INSTITUTO TECNOLÓGICO DE COSTA RICA

ESCUELA DE INGENIERÍA FORESTAL

VINAGRE DE MADERA PROCEDENTE DE LA PIRÓLISIS LENTA DE RESIDUOS DE MADERA DE *Gmelina arborea*: PRODUCCIÓN, COMPOSICIÓN QUÍMICA Y EFECTOS BIOLÓGICOS EN CULTIVOS

TESIS PARA OPTAR POR EL TÍTULO DE MAGISTER SCIENTIAE EN CIENCIAS FORESTALES CON EL GRADO ACADÉMICO DE MAESTRÍA

JAIR GRANADOS CHACÓN

CARTAGO, COSTA RICA, 2024

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Jair Granados-Chacón

RESUMEN GENERAL

La pirólisis lenta es una solución innovadora para la gestión de residuos forestales, ya que permite obtener productos energéticos como el biocarbón, gases de síntesis, alquitrán y vinagre de madera. El vinagre de madera (VM) presenta altos rendimientos y ofrece potenciales aplicaciones para la agricultura y el sector forestal. En Costa Rica hay una alta generación de residuos de madera de *Gmelina arborea* Roxb. ex Sm; sin embargo, existe poca información sobre los rendimientos de los productos de la pirólisis lenta de residuos forestales y sus potenciales usos en el país. Por lo tanto, los objetivos de este trabajo fueron: (i) determinar el proceso y los rendimientos de los diferentes productos (carbón vegetal, VM, bio-aceite, y gases no condensables) de la pirólisis lenta de dos formas de residuos (astillas de madera y madera maciza) de *G. arborea* en un prototipo de reactor semiindustrial; (ii) determinar las propiedades físicas y la composición química del VM de residuos de *G. arborea;* (iii) determinar los efectos del VM sobre el crecimiento, la biomasa, la clorofila, los nutrientes y el color de las plantas de lechuga (*Lactuca* sativ*a* L); y (iv) evaluar la eficacia, en tres dosis diferentes del VM para controlar arvenses en una plantación de árboles de Navidad de *Cupressus lusitanica* Mill., evaluando el daño visual y la reducción de biomasa. Los resultados no mostraron diferencias significativas en los rendimientos de carbón vegetal (26-28%), VM (28-30%) y gases no condensables (37%), pero el rendimiento de bio-aceite fue superior en el caso de los residuos de madera maciza (7.7%). Los fenoles (32,9%) fueron identificados como la clase química dominante en el VM. El fenol 2,6-dimetoxifenol (siringol, 14,8%) fue el principal componente. Las plantas de lechuga tratadas con VM al 0,25% mostraron un aumento del contenido de clorofila; sin embargo, no se observó ninguna mejora en el crecimiento ni en la producción de biomasa. En cambio, el VM al 0,50 % produjo efectos perjudiciales en el diámetro de la lechuga, el contenido de clorofila, la producción de biomasa y causó daños visuales. Las aplicaciones de VM diluido al 75% mostraron una rápida desecación de gramíneas y arvenses de hoja ancha, proporcionando un 85-88% de lesión visual y una significativa reducción de biomasa aérea (< 40%) tras el tratamiento con 3000 y 5000 L ha^{-1}. En general los resultados sugieren que: (i) los residuos de madera maciza son recomendables para completar el proceso en un menor tiempo y consumiendo menor energía, pero las astillas producen carbón con mejores propiedades energéticas; (ii) el uso del VM como fertilizante foliar puede ser perjudicial y no aumenta los rendimientos de las plantas de lechuga; (iii) el VM es una potencial alternativa natural para el control de arvenses, pero se necesita mayor investigación sobre la frecuencia y momento de aplicación para mejorar su eficacia.

Palabras clave: ácidos piroleñosos, destilado de madera, siringol, control de malezas, abono foliar

ABSTRACT

Slow pyrolysis is an innovative solution for the management of forest residues, as it allows obtaining energy products such as biochar, syngas, tar and wood vinegar. Wood vinegar (WV) presents high yields and offers potential applications for agriculture and forestry. In Costa Rica there is a high generation of wood residues from *Gmelina arborea* Roxb. ex Sm; however, there is little information on the product yields of slow pyrolysis of forest residues and their potential uses in the country. Therefore, the objectives of this work were to: (i) determine the process and yields of the different products (charcoal, WV, bio-oil, and non-condensable gases) from the slow pyrolysis of two forms of residues (wood chips and solid wood) of *G. arborea* in a prototype semi-industrial reactor; (ii) determine the physical properties and chemical composition of the WV from *G. arborea* wood residues; (iii) determine the effects of WV on growth, biomass, chlorophyll, nutrients and color of lettuce (*Lactuca sativa* L) plants; and (iv) evaluate the efficacy of three different doses of WV to control weeds in a Christmas tree plantation of *Cupressus lusitanica* Mill. by assessing visual damage and biomass reduction. Results showed no significant differences in charcoal (26-28%), WV (28-30%) and non-condensable gas (37%) yields, but bio-oil yield was higher for solid wood residues (7.7%). Phenols (32.9%) were identified as the dominant chemical class in the WV. Phenol 2,6-dimethoxyphenol (syringol, 14.8%) was the major component. Lettuce plants treated with 0.25% WV showed an increase in chlorophyll content; however, no improvement in growth or biomass production was observed. In contrast, 0.50% WV produced detrimental effects on lettuce diameter, chlorophyll content, biomass production and caused visual damage.

Applications of 75% diluted WV showed rapid desiccation of grasses and broadleaf weeds, providing 85-88% visual injury and significant aerial biomass reduction (< 40%) after treatment with 3000 and 5000 L ha^{-1}. Overall the results suggest that: (i) solid wood residues are recommended to complete the process in less time and consuming less energy, but wood chips produce charcoal with better energy properties; (ii) the use of WV as a foliar fertilizer can be detrimental and does not increase lettuce plant yields; (iii) WV is a potential natural alternative for weed control, but more research is needed on the frequency and timing of application to improve its efficacy.

Keywords: pyroligneous acids, wood distillate, syringol, weed control, foliar fertilizer

CONSTANCIA DE DEFENSA PÚBLICA DE TRABAJO FINAL DE GRADUACIÓN

Trabajo final de graduación defendido públicamente ante el Tribunal Evaluador integrado por Dr. Roger Moya Roque, Dr. Alexander Berrocal Jiménez, PhD. Dagoberto Arias Aguilar y M.Sc. Mario Guevara Bonilla como requisito parcial para optar por el grado de Maestría en Maestría en Ciencias Forestales

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Director de Tesis

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INTRODUCCIÓN GENERAL

En Costa Rica, algunas tecnologías de transformación de la madera con propósitos energéticos han ganado popularidad a través de la modificación física para producción de pellets [1], o la transformación térmica y termoquímica como la gasificación [2], torrefacción [3] y pirólisis [4], [5]. Como consecuencia, se han establecido plantaciones dendroenergéticas de rotación corta de *Gmelina arborea* Roxb. ex Sm (melina) con rendimientos de biomasa similares a otros cultivos energéticos de rotación corta [6]

El árbol de melina es la segunda especie forestal más plantada en Costa Rica con fines comerciales [7]. Es la principal especie para la producción de tarimas, actividad que domina el mercado nacional de madera y a la cual se destina más del 50% del total de madera en rollo producida en el país [8]. Según Espinoza-Durán & Moya [9], en la corta y aserrío de melina se producen una alta cantidad de residuos, alcanzando valores de hasta el 78% del volumen del árbol en pie.

Los residuos forestales pueden ser encontrados en distintas formas y tamaños; no obstante, sobresalen los residuos del aserrío de la madera como despuntes, tablas y recortes [5], y las astillas, a partir de la transformación física de los residuos forestales para reducir su tamaño y facilitar el secado, almacenamiento, carga y transporte [10], [11]. Según la Oficina Nacional Forestal [12], estos residuos representan una oportunidad para ser aprovechados con fines energéticos.

La pirólisis lenta, considerada una tecnología de emisiones negativa [13], ofrece una solución innovadora para la gestión de residuos lignocelulósicos, al fijar carbono en el carbón vegetal producido [14]. La pirólisis lenta es el proceso de degradación térmica de biomasa en ausencia o con una presencia mínima de oxígeno [15], [16]. Es clasificada por su baja tasa de calentamiento (0.1-10 °C min^{-1}), baja temperatura (300-700 °C) y tiempos de permanencia largos (de minutos a horas) [17], [18], [19]. Este proceso maximiza la producción de carbón, con rendimientos típicos del 35% de la biomasa seca [20]. No obstante, los subproductos *syngas* (gases no condensables) y vinagre de madera (VM; fase acuosa a partir de los gases condensables), suponen una oportunidad de aprovechamiento debido a sus considerables rendimientos de 35% [20] y 25% [21], respectivamente. Además, el proceso genera alquitrán (mezcla viscosa de compuestos orgánicos), en una proporción entre 3-5% [21], [22], [23]. En el caso de la melina, se han observado rendimientos del carbón entre 30.5-56.6%, de líquidos piroleñosos (alquitrán y vinagre de madera) de 17.8-32.9%, y de *syngas* de 25.6-36.6% [4]

El carbón vegetal es un producto rico en carbono y, debido a sus propiedades químicas y físicas, es adecuado para una amplia gama de aplicaciones: producción de calor y energía, metalurgia, agricultura, usos médicos, entre otros [24]. No obstante, los subproductos también pueden ser utilizados. El *syngas* es una mezcla de gases no condensables como el monóxido de carbono (CO), dióxido de carbono (CO_2), hidrógeno (H_2), metano (CH_4), entre otros, que pueden ser utilizados como combustible para generar calor o electricidad [25]

Por otro lado, el VM consiste principalmente en agua (80-90%) y más de 200 compuestos orgánicos clasificados como ácidos, alcoholes, cetonas, aldehídos, ésteres, furanos, fenoles y nitrogenados [35, 36]. El VM ha ganado popularidad en la agricultura y sector forestal [26], [27], [28]. Estudios han demostrado que posee actividad antioxidante y tiene potencial como biocida [29], incluyendo como fungicida [30]. En el suelo, puede mejorar su salud [31], aumentar la diversidad microbiana y aumentar la abundancia de bacterias promotoras del crecimiento vegetal [32] y puede ser utilizado para remediación por contaminación de cadmio y zinc [33]. Además, distintos autores destacan la capacidad del VM a bajas concentraciones (0.2% o 0.25%) para promover el crecimiento de los plantas, aumentar el rendimiento y mejorar la calidad de distintos cultivos como tomate [34], lentejas [35], canola [36], arándanos [37] y pepino [38]. Estos resultados son atribuidos a los ácidos (especialmente el ácido acético y el ácido butírico) y los fenoles [36]. El catión hidrógeno presente en los ácidos puede penetrar en los tejidos foliares y potenciar la actividad celular, aumentando el vigor de la planta [26]. Por otro lado, Vannini *et al*. [33] sugieren que los polifenoles pueden provocar un aumento del contenido de clorofila, con lo que se mejora la fotosíntesis y el crecimiento de las plantas.

La lechuga (*Lactuca sativa* L.) ha sido utilizada como cultivo modelo para evaluar la eficacia de aplicaciones foliares de VM producido a partir de castaña (*Castanea sativa* Mill.) [39], [40], [41]. Vannini *et al.* [39] demostraron la efectividad de VM al 0.2% para aumentar el rendimiento fotosintético y crecimiento de las plantas de lechuga, y se observó un aumento de casi el 50% en el contenido de clorofila y la producción de biomasa cuando se combinó con lecitina de soja. De forma similar, Fedeli *et al.* [40] encontraron un incremento en la biomasa de lechuga y una mejora de parámetros cualitativos como azúcar soluble y dulzor total con aplicaciones foliares de VM al 0,25%. Además, el VM al 0,2% ha mostrado un mayor poder antioxidante y moléculas antioxidantes, y la capacidad para proteger las plantas de lechuga de los daños inducidos por el ozono, contrarrestando el estrés oxidativo en el sistema fotosintético [41].

Por otro lado, autores han destacado el uso de VM para el control de malezas, con efectos similares a herbicidas no selectivos, los cuales se manifiestan rápidamente (menos de 24 h) a través de la marchitez y decoloración de las plantas [42], [43], [44], [45], [46]. Sin embargo, solo se han realizado unos pocos estudios en condiciones naturales [43]. Según Aguirre *et al.* [42], la biomasa en plantas tratadas con VM puede reducirse hasta un 70% en siete días, mientras que en 42 días puede ser hasta cuatro veces menor que el control. Además, mencionan que diluciones de VM al 25% puede ser suficientes para el control de arvenses [42]. Por otro lado, Liu *et al.* [45] obtuvieron un adecuado control del "zacate cabezón" (*Poa annua* L.) con VM sin diluir y aplicaciones al suelo de 100 L m^{-3} o foliares de 2800 L ha^{-1}. Sin embargo, para el control de malezas de hojas ancha, se recomienda aplicar dosis de 4000 L ha^{-1} de VM sin diluir [44]. Estas propiedades herbicidas son atribuidas a la gran cantidad de fenoles y ácidos orgánicos presentes en el VM, especialmente el ácido acético, el cual suele representar la mayor proporción del vinagre [42], [44], [46].

Por lo tanto, *G. arborea* es una especie potencial para la producción de carbón vegetal a base de residuos y el aprovechamiento de los subproductos del proceso de pirólisis. Sin embargo, es necesario determinar el rendimiento de los diferentes productos de pirólisis, en un prototipo de reactor semi-industrial que se ajuste a las necesidades nacionales. Además, se debe caracterizar y determinar la composición del VM de *G. arborea* y evaluar potenciales usos para su aprovechamiento en la agricultura y sector forestal. Por esta razón, este trabajo tiene como objetivos: (i) Determinar los rendimientos de los diferentes productos (carbón vegetal, VM, alquitrán y *syngas*) del proceso de pirólisis lenta de dos formas de residuos de madera (astillas y extremos de tablas) de *G. arborea*, a partir de un prototipo de reactor semi-industrial. (ii) Determinar las propiedades físicas y la composición química del VM de residuos de madera de *G. arborea* producidos con pirólisis lenta. (iii) Determinar los efectos del VM de residuos de madera de *G. arborea* sobre el crecimiento, la biomasa, la clorofila, los nutrientes y el color de plantas de *Lactuca sativa.* (iv) Evaluar la eficacia, en tres dosis diferentes, del VM de residuos de madera de *G. arborea*, para controlar arvenses en una plantación de árboles de Navidad de *Cupressus lusitanica*, evaluando el daño visual y la reducción de biomasa.

CAPÍTULO I. Effect of shape of residues of *Gmelina arborea* wood on yields and process of slow pyrolysis using a semi-industrial reactor prototype.

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Abstract

Pyrolysis of biomass residues can generate savings in the value chains of forest products due to the potential uses of its products in the forestry sector. The aim of this study was to know performance during slow pyrolysis process and their yields of different products (charcoal, wood vinegar, bio-oil, and non-condensable gases) of two shapes of residues, wood chips and solid wood board-ends from *Gmelina arborea*. Results showed no significant differences in yields of charcoal (26-28%), wood vinegar (28-30%) and non-condensable gases (37%), but bio-oil yield was higher for the solid wood board-ends residues (7.7%). The evaluation of energy charcoal characteristics and wood vinegar was similar for two shapes of parentwood. So, results suggest that the shape of parentwood provided similar charcoal, condensable and non-condensable gases yields, but solid board-ends are recommended to obtain higher yield of bio-oil and complete the process in less time and charcoal and vinegar characteristic were affected by shape of parentwood.

Keywords: pyrolysis; biochar; pyroligneous acids; wood tar; wood vinegar

1. Introduction

Biomass pyrolysis is the thermal degradation of biomass in absence of oxygen and is one of the most common thermochemical biomass conversion processes for energy production [16], [19]. Biomass decomposition occurs at elevated temperatures (400 – 900 ºC) by breaking down the long-chain hydrocarbons in the wood biopolymers [19]. Pyrolysis can be categorized as slow pyrolysis or fast pyrolysis based on the heat rate and maximum reaction temperature [19]. Slow pyrolysis occurs at temperatures between 300-700 °C with residence times of minutes to hours and heating rates of 0.1-10 °C min^{-1} [19].

Biomass from wood (chips or fiber) can be produced different shape during its size reduction and then they can be affects can affect the pyrolysis process and some problem can be presented [47]. Particle size or its shape of biomass affects the pyrolysis products yields, as higher yields of char have been observed for pyrolyzing biomass at temperatures lower than 400 °C, while at higher temperatures char and gas yields are favored by smaller particles [48]. In addition, cubic-shaped biomass pyrolyze longer and have higher char yields than slender particles which produce more volatile compounds [49]. Another parentwood characteristic related to shape is its moisture. Smaller dimensions present lower values of moisture content and high dimensions presents high moisture content. High moisture content affects the heat transfer, the pyrolysis reactions and product distribution in pyrolyzing biomass [50] and is one of the main reasons for poor quality of crude bio-oil [51]. Moreover, high moisture content leads to more energy consumption [52]

On the other hand, 88 % of the total roundwood produced in Central America and the Caribbean is used as fuel [53] showing the importance of fuel in part of the world. Moreover, in 2020, 90% of global bioenergy demand was produced in a traditional way in open fires or rustic kilns [54].

The Central America need for a structural transition towards the valorization of forest resources is evident. Feedstock of forest resources can be obtained from: short rotation energy plantation, forest residues from logging of plantation or natural forest trees, clear-cut of tree in agriculture areas or cities [55]. These resources had a common process, the size reduction in situs is necessary by chips production [56]. The sawmill process produced different residues, such as board-ends, boards of small dimensions, sawdust and other solid materials, which must be too reduced their size by chips production [57]. So, several shapes or sizes of forest feedstock can be found for energy production: boards of small dimensions (solid wood board-ends) and chips. So, increasing the profitability of wood energy can be obtained by managing the wood residues and generating savings in the process [58]

Among the different methods or reactors used for slow pyrolysis in developing countries [59]; such as flame curtain kilns, drum kilns, among other equipment [60]. Drum kilns reactor is used in many developments' country, due to low investment costs and minimal knowledge required [60], however the new model consider smoke condensation for wood vinegar productor and utilizations of combustible gasses [61]. The yield of biochar is maximized (~35%) through slow pyrolysis [20] and when the gasses are condensed one proportion is called as bio-oil (3-5%) and is a viscous mixture of organic compounds, mostly oxygenated hydrocarbons [23] and other proportion of gases condensed (~25%) are called wood-vinegar [61]. Finally, a proportion (~35%) are not condensed and named as syngas production [20].

In Costa Rica, fuelwood has gained popularity in recent years as a renewable alternative to the growing energy demand and an aid to sustainable rural development [10]. In that sense, energy production from forest biomass has been found viable for self-consumption [21]. Short rotation energy plantations produce feedstock for energy production [20], [22]. But, some studies too recommend the use of forest residues as an innovative solution for different thermal and thermo-chemical process for energy productions, as gasification [23], torrefaction [24], pyrolysis [25], besides the physical modification of this feedstock as pellet fabrication [26]. According with different studies, sawnwood yields are low and residues of up to 78% of the standing tree volume have been reported, suggesting the need to find alternatives for the non-marketable volume [27]. Then, as it was indicated, the residues can be found in two different shapes: solid wood board-ends from industrial process and chips from forest residues and solid residues in sawmill [25].

Gmelina arborea Roxb. ex Sm. (melina), is the second most reforested forest species in Costa Rica [28]. It is the main species used in the manufacture of pallets, a product that dominates the national timber market [29] and high quantity of residues are produced during logging and sawmill [27]. *G. arborea* wood has desirable physical and chemical properties for different products of pyrolysis [30]. However, *G. arborea* wood can present high moisture content and cellulose percentage (47%) in solid wood or parentwood [31]. High moisture content and slow dried rate are main problem of *G. arborea* wood and affect many industrial process [30]. During pyrolysis process, the high moisture and cellulose lead to tar production and high cellulose influence the production of high char at low temperatures and the production of volatile products at high temperatures [32]. These findings suggest that pyrolysis products of *G. arborea* can be optimized based on the parentwood and the type of pyrolysis. In fact, Moya *et al.* [30] showed the main variables of pyrolysis process and yields of different products were affected by pyrolysis temperature.

So, *G. arborea* is a potential species for residues-based charcoal production, however it necessary to determine the performance and yields of different products of pyrolysis in semi-industrial drum kilns prototype according to social conditions of Costa Rica. This study aims to (i) determine the yields of the different products (charcoal, wood vinegar, bio-oil, and non-condensable gases) and

(ii) evaluate the conditions in three stages of the slow pyrolysis process of two shape of wood residues (wood chips and solid wood board-ends) of *Gmelina arborea*, from a semi-industrial prototype reactor. Results are going to be useful to estimate the production of biochar, condensable liquids (vinegar and bio-oil) and non-condensable gases from wood residues of *Gmelina arborea*.

