

## Boosting the resilience to drought of crop plants using wood distillate: A pilot study with lettuce (*Lactuca sativa* L.)

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### ABSTRACT

Increasingly severe drought driven by climate change is harming crop quality and productivity, making it crucial to find nature-based solutions like wood distillate (WD) to aid crops withstand these stressful conditions. This study investigated the effects of WD application on the growth and stress responses of lettuce (*Lactuca sativa* L.) under drought conditions. The experiment included periodic measurements of plant growth-related parameters and stress indices to evaluate the effectiveness of WD in mitigating drought-induced damage. The results showed a significant increase (13–26 %) in fresh biomass of the aboveground portion of lettuce treated with WD, indicating the potential of WD as a biostimulant to promote plant growth. In addition, treatment with WD led to a significant increase in total soluble protein content (14–28 %), showing a possible positive effect on protein synthesis. The enhancement of anti-radical activity (measured in terms of DPPH) following the application of WD up to 42 % reflected the ability of the plant to scavenge harmful reactive oxygen species and alleviate oxidative stress. The observed reduction (up to 19 % in comparison with control) in MDA content also confirmed the effectiveness of WD in protecting the integrity of plant cell membranes from oxidative damage. Despite the beneficial effects on anti-radical activity, the total content of the antioxidant compounds (phenols and flavonoids) decreased with the use of WD (5–14 %), most likely suggesting complex interactions between WD and the biosynthesis of these secondary metabolites. The study showed the positive effect of WD application on the growth and stress tolerance of lettuce plants under drought conditions. These results can provide new insights into sustainable agricultural practices and the potential application of WD as a nature-based and effective means to improve crop productivity, especially in water scarce areas.

### Introduction

Drought is exerting a profound impact on the physiological well-being of plants even in natural ecosystems. This has far-reaching implications for ecosystem management and conservation efforts (Munné-Bosch and Villadangos, 2023). Alarming trends show that the intensity and frequency of drought events have surged in recent decades, and climate change continues to exacerbate this trend (Jafari et al., 2018; González-Villagra et al., 2022; Yang et al., 2023a). Climate change is exacerbating the risk of expansion in arid and semi-arid zones, leading to more rapid and severe drought occurrences (Li et al., 2024). These sensitive areas, with fragile ecosystems, are particularly vulnerable to

the impacts of climate-induced drought (Yang et al., 2023b).

Such extreme drought events have adverse effects on plant physiology and growth, leading to reduced productivity and increased mortality (Yang et al., 2023a). In addition, the sensitivity of vegetation to soil moisture and drought is generally increasing (Li et al., 2024). Thus, it has become crucial to unravel the physiological mechanisms behind how plants respond to extreme drought, as this knowledge is paramount in predicting plant performance under the looming specter of climate change. Therefore, the quest for novel and effective methodologies to combat these unfavorable conditions becomes ever more pressing. Although water scarcity presents itself as a shared challenge, certain regions (especially those in the Mediterranean area in addition to arid

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and semi-arid areas) exhibit higher susceptibility to drought, therefore, the quest for novel and effective solutions to combat these unfavorable adverse conditions becomes ever more pressing. In this context, the utilization of eco-friendly processes, providing renewable energy and green products, and the adoption of sustainable practices in agriculture have attracted substantial global attention (Cândido et al., 2023). One such promising nature-based solution is wood distillate (WD), commonly known also as wood vinegar or pyrolygneous acid.

Wood distillate, a by-product of woody biomass pyrolysis for green energy production, boasts a diverse composition, encompassing organic acids, aldehydes, ketones, phenols, and furans (Zhang et al., 2020). While the chemical composition of WD mainly depends on the heating rate, temperature, and raw materials (Wang et al., 2019), the intricate richness in WD composition has opened possibilities for its application in various sectors, particularly in agriculture, where it serves as a sustainable additive and alternative to conventional synthetic agrochemicals, such as pesticides and fertilizers (Tiilikkala et al., 2011). The natural acidity of WD can help balance soil pH, prevent ammonium loss, reduce N<sub>2</sub>O and CH<sub>4</sub> emissions, enhance nutrient availability, and change the composition of soil microbes (Lee et al., 2021; Yuan et al., 2022). Wood distillate gained more attention recently in different sectors such as in reducing water pollution, mitigating soil contamination, promoting environmental sustainability, and food and health industries (Mhamdi, 2023). Although its relative high application cost is a limitation to use it in agriculture, increasing biochar production with developed technologies and expansion of the use will make it more accessible and cost-effective (Liu et al., 2021). The wood distillate market is expected to reach USD 80 million by 2030, with a compound annual growth rate (CAGR) of 6.80 %, starting at USD 53.4 million in 2023 (Singh, 2024).

Previous research has demonstrated numerous agricultural benefits from WD application, with notable effects including heightened crop productivity, stimulated nutrient uptake, and enhanced photosynthetic rates (Aremu et al., 2012; Grewal et al., 2018; Jamil et al., 2014; Zhu et al., 2021; Vannini et al., 2022; Ofoe et al., 2022). The versatile application of WD can span from seeds immersion to leaf spraying, soil spraying, and drip methods such as fertigation (Cândido et al., 2023). These studies have predominantly focused on assessing the final effect of WD at the later stages of experimentation, with less emphasis on monitoring changes over time in measured parameters. Consequently, an important research gap lies in understanding the effect of WD on plant drought tolerance, which warrants a more comprehensive investigation.

In this study, we have selected lettuce (*Lactuca sativa* L.) as model plant species due to its global significance as one of the most widely cultivated leafy vegetables (Cassetari, 2010). Additionally, lettuce is an ideal candidate for studying the effect of WD under varying drought conditions since it exhibits sensitivity to drought stress (Sorrentino et al., 2020) and needs ample water and nutrient supply to growth, especially in warmer conditions (Dilbar et al., 2021). The aim of the study is to examine the effects of WD application at various time points on lettuce (*Lactuca sativa* L.) performance and growth under different drought stress scenarios.

## Material and methods

### Wood distillate

The WD used in this study was provided by BioDea® (Arezzo, Italy) and derived from the steam distillation of sweet chestnut (*Castanea sativa* Mill.) sap. The WD exhibited the following characteristics: pH of 3.5 – 4.5, density of 1.05 kg L<sup>-1</sup>, acetic acid content of 2 – 2.3 %, polyphenol content of 23 – 26 g L<sup>-1</sup>, and <1 mg L<sup>-1</sup> of potentially toxic elements. The element concentration in pure WD determined by atomic absorption spectroscopy was as follows: Fe 3.2 ± 0.05 mg L<sup>-1</sup>, Na 4.9 ± 0.4 mg L<sup>-1</sup>, K 32.9 ± 0.6 mg L<sup>-1</sup>, Ca 944.2 ± 5.3 mg L<sup>-1</sup>, Zn 3.6 ± 0.1

mg L<sup>-1</sup>, and Mg 16.0 ± 1.0 mg L<sup>-1</sup> (Carril et al., 2023).

### Plant growth and treatments

Lettuce (*Lactuca sativa* L., cv. Ranger) seedlings of uniform size, were sourced from a local nursery and transplanted individually into black plastic pots, each containing 140 g of a commercial growing medium (VigorPlant Italia srl).

