Wood vinegar inhibits emergence and initial growth of leucaena (*Leucaena leucocephala* /Lam./ de Wit) seedlings

Stephanni Ingrid DE SOUZA SILVA[1]
Alexandre SANTOS PIMENTA[2]
Neyton DE OLIVEIRA MIRANDA[3] (✉)
Yanka Beatriz COSTA LOURENÇO[2]
Elias COSTA DE SOUZA[4]

Summary

The need to investigate species used in forest exploitation and the disposal of their residues and byproducts motivated the evaluation in a greenhouse of the effect of wood vinegar (WV) on seed germination and seedling growth of leucaena (*Leucaena leucocephala* (Lam.) de Wit) in two substrates. The studied factors were WV concentrations in irrigation water (0.0, 0.4, 0.8, 1.2 and 1.6%) and substrate (sand or vermiculite). Each treatment had four repetitions of 30 seeds in plastic trays. The variables evaluated up to 27 days after sowing were: emergence percentage, emergence speed index, percentage of ungerminated seeds, total seedling length, shoot height, shoot and root dry weight. The concentrations of wood vinegar used in this study had an inhibitory effect on the germination, emergence and initial development of leucaena at all studied concentrations. A large decrease in percentage and speed of germination of leucaena was observed, mainly in the sand substrate. The more intense effect on the germination stage than in later stages of development was caused by substances contained in WV, such as acetic acid, furfural and methanol. The germination, emergence and initial growth of leucaena were less impaired when the substrate was vermiculite. For effective control of leucaena it is necessary to test higher concentrations of wood vinegar.

Key words

forestry, substrate, slow pyrolysis byproducts, biocide

[1] Forest Sciences Graduate Program, Federal University of Rio Grande do Norte—UFRN, Natal, RN, Brazil

[2] Agricultural Sciences Academic Unit, Federal University of Rio Grande do Norte—UFRN, Natal, RN, Brazil

[3] Department of Agronomic and Forest Sciences, Federal Rural University of Semiarid Region - UFERSA, Mossoró, RN, Brazil

[4,5] Forest Resources Graduate Program, University of São Paulo -USP, Piracicaba, SP, Brazil

✉ Corresponding author: neyton.miranda@ufersa.edu.br

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Introduction

Forestry and the processing of its products generate large amounts of waste and byproducts with economic potential. In Brazil, this activity has great economic and environmental importance, as it generates products such as lumber, firewood, cellulose and charcoal, protects vulnerable areas and recovers degraded areas. Among the 7.8 million hectares of planted forests, 5.6 million is planted with eucalyptus, of which 1.1 million is to produce charcoal, which is important in steelmaking metallurgy (Pimenta et al., 2018).

Among the aspects of forest activity that raise concern are the economic use and environmental impact of cultivated species like leucaena and the destination of residues and byproducts like wood vinegar, which is generated in large volumes in charcoal production.

Leucaena is an exotic perennial leguminous tree, introduced voluntarily in Brazil. The expansion of its cultivation is due to its multiple uses. As a green manure, this species can associate with nitrogen-fixing and phosphorus-absorbing soil microorganisms. It is useful for erosion control, soil enrichment and improvement, remediation of degraded areas, and restoration of vegetation cover. It is used for forage and intercropped with other plants in agrosilvopastoral systems, favouring the establishment of forage grasses and native tree species (Drumond and Ribanski, 2010; Oliveira et al., 2010; Santos et al., 2011; Couto, 2012).

As forage, leucaena is very palatable and has good nutritional value, containing from 20 to 35% crude protein, and is supplied fresh or as hay to several livestock species. But it contains tannins, which reduce dry matter digestibility, and mimosine, which can cause intoxication in non-ruminant animals (Drumond and Ribanski, 2010; Couto, 2012).

As a planted forest species, leucaena is an alternative to eucalyptus to produce low-cost biomass in regions such as the Brazilian semiarid zone, with restrictive thermal and hydric conditions. In this region, leucaena stands out in relation to native species for its wood density, calorific value and carbonization gravimetric yield. Its wood is also used for pulp and particleboard production (Drumond and Ribanski, 2010; Couto, 2012).

However, leucaena is considered one of the hundred most aggressive invasive species by the International Union for Nature Conservation (Lowe et al., 2000). As such, it is on the Global Invasive Species Program list (Matthews and Brand, 2005), and it is classified as invasive in many countries, including European ones (Dana et al., 2003).

