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Wood Distillate as a Solution for Growing Crops Under Water Deficiency

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Abstract: This study investigated if the foliar application of wood distillate (WD, a by-product of biomass pyrolysis, containing bioactive compounds, including organic acids and phenols) influences some key parameters (fresh weight, photosynthetic efficiency, antioxidant compounds, stress-related biochemical markers, and mineral content) of basil plants, used as a model crop, grown under water-limited conditions. The experimental setup included control and WD treatments (applied via foliar application at 0.2%) under three drought levels: no stress, moderate, and high stress. The results indicated that the application of WD contributed to improving the fresh weight, chlorophyll, reduced oxidative stress, and stable levels of essential nutrients across varying drought intensities. These outcomes highlight the potential of WD as an effective biostimulant for enhancing drought tolerance in basil plants under water deficiency.

Keywords: basil plants; drought stress; oxidative response; pyroligneous acid; sustainable agriculture

1. Introduction

Drought, i.e., a prolonged period of water deficiency, poses a critical challenge to plant growth and development [1–4]. Water is essential for plant physiological processes, including photosynthesis, nutrient uptake, and transpiration [5,6]. Under drought conditions, these processes are disrupted, leading to reduced plant productivity, slower growth rates, and, in severe cases, plant death [7,8]. In response to water stress, plants undergo a series of physiological and biochemical changes, including stomatal closure, altered hormone levels, and the activation of stress-related genes [9,10]. These adaptations, although beneficial for short-term survival, often come at the cost of growth and yield [11,12]. For crops such as basil, which require a consistent water supply, drought conditions can severely impact biomass production and nutritional quality [13,14]. As global temperature rises and water scarcity becomes more prevalent, the need for sustainable solutions that allow the cultivation of crops under water-limited conditions while minimizing the impact on the environment is crucial [15,16]. The Mediterranean basin, a key zone for agriculture, is particularly vulnerable due to its arid and semi-arid climate, where water availability is critical for sustaining both natural ecosystems and agricultural production [17,18]. As drought conditions intensify, the need for innovative, sustainable solutions to improve plant resilience has become increasingly urgent. One promising nature-based solution that has recently attracted attention is wood distillate (WD, also known as wood vinegar or pyroligneous acid), a by-product of plant biomass pyrolysis for green energy production [19],

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which has shown potential for enhancing resilience against various abiotic stressors, including drought. Fang et al. [20] found that WD may improve plant water use efficiency by promoting root growth and enhancing the ability of plants to access deeper soil water reserves. Ghorbani et al. [21] found that applying WD via fertirrigation reduces the oxidative stress in lettuce plants, also increasing some key parameters like fresh weight and antioxidant compounds, showing a possible use of this product in a water deficit situation.

Additionally, the bioactive compounds of WD, such as phenols and organic acids, may play a role in modulating plant stress responses, including the activation of antioxidant defenses that help mitigate the damaging effects of drought-induced oxidative stress [22,23]. Despite these few studies suggesting that WD could be a valuable tool for improving crop resilience to drought, especially in water-sensitive species, its potential for growing crops under water-limited conditions remains largely unexplored. Notably, no prior research has examined the effects of WD through foliar applications, despite this being one of the most efficient delivery methods for improving crop yield and enhancing resistance to abiotic stress. This study, therefore, represents a significant advancement in scientific understanding by exploring a novel application approach that could maximize the agronomic benefits of WD, offering new insights into sustainable strategies for mitigating water stress in crops. In particular, foliar application is especially advantageous under drought conditions, as it allows for the direct uptake of bioactive compounds by the leaves, bypassing the limitations of reduced soil moisture that can impair root absorption. Given that WD contains highly mobile and readily absorbable compounds, its foliar application ensures rapid assimilation and targeted action, reducing losses due to microbial degradation in the soil and enhancing treatment efficiency in water-limited environments.

Basil (*Ocimum basilicum* L.) is a highly valued herb, cultivated globally for its culinary, medicinal, and aromatic properties. In Mediterranean climates, basil is a staple crop, but it is also highly sensitive to water shortage since drought can greatly reduce its biomass production, as well as the quality and quantity of its antioxidant molecules [13,14]. Due to its sensitivity to water availability, basil is an ideal candidate for investigating the effects of WD on plant growth and productivity under water-limited conditions.

The aim of this study is to test if, under water-limited conditions, foliar application of WD influences some key parameters, such as fresh weight, chlorophyll content, photosynthetic efficiency, antioxidant compounds, stress-related biochemical markers, and mineral content, using basil as a model plant.

