucine, and valine (Matityahu, Godo, Hacham, & Amir, 2013), and of secondary metabolites, specifically antioxidant compounds such as glutathione (Levine, Mosoni, Berlett, & Stadtman, 1996; Matityahu et al., 2013). Therefore, a decrease in the content of methionine could be linked to a differential increase in the content of other FAAs, soluble proteins, and free scavenging antiradical activity in WD-treated plants. Among those FAAs whose content was increased, valine, isoleucine and leucine are used as alternative energy sources by plants (Binder, 2010), thus an increase in their content indicates a successful plant performance.

Proteins are a main class of nutrients in chickpea seeds, which usually have a soluble protein content of 12–13% up to 29%, generally twice and even thrice than in cereals (Salimath et al., 2007). In our study, the treatment with WD increased the total content of soluble proteins by 12.9%. Similar effects were also reported for tobacco (*Nicotiana tabacum* L.) leaves and rapeseed seeds treated with 0.25–0.3% WD (Mao et al., 2019; Zhu et al., 2021). Since the consumption of legume proteins is an effective and sustainable alternative to those of animal origin, boosting the protein content of legumes could help to feed an exponentially growing population, compensating for the forecast shortage of animal proteins, (Śmiglak-Krajewska & Wojciechowska-Solis, 2021), and alleviating the problem of human famine and malnutrition (Maphosa & Jideani, 2017). At the same time, since legume production has a low environmental impact, allocating agricultural land to the cultivation of legumes rather than to animal breeding would reduce water consumption, greenhouse gas emissions, and preserve soil health and quality (Aleksandrowicz, Green, Joy, Smith, & Haines, 2016; McDermott & Wyatt, 2017; Szczebyło, Rejman, Halicka, & Laskowski, 2020).

There is evidence that an increased antioxidant activity is directly correlated with an increase in TPC (Chedea & Pop, 2019). Indeed, polyphenols are biologically active substances, commonly contained in bio-based products, with anti-inflammatory and antioxidant properties (Han, Shen, & Lou, 2007). Our chickpea plants treated with WD produced seeds with a higher TPC (+16.4%) and ARA% (+28.4%) compared to the controls. Studies concerning the effects of WD on these two parameters in plants are scanty. Benzon and Lee (2016) showed that applications of WD at concentrations 0.2% and 0.4% to tomato plants increased TPC in fruits, while only 0.4% WD increased ARA%. Kårlund et al. (2014) evaluated the effect of WD on strawberry (*Fragaria* × *ananassa*), but, in this case, it was not found an increase in TPC in fruits, except for chlorogenic acid, whose level doubled compared to the control. Nevertheless, TPC levels found in our control and treated seeds are consistent with those reported for several varieties of chickpea (Salgado et al., 2001; Segev et al., 2010). The increase in antioxidant power observed in WD-treated samples may be related to a stimulatory effect, known as eustress, induced by the WD treatment. The application of natural, chemical, and physical agents for increasing the quality of crop plants is indeed a well-known and common horticultural practice (Marchica et al., 2021), based on the induction of defence mechanisms with the consequent enhanced production of antioxidant compounds (Baenas, García-Viguera, & Moreno, 2014). Recently, Vannini, Fedeli, Guarnieri, and Loppi (2022), showed that foliar application of 0.2% WD protects lettuce plants exposed to concentrations of 60 ppb of O_3, by increasing the antioxidant power as well as the content of caffeic acid and quercetin in WD-treated compared to control plants. Also, other studies showed an increase in the content of antioxidant compounds in crop plants after WD application (Benzon & Lee, 2016; Kårlund et al., 2014), further suggesting the possible role of WD as eustressor.

Overall, the foliar treatment with WD did not cause any change in the mineral composition of chickpea seeds, and only the contents of K and Mg showed a significant reduction. A reduction of K and Mg in plants is often associated with Na excess and salinity (Renault, Paton, Nilsson, Zwiazek, & MacKinnon, 1999), and there is evidence that the content of sugar and starch decreased significantly during K and Mg deficiencies (Koch et al., 2019). However, the concentration of Na in the pure WD used in this study is 0.33 g L^{-1} (unpublished data), which is below the 0.5 g L^{-1} 'no risk' limit set by FAO (Brouwer, Goffeau, & Heibloem, 1985) for irrigation water, and our results showed that sugars were not reduced while starch was increased in seeds from treated plants. Moreover, the concentrations of K and Mg found in our samples fall in the ranges reported in the literature for chickpea seeds, 8,160–15,800 mg kg^{-1} for K and 1,525–1,902 mg kg^{-1} for Mg (Ray et al., 2014; Wang & Daun, 2004). Furthermore, it has recently been shown that the application of 1% WD can significantly reduce the concentration of K in the leaves of citrus rootstocks, although the roots and stems are not affected (Najafi-Ghiri, Mirsoleimani, Boostani, & Amin, 2022). This evidence suggests a negative effect of WD on the content of K in leaves of treated plants.