In northeastern Brazil, leucaena presents a maximum risk of invasion (Leão et al., 2011), and in the state of Paraná, it is on the official list of Invasive Alien Species (Cunha et al., 2013). Its expansion threatens biodiversity in the Fernando de Noronha Archipelago (Mello, 2013), it is encroaching on a native riparian forest in Maringá, Paraná (Cunha et al., 2011), and it is considered an invasive weed on the banks of the Itaipu Dam reservoir (Scherer et al., 2005).

In turn, wood vinegar (WV), the aqueous fraction obtained from slow pyrolysis of lignocellulosic materials such as wood (Pimenta et al., 2018), is not exploited in traditional charcoal production and is lost to the atmosphere in the fraction of condensable gases, or it is discharged, with risks to environmental and human health (Mmojieje, 2016; Grewal et al., 2018). If collected, it composes 30 to 40% of the initial dry mass of wood after decantation (Silva et al., 2017) and 27% after distillation (Pimenta et al., 2018).

Pyrolysis technologies have generated a production chain to produce biochar and its byproduct, wood vinegar, which is considered a “green chemical substance” and organic product in agriculture. Its potential as a sustainable substitute for environmentally harmful synthetic agrochemicals is due to it being a natural product, nontoxic to most cultivated plants, beneficial to soil microbiota and advantageous as a biocide, herbicide and/or weed growth inhibitor. Other uses of WV are as food preservatives and flavorings, traditional medicines, anti-parasitic and feed additives for animals, wood preservatives, soil conditioners, plant growth stimulants and/or regulators, and seed germination promoters (Tiilikkala et al., 2010; Silva et al., 2017; Grewal et al., 2018; Pimenta et al., 2018).

Given the above, this study aims to evaluate wood vinegar as a promoter or inhibitor of seed germination, emergence and initial seedling development of leucaena in two substrates.

Material and Methods

The experiment was conducted in 2018 in the Wood Technology Laboratory and a greenhouse, with raw material obtained from the Forest Experimentation Area, all located in the Agricultural Sciences Unit of the Federal University of Rio Grande do Norte, in Macaiba, RN, Brazil (5º53'04” S, 35º48” W).

The wood of the eucalyptus clone GG 100 (*Eucalyptus urophylla* x *grandis*) was cut into 2 cm thick discs and divided into four wedges, which were dried for 24 hours in an oven at 103 ± 1°C. Next, carbonization runs were performed with wood samples weighing 500 g. The samples were placed in a metal container and inserted into a laboratory muffle furnace equipped with a tubular metallic condenser kept at room temperature. The heating rate was 1.25°C min^{-1} until the final temperature of 450°C, which was maintained for 30 minutes. The resulting condensed liquids were kept at room temperature in a glass bottle, and at the end of the carbonizations, the accumulated liquid was distilled twice at 100°C, and the process was stopped at 105°C to obtain WV free of oil and tar.

After carbonization, the gravimetric yields of charcoal and crude pyroligneous liquor (total condensed liquids) were determined based on dry wood. Non-condensable gases were calculated by difference. These gravimetric yields were 39.3% charcoal, 37.8% condensed liquids and 22.9% non-condensable gases. After distillation of the initial mass of condensed liquids, a yield of 70.5% purified WV was obtained, which corresponds to 26.7% of the initial mass of dry wood.

The evaluation of wood vinegar’s effect on leucaena was carried out in 13 x 13 x 5 cm plastic trays, where 30 seeds were sown. The experimental design was completely randomized in a factorial scheme with four replications, whose factors were WV concentrations in the irrigation water (0.0, 0.4, 0.8, 1.2 and 1.6%) and substrate type (sand or vermiculite).

Leucaena seeds, donated by Techflora Ltda., were disinfected in sodium hypochlorite. The procedure for breaking dormancy was to cut off the opposite side of the micropyle. The sowing was done in polystyrene trays with 128 cells on May 8th. Emergence assessments began on April 19th and were carried out daily until May 16th (27 days), when the plants were removed with shoots and roots. The trays were irrigated daily with 50 ml of distilled water containing the WV concentrations. The dry masses were determined after drying in a forced-air oven at 65°C for 72 hours.

The evaluated variables were: percentage of emergence (PE (%) = (n / 30) x 100), where *n* is the number of seeds germinated at the end of the test; emergence speed index (ESI = ∑ (ni / ti), in which *ni* is the number of seedlings emerged on day *i*, and *ti* is the time in days after the beginning of counting; percentage of non-germinated seeds (NGS) at the end of the test; total length of shoots plus roots (TOL) in cm; height of shoots (HES) in cm; shoot dry mass (MSH) in g per plant; and root dry mass (MRT) in g per plant.