2. Materials and methods

2.1.Materials

Wood residues with shape from the sawmill process and secondary process of *Gmelina arborea* were used. The company Maderas Cultivadas de Costa Rica (MCC) provided the wood from fast-growing plantations between 9 and 15 years old. Two shapes of wood residues were used: wood chips and solid wood board-ends without bark (Figure 1a-b).

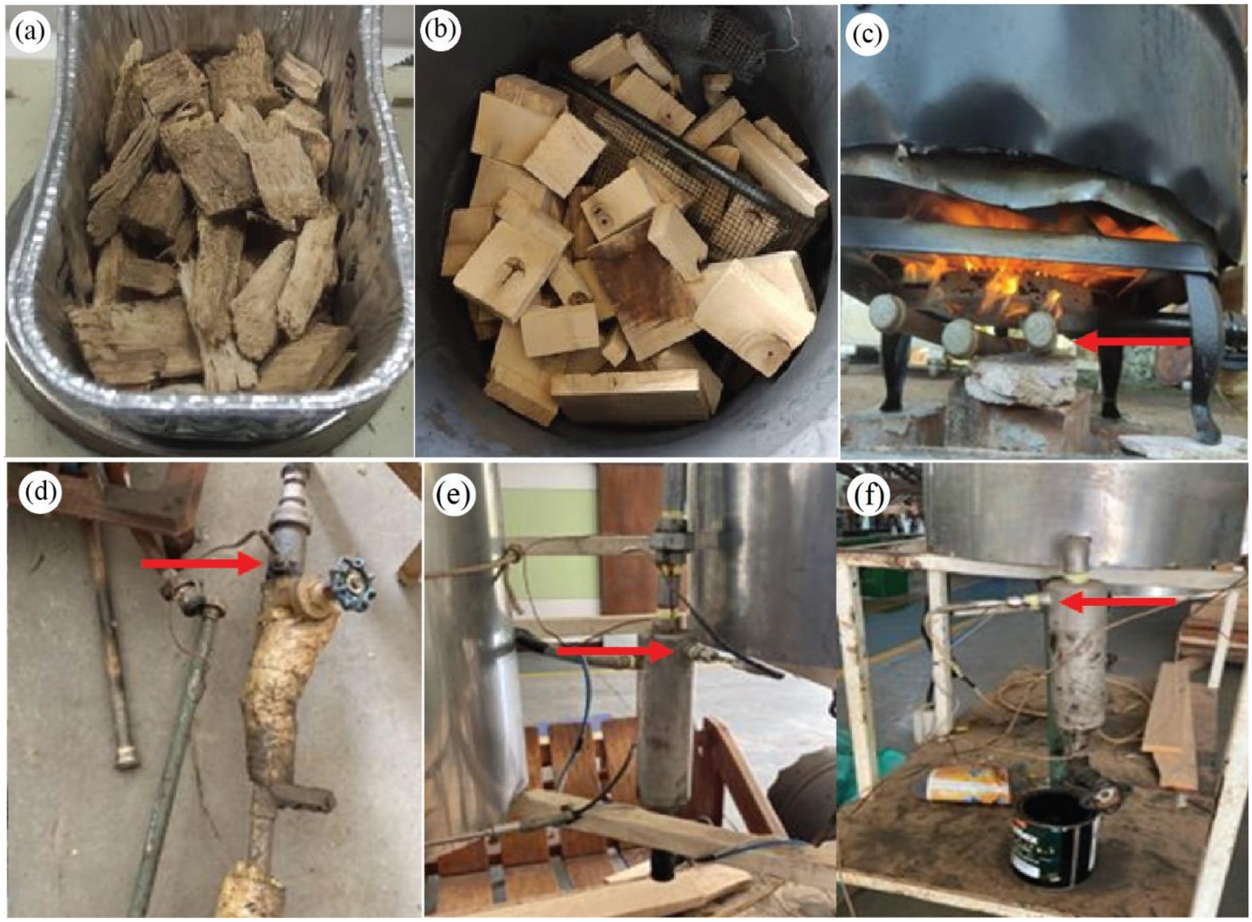

Figure 1. Wood residues of *G. arborea* used in the pyrolysis process: chip parentwood (a) and board-ends solid parentwood (b). Different parts in the rector: Non-condensable gases outlet (c) and temperature meter probes at three stages of the wood residues pyrolysis process: pyrolizer gas outlet (d), after the first cooler (e) and after the second cooler (f).

2.2.Raw material characterization

Chip parentwood were residues from sawlog processing with dimensions of 5 to 10 cm long x 2-5 cm width and air-dried. Solid parentwood board-ends were residues from the secondary wood processing with dimensions of 4-25 cm long and 12-32 mm thick. Moisture content (MC%) was calculated according to the oven-dried secondary method of ASTM D4442-20 standard [33]. Three samples per batch of type of residue were extracted and the capacity varied for shape of parentwood (Table 1). Parentwood presented MC% differences as well, solid wood board-ends had a statistically lower MC% than chip parentwood.

Table 1. Moisture content (MC%) and pyrolizer capacity of parentwood.

Parentwood	MC (%)	Weight (kg)
Chips	16.11^{A}	6.50^{B}
Board-ends	10.20^{B}	10.79^{A}

Legend: Different letters between residues indicate statistical differences (p-value<0.05)

2.3.Slow pyrolysis process

The pyrolysis of wood residues was carried out in a cylindrical reactor of 58 cm diameter, 88 cm long, and 232 L capacity (Figure 2). The reactor was covered with another cylinder with a glass fiber insulating jacket. First, the reactor was heated with liquefied petroleum gas (LPG) until pyrolysis. Then the pyrolytic gases were cooled through a system of coolers made of two helical coil heat exchangers. The first heat exchanger was cooled with air at room temperature and the second cooler with a closed water circuit moved by a pump. Liquids were collected after each cooler. Non-condensable gases (syngas) were utilized to heat the reactor (Figure 1c). The slow pyrolysis was finished when the syngas flame was over. The pyrolysis process was executed five batches or running for chip parentwood and four batches or running for solid parentwood board-ends.

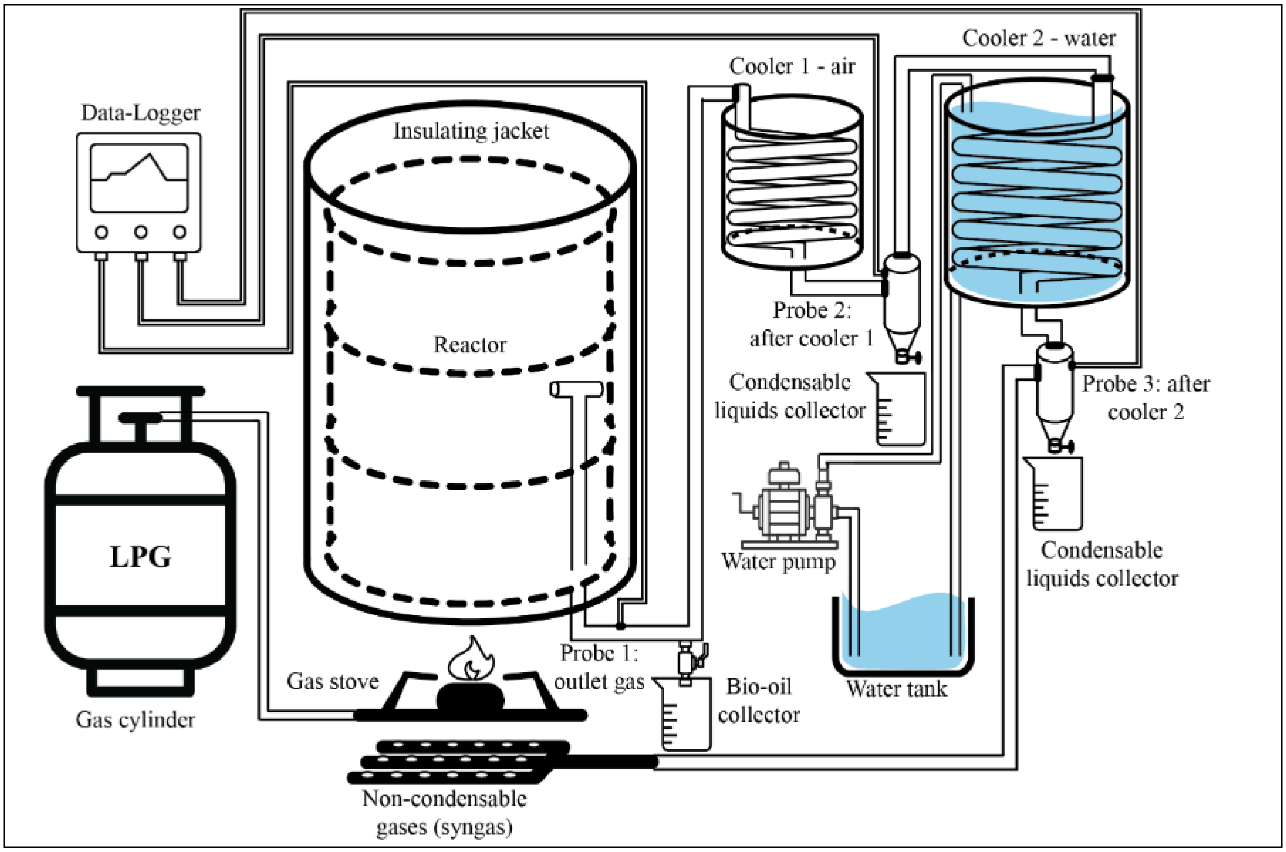

Figure 2. Cylindrical reactor designed and utilized for evaluation of the slow pyrolysis process.

Temperature was registered in three different stages of the process and are showed in the Figure 1d-f: pyrolizer gas outlet temperature (Probe 1, Figure 1d), temperature of gases after the first cooler (Probe 2, Figure 1e) and temperature of non-condensable gases after the second cooler (Probe 3, Figure 1f). Temperatures were measured each minute with a datalogger Testo model 176/T4 (Testo SE & Co., Titisee-Neustadt, Germany) and were registered for each running of the pyrolysis process.

2.4.Evaluation of the yield of pyrolysis products

The yields of the different products were calculated as Moya *et al* [30]: charcoal, bio-oil, wood vinegar and non-condensable gases. Parentwood was weighted before running the pyrolysis process and at the end of this, charcoal, bio-oil and vinegar were weighed to calculate each yield.

The yields of charcoal, bio-oil and vinegar were calculated according to Eq. (1). Non-condensable gases yield was calculated according to Eq. (2)

$$Charcoal, vinegar\ or\ bio-oil\ \ yield\ (\%) = \frac{Charcoal,\ \ vinegar\ or\ bio-oil\ weight\ (kg)}{Parentwood\ weight\ (kg)} * 100 \qquad (1)$$

$$Non-condensable\ \ yield\ (\%) = 100 - (charcoal + vinegar + tar\ yields) \qquad (2)$$

2.5.Evaluation of conditions in three stages of the pyrolysis process

The temperature and time data were used to evaluate the pyrolysis process according to Moya *et al* [30] with some modifications. Temperature was recorded in three stages using probes: (1) outlet pyrolysis gas reactor, (2) outlet pyrolysis gas of cooler 1 and (3) outlet pyrolysis gas of cooler 2 (Figure 1d-f). Four parameters for the outlet pyrolysis gas reactor and outlet pyrolysis gas of cooler 1, and three parameters for the outlet pyrolysis gas of cooler 2. Figure 3 presents the points and abbreviations of the parameters in the different stages and Table 3 describes the parameters evaluated. Finally, the duration of the pyrolysis process was measured.

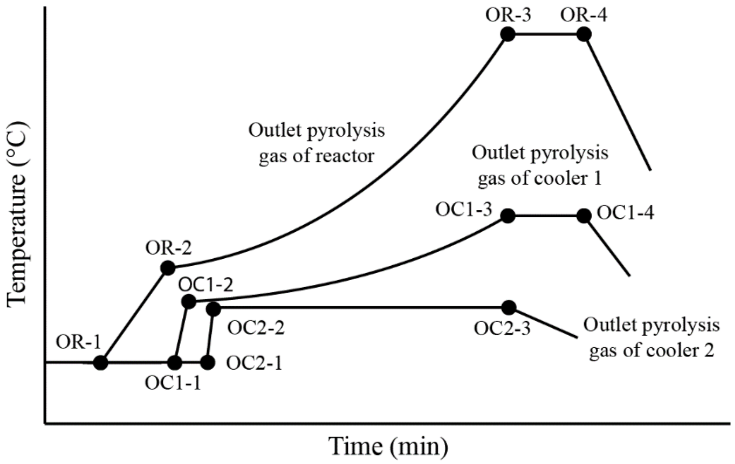

Figure 3. Different stages of the pyrolysis process of *Gmelina arborea* wood residues in four points of the outlet pyrolysis gas reactor and outlet pyrolysis gas of cooler 1, and three points of the outlet pyrolysis gas of cooler 2.

2.6.Condensable products and charcoal characteristics

The physical properties of condensable products were determined, specifically color, odor, insoluble particles, density, electric conductivity (EC) and pH of wood vinegar and bio-oil. Insoluble particles were obtained by filtering the products with a paper filter, and the value was calculated as the difference between the initial and final weight. The density was determined by dividing the liquid mass by its volume. The pH was measured with a pH meter (PHS-3C) and EC was determined only for wood vinegar with a Hanna Instruments HI98312, R.I, USA, water conductivity meter.

Table 2. Parameters of time and temperature of the different stages evaluated during pyrolysis process of *Gmelina arborea.*

Stage	Parameters	Abbreviations of points in figure 3
Outlet pyrolysis gas of reactor	1. Time when temperature began to increase	OR-1
	2. Time when water evaporation began	OR-2
	3. Temperature when water evaporation began	OR-2
	4. Time of stabilization in maximum temperature	OR-3
	5. Temperature of stabilization in maximum temperature	OR-3
	6. Time when temperature began to decrease	OR-4
	7. Temperature when it began to decrease	OR-4

Stage	**Parameters**	**Abbreviations of points in figure 3**
Outlet pyrolysis gas of cooler 1	1. Time when the temperature begins to increase	OC1-1
	2. Time when water evaporation began	OC1-2
	3. Temperature when water evaporation began	OC1-2
	4. Time of stabilization in maximum temperature	OC1-3
	5. Temperature of stabilization in maximum temperature	OC1-3
	6. Time when temperature began to decrease	OC1-4
	7. Temperature when it began to decrease	OC1-4
Outlet pyrolysis gas of cooler 2	1. Time when temperature began to increase	OC2-1
	2. Time when water evaporation began	OC2-2
	3. Temperature when water evaporation began	OC2-2
	4. Time when temperature began to decrease	OC2-3
	5. Temperature when it began to decrease	OC2-3

And charcoal characteristics determined were physical, energetic and chemical properties for charcoal produced with two shapes of parentwood. The physical properties determined were color and visual aspects of charcoal, bulk and apparent density and moisture content. For energy characteristics measured were gross caloric value, ash, and volatile content. GCV was determined at 0% of moisture content according to ASTM D5865M-19 standard and using Parr's calorimetric test [34]; for each material, ten samples of 300 mg per pyrolysis temperature were tested. Ash content was determined in three samples (2 g each) per material of each temperature/species, according to ASTM D3173 standard [35]. Three samples weighing 3 g each per temperature/species were used following the ASTM D1762 [36]

Another analysis was thermogravimetric analysis (TGA) using Thermogravimetric analyzer (TA Instruments Q500, New Castle, Denver, USA). An inert atmosphere was provided by ultra-high purity nitrogen with glow rates of 90.0 mL min^{-1}. One sample of 5 mg of charcoal was used for each temperature and parent wood. Each analysis was developed beginning with a thermal stabilization and isothermal period at 30 °C and 10 min. The heat rate was 25 °C min^{-1} until 750 °C. TA Instruments Universal Analysis 2000 software was used in data acquired.

2.7.Statistical analysis

The assumptions of normal distribution and homogeneity of variances were confirmed for the different product yields, the parameters evaluated during the pyrolysis process and charcoal characteristics. Two-sample t-tests for independent groups were carried out to determine statistical differences between the average of the variables measured of the wood residues and characoal characteristics. Principal component multivariate analysis (PCA) was applied for reactor condition. PCA was computed to explain the relationship between the first group of variables, consisting of the feedstock MC%, the pyrolyzer capacity and the yields of the different products. Two PC were established for each analysis and the type of parentwood was used to analyze the clustering of the observations. The t-tests and multivariate analyses were conducted using the R programming language v.4.3.1 in the integrated development environment RStudio v.2023.16.0-421 [37]

3. Results

3.1.Evaluation of the yield of pyrolysis products

The different products obtained during the pyrolysis process are presented in Figure 4a. Charcoal and vinegar presented similar yields, that varied from 26 to 31% and from 27 to 32%, respectively (Figure 4b and Figure 4c). The yield of condensable (sum of vinegar and bio-oil) was statistically equal in two types of shape of parentwood, which varied between 33 and 38% (Figure 4d). Bio-oil had the lowest yield among the different products (Figure 4a), presenting the highest percentage when solid parentwood board-ends was used (7.7%) (**Error! Reference source not found.**e). The product with the highest yield was the non-condensable (syngas), it varied between 34 to 40% (**Error! Reference source not found.**f).

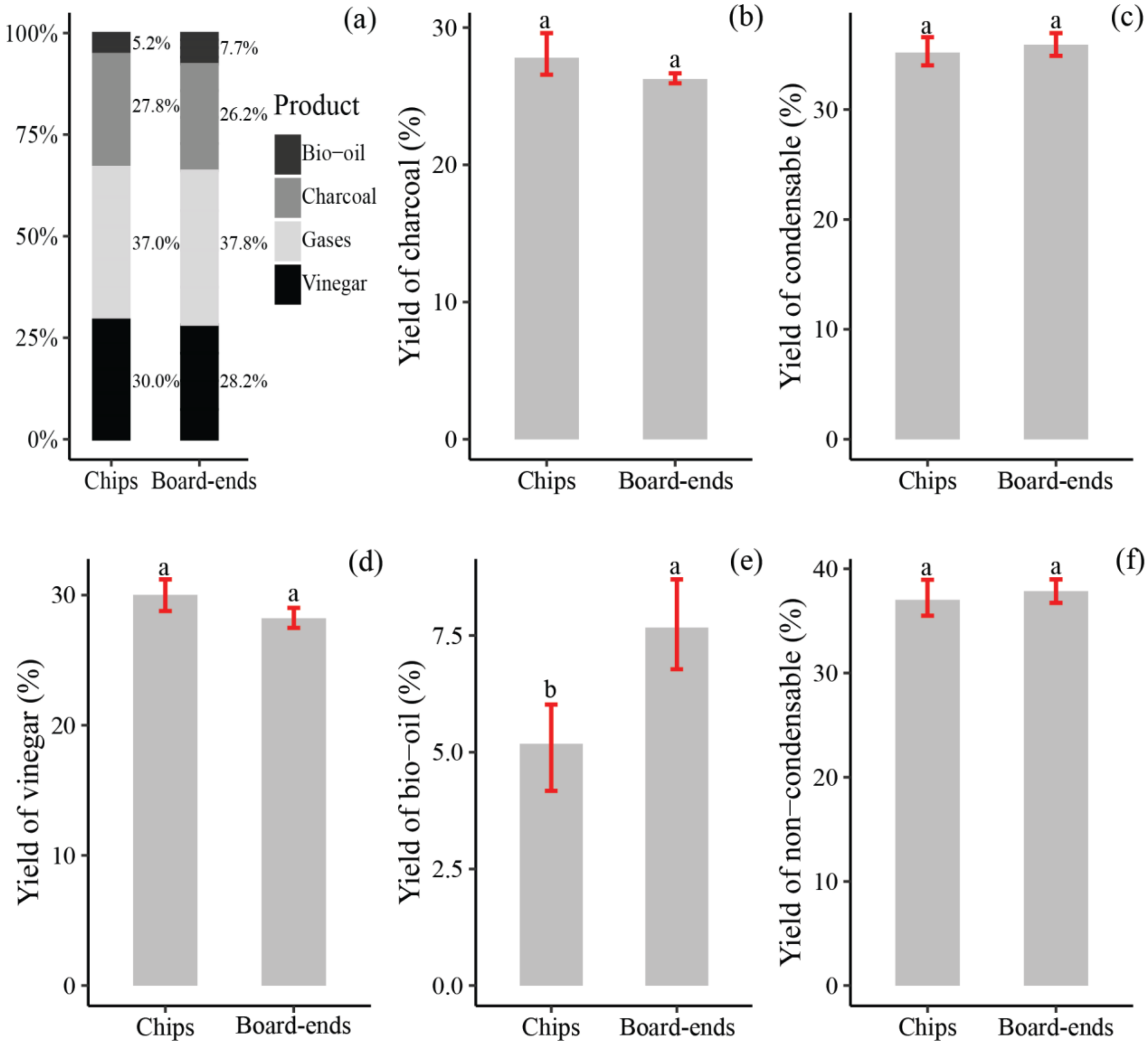

Figure 4. Distribution of the products (a) and yields of charcoal (b), condensable gases (c), wood vinegar (d), bio-oil (e), and non-condensable gases (f) from the pyrolysis process of two shapes of wood residues of *Gmelina arborea*.