Although not statistically significant, plant biomass also increased (+37%) following the application of 0.25% WD. This trend is consistent with the results reported in the literature for several crop plants:

lettuce (*Lactuca sativa* L.) +39% (Vannini et al., 2021), +49% (Fedeli et al., 2022), +42% (Mu et al., 2006), tomato (*Solanum lycopersicum* L.) +27% (Mungkunkamchao et al., 2013), rice (*Orzya sativa* L.) +30% (Zulkarami et al., 2014).

5 | CONCLUSIONS

The present study clearly showed a beneficial effect of weekly foliar application of 0.25% WD on chickpea performance. Specifically, plants treated with WD showed an increased yield in terms of seed weight and diameter, and improved nutritional properties in terms of starch, soluble proteins, polyphenols, and total antioxidant power, as well as many amino acids. Moreover, the treatment with WD did not affect the content of mineral elements, except for K and Mg whose content was reduced. Being chickpea seeds an excellent source of proteins, carbohydrates, and minerals, they provide an effective and sustainable alternative to animal consumption for human nutrition. The application of this eco-safe, bio-based product in agriculture can thus potentially contribute to the achievement of United Nations sustainable development goals related to food security, ending world hunger, promoting sustainable agriculture and counteracting climate change.

AUTHOR CONTRIBUTIONS

Stefano Loppi, Riccardo Fedeli and Andrea Vannini conceived and designed the experiments; Riccardo Fedeli, Andrea Vannini, Viviana Maresca, Dmitry Alexandrov and Massimo Guarnieri performed the experiments; Riccardo Fedeli and Silvia Celletti analysed the data and wrote the paper; Stefano Loppi, Silvana Munzi and Cristina Cruz supervised the text. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

Thanks are due to Fattoria Santa Vittoria, Foiano della Chiana, Italy, for kindly providing chickpea seeds, and to Francesco Barbagli (BioDea and BioEsperia, Arezzo, Italy) for kindly providing the wood distillate.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data presented in this study are available on request from the corresponding author.

ORCID

Riccardo Fedeli https://orcid.org/0000-0002-8736-1313
Andrea Vannini https://orcid.org/0000-0003-3789-6691
Silvia Celletti https://orcid.org/0000-0002-5393-6465
Stefano Loppi https://orcid.org/0000-0002-3404-1017

REFERENCES

Aleksandrowicz, L., Green, R., Joy, E. J. M., Smith, P., & Haines, A. (2016). The impacts of dietary change on greenhouse gas emissions, land use, water use, and health: A systematic review. *PLoS One*, *11*, e0165797.

Alewu, B., & Nosiri, C. (2011). Pesticides and human health. In M. Stoytcheva (Ed.), *Pesticides in the modern world–effects of pesticides exposure* (pp. 231–250). London, UK: InTech. http://www.intechopen.com/books/pesticides-in-the-modern-world-effects-of-pesticides-exposure/pesticide-and-human-health

Amir, R., Cohen, H., & Hacham, Y. (2019). Revisiting the attempts to fortify methionine content in plant seeds. *Journal of Experimental Botany*, *70*(16), 4105–4114.

Baenas, N., García-Viguera, C., & Moreno, D. A. (2014). Elicitation: A tool for enriching the bioactive composition of foods. *Molecules*, *19*(9), 13541–13563.

Becagli, M., Santin, M., & Cardelli, R. (2021). Co-application of wood distillate and biochar improves soil quality and plant growth in basil (*Ocimum basilicum*). *Journal of Plant Nutrition and Soil Science*, *185*(1), 120–131. https://doi.org/10.1002/jpln.202100239

Benzon, H. R. L., & Lee, S. C. (2016). Potential of wood vinegar in enhancing fruit yield and antioxidant capacity in tomato. *Korean Journal of Plant Resources*, *29*(6), 704–711.