Data were subjected to analysis of variance by the generalized linear model's procedure and the F test. The means of substrates were compared by the Tukey test ($p < 0.05$). Regression analysis was used to express the behaviour of variables as a function of the concentration of WV in water. The statistical software R (R Core Team, 2019) was used in data processing.

Results

The variables related to germination and emergence, PE, NGS and ESI were significantly affected by substrate type ($p < 0.01$) and WV concentration ($p < 0.01$). No significant effect of the interaction between the factors was observed in these variables.

In general, seedlings resulting from sowing in vermiculite presented higher germination and emergence values than those sown in the sand. In comparison to sand, the variables PE and ESI were 34% and 45% higher, respectively, when the substrate was vermiculite, while NGS values were 27% lower (Table 1).

Table 1. The effect of different wood vinegar concentrations on the percentage of emergence (PE), non-germinated seeds (NGS), emergence speed index (ESI), total length (TOL), seedling height (HES), shoot mass (MSH) and root mass (MRT) of leucaena

Substrate	PE (%)	NGS (%)	ESI	TOL (cm)	HES (cm)	MSH (g)	MRT (g)
Sand	44.21 b	55.09 a	3.42 b	12.77 b	6.06 b	0.06 b	0.14 b
Vermiculite	59.30 a	40.00 b	4.97 a	22.45 a	9.20 a	0.09 a	0.22 a

Means of substrates followed by different letters are different according to the Tukey test ($p < 0.05$).

The variables PE and ESI presented a linear negative behaviour as a function of WV concentration, while NGS increased linearly with increasing WV concentration (Fig. 1). The results of the treatment without WV and the treatment with the highest WV concentration (1.6%) revealed a 66% decrease in PG and 85% in ESI, while NGS increased by 125%.

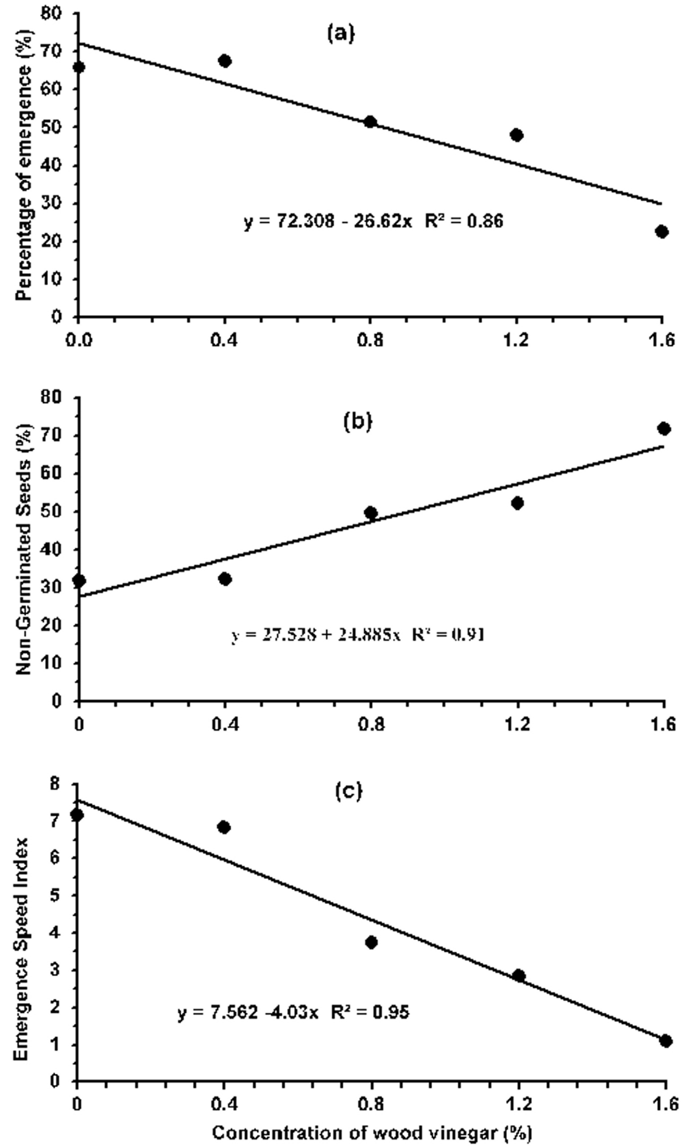

Figure 1. Regressions of the variables percentage of emergence (a), non-germinated seeds (b) and emergence speed index (c) of leucaena as a function of wood vinegar concentrations

The growth variables of leucaena, TOL, HES, MSH, and MRT showed a significant effect of the type of substrate used ($p < 0.01$), but only TOL and HES showed a significant effect of WV concentration ($p < 0.01$). However, all these variables were significantly affected by the interaction between substrate and WV concentration.

