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Wood Distillate Enhances Seed Germination of Chickpea, Lettuce, and Basil

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Abstract: Seed priming with synthetic chemicals may be harmful to the environment and human health. Their replacement with bio-based compounds may overcome these concerns. In this study, we assessed the effectiveness of wood distillate (WD) in enhancing in vitro germination of crop plants using basil, chickpea, and lettuce as case studies. Seeds of the three species were soaked for 24 h in 0.25% and 0.17% WD solutions and then left to germinate for 7 days at 20 °C in a dark germination chamber. Seed pre-treatment with 0.25% WD enhanced germination in all tested species, while 0.17% WD stimulated germination in lettuce and chickpea, but not in basil. For lettuce, 0.17% WD worked better than 0.25% WD. Radicle length of basil and chickpea increased following pre-treatment with 0.25% WD, while in lettuce, it increased after pre-treatment with 0.17% WD. Treating seeds with appropriate WD solutions is a potential strategy to improve germination of crop plants.

Keywords: bio-based fertilizers; biostimulant; pyroligneous acid; wood vinegar; radicle length; seed priming

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1. Introduction

Seed germination, the fundamental process regulating plant propagation, is the bedrock of biodiversity conservation and the linchpin of productive agriculture. Irregular or suboptimal germination reverberates across the agricultural landscape, resulting in uneven seedling growth and substantial financial losses, findings underscored by extensive research [1].

In the relentless pursuit of optimizing germination, with an unwavering commitment to both uniformity and celerity, seed priming has emerged as a new technique poised to redefine agricultural practices [1,2]. This technique represents the hydration of seed levels, a balance that creates conditions conducive to the vital metabolic processes required for germination while limiting root emergence. The results achieved with seed priming are very interesting: primed seeds emerge from the soil with unprecedented speed, show vigorous growth patterns, and achieve early flowering—a feature of paramount importance especially in regions besieged by recurring drought conditions. Moreover, in an epoch marked by heightened environmental consciousness and mounting concerns over the ecological footprint of synthetic chemical applications, this technique assumes the mantle of a sustainable and ecologically responsible alternative. It serves as a powerful means to ameliorate plant stress, elevate overall plant vitality, and mitigate the environmental repercussions associated with conventional agricultural methods.

Significantly, seed priming presents itself as a pragmatic, cost-effective methodology replete with a plethora of benefits. It stands as a reliable avenue for ensuring synchronized seed germination, enhancing seedling emergence rates, and nurturing seedlings imbued with heightened resilience. Most significantly, this approach contributes to a reduction in excessive chemical fertilizer usage, amplifies crop yields, and instills systemic resistance in plants—a confluence of advantages that seamlessly align with contemporary agricultural sustainability paradigms [3]. Different types of techniques are at our disposal, ready to expedite and enhance seed germination. Liquid and osmotic priming methods, celebrated for their well-established efficacy, have rightfully ascended to preeminence as the most widely embraced approach [4]. Furthermore, innovative pre-treatment strategies employing agents such as $Si/As_{(III)}$, sodium selenate, selenium, sodium nitroprusside, and even conventional commercial fertilizers have boldly demonstrated their efficacy in enhancing overall plant performance, particularly when confronted with the crucible of challenging environmental conditions. These include water scarcity, soil salinity, metal toxicity, and temperature fluctuations [5–9].

In the contemporary context, where environmental protection and the imperative of nourishing a burgeoning global population converge, the search for practical, ecologically conscientious alternatives to the indiscriminate use of agrochemicals is of paramount importance. Against this background, wood distillate (WD) emerges as an exceptionally promising solution, emblematic of the convergence of ecological responsibility and agricultural productivity. Wood distillate holds the transformative potential to reshape the agricultural paradigm, forging a sustainable coexistence between agriculture and the environment while simultaneously addressing the pressing challenges of food security and ecological responsibility in a rapidly evolving world.

Wood pyrolysis, a pivotal thermochemical process, engenders the formation of two primary residual constituents: a solid phase recognized as biochar, and pyroligneous acid, a liquid component also known as WD [10]. For optimal WD yield when compared to biochar, the most effective production method involves utilizing fast pyrolysis at temperatures ranging from 350 to 500 °C.

