Research Article

Effects of wood distillate and soy lecithin on the photosynthetic performance and growth of lettuce (*Lactuca sativa* L.)

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Abstract

This study investigated the effectiveness of foliar application of sweet chestnut wood distillate on the photosynthetic performance and growth of lettuce (*Lactuca sativa* L. cv 'Canasta'), both alone and in combination with soybean lecithin used as biosurfactant. Seedlings of lettuce were treated with a foliar application of either mineral water (control), or 1:500 solutions of 3% soy lecithin, wood distillate and wood distillate plus 3% soy lecithin. After 1 week, the chlorophyll content, chlorophyll fluorescence, chlorophyll reflectance and dry biomass were measured. The results showed that both wood distillate and lecithin exerted a positive effect on chlorophyll content and biomass production, but the joint use of wood distillate and lecithin quickly stimulated a ca. 50% increase in these parameters and is thus recommended.

Keywords Bio-based product · Biomass · Chlorophyll content · Photosynthesis · Pyroligneous acid · Wood vinegar

1 Introduction

Many chemicals commonly used in conventional agriculture are often associated with hazardous effects, and there is growing concern for human health and the environment. This is giving rise to growing interest toward green chemistry, but these bio-based and renewable products still occupy a small portion of modern agriculture. It must be emphasized that there is no correlation between natural and non-toxic as some natural compounds show a much greater toxicity than synthetic ones (i.e., botulin). Nevertheless, at routine usage doses, bio-based products have been shown to be safe, even for sensitive nontarget organisms [1, 2].

Wood distillate, also known as pyroligneous acid or wood vinegar, is one of these bio-based products which is empirically known for providing several benefits to agricultural plants, e.g., limiting the action of pathogens and increasing their productivity [3]. Among the most investigated effects related to the application of wood distillate to plants is the increase in biomass [4, 5] and in the number of fruits along with their size [6, 7]. In addition, also positive effects on the chlorophyll content and the photosynthetic rate are reported [4, 8]. Although the mechanism of action of wood distillate is still unclear, the beneficial effects for plants are supposed to be related to its particular chemical composition rich in antioxidants which are regarded as responsible for the stimulation of some plant enzymes [3].

Soy lecithin is a product rich in phospholipids [9], obtained from the degumming of the soybean oil and the removal of the residual water pool [10]. Given its ability to have both hydrophilic and hydrophobic characteristics, soybean lecithin has emulsifying characteristics [11] exploited in pharmaceutical contexts and as a food additive [12]. In agriculture, this product is well known as an additive in spray applications on crops, being able to limit spray drifting and the evaporation of droplets thanks to its

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role to isolate the active ingredient in the internal parts of each droplet (liposomes), favoring assimilation by leaves [13]. In addition, its action as a fungicide is recognized [14], due to its inhibiting power of the development of fungal pathogens on leaf surfaces [15, 16].

The aim of this study was to investigate if foliar application of wood distillate enhances the photosynthetic performance and growth of lettuce, a common agricultural plant. Additionally, the enhancement effect of adding soy lecithin as biosurfactant was also evaluated.

2 Materials and methods

2.1 Experimental design

Seedlings of *Lactuca sativa* cv. 'Canasta' cultured in potting soil and characterized by three leaves of an approximate height of 15 cm were obtained from a local plant nursery. In the laboratory, the seedlings were left to acclimate for 4 days in a climatic chamber at temperature = 20 ± 1 °C, relative humidity (RH) = 60 ± 2%, light = 80 ± 5 μM m^{-2} s^{-1} PAR, photoperiod = 12 h. Afterward, the seedlings were sprayed with either mineral water (control), or 1:500 solutions of 3% soy lecithin, wood distillate and wood distillate plus 3% soy lecithin. The dilution of 1:500, as well as the amount of 100 mL per treatment, and the addition of 3% lecithin were selected following the instructions of the wood distillate distributor [17]. The volume of 100 mL was adequate to hydrate the surface of six plants (statistical replicates) for each treatment. Spray applications were performed in the late afternoon. Treatment solutions were mainly sprayed on the upper surface of the leaves, trying to reproduce a field treatment as much as possible. In this work, we investigated the effectiveness of the foliar treatment as a whole, and the actual contribution of the stomatal versus the cuticular route was not explored.

After the treatment, the seedlings were left for 1 week in the climatic chamber at the same conditions as above, taking care to randomly rotate their position every day in order to minimize possible microenvironmental effects. Each seedling was watered daily with 10 mL of mineral water.