The values of the four leucaena growth variables analyzed were higher when the substrate was vermiculite than sand. This superiority of vermiculite was represented by 76%, 52%, 50% and 57% higher values of TOL, HES, MSH and MRT, respectively (Table 1).

When the substrate was sand, there was a decreasing trend in the values of the variables with increasing concentration of WV. Between the treatments without WV application and those that received the highest (1.6%) concentration, the variables TOL, HES and MSH decreased by 79, 67 and 30%, respectively, while MRT decreased by 65% until the concentration of 1.33% WV (Fig. 2).

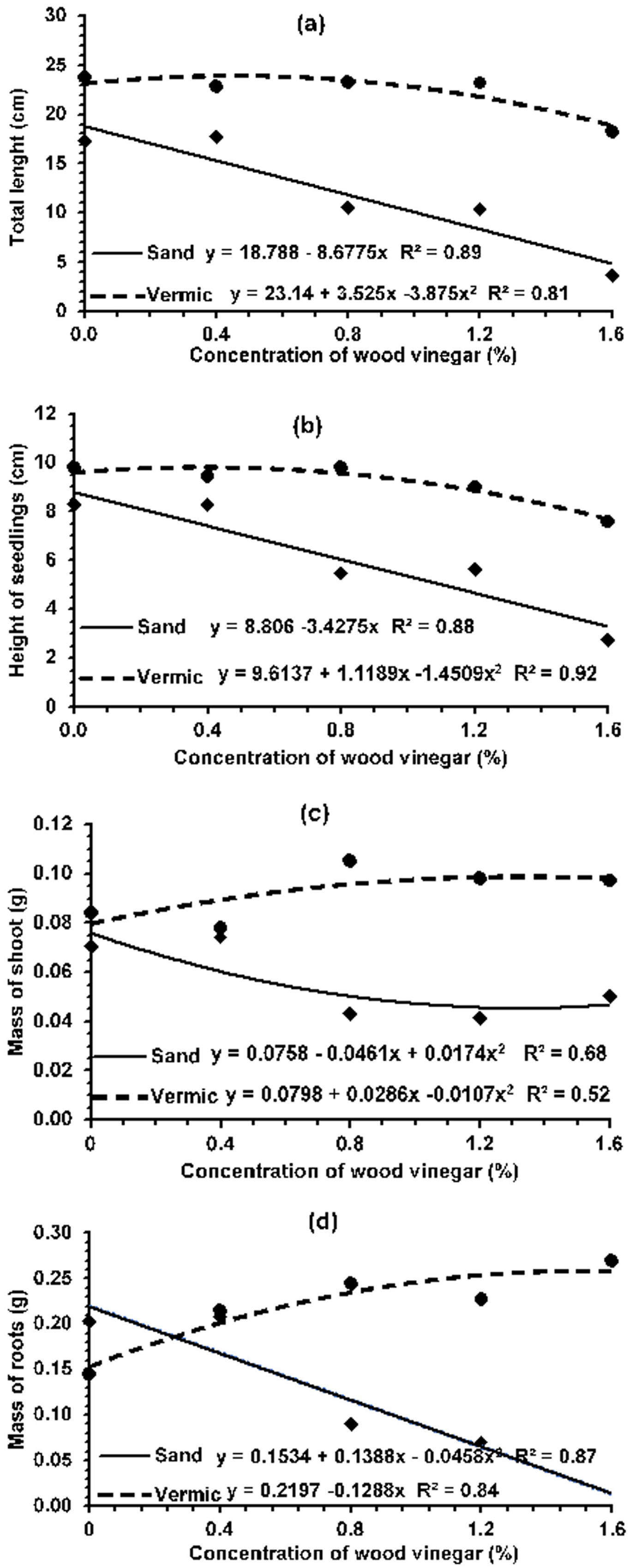

Figure 2. Regressions of the variables total length (a), seedling height (b), shoot mass (c) and root mass (d) of leucaena as a function of wood vinegar concentrations

When the substrate was vermiculite, the variables TOL and HES showed stable values up to the concentrations between 0.4 and 0.5%, after which a decrease in values was observed as the concentrations of WV increased. However, the variables MSH and MRT showed a slight positive effect of WV at lower concentrations, and the tendency to decrease in their values started from concentrations between 1.4 and 1.5%.