This process initiates with the rapid and precise heating of woody biomass, which induces a cascade of thermal decomposition reactions, leading to the desired products. Subsequently, the process culminates in an expeditious cooling phase, finalizing the transformation. Remarkably, the resultant product displays a dynamic and multifaceted composition: an estimated 60–75% of the resulting material comprises the liquid phase, while the solid counterpart encompasses a substantial proportion, typically ranging from 15 to 25%. The gaseous fraction, though comparatively smaller, contributes to approximately 10–20% of the overall composition. The production of WD also yields positive environmental benefits. The materials employed in WD production comprise woody biomass sourced from the second cutting of the forest. This results in a valuable product for fostering the growth of crops, effectively utilizing forest waste and aligning to reduce reliance on synthetic chemical fertilizers. Additionally, it is approximated that each liter of WD sequesters 1.5 kg of CO_2. This carbon capture occurs throughout the entire production process, which not only generates the two by-products but also thermal energy. This thermal energy, in turn, powers the system, creating a sustainable and integrated approach to resource utilization.

Wood distillate exhibits a compositional profile rich in bioactive constituents, notably encompassing polyphenols, alcohols, acids, and esters. These compounds collectively have an important bio-stimulatory action, exerting profound influence within the domain of plant biology [11,12]. For these reasons, this product has recently been included in the list of natural products that can be used in organic farming due to its composition [13]. It is worth noting that the chemical properties of WD are dependent not only on the plant feedstock, but also on the operating parameters used during pyrolysis, and the resulting WD may have quite different characteristics. Wood distillate unravels the multifaceted effects it exerts across various dimensions of plant physiology. These effects span a broad spectrum, encompassing enhancements in germination rates [14], the augmentation of crop yields, and the elevation of the nutritional quality of crops [15–19]. Moreover, they emerge as an indispensable ally in the endeavor for sustainable and ecologically conscientious agricultural practices. It is noteworthy that WD's benign footprint extends to human safety, having undergone rigorous scrutiny and conclusive validation as non-toxic to human health [20]. From an environmental point of view, WD was found to be safe even for non-target organisms [21–24]. This is also fundamental for the sustainable development goals (SDGs) issued by the European Commission, given that objective 15 of the 2030 Agenda aims to "*protect, restore and promote the sustainable use of terrestrial ecosystems and biodiversity*". These results are also of interest as the SDGs have also played a fundamental role in reducing both pesticides and synthetic fertilizers by 50% by 2030.

As far as we know, there is currently no information available regarding the impact of WD on seed priming. In this study, we explore the influence of WD on priming seeds from three distinct crop plants: lettuce (*Lactuca sativa* L.), basil (*Ocimum basilicum* L.), and chickpea (*Cicer arietinum* L.). Our working hypothesis is that seeds pre-treated with WD perform better in terms of germination and early growth parameters.

2. Materials and Methods

2.1. Species Description

The wood distillate was tested on the seeds of plants of relevant agronomic interest for the Mediterranean area. Lettuce (*Lactuca sativa* L., var. Salanova) is an herbaceous plant belonging to the Asteraceae family whose leaves are eaten, generally raw, because they are rich in water, vitamins, and mineral salts [25,26]. Basil (*Ocimum basilicum* L., cv "Riviera Ligure") is an annual herbaceous plant belonging to the Lamiaceae family, normally cultivated as an aromatic plant [27,28]. Chickpea (*Cicer arietinum* L., cv "small-seeded from Arezzo") is the third most prevalent legume in world production, after soybean and bean. Its cultivation takes place mainly in India and Australia and it is poised to become an important vegetable due to the exponential growth of the human population and the lack of land for animal breeding. Thus, it is expected that chickpeas will replace animal proteins in the human diet [29,30]. All seeds were taken from a local farm, and the species were selected since basil and lettuce are our model plants for leaf analysis, and chickpea for seed.