Binder, S. (2010). Branched-chain amino acid metabolism in *Arabidopsis thaliana*. *The Arabidopsis Book*, 8, e0137.

Brauer, M., & Curtin, J. J. (2018). Linear mixed-effects models and the analysis of nonindependent data: A unified framework to analyze categorical and continuous independent variables that vary within-subjects and/or within-items. *Psychological Methods*, *23*(3), 389–411.

Brouwer, C., Goffeau, A., & Heibloem, M. (1985). *Irrigation water management: Training manual no. 1–introduction to irrigation, chapter 7: Salty soils*. Rome: FAO-Food and Agriculture Organization of the United Nations.

Cabrera, C., Lloris, F., Giménez, R., Olalla, M., & López, M. C. (2003). Mineral content in legumes and nuts: Contribution to the Spanish dietary intake. *Science of the Total Environment*, *308*(1–3), 1–14.

Celletti, S., Bergamo, A., Benedetti, V., Pecchi, M., Patuzzi, F., Basso, D., … Mimmo, T. (2021). Phytotoxicity of hydrochars obtained by hydrothermal carbonization of manure-based digestate. *Journal of Environmental Management*, *280*, 111635.

Celletti, S., Lanz, M., Bergamo, A., Benedetti, V., Basso, D., Baratieri, M., … Mimmo, T. (2021). Evaluating the aqueous phase from hydrothermal carbonization of cow manure digestate as possible fertilizer solution for plant growth. *Frontiers in Plant Science*, *12*, 1317.

Chedea, V. S., & Pop, R. M. (2019). Total polyphenols content and antioxidant DPPH assays on biological samples. In R. R. Watson (Ed.), *Polyphenols in plants* (pp. 169–183). New York: Academic Press.

Cohen, S. A., & Michaud, D. P. (1993). Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Analytical Biochemistry*, *211*(2), 279–287.

Fačkovcová, Z., Vannini, A., Monaci, F., Grattacaso, M., Paoli, L., & Loppi, S. (2020a). Effects of wood distillate (pyroligneous acid) on sensitive bioindicators (lichen and moss). *Ecotoxicology and Environmental Safety*, *204*, 111117.

Fačkovcová, Z., Vannini, A., Monaci, F., Grattacaso, M., Paoli, L., & Loppi, S. (2020b). Uptake of trace elements in the water fern *Azolla filiculoides* after short-term application of chestnut wood distillate (Pyroligneous acid). *Plants*, *9*(9), 1179.

Fait, A., Angelovici, R., Less, H., Ohad, I., Urbanczyk-Wochniak, E., Fernie, A. R., & Galili, G. (2006). Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. *Plant Physiology*, *142*(3), 839–854.

FAOSTAT. (2011). https://www.fao.org/faostat/en/#home.

Fedeli, R., Vannini, A., Guarnieri, M., Monaci, F., & Loppi, S. (2022). 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*, *11*(3), 404. https://doi.org/10.3390/biology11030404

Filippelli, A., Ciccone, V., Loppi, S., & Morbidelli, L. (2021). Characterization of the safety profile of sweet chestnut wood distillate employed in agriculture. *Safety*, *7*(4), 79.

Fincher, G. B. (1989). Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annual Review of Plant Biology*, *40*(1), 305–346.

Garcia, J., Barker, D. G., & Journet, E. P. (2006). Seed storage and germination. In U. Mathesius, E. P. Journet, & L. W. Sumner (Eds), *The Medicago truncatula handbook* (pp. 1–9). Ardmore, OK: Noble Research Institute.

Grewal, A., Abbey, L., & Gunupuru, L. R. (2018). Production, prospects and potential application of pyroligneous acid in agriculture. *Journal of Analytical and Applied Pyrolysis*, *135*, 152–159.

Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance. *International Journal of Molecular Sciences*, *8*(9), 950–988.

Henríquez, C., Almonacid, S., Chiffelle, I., Valenzuela, T., Araya, M., Cabezas, L., … Speisky, H. (2010). Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile. *Chilean Journal of Agricultural Research*, *70*(4), 523–536.

Italian Ministerial Decree 6793. (2018). https://www.gazzettaufficiale.it/eli/id/2018/09/05/18A05693/sg.