Discussion

The results demonstrate the influence of wood vinegar concentrations on the germination, emergence and initial growth of leucaena, which differed between sand and vermiculite. This is in line with studies with several other species. In this sense, Hagner (2013) reported the use of WV as a non-selective herbicide for important invasive species in Europe, mainly dicotyledons, and recommended its application before the emergence of cultivated plants, due to its contact effect.

The better germination and initial growth of leucaena was observed in this study when the substrate was vermiculite has also been observed for other plant species, although sand is widely used as a substrate for germination tests and seedling production. According to Pacheco et al. (2006), the proper substrate should promote a balance between water availability and aeration, but sand has a low capacity to retain nutrients and make them available, and its low water retention requires frequent water replacement and causes dryness of the soil surface. In turn, vermiculite benefits seed germination and early plant growth by retaining more water, due to its high porosity and low density (Lima et al., 2010; Oliveira et al., 2012). The faster uptake of water by seeds in vermiculite is due to the larger seed contact area with the substrate (Gonçalves et al., 2007), which also releases Mg ions into the soil and retains phosphorus and ammoniacal nitrogen (Moreira, 2018).

The negative effect of WV on germination variables and initial growth of leucaena was greater at higher concentrations of WV in both substrates, likely caused by substances contained in WV, such as acetic acid, which according to Mu et al. (2004) was responsible for the strong inhibitory effect of WV on the root and hypocotyl growth of *Crysanthemum coronarium* and *Rorippa nasturtium-aquaticum*. Other harmful substances are furfural and methanol (Hagner, 2013), phenols, organic acids and carbonyls (Grewal et al., 2018). The effect of WV also depends on the process of obtaining it, the raw material used and the plant species treated (José et al., 2016). In our study, WV was distilled twice, so it had a higher content of volatile compounds compared to decanted WV, according to Alves et al. (2007). In this regard, other studies have used different formulations and processes for obtaining WV, such as decantation and distillation, as well as ether extraction techniques (Mu et al., 2003). The temperature in the condenser where WV is collected after carbonization also influences the concentration of inhibitory substances. The temperature that provided the highest concentration of these substances was between 250 and 400°C in the study by Mu et al. (2004).

The detrimental effect of WV was more intense on leucaena germination and emergence variables (PE and ESI). In this sense, Souza Filho et al. (2000) reported that ESI is the variable most sensitive to stresses like the effect of substances contained in WV. The greater inhibitory effect of WV in the early stages of germination than in phases after germination was also observed by Mmojieje (2016). According to Arruda et al. (2012), the embryo and endosperm membrane cells are very sensitive to toxic effects like those promoted by the substances contained in WV, with reduction in enzymatic activity and lower meristematic development. Therefore, it can be inferred that leucaena is more impaired by WV in its germination stage than in later stages of development. Thus, the WV can control leucaena in the wild, where according to Mariano et al. (2016), it is hampered by problems in germination, emergence and early development.

Although Mmojieje (2016) states that WV concentrations above 10% are necessary to inhibit seed germination and seedling growth, the concentrations used in this study provided a large decrease in percentage and speed of germination of leucaena. In other studies, WV concentrations of approximately 1 and 2%, as in our study, caused effects such as: delayed plant development and injuries in different species (Silveira, 2010); strong inhibition of root germination and development in *Lactuca sativa, Rorippa nasturtium-aquaticum, Cryptotaenia japonica* and *Chrysanthemum coronarium* (Mu et al., 2004); inhibition of corn germination (Ling et al., 2009); severe decrease in germination percentage and ESI of *Eugenia dysenterica* and *Handroanthus serratifolius* (José et al., 2016); and reduction of mass per area of the total weed community in a wheat field (Koc et al., 2019). On the other hand, WV concentrations above 25% inhibited the sprouting of *Cyperus rotundus* tubers and more severe effects occurred at concentrations of 50 to 100% (Costa et al., 2010).