2. Materials and Methods

2.1. Experimental Design

Basil (*Ocimum basilicum* L.) seedlings of uniform size were obtained from a local nursery (2 week growing period from the sowing of the seeds) and transplanted individually into black plastic pots, each containing 120 g of a commercial growing medium (Vigor-Plant Italia srl, Fombio, Italy). The experimental design included two primary treatments: control (C, leaves sprayed weekly with water) and wood distillate (WD, leaves sprayed weekly with 0.2% WD). Wood distillate characteristics are reported in Fedeli et al. [24]. The choice of a 0.2% concentration was chosen based on previous literature articles [24,25]. Higher concentrations (>0.2%) have the potential to induce phytotoxic effects, as observed in several articles, while lower concentrations may not provide sufficient physiological benefits. This specific concentration was selected to ensure optimal plant response without causing stress or adverse side effects. Additionally, foliar application at this level allows for the efficient absorption of bioactive compounds while avoiding excessive accumulation that could interfere with normal leaf physiology. Each treatment was further divided into three levels of stress by water deficiency (Figure 1): no stress (NS, irrigation at 70% of

water holding capacity—WHC), moderate stress (MS, irrigation at 50% of WHC), and high stress (HS, irrigation at 30% of WHC). Soil moisture levels were maintained throughout the six-week experimental period by weighing each pot daily and adjusting the water input to match the designated drought stress levels.

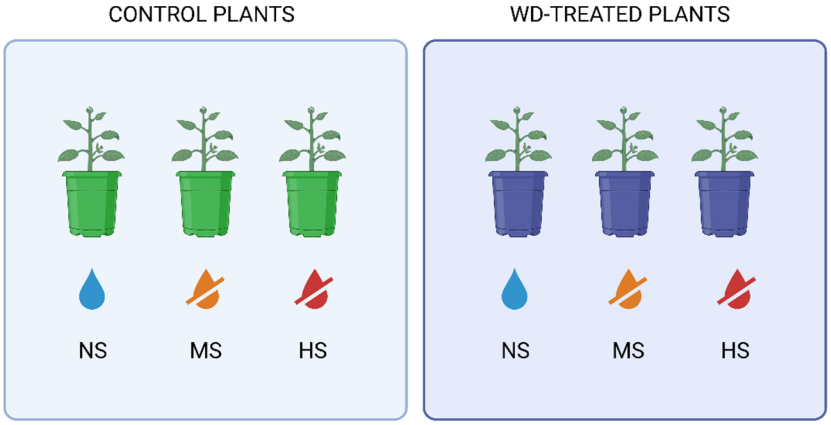

Figure 1. Experimental design. NS = no stress; MS = moderate stress; HS = high stress.

The seedlings were grown in a climatic chamber with a 16/8 h day/night photoperiod, temperature set at 24 °C during the day and 20 °C at night, photosynthetically active radiation (PAR) of 250 µmol m^{-2} s^{-1}, and 70% relative humidity. At the conclusion of the 4-week growth period, the photosynthetic parameters of the leaves were measured (*see Section 2.2*) and then the above-ground portion of basil plants was harvested and weighed to determine the fresh weight. Subsequently, the leaves were dried at 40 °C using a food dehydrator, pulverized using a professional mixer to obtain a uniform powder, and stored at −20 °C for later biochemical analyses.

2.2. Photosynthetic Parameters

The quantification of total chlorophyll content on a leaf area basis (mg/m^2) was conducted in intact basil leaves utilizing a portable, noninvasive chlorophyll content meter (CCM-300, Opti-Sciences Inc., Hudson, NH, USA). For each plant, chlorophyll content was assessed by performing three separate measurements per leaf—one near the apex, one at the central region, and one proximal to the base—focusing on the three most fully expanded upper leaves [25].

Following a 15 min period of dark adaptation, the leaves were exposed to a 1 s pulse of saturating red light (650 nm) at an intensity of 2400 µmol mm^{-2} s^{-1}. The resulting fluorescence emission was recorded using a plant efficiency analyzer (Handy PEA, Hansatech Ltd., Norfolk, UK) in accordance with the protocol outlined by Fedeli et al. [26]. Two key parameters indicative of photosynthetic performance were analyzed: F_V/F_M, which denotes the maximum quantum efficiency of photosystem II (PSII), and PI_{abs}, an index reflecting plant vitality and overall physiological status.

2.3. Malondialdehyde

The determination of malondialdehyde (MDA) content was performed following a modified protocol adapted from Fedeli et al. [27]. In brief, 0.5 g of frozen plant material was homogenized in 5 mL of an extraction solution containing 0.25% (*w*/*v*) 2-thiobarbituric acid (TBA) dissolved in 10% (*w*/*v*) trichloroacetic acid. The homogenate was incubated at 95 °C for 30 min in a thermoblock, followed by rapid cooling on ice to terminate the reaction. Subsequently, the mixture was centrifuged at 4000 rpm for 10 min. The supernatant was analyzed spectrophotometrically at 532 nm and 600 nm using a UV-Vis spectropho-

tometer. The absorbance reading at 600 nm was subtracted from that at 532 nm, and the concentration of the MDA–TBA complex was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4. Proline

Proline content was quantified according to the method described by Azarnejad et al. [28]. Briefly, 0.1 g of frozen sample was homogenized in 2 mL of 3% (*w*/*v*) 5-sulfosalicylic acid dihydrate, followed by centrifugation at 4000 rpm for 10 min. A 0.5 mL aliquot of the supernatant was then mixed with 0.5 mL of acetic acid and 0.5 mL of acid-ninhydrin reagent, which was prepared by dissolving 1.25 g of ninhydrin in 30 mL of acetic acid and 20 mL of 6 M phosphoric acid. The reaction mixture was incubated at 100 °C for 1 h, then rapidly cooled on ice to halt the reaction. Subsequently, 1.5 mL of toluene was added to extract the colored complex. The absorbance of the clear organic phase was measured at 520 nm using a UV-Vis spectrophotometer.