The experimental growth design consisted of two main treatments, namely control (C, only water) and WD application. Each main condition was subjected to three different drought levels: 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), as shown in Fig. 1. Seedlings were cultivated in a climate chamber under controlled conditions, with a photoperiod of 16/8 h (day/night), temperatures of 24/20 °C (day/night), a photosynthetically active radiation (PAR) light intensity of 250 μmol m<sup>-2</sup> s<sup>-1</sup>, and a relative humidity of 70 %.

Pots were initially irrigated at 70 % of WHC with either water or 0.5 % (v/v) WD, depending on the treatment. Wood distillate was applied fertigating soil once per week, instead of regular irrigation, for four weeks. The desired concentration of WD was chosen based on the results of Ofoe et al. (2022) and Celletti et al. (2023a) showing WD efficiency on plant growth by fertigation. Throughout the experimental growth period (lasted four weeks), the soil moisture was maintained by daily weighing each pot and adjusting water additions to the desired stress conditions. The drought stress was initiated 15 days after transplanting, and subsequent sampling and analyses were performed at specific time intervals: first harvest (T1) at the beginning of the imposition of drought stress (15 days after transplanting - DAT), second harvest (T2) after one week of stress (22 DAT), and third harvest (T3) after two weeks of stress (30 DAT).

### Growth and physiological analyses

Leaf chlorophyll content was measured using a non-destructive portable chlorophyll content meter (CCM, model 300, Opti-Science Inc., Hudson, NH, USA), and expressed on a surface basis (mg.m<sup>-2</sup>). Twelve measurements were randomly taken on the three youngest fully expanded leaves (four measurements per leaf) and averaged for each plant. The fresh biomass of lettuce leaves was weighed and immediately frozen at -20 °C until further analyses.

### Biochemical analyses

The total content of soluble proteins was determined in lettuce leaf extracts using the Bradford method (Bradford, 1976), with bovine serum albumin as standard. The extracts were obtained following the method reported by Celletti et al. (2023b). The absorbances of samples were read at 595 nm using a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA).

Anti-radical activity (ARA) in lettuce leaves indicative of total antioxidant power, was spectrophotometrically measured at 517 nm via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Fedeli et al. (2023).

Lipid peroxidation level was estimated as malondialdehyde (MDA) content, a biomarker of cell membrane oxidative damage, according to Zhang et al. (2021). The absorbance was measured in the leaf supernatants at 450, 532 and 600 nm using a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA).

The contents of total phenolics (TPC) and flavonoids (TFC) were determined in the extracts of lettuce leaves, previously dried in the dark, according to Borella et al. (2023). TPC and TFC were quantified with the Folin-Ciocalteu method (Al-Duais et al., 2009) and an aluminum chloride colorimetric method (Chang et al., 2002), respectively. Absorbances of the samples were read at 760 and 415 nm, respectively, using a

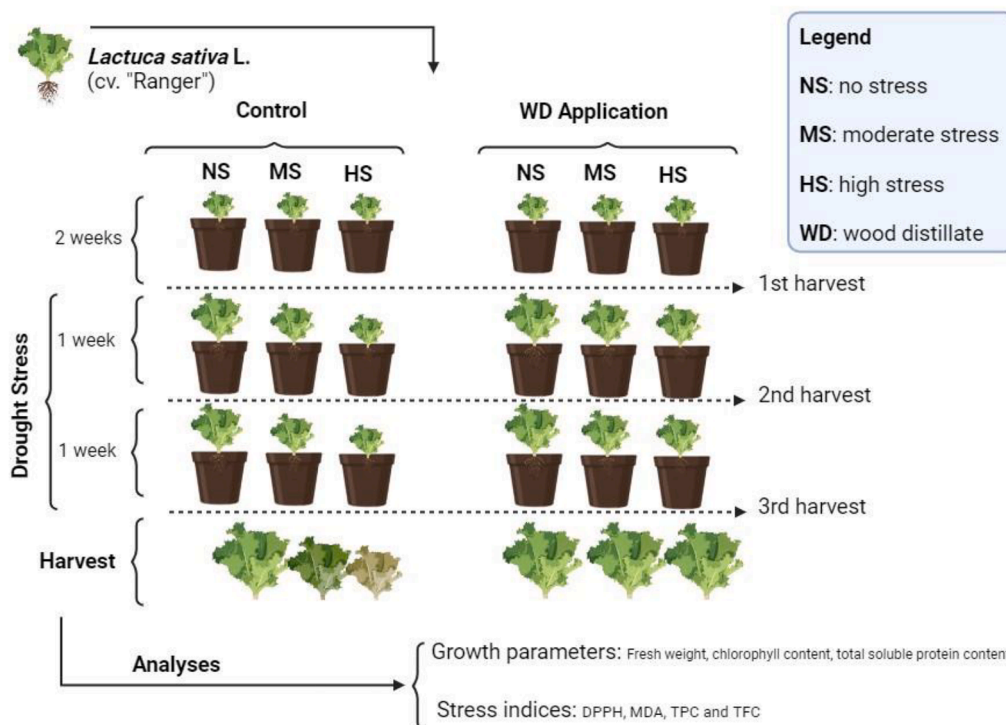


Fig. 1. Experimental growth scheme, showing WD application, different drought levels and sampling/analyses times. (created by Biorender.com).

UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA).

Statistical analysis

To assess the significance of differences between drought conditions in the WD treatment and corresponding conditions in the C treatment at each harvest time, a paired student's *t*-test for normally distributed data and Wilcoxon's test for non-normally distributed data were performed. Normality of the data was verified with the Shapiro-Wilk test. LSD-test

(Least significant difference) and Kruskal-Wallis's along with Conover-Iman tests were used as a post-hoc test for the one-way analysis of variance (ANOVA) between different treatments in each harvest time for normally and non-normally distributed data, respectively. Additionally, a three-way analysis of variance (3-way ANOVA) was conducted to explore the relationships between multiple variables (treatment, drought, and time). Pearson correlation coefficient analysis was also performed to test correlation between different measured parameters. Statistical analyses and heatmap illustrations were performed using the

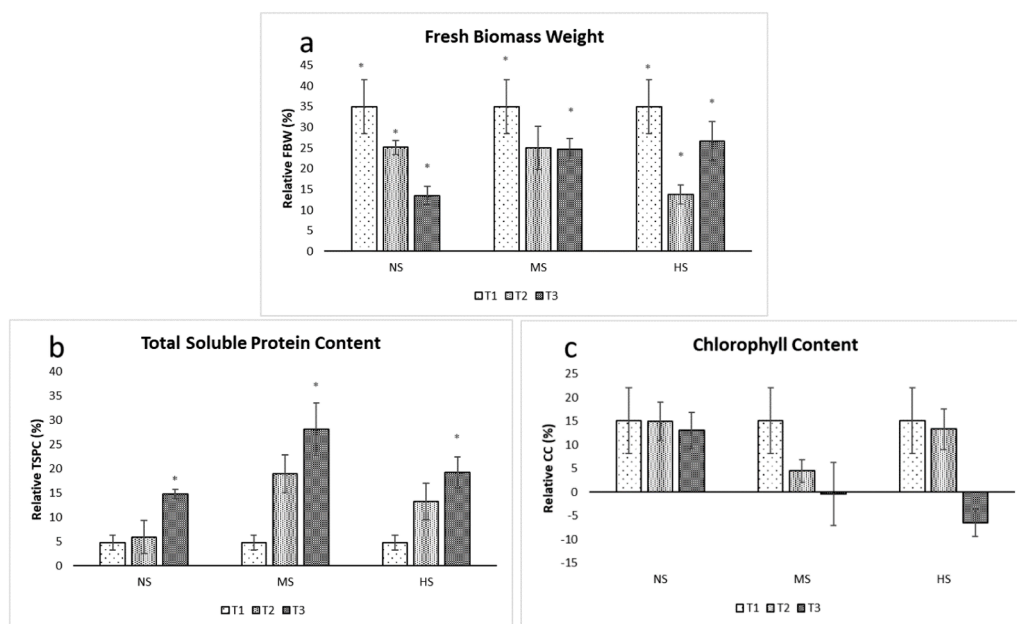


Fig. 2. Relative change (%) of growth parameters in WD treatment in comparison with C treatment. \* = significant ( $p < 0.05$ ) difference between WD treatments with the same control treatments in terms of time and drought conditions. NS= no stress, MS= moderate stress, and HS= high stress. Line "0" is considered as control treatment. Error bars showed standard errors for replicates of each treatment.