2.2. Seed Treatment with Wood Distillate

Commercial seeds of basil, chickpea, and lettuce were surface-sterilized for 5 min with a 3% $CaClO_3$ solution and then vigorously washed with UV-sterilized distilled water. Seeds were soaked for 24 h in three different solutions containing WD at 0% (control; sterilized distilled water), 0.17%, and 0.25% and successively closed inside Petri dishes equipped with a Whatman filter (11 cm in diameter) saturated with 5 mL of distilled water. All Petri dishes were then incubated for seven days in the dark in a germination chamber at 20 ± 1 °C. Fifteen seeds were placed in each Petri dish. All treatments were performed in triplicate. These concentrations were chosen based on results obtained by authors on other species [31]. Sweet chestnut (Castanea sativa Mill) wood distillate (BioDea®), produced from waste wood from forest management, was kindly provided by a local company (Bio-Esperia srl, Umbertide, PG, Italy). The main features of the WD used are shown in Table 1.

Table 1. Characteristics of wood distillate (BioDea®), as indicated by the producer (Bio-Esperia srl).

pH	Density (kg/L)	Acetic Acid (% *v*/*v*)	Total Phenols (g/kg)	Total Polyphenols (g/kg)	Heavy Metals (mg/kg)
4 ± 0.5	1.05 ± 0.02	2.1 ± 0.1	3 ± 0.2	24 ± 2	<1

2.3. Germination and Radicle Length

Both germination and radicle length were recorded every 24 h. Seeds were considered germinated when the radicle protruded from the seed coat (≥2 mm). Specifically, germination was assessed by means of four parameters: Germination Percentage (GP), Germination Energy (GE; [8]), Germination Rate Index (GRI; [32]), and Mean Germination Time (MGT; [33]), calculated according to the following equations:

$$\boldsymbol{GP} = (\boldsymbol{Number\ of\ germinated\ seeds / Total\ number\ of\ seeds}) \times \mathbf{100}$$

$$\boldsymbol{GE\%} = \frac{\boldsymbol{Number\ of\ germinated\ seeds\ at\ 4\ DAS}}{\boldsymbol{Total\ number\ of\ seeds\ tested}} \times \mathbf{100}$$

where *DAS* represents days after sowing;

$$\boldsymbol{GRI} = \boldsymbol{G1/1} + \boldsymbol{G2/2} + \cdots + \boldsymbol{Gi/i}$$

where $G1$ is the Germination Percentage on day 1, $G2$ is the Germination Percentage on day 2, and so on until Gi is the last day;

$$\boldsymbol{MGT} = \sum(\boldsymbol{D} \times \boldsymbol{n}) / \sum \boldsymbol{n}$$

where n is the number of seeds newly germinated on day D, and D is the number of days counted from the beginning of the test;

Radicle length was measured using PixelStick v.2.5 software [34].

2.4. Statistical Analysis

Data normality was verified with the Shapiro–Wilk test. The significance of differences ($p < 0.05$) between treatments was assessed through the use of a pairwise Student's *t*-test, correcting for multiple testing according to Benjamini and Hochberg [35]. All results are presented as mean ± standard deviation. The statistical analysis was performed using the free software R v.4.0.3 [36].

3. Results

3.1. Basil Seeds

Basil seeds soaked in 0.25% WD showed an increase in GP, GE, GRI, and MGT values compared to the control values. Similarly, seeds soaked in 0.17% WD showed increased GP and MGT, but decreased GE and GRI. Statistically significant differences between the 0.25% and 0.17% WD treatments emerged for all parameters (Figure 1). The radicles exhibited consistent growth during the initial three days. From the fourth day onward, the application of 0.25% WD resulted in a statistically significant enhancement of the radicle growth (Figure 2).

3.2. Lettuce Seeds

Both WD treatments enhanced the germination performance of lettuce seeds, except for MGT at 0.25%, which showed no difference compared to the control (Figure 3). Seeds treated with 0.17% WD always showed higher values than those treated with 0.25% WD. In the case of lettuce, the application of both WD treatments enhanced the radicle length starting from day 3 and 2, respectively (Figure 4). Upon conclusion of the week-long observation period, the effects of the 0.17% treatment were more marked than those of the 0.25% treatment.

