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PYROLIGNEOUS EXTRACT FOR PRODUCTION OF *Pinus taeda* L. SEEDLINGS

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ABSTRACT - Production of well-developed *Pinus taeda* L. seedlings is essential for satisfactory development of planted forests. Biostimulant compounds, such as pyroligneous extract, have shown great potential to improve plant growth of some agronomic crops, and therefore should be tested in forestry species as well. Hence, the goal of this study was to evaluate the effects of pyroligneous extract on *P. taeda* seedlings under nursery conditions. The experiment was carried out under completely randomized conditions, studying three concentrations of extract added to the substrate. Plant height and diameter were measured monthly, and data regarding plant biomass and development were collected after six months. Pyroligneous extract had no effect on seed germination. Plant height was improved in 12.5% only at 30 days after sowing with 2.5% extract added to the substrate. Fresh mass or volume of roots, as well as shoot mass, were not affected by pyroligneous extract. However, root dry mass was increased from 0.258g to 0.335g with 1.0% pyroligneous extract. Root production by *Pinus taeda* seedlings is significantly improved by adding 1.0% pyroligneous extract in the substrate before sowing. Therefore, addition of 1.0% pyroligneous extract to substrate is recommended to improve root development of *Pinus taeda* seedlings at nursery.
Keywords: pine trees, pyroligneous acid, wood charcoal residues.

UTILIZAÇÃO DE EXTRATO PIROLENHOSO NA PRODUÇÃO DE MUDAS DE Pinus taeda L.

RESUMO - A produção de mudas de *Pinus taeda* L. bem desenvolvidas é essencial para o desenvolvimento satisfatório das florestas plantadas. Compostos bioestimulantes, como o extrato pirolenhoso, têm demonstrado grande potencial para melhorar o crescimento de plantas de algumas culturas agronômicas e, portanto, devem ser testados também em espécies florestais. Assim, o objetivo desse estudo foi avaliar os efeitos do extrato pirolenhoso em mudas de *P. taeda* em condições de viveiro. O experimento foi conduzido em delineamento completamente casualizado, estudando três concentrações de extrato pirolenhoso adicionado ao substrato. Altura e diâmetro das plantas foram mensurados mensalmente, e dados de massa e desenvolvimento foram coletados ao final de seis meses. O extrato pirolenhoso não teve efeito sobre a germinação das sementes. A altura das plantas foi melhorada em 12,5% apenas aos 30 dias após a semeadura com 2,5% de extrato adicionado ao substrato. A massa fresca ou o volume das raízes, assim como a massa da parte aérea, não foram afetados pelo extrato pirolenhoso. No entanto, a massa seca da raiz aumentou de 0,258g para 0,335g com 1,0% de extrato pirolenhoso. A produção de raízes por mudas de *Pinus taeda* é significativamente melhorada com a adição de 1,0% de extrato pirolenhoso no substrato antes da semeadura. Portanto, a adição de extrato pirolenhoso a 1,0% é recomendada para melhorar o desenvolvimento radicular de mudas de *Pinus taeda* em viveiro.
Palavras-chave: ácido pirolenhoso, pinheiro, resíduos de carvão vegetal.

INTRODUCTION

Production of *Pinus* in Brazil has risen through the past decades, and the currently cultivated area corresponds to 1.7 million hectare (IBÁ, 2021). Most of this volume is destined to production of paper and wood products, which are mostly exported. As demands of *Pinus* trees have increased, efforts have been made towards successful establishment of seedlings in the field. Low levels of mortality, fast growth and good biomass production are desirable plant traits that contribute to better formation of planted forests and accelerate *Pinus* development in the field (CARNEIRO et al., 2007; MEZA et al., 2020).

A key step in this process is to choose seedlings of good quality and hence, nursery practices are fundamental. Throughout the past decades, some silvicultural practices include the use of different substrates such as fresh sawdust, pine bark, rice hull, coconut husk and sandy soil (FREITAS et al., 2005; FAJARDO-MEJÍA et al., 2016; GONZÁLEZ-OROZCO et al., 2018) and inoculation with plant growth-promoting microorganisms (SANTOS et al., 2018; KONDO et al., 2020; WEN et al., 2022). Addition of some organic residues with bioestimulant properties, such as pine needles, peat moss and pine bark have also shown very

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positive results on seedling development (GONZÁLEZ-OROZCO et al., 2018; CECCAGNO et al., 2019).

Pyroligneous extract (PE) is one example of a liquid residue generated by the production of wood charcoal and has been tested for its nutrition and growth benefits at several countries (OFOE et al., 2022, ZENG et al., 2022). In Brazil, however, its applications are mostly related to food industry (WAZLAWIK, 2000; SOUTO et al., 2017) and its properties as fertilizer or bioestimulator have been poorly understood and limited to a few species such as tomato, maize, soybean, orchid, eucalyptus and pine trees (PORTO et al., 2007; SOUZA-SILVA et al., 2006; SILVA et al., 2017; SILVA et al., 2021). Some results indicate positive effects on plant height root and shoot biomass. All of those studies, however, have been conducted only at the first days or weeks of plant growth, lacking a more complete spectrum of effects and applicability of PE.