dplyr, agricolae, vegan, ggplot2, fmsb, outliers, plotly and corplot packages of the R statistical software (R Core Team, 2023). A  $p$ -value < 0.05 was considered statistically significant.

## Results

### Growth and biochemical parameters

In general, the results of the experiment revealed that wood distillate (WD) application significantly increased biomass and anti-radical activity while mitigating biomass loss and malondialdehyde (MDA) content under high drought stressful conditions. The results of the experiment, as depicted in Figs. 2-4, reveal the dynamics of fresh biomass in the aerial part of lettuce plants over time (Fig. S.1). Notably, the application of WD under different stressful conditions led to a significant increase in biomass compared to the control (C) (Fig. 2.a). Particularly promising was the observation that WD application mitigated biomass loss under high stressful conditions (WD-HS-T3).

The total soluble protein content exhibited a similar increasing trend in both WD and C treatments, with a significant rise observed at the end of the experiment (Fig. 2.b). However, it is noteworthy that the total soluble protein content increased over time but decreased with the intensity of drought stress (Fig. S.1).

In terms of total chlorophyll content, while results showed a slight increase in WD-treated plants in no stress conditions, no significant change was observed in the WD-treated plants compared to the control under stressful conditions (Figs. 2.c, 4 and S.1).

The stress indices obtained from the experiment are presented in Figs. 3 and 4, and they reveal interesting insights into the effects of WD application on various stress-related parameters.

The anti-radical activity, expressed as ARA (%), displayed a significant increase in WD-treated plants (Fig. 3.a). Moreover, the difference between WD-treated and control plants became more pronounced with increasing drought stress intensity. Similar to total soluble protein content, anti-radical activity exhibited an increasing trend over time and decreased with the severity of drought stress (Fig. S.1).

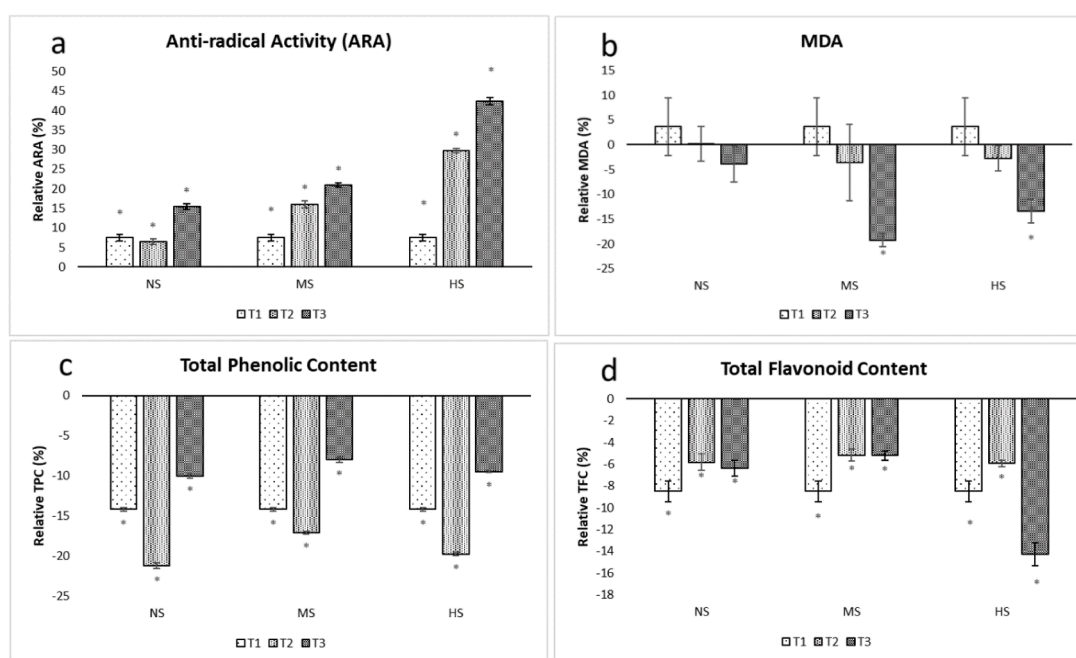
The content of malondialdehyde (MDA) was significantly reduced at

the end of the experiment by WD application (Fig. 3.b). Notably, the differences between WD treatments and their respective control became more apparent in the later stages of the experiment, while no significant differences were observed in the first two harvests (T1 and T2). Additionally, MDA content showed no significant difference between treatments without drought stress. On the other hand, total phenol and flavonoid contents displayed a significant decrease in WD-treated plants (Figs. 3.c, 3.d). However, both these compounds increased over time and with increasing drought intensity (Fig. S.1).

Overall, the results indicated that WD application influenced growth parameters positively, leading to increased biomass and enhanced anti-radical activity; moreover, WD application mitigated the adverse effects of drought stress, as evidenced by the reduced levels of MDA content (Fig. 4). In addition, the same trend as MDA has been observed in total phenol and flavonoid contents.

### Interactions of treatment, time, and drought conditions

The results of the 3-way ANOVA analysis highlighted significant effects of various factors on the investigated parameters (Tab. S.1 and Fig. 5). The WD treatment showed a substantial influence on most parameters, including fresh weight, protein content, DPPH, MDA, TPC, and TFC. Drought stress also exhibited a considerable impact on the plant responses, significantly affecting all parameters. On the other hand, time also played a critical role, showing significant effects on all analyzed parameters. The interactions among these factors further shape the plant responses. The interaction between treatment and drought significantly affected DPPH and TFC. The interaction between treatment and time significantly influenced DPPH and TPC. In addition, the interaction between time and drought significantly affected chlorophyll, proteins, DPPH, MDA, TPC and TFC. It is evident that the experimental conditions had a profound impact on the physiology, biochemistry, and antioxidant activity of lettuce plants. Furthermore, the results of Pearson correlation analysis between the investigated parameters (Fig. S.2) indicated a positive correlation between fresh weight and protein content and DPPH, as well as a negative correlation of DPPH with MDA, TPC and TFC.



**Fig. 3.** Relative change (%) of stress indices in WD treatment in comparison with C treatment. \* = significant ( $p < 0.05$ ) difference between WD treatments with the same control treatments in terms of time and drought conditions. NS= no stress, MS= moderate stress, and HS= high stress. Line "0" is considered as control treatment. Error bars showed standard errors for replicates of each treatment.