Note: Confidence limits α=0.05 and different letters between residues indicate statistical differences (p-value<0.05).

3.2.Evaluation of conditions in three stages of the pyrolysis process

The total duration of pyrolysis process presented significant differences among wood residues. Chips parentwood lasted longer with 118 min, compared to the 93 min for solid parentwood board-ends. In the three stages evaluated of the pyrolysis process, the time when the temperature started to increase (OR-1, OC1-1 and OC2-1) and time and temperature when the water started to evaporate (OR2, OC1-2 and OC2-2) did not presented significant differences between two shape of residues (

Multivariate analysis

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Table 3. Conditions of temperatures and times of the different stages during pyrolysis process of wood residues of *Gmelina arborea.*

Stage	**Abbreviations of points in figure 4**	**Time (min)**		**Temperature (°C)**	
		Board-ends	**Chips**	**Board-ends**	**Chips**
Outlet pyrolysis gas of reactor	OR-1	3.0^{A}	8.7^{A}	-	-
	OR-2	12.7^{A}	15.0^{A}	73.4^{A}	75.8^{A}
	OR-3	80.0^{B}	103.7^{A}	262.4^{A}	162.2^{B}
	OR-4	85.3^{B}	109.0^{A}	240.4^{A}	153.7^{B}
	OC1-1	14.3^{A}	17.0^{A}	-	-

Stage	Abbreviations of points in figure 4	Time (min)		Temperature (°C)	
		Board-ends	Chips	Board-ends	Chips
Outlet pyrolysis gas of cooler 1	OC1-2	18.0^A	21.7^A	76.8^A	76.6^A
	OC1-3	82.0^B	104.7^A	182.1^A	97.5^B
	OC1-4	87.0^B	110.7^A	174.8^A	92.3^B
Outlet pyrolysis gas of cooler 2	OC2-1	21.3^A	31.7^A	-	-
	OC2-2	25.0^A	37.0^A	73.1^A	64.9^A
	OC2-3	77.3^B	103.0^A	64.8^A	59.9^A

Legend: Different letters between parentwood (board-ends and chips) of the corresponding variable (time and temperature) are statistically different at 95%.

Outlet of the reactor. The time when the temperature started to increase (OR-1) varied between 1 to 13 min after starting to heat the reactor and any difference was found between two types of parentwood (Table 3). The time and temperature when the water started to evaporate (OR-2) varied between 12 to 20 min and 67.5 to 89.6 °C, respectively (Table 3) and again not difference was observed between two types of parentwood. The time and temperature when the process (OR-3) reached the maximum temperature and when it began to decrease varied among two types of residues of parentwood (Table 3). Solid parentwood board-ends presented higher values of maximum temperature and shorter time than chips parentwood. After 5 min of stabilization of the maximum temperature, it began to decrease (OR-4) at between 205.5 and 273.0 °C for solid parentwood board-ends and 144.8 and 170.3 °C for wood chips, with statistical differences between two types of parentwood (

Multivariate analysis

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Outlet of the cooler 1. Temperature began to increase in cooler 1 (OC1-1) after 10 min later than the outlet of the reactor for two types of residues, between 12 to 18 min. Then the evaporation of water in the reactor was registered in the cooler (OC1-2) at a time between 18 and 21 min and temperature between 75.1-78.2 °C for two types of residues and any statistical differences. Later, maximum temperatures (OC1-3) were reached, and solid wood board-ends presented statistically higher temperature and shortest time than wood chips (

Multivariate analysis

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Approximately 5 min later, the temperature began to decrease at 173 to 177.3 °C for solid wood board-ends and 90.9 to 95 °C for chips parentwood (OC1-4) and both types of residues were statistically different (

Multivariate analysis

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Outlet of cooler 2. The time when the temperature started to increase (OC2-1) varied between 19 and 40 min, the temperature when water started to evaporate in the reactor (OC2-2) from 59.2 and 75.1 °C and the range of time of 22 to 50 min showed no statistical difference. The temperature stabilized at water evaporation (OC2-2) and then started to decrease (OC2-3) at 71-86 min for solid parentwood board-ends and this time was statistically lower than chips parentwood (Table 3). Temperatures did not present statistical differences (

Multivariate analysis

).

3.3.Multivariate analysis

The multivariate analysis showed that first PCA applied for the moisture content (MC%), the pyrolyzer capacity and the products yields showed the first two principal components (PC) explaining approximately 82% of the accumulated variability (**Error! Reference source not found.**4). The PC 1 was mainly influenced by MC% and the pyrolyzer capacity, and in less proportion by the yields of vinegar and bio-oil (**Error! Reference source not found.**). The PC 2 explained the 29% of the variation between observations (**Error! Reference source not found.**) and it was mostly influenced by the charcoal yield (**Error! Reference source not found.**4). The PCA using the principal component 1 and 2 of the observations showed that the relationship between variables of the MC%, pyrolyzer capacity and pyrolysis products yields (**Error! Reference source not found.**). It was also possible to distinguish two groups that represent the observations: one group for solid wood board-ends and the other one for chips parentwood (**Error! Reference source not found.**). Solid parentwood board-ends were more correlated to PC 1, and the factors associated with this grouping were pyrolyzer capacity and bio-oil yield. On the other hand, chips parentwood are associated more with the PC 2 and the association factor were the parameter of MC%, and vinegar and charcoal yields (**Error! Reference source not found.**).

Table 4. Correlation matrix for the moisture content of parentwood, pyrolyzer capacity and pyrolysis products yields (PCA 1) and Proportion of variance of the principal components of the multivariate analysis.

Variable	**PC 1**	**PC 2**
Moisture content	0.93**	0.08
Pyrolyzer capacity	-0.92**	-0.31
Vinegar yield	0.74	-0.59
Bio-oil yield	-0.76*	0.03
Charcoal yield	0.42	0.83**
Gases yield	-0.52	0.48
Proportion of variance	0.53	0.53
Accumulated proportion	0.29	0.82

Note: * indicates statistical differences at 95% (p-value<0.05), ** at 99% (p-value<0.01) and *** at 99.9% (p-value<0.001) in Student's t-test.

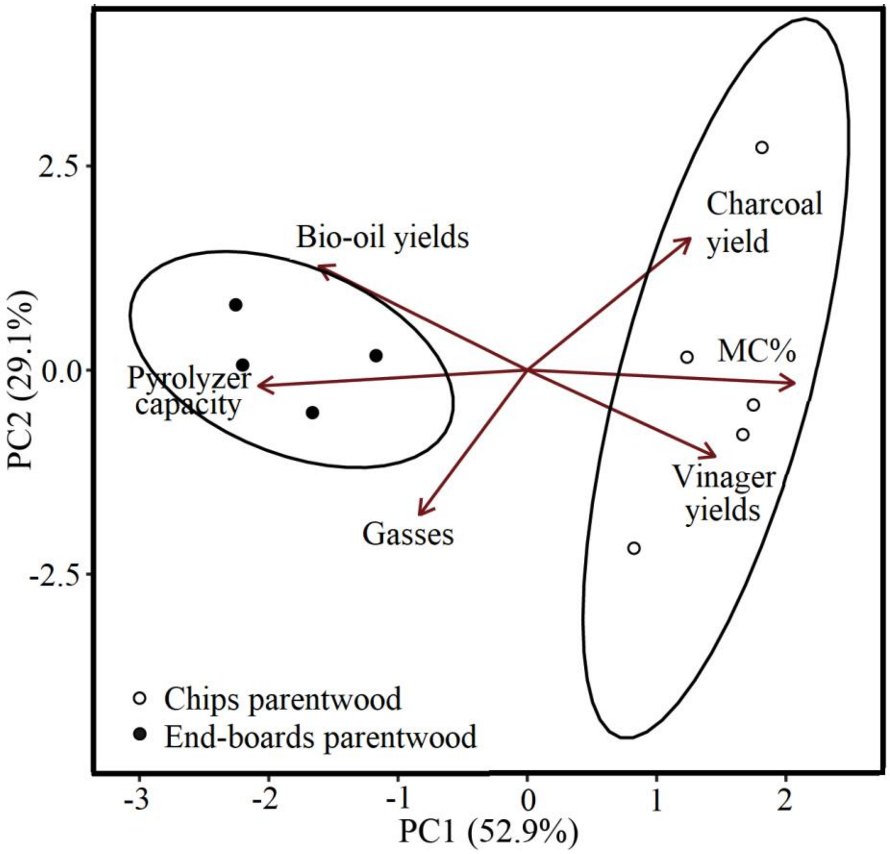

Figure 5. PCA of the weight and moisture content and pyrolysis products yields for pyrolysis process.

Note: Ellipses at 95% of normal probability group the observations by parentwood.

3.4.Charcoal and wood vinegar characteristics

The charcoal produced with two different shapes of parentwood varied with dimensions of charcoal pieces (Figure 6a-b). Board-ends shape (Figure 6a) produced bigger dimensions than chips shape (Figure 6b). The color of wood vinegar presented a similar color and visual color between two shapes and the color looks reddish (Figure 6c).

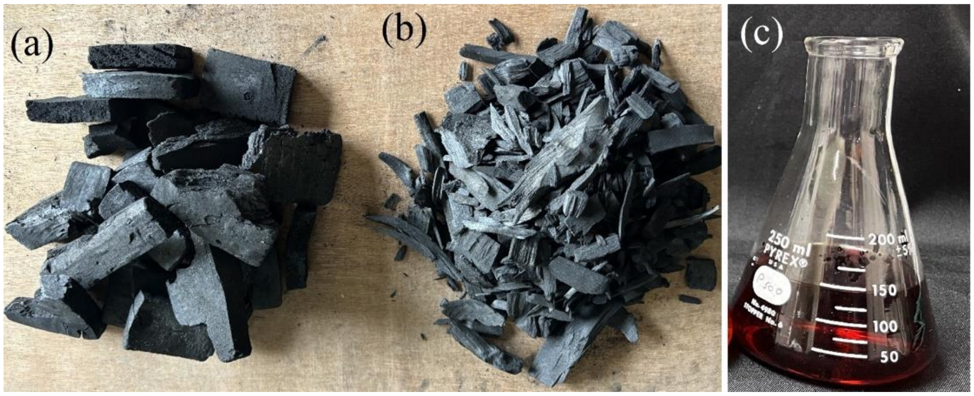

Figure 6. Visual aspects of charcoal (a-b) and wood vinegar (b) produced with two shapes of wood residues of *Gmelina arborea*

Physical characteristics of vinegar and bio-oil are presented in (Table 5), where it can observe that vinegar and bio-oil are different among them. The evaluation of other physical and energy characteristics is presented in (Table 6). Many statistical differences were observed in charcoal characteristics of two shapes of parentwood, Charcoal produced with chips shapes presented the highest values in gross caloric values, carbon content, and pH, but this carbon presented the lowest values in apparent density, volatile matter and oxygen content.

Table 5. Physical characteristics of vinegar and bio-oil produced with two shapes of wood residues of *Gmelina arborea*

Properties	Vinegar	Bio-oil
Color	Yellowish-brown	Black
Odor	Vinegar	Smoke
Insoluble particles (%)	0.16 w/w%	13.5 w/w%
Density (g/mL)	1.02	1.10
Electric conductivity (mS/cm)	2.3	-
pH	2.9	3.1

Table 6. Physical, chemical and energy characteristics produced with two shapes of wood residues of *Gmelina arborea*

Type of characteristics	Parameters	Board-ends	Chips
Physical	Bulk density (g cm^{-3})	0.23A	0.23A
	Apparent density (g cm^{-3})	0.237A	0.107B
	Moisture content (%)	4.44A	4.13A
Energy	Gross caloric value (MJ kg^{-1})	28.33A	31.36B
	Volatile matter (%)	39.36A	33.51B
	Ash (%)	3.23A	3.21A
Chemical	Carbon content (%)	79.69A	86.53B
	Hydrogen content (%)	2.97A	2.48A
	Oxygen content (%)	14.11A	7.78B
	pH	8.91A	10.03B

Note: Confidence limits α=0.05 and different letters between residues indicate statistical differences (p-value<0.05).

TGA analysis (Figure 7a) of charcoal produced with two shapes of residues of *G. arborea* showed typical behavior: in the first type (Figure 7a), a slight weight decrease was presented between 25 °C and 100 °C. After, a stable weight loss occurred between 100 °C and 200 °C and then comes a phase of ample temperature range, between 200 °C and 800 °C (Figure 7b) where maximum charcoal decomposition occurred. The maximum peak between 450 °C and 550 °C. There was decomposition differences between charcoal produced with two shapes of charcoal: charcoal from board-ends wood presented faster decomposition in relation to charcoal from chips (Figure 7). This can be observed in slope of mass remanent and derived mass, charcoal from chips presented stable values of derived mass, however for board-ends there is a higher value of derived mass (Figure 7b).

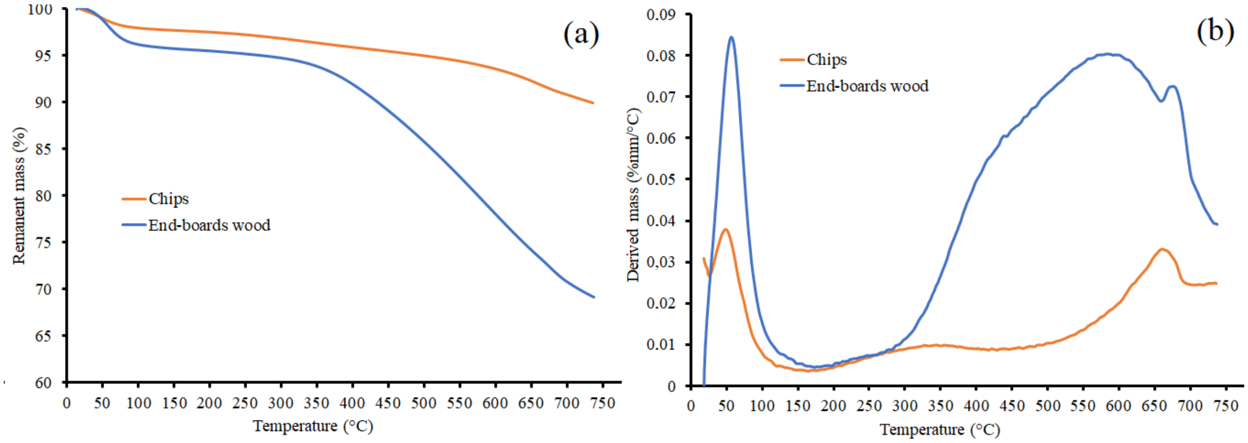

Figure 7. TGA and DTG of charcoal produced with two shape of wood residues of *Gmelina arborea.*

4. Discussion

4.1.Evaluation of conditions in three stages of the pyrolysis process

Different particle shapes were used in this study (Figure 1a-b). It is expected to obtain higher charcoal production using larger particles due to low heat transfer rate [32]. However, no significant difference of charcoal yield between the chips and board-ends were observed in this study (Figure 4b). Instead, in this study larger particles (solid wood board-ends) reached the maximum temperature faster than the smaller particles (wood chips) (

Multivariate analysis

). On the other hand, moisture content of biomass increases the energy required to reach the pyrolysis temperature [32]. In this study feedstock presented MC% below the fiber saturation point (**Error! Reference source not found.**), being suitable for pyrolysis [32]. However, chips presented higher moisture content (**Error! Reference source not found.**), which means that more energy supplied to the pyrolyzer through the stove is consumed to remove the moisture and less is used to raise the temperature [32] and this humidity condition probably produced longer time and lower temperatures at the different points where these parameters were measured (

Multivariate analysis

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No differences were observed for these products among the type of residue (Figure 4a) and the yields of charcoal, condensable and non-condensable gases agreed with percentages reported by Moya *et al*. [30] for *G. arborea* pyrolyzed at 450-500 °C. Tripathi *et al* [32] mentioned that a high heating rate enhances biomass fragmentation and gaseous and liquid yield, and that at low temperatures contributes to high char yields while at high temperatures produce highest volatiles by cellulose decomposition and condensable products increased. But according to the percentage of yield, the utilization of parentwood with two different shapes had little effects in these percentages, except for yield of bio-oil, which chips parentwood produced the lowest percentage (Figure 4e).

Wood chips pyrolysis had similar production of vapors (gases) as solid wood board-ends, probably because board-ends produced more gases by direct decomposition and less by secondary decomposition of wood tar due to a higher heating rate [38], contrary to wood chips. However, wood chips did not produce higher temperatures inside the reactor (OR-2 and OR-3), that help cellulose decomposition to increase the biol-oil production. At higher surface area to volume ratio, it is expected to enhance the production of bio-oil, due to a faster decomposition of the wood and shorter transportation of tar through the hot porous solids [38]. However, the smallest particle in this experiment (i.e. wood chips) contained higher moisture that slowed down the heating rate of feedstock (

Multivariate analysis

), which favors the production of char rather than tar. The bio-oil from solid wood board-ends (Figure 4e) can be produced from a higher volatilization of materials due to higher temperatures (

Multivariate analysis

) in this shape of feedstock [39]. In fact, PCA showed that yields of different products were related to the shaped of parentwood (**Error! Reference source not found.**a), solid wood board-ends increased the bio-oil yield and greater weight of biomass can be placed inside the reactor, while chips parentwood increased vinegar and charcoal yields.

Compared to other tropical wood species, *G. arborea* WV presented a low pH (2.9), but still within the range of 2.9-3.5, and higher density (1.02 g mL^{-1}) than the reported range of 1.005–1.016 g mL^{-1} [40]. The primary organic compound found in *G. arborea* WV was 2,6-dimethoxyphenol (syringol) with 14.8 % of the total area, similarly syringol has been reported as the major component of *Litchi chinensis* WV, representing 29.54 % of its composition [41]. Other studies have reported WV with higher acid and lower phenols content [42], [43], however these studies are often focused on temperate climate species, rather than tropical species.

The temperature of stabilization in maximum temperature of the reactor outlet (OR-3;

Multivariate analysis

) for chips and board-ends parentwood presented similar performance as Moya *et al*. [30] when pyrolysis was conducted at 450 °C and 500 °C, respectively. This behavior suggests that in this study, the maximum reached temperatures inside the reactor were higher. In addition, the temperatures and the product yields obtained (Figure 4) correspond with the slow pyrolysis values

[18]. However, the time and temperatures varied with the shape of parentwood, especially inside the reactor in the outlet of pyrolysis gases (OR), but not when gases were cooled (

Multivariate analysis

). Wood chips extended the time of pyrolysis gas outlet, time of reaching the maximum temperature, time when temperature began to decrease, and temperatures were lower inside the reactor and for the gases produced by pyrolysis; these conditions produce different chemical reactions for different feedstock [44]

Atreya *et al.*, [5] found that temperature when pyrolysis occurs influences the pyrolysis duration, which also vary with different shapes and size of the particles and follows the mass of the decomposing particle. For moisture free feedstock, large particles with cubic or spherical shapes pyrolyze slower than small and thin particles [5]. Similarly, Peters and Bruch [45] indicate that the start of the pyrolysis depends on the particle size and the heating temperature, and Bennadji *et al*. [46] found that the time of heating and devolatilization increase with increasing the particle size. These findings of feedstock size and shape were conducted with moisture free particles which can explain the contrast with our results, where the larger and like cubic shape, but drier particles (board-ends) presented the higher temperatures and the shorter pyrolysis duration.

4.2.Charcoal and wood vinegar characteristics

The results of the analysis of the physical properties of wood vinegar and bio-oil are described in Table 5. Bio-oil was more viscous, with higher density and pH, stronger smell and darker color than vinegar. Wood vinegar presented higher transparency and less suspended solids as insoluble particles. The comparison of these liquid products with other tropical wood species, *G. arborea* WV presented a low pH (2.9), but still within the range of 2.9-3.5, and higher density (1.02 g mL^{-}

^{1}) than the reported range of 1.005–1.016 g mL^{-1} [40]. The primary organic compound found in *G. arborea* WV was 2,6-dimethoxyphenol (syringol) with 14.8 % of the total area, similarly syringol has been reported as the major component of *Litchi chinensis* WV, representing 29.54 % of its composition [41]. Other studies have reported WV with higher acid and lower phenols content [42], [43], however these studies are often focused on temperate climate species, rather than tropical species.