The different behaviour of the variables TOL, HES, MSH and MRT, when the substrate was sand or vermiculite, indicates that the initial development of leucaena is affected by the interaction between WV concentration and substrate. In sand, negative linear behaviour was observed as a function of WV concentration, as occurred with the germination and emergence variables. In vermiculite, the values of TOL and HES decreased from concentrations between 0.4 and 0.5%, while MSH and MRT showed a slight positive effect at lower concentrations and their values only tended to decrease starting from concentrations between 1.4 and 1.5%. This was explained by Andreazza et al. (2013), who observed that leucaena shoots and roots grow differently in response to the substrate, with compensation in growth of one of these parts in response to the growth of the other.

The benefit to the variables MSH and MRT at the lowest WV concentrations and the harm at the highest concentrations was similar to the findings of Mu et al. (2003), who reported that WV concentrations of approximately 0.1% promoted the germination of *Rorippa nasturtium-aquaticum, Cryptotaenia japonica* and *Chrysanthemum coronarium*, but inhibition occurred at higher concentrations. Similarly, Ling et al. (2009) observed that WV concentrations of approximately 0.3% promoted corn germination, but concentrations of 1% and higher impaired germination. According to the authors, at lower concentrations WV favours germination because it destroys the outer layers of seeds, increasing aeration and water absorption, but at higher concentrations the growing tissues are destroyed.

The detrimental effect of WV on MSH and MRT when the substrate was vermiculite was only evident at the higher concentrations. This is due to the ability of vermiculite of adsorbing WV and thus decreasing its detrimental effects at the lower doses. According to Togoro et al. (2014), WV is an organic acid and tends to be adsorbed by clays in the soil. The very small size of vermiculite particles, their large specific surface area, porosity, cation exchange capacity and chemical and mechanical stability make it a material with high chemical and physical adsorption capacity for molecules of varying sizes and charges, such as pollutants and pesticides, metals (heavy or not), cationic organic dyes, phenolic organic compounds and oily effluents (Teodoro, 2018; Francisco et al., 2019; Leiva et al., 2019).

Although higher WV concentrations than those studied here are required for the effective control of leucaena, the results obtained support the use of wood vinegar in substitution to synthetic herbicides, depending on the species to be controlled, as stated by Grewal et al. (2018). Even the use of WV as an additive for mixture with synthetic herbicides can increase their efficiency and thus reduce the volume of synthetic pesticides applied (Hagner, 2013). Due to its action as a biocide/pesticide replacing synthetic agricultural agrochemicals, the use of WV can reduce soil and water contamination, pesticide leaching into groundwater and watercourses, and risks to human health. Reducing dependence on synthetic pesticides for pest and disease control is an alternative plant protection technology and is very useful in organic agriculture (Tiilikkala et al., 2010; Togoro et al., 2014).

Conclusion

Wood vinegar had an inhibitory effect on the germination, emergence and initial development of leucaena at the studied concentrations. This effect was greatest when leucaena was sown in sand substrate. However, it is necessary to test higher concentrations of wood vinegar for effective control of leucaena.

References

Alves A.S., Lima V.L.A., Farias M.S.S., Firmino M.C., Medeiros S.S. (2012). Desempenho germinativo de sementes de leucena: avaliação de substratos e lâminas de água. Irriga: 105-119. doi: 10.15809/irriga.2012v1n01

Alves M., Cazetta J.O., Nunes M.A., Oliveira C.A.L., Colombi C.A. (2007) Ação de diferentes preparações de extrato pirolenhoso sobre *Brevipalpus phoenicis* (geijskes). Rev Bras Frut 29: 382-385. doi: 10.1590/S0100-29452007000200037

Andreazza R., Antoniolli Z.I., Silva R.F., Schirmer G.K., Scheid D.L., Quadro M.S., Barcelos M.A. (2013). Efeito de vermicomposto no crescimento inicial de ipê amarelo (*handroanthus chrysotrichus*) e leucena (*leucaena leucocephala*). Nativa 1: 29-33. doi: 10.14583/2318-7670.v01n01a06

Arruda Y.M.B.C., Ferraz I.D.K., Albuquerque M.C.F. (2012). Fontes e concentrações de águas de fumaça na germinação de sementes e no vigor de plântulas de tomate. Hort Bras 30: 293-299. doi: 10.1590/S0102-05362012000200018

Costa M.A.R., Togoro A.H., Silva J.A.S., Cazetta J.O. (2010). Uso do extrato pirolenhoso no controle de *Cyperus rotundus* L.. In: Anais do XXII Congresso de Iniciação Científica da Unesp, Universidade Estadual Paulista – UNESP, Jaboticabal, Brazil.