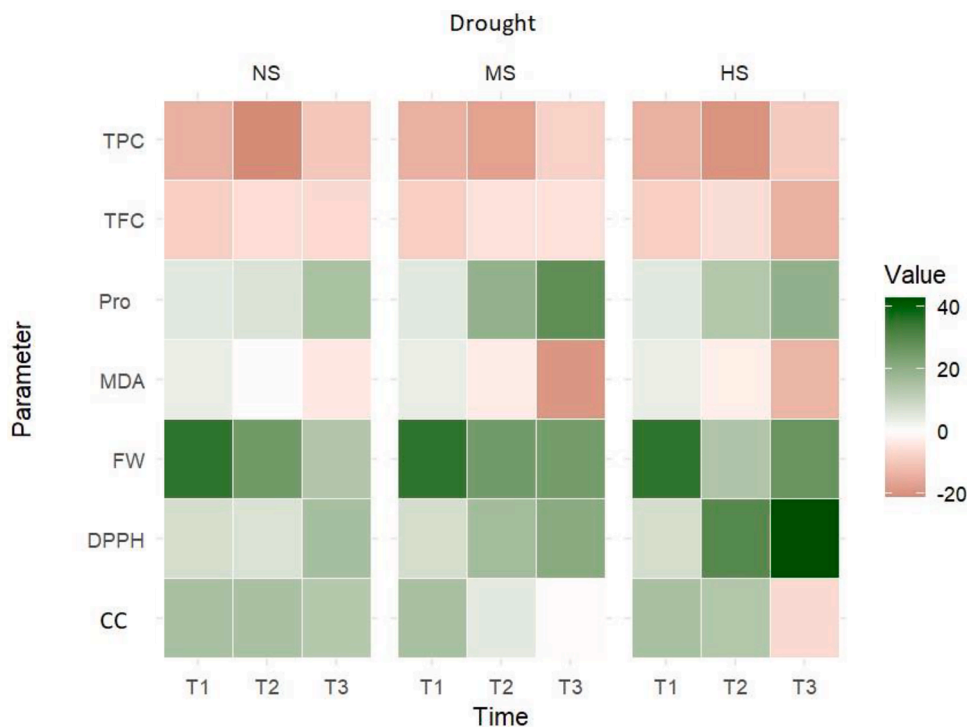


Fig. 4. Heatmap of the change percentage in different time and drought conditions in WD treatments in comparison with control treatment. FW= fresh weight, Pro= Protein, CC= chlorophyll, DPPH = anti-radical activity, MDA= malondialdehyde, TPC= total phenol content, and TFC= total flavonoid content. NS= no stress, MS= moderate stress, and HS= high stress. Green color showed increase and red color showed decrease of WD-treated plants in comparison with control. Intensity of the color showed the amount of difference.

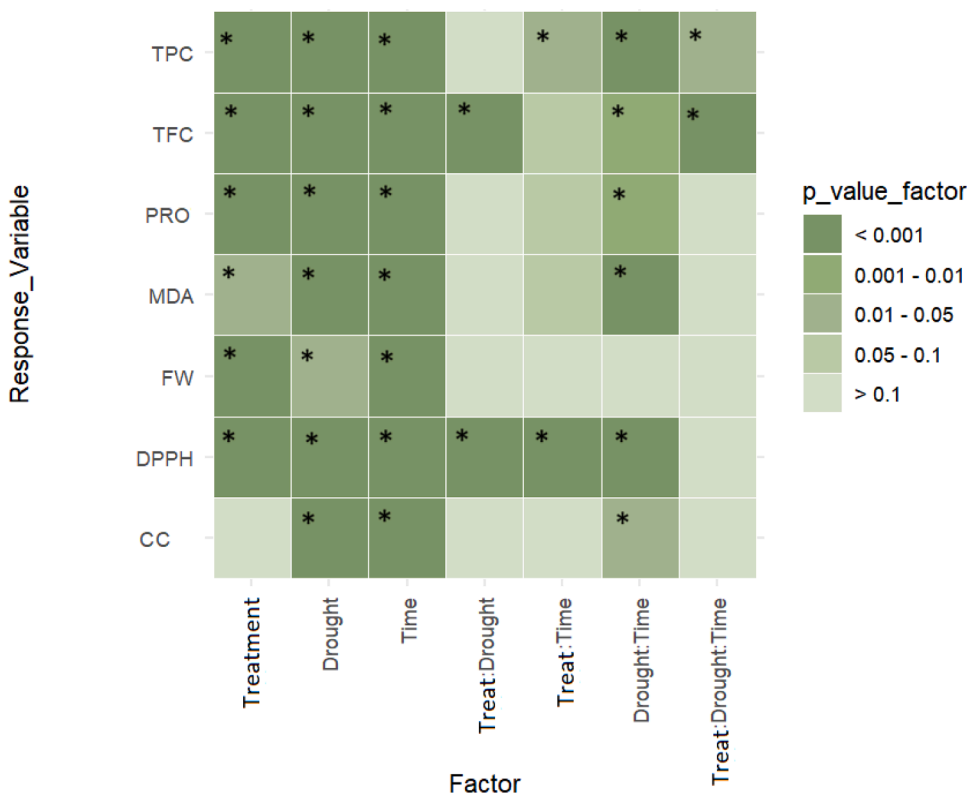


Fig. 5. Heatmap of p-values for 3-Way ANOVA in interactions of treatment, time, and drought conditions. FW= fresh weight, Pro= Protein, CC= chlorophyll, DPPH = anti-radical activity, MDA= malondialdehyde, TPC= total phenol content, and TFC= total flavonoid content. \* = significant ( $p < 0.05$ ) difference in interactions of different conditions.



## Discussion

The observed results can be explained by understanding the potential mechanisms and physiological responses of lettuce plants to WD application under drought stress conditions. In the following, there are some possible reasons for the observed outcomes.

### *Physiological and biochemical parameters*

The negative effects of drought stress on both wild and cultivated plants are widely documented in the literature (e.g., Griesser et al., 2015; Dehghani Bidgoli et al., 2018; Liu et al., 2023; Yavuz et al., 2023). According to the present results, drought stress decreased the biomass production of lettuce, while WD application determined a significant increase in plant growth. Several studies mentioned and confirmed the positive effects of WD application on plant growth and biomass production (Wang et al., 2019; Vannini et al., 2022; Jindo et al., 2022; Fedeli et al., 2022; Celletti et al., 2023a). WD contains a wide array of organic compounds, such as organic acids, phenols, and plant growth regulators (Wei et al., 2010). These compounds might act as biostimulants, enhancing nutrient uptake, and promoting cell division and elongation, resulting in increased biomass even under drought stress conditions. In addition, WD may trigger stress-related signaling pathways in plants (Xia et al., 2015), leading to the activation of stress tolerance mechanisms, such as the production of osmoprotectants, antioxidants, and heat shock proteins. These mechanisms can help the plant to cope with drought-induced oxidative damage and maintain better growth.

The significant increase in fresh biomass of the aerial part of lettuce plants with WD application, particularly under drought stress conditions, highlights the potential of WD as a biostimulant for enhancing lettuce growth. The observed prevention of biomass loss in WD treatment under high stress compared to the control without drought stress (WD-HS-T3 vs. C—NS-T3) indicated that WD might play a crucial role in mitigating the adverse effects of water scarcity on plant development. This finding suggests that WD could be an effective tool to improve crop productivity in regions vulnerable to drought, especially arid and semi-arid regions.