A slight weight decrease was presented between 25 °C and 100 °C in charcoal from two shape of residues is attributed to water loss [47]. After, a stable weight loss occurred between 100 °C and 200 °C and is due to due to evaporation of the organics trapped on the surface of the samples Moya *et al.*, [30]. Maximum charcoal decomposition occurred 200 °C and 800 °C is due to the decomposition of the side groups with low thermal stability and volatile matter emission due to oxidation of the carbonaceous materials, such as carboxyl, carbonyl, and aliphatic hydrocarbon groups [48]. The maximum peak between 450 °C and 550 °C, represented the splitting-off of the more resistant side groups and the formation of the aromatic rings. At the final stage of the TGA curve (Figure 7), before ash, it represents the decomposition of the heat resistant heteroaromatic structures and the formation of polyaromatic structures [48].

This can be observed in slope of mass remanent and derived mass, charcoal from chips presented stable values of derived mass, however for board-ends there is a higher value of derived mass (Figure 7b). The charcoal from board-ends it was observed an inappropriate carbonization process, the inflexion that occurred between 650 °C and 700 °C (Figure 7b) evidences the presence of still unchanged wood components, hemicelluloses celluloses or lignin during pyrolysis [49].

In relation to visual evaluation, it was difficult to observe the differences, besides their shapes (Figure 6). However, charcoal properties were different for two shapes of parentwood. The parentwood chips are smaller dimensions than parentwood of board-ends (Figure 1a-b) and according to ours results, smaller dimensions favored the productions of charcoal with the best energy properties. Charcoal from chips parentwood produced the best energy properties due to higher gross caloric value and carbon content (Table 6). In fact, TGA showed that board-ends parentwood presented an incomplete pyrolysis (Figure 7b).

5. Conclusion

The conversion of *Gmelina arborea* wood residues into different products through the slow pyrolysis process, could provide an opportunity for Costa Rican forestry stakeholders revalue this material. So, it is of interest to know the products yields and performance of the pyrolysis process of different wood residues, using a small-scale reactor prototype and utilizing the non-condensable gases in the same reactor. This study suggests that wood chips and solid wood board-ends solid from *G. arborea* provide similar yields of charcoal, condensable and non-condensable gases. Some differences in pyrolysis process were found, the use of board-ends is recommended to obtain higher yield of bio-oil (Figure 4**Error! Reference source not found.**e) and complete the process in less time, making it energetically more efficient (

Multivariate analysis

). But charcoal characteristics were different, charcoal from chips parentwood produced best energy properties due to higher gross caloric value and carbon content. TGA showed that board-ends parentwood presented an incomplete pyrolysis.

Credit authorship contribution statement

Jair Granados-Chacón and **Pablo Valencia-González:** data curation, formal analysis, investigation, methodology, software, visualization, writing-original draft. **Roger Moya:** conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing-review and editing.

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.

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Appendix A. Supplementary material

The online version does not contain supplementary material.

Data availability of data and materials

Data will be made available on request.

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CAPÍTULO II. Effect of wood vinegar from residues of *Gmelina arborea* on the growth, chlorophyll, biomass and color of *Lactuca sativa.*

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Abstract

Wood vinegar (WV) has been proven effective as a biostimulant to promote plant growth, increase biomass and fruit production and quality. The objective of this study were to: (i) determine the physical properties and chemical composition of WV from *G. arborea* wood residues, and (ii) determine the effects of WV on the yield, growth, chlorophyll, nutrients, and color of *Lactuca sativa* (lettuce) plants. Plants were treated weekly for 28 days (four applications) and harvested on day 40. Leaves were sprayed with 100 mL of WV at doses of 0.25% and 0.50%, Bayfolan® Forte (BF) at 0.25% and distilled water (control). The results showed that WV is formed by phenols components (32.9%) were identified as the dominant chemical class. Phenol 2,6-dimethoxyphenol (syringol, 14.8%) was the major component. Plants treated with WV at 0.25 % showed an increase in chlorophyll content (SPAD units);

however, no improvement was observed in growth and biomass production. Moreover, WV at 0.50% led to detrimental effects on lettuce diameter, chlorophyll content, biomass production and caused visual damage. These results may be attributed to the high concentrations of phenolic compounds (33%), known to have allelopathic effects, high contents of iron (Fe 1035 mg L^{-1}) and zinc (Zn 1074 mg L^{-1}), and low nitrogen input (0.02%) in pure *G. arborea* WV. Finally, treatments did not change the nutrient concentration or content of potentially toxic elements, suggesting its safety for consumption

Keywords: slow pyrolysis, pyroligneous acids, fertilizer, wood distillate

1. Introduction

Pyrolysis is a thermochemical transformation of biomass under the absence, or at low concentrations, of oxidizing agents to produce usable energy products (biochar, bio-oil and syngas) [1]. The quality and yields of each pyrolytic product is highly dependent on the feedstock and pyrolysis parameters, as temperature, heating rate and residence time [1, 2]. Slow pyrolysis is the best method to maximize biocarbon production and is categorized by a slow heating rate (0.1-10 °C min^{-1}) , lower temperature (300-700 °C) and higher residence time (from minutes to hours) [3, 4]; however, this process of pyrolysis also produce pyroligneous acids and syngas that can be produced in adequate proportion [4, 5].

Slow pyrolysis is considered a negative emissions technology [6], and it is used to provide a solution to biomass residues management while fixing carbon in the produced biochar and secondary products, for example syngas, can be utilized as well [7]. Syngas is a mixture of non-condensable gases like carbon monoxide (CO), carbon dioxide (CO_2), hydrogen (H_2), methane (CH_4), and a small proportion of other gases, which can be used as fuel for heat or to generate electricity [8]. On the other hand, the pyrolytic liquid, also known as pyroligneous acids or wood vinegar (WV), has potential benefits, with many different uses in agriculture and forestry [9-12]. Moreover, due to its benefits, composition and origin, WV is allowed in organic farming in many countries, such as United States [13] and Italy [14].

WV has gained popularity as a natural alternative to synthetic herbicides, providing weed control by limiting its development, due to its high content of acids and phenols [15-19]. Noteworthily, researchers have found antioxidant and antimicrobial activity on WV [20], including fungicidal properties [21, 22]. Moreover, WV has shown excellent results when used to improve soil health [23], enhance soil microbial diversity [24], and for soil remediation [25]. In addition, studies have shown that WV can increase the abundance of beneficial soil microorganisms such as plant growth-promoting bacteria, which increase the availability of soil nutrients to plants [24, 25].

Studies highlight the ability of WV at low concentrations to promote plant growth [26, 27] and to increase crop yields and quality [28-30]. Ofoe *et al.* [29] observed an improvement of the number fruits and yields of tomato (*Solanum lycopersicum* ‘Scotia’), but a detriment of the fruit quality, with WV applications of 0.50% and an increase of elemental composition of fruits at 0.25%. WV at 0.2% applied in lentil plants (*Lens culinaris* L.) increased the plant and shoot biomass, the number and weights of pods and seeds and the total seed protein [30]. Zhu *et al*. [26] reported significant increments of plant height, total leaf number, green leaf number, leaf area, effective branch number, and pod number per plant, on rapeseed (*Brassica napus* L.) plants treated with WV at 0.25%. Moreover, co-applications of WV and biochar have also shown beneficial effects in crops, for instance blueberry (*Vaccinium corymbosum* L.) fruits accumulated more vitamin C with soil amendments of 1.5% biochar and 0.2% WV

[28]. Pan *et al.* [27] observed that soil amendments of 1.5% biochar combined with foliar applications of WV at 0.02% on cucumber (*Cucumis sativus L*) resulted in increments of ca. 30% plant height, 117% root length, 121% root volume and 76% root tips.

Moreover, lettuce (*Lactuca sativa* L.), has been used as a model crop to evaluate the efficacy of foliar applications of WV produced from sweet chestnut (*Castanea sativa* Mill.) [31-33]. For instance, Vannini *et al.* [31] demonstrated its effectiveness at 1:500 (0.2%) on the photosynthetic performance and growth of lettuce plants, and an increase of almost 50% on chlorophyll content and biomass production was observed when combined with soy lecithin. Similarly, Fedeli *et al.* [32] found an increase in lettuce biomass and improvement of qualitative parameters as sugar and total sweetness with foliar applications of WV at 0.25%. Moreover, WV at 0.2% had exhibited higher antioxidant power and antioxidant molecules and the ability of WV to protect lettuce plants from ozone-induced damage by counteracting oxidative stress on the photosynthetic system [33].

These results are attributed to WV chemical composition, consisting of bioactive compounds like organic acids, phenols, alcohols, alkanes and ester [26, 29, 34, 35]. According to Zhu *et al.* [26] acids (especially acetic acid and butyric acid) and phenols play a major role in the promotion of plant growth. The hydrogen cation present in acids can penetrate the leaf tissues and enhance cellular activity, increasing the vigor of plant [26]. Although the action mechanism is unknown, Vannini *et al.* [31] speculate that plants exposure to polyphenols leads to an increase in chlorophyll content, thereby photosynthesis is improved leading to higher yields.

In Costa Rica, the slow pyrolysis for the biochar production can be a sustainable solution to the great proportion of *G. arborea* wood residues produced in the country [36]. In fact, as Granados-Chacón *et al*. [37] and Moya *et al.* [38] had investigated, the slow pyrolysis of *G. arborea* wood residues presents yields of charcoal were 26-28%, WV of 28-30% and non-condensable gases of 37%. These results represent an opportunity to use the WV as a fertilizer in organic production, since it is one of the substances allowed by the Organic Agriculture Regulations of Costa Rica as natural acids (vinegar) [39]. However, no information has been presented about its efficacy as a natural fertilizer. Therefore, the objectives of this study were: (i) to determine the physical properties and chemical composition of WV, and (ii) to determine the effects of WV from *G. arborea* wood residues on the yield, growth, chlorophyll, nutrients, and color of *Lactuca sativa* plants.

2. Materials and methods

This experiment was carried out in a greenhouse at the Instituto Tecnológico de Costa Rica (ITCR), located in the province of Cartago, Costa Rica (9° 50'59.23" N latitude and 83° 54'36.27" W longitude). Located at an altitude of 1360 masl, with a temperature range of 17-24 °C, and an average annual rainfall of 2300 mm, the area corresponds to a Very Humid Forest life zone [40]. On site and during the experimental period (February-March), the Instituto Metereológico Nacional (IMN) reported temperatures between 14.7 and 21.4 °C, an average monthly rainfall of 7.2 mm and an average monthly solar radiation of 22 MJ m^{-2}.

2.1.Plant material and wood vinegar source

Seedlings of *Lactuca sativa* (cv ‘Bergam’s Green’) with three leaves and an approximate height of 15 cm were bought from a local plant nursery (Figure 1a). Wood vinegar (WV) was produced from the slow pyrolysis of wood residues of *Gmelina arborea* in a semi-industrial reactor prototype. Pyrolytic gases and pyroligneous acids collected from a pyrolysis reactor were condensed in two helical coil heat exchangers and WV was produced. This reactor presented a charcoal yield of 26 to 31% and a WV yield from 27 to 32%.

2.2.Wood vinegar physical properties and chemical composition

The physical properties (pH, electric conductivity (EC) and density) of the WV were determined by the Centro de Investigaciones Agronómicas (CIA) of the Universidad de Costa Rica (UCR). The concentration of elements (N, Cu, Fe, Zn, Mn, B, P, Ca, Mg, K and S) in pure WV was determined with an analysis of organic fertilizers. Nitrogen (N) was determined by MicroKjeldahl wet digestion with H_2SO_4 and colorimetric determination in the Flow Injection Analyzer (FIA) and P, Ca, Mg, K, S, Fe, Cu, Zn, Mn, B by digestion with HNO_3 and determination by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

Chemical composition of WV was determined by gas chromatography–mass spectrometry (GC-MS) in the Centro de Investigación y de Servicios Químicos y Microbiológicos (CEQIATEC) of the Instituto Tecnológico de Costa Rica (TEC). The WV samples were diluted by 8 in GC-grade toluene and analyzed on a Thermo Scientific Trace 1310 gas chromatograph with a TSQ 8000 Evo MS detector and a 30 m long TG5-SILMS Thermo Scientific capillary column. Injection volume was 1 µL by means of a TriPlus RSH autosampler for liquids. The injection was splitless, at 250°C, with a vacuum compensated for carrier flow of 1 mL min^{-1}. The initial temperature of the oven was held for 5 minutes at

90°C, then it was raised to 25°C min^{-1} until reaching 180°C, then at 5°C min^{-1} until reaching 255°C, which was held for 5 minutes.

2.3.Soil conditions and experimental design

Plants were grown in a soil characterized by 15% clay, 57% sand, 28% silt, 6.4% organic matter, 15.4 cmol (+) L^{-1} CEC, pH of 5.7, 0.1 acidity, Ca 12.7 cmol (+) L^{-1}, Mg 2.4 cmol (+) L^{-1}, K 0.3 cmol (+) L^{-1}, Cu 16 cmol (+) L^{-1}, Fe 124.7 mg L^{-1}, Zn 8.4 mg L^{-1}, Mn 5.3 mg L^{-1}, 4.5% C, 0.4% N and a C:N ratio of 10.4. Soil was characterized by the Centro de Investigaciones Agronómicas (CIA) of Universidad de Costa Rica (UCR).

In the greenhouse, seedlings were transplanted into plastic pots of 15 × 15 × 12 cm (Figure 1a). After the transplanting (day 0), seedlings were treated on days 7, 14, 21, and 28 (four applications). The treatments were: WV at doses of 0.25% (WV 0.25%) and 0.50% (WV 0.50%), Bayfolan® Forte at 0.25% (BF 0.25%) (Bayer AG, Frankfurt, Germany) and distilled water (Control). Treatment solutions of approximately 100 mL were sprayed over the surface of six seedlings (statistical replicates). After the treatment, seedlings were randomly rotated to minimize microenvironmental effects, as described by Vannini *et al.* [31]. The experiment lasted 40 days; after the last treatment (on day 28), lettuce plants were not treated for the next 12 days and then harvested on day 40 (Figure 1b). A completely randomized design was used, and the experiment was replicated three times (experimental replicates; Figure 2). Therefore 72 plants (experimental units) were sampled (6 lettuce plants × 4 treatments × 3 experimental replicates = 72 experimental units)

Figure 1. Transplanted lettuce plants (*Lactuca sativa*) on day 0 (a) and on day 40 ready to harvest (b) after 12 days of the last treatment of *G. arborea* wood vinegar (WV) at 0.25 % and 0.50 %, Bayfolan® Forte (BF) at 0.25 % and distilled water (control).

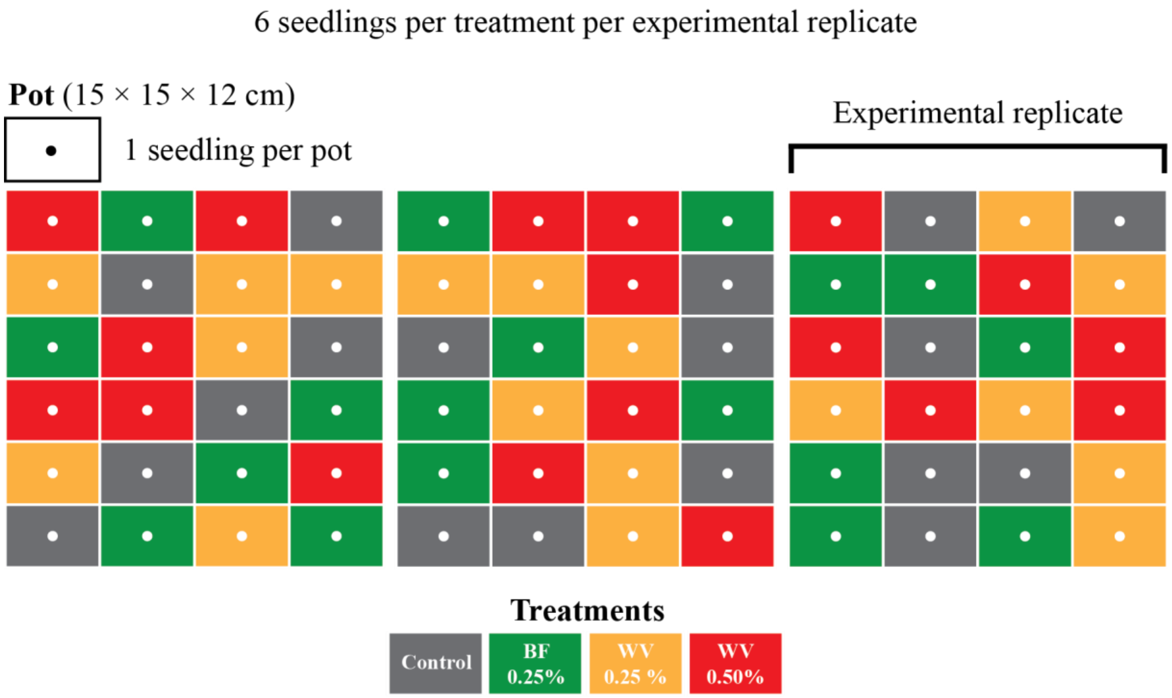


Figure 2. Completely randomized experimental design of lettuce plants (*Lactuca sativa*) treated with distilled water (control), Bayfolan® Forte (BF) at 0.25 % and *G. arborea* wood vinegar (WV) at 0.25 % and 0.50 %.

2.4.Evaluation of growth parameters and chlorophyll over time

The growth parameters and chlorophyll content were evaluated on day 0, 7, 14, 21, 28 and 40 for all (72) lettuce plants.

2.4.1. Growth parameters

The growth variables evaluated were: (i) height of plant (cm), which represents the measure from the soil level to the highest point of the plant; (ii) leaves, as the number of open leaves and (iii) head diameter (cm), which is the average of two perpendicular measurements of the diameter of the plant. Moreover, the increment of the values of these variables was calculated based on the last measurement.

2.4.2. Chlorophyll content

The chlorophyll content was measured with a MC-100 Chlorophyll Meter (Apogee Instruments, Utah, USA), which provides SPAD units that are relative indicators of chlorophyll concentration [41]. All plants were sampled and six measurements per plant were taken at the apical parts of three major leaves, avoiding leaf nerves.

2.5.Evaluation at the time of lettuce was harvested for sale

At the end of the experiment, on day 40, color was determined, and a destructive assessment of shoots and roots biomass was conducted for all lettuce plants. A sample of lettuce leaves per treatment was used to conduct a chemical analysis of nutrients.

2.5.1. Color determination

Color coordinates L^*, a^*, b^* were recorded according to CIELab's chromaticity system with a Miniscan XE plus colorimeter (HunterLab, VA, USA). Six measurements per plant were taken at the apical parts of three major leaves, avoiding the leaf nerves.

2.5.2. Biomass

The plants were harvested and separated into shoots and roots. Shoots were used to measure the leaf area (cm^2) using a LI-COR LI-3100C) leaf area meter (Lincoln, Nebraska, USA). The roots were washed to remove soil particles, so it was not possible to measure the weight of fresh roots. Fresh shoots (g) were weighed with a precision balance and then, shoots and roots were dried in an oven at 60 °C for 48 h. Afterwards, dry weights were measured again with a precision balance. Dry matter of shoots was calculated as the percentage of dry weight divided by fresh weight. The total dry biomass was calculated as the sum of dry shoots and roots weights.

2.5.3. Chemical leaf analysis

At the end of the experiment, three samples per treatment were used to perform a complete chemical leaf analyses in the CIA of the UCR to determine the element concentrations (%) of nutrient contents (N, P, Ca, Mg, K and S) and the contents (mg kg^{-1}) of potentially toxic elements (Fe, Cu, Zn, Mn and B). Nitrogen (N) was determined by dry combustion in an N Autoanalyzer, while the other elements by wet digestion with HNO_3 and determination by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; PerkinElmer Inc., Waltham, Massachusetts, USA).