2.5. Antioxidant Compounds

The total phenolic content (TPC) and total flavonoid content (TFC) were assessed following a modified version of the method described by Borella et al. [29]. Approximately 1 g of dried plant material was extracted in 10 mL of 80% (*v*/*v*) methanol. The extraction was carried out under continuous orbital shaking for 30 min, followed by incubation in the dark at 4 °C for 48 h. The extracts were then filtered using Whatman No. 1 filter paper, and the resulting filtrates were used for subsequent TPC and TFC determinations.

For TPC quantification, the procedure described by Fedeli et al. [30] was followed. A 0.125 mL aliquot of the filtrate was mixed with 2 mL of deionized water, followed by the addition of 0.125 mL of Folin–Ciocalteu reagent. After incubating in darkness for 3 min, 1.250 mL of 7% (*w*/*v*) sodium carbonate (Na_2CO_3) and 1 mL of deionized water were added. The solution was vigorously mixed and further incubated in the dark for 90 min. The absorbance was subsequently recorded at 760 nm using an Agilent UV-Vis spectrophotometer.

The TFC was determined following the protocol outlined by Lamaro et al. [31]. A 0.250 mL aliquot of the filtrate was mixed with 0.075 mL of 5% (*w*/*v*) sodium nitrite ($NaNO_2$), followed by the addition of 0.075 mL of 10% (*w*/*v*) aluminum chloride ($AlCl_3$) after 5 min. The mixture was shaken and incubated in darkness for another 5 min before the addition of 0.5 mL of 1 M sodium hydroxide (NaOH). After an additional 15 min incubation in the dark, absorbance was measured at 415 nm using an Agilent UV-Vis spectrophotometer.

Vitamin C content was analyzed using the protocol outlined by Fedeli et al. [32]. Approximately 200 mg of fresh plant material was homogenized in 0.8 mL of 10% (*w*/*v*) trichloroacetic acid (TCA). The homogenate was filtered through gauze, chilled at −20 °C for 5 min, and centrifuged at 3000 rpm for 5 min. A 0.4 mL aliquot of the supernatant was subsequently mixed with 1.6 mL of distilled water (dH_2O) and 0.2 mL of 0.2 M Folin–Ciocalteu reagent. After a 10 min incubation in the dark, absorbance was measured at 760 nm using a UV-Vis spectrophotometer.

2.6. Mineral Content

The elemental composition of basil leaves was assessed using a portable X-ray fluorescence (XRF) spectrometer, following the protocol established by Fedeli et al. [33]. Approximately 1 g of dried plant material was placed in a plastic sample cup and positioned within the instrument's designated compartment for analysis. The concentrations of aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), potassium (K), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn) were quantified under the Geochem analytical

mode. Measurements were performed with an acquisition time of 20 s per beam, utilizing a total of three beams per sample analysis. The accuracy and reliability of the obtained data were validated using 14 certified plant reference materials, as described in Fedeli et al. [33]. The mineral content is expressed as mg of each element per kg of dry weight.

2.7. Statistical Analysis

Since the data followed a normal distribution (Shapiro–Wilk test, $p > 0.05$), parametric tests were used. A two-way ANOVA was run to check for differences ($p < 0.05$) in the effects of water stress levels and treatment, as well as for their interaction, followed by a Tukey HSD post hoc test ($p < 0.05$). The data are presented as mean values of 10 replicates, along with the associated standard error. Multivariate correlations analysis was conducted by normalizing the data and calculating the percentage change in parameters in the NS + WD, MS, MS + WD, HS, and HS + WD treatments relative to NS. A heatmap was then generated using the ggplot2 package to visualize the variations. Positive values (increase compared to NS) were represented in blue, while negative values (decrease compared to NS) were displayed in red, using an inverted "*coolwarm*" color scale for better interpretation. All statistical analyses were performed using the R software [34].

3. Results

The results of two-way ANOVA (Table 1) showed a statistically significant effect of water stress level and treatment, while there was no statistically significant interaction between them.

Table 1. Results of two-way ANOVA.