Proteins play vital roles in various physiological processes, including cell structure, enzymatic activities, and stress response (Chi et al., 2019). WD might stimulate the expression of genes involved in protein synthesis and increase the availability of amino acids, leading to enhanced protein production in plants (Fačková et al., 2020). In addition, antioxidant properties of WD may reduce oxidative damage to proteins, preserving their structure and functionality, and consequently increasing the total soluble protein content, as observed in this study. The significant increase in total soluble protein content with WD application supports the idea that WD acts as a growth promoter and aids in the accumulation of essential plant proteins. The improvement in total soluble protein content with WD treatment further underlines its potential as a beneficial biostimulant to enhance crop performance. Similar results are reported by Zhu et al. (2021), Celletti et al. (2023a), and Ofoe et al. (2023), who studied the effects of WD on rapeseed, basil, and tomato, respectively, reporting that WD application increased the protein content.

While WD showed positive effects on other growth parameters, its impact on chlorophyll content, if any, was minimal, possibly due to the specific nature of the drought stress applied in the experiment, fertirrigation application of WD or the specific interactions between WD and the chlorophyll biosynthesis pathways. On the other hand, plant efforts to deal with stressful conditions can lead to increased chlorophyll concentrations in the lettuce leaves under the drought stress (Urban et al., 2017; Ofoe et al., 2022). Although, a lot of studies showed the positive impact of WD (mostly foliar) application on chlorophyll content (e.g., Vanini et al., 2022; Fedeli et al., 2022), but there was no significant difference between WD and related control conditions for chlorophyll

content in this study. The same results are observed by Becagli et al. (2022), Ofoe et al. (2022), and Celletti et al. (2023a) that WD application as fertirrigation has no significant impact on the chlorophyll concentration in beans cultivated in open field, and in tomato and basil plants cultivated under controlled conditions, respectively.

WD likely contains compounds with strong antioxidant properties (Mathew et al., 2015), such as phenols and other bioactive molecules (Rabiu et al., 2021). These antioxidants can scavenge free radicals and reactive oxygen species (ROS) (Grewal et al., 2018), reducing oxidative stress and enhancing the overall antioxidant capacity of the plant. Drought stress is known to increase the production of ROS, leading to plant cell membrane oxidative damage (Miller et al., 2010). WD application might help neutralize these ROS, thereby preventing cellular damage (Loo et al., 2007) and maintaining higher antioxidant activity in lettuce plants. The observed increase in anti-radical activity, as measured by ARA (%), with WD application indicates that WD has strong antioxidant properties. This property can scavenge harmful free radicals and ROS, reducing oxidative stress in lettuce plants exposed to drought stress conditions. These results are in line with those obtained by Kang et al. (2012) on rice, Vannini et al. (2022) on lettuce, Ofoe et al. (2023) on tomato, and Celletti et al. (2023a) on basil. The cumulative effect of WD on antioxidant activity over time and under drought stress intensity suggests a consistent positive response, making it a promising natural solution to combat oxidative stress-induced damage in crops.

Malondialdehyde is a by-product of cell membrane lipid peroxidation, which occurs due to oxidative stress (Giera et al., 2012). The observed decrease in MDA content in WD treatments suggested that WD may have suppressed lipid peroxidation and thus cell membrane damage (Mahmud et al., 2020), possibly due to its antioxidant properties. WD may also activate specific enzymatic pathways that scavenge lipid peroxides and protect cellular membranes from oxidative destruction (Mathew et al., 2015). Under stressful conditions, such as drought, plant cells will generate excessive free radicals through various ways, which can trigger membrane lipid peroxidation and damage the membrane system (Bolwell and Wojtaszek, 1997). The main reaction is by ROS promoting the peroxidation of unsaturated fatty acids in membrane lipids to produce MDA (Chen et al., 2020). MDA content increased by drought stress and over time (Hosseini et al., 2018). Fan et al. (2022) documented a similar MDA increase in tomato under drought stress. On the other hand, Chen et al. (2020) showed a decrease in MDA content by WD application in grapevine. As well, Wang et al. (2019) expressed that WD application can decrease H<sub>2</sub>O<sub>2</sub> and MDA content in wheat under drought stress. Increasing drought stress causes a steady increase in MDA content (Zuo et al., 2013). The significant decrease in MDA content with WD application, particularly at the end of the experiment, highlights the ability of WD to mitigate lipid peroxidation and oxidative stress under drought conditions. The reduced MDA content suggests that WD application may effectively protect cellular membranes from oxidative damage, further contributing to improved plant health and stress tolerance.

Although drought stress caused a significant increase in TPC and TFC (Shang et al., 2002; Yang et al., 2018; Dehghani Bidgoli et al., 2019), the observed decrease in TPC and TFC with WD treatment indicates a complex response. While WD positively influences antioxidant activity, the reduction in these secondary metabolites may suggest their involvement in other metabolic processes triggered by WD application. Further research is needed to understand the intricate interactions between WD and the biosynthesis pathways of these compounds. Nevertheless, there are some studies indicating a reduction in TPC and TFC in plant tissues when macro- and micronutrient availability is high (Ibrahim et al., 2010; Bustamante et al., 2020). The decrease in total phenolic and flavonoid contents in WD treatments could be due to the altered metabolic pathways induced by WD. Some phenolics and flavonoids might be utilized in other physiological processes operated by WD, leading to lower accumulations in leaves. In addition, there is another possible hypothesis: since WD is rich in phenols and flavonoids and these

compounds could be absorbed by the plant, it did not need to produce these compounds to deal with the stressful conditions. Although this issue is not yet fully investigated, [Ofuo et al. \(2022\)](#) found similar reductions in TPC and TFC of tomato following 0.5 % WD application.

It is important to note that the exact mechanisms underlying the observed results may be complex and multifaceted. Plant responses to WD application and drought stress can vary depending on plant species, the concentration of WD used, and the severity of drought stress applied. Further research and analysis are thus necessary to delve deeper into the specific molecular pathways and mechanisms responsible for these outcomes.

#### Interaction of treatment, time and drought conditions

The results of the 3-way ANOVA analysis provide valuable insights into the impact of different experimental conditions on plant-related parameters. The treatments applied to the plants significantly affected various aspects, including fresh weight, protein content, antioxidant activity (DPPH), and the production of phenolic and flavonoid compounds. These findings suggest that specific treatments can influence plant growth, protein synthesis, and antioxidant defenses, leading to potential health benefits through enriched bioactive compounds, as mentioned by [López-Galiano et al. \(2019\)](#) and [Seleiman et al. \(2021\)](#).

Drought stress emerges as a prominent factor affecting plant response, as it significantly impacted most analyzed parameters, indicating hampered growth, increased protein content, and enhanced antioxidant activity under water-limited conditions ([Kapoor et al., 2020](#)). Moreover, the time factor also played a critical role ([Vicente-Serrano et al., 2012](#)), showing significant effects on all parameters studied. The observed variations over time reflect the dynamic nature of plant responses during the different stages of the experiment. Additionally, the interactions between treatment, drought stress, and time further shape the plant behavior, underscoring the complexity of their interactions. The similar results are observed by [Jurado-Mañogil et al. \(2024\)](#) studying hormonal, enzymatic and osmoregulatory response to drought in *Prunus* species over the time. Understanding these intricate relationships can aid in devising targeted approaches to optimize plant growth, nutrient content, and antioxidant potential, with potential implications for agricultural and health-related sectors. However, further research is necessary to understand the underlying molecular mechanisms behind these observed effects and to generalize the findings across different plant species and experimental settings.