2.6.Statistical analysis

The assumptions of normality and homogeneity of variances were checked. To determine the effects of the fertilizers on each plant parameter, one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference test was performed on parametric data. Kruskal-Wallis's test and Wilcoxon signed-rank test were performed for non-parametric data. A principal component analysis (PCA) was applied to determine the relationships among treatments and the plant parameters that resulted with significant differences on day 40: height, diameter, chlorophyll content, fresh shoot biomass, dry shoot biomass, total dry biomass and leaf area. The analyses were conducted using the statistical computing software R v.4.3.2 (Vienna, Austria) in the integrated development environment RStudio v.2023.16.0-421 [42]

3. Results

3.1. Wood vinegar chemical composition

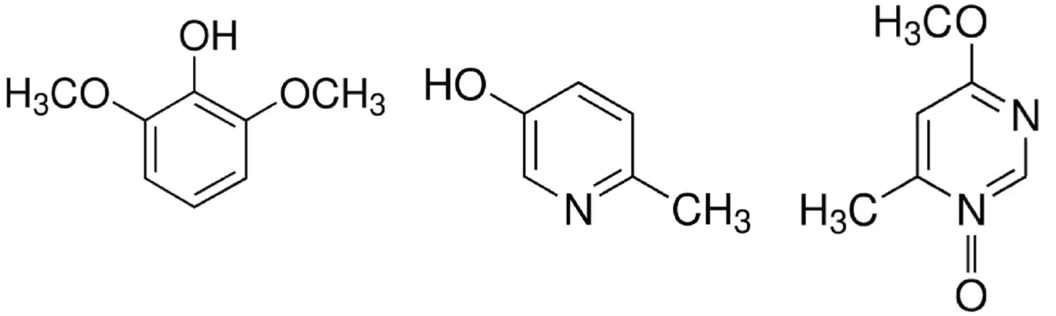
The WV was characterized with visually transparency and yellowish-brown color, a vinegary and smoky odor and the physical parameters presented the following values: pH 2.9, density 1.02 g mL^{-1} and EC 2.3 mS cm^{-1}. The elemental analysis showed the following values: N 0.02% (w/w), Cu 7 mg kg^{-1}, Fe 1015 mg kg^{-1}, Zn 1053 mg kg^{-1}, Mn 3 mg kg^{-1}, B 4 mg kg^{-1}; while P, Ca, Mg, K and S were not detected by the analysis.

Table 1. Most abundant components (>1% peak area) identified in the GC-MS chromatogram of the *Gmelina arborea* wood vinegar.

Chemical class	**RT (min)**	**Name (and % probability)**	**Molecular Formula**	**Molecular Weight**	**% Area**
Ketone	6.14	4.4-dimethyl-2-cyclohexen-1-one (36%)	$C_8H_{12}O$	124.18	2.4
Nitrogenous	7.62	5-hydroxy-2-methylpyridine (6%)	C_6H_7NO	109.13	12.3
Nitrogenous	8.22	Methyl 6-methyl-1-oxido-4-pyrimidinyl ether (26%)	$C_6H_8N_2O_2$	140.14	8.4
Ketone	8.33	Piperitone (51%)	$C_{10}H_{16}O$	152.23	5.9
Phenol	8.47	4-methylcatechol (42%)	$C_7H_8O_2$	124.14	4.9
Phenol	8.92	2,6-dimethoxyphenol (Syringol) (36%)	$C_8H_{10}O_3$	154.16	14.8
Phenol	9.19	4-ethyl-resorcinol (25%)	$C_8H_{10}O_2$	138.16	5.3
Amide	9.34	Diazobicyclo(4.4.0)dec-5-en-2-one (29%)	$C_8H_{12}N_2O$	152.19	1.7
Phenol	9.66	4-methoxy-3-(methoxymethyl) phenol (24%)	$C_9H_{12}O_3$	168.19	4.7
Ester	9.92	3,7-dimethyl-6-nonen-1-ol acetate (55%)	$C_{13}H_{24}O_2$	212.33	1.2
Alcohol	10.03	5-isopropyl-6-methyl-5-hepten-3-yn-2-ol (14%)	$C_{11}H_{18}O$	166.26	1.2
Nitrogenous	10.29	2,4,5-Trimethoxyamphetamine (19.5%)	$C_{12}H_{19}NO_3$	225.28	3.3

Chemical class	RT (min)	Name (and % probability)	Molecular Formula	Molecular Weight	% Area
Ketone	10.37	Guaiacylacetone (44%)	$C_{10}H_{12}O_3$	180.20	3
Aldehyde	11.58	Syringaldehyde (20%)	$C_9H_{10}O_4$	182.17	1.4
Phenol	12.36	Acetosyringone (47%)	$C_{10}H_{12}O_4$	196.2	2.1
Ketone	12.73	Desaspidinol (32%)	$C_{11}H_{14}O_4$	210.23	2.5
Phenol	14.3	5-(3-hydroxypropyl)-2,3-dimethoxyphenol (89%)	$C_{11}H_{16}O_4$	212.24	1.1

The GC-MS analysis of the WV identified 17 components as the most abundant (>1% peak area) organic compounds, representing more than 76.2% of the total composition of WV (Table 1). Among the 17 organic compounds, the three major components (Figure 3) were 2,6-dimethoxyphenol (syringol, 14.8%), 5-hydroxy-2-methylpyridine (12.3%) and methyl 6-methyl-1-oxido-4-pyrimidinyl ether (8.4%). Phenols was the most abundant functional group of compounds with almost 33% of the peak area, followed by the nitrogenous group (24%) and ketones (13.8%). It was not possible to identify any compound of the acid chemical class with a >1% peak area (Table 1).


2,6-dimethoxyphenol

5-hydroxy-2-methylpyridine

Methyl 6-methyl-1-oxido-4-pyrimidinyl ether

Figure 3. Three major components structures of the wood vinegar analyzed by GC-MS

3.2.Evaluation of growth parameters and chlorophyll over time

Foliar application of WV showed no significant effect on growth parameters during the first 20 days (Table 2). Differences in plant height and head diameter among treatments were observed until day 21 and 28, respectively (Figure 4a-b), due to a significant increment (Table 2, Figure 5a-b). No significant differences in the number of leaves or their increment were found at any stage of growth (Table 2, Figure 4c, Figure 5c). On the other hand, significant effects on chlorophyll content among treatments were found from day 28 onwards (Table 2, Figure 7b-c).

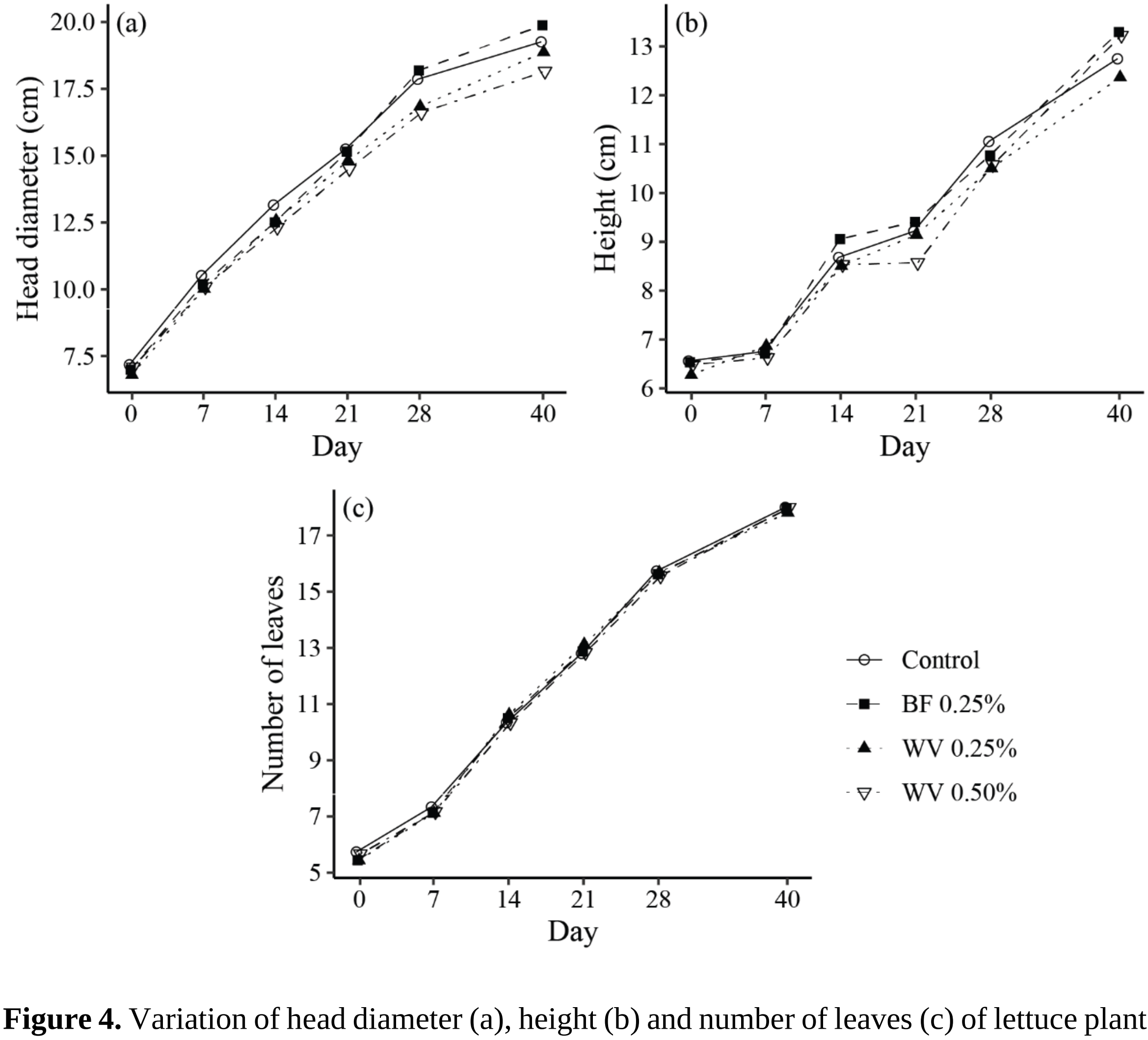


Figure 4. Variation of head diameter (a), height (b) and number of leaves (c) of lettuce plants at 40 days treated weekly with distilled water (Control), Bayfolan® Forte at 0.25% (BF 0.25%) and *G. arborea* wood vinegar at 0.25% (WV 0.25%) and 0.50% (WV 0.50%).

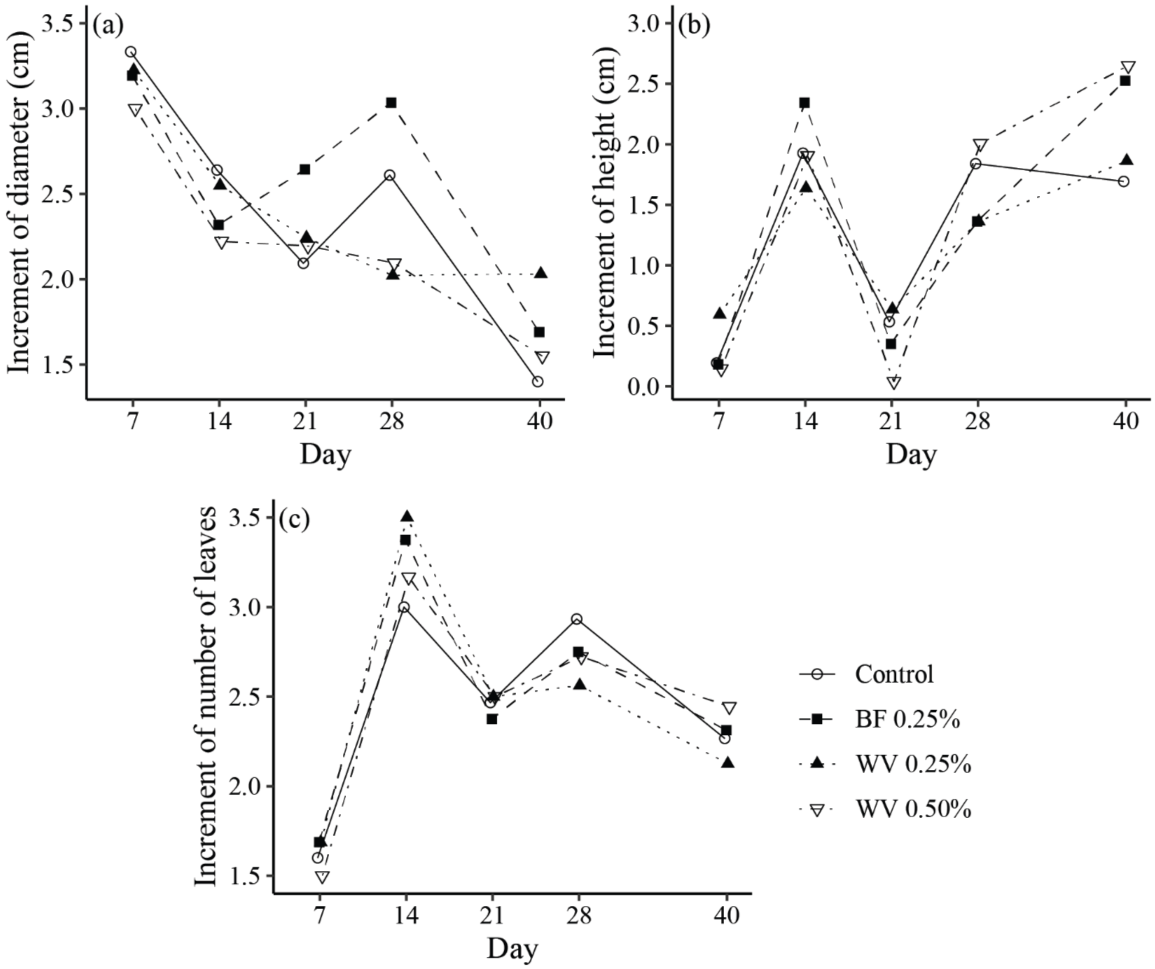


Figure 5. Variation of the increment of the head diameter (a), plant height (b) and the number of leaves (c) of lettuce plants, treated weekly with distilled water (Control), Bayfolan® Forte (BF 0.25%) and two wood vinegar (WV) applications.

Table 2. F value obtained from analysis of variance for the different parameters evaluated of lettuce plants treated with different doses of WV.

Day	Diameter	Height	Leaves*	Increment of Diameter	Increment of Height	Increment of Leaves*	Chlorophyll	Increment of Chlorophyll
0	0.34	0.33	2.43	-	-	-	-	-
7	0.61	0.19	1.42	0.24	1.35	0.99	0.06	0.25
14	1.34	1.32	0.94	0.71	1.34	3.64	1.78	1.60
21	1.15	3.34 **	0.43	1.04	2.89 **	0.15	5.09	1.08
28	7.16 **	1.01	0.41	4.20 **	1.75	1.41	22.50 **	4.56 **
40	8.40 **	3.50 **	0.23	1.96	2.15	0.48	35.71 **	3.88 **

Leaf area and biomass

	Leaf area	Fresh shoot biomass	Dry shoot biomass	Shoot dry matter*	Dry root biomass	Dry total biomass
40	3.23 **	4.26 **	3.26 **	9.48 **	3.22 **	3.98 **

Legend: *parameter subjected to the non-parametric test Kruskal-Wallis (chi-squared value), ** there are significant differences (p-value < 0.05).

3.3.Evaluation at the time of lettuce was harvested for sale

3.3.1. Diameter, height and number of leaves

At the time of lettuce was for sale (40 days), the application of BF 0.25% showed better results of head diameter, height and leaf area over WV doses (Figure 6a,b,d). WV 0.50% showed better results in plant heigh over the dose of WV 0.25% (Figure 6b). However, a negative impact of WV 0.50% was observed for the head diameter at the end of the experiment (Figure 6a). Moreover, no difference was found in the number of leaves (Figure 6c)

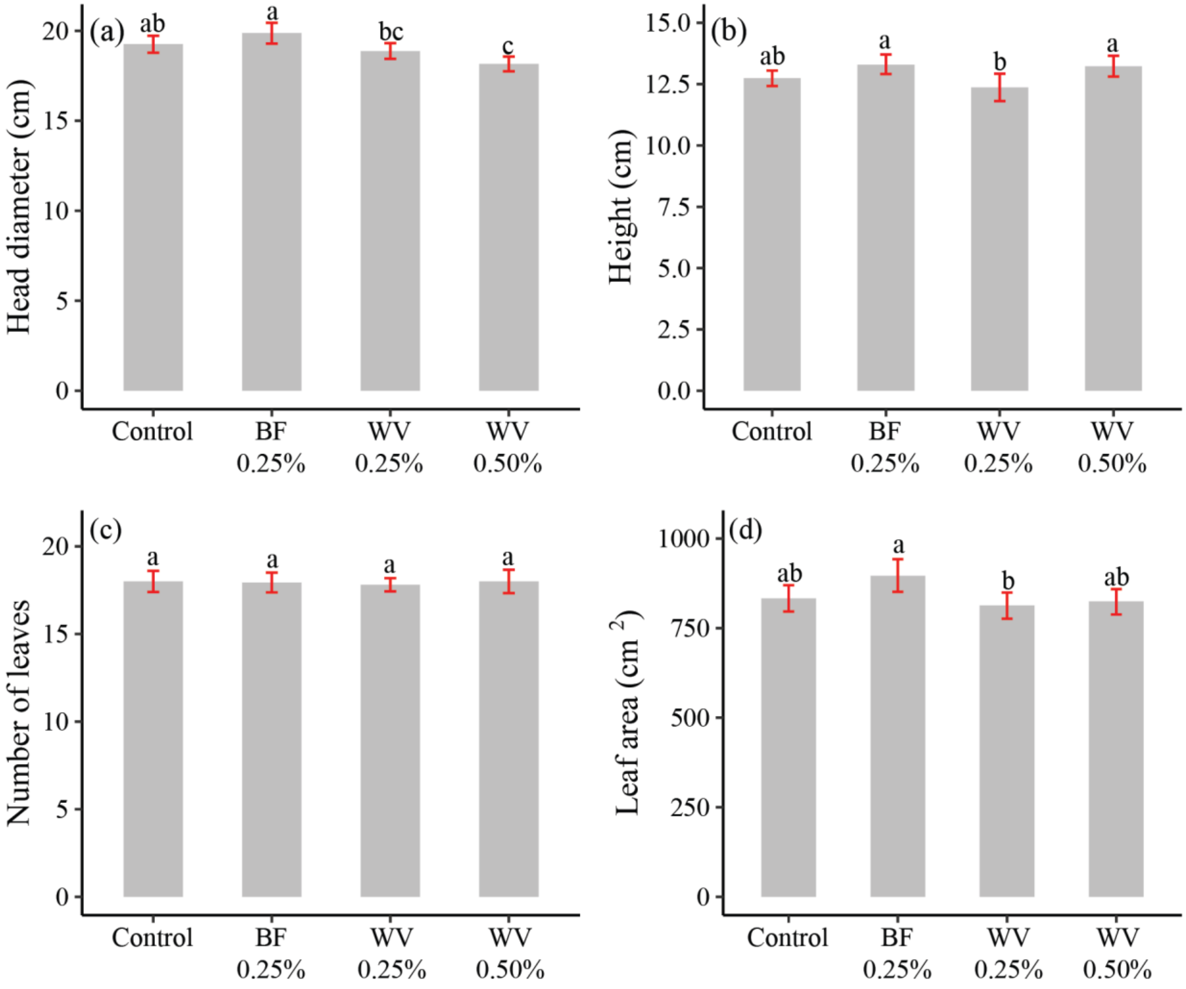


Figure 6. Head diameter (a), height (b), number of leaves (c) and leaf area (d) of 40-day-old lettuce plants treated weekly with distilled water (Control), 0.25 % BF (Bayfolan® Forte) and 0.25 % and 0.50 % WV (*G. arborea* wood vinegar).

3.3.2. Chlorophyll content

Plants treated with BF 0.25% and WV 0.25% showed higher SPAD units compared with control readings at day 40 (+6.56% and +4.54%, respectively). Meanwhile plants treated with WV 0.50% showed significantly lower values (-5.07%) than the control (a).