		Fresh Weight		**Chlorophyll**		**F_v/F_m**	
	df	F	*p*	F	*p*	F	*p*
WS	2	5.43	0.012	4.12	0.014	3.87	0.532
WD	1	6.25	0.021	7.02	0.018	5.1	0.487
WS × WD	1	0.89	0.041	1.1	0.033	0.75	0.478
		Pi_{abs}		**MDA**		**Proline**	
	df	F	*p*	F	*p*	F	*p*
WS	2	2.96	0.874	6.3	0.024	5.68	0.451
WD	1	4.55	0.647	5.98	0.145	4.88	0.024
WS × WD	1	1.03	0.364	0.92	0.401	1.15	0.321
		TPC		**TFC**		**Vitamin C**	
	df	F	*p*	F	*p*	F	*p*
WS	2	8.75	0.001	3.15	0.146	1.89	0.154
WD	1	9.5	0.004	4.32	0.198	2.65	0.109
WS × WD	1	0.68	0.049	0.82	0.443	1.05	0.351
		Al		**Ca**		**Cu**	
	df	F	*p*	F	*p*	F	*p*
WS	2	4.21	0.02	6.35	0.31	3.77	0.35
WD	1	5.55	0.108	4.88	0.24	6.32	0.14
WS × WD	1	1.22	0.272	0.92	0.34	1.05	0.312
		Fe		**K**		**Mn**	
	df	F	*p*	F	*p*	F	*p*
WS	2	5.12	0.24	7.42	0.29	4.88	0.27
WD	1	7.2	0.12	6.5	0.18	5.75	0.37
WS × WD	1	0.87	0.369	0.78	0.398	1.02	0.318
		P		**S**		**Zn**	
	df	F	*p*	F	*p*	F	*p*
WS	2	6.1	0.4	3.95	0.28	5.7	0.41
WD	1	6.4	0.51	5.3	0.22	6.8	0.25
WS × WD	1	0.95	0.353	1.15	0.289	1.08	0.307

Pi_{abs}: performance index; **MDA**: malondialdehyde content; **TPC**: total polyphenol content; **TFC**: total flavonoid content; **Al**: aluminum; **Ca**: calcium; **Cu**: copper; Fe: iron, K: potassium; **Mn**: manganese; **P**: phosphorus; **S**: sulfur; **Zn**: zinc.

3.1. Effect of Water Deficiency

Control plants did not show any difference in fresh weight across water stress levels, while the content of chlorophyll decreased progressively with stress; a similar trend was observed also for WD-treated plants (Table 2, Figure 4).

The content of MDA increased in control plants with drought stress, while, in WD-treated plants, no significant difference was observed (Figures 2A and 4). Proline was unchanged in control plants, but a remarkable decrease was observed in WD-treated plants with increasing stress (Figures 2B and 4).

Table 2. Fresh weight, chlorophyll content (Chl), F_V/F_M, and PI_{ABS} of basil (mean ± standard error). NS = no stress; MS = medium stress; HS = high stress. C = control plants; WD = wood distillate. Different letters (lowercase for C and uppercase for WD) indicate significant differences between the same treatment; * indicates significant differences ($p \leq 0.05$) in pairs relative to the same treatment.

	NS		**MS**		**HS**	
	C	***WD***	***C***	***WD***	***C***	***WD***
Fresh weight (g)	9.8 ± 0.5 *	11.2 ± 0.3	9.7 ± 0.3 *	11.0 ± 0.4	9.1 ± 0.4	9.8 ± 0.6
Chl (mg m^{-2})	295 ± 9 *	369 ± 6 ^{A}	297 ± 11 *	338 ± 9 ^{AB}	272 ± 8 *	332 ± 11 ^{B}
F_V/F_M	0.821 ± 0.003	0.815 ± 0.009	0.811 ± 0.009	0.820 ± 0.003	0.808 ± 0.010	0.818 ± 0.004
PI_{ABS}	2.82 ± 0.25	2.60 ± 0.21	2.57 ± 0.22	2.48 ± 0.23	2.75 ± 0.29	2.63 ± 0.14