#### Conclusions

The outcomes of this study demonstrated that the application of 0.5 % WD had a significantly positive impact on the growth and stress tolerance of lettuce plants exposed to drought conditions. This treatment leads to increased fresh biomass, preventing biomass loss in stressed plants and showcasing its potential as a stress alleviator with economic relevance.

WD acts as a growth promoter, enhances antioxidant activity, and helps mitigate oxidative stress, contributing to improved plant performance. These findings could have significant implications for sustainable agriculture by providing a nature-based and effective approach to enhance crop productivity and stress tolerance. Future research could focus on unraveling the molecular mechanisms behind the effects of WD, optimizing application rates, investigating its use on different crop varieties, and in field settings, studying its interaction with soil microbiota and exploring long-term effects.

Overall, these findings highlight the potential of WD as a valuable biostimulant in sustainable crop management and agriculture in the face of an ever-changing climate, particularly in arid, semi-arid or drought-prone regions, where it can enhance plant growth and alleviate the detrimental effects of drought stress.

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#### CRediT authorship contribution statement

**Majid Ghorbani:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Nazanin Azarnejad:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pablo Carril:** Writing – review & editing, Investigation, Data curation. **Silvia Celletti:** Writing – review & editing. **Stefano Loppi:** Writing – review & editing, Supervision, Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.stress.2024.100450](https://doi.org/10.1016/j.stress.2024.100450).

#### References

- Al-Duais, M., Müller, L., Böhm, V., et al., 2009. Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing: use of different assays. *Eur. Food Res. Technol.* 228, 813–821. <https://doi.org/10.1007/s00217-008-0994-8>.
- Aremu, A.O., Bairu, M.W., Finnie, J.F., van Staden, J., 2012. Stimulatory role of smoke-water and karrikinolide on the photosynthetic pigment and phenolic contents of micro propagated “Williams” bananas. *Plant Growth Regul.* 67, 271–279. <https://doi.org/10.1007/s10725-012-9685-3>.
- Becagli, M., Arduini, I., Cantini, V., Cardelli, R., 2022. Soil and foliar applications of wood distillate differently affect soil properties and field bean traits in preliminary field tests. *Plants (Basel)* 12 (1), 121. <https://doi.org/10.3390/plants12010121>.
- Bolwell, G.P., Wojtaszek, P., 1997. Mechanisms for generation of reactive oxygen species in plant defence—a broad perspective. *Physiol. Mol. Plant Pathol.* 51, 347–366. <https://doi.org/10.1006/pmpp.1997.0129>.
- Borella, M., Baghdadi, A., Bertoldo, G., Della Lucia, M., Chiodi, C., Celletti, S., Deb, S., Baglieri, A., Zegada-Lizarazu, W., Pagani, E., Monti, A., Mangione, F., Magro, F., Hermans, C., Stevanato, P., Nardi, S., 2023. Transcriptomic and physiological approaches to decipher cold stress mitigation exerted by brown-seaweed extract (BSE) application in tomato. *Front. Plant Sci. Sec. Crop Product Physiol.* 14 <https://doi.org/10.3389/fpls.2023.1232421>.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. <https://doi.org/10.1006/abio.1976.9999>.
- Bustamante, M.Á., Michelozzi, M., Barra Caracciolo, A., Grenni, P., Verbokkem, J., Geerdink, P., Safi, C., Nogues, I., 2020. Effects of soil fertilization on terpenoids and other carbon-based secondary metabolites in *Rosmarinus officinalis* plants: a comparative study. *Plants* 9 (7), 830. <https://doi.org/10.3390/plants9070830>.
- Cândido, N.R., Duarte Pasa, V.M., Vilela, A.O., Campos, A.D., Fátima, Á., Modolo, L.V., 2023. Understanding the multifunctionality of pyrolytic acid from waste biomass and the potential applications in agriculture. *Sci. Total Environ.* 881 (2023), 163519 <https://doi.org/10.1016/j.scitotenv.2023.163519>. ISSN 0048-9697.
- Carril, P., Bianchi, E., Cicchi, C., Coppi, A., Dainelli, M., Gonnelli, C., Loppi, S., Pazzagli, L., Colzi, I., 2023. Effects of wood distillate (pyrolytic acid) on the yield parameters and mineral composition of three leguminous crops. *Environments* 10, 126. <https://doi.org/10.3390/environments10070126>.
- Cassatari, L.S., 2010. Teorde Clorofilase B-Carotenom Cultivarese Linhagemde Alfases. Dissertação (Mestrado). Universidade Federal de Lavras, p. 68p. <https://doi.org/>