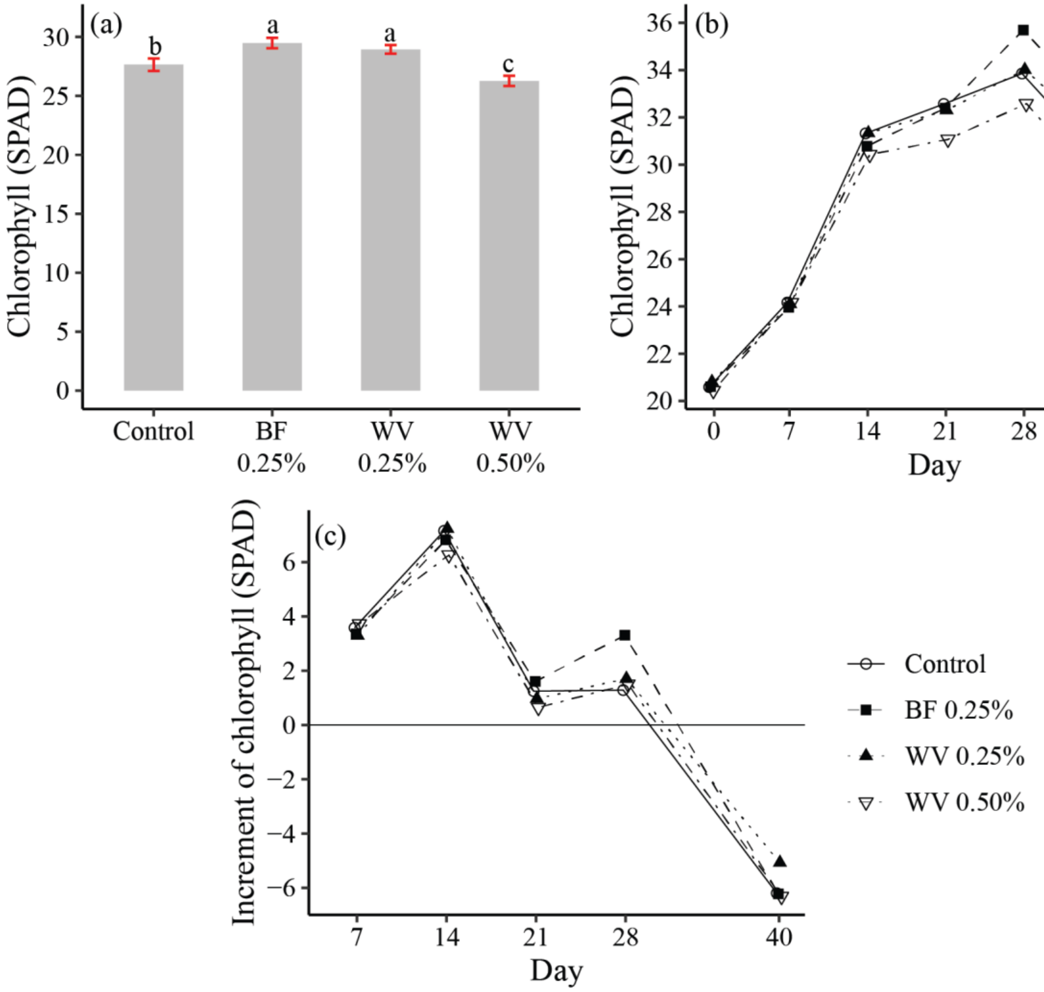


Figure 7. Chlorophyll SPAD units on day 40 (a) and chlorophyll variation (b) and increment (c) of lettuce plants treated weekly with distilled water (Control), Bayfolan® Forte at 0.25% (BF 0.25%) and *G. arborea* wood vinegar at 0.25% (WV 0.25%) and 0.50% (WV 0.50%).

3.3.3. Biomass

The different doses of *G. arborea* WV showed no improvement in biomass production over the control (Figure 8). Instead, WV 0.50% produced less fresh and dry shoot biomass and total biomass than BF 0.25% (Figure 8a,b,e) and less shoot dry matter an total biomass than the control (Figure 8c,e). On the other hand, BF 0.25% presented no differences with the control.

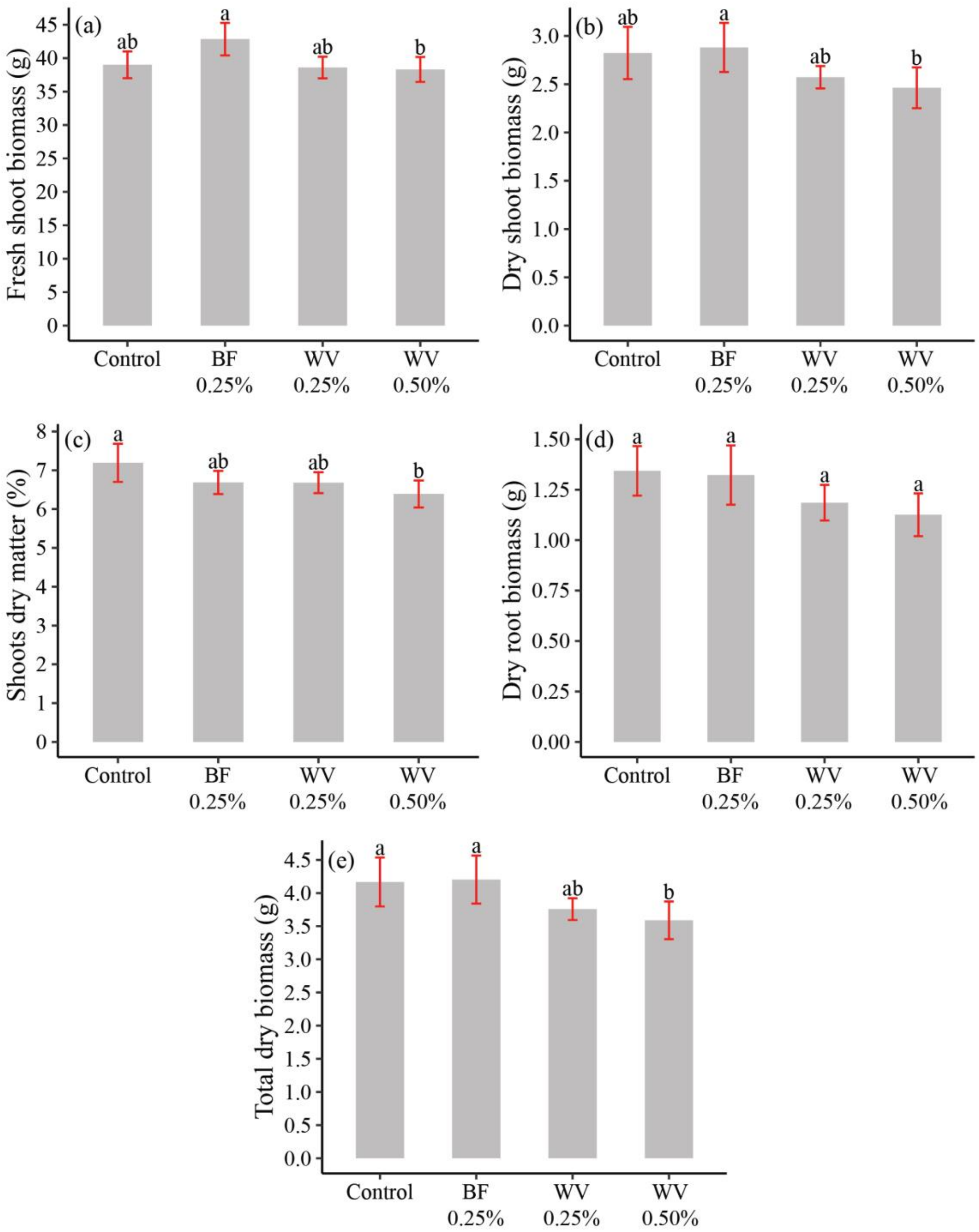


Figure 8. Fresh shoot biomass (a), dry shoot biomass (b) shoots dry matter (c), dry root biomass (d) and total dry biomass (e) of 40-day-old lettuce plants, treated weekly with distilled water (Control), 0.25 % BF (Bayfolan® Forte) and 0.25 % and 0.50 % WV (*G. arborea* wood vinegar).

3.3.4. Color parameters

At the harvest day (day 40), no differences differences of color coordinates among the treatments were found (Table 3). Instead, leaves of some plants treated with WV 0.50% presented dark green spots, indicating phytotoxicity (Figure 9)

Table 3. Color parameters values (mean ± error) of lettuce (*Lactuca sativa*) leaves on day 40, treated weekly with distilled water (Control), Bayfolan® Forte at 0.25% (0.25% BF) and 0.25% and 0.50% of *G. arborea* wood vinegar (WV).

Treatment	***L****	***a****	***b****
Control	49.56 ± 0.08 ns	-10.78 ± 0.03 ns	28.36 ± 0.06 ns
BF 0.25%	49.73 ± 0.07 ns	-10.72 ± 0.04 ns	28.35 ± 0.06 ns
WV 0.25%	49.13 ± 0.06 ns	-10.65 ± 0.04 ns	28.47 ± 0.06 ns
WV 0.50%	49.2 ± 0.06 ns	-10.74 ± 0.04 ns	28.51 ± 0.07 ns

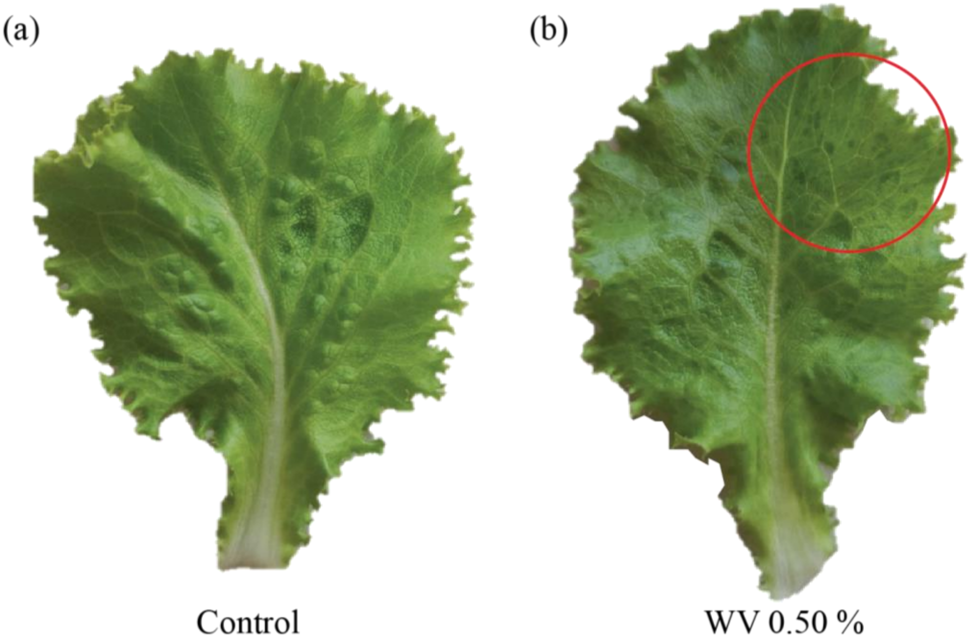


Figure 9. Color of lettuce leaves treated with control (a) and wood vinegar (WV) at 0.50% (b). The red circle indicates dark green spots caused by the treatment.

3.3.5. Chemical leaf analysis

The different dose of WV did not alter the different nutrient concentrations or content of potentially toxic elements on leaves (Table 4) as no significant differences were observed between treatments for any macro or microelements.

Table 4. Nutrient concentration or contents (mean ± error) resulting from the foliar chemical analysis of the harvested lettuce (*Lactuca sativa*) leaves, treated weekly with distilled water (Control), Bayfolan® Forte at 0.25% (0.25% BF) and 0.25% and 0.50% of *G. arborea* wood vinegar (WV).

Element	Control	BF 0.25%	WV 0.25%	WV 0.50%
N (% w/w)	1.60 ± 0.07 ns	1.82 ± 0.06 ns	1.69 ± 0.11 ns	1.92 ± 0.04 ns
P (% w/w)	0.20 ± 0.00 ns	0.22 ± 0.02 ns	0.21 ± 0.01 ns	0.21 ± 0.01 ns
Ca (% w/w)	1.23 ± 0.04 ns	1.31 ± 0.10 ns	1.12 ± 0.03 ns	1.29 ± 0.06 ns
Mg (% w/w)	0.29 ± 0.01 ns	0.30 ± 0.02 ns	0.26 ± 0.01 ns	0.28 ± 0.02 ns
K (% w/w)	4.96 ± 0.15 ns	5.11 ± 0.33 ns	4.62 ± 0.15 ns	5.1 ± 0.11 ns
S (% w/w)	0.14 ± 0 ns	0.16 ± 0.01 ns	0.14 ± 0.01 ns	0.16 ± 0.01 ns
Fe (mg kg^{-1})	129 ± 5.24 ns	135 ± 16.26 ns	121 ± 8.33 ns	155 ± 9.50 ns
Cu (mg kg^{-1})	5 ± 0.00 ns	6 ± 0.67 ns	5 ± 0.00 ns	6 ± 0.33 ns
Zn (mg kg^{-1})	32 ± 0.33 ns	35 ± 3.00 ns	33 ± 0.88 ns	44 ± 2.19 ns
Mn (mg kg^{-1})	37 ± 1.33 ns	40 ± 5.17 ns	35 ± 0.67 ns	37 ± 4.16 ns
B (mg kg^{-1})	24 ± 0.33 ns	28 ± 3.18 ns	23 ± 0.88 ns	26 ± 2.65 ns

Note: ns means no significant difference between treatments ($p \geq 0.05$).

3.4.Multivariate analysis

The first two principal components (PC) of the PCA explained almost 74% of the total variance (Figure 10). The PC 1 explained almost 56% of the variability and was more correlated with the biomass parameters, shoot dry matter and less percentage by leaf area (Table 5). On the other hand, the PC 2 explained about 18% of the variance and was mostly influenced by the plant height (Table 5). The significant (p-value < 0.05) and strongest correlations found among variables were total dry biomass-dry shoot biomass (R=0.96), leaf area-fresh shoot biomass (R=0.90) and fresh shoot biomass-dry shoot biomass (R=0.81; Table 5). Despite the similitudes found in the univariate analysis, the PCA showed a clustering pattern of the treatments: control and WV 0.25% are grouped together and related with BF 0.25% and WV 0.50%, however the last two are grouped apart from each other (Figure 10). The plants treated with BF 0.25% were more associated with the PC1 correlated variables (biomass parameters, leaf area and chlorophyll) and height, while with WV 0.50% plants only correlated to plant height (Figure 10).

Table 5. Eigenvectors (e) and correlations of the principal components (PC) with the parameters of the multivariate analysis for 40-day-old lettuce plants treated weekly with distilled water (Control), 0.25% BF (Bayfolan® Forte) and 0.25% and 0.50% WV (*G. arborea* wood vinegar).

Parameter	**e 1**	**e 2**	**PC 1**	**PC 2**
Head diameter	0.37	-0.06	0.77**	-0.08
Height	0.10	0.71	0.22	0.85**
Chlorophyll	0.12	-0.06	0.26*	-0.08
Leaf area	0.39	0.38	0.83**	0.46***
Fresh shoot biomass	0.42	0.25	0.89**	0.31*
Dry shoot biomass	0.46	-0.13	0.96**	-0.16
Total dry biomass	0.44	-0.19	0.94**	-0.23
Shoots dry matter	0.32	-0.48	0.67***	-0.58***

Note: * indicates statistical significance of Pearson correlation coefficient at 95% (p-value<0.05), ** at 99% (p-value<0.01) and *** at 99.9% (p-value<0.001).

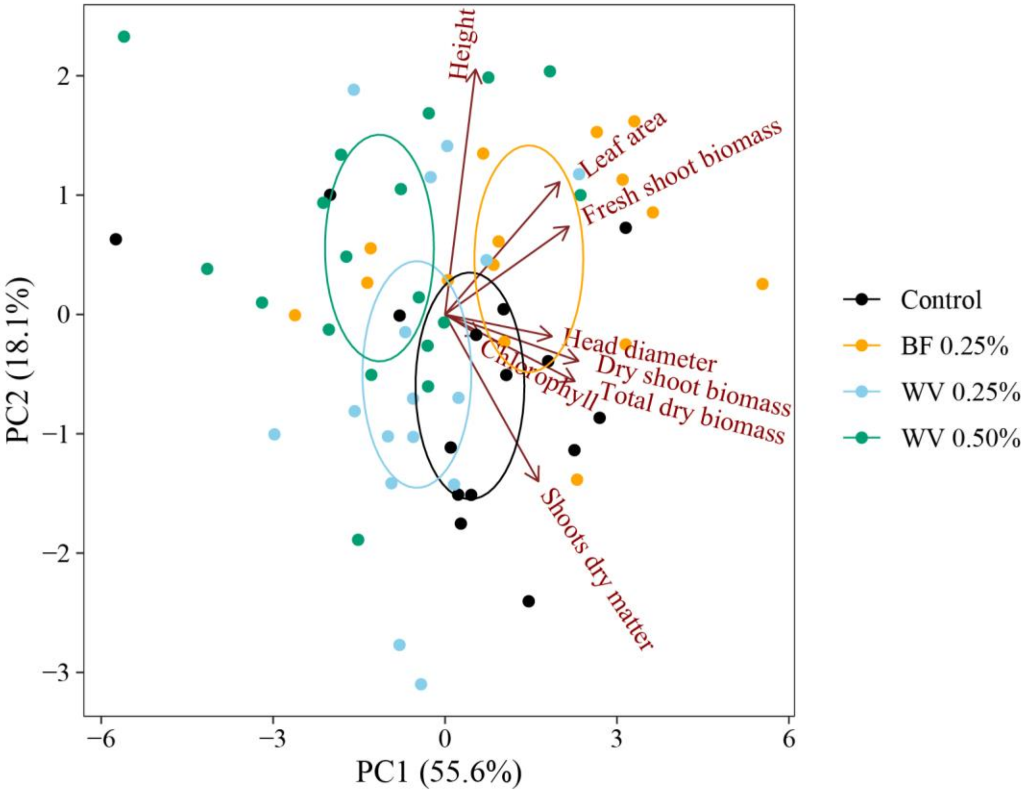


Figure 10. Principal components analysis (PCA) for the chlorophyll SPAD values, head diameter, height, leaf area, biomass (fresh shoots, dry shoots and total dry) and total dry matter of 40-day-old lettuce plants treated weekly with water (control), 0.25% BF (Bayfolan® Forte) and 0.25% and 0.50% WV (*G. arborea* wood vinegar).

Note: Euclidean ellipses represent 95% confidence levels.

4. Discussion

In this study, WV from *G. arborea* wood residues fulfill the quality parameters of specific gravity (between 1.010–1.050 g mL^{-1}), color (from pale yellow to light brown and reddish), odor (smoky), pH (around 3), and transparency with no suspended solids, established by the Japan Pyroligneous Liquor Association [43]. In addition, several studies have reported pH values below 3 [44-47]

Organic acids are often the main components of WV, with acetic acid as the major constituent [47]. However, WV composition of this study disagreed with other studies, where phenols accounted for 33% of the most abundant compounds (Table 1) and many studies have reported that WV reached high concentrations of phenolic compounds [16, 20, 47, 48]. Yang *et al*. [20] mentioned that phenols that usually are found in WV are derived from the breakdown of lignin and hemicellulose, and comprise a range of 30-60% of the total organic compounds, therefore it was evident that parentwood of *G. arborea* was depolymerized during pyrolysis process. Syringol (2,6-dimethoxyphenol) was other important dominant organic compound found in *G. arborea* WV (Table 1, Figure 3), that can have a proportion greater than 29% in WV [20], and is considered a powerful antioxidant [49, 50]. Then, WV of *G. arborea* presents important potential in uses with antioxidant properties.

The elemental analysis of WV from *G. arborea* wood residues showed high element contents of iron (Fe 1035 mg L^{-1}) and Zinc (Zn 1074 mg L^{-1}) compared to WV from orchards woody pruning residues (Fe 183 mg L^{-1}) [51] and sweet chestnut (*Castanea sativa* Mill.) (Fe 3.2 ± 0.05 mg L^{-1} and Zn of 3.6 ± 0.1 mg L^{-1}) [30]. Fe and Zn are considered potential toxic elements, then exhibited high content in WV of *G. arborea* can lead to phytotoxicity or to an uptake accumulation in the plant [52].

WV showed an improvement in chlorophyll content (Figure 7a), comparable with synthetic fertilizer (Figure 7). WV at 0.25% increased the chlorophyll values of lettuce plants as compared to the control (Figure 7a). This result agrees with the findings by Vanini *et al* [31] and Fedeli *et al* [32], when *Castanea sativa* wood distillated was applied on 'Canasta' and 'Adela' cultivars and chlorophyll content was increased. Although the mechanism of action of WV on plant photosynthesis is unknown, Vannini *et al.* [31] suggested that polyphenol exposure leads to an increase in chlorophyll, which may explain our results.

On the other hand, Liu *et al.* [53] mentioned that, an increment of chlorophyll is expected to lead to an increase of aboveground biomass, so growth parameters, fresh and dry biomass, due to improvements in the photosynthetic system.. However, WV 0.25% showed no differences in growth or biomass production compared with the control (Figure 8), this may be due to the low concentration of nitrogen (N) in WV (0.02%). Nitrogen is one of the most essential nutrients that plants need to grow and limited availability of N reduce the photosynthetic efficiency of lettuce [54], thereby energy production, plant growth and biomass production is affected [53]. Roots biomass showed no changes (Figure 8d) probably because WV applications were not done by soil irrigation. Yang *et al*. [55] observed and increase in cotton root vigor higher than 50% when WV was combined with biochar applications, due to an improvement of the soil environment through acid-based neutralization. Akely *et al.* [23], suggest that WV can stimulated the plant roots to discharge more exudates, enhancing the soil microenvironment and improving the uptake of resources and the production of shoot, root and dry matter. Then, the foliar application dose of 0.25% of WV was not adequate for improving lettuce growth or increasing the lettuce biomass.