The selected light intensity (80 μM m^{-2} s^{-1} PAR) is higher than the minimum light intensity measured in greenhouses, i.e., the minimal light intensity at which plants can be cultivated [18]. It is possible that the low light intensity may have reduced stomatal opening, limiting the effect of wood distillate on lettuce plants. However, we compared our treatments with a reference control and we have no reason to assume that net photosynthetic rate and stomatal conductance, although not approached experimentally, were not similar in treated and control samples.

All analytical measurements were carried out after 7 days from treatment.

The sweet chestnut (*Castanea sativa* Mill.) wood distillate and the soybean (*Glycine max* (L.) Merr.) lecithin were made available by the company Esperia s.r.l. (RM Energy Solutions Group). The wood distillate was produced by steam distillation over a wide range of temperatures, using wet virgin biomass from the management of sweet chestnut as feedstock. Analysis provided by the producer indicates polyphenols in the range 13–16 g/L.

2.2 Photosynthetic parameters

In order to assess the effectiveness of wood distillate treatments on the photosynthetic performance of lettuce, as well as the ability of soy lecithin to enhance such effects, we investigated the chlorophyll content, chlorophyll fluorescence and chlorophyll reflectance. Fifteen measurements were taken for each plant.

The total chlorophyll content was quantified by a nondestructive chlorophyll content meter (CCM-300, Opti-Science, Hudson, USA) which provides results on a surface basis (mg m^{-2}) according to Gitelson et al. [19].

The chlorophyll a fluorescence is an effective methodology to investigate the functionality of the photosynthetic machinery [20]. Chlorophyll fluorescence was investigated through the use of the potential quantum efficiency of PSII, i.e., the indicator of photosynthetic efficiency F_V/F_M, where F_V indicated the difference between the maximal (F_M) and the minimal (F_0) fluorescence, and the general indicator of the photosystem I and II functionality, i.e., the performance index (PI_{ABS}). In addition, the analysis of the fluorescent transients (OJIP test) and the relative variable fluorescence (V_{OP} test; $V_{OP} = (F_t - F_0)/(F_M - F_0)$, where F_t indicated the fluorescence at '*t*' time [21], were also investigated in order to check for differences in the expression of each OJIP step between treatments. Prior to the analysis, samples were dark-adapted for 15 min in a climatic chamber (16 °C, RH = 90%). The analyses were carried out by flashing samples for 1 s with a saturated (1800 μmol m^{-2} s^{-1}) red light pulse (650 nm), using a Plant Efficiency Analyzer (Handy PEA, Hansatech Ltd., Norfolk, UK).

The normalized difference vegetation index (NDVI), defined as the ratio between the difference and the sum of the infrared spectral reflectance (NIR) and visible red light (red), is a common indicator of plant health and was measured with a PlantPen NDVI (Photon Systems Instruments, Czech Republic).

2.3 Dry biomass

The aboveground portions of each plant were cut and oven-dried at 105 °C for 1 h. After cooling, the plants were left to stabilize for five minutes and then weighed with a precision balance.

2.4 Statistical analysis

To check for the significance of differences in photosynthetic parameters between treated and control samples, as well as between treatments, a generalized linear mixed model (GLMM) was fitted with all the 90 measurements for each parameter, with treatment as fixed effect and plant as random effect. The significance of the model was checked by the analysis of deviance (type II Wald chi-square test). Post hoc pairwise comparisons were run with the Tukey test. The significance of differences in biomass ($N = 6$) was checked with a pairwise *t* test, correcting for multiple comparisons according to Benjamini and Hochberg [22]. All calculations were run using the R software [23].

3 Results

Samples treated with wood distillate, added or not with soy lecithin, showed a statistically higher chlorophyll content compared with control samples (+48–54%) and soy lecithin alone (+16–21%); plants treated with lecithin only also showed significantly higher values (+27%) than controls (Table 1). Photosynthetic efficiency (F_V/F_M) and performance index (PI_{ABS}) did not show any significant variation among treatments (Table 1).

The analysis of the fluorescence transient curves (Fig. 1) did not reveal alterations in the OJIP steps. Although samples treated with wood distillate indicated a slight increase in their fluorescent profile starting from the J step, and those treated with lecithin showed a similar modest decrease, the results of the V_{OP} analysis did not indicate differences between treatments. The NDVI showed a statistically significant increase only for the plants treated with wood distillate; the addition of lecithin to wood distillate, although causing higher values than controls, was not statistically effective (Table 1).