- 10.1016/j.scienta.2020.109578. Programade Pós Graduaçãoem Agronomia-FitotecniaPaim et al., 2020. Mild drought stress has potential to improve lettuce yield and quality, *Scientia Horticulturae*, Volume 272, 2020, 109578, ISSN 0304-4238.
- Celletti, S., Fedeli, R., Ghorbani, M., Aseka, J.M., Loppi, S., 2023a. Exploring sustainable alternatives: wood distillate alleviates the impact of bioplastic in basil plants. *Sci. Total Environ.* 900, 166484 <https://doi.org/10.1016/j.scitotenv.2023.166484>.
- Celletti, S., Fedeli, R., Ghorbani, M., Loppi, S., 2023b. Impact of starch-based bioplastic on growth and biochemical parameters of basil plants. *Sci. Total Environ.* 856 (2) <https://doi.org/10.1016/j.scitotenv.2022.159163>.
- Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10 (3), 178–182. <https://doi.org/10.38212/2224-6614.2748>.
- Chen, Y.H., Li, Y.F., Wei, H., Li, X.X., Zheng, H.T., Dong, X.Y., Xu, T.F., Meng, J.F., 2020. Inhibition efficiency of wood vinegar on grey mould of table grapes. *Food Biosci.* 38 <https://doi.org/10.1016/j.fbio.2020.100755>.
- Chi, Y.H., Koo, S.S., Oh, H.T., Lee, E.S., Park, J.H., Phan, K.A.T., Wi, S.D., Bae, S.B., Paeng, S.K., Chae, H.B., Kang, C.H., Kim, M.G., Kim, W.-Y., Yun, D.-J., Lee, S.Y., 2019. The physiological functions of universal stress proteins and their molecular mechanism to protect plants from environmental stresses. *Front. Plant Sci.* 10, 750. <https://doi.org/10.3389/fpls.2019.00750>.
- Dehghani Bidgoli, R., Azrnejad, N., Akhbari, M., 2018. Reducing the effects of drought stress in rosemary (*Rosmarinus officinalis* L.) plant by using PGPR. *J. Appl. Res. Plant Physiol.* 4 (2), 67–80. <http://arpe.gonbad.ac.ir/article-1-281-en.html>.
- Dehghani Bidgoli, R., Azarnejad, N., Akhbari, M., Ghorbani, M., 2019. Salinity stress and PGPR effects on essential oil changes in *Rosmarinus officinalis* L. *Agric. Food Secur.* 8, 2. <https://doi.org/10.1186/s40066-018-0246-5>.
- Dilbar, J.K., Narimanov, A., Wirth, S., 2021. Beneficial effects of biochar application on lettuce (*Lactuca sativa* L.) growth, root morphological traits and physiological properties. *Ann. Phytomed.* 10, 93–100. <https://doi.org/10.21276/ap.2021.10.2.13>.
- Fačková, Z., Vannini, A., Monaci, F., Grattacaso, M., Paoli, L., Loppi, S., 2020. Uptake of trace elements in the water fern *Azolla filiculoides* after short-term application of chestnut wood distillate (pyrolygneous acid). *Plants* 9 (9), 1179. <https://doi.org/10.3390/plants9091179>.
- Fan, S., Wu, Hong, Gong, Haijun, Guo, Jia, 2022. The salicylic acid mediates selenium-induced tolerance to drought stress in tomato plants. *Sci. Hortic.* 300, 111092 <https://doi.org/10.1016/j.scienta.2022.111092>.
- Fedeli, R., Vannini, A., Guarneri, 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* (Basel) 11 (3), 404. <https://doi.org/10.3390/biology11030404>.
- Fedeli, R., Celletti, S., Loppi, S., Vannini, A., 2023. Comparison of the effect of solid and liquid digestate on the growth of lettuce (*Lactuca sativa* L.) plants. *Agronomy* 13, 782. <https://doi.org/10.3390/agronomy13030782>.
- Giera, M., Lingeman, H., Niessen, W.M.A., 2012. Recent advancements in the LC- and GC-based analysis of malondialdehyde (MDA): a brief overview. *Chromatographia* 75, 433–440. <https://doi.org/10.1007/s10337-012-2237-1>.
- Grewal, A., Abbey, L., Gunupuru, L.R., 2018. Production, prospects and potential application of pyrolygneous acid in agriculture. *J. Anal. Appl. Pyrolysis* 135, 152–159. <https://doi.org/10.1016/j.jaap.2018.09.008>.
- Griesser, M., Weingart, G., Schoedl-Hummel, K., Neumann, N., Becker, M., Varmuza, K., Liebner, F., Schuhmacher, R., Forneck, A., 2015. Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera* cv. Pinot noir). *Plant Physiol. Biochem.* 88, 17–26. <https://doi.org/10.1016/j.plaphy.2015.01.004>.
- Hosseini, M.S., Samsampour, D., Ebrahimi, M., Abadía, J., Khanahmadi, M., 2018. Effect of drought stress on growth parameters, osmolyte contents, antioxidant enzymes and glycyrrhizin synthesis in licorice (*Glycyrrhiza glabra* L.) grown in the field. *Phytochemistry* 156, 124–134. <https://doi.org/10.1016/j.phytochem.2018.08.018>.
- Ibrahim, M.H., Jaafar, H.Z., Rahmat, A., Rahman, Z.A., 2010. The relationship between phenolics and flavonoids production with total non-structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. Under high CO<sub>2</sub> and nitrogen fertilization. *Molecules* 16, 162–174. <https://doi.org/10.3390/molecules16010162>.
- Jafari, M., Tavili, A., Panahi, F., Zandi Esfahan, E., Ghorbani, M., 2018. Reclamation of Arid Lands. Springer International Publishing. <https://doi.org/10.1007/978-3-319-54828-9>.
- Jamil, M., Kanwal, M., Aslam, M.M., Khan, S.U., Malook, I., Tu, J., Rehman, S., 2014. Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition. *Aust. J. Crop Sci.* 8, 159–170. <https://doi.org/10.3316/informit.197742907597884>.
- Jindo, K., Goron, T.L., Kurebito, S., Matsumoto, K., Masunaga, T., Mori, K., Miyakawa, K., Nagao, S., Tokunari, T., 2022. Sustainable plant growth promotion and chemical composition of pyrolygneous acid when applied with biochar as a soil amendment. *Molecules* 27 (11), 3397. <https://doi.org/10.3390/molecules27113397>.
- Jurado-Mañogil, C., Martínez-Melgarejo, P.A., Martínez-García, P., Rubio, M., Hernández, J.A., Barba-Espín, G., Diaz-Vivancos, P., Martínez-García, P.J., 2024. Comprehensive study of the hormonal, enzymatic and osmoregulatory response to drought in Prunus species. *Sci. Hortic.* 326, 112786 <https://doi.org/10.1016/j.scienta.2023.112786>.
- Kang, M., Heo, K., Kim, J., Cho, S., Seo, P., Rico, C., Lee, S., 2012. Effects of carbonized rice hull and wood charcoal mixed with pyrolygneous acid on the yield, and antioxidant and nutritional quality of turk. *Turkish J. Agric. Forestry* 36 (1), 45–53. <https://doi.org/10.3906/tar-1001-640>.
- Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., Sharma, A., 2020. The impact of drought in plant metabolism: how to exploit tolerance mechanisms to increase crop production. *Appl. Sci.* 10, 5692. <https://doi.org/10.3390/app10165692>.
- López-Galiano, M.J., García-Robles, I., González-Hernández, A.I., Camañes, G., Vicedo, B., Real, M.D., Rausell, C., 2019. Expression of miR159 is altered in tomato plants undergoing drought stress. *Plants* 8, 201. <https://doi.org/10.3390/plants8070201>.
- Lee, J.K., Park, H.J., Cha, S.J., Kwon, S.J., Park, J.H., 2021. Effect of pyrolygneous acid on soil urease, amidase, and nitrogen use efficiency by Chinese cabbage (*Brassica campestris* var. Pekinensis). *Environ. Pollut.* 291, 118132 <https://doi.org/10.1016/j.envpol.2021.118132>.
- Li, P.Q., Ye, A., Wada, Y., Zhang, Y., Zhou, J., 2024. Climate change leads to an expansion of global drought-sensitive area. *J. Hydrol.* 632, 130874 <https://doi.org/10.1016/j.jhydrol.2024.130874>.
- Liu, X., Wang, J., Feng, X., Yu, J., 2021. Wood vinegar resulting from the pyrolysis of apple tree branches for annual bluegrass control. *Ind. Crops. Prod.* 174, 114193 <https://doi.org/10.1016/j.indcrop.2021.114193>.
- Liu, X., Li, Yue, Micallef, Shirley A., 2023. Natural variation and drought-induced differences in metabolite profiles of red oak-leaf and Romaine lettuce play a role in modulating the interaction with *Salmonella enterica*. *Int. J. Food Microbiol.* 385 (2023), 109998 <https://doi.org/10.1016/j.ijfoodmicro.2022.109998>.
- Loo, A., Jain, K., Darah, I., 2007. Antioxidant and radical scavenging activities of the pyrolygneous acid from a mangrove plant, *rhizophora apiculata*. *Food Chem.* 104, 300–307. <https://doi.org/10.1016/j.foodchem.2006.11.048>.
- Mahmud, K.N., Hashim, N.M., Ani, F.N., et al., 2020. Antioxidants, toxicity, and nitric oxide inhibition properties of pyrolygneous acid from palm kernel shell biomass. *Waste Biomass Valor* 11, 6307–6319. <https://doi.org/10.1007/s12649-019-00857-w>.
- Mathew, S., Zakaria, Z.A., Musa, N.F., 2015. Antioxidant property and chemical profile of pyrolygneous acid from pineapple plant waste biomass. *Process Biochem.* 50 (11), 1985–1992. <https://doi.org/10.1016/j.procbio.2015.07.007>, 2015.
- Mhamdi, R., 2023. Evaluating the evolution and impact of wood vinegar research: a bibliometric study. *J. Anal. Appl. Pyrolysis* 175, 106190. <https://doi.org/10.1016/j.jaap.2023.106190>.
- Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* 33 (4), 453–467. <https://doi.org/10.1111/j.1365-3040.2009.02041.x>.
- Munné-Bosch, S., Villadangos, S., 2023. Cheap, cost-effective, and quick stress biomarkers for drought stress detection and monitoring in plants. *Trends Plant Sci.* <https://doi.org/10.1016/j.tplants.2023.01.004>. ISSN 1360-1385.
- Ofoe, R., Qin, D., Gunupuru, L.R., Thomas, R.H., Abbey, L., 2022. Effect of pyrolygneous acid on the productivity and nutritional quality of greenhouse tomato. *Plants* 11 (13), 1650. <https://doi.org/10.3390/plants11131650>.
- Ofoe R., Mousavi S.M.N., Thomas R.H., Abbey L., 2023. Foliar application of pyrolygneous acid acts synergistically with fertilizer to improve the productivity and phytochemical properties of greenhouse-grown tomato. <https://doi.org/10.21203/rs.3.rs-2640142/v1> (preprint).
- Rabiu, Z., Hamzah, M.A.A.M., Hasham, R., et al., 2021. Characterization and anti-inflammatory properties of fractionated pyrolygneous acid from palm kernel shell. *Environ. Sci. Pollut. Res.* 28, 40535–40543. <https://doi.org/10.1007/s11356-020-09209-x>.
- Seleman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H.H., Battaglia, M.L., 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* 10, 259. <https://doi.org/10.3390/plants10020259>.
- Shang, X., Wang, Y., Yan, X., 2002. Effect of soil moisture on growth and root-salidroside content in *Rhodiola sachalinensis*. *Plant Physiol. Commun.* 39, 335–336. <https://doi.org/10.5555/200403091573>.
- Singh, S., 2024. Wood vinegar Market Research Report information by pyrolysis type (slow pyrolysis, fast pyrolysis and intermediate pyrolysis), by application (agriculture, animal feed, food & beverages, and others), and by region (North America, Europe, Asia Pacific, and rest of the world). Market forecast till 2030. Market Res. Future. ID: MRRFR/AGR/5795-HCR.
- Sorrentino, M., Colla, G., Rouphael, Y., Panzarová, K., Trtlík, M., 2020. Lettuce reaction to drought stress: automated high-throughput phenotyping of plant growth and photosynthetic performance. *Acta Hort.* 1268, 133–142. <https://doi.org/10.17660/ActaHortic.2020.1268.17>.
- Tiilikkala, K., Lindqvist, I., Hagner, M., Setälä, H., Perdikis, D., 2011. Use of Botanical Pesticides in Modern Plant Protection. *Pesticides in the Modern World - Pesticides Use and Management*, pp. 259–272. <https://doi.org/10.5772/17737>.
- Urban, J., Ingwers, M., McGuire, M.A., Teskey, R.O., 2017. Stomatal conductance increases with rising temperature. *Plant Signal. Behav.* 12, e1356534 <https://doi.org/10.1080/15592324.2017.1356534>.
- Vannini, A., Fedeli, R., Guarneri, M., Loppi, S., 2022. Foliar application of wood distillate alleviates ozone-induced damage in lettuce (*Lactuca sativa* L.). *Toxics* 10 (4), 178. <https://doi.org/10.3390/toxics10040178>.
- Vicente-Serrano, S.M., Gouveia, C.M., Camarero, J.J., Begueria, S., Trigo, R.M., López-Moreno, J.I., Azorín-Molina, C., Pasho, E., Lorenzo-Lacruz, J., Revuelto, J., Morán-Tejeda, E., Sanchez-Lorenzo, A., 2012. Response of vegetation to drought time-scales across global land biomes. *Proc. Natl. Acad. Sci.* 110, 52–57. <https://doi.org/10.1073/pnas.1207068110>.
- Wang, Y., Qiu, L., Song, Q., Wang, S., Wang, Y., Ge, Y., 2019. Root proteomics reveals the effects of wood vinegar on wheat growth and subsequent tolerance to drought stress. *Int. J. Mol. Sci.* 20 (4), 943. <https://doi.org/10.3390/ijms20040943>.
- Wei, Q., Ma, X., Zhao, Z., Zhang, S., Liu, S., 2010. Antioxidant activities and chemical profiles of pyrolygneous acids from walnut shell. *J. Anal. Appl. Pyrolysis.* 88 (2), 149–154. <https://doi.org/10.1016/j.jaap.2010.03.008>.