The application of wood vinegar at 0.50% caused visual injuries on plant leaves (Figure 9) and led to poor efficiency on lettuce diameter, chlorophyll content and biomass (Figure 6-8), as observed in the PCA (Figure 10), where all the plant parameters (except height) have a negative association with the 0.50% samples. Fedeli *et al*. [32] reported that WV at 0.50% reduced the content of glucose, fructose and TSI by ca 30% on lettuce plants. Then doses of 0.50% of WV of *G. arborea* agreed with a detriment in plant parameters observed by Fedeli *et al*. [32]. Although WV doses were not pure but diluted, Fe and Zinc contents in *G. arborea* WV were excessively high compared to other studies, as we mentioned before, and compared to Bayfolan® Forte (Fe 500 mg L^{-1} and Zn 800 mg L^{-1}). Fe is essential to plants as it is involved in chlorophyll synthesis [56]; however, at high concentrations, as presented in this study, it can cause phytotoxicity, growth inhibition and decreased chlorophyll content [57]. Excessive Zinc significantly decreases crop biomass, growth rate and photosynthesis [58]. Moreover, phenolic compounds are among the most important and common plant-derived allelochemicals, providing environmentally friendly alternatives for pesticides and herbicides. In addition, WV rich in phenolic compounds, as found in WV of *G. arborea*, including syringol (12%) [17], can result in observed 50% control on weed seedlings when applied at concentrations of 0.42% [18], and numerous studies have found WV to be phytotoxic at higher concentrations [15, 16, 49]. These results may be aggravated due to the acidic conditions of WV increasing the phytotoxicity of acids and phenols [51]. Therefore, these conditions led to problems on lettuce plants treated with WV at 0.50%

Zhu *et al*., [26] observed that WV tend to increase the number of total leaves and green leaves of of canola by delaying the plant senescence; however, they did not delve into the mechanisms of action of WV. Element content of lettuce leaves was not altered by any treatment (Table 4), as observed by Fedeli *et al.* [32] with WV from sweet chestnut, suggesting its safety for consumption. However, the average values of the nutrients P (2.11 mg kg^{-1}) and K (49.47 mg kg^{-1}) were 3.3 and 1.8 times lower than the typical values [59]. On the other hand, the content of Fe (135 mg kg^{-1}), a potential toxic element on lettuce leaves [32], was found more than 2 times higher than values reported by Song *et al.* [59] (ca. 60 mg kg^{-1}), and even higher than Fe-biofortified lettuce plants (from 66.0 to 93.0 mg kg^{-1}) [60]. This situation can be attributed to the soil conditions with high iron content (124.7 mg L^{-1}) and the fact that lettuce can hyper-accumulate heavy metals due to specialized ion absorption and transport mechanisms [52].

5. Conclusion

The application of WV from *G. arborea* wood vinegar on lettuce increased the chlorophyll SPAD units, however, its use as foliar fertilizer is not recommended at the concentrations of 0.25% and 0.50%, due to its low performance on growth and biomass parameters. Moreover, negative effects can be obtained on lettuce plant growth, biomass and visual damage when WV 0.50% is applied. The predominant phenol composition and high concentrations of iron and zinc, and low nitrogen input of *G. arborea* WV may be responsible for its poor efficiency as a foliar fertilizer. Therefore, the foliar applications of WV at 0.25% and 0.50% are not recommended. The low efficacy on promoting growth, biomass production, and the visual damage observed provide evidence of allelopathic effects of WV from *G. arborea*, suggesting it has potential to be used as a natural alternative to synthetic herbicide. However, further and specific research is needed in this regard to elucidate the herbicidal effects of *G. arborea* WV.

CRediT authorship contribution statement

Jair Granados-Chacón: data curation, formal analysis, investigation, methodology, software, visualization, writing-original draft. **Roger Moya:** conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing-review and editing. **Jaime F. Quesada-Kimzey:** methodology, visualization.

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.

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Appendix A. Supplementary material

The online version does not contain supplementary material.

Data availability of data and materials

Data will be made available on request.

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CAPÍTULO III. Evaluation of wood vinegar from the pyrolysis of wood residues of *Gmelina arborea* as an herbicide for weed control

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Abstract

Wood vinegar (WV) produced via biomass pyrolysis, presents an organic alternative for weed control. This study aimed to: (i) determine the physical properties and chemical composition of WV, and (ii) evaluate the efficacy of WV from wood residues of *Gmelina arborea* produced with slow pyrolysis to control weeds under a *Cupressus lusitanica* Christmas tree plantation. WV were dissolved in water at 75% and applied in doses of 1000, 3000 and 5000 L ha^{-1}, with synthetic herbicide Basta® (glufosinate-ammonium) as a control. The efficacy of WV on weeds was visually evaluated from 0 (not injury) to 100 (plant death). WV primarily comprised phenolic compounds (32.9%), with syringol (2,6-dimethoxyphenol) as the major component (14.8%). Treatments with 3000 and 5000 L ha^{-1} doses caused rapid desiccation of grass and broadleaf weeds, achieving 85-88% visual injury.

Biomass reduction of 40% can be obtained when WV is applied at high doses (3000 and 5000 L ha^{-1}) on mature grass-dominated weeds, showing effects comparable to synthetic herbicide. While WV effectively reduced weed biomass, it was insufficient to completely remove matured weeds.

Keywords: pyroligneous acids, organic herbicide, christmas tree plantation, grass weeds, syringol, glufosinate-ammonium.

1. Introduction

Herbicides rank as the third most toxic group of pesticides, after insecticides and fungicides [1]. The most widely used herbicides in the world are the broad-spectrum and non-selective chemicals glyphosate, glufosinate-ammonium and paraquat [2]. Glyphosate inhibits enzymes essential for aromatic amino acid biosynthesis, ultimately causing plant death through starvation [3]. It is commonly applied to eliminate vegetation after harvest or before crop establishment [4]. Similarly, glufosinate is used for post-emergence and pre-plant burndown [5]. Its rapid phytotoxicity results from the accumulation of reactive oxygen species, which trigger lipid membrane peroxidation [6-7]. Paraquat exhibit very fast uptake producing reactive oxygen species that cause desiccation of plant tissues [8]. Although, paraquat has been banned in several countries due to health risks [9-11].

The use of pesticides has been increased in recent decades due to the increment of crops production; unfortunately, the risks associated with their use have outweighed their beneficial effects [1]. Latin America is not exception, this region represents the world main agricultural area with a very intensive use of pesticides [12]. This situation is aggravated by the fact that research and legislative efforts on pesticide use and management in the different countries are not as exhaustive as their temperate counterparts [14]. Extensive research has been conducted on the problems associated with synthetic herbicides, among these issues are: weed resistance, exposure of workers and health issues [14-16], residues on food [17] and animal feed [18], and the effects on the environment [13, 19], biodiversity [20, 21], water [22, 23] and soils [24, 25].

Costa Rica, a small country in Central America, despite being known worldwide for its environmental policies, is one of the largest consumers of pesticides [26]. Costa Rica presents an intensive use of the synthetic herbicides glyphosate and paraquat [27-30] which are the second and third most imported pesticide by volume, respectively [31]. Training of pesticide applicators on proper use practices and protection should be encouraged to reduce health and safety risks to workers and the environment [12]. However, training and awareness programs may not effectively translate into behavioral changes [32, 33].

On the other hand, there is a growing need for sustainable weed management that allows economic profitability, reduces environmental impact and meets social demands for food security [34]. Bioherbicides, products of natural origin for weed control [35], promise to be more environmentally friendly [36] and have the potential to cause rapid plant degradation [34]. For example, in Costa Rica, D-limonene, pine and rosemary extracts were found to be effective and even faster than a synthetic herbicide in post-emergence weed control [37]. Organic compounds used for weed control include flavonoids, terpenoids, alkaloids and quinones, and phenolic acids. [4].

Recently, wood vinegar (WV), produced by pyrolysis of biomass, has attracted interest for its use as a bioherbicide [38]. WV consists mostly of water (80-90%) and more than 200 organic compounds categorized as acids, alcohols, ketones, aldehydes, esters, furans and nitrogenates [38, 39]. The herbicidal properties of WV are attributed to the large amount of acids, especially acetic acid, which usually occupies the largest proportion, and phenols [2], [38, 40]. It has been shown that WV at high concentrations and application doses, presents effective herbicidal effects for the management of weeds [38, 40-42]. However only a few studies as an herbicide in natural conditions have been conducted [39]. In China, WV from elm (*Ulmus* spp.) and apple (*Malus* × *domestica* Borkh) tree branches pruning waste has been proven to control weed species under field conditions, similar to a non-selective herbicide, by causing fast desiccation on plants due to high content of acids [40, 42]. Similarly, in Spain, Aguirre *et al* [38] concluded that WV helps to control the development of annual plants by damaging the entire epidermis and its stomatal cells.

In Costa Rica, pyrolysis of biomass has the potential to provide a solution to wood waste management [39] while producing WV, as it is the major constituent of pyrolysis liquid [43]. The second most planted tree in Costa Rica is *Gmelina arborea* Roxb. ex Sm (melina) [44]. The wood processing of this tree has been reported to be inefficient and producing great amount of residues [45]. For this reason, the yields and process of slow pyrolysis of melina wood residues has been investigated. Recent research of *G. arborea* residues [46] showed that yields of charcoal were 26-28%, WV of 28-30% and non-condensable gases of 37%. However, these studies presented the importance and characteristics of solid products (charcoal) as energy and agriculture applications.

WV, produced by the slow pyrolysis of *G. arborea* wood residues, shows a high yield but no information has been presented about its potential as a natural alternative of weed control due to its herbicidal properties. So, further research is needed to elucidate its efficacy to control weeds. Then the objectives were established: (i) to determine the physical properties and chemical composition of WV, and (ii) to evaluate the efficacy of WV in three different doses from wood residues of *Gmelina arborea* produced with slow pyrolysis to control weeds under a *Cupressus lusitanica* Christmas tree plantation, evaluating visual injury and biomass reduction of weeds.

2. Materials and methods

2.1. Wood vinegar preparation

Wood vinegar (WV) was produced from the slow pyrolysis of wood residues of *Gmelina arborea* in a semi-industrial reactor prototype. Pyrolytic gases and pyrolytic acids collected from the pyrolysis reactor were condensed in two helical coil heat exchangers and WV was produced. This reactor presented a charcoal yield of 26 to 31% and a WV yield from 27 to 32%.

2.2. Wood vinegar physical properties and chemical composition

The WV physical properties of pH, electric conductivity (EC) and density were determined by the Centro de Investigaciones Agronómicas (CIA) of the Universidad de Costa Rica (UCR). The concentration of elements (N, Cu, Fe, Zn, Mn, B, P, Ca, Mg, K and S) in pure WV was determined with an analysis of organic fertilizers. Nitrogen (N) was determined by MicroKjeldahl wet digestion with H_2SO_4 and colorimetric determination in the Flow Injection Analyzer (FIA) and P, Ca, Mg, K, S, Fe, Cu, Zn, Mn, B by digestion with HNO_3

and determination by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

Chemical composition of WV was determined by gas chromatography–mass spectrometry (GC-MS) in the Centro de Investigación y de Servicios Químicos y Microbiológicos (CEQIATEC) of the Instituto Tecnológico de Costa Rica (TEC). The WV samples were diluted by 8 in GC-grade toluene and analyzed on a Thermo Scientific Trace 1310 gas chromatograph with a TSQ 8000 Evo MS detector and a 30 m long TG5-SILMS Thermo Scientific capillary column. Injection volume was 1 µL by means of a TriPlus RSH autosampler for liquids. The injection was splitless, at 250°C, with a vacuum compensated for carrier flow of 1 mL min^{-1}. The initial temperature of the oven was held for 5 minutes at 90°C, then it was raised to 25°C min^{-1} until reaching 180°C, then at 5°C min^{-1} until reaching 255°C, which was held for 5 minutes.

2.3. Site and soil conditions

This experiment was conducted during the first months of the wet season (from May to June of 2024) in a *Cupressus lusitanica* christmas tree plantation (9°50'29.2" N 83°47'05.2" W, (Figure 1a) of approximately 1500 m^2 and 18 months old located in Paraíso, Cartago province, at 1130 masl. Grass was the dominant weed group.

Soil was characterized as clay loam with 32.33 % clay, 36.33 % sand and 31.33% silt, and 4.23 % organic matter. Soil chemistry was characterized by 5.20 pH, acidity 1.12 cmol (+) L^{-1}, EC 0.20 mS cm^{-1}, Ca 10.95 cmol (+) L^{-1}, Mg 2.05 cmol (+) L^{-1}, K 0.53 cmol (+) L^{-1}, CEC 14.66 cmol (+) L^{-1}, acid saturation 7.77 %, P 44.67 mg L^{-1}, Zn 3.87 mg L^{-1}, Cu 22.67 mg L^{-1}, Fe 389.67 mg L^{-1}, Mn 26.00 mg L^{-1}, C 2.96 %, N 0.34 % and a C:N ratio of 8.83.

Figure 1. *Cupressus lusitanica* christmas tree plantation before treatment (a) and plot treated with wood vinegar (3000 L ha^{-1}, three days after treatment) (b)

2.4.Treatment and experimental test

Three different doses were applied using diluted wood vinegar at 75%: treatment applying doses of 1000 L ha^{-1} (WV 1000 L ha^{-1}), 3000 L ha^{-1} (WV 3000 L ha^{-1}), and 5000 L ha^{-1} (WV 5000 L ha^{-1}). After other two treatments for comparisons: one applying a commercial herbicide, specifically Basta® 14 SL of BASF (Bayer AG, Frankfurt, Germany) at 0.75% (0.375 kg ha^{-1}) at the rate of 250 L ha^{-1} and a treatment without applications of WV or commercial herbicide (control). This commercial herbicide was selected due to its popularity, and it is considered moderately hazardous in Costa Rica. The applications of WV and commercial herbicide were using a manual sprayer equipped with a flat-fan nozzle on a sunny day during the first morning hours. Personal protective equipment was used. Trees were covered with plastic sheeting to protect them from drift related damage.

Before application, weed dominant species in the tree plantation were identified and classified as grass, broadleaf and cyperaceous. Grass weed was the most dominant group. A completely randomized design was utilized for the experimental test with nine replications (0.5 × 0.5 m plots) established where grass covered > 90 % of the area (Figure 1b). Grass coverage area (%) was determined by dividing the plot into 100 subplots (5 x 5 cm) and counting the subplots with grass as dominant weed group.

2.5.Evaluation of treatment

Weed control was visually evaluated at 1, 3, 7, 14 and 21 days after doses application treatment (DAT) in each plot; on a percentage scale, where 0 represents no injury and 100 plant death using the method proposed by Liu *et al.* [40]. At 27 DAT, aboveground biomass was evaluated. A random sample of 162.15 cm^2 of aboveground biomass was harvested and fresh biomass immediately weighed, then oven-dried at 105 °C for 24 hours and again weighed

2.6.Statistical analysis

Homogeneity of equal variance was tested using Levene's test and normality was tested using Shapiro-Wilk's test on the residuals of the one-way analysis of variance (ANOVA) model. To determine the effects of the application rates on the visual injury (%) and biomass reduction (kg ha^{-1}), one-way analysis of variance (ANOVA) with Tukey’s Honestly Significant Difference test was performed on parametric data, and Kruskal-Wallis’s test and Wilcoxon signed-rank test were performed for non-parametric data. All the analyses were conducted using the statistical computing software R v.4.3.2 in the integrated development environment RStudio v.2023.16.0-421 [47].

3. Results

3.1.Wood vinegar physical properties and chemical composition

WV was characterized with visually transparency and yellowish-brown color, a vinegar and smoky odor and the physical parameter presented the following values: pH 2.9, density 1.02 g mL^{-1} and EC 2.3 mS cm^{-1}. The elemental analysis showed the following values: N 0.02 % (w/w), Cu 7 mg kg^{-1}, Fe 1015 mg kg^{-1}, Zn 1053 mg kg^{-1}, Mn 3 mg kg^{-1}, B 4 mg kg^{-1}; while P, Ca, Mg, K and S were not detected by the analysis.

Table 1. Most abundant components (>1 % peak area) identified in the GC-MS chromatogram of the *Gmelina arborea* wood vinegar.

Chemical class	**RT (min)**	**Name (and % probability)**	**Molecular Formula**	**Molecular Weight**	**% Area**
Ketone	6.14	4.4-dimethyl-2-cyclohexen-1-one (36%)	$C_8H_{12}O$	124.18	2.4
Nitrogenous	7.62	5-hydroxy-2-methylpyridine (6%)	C_6H_7NO	109.13	12.3
Nitrogenous	8.22	Methyl 6-methyl-1-oxido-4-pyrimidinyl ether (26%)	$C_6H_8N_2O_2$	140.14	8.4
Ketone	8.33	Piperitone (51%)	$C_{10}H_{16}O$	152.23	5.9
Phenol	8.47	4-methylcatechol (42%)	$C_7H_8O_2$	124.14	4.9
Phenol	8.92	2,6-dimethoxyphenol (Syringol) (36%)	$C_8H_{10}O_3$	154.16	14.8
Phenol	9.19	4-ethyl-resorcinol (25%)	$C_8H_{10}O_2$	138.16	5.3
Amide	9.34	Diazobicyclo(4.4.0)dec-5-en-2-one (29%)	$C_8H_{12}N_2O$	152.19	1.7
Phenol	9.66	4-methoxy-3-(methoxymethyl) phenol (24%)	$C_9H_{12}O_3$	168.19	4.7
Ester	9.92	3,7-dimethyl-6-nonen-1-ol acetate (55%)	$C_{13}H_{24}O_2$	212.33	1.2
Alcohol	10.03	5-isopropyl-6-methyl-5-hepten-3-yn-2-ol (14%)	$C_{11}H_{18}O$	166.26	1.2
Nitrogenous	10.29	2,4,5-Trimethoxyamphetamine (19.5%)	$C_{12}H_{19}NO_3$	225.28	3.3

Chemical class	RT (min)	Name (and % probability)	Molecular Formula	Molecular Weight	% Area
Ketone	10.37	Guaiacylacetone (44%)	$C_{10}H_{12}O_3$	180.20	3
Aldehyde	11.58	Syringaldehyde (20%)	$C_9H_{10}O_4$	182.17	1.4
Phenol	12.36	Acetosyringone (47%)	$C_{10}H_{12}O_4$	196.2	2.1
Ketone	12.73	Desaspidinol (32%)	$C_{11}H_{14}O_4$	210.23	2.5
Phenol	14.3	5-(3-hydroxypropyl)-2,3-dimethoxyphenol (89%)	$C_{11}H_{16}O_4$	212.24	1.1

The GC-MS analysis of the WV identified 17 components as the most abundant (>1% peak area) organic compounds, representing more than 76.2 % of the total composition of WV (Table 1). Among the 17 organic compounds, the three major components (Figure 2) were 2,6-dimethoxyphenol (syringol, 14.8 %), 5-hydroxy-2-methylpyridine (12.3 %) and methyl 6-methyl-1-oxido-4-pyrimidinyl ether (8.4 %). Phenols was the most abundant functional group of compounds with almost 33% of the peak area, followed by the nitrogenous group (24 %) and ketones (13.8 %). It was not possible to identify any compound of the acid chemical class with a >1% peak area (Table 1).

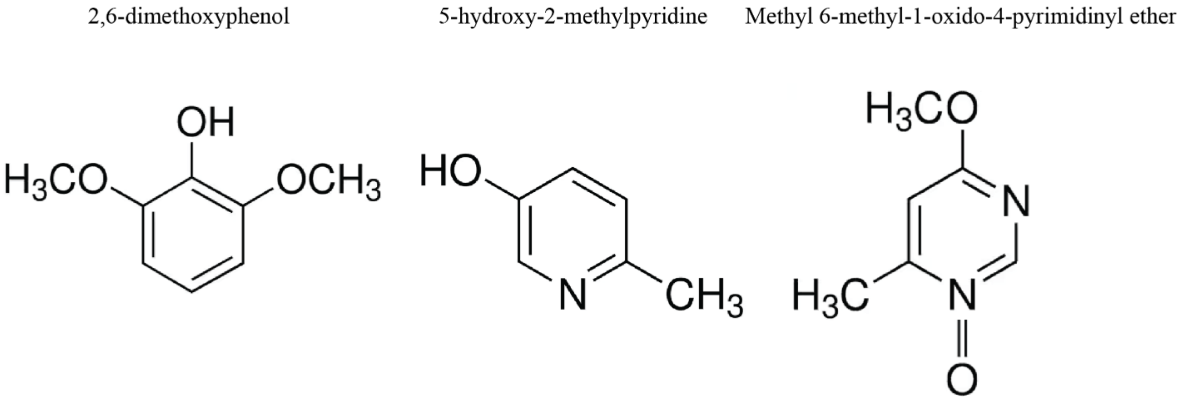

Figure 2. Three major components structures of the wood vinegar analyzed by GC-MS

3.2.Visual injury

The main grass species were *Paspalum conjugatum* P.J. Bergius, *Paspalum* sp. L., *Digitaria* sp. Haller and in lower proportion *Cynodon nlemfuensis* Vanderyst; broadleaf weeds were identified as *Drymaria cordata* (L.) Willd. ex Schult., *Oxalis debilis* Kunth and *Ipomoea* sp. L.; and for the Cyperaceae family only *Cyperus* sp. L. was identified.