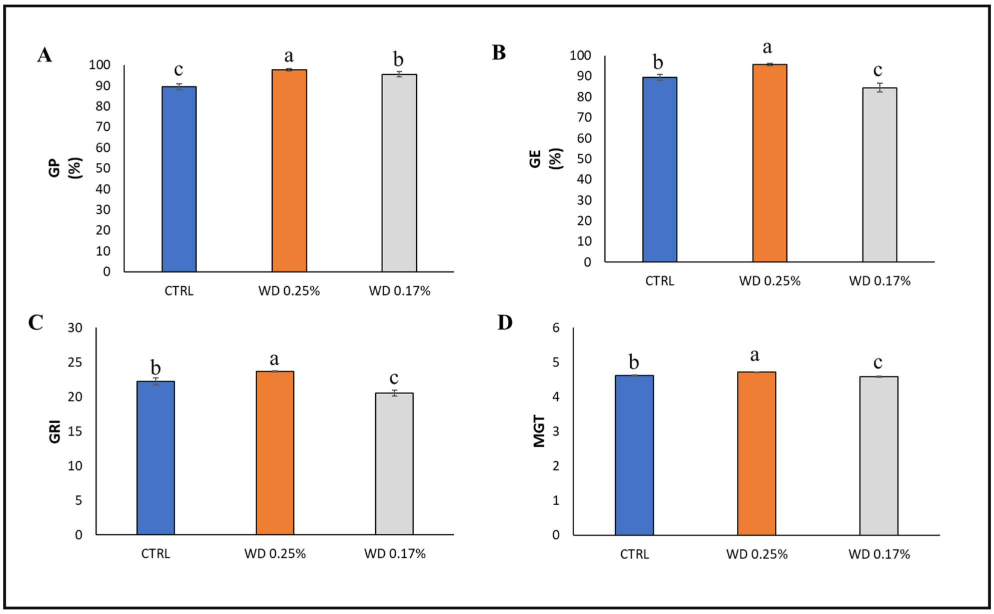

Figure 1. Mean ± standard deviation of Germination Percentage (GP%) (**A**), Germination Energy (GE%) (**B**), Germination Rate Index (GRI) (**C**), and Mean Germination Time (MGT) (**D**) of basil seeds soaked with distilled water (CTRL), 0.25% WD, and 0.17% WD; different letters indicate statistically significant differences between treatments ($p < 0.05$).

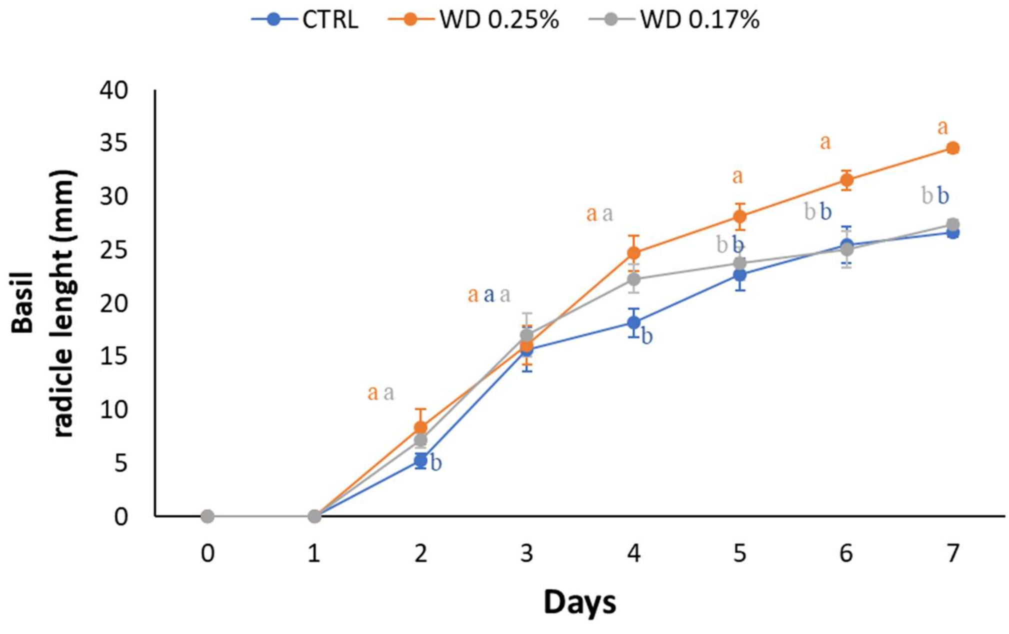

Figure 2. Radicle length of basil seedlings primed with water (CTRL), 0.25% WD, or 0.17% WD. The length of the radicle was measured daily for 7 days. Different letters indicate statistically significant differences between treatments ($p < 0.05$).