- Xia, X.J., Zhou, Yan-Hong, Shi, Kai, Zhou, Jie, Foyer, Christine H., Yu, Jing-Quan, 2015. Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *J. Exp. Bot.* 66 (10), 2839–2856. <https://doi.org/10.1093/jxb/erv089>.
- Yang, L., Wen, K.S., Ruan, X., Zhao, Y.X., Wei, F., Wang, Q., 2018. Response of plant secondary metabolites to environmental factors. *Molecules* 23 (4), 762. <https://doi.org/10.3390/molecules23040762>.
- Yang, D., Wang, Yang-Si-Ding, Wang, Qin, Ke, Yan, Zhang, Yun-Bing, Zhang, Shi-Bao, Zhang, Yong-Jiang, McDowell, Nate G., Zhang, Jiao-Lin, 2023a. Physiological response and photosynthetic recovery to an extreme drought: evidence from plants in a dry-hot valley savanna of Southwest China. *Sci. Total Environ.* 868, 161711 <https://doi.org/10.1016/j.scitotenv.2023.161711>.
- Yang, S., Zhao, B., Yang, D., Wang, T., Yang, Y., Ma, T., Santisirisoombon, J., 2023b. Future changes in water resources, floods and droughts under the joint impact of climate and land-use changes in the Chao Phraya basin, Thailand. *J. Hydrol.* 620 (Part A), 129454 <https://doi.org/10.1016/j.jhydrol.2023.129454>.
- Yavuz, D., Seymen, Musa, Kal, Ünal, Atakul, Zeliha, Tanriverdi, Ömer Burak, Türkmen, Önder, Yavuz, Nurcan, 2023. Agronomic and physio-biochemical responses of lettuce to exogenous sodium nitroprusside (SNP) applied under different irrigation regimes. *Agric. Water. Manage* 277, 108127. <https://doi.org/10.1016/j.agwat.2022.108127>. ISSN 0378-3774.
- Yuan, Y., Kong, Q., Zheng, Y., Zheng, H., Liu, Y., Cheng, Y., Zhang, X., Li, Z., You, X., Li, Y., 2022. Co-application of biochar and pyrolytic acid improved peanut production and nutritional quality in a coastal soil. *Environ. Technol. Innov.* 28, 102886 <https://doi.org/10.1016/j.eti.2022.102886>.
- Zhang, Y.C., Wang, X., Liu, B.J., Liu, Q., Zheng, H., You, X.W., et al., 2020. Comparative study of individual and co-application of biochar and wood vinegar on blueberry fruit yield and nutritional quality. *Chemosphere* 246. <https://doi.org/10.1016/j.chemosphere.2019.125699>.
- Zhang, Y., Wang, L., Li, T., Liu, W., 2021. Mutual promotion of LAP2 and CAT2 synergistically regulates plant salt and osmotic stress tolerance. *Front. Plant Sci.* 12. <https://doi.org/10.3389/fpls.2021.672672>.
- 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. <https://doi.org/10.3390/agronomy11030510>.