Statistically significant biomass increases compared to control values (Table 1) were recorded for plants treated

Table 1 Photosynthetic parameters and biomass (mean ± standard error) in samples of *Lactuca sativa* after 7 days from foliar applications of water (CTRL), soy lecithin (LC), wood distillate (WD) and wood distillate plus soy lecithin (WD + LC)

	Chlorophyll (mg m^{-2})	F_V/F_M	PI_{ABS}	NDVI	Biomass (mg dw)
CTRL	155 ± 7 a	0.785 ± 0.004 a	1.01 ± 0.04 a	0.47 ± 0.01 a	82.1 ± 0.8 a
LC	197 ± 7 b	0.787 ± 0.005 a	0.96 ± 0.05 a	0.47 ± 0.01 a	103.7 ± 1.0 b
WD	238 ± 7 c	0.788 ± 0.004 a	1.02 ± 0.04 a	0.50 ± 0.01 b	113.5 ± 1.0 c
WD + LC	229 ± 7 c	0.788 ± 0.004 a	1.01 ± 0.04 a	0.48 ± 0.01 ab	124.1 ± 1.3 d

Different letters indicate statistically significant ($p < 0.05$) differences between treatments

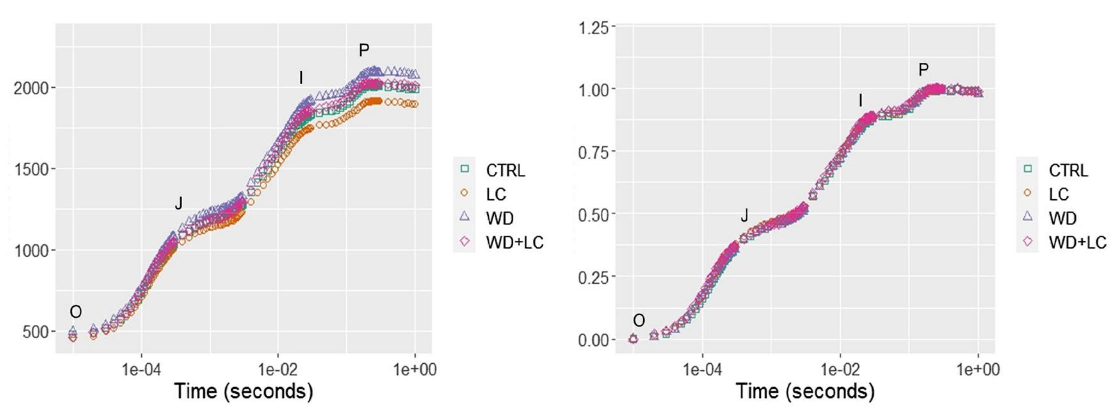

Fig. 1 Fluorescent transient curves (OJIP) (left) and V_{OP} transients (right) in samples of *Lactuca sativa* after 7 days from foliar applications of water (CTRL), soy lecithin (LC), wood distillate (WD) and wood distillate plus soy lecithin (WD + LC)

with lecithin (+ 26%), wood distillate (+ 38%) and wood distillate plus lecithin (+ 51%).

4 Discussion

Foliar application of wood distillate diluted 1:500 to lettuce plants, both alone and in combination with lecithin, determined an early (1 week) chlorophyll increase of ca. 50%. This is consistent with the 11%, 28% and 61% increase in chlorophyll content observed in rice (*Oryza sativa*) plants after 1, 2 and 3 foliar applications of 1:500 wood distillate, respectively [4]. Similarly, chlorophyll increases > 50% compared to controls were observed in 4-week-old rice seedlings after priming seeds with 1:100, 1:300 and 1:1000 wood distillate [5]. In addition, mustard (*Sinapis arvensis*) plants grown in soils added biweekly with 1:500 wood distillate showed increases in chlorophyll content of ca. 20% [24]. However, soil and foliar applications of 1:800 wood distillate did not statistically enhance the chlorophyll content of tomato (*Solanum lycopersicum*) plants [6]. The ability of lecithin to enhance the chlorophyll content of plants is unknown, but being a source of nitrogen and phosphorous [25], it is well possible that lecithin may enhance the chlorophyll content acting as a fertilizer [26, 27].

The application of wood vinegar and soy lecithin did not influence the photosynthetic efficiency and performance index of lettuce, which were otherwise similar to those of healthy lettuce plants [28]. There is limited and contrasting information in the literature about the effects of wood distillate application on the photosynthetic performance. Treatments with 1:300 and 1:200 solutions determined increased F_V/F_M values in cucumber (*Cucumis satuivus*) plants [29] and rice plants, respectively [4], whereas treatments with more diluted solutions of 1:2000 and 1:4000 caused reductions in the photosynthetic rates in lettuce [8]. Studies about the effects of soybean lecithin on photosynthesis are very scanty: Soil application of 10 g/L lecithin solution caused a decrease in the photosynthetic efficiency of maize (*Zea mays* L.) plants [30].