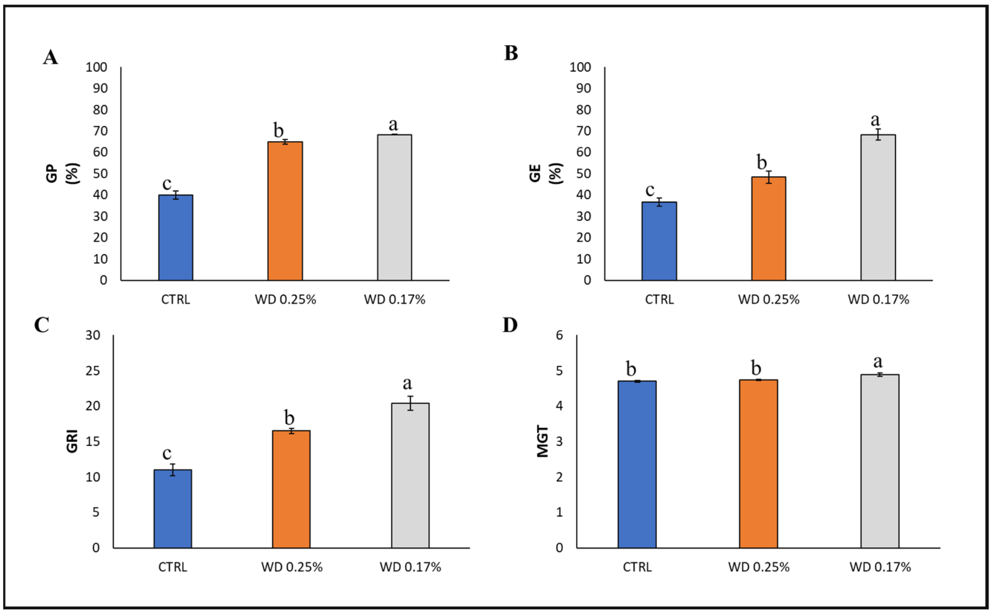

Figure 3. Mean ± standard deviation of Germination Percentage (GP%) (**A**), Germination Energy (GE%) (**B**), Germination Rate Index (GRI) (**C**), and Mean Germination Time (MGT) (**D**) of lettuce seeds soaked with distilled water (CTRL), 0.25% WD, and 0.17% WD; different letters indicate statistically significant differences between treatments ($p < 0.05$).