Jee, S.-O., & Cho, D.-H. (2005). Effect of pyroligneous liquor on the content and activity of endogenous substances of *Neofinetia falcata* cultured *in vitro*. *Journal of Life Science*, *15*, 673–677.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *British Journal of Nutrition*, *108*(S1), S11–S26.

Kårlund, A., Salminen, J. P., Koskinen, P., Ahern, J. R., Karonen, M., Tiilikkala, K., & Karjalainen, R. O. (2014). Polyphenols in strawberry (*Fragaria* × *ananassa*) leaves induced by plant activators. *Journal of Agricultural and Food Chemistry*, *62*(20), 4592–4600.

Koch, M., Busse, M., Naumann, M., Jákli, B., Smit, I., Cakmak, I., … Pawelzik, E. (2019). Differential effects of varied potassium and magnesium nutrition on production and partitioning of photoassimilates in potato plants. *Physiologia Plantarum*, *166*(4), 921–935.

Levine, R. L., Mosoni, L., Berlett, B. S., & Stadtman, E. R. (1996). Methionine residues as endogenous antioxidants in proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(26), 15036–15040.

Loppi, S., Fedeli, R., Canali, G., Guarnieri, M., Biagiotti, S., & Vannini, A. (2021). Comparison of the mineral and nutraceutical profiles of elephant garlic (*Allium ampeloprasum* L.) grown in organic and conventional fields of Valdichiana, a traditional cultivation area of Tuscany, Italy. *Biology*, *10*(10), 1058.

Luke, S. G. (2017). Evaluating significance in linear mixed-effects models in R. *Behavior Research Methods*, *49*(4), 1494–1502.

Mahadevamma, S., Shamala, T. R., & Tharanathan, R. N. (2004). Resistant starch derived from processed legumes: *In vitro* and *in vivo* fermentation characteristics. *International Journal of Food Science & Nutrition*, *55*, 399–405. https://doi.org/10.1080/09637480400002891

Mao, K., Li, S., Li, B., Wu, W., Yuan, S., Niu, Y., … Zhang, L. (2019). Effects of wood vinegar on the growth, yield and quality of upper leaves of Nanzheng flue-cured tobacco. *Journal of Southwest Agriculture*, *3*, 645–652.

Maphosa, Y., & Jideani, V. A. (2017). The role of legumes in human nutrition. In M. C. Hueda (Ed.), *Functional food - improve health through adequate food*. [Internet]. London: IntechOpen. https://www.intechopen.com/chapters/55808 doi: https://doi.org/10.5772/intechopen.69127

Marchica, A., Ascrizzi, R., Flamini, G., Cotrozzi, L., Tonelli, M., Lorenzini, G., … Pellegrini, E. (2021). Ozone as eustress for enhancing secondary metabolites and bioactive properties in *Salvia officinalis*. *Industrial Crops and Products*, *170*, 113730.

Matityahu, I., Godo, I., Hacham, Y., & Amir, R. (2013). Tobacco seeds expressing feedback-insensitive cystathionine gamma-synthase exhibit elevated content of methionine and altered primary metabolic profile. *BMC Plant Biology*, *13*, 206.

McDermott, J., & Wyatt, A. J. (2017). The role of pulses in sustainable and healthy food systems. *Annals of the New York Academy of Sciences*, *1392*(1), 30–42.

Mourant, D., Yang, D. Q., Lu, X., & Roy, C. (2005). Anti-fungal properties of the pyroligneous liquors from the pyrolysis of softwood bark. *Wood and Fiber Science*, *37*(3), 542–548.

Mu, J., Yu, Z. M., Wu, W. Q., & Wu, Q. L. (2006). Preliminary study of application effect of bamboo vinegar on vegetable growth. *Forestry Studies in China*, *8*(3), 43–47.

Mungkunkamchao, T., Kesmala, T., Pimratch, S., Toomsan, B., & Jothityangkoon, D. (2013). Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (*Solanum lycopersicum* L.). *Scientia Horticulturae*, *154*, 66–72.

Najafi-Ghiri, M., Mirsoleimani, A., Boostani, H. R., & Amin, H. (2022). Influence of wood vinegar and potassium application on soil properties and Ca/K ratio in citrus rootstocks. *Journal of Soil Science and Plant Nutrition*, *22*(1), 334–344.

R Core Team. (2021). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Ratanapisit, J., Apiraksakul, S., Rerngnarong, A., Chungsiriporn, J., & Bunyakarn, C. (2009). Preliminary evaluation of production and characterization of wood vinegar from rubberwood. *Songklanakarin Journal of Science & Technology*, *31*(3), 343–349.