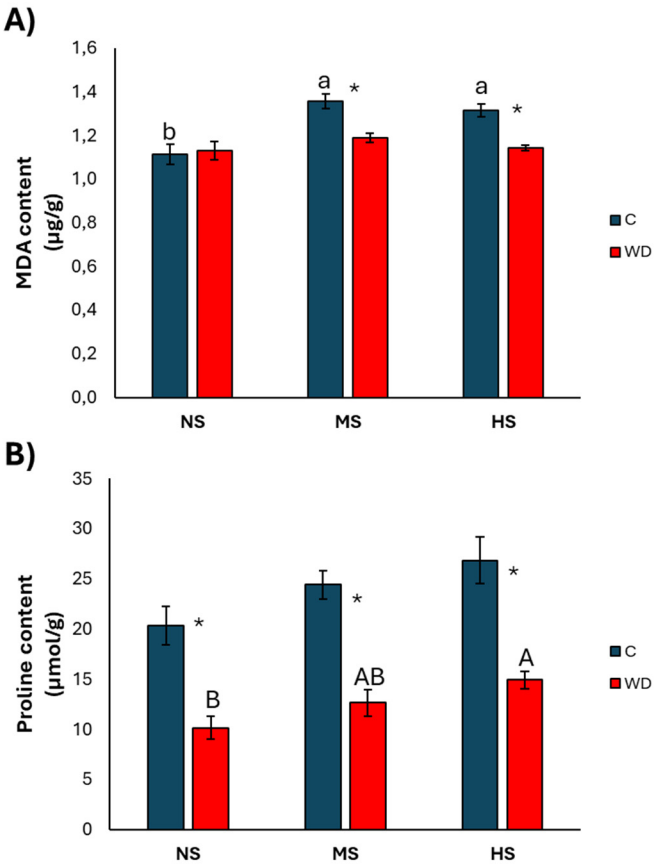

Figure 2. Malondialdehyde (MDA) content (**A**) and proline content (**B**) of basil (mean ± standard error). NS = no stress; MS = medium stress; HS = high stress. C = control plants; WD = wood distillate. Different letters (lowercase for C and uppercase for WD) indicate significant differences between the same treatment; * indicates the significant differences ($p \leq 0.05$) in pairs relative to the same treatment.

As far as antioxidant compounds are concerned (Figures 3 and 4), the only significant variations were observed for TPC in control plants, which decreased along the water stress.

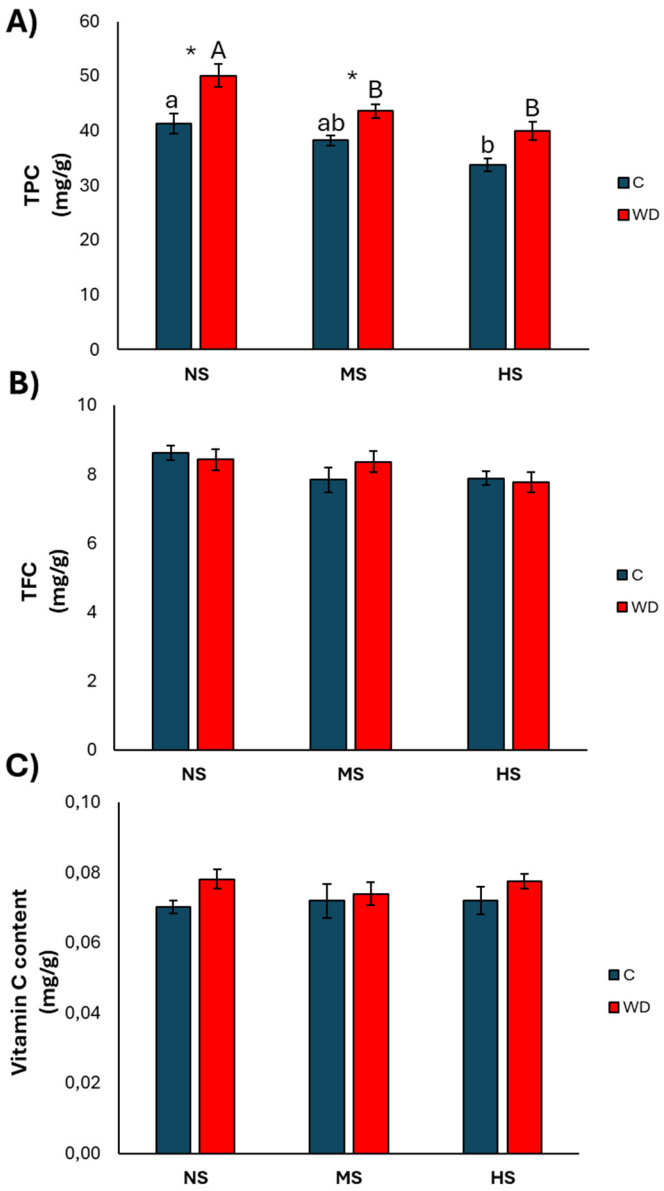

Figure 3. Total polyphenol content (TPC) (**A**), total flavonoid content (TFC) (**B**), and vitamin C content (**C**) of basil (mean ± standard error). NS = no stress; MS = medium stress; HS = high stress. C = control plants; WD = wood distillate. Different letters (lowercase for C and uppercase for WD) indicate significant differences between the same treatment; * indicates the significant differences ($p \leq 0.05$) in pairs relative to the same treatment.

The mineral content of control plants was unchanged with increasing water stress, with the only exception of a reduction in Al at high water stress (Table 3, Figure 4). Similarly, WD-treated plants also did not show significant differences across stress levels, with the only exception of P, which increased at high water stress (Table 3, Figure 4).