Couto L. (2012). Potencial da leucena para produção de biomassa para energia no Brasil. Jornal das Indústrias Brasileiras de Biomassa. Available at: http://www.biomassabr.com/bio/resultado noticias.asp?id=1827 [Accessed 25 June, 2019].

Cunha F.R.P., Fernandes A., Silva H.P. (2013). A dispersão da *Leucaena leucocephala* (Lam.) de Wit nos córregos urbanos de Maringá (PR). Arquivos do MUDI, 17 (1): 3-4.

Dana E.D., Randall R.P., Sanz-Elorza M., Sobrino, E. (2003). First evidences of the invasive behavior of *Leucaena leucocephala* in Europe. Oryx, 37(1), 14.

Drumond M.A., Ribaski J. (2010). Leucena (*Leucaena leucocephala*): leguminosa de uso múltiplo para o semiárido brasileiro. Embrapa Semiárido, Petrolina, Brazil, 8pp.

Francisco L.V.S., Oliveira I.M., Silva J.F., Castro V.D. (2019) Utilização da vermiculita organofilizada com surfactantes catiônicos como potencial material adsorvente de contaminantes emergentes. Braz J of Develop 5: 19381-19388. doi: 10.34117/bjdv5n10-164

Gonçalves E.P., Alves E.U., Bruno R.L.A., França P.R.C., Silva K.B., Galindo, E.A. (2007). Germinação e vigor de sementes de *Crataeva tapia* L. em diferentes substratos. Acta Sci Biol Sci 29: 363-367. doi: 10.4025/actascibiolsci.v29i4.874

Grewal A., Abbey L., Gunupuru L.R. (2018). Production, prospects and potential application of pyroligneous acid in agriculture, J Anal Appl Pyrol 135: 152-159. doi: 10.1016/j.jaap.2018.09.008

Hagner M. (2013). Potential of the slow pyrolysis products birch tar oil, wood vinegar and biochar in sustainable plant protection - pesticidal effects, soil improvement and environmental risks. PhD. Faculty of Biological and Environmental Sciences of the University of Helsinki, Helsinki, Finland.

José A.C., Andrade R.J., Pereira W.V.S., Silva N.C.N., Faria J.M.R. (2016). Efeito do extrato pirolenhoso sobre a germinação de espécies do Cerrado Brasileiro. Cad Ciênc Agrá 8: 62-69.

Koc I., Namli A., Mendes M., Pinar S.M., Cig F., Yardim E.N. (2019). A study on the effects of wood vinegar on weeds and cultivated plants in the wheat agro-ecosystemns. Fresenius Environ Bull 28: 2747-2753.

Leão T.C.C., Almeida W.R., Dechoum M., Ziller S.R. (2011). Espécies exóticas invasoras no nordeste do brasil: contextualização, manejo e políticas públicas. Centro de Pesquisas Ambientais do Nordeste – CEPAN, Recife, Brazil, 99pp.

Leiva J.A., Wilson P.C., Albano J.P., Nkedi-Kizza P., O'connor G.A. (2019). Pesticide sorption to soilless media components used for ornamental plant production and aluminum water treatment residuals. ACS Omega 4: 17782-17790. doi: 10.1021/acsomega. 9b02296

Lima J.F., Silva M.P.L., Teles S., Silva F., Martins G.N. (2010). Avaliação de diferentes substratos na qualidade fisiológica de sementes de melão de caroá [Sicana odorifera (Vell.) Naudim]. Rev Bras Plantas Med 12: 163-167. doi: 10.1590/S1516-05722010000200007

Ling Z., Enchen J., Bosong L. (2009). Effect of wood vinegar on seed germination and water implantation of corn. J Northeast Agric Univ 16: 6-11.

Lowe S., Browne M., Boudjelas S., De Poorter M. (2000). 100 of the world's worst invasive alien species. A selection from the global invasive species database. The Invasive Species Specialist Group (ISSG), Species Survival Commission (SSC), World Conservation Union (IUCN), Auckland, New Zealand, 12pp.

Mariano L.G., Somavilla A., Silveira A.G., Salamoni A.T. (2016). Análise de superação de dormência de sementes de *Leucaena leucocephala* e desenvolvimento inicial de plântulas. Rev Eletrônica Gest Educ Tecnol Ambient 20: 398–404.

Matthews S., Brand K. (2005). América do Sul invadida: a crescente ameaça das espécies exóticas invasoras. Global Invasive Species Programme – GISP, Nairobi, Kenya, 80pp.