Ray, H., Bett, K., Tar'an, B., Vandenberg, A., Thavarajah, D., & Warkentin, T. (2014). Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. *Crop Science*, *54*(4), 1698–1708.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J. J., & MacKinnon, M. D. (1999). Responses of boreal plants to high salinity oil sands tailings water. *Journal of Environmental Quality*, *28*(6), 1957–1962.

Salgado, P., Lalles, J. P., Toullec, R., Mourato, M., Cabral, F., & Freire, J. P. (2001). Nutrient digestibility of chickpea (*Cicer arietinum* L.) seeds and effects on the small intestine of weaned piglets. *Animal Feed Science and Technology*, *91*(3–4), 197–212.

Salimath, P. M., Toker, C., Sandhu, J. S., Kumar, J., Suma, B., Yadav, S. S., & Bahl, P. N. (2007). Conventional breeding methods. In S. S. Yadav, R. J. Redden, W. Chen, & B. Sharma (Eds.), *Chickpea breeding and management* (pp. 369–390). Wallingford, UK: CAB International.

Segev, A., Badani, H., Kapulnik, Y., Shomer, I., Oren-Shamir, M., & Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). *Journal of Food Science*, *75*(2), S115–S119.

Śmiglak-Krajewska, M., & Wojciechowska-Solis, J. (2021). Consumption preferences of pulses in the diet of Polish people: Motives and barriers to replace animal protein with vegetable protein. *Nutrients*, *13*(2), 454.

Szczebyło, A., Rejman, K., Halicka, E., & Laskowski, W. (2020). Towards more sustainable diets—Attitudes, opportunities and barriers to fostering pulse consumption in Polish cities. *Nutrients*, *12*(6), 1589.

Thalmann, M., & Santelia, D. (2017). Starch as a determinant of plant fitness under abiotic stress. *New Phytologist*, *214*(3), 943–951.

Theerakulpisut, P., Kanawapee, N., & Panwong, B. (2016). Seed priming alleviated salt stress effects on rice seedlings by improving Na^+/K^+ and maintaining membrane integrity. *International Journal of Plant Biology*, *7*(1), 53–58.

Vannini, A., Fedeli, R., Guarnieri, M., & Loppi, S. (2022). Foliar application of wood distillate alleviates ozone-induced damage in lettuce (*Lactuca sativa* L.). *Toxics*, *10*(4), 178.

Vannini, A., Moratelli, F., Monaci, F., & Loppi, S. (2021). Effects of wood distillate and soy lecithin on the photosynthetic performance and growth of lettuce (*Lactuca sativa* L.). *SN Applied Sciences*, *3*(1), 113.

Wang, N., & Daun, J. K. (2004). *The chemical composition and nutritive value of Canadian pulses* (pp. 19–29). Winnipeg, MB: Canadian Grain Commission (CGC).

Weber, H., Borisjuk, L., & Wobus, U. (2005). Molecular physiology of legume seed development. *Annual Review of Plant Biology*, *56*, 253–279.

World Health Organization. (1990). *Public health impact of pesticides used in agriculture*. England; Geneva, Switzerland: World Health Organization.

Zhu, K., Gu, S., Liu, J., Luo, T., Khan, Z., Zhang, K., & Hu, L. (2021). Wood vinegar as a complex growth regulator promotes the growth, yield, and quality of rapeseed. *Agronomy*, *11*(3), 510.

Zulkarami, B., Ashrafuzzaman, M., Husni, M. O., & Ismail, M. R. (2011). Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. *Australian Journal of Crop Science*, *5*, 1508–1514.

Zulkarami, B., Panhwar, Q. A., Ismail, M. R., Mohd Saud, H. M., Mondal, M., Naher, U. A., & Islam, M. R. (2014). Rice yield improvement by foliar application of phytohormone. *Journal of Food, Agricuture & the Environment*, *12*(2), 399–404.

How to cite this article: Fedeli, R., Vannini, A., Celletti, S., Maresca, V., Munzi, S., Cruz, C., Alexandrov, D., Guarnieri, M., & Loppi, S. (2022). Foliar application of wood distillate boosts plant yield and nutritional parameters of chickpea. *Annals of Applied Biology*, 1–8. https://doi.org/10.1111/aab.12794