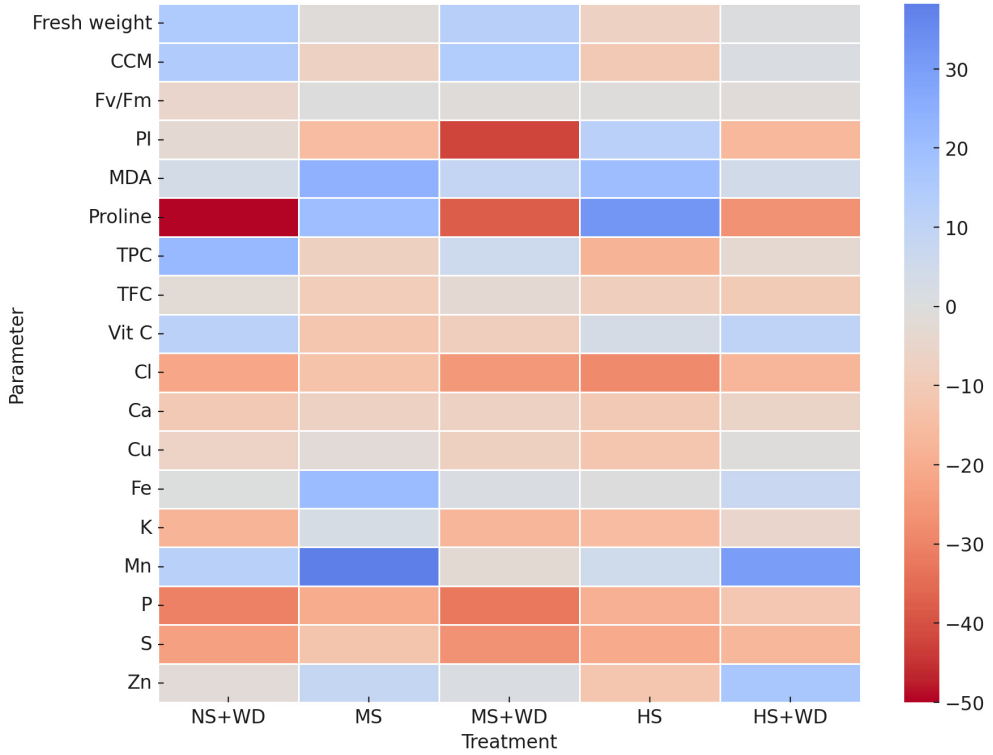

Figure 4. Heatmap showing the percentage change in physiological (fresh weight, chlorophyll content (CCM), F_v/F_m, and performance index (PI)) and biochemical parameters (malondialdehyde content (MDA), proline, total polyphenol content (TPC), total flavonoid content (TFC), vitamin C (Vit C), calcium (Ca), copper (Cu), iron (Fe), potassium (K), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn)) under different water stress treatments alone (MS and HS) and in combination with wood distillate (WD) application (NS + WD, MS + WD, and HS + WD) relative to the control (NS). Blue shades indicate an increase, while red shades represent a decrease compared to the control. The intensity of the color reflects the magnitude of the variation.

Table 3. Mineral content of basil (mean ± standard error). NS = no stress; MS = medium stress; HS = high stress. C = control plants; WD = wood distillate. Different letters (lowercase for C and uppercase for WD) indicate significant differences between the same treatment; symbol * indicates the significant differences ($p \leq 0.05$) in pairs relative to the same treatment.

	NS		**MS**		**HS**	
	C	***WD***	***C***	***WD***	***C***	***WD***
Al (mg/kg)	475 ± 15 ^{ab}	505 ± 20	582 ± 38 ^{a}	514 ± 17	518 ± 24 ^{b}	507 ± 30
Ca (%)	1.4 ± 0.1	1.3 ±0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
Cu (mg/kg)	3.4 ± 0.4	3.2 ± 0.3	3.4 ± 0.3	3.2 ± 0.2	3.0 ± 0.2	3.5 ± 0.3
Fe (mg/kg)	64 ± 6	64 ± 3	77 ± 4	65 ± 3	64 ± 3	69 ± 6
K (%)	2.3 ± 0.1 *	1.9 ± 0.1	2.4 ± 0.2 *	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
Mn (mg/kg)	49 ± 6	55 ± 5	68 ± 6 *	49 ± 3	52 ± 3	64 ± 7
P (mg/kg)	6296 ± 309 *	4382 ± 293 ^{B}	5017 ± 496	4226 ± 260 ^{B}	5106 ± 251	5569 ± 297 ^{A}
S (mg/kg)	1674 ± 142 *	1282 ± 64	1471 ± 145	1218 ± 74	1328 ± 83	1389 ± 99
Zn (mg/kg)	33 ± 2	32 ± 2	35 ± 1	33 ± 2	29 ± 1 *	38 ± 2

3.2. Effect of Treatment with Wood Distillate

The fresh weight of basil was higher in WD-treated plants at no and moderate water stress but similar at high water stress, while the content of chlorophyll was always higher in WD-treated plants, irrespective of water stress level (Table 2, Figure 4). Differences did not emerge for F_V/F_M and PI_{ABS} (Table 2, Figure 4).

The level of MDA was higher in control plants at both moderate and severe water stress, while the level of proline was always higher in control plants, irrespective of water stress level (Figures 2 and 4).

The level of TPC was always higher in WD-treated plants, irrespective of water stress level, while differences did not emerge for TFC and vitamin C (Figures 3 and 4).