Grass and broadleaf weeds presented the highest visual injuries on day 1 and day 3 (**Figure 3**a, b). WV 5000 and 3000 L ha^{-1} showed the highest grass control on the first 3 DAT, with 89 % and 85 % visual injury, respectively (**Figure 3**a). On the other hand, the synthetic herbicide Basta® at 250 L ha^{-1} acted slowly and the effects were observable at 3 DAT and thereafter (**Figure 4**).

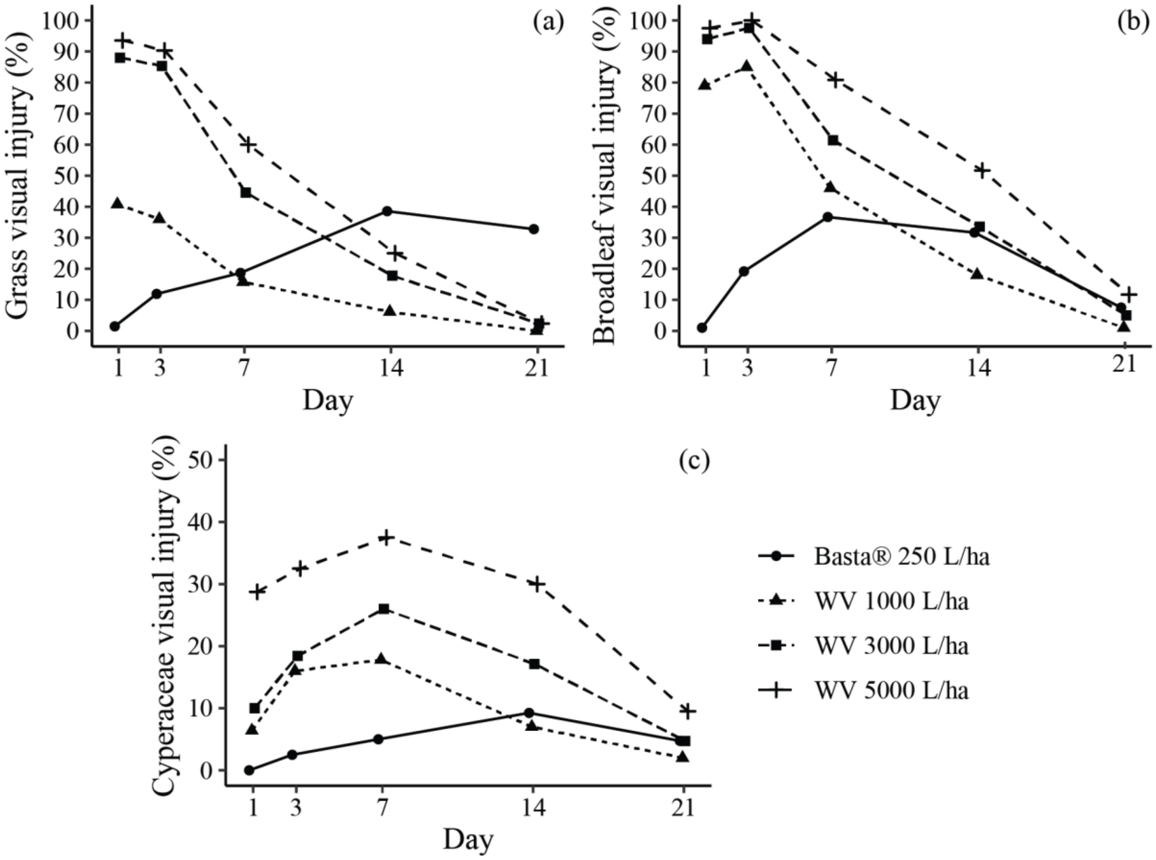

Figure 3. Visual injury of the weed groups: grass (a), broadleaf (b) and cyperaceae (c); treated with synthetic herbicide Basta® and three wood vinegar (WV) applications volumes over 21 days, in a *Cupressus lusitanica* christmas tree plantation.

The application of Basta® 250 L ha^{-1} showed an incrementing trend on plant injury until 14 DAT for grass and cyperaceous weeds (**Figure 3**a,c) and kept stable until 21 DAT, for broadleaf weeds a considerable decrease was observed between 14 and 21 DAT (**Figure 3**b). Cyperaceous weeds were the least visually injured plants, with a maximum visual injury of 50 % (**Figure 3**c). Overall, plant desiccation followed a decreasing trend for wood vinegar treatments as the days passed, until almost reach fully foliage recovery on day 21 (**Figure 4**).

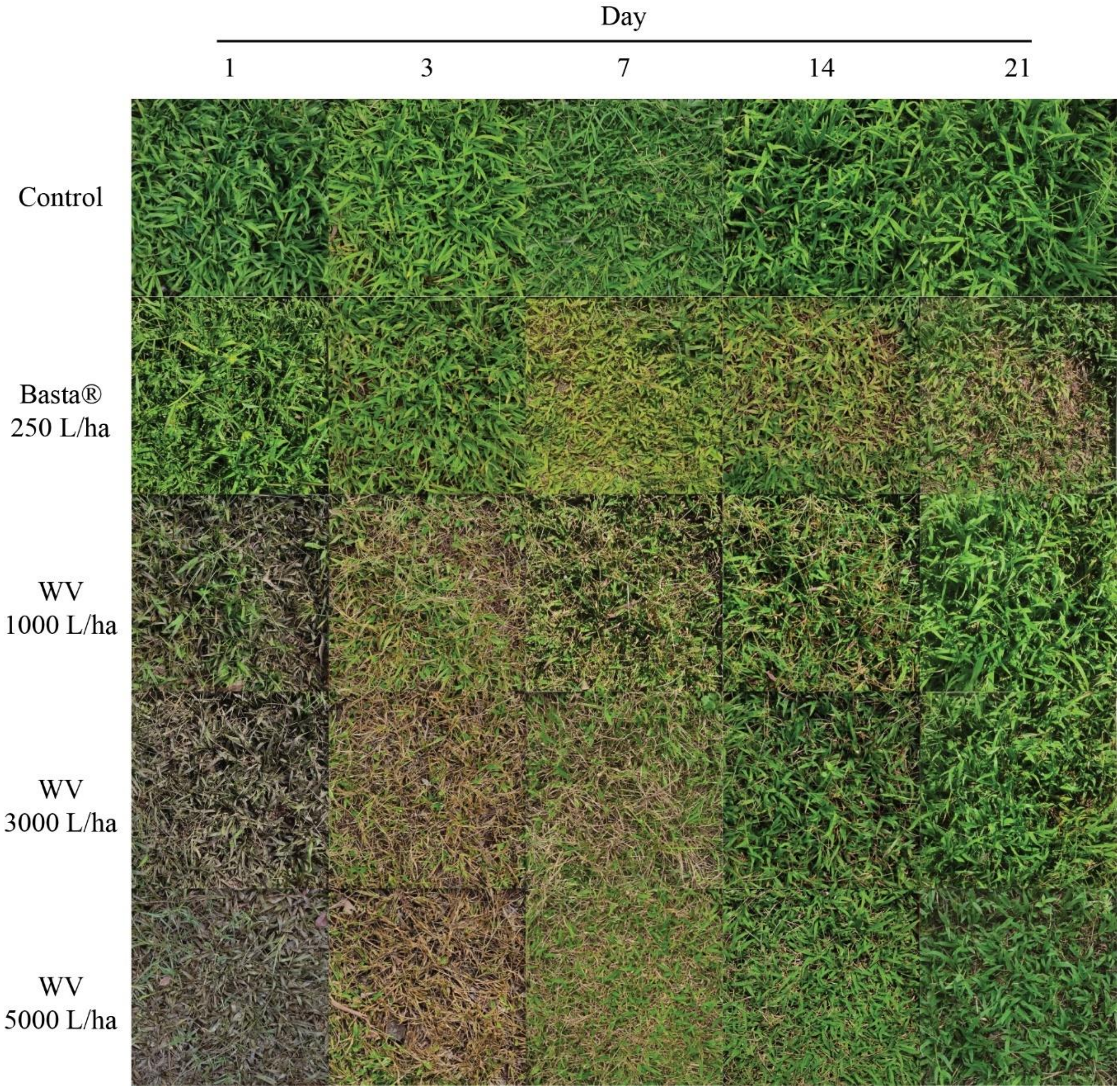

Figure 4. Injury of weed caused by synthetic herbicide Basta® and three wood vinegar (WV) applications volumes over 21 days, in a *Cupressus lusitanica* christmas tree plantation.

3.3.Biomass

WV 3000 and 5000 L ha^{-1} and Basta® 250 L ha^{-1} showed no significant differences in aboveground biomass and were significantly lower than the control and WV 1000 L ha^{-1} (**Figure 5**). Basta® treatment reduced the fresh and dry aboveground biomass by 51.2 and 33 % respectively in relation to control treatment. Similarly, WV 3000 L ha^{-1} by 40.4 and 31.6% and WV 5000 L ha^{-1} by 37.4 and 33.4%, respectively. No biomass differences were found between the control treatment and WV 1000 L ha^{-1} (**Figure 5**).

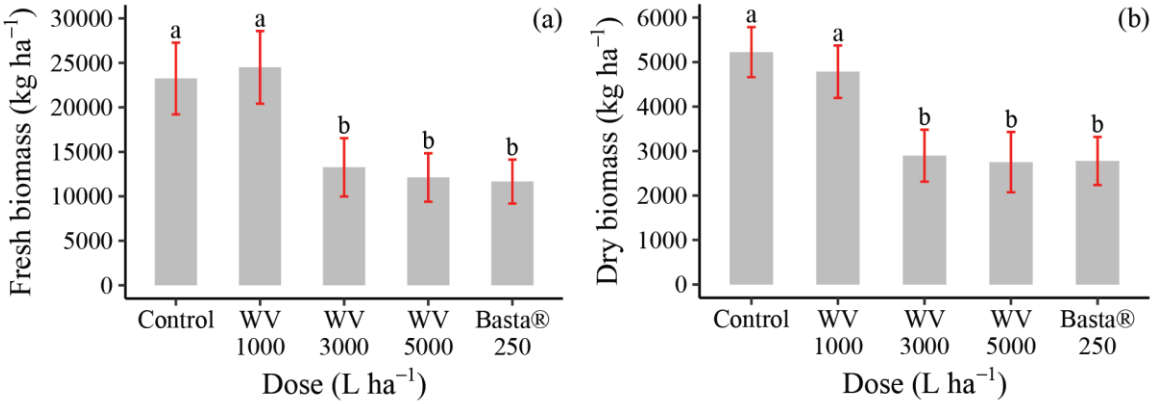

Figure 5. Fresh (a) and dry (b) biomass of weeds 27 days after treatment with synthetic herbicide Basta® and three wood vinegar (WV) applications volumes in a *Cupressus lusitanica* christmas tree plantation.

4. Discussion

According to Wada [48], the Japan Pyroligneous Liquor Association defined seven parameters to assess the quality of WV, which should have a pH around 3, a specific gravity in the range of 1.010–1.050 g mL^{-1}, a color ranging from pale yellow to light brown and reddish, a distinct smoky odor, transparency with no suspended solids, ignition residue less than 0.2% and dissolved tar content of less than 3%. Therefore, the five determined variables in this study meet these criteria. In addition, different authors have reported pH values below 3 for WV from other species [38, 39, 49, 50].

A large proportion of acid organic compounds is usually reported in WV, while acetic acids are generally considered its main active ingredient [38, 41, 42]. However, the results of this study disagreed with these reports, in the WV from *G. arborea* residues no abundant acids or acetic acid were identified through the GC-MS analysis (**Table 1**). Instead, phenols were the most abundant functional group, representing 33% of the identified organic compounds, and the major component 2,6-dimethoxyphenol (syringol) stood out with 14.8% (**Table 1**). Through the comparison with other studies, it was found that WV derived from *Populus* or *Ulmus* presented a composition of phenols of 23.2% [40] and 39.33% [50], percentages lower and higher, respectively, than WV from *G. arborea* wood. According to Yang *et al*. [51], phenolic compounds can reach 30-60% of the total organic compounds. Syringol is a powerful antioxidant [52, 53], which can comprise up to 30% of WV organic components [2, 40, 42, 50, 51], values higher than found in WV of *G. arborea*.

High phenolic composition in WV, as found in WV of *G. arborea*, has been associated with antioxidant and antibacterial activity [51, 54]. Plant derived phenolic compounds have shown acute phytotoxicity in seed germination and seedlings growth [55-57]. WV tested in this study showed control on different weed groups (grass, broadleaf and cyperaceae, Figure 4). Some effects associated to WV in weed control are: (i) phenols components in WV can reduce amylase activity in weeds, slowing the starch hydrolysis; therefore, delaying seed germination [58]. (ii) the efficacy of wood vinegar increases with the increasing of phenols and acetic acids [2]. (iii) acidic conditions of WV (pH = 2.9) increase the phytotoxicity of organic acids and phenols [59]. (iv) then these three effects can be helped to produce injury in the different weed groups in this study.

Other researches [2, 38] established the importance of acetic acids and phenolic compounds mixed in WV on weed control, which can indicate its efficacy for weed control in *Cupressus lusitanica* christmas tree plantation. Acetic acid is known to be an organic but costly alternative for herbicides, however, acetic acids and phenolic compounds in WV have shown a greater mixed effect compared to the effects of acetic acids alone [2], suggesting a synergistic effect with other components of WV like phenols. According to Aguirre *et al*. [38], phenols can increase the phytotoxicity of WV by enabling acids to stick to the plant, explaining the synergistic effect of these compounds. However, the interactions and action mechanism of acids and phenols on plants are not fully understood and still need to be further investigated.

WV has been proven effective as a natural derived herbicide [38, 40, 41]. The results of this study confirmed the effectiveness of WV from *Gmelina arborea* residues for weed control, especially grass and broadleaf species (**Figure 4**a-b). WV presented an immediate effect on grass weeds (**Figure 3**), providing more than 88% of visual injury 1 DAT and 85% 3 DAT with the higher doses (3000 and 5000 L ha^{-1}), but low efficiency over time. Other studies confirmed this effect, reporting this pattern of great initial weed control but decreasing efficiency over time [38, 40]. Similarly, Domenghini [60] reported that horticulture grade vinegar (20-30% acetic acid, an important component of WV), show a fast initial control of weeds but insufficient for a prolonged period of time, results similar to the ones found for the WV of *G. arborea* wood. Another effect that affected the decreasing efficiency of WV control on weeds over time was the presence of rain. WV was applied in the rainy season in tree plantation. According to Aguirre *et al.* [39], the effectiveness of WV can be reduced by the dilution effect caused by rains, therefore it is recommended to apply wood vinegar during dry season, given the speed of action.

On the other hand, an inconvenient was found in WV uses in weed control; the weed *Cyperus* sp. was not effectively controlled with WV, showing a control of 30% (WV 5000 L ha^{-1}) three weeks after treatment (**Figure 3**c). Maliang *et al* [61] found that the herbicidal effects of pyroligneous acids (including wood-derived vinegar) on *Cyperus rotundus* increased with higher acid and tar content, reaching up to 80 % of fresh weight control effect. The low content of acids identified in *G. arborea* wood vinegar may explain the low control of the cyperaceous weeds.

However, the results of low herbicidal effects cannot be attributed solely to WV, since some *Cyperus* genera are known for being difficult to control and only a few herbicides are recommended for its effective control, often selective and systemic herbicides [62]. Some *Cyperus* species have evolved resistance to selective herbicides as acetolactate synthase (ALS) or photosynthesis II (PSII)-inhibiting herbicides [63, 64], therefore, there is a need for alternative solutions. WV, despite not being able to provide effective control with a unique application, could be able to control cyperaceous weeds with repeating applications, as it recommended for other non-selective and contact herbicides [62, 65].

As it resulted for visual injury, weeds showed greater biomass reduction at higher application doses of WV (3000 and 5000 L ha^{-1}) at 27 DAT, reaching up to 40 % mass reduction, with similar effect of the synthetic herbicide Basta® at a conventional dose (**Figure 5**). Which coincides with other studies that have reported greater weed control and biomass reduction with the increase of WV concentrations and application rates [2, 41, 42, 61]. However, no significant difference was found between the herbicidal effects of WV 3000 and 5000 L ha^{-1}. Hence, the recommended application rate of *G. arborea* WV to inhibit weed growth is 3000 L ha^{-1}, since it can provide the same effect as the higher dose tested, but with lower investment of resources, time and effort. As we observed in our experiment, the efficacy of WV depends on the objective as WV is effective to limit biomass development, thus competition for resources with crops, but it is inadequate to completely remove the weed cover [38].

To improve the efficacy, wood vinegar as a contact herbicide should have more effect on seedlings and annual weeds compared to perennials and more mature plants [63]. Additionally, multiple applications in the same area could be performed; nevertheless, this approach may be costly [35] and further investigation is needed in this regard. Also, further investigation is needed to evaluate the environmental effects of the application of WV as a natural alternative to synthetic herbicides.

5. Conclusion

Wood vinegar produced from *Gmelina arborea* residues presented adequate quality physical properties according to the Japan Pyroligneous Liquor Association. WV is composed of many chemical compounds, mostly phenols. High application doses of wood vinegar produced a rapid control of weeds, with a desiccation effect observed in a few hours after application. Biomass reduction of 40% can be obtained when WV is applied at high doses on mature grass-dominated weeds, thereby reducing competition for resources with the main crop. However, WV is not sufficient to eliminate weeds. Therefore, *G. arborea* WV is a potential organic alternative for weed control, but further investigation on the frequency and time of application to improve its effectiveness and the mixing with other non-selective and contact herbicides.

CRediT authorship contribution statement

Jair Granados-Chacón: data curation, formal analysis, investigation, methodology, software, visualization, writing-original draft. **Roger Moya:** conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing-review and editing. **Jaime F. Quesada-Kimzey:** methodology, formal analysis, visualization.

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.

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Appendix A. Supplementary material

The online version does not contain supplementary material.

Data availability of data and materials

Data will be made available on request.

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CONCLUSIONES GENERALES

La transformación de los residuos de madera de *Gmelina arborea* a los distintos productos del proceso de pirólisis, ofrece nuevas oportunidades a la agricultura y el sector forestal de Costa Rica. Este estudio sugiere que la pirólisis lenta de los residuos *G. arborea* en forma de astillas o madera sólida en un prototipo de reactor semiindustrial, resulta en rendimientos similares de carbón vegetal, gases no condensables, y VM. No obstante, los residuos de madera sólida resultan en una mayor producción de alquitrán o bio-aceite y el tiempo de pirólisis es menor, lo cual significa mayor eficiencia energética; aunque los resultados del TGA indican una pirólisis incompleta. Por otro lado, las astillas de madera producen un biocarbón con mejores propiedades energéticas, en términos de poder calórico y contenido de carbono.

El VM de residuos forestales de *G. arborea*, presentó propiedades físicas adecuadas de peso específico, color, olor y transparencia que satisfacen los parámetros de calidad de la Asociación Japonesa de Licores Piroleñosos. El pH (2.9) no cumplió el criterio de 3.0; no obstante, múltiples autores indican valores típicos menores a este umbral. El VM de *G. arborea* se encontró constituido en su mayoría por fenoles (32,9%), principalmente por el fenol antioxidante 2,6-dimetoxifenol (siringol, 14,8%).

La utilización del vinagre de residuos de madera de *G. arborea* como fertilizante foliar para promover el crecimiento y producción de biomasa en plantas de lechuga (*Lactuca sativa* L.), no presentó resultados satisfactorios. Por el contrario, aplicaciones de VM al 0.50% causaron un detrimento en el diámetro y producción de biomasa y se observó un daño visual en las hojas de algunas plantas, sugiriendo efectos alelopáticos. Por lo tanto, no se recomienda su aplicación en las dosis de 0.25% 0.50% o mayores.

La aplicación de VM diluido al 75% y dosis altas de 3000 y 5000 L ha^{-1} producen un rápido control de arvenses, causando efectos de desecación en las plantas pocas horas después de la aplicación. Estas dosis pueden producir una reducción de la biomasa de hasta un 40% en arvenses dominados por gramíneas, que compiten por recursos con los cultivos. Por lo tanto, el VM es efectivo para reducir la biomasa de arvenses, pero insuficiente para eliminarlas por completo. Se requiere más investigación en la frecuencia y el tiempo de aplicación para mejorar la efectividad del VM de residuos de *G. arborea* para el control de arvenses.

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