Brazil is a major producer of wood charcoal and, therefore, has great potential to produce significant amounts of PE that could be used in agricultural or forestry systems to improve plant nutrition and growth (CANAL et al., 2020). However, basic research on PE and forestry species is currently inexistent regarding aspects like levels, mode of application and effects on plant development. Therefore, the goal of this study was to evaluate application of pyroligneous extract on seedlings of *Pinus taeda* produced in nursery conditions.

MATERIAL AND METHODS

This research was conducted in Curitibanos/SC, Brazil (27°16’60’’ S, 50°35’7’’ W) in cooperation with the companies “ATO Participações” and “Primon Mudas Florestais”. The former has pyroligneous extract producer activities, and the latter is a nursery that produces Pinus seedlings to be sold to several forestry companies in the state. The experiment was carried on in a completely randomized design and followed Federal Regulations presented at Normative Instruction DAS 53 of October 15, 2013, established by the Brazilian Ministry of Agriculture (Ministério de Agricultura, Pecuária e Abastecimento - MAPA, 2013). Characterization of the pyroligneous extract used in this study is presented in Table 1.

TABLE 1 - Characterization of pyroligneous extract produced by ATO Participações, originated from Eucalyptus wood charcoal.

Characteristics	Values	Characteristics	Values
pH in $CaCl_2$	2.75	Total N	0.16%
Electric conductivity	1,168 Us CM^{-1}	Soluble K (K_2O)	0.05%
Saline index	0.01	Total B	9.63 mg kg^{-1}
Density	1.01 g $cm^{3\ -1}$	Total Cu	1.69 mg kg^{-1}
Organic C	1.24%	Total Fe	120.60 kg kg^{-1}
OrgC/N ratio	7.75	Total Mn	0.68 mg kg^{-1}
Total Na	30.36 mg kg^{-1}	Total Ni	5.90 mg kg^{-1}
Total Zn	3.62 mg kg^{-1}	Total Pb	1.54 mg kg^{-1}
Total Cr	1.24 mg kg^{-1}	Total Co	0.37 mg kg^{-1}

Treatments tested were T1: control, T2: 0.5% PE, T3: 1.0% PE, T4: 2.5% PE. Forty replicates per treatment were initially established. After 30 days, tubes with non-germinated seeds or dead seedlings were excluded. Evaluations of height, diameter and plant mass were, from then on, made with 24 replicates per treatment.

Fifty-milliliter tubes were used and filled with standard substrate used in this nursery (Table 2). For each 50kg of this substrate, a 1:1:1 mixture of NPK fertilizers was added, following routine nursery practices. Commercial fertilizers were Osmocote 19-6-10 Mini Pril 3M, Osmocote 18-5-9 Mini Pril 5M and Plantacote® Supra P 4M 14-25-6.

TABLE 2 - Characterization of substrate used to produce *Pinus taeda* seedlings.

Characteristics	Values	Characteristics	Values
Soluble P (P_2O_5)	3.32 mg kg^{-1}	pH	7.21
Soluble K (K_2O)	181.56 mg kg^{-1}	Electric conductivity	0.23 mS cm^{-1}
Ca	11.10 mg kg^{-1}	Density	0.53 g $cm^{3\ -1}$
Mg	4.82 mg kg^{-1}	CEC	410.00 $mmol_c$ kg^{-1}
S	25.77 mg kg^{-1}	Organic carbon	8.45%
B	0.05 mg kg^{-1}	Total carbon	16.43%
Cu	0.13 mg kg^{-1}	Water loss at 65ºC	50.82%
Fe	18.31 mg kg^{-1}	Water loss at 110ºC	3.65%
Mn	0.12 mg kg^{-1}	Total fixed solids at 110ºC	96.35%
Mo	0.01 mg kg^{-1}	Total fixed solids at 550ºC	36.22%
Na	54.00 mg kg^{-1}	Total volatile solids	60.13%

PE was diluted at different percentages and added to the substrate before sowing. First, the extract was diluted in water at different rates to achieve concentrations defined in treatments 2-4. Five mL of the solution were applied to the substrate of each tube using a sterile pipette. Only water was added to T1 tubes. Then, three 1,5-generation *Pinus taeda* seeds were placed in each tube and those were covered with a layer of substrate without PE. Tubes were kept at greenhouse conditions at the nursery for 180 days, with automated irrigation, as applied to other seedlings that are produced for purchase by reforestation companies.

At 20 and 30 days after sowing, the percentage of emerged seedlings in each tube was calculated. At 30 