The mineral content was mostly similar in control and WD-treated plants across all water stress levels, with the exceptions of K being higher in control plants at no and moderate water stress, P and S being higher in control plants without water stress, and Zn being higher in WD-treated plants at severe water stress (Table 3, Figure 4).

4. Discussion

4.1. Effect of Water Deficiency

Water deficiency is a well-documented environmental stressor that disrupts plant growth, physiology, and biochemical processes, leading to decreased biomass and heightened oxidative damage from reactive oxygen species (ROS) accumulation [35–37]. Several studies showed that drought can markedly decrease the photosynthetic capacity of plants due to chlorophyll degradation and impaired biosynthesis [38,39], often affecting crops like maize and wheat [40,41]. Our results are consistent with these outcomes and showed a significant reduction in the content of chlorophyll as water stress increased. No variations are reported in Fv/Fm and PI_{abs} values, which are two important parameters for the evaluation of the photosynthetic responses. On one hand, Fv/Fm, the maximum quantum yield of PS_{II} photochemistry, is a widely used indicator of plant photosynthetic efficiency and stress status. In non-stressed higher plants, Fv/Fm typically ranges between 0.78 and 0.84, with values below this threshold indicating photoinhibition or stress-induced damage to the photosynthetic apparatus. On the other hand, PI_{abs} is a comprehensive indicator of photosynthetic performance, integrating multiple parameters related to energy absorption, electron transport, and overall plant vitality [42,43].

Oxidative damage is another key issue of water deficiency, often manifested as increased MDA levels due to lipid peroxidation. Several studies (e.g., [44,45]) reported that MDA content rises significantly under water stress, indicating enhanced oxidative damage to cell membranes. This is consistent with our results, which showed higher MDA levels in control plants as water stress increased, underscoring the role of ROS-induced lipid peroxidation in plant damage under water deficiency. High ROS levels compromise membrane stability and cellular integrity, as shown in other studies on tomato and barley under similar stress conditions [44,45].

Proline accumulation, an adaptive response to osmotic stress, helps stabilize cell structure and protect proteins, often serving as a measure of tolerance to drought stress [46]. However, in this study, the content of proline in control plants remained relatively stable across the water stress levels, possibly reflecting species-specific or even genotype-specific responses to drought. Other studies, such as those on rice and wheat, indicate that proline accumulation may vary based on the severity of the stress and the species' ability to compensate through alternative pathways [47,48].

Water stress also disrupts nutrient uptake and transport, altering the concentration of essential nutrients such as K, P, and Zn, which play key roles in enzyme activation, cellular regulation, and stress mitigation. Reduced soil water limits nutrient mobility and uptake efficiency [49], leading to nutrient deficiencies that can worsen drought impact on metabolic and structural functions. Our findings of decreased K, P, and Zn in control plants as water stress increased align with previous studies, which suggest that nutrient scarcity under drought impairs plant growth, metabolism, and overall health [50,51].

4.2. Effect of Treatment with Wood Distillate

Studies on the effect of foliar application of wood distillate to plants under water deficiency are scanty. Treatment with WD mitigated many negative drought effects, showing a remarkable potential as a biostimulant that enhances resilience to drought.

A significant effect of WD application was the increase in fresh weight observed in treated plants across stress levels. Plants subjected to NS and MS conditions and treated with WD exhibited a notable improvement in fresh weight accumulation compared to untreated controls. This suggests that WD may enhance water retention, cell expansion, and overall plant growth even under limited water availability. The positive effect of WD on fresh weight is likely attributed to its biostimulant properties, which include improved nutrient uptake, enhanced photosynthetic efficiency, and reduced oxidative stress. Similar results were reported by Ghorbani et al. [21], who documented a significant increase in lettuce biomass under drought stress following WD application via fertirrigation. Additionally, the presence of bioactive compounds in WD, such as phenolic compounds and organic acids [52], may contribute to the enhancement of metabolic processes involved in growth and biomass production. By improving water-use efficiency and nutrient availability, WD-treated plants were better able to sustain growth, leading to higher fresh weight accumulation even under stress conditions. Another prominent effect observed is the ability of WD to preserve chlorophyll content across all stress levels, suggesting improved chlorophyll homeostasis. Consistent with our findings, Wang et al. [53] reported a similar chlorophyll maintenance in wheat treated with WD and attributed this effect to the bioactive compounds of WD, which may enhance chlorophyll biosynthesis or delay degradation. Similarly, Ghorbani et al. [21] observed a 15% increase in chlorophyll content in WD-treated lettuce plants under moderate drought conditions, reinforcing the idea that WD supports chlorophyll stability even under limited water availability. This chlorophyll-preserving effect can be attributed to the bioactive compounds present in WD, which may act through multiple mechanisms to enhance chlorophyll biosynthesis and delay its degradation under water stress conditions. Phenolic compounds and organic acids present in WD are known to have antioxidant properties, which can mitigate oxidative stress, a key factor in chlorophyll breakdown during drought [54,55]. By reducing ROS accumulation, WD likely helps maintain chlorophyll integrity and prolongs its functionality. Additionally, WD may influence hormonal regulation, particularly by modulating cytokinin and abscisic acid (ABA) pathways. Cytokinins are known to delay leaf senescence by stabilizing chlorophyll levels [56,57], while ABA plays a crucial role in drought response [58–60]. The balance between these hormones could contribute to improved chlorophyll retention and overall stress tolerance. Furthermore, WD may enhance the activity of antioxidant enzymes such as SOD, CAT, and POD, which help neutralize oxidative damage and prevent chlorophyll degradation. This aligns with previous findings indicating that WD-treated plants exhibit higher antioxidant capacity, supporting their ability to withstand abiotic stress conditions more effectively [24,27].