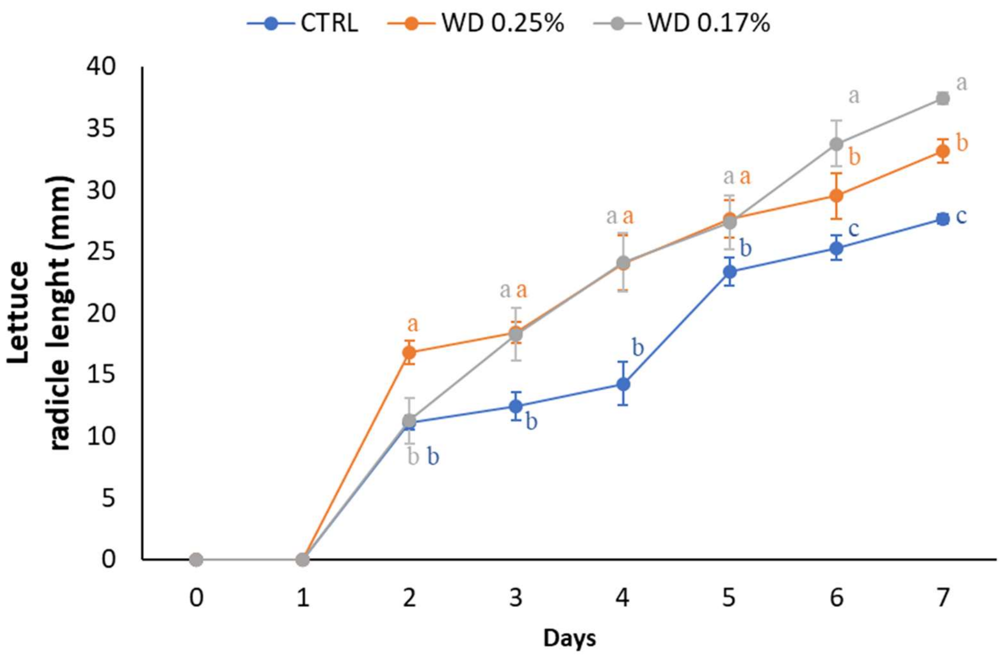

Figure 4. Radicle length of lettuce seedlings primed with water (CTRL), 0.25% WD, or 0.17% WD. The length of the radicle was measured daily for 7 days. Different letters indicate statistically significant differences between treatments ($p < 0.05$).

3.3. Chickpea Seeds

Chickpea seeds treated with both 0.17% and 0.25% WD showed increased GP, GE, GRI, and MGT when compared to control values (Figure 5), with greater effects seen using the 0.17% on GP and GRI. Finally, both treatments significantly increased radicle length from day 4 onwards, with 0.25% WD showing the greatest impact (Figure 6).

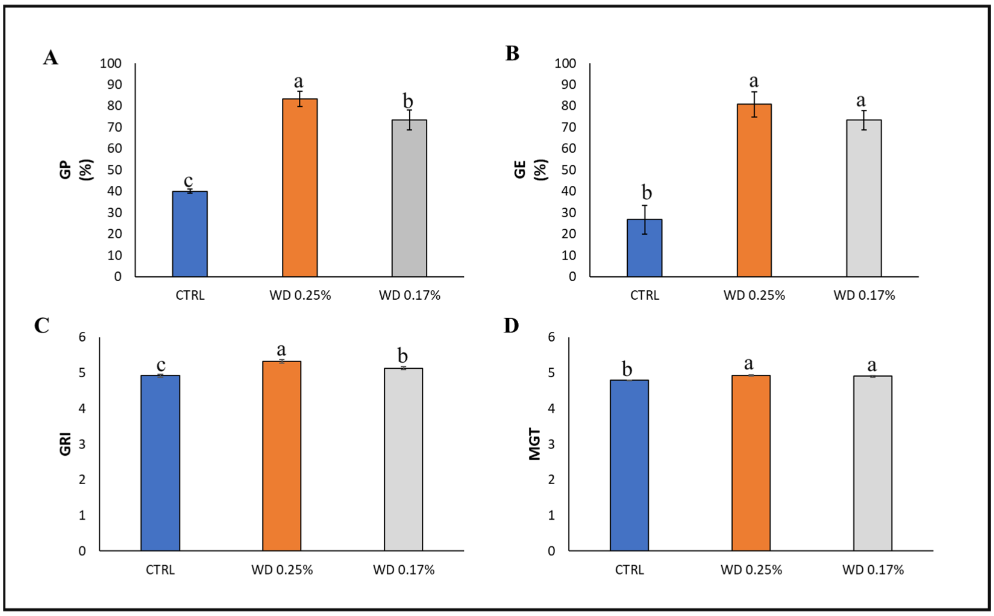

Figure 5. Mean ± standard deviation of Germination Percentage (GP%) (**A**), Germination Energy (GE%) (**B**), Germination Rate Index (GRI) (**C**), and Mean Germination Time (MGT) (**D**) of chickpea seeds soaked with distilled water (CTRL), 0.25% WD, and 0.17% WD; different letters indicate statistically significant differences between treatments ($p < 0.05$).

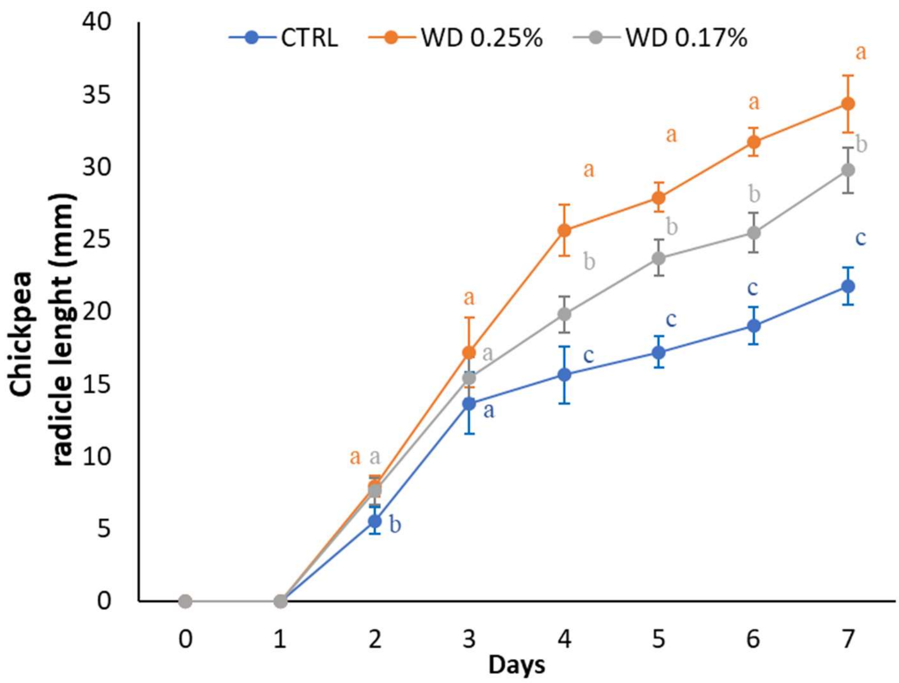

Figure 6. Radicle length of chickpea seedlings primed with water (CTRL), 0.25% WD, or 0.17% WD. The length of the radicle was measured daily for 7 days. Different letters indicate statistically significant differences between treatments ($p < 0.05$).

4. Discussion

The application of WD in a concentration range of 0.17% to 0.25% (v/v) has shown a positive effect in significantly improving both Germination Percentage and rootlet length in several plant species. These remarkable improvements are supported by WD's unique ability to effectively interrupt seed dormancy, bringing seeds into an active and accelerated state of growth and development.

Seed germination, a milestone in the life cycle of plants, has long been recognized as susceptible to the influences of pre-germination techniques that possess the power to circumvent the innate dormancy mechanisms inherent to seeds [37]. A profound understanding has emerged through extensive research, revealing the underlying mechanisms of seed priming, which is one such technique. Pre-germinative processes facilitate both quantitative and qualitative biochemical changes within the seeds. These processes encompass the repair of cellular membranes, the robust synthesis and activation of enzymes critical for the degradation and mobilization of stored reserves, and the initiation of endo-β-mannase, the enzyme responsible for ethylene synthesis [38]. Notably, de Castro et al. [39] have shed light on how seed priming facilitates and synchronizes DNA replication across all embryonic cells, showing a seamless progression of the cell cycle from *G*1 to *G*2. This process is promoted by the activation of cell cycle proteins such as β-tubulin, cyclins, and cyclin-dependent protein kinases. This pre-activation of the cell cycle represents a key mechanism through which priming amplifies germination performance [40]. Moreover, the augmented germination observed in primed seeds is often attributed to the heightened activity of antioxidant enzymes, further enriching the narrative of seed priming [41].

Based on this knowledge, a number of interesting studies have highlighted the potential of WD as a powerful germination stimulator in various crop species [12,14,41]. Its biostimulating capacity is based on the presence of phenolic compounds, organic acids (including citric acid), and various alcohol compounds, each of which is able to exert a favourable influence on seed germination rates [12]. Of particular interest is the presence of butanolide, a biologically active compound belonging to the karrikine family of phytohormones [42,43]. This compound, armed with its stimulatory effects on germination and its pivotal role in regulating seedling photomorphogenesis, emerges as a keystone in WD's biostimulatory action [44,45]. Furthermore, its multifaceted chemical composition, boasting various alcohol forms such as ethanol, methanol, and propanol, unfolds as a compendium of germination triggers, particularly efficacious in propelling the germination of select grass seeds, as reported in the scientific literature [46,47]. However, the effectiveness of WD is highly dependent on the concentrations used. Concentrations above the 0.5% threshold have been unequivocally identified as inhibiting germination and growth in a pantheon of plant species [48]. In our study, the two concentrations tested—0.17% and 0.25%—not only proved effective in increasing germination rates, but also showed a remarkable propensity to promote robust radicle development in all the seeds tested. These results are similar to the conclusions drawn from the study conducted by Kulkarani et al. [49], where a 0.2% WD treatment showed increased germination rates and rootlet elongation in rice seeds.

In essence, when used precisely at the right concentrations, WD emerges as a product to stimulate seed germination, remove dormancy barricades, and promote significant rootlet growth. Recently, it has been demonstrated that the efficacy of WD utilization is both dose-dependent and species-specific, as highlighted by Fedeli et al. [31]. Wood distillate was used at varying concentrations to the diaspores of three endangered cultivable plant species: *Bromus secalinus*, *Centaurea cyanus*, and *Legousia speculum-veneris*. The results show that its impact is contingent on the dosage applied and varies from one species to another. In the case of *Bromus secalinus*, the Germination Percentage (GP) exhibited a decrease at a WD concentration of 0.125%, after which it stabilized with increasing concentrations reaching 1%. With a 2% concentration of WD, germination was virtually nonexistent. Mean germination time (MGT) showed no impact for concentrations lower than 1%, but, at 2%, a significant increase was observed. Likewise, the Germination Rate Index (GRI) and Germination Energy (GE) demonstrated no relevant changes up to 1% WD, but exhibited a noticeable decrease at 2%. Contrastingly, in *Centaurea cyanus*, no impact of WD treatment was observed on GP and GE across all concentrations. MGT was not affected by treatments with WD concentrations lower than 1%, while 2% WD significantly enhanced MGT in comparison to control samples. The Germination Rate Index only showed an increase at the lowest concentrations. All the parameters tested were unaltered in *Legousia speculum-veneris* at low WD concentration, while the 0.5% WD treatment affected the parameters differently, decreasing GP, GRI, and GE, and increasing MGT. The 2% WD treatment caused complete inhibition of seed germination.

These findings draw attention to the pronounced species-specific variability in response to WD, which may also be attributed to seed size and the composition of the pericarp [50]. The pericarp is pivotal in protecting the seed during dormancy and ensuring its viability in unfavorable conditions [51]. The permeability of the seed's pericarp defines to which extent it can absorb certain substances. Scarcely permeable pericarps can hinder or restrict the penetration of a substance into the seed, while highly or moderately permeable pericarps can simplify the uptake of the same substance. Therefore, a diverse permeability to WD can be related to the different effects of the treatments on plant subjects. Notably, not only can different plant species have different pericarp permeability, but specific characteristics of the seeds can also have a role in defining the degree of permeability within seeds of the same plant species.

In our study, it was decided to exclusively test two concentrations, which were the most pertinent from an agronomic perspective. This decision was made as the primary aim of our work was to determine the most suitable concentration for highlighting a biostimulant effect on the germination of agriculturally relevant seeds. It appears to be species-specific, as the most pronounced stimulating effect was observed with a concentration of 0.25% WD for basil and chickpeas, while lettuce exhibited the highest stimulation at a concentration of 0.17% WD.

Overall, these findings confirm the potential of WD as a sustainable and environmentally friendly solution for stimulating and promoting seed germination, at a specific concentration, without the need for synthetic chemicals. These results also encourage further research to investigate whether priming with WD promotes seedling growth during post-germination stages. Further studies are needed to validate the effects of this product on other species of commercial interest. Moreover, further tests with other types of WD are necessary to validate whether using products derived from different feedstocks and different operational parameters can achieve the same important results, from a seed germination point of view, as those that emerged from this study.

The use of WD has larger implications beyond agriculture: the full valorization of waste plant biomass from forest management is a formidable example of circular economy and an important opportunity to combat climate change and protect biodiversity.

5. Conclusions

The use of the wood distillate at concentrations in the range of 0.17–0.25% (v/v) increased both the Germination Percentage and the length of the radicle both in chickpeas and in basil and lettuce seeds. This confirms the potential of WD, a product of natural origin, as a sustainable and environmentally friendly solution for stimulating and promoting seed germination in place of synthetic chemicals. The energy valorization and effective reuse in agriculture of the by-products of the pyrolysis of waste wood biomass represent a formidable example of the circular economy that can contribute to reducing the impact on the environment and combatting climate change.

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