The OJIP step amplitudes were not modified by the wood distillate treatment. In fact, although the treatment with wood distillate seemed to enhance some transient steps (J, I and P), most likely indicating a major ability of electrons to chemically reduce plastoquinone A (Q_A), plastoquinone B (Q_B) and a free plastoquinone (PQ) [31], these variations were not as important for being visible in their V_{OP} profile [21]. Also, low light intensity can increase the amount of QA—reoxidation in PSII centers, improving the openness of PSII and promoting electron transfer, but we are confident that the influence on fluorescent transient curves was caused by the chemicals sprayed rather than by the low light intensity since we have based our results on comparison with a reference control, so we argue that the effect of low light intensity is the same in both treated and control samples. Also, the application of soy lecithin negatively affected the same JIP steps, but again these changes were not apparent in the V_{OP} profile. The combined application of wood distillate and lecithin showed no effect on the OJIP transients, probably as a result of the above contrasting effects. As a matter of fact, all treatments indicated overlapping V_{OP} trends. Consistently, treatment with wood distillate increased the reflectance (NDVI) of lettuce plants, indicating a better photosynthetic performance; however, simultaneous treatment with wood distillate and soy lecithin did not show this beneficial effect, even if the reduction was an insignificant 4% compared with samples treated only with wood distillate. We may speculate that this reduction is related to the mode of action of this biosurfactant, which increases spray droplet retention by plant leaves [32].

Soy lecithin, wood distillate and especially their combination stimulated biomass production in lettuce. Consistently with our results, foliar application of 1:500 bamboo wood vinegar increased (+ 42%) the biomass of lettuce plants; treatment with different concentrations of 1:300 and 1:800 was less effective [33]. Biomass increase (+ 20–45%) following foliar application of wood distillate was also found in rice [4] and tomato plants [6]. Dry weight increases (+ 33%) in shoots of rice plants were also observed when 1:300 wood distillate was added to a culture hydroponic medium [5]. Moreover, soil addition of wood vinegar caused 8–42% biomass increase in sugarcane (*Saccharum officinarum* L.) crops [34, 35]. Studies investigating soybean lecithin on plant growth are infrequent and the results are inconclusive: Soil application of 10 g/L lecithin solution did not determine any change in maize plant biomass [30], while biomass increase has been reported for endive (*Cichorium intybus* L.) plants sprayed five times with 15% soy lecithin [16]. However, it should be considered that soy lecithin is a source of nitrogen and phosphorous for plants [25]. Based on the present results, we can suggest that soy lecithin enhances the beneficial action of wood distillate in stimulating biomass production in lettuce; however, we cannot rule out that this positive combination is just the outcome of a mere additive effect.

Yield increase following wood vinegar application was suggested to be related to its ability to simulate or stimulate plant phytohormones or to increase plant photosynthesis [33], and our results are consistent with this view. Wood distillate has a very complex chemical composition, which usually includes a mixture of guaiacols, catechols, syringols, phenols, vanillins, furans, pyrans, carboxaldehydes, hydroxyketones, sugars, alkylaryl ethers, nitrogenated derivatives, alcohols, acetic acid, and other carboxylic acids [36]. Among the many compounds reported in the chemical composition of wood vinegar, polyphenols are probably the most important for their antioxidant, antimicrobial and growth enhancer properties [3, 37]. Moreover, polyphenols are linked to the chlorophyll content of different plants [38, 39], and although information about the mechanisms of action of wood distillate on plant photosynthesis is unknown, we speculate that chlorophyll increase in lettuce plants is a consequence of polyphenol exposure. In support of this hypothesis, foliar applications of solution containing very high polyphenol concentrations (i.e., 130 mg GAE/L), approximately four times higher than those estimated in our wood distillate dilution (30 mg/L), were able to increase (up to 150%) the concentration of chlorophyll a, chlorophyll b and carotenoids in sunflowers (*Helianthus annuus* L.) after 30 and 60 days from foliar application [40]. An increase in chlorophyll content is an indication of the possibility of increasing the biomass, and in the case of lettuce this leads to a higher yield [41]. Nevertheless, polyphenols themselves may also play a direct role in enhancing biomass production in plants, as recently highlighted by [40, 42], a factor probably linked to the ability of these compounds to increase cell division [42], as a consequence of the activation of specific transcription genes responsible for the plant growth [43].

5 Conclusions

This study demonstrated the effectiveness of foliar application of sweet chestnut wood distillate diluted 1:500 on the photosynthetic performance and growth of lettuce, both alone and in combination with 3% soybean lecithin used as biosurfactant. Both wood distillate and lecithin exerted a positive effect, but the joint use of wood distillate and lecithin quickly stimulated a ca. 50% increase in chlorophyll content and biomass production and is thus recommended.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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