Indeed, another critical benefit of WD is its ability to reduce oxidative damage, as indicated by the lower MDA levels in WD-treated plants. This aligns with the findings of Grewal et al. [52] and Loo et al. [61], who documented that WD enhances antioxidant defense, leading to better ROS scavenging and reduced lipid peroxidation. Studies by Saeed et al. [51] showed that WD decreases MDA levels in tomato under drought stress, as antioxidant enzymes like SOD and CAT were activated, thus protecting plants from oxidative damage. In agreement, Ghorbani et al. [21] demonstrated that WD drench application significantly reduced MDA levels in lettuce by up to 19% under high drought stress, indicating WD's efficacy in maintaining cell membrane integrity and reducing oxidative stress.

A notable reduction in the content of proline has been observed in WD-treated plants, especially under high-stress conditions. This trend suggests that WD application may reduce osmotic stress, thus reducing the need for proline synthesis. This observation is consistent with the findings of Rathore et al. [62], who demonstrated that seaweed extracts and similar biostimulants help maintain osmotic balance, reducing the need for proline accumulation. Ghorbani et al. [21] also reported reduced proline levels in WD-treated plants, highlighting that WD supports osmotic stability, potentially reducing reliance on proline as an osmoprotectant.

The enhanced TPC observed in WD-treated plants across stress levels indicates that WD may trigger antioxidant pathways, increasing protective secondary metabolites. Phenolic compounds are critical in plant defense, as they scavenge ROS and reduce oxidative damage. Khan et al. [63] and Liang et al. [64] found increased TPC in biostimulant-treated plants under drought stress, which helped mitigate oxidative damage through elevated antioxidant pathways. Likewise, Aremu et al. [22] and Ghorbani et al. [21] observed elevated antioxidant activity in WD-treated plants, effectively enhancing ROS scavenging capacity under drought conditions.

WD-treated plants also exhibited relatively stable nutrient levels across stress conditions, contrasting with the nutrient depletion seen in control plants. The effect of WD on nutrient stability may be due to its ability to enhance nutrient-use efficiency or stimulate root uptake under water-limited conditions. This observation is consistent with the findings by Akhtar et al. [65], where WD-treated crops maintained higher levels of essential nutrients like K and P, which are crucial for drought resilience. The outcomes of Jindo et al. [66] and Fang et al. [20] further support this, demonstrating that WD-treated plants retain higher nutrient levels, such as K, which is essential for osmotic regulation under drought stress. Our findings of increased K and Zn levels in WD-treated plants align with these studies, suggesting that WD improves nutrient acquisition, thereby enhancing plant resilience to drought. The significant increase in Zn content in WD-treated plants under high drought stress is particularly interesting, as Zn plays a key role in stress tolerance by activating enzymes and maintaining membrane stability. Similar results were observed by Chen et al. [67], with rice treated with WD exhibiting improved Zn uptake under drought, supporting essential metabolic processes. This parallels our findings, where increased Zn in WD-treated plants likely supported antioxidant defenses and overall stress tolerance.

5. Conclusions and Future Perspectives

In conclusion, the findings from this study demonstrate the potential of wood distillate (WD) as a biostimulant for enhancing drought tolerance in basil plants. WD application was associated with increased fresh weight, improved chlorophyll content, reduced oxidative stress, and more stable nutrient levels across different drought intensities. These benefits, likely mediated by the bioactive compounds in WD, suggest a supportive role in maintaining key physiological processes such as photosynthesis and antioxidant defenses under water-limited conditions. However, these results are derived from a single crop species, one WD concentration, and a short-term experimental period. Therefore, further research is needed to confirm the consistency and generalizability of these effects. Future studies should include (*i*) dose–response trials to identify optimal application rates, (*ii*) absorption and translocation profiling to understand the movement and fate of WD within plant tissues, (*iii*) residue analyses to ensure safety and sustainability, and (*iv*) longer-term evaluations to assess the persistence and repeatability of the observed effects. Additionally, expanding this research to other crops with varying drought sensitivities and to different environmental conditions will be essential for validating the broader applicability of WD. Investigations into other abiotic stressors (e.g., salinity, heat,

and nutrient imbalance), as well as molecular and physiological analyses, could further clarify the underlying mechanisms involved. Taken together, these efforts will help define the full potential and limitations of WD as a sustainable input for improving crop resilience under stress.

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