Mello T.J. (2013). Invasão biológica em ilhas oceânicas: o caso de *Leucaena leucocephala* (Leguminosae) em Fernando de Noronha. Master of Science. Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

Mmojieje J. (2016). Pyroligneous Acid: A Farmer's Flexible Friend? J Crop Improv 30: 341-351. doi: 10.1080/15427528.2016.1160462

Moreira S.S. (2018). Germinação e vigor de sementes de quiabo-de-metro (*Trichosanthes cucumerina* L.). Bachelor of Science. Universidade Federal Rural da Amazônia, Belém, Brazil.

Mu J., Uehara T., Furuno T. (2003). Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants. J Wood Sci 49: 262–270. doi: 10.1007/s10086-002-0472-z

Mu J.,·Uehara T., Furuno T. (2004). Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants II: composition of moso bamboo vinegar at different collection temperature and its effects. J Wood Sci 50: 470–476. doi: 10.1007/s10086-003-0586-y

Oliveira P.D., Maneschy R.Q., Costa K.C.G., Guimarães T.P, Oliveira I.K.S. (2010). Estabelecimento de banco forrageiro com leucena em unidade de produção familiar no Sudeste do Pará. Agroecossistemas 2: 32-28.

Oliveira K.S., Oliveira K.S., Aloufa M.A.I. (2012). Influência de substratos na germinação de sementes de Anadenanthera colubrina (Vell.) Brenan em condições de casa de vegetação. Rev Arvore 36: 1073-1078. doi: 10.1590/S0100-67622012000600008

Pacheco M.V., Matos V.P.; Ferreira R.L.C.; Feliciano A.L.P., Pinto K.M.S. (2006). Efeito de temperaturas e substratos na germinação de sementes de *Myracrodruon urundeuva* fr. All. (anacardiaceae). Rev Arvore 30: 359-367. doi: 10.1590/S0100-67622006000300006

Pimenta A.S., Fasciotti M., Monteiro T.V.C., Lima K.M.G. (2018). Chemical Composition of Pyroligneous Acid Obtained from Eucalyptus GG100 Clone. Molecules 23: 426. doi: 10.3390/molecules23020426

R Core Team (2019). R: A language and environment for statistical computing. Vienna, Austria. Available at: https://www.R-project.org/. Accessed 23 March, 2019.

Santos N.A.V., Teixeira N.C., Silva P.L.P., Ribeiro R.S., Maurício R.M. (2011). Utilização da leucena (*Leucaena leucocephala*) e de duas variedades do feijão guandu (Cajanus cajan) em sistemas silvipastoris para formação de banco proteico. In: Anais do X Congresso de Ecologia do Brasil, Sociedade de Ecologia do Brasil, São Lourenço, Brazil.

Scherer L.M., Zucareli V., Zucareli C.A., Fortes A.M.T. (2005). Allellopatic effects of aqueous extracts of leucena (*Leucaena leucocephala* Wit.) leave and fruit on germination and root growth of canafístula (*Peltophorum dubium* Spreng). Semina Cienc Agrar 26: 161-166. doi: 10.5433/1679-0359.2005v26n2p161

Silva M.K.A.G., Pimenta A., Souza E.C., Nóbrega R.C.S., Silva S.I.S., Lourenço Y.B.C., Caetano V., Medeiros L.C.D. (2017). Produção, composição e utilização do extrato pirolenhoso na agricultura. In: Anais do III Congresso brasileiro de Ciência e Tecnologia da Madeira, Sociedade Brasileira de Ciência e Tecnologia da Madeira, Florianópolis, Brazil.

Silveira C.M. (2010). Influência do extrato pirolenhoso no desenvolvimento e crescimento de plantas de milho. PhD. Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, Brazil.

Souza Filho A.P.S. (2000). Influência da luz, temperatura e estresses osmótico e salino na germinação de sementes de *Leucaena leucocephala*. Pasturas tropicales, 22(2): 47-53.

Teodoro L. (2018). Caracterização da argila vermiculita expandida para possível aplicação como adsorvente. Bachelor of Science. Universidade Tecnológica Federal do Paraná, Pato Branco, Brazil.

Tiilikkala K., Fagernäs L., Tiilikkala J. (2010). History and use of wood pyrolysis liquids as biocide and plant protection product. Open Agr J 4: 111-118. doi: 10.2174/1874331501004010111

Togoro A.H., Silva J.A.S., Cazetta J.O. (2014). Chemical changes in an oxisol treated with pyroligneous acid. Cienc Agrotec 38: 113-121. doi: 10.1590/S1413-70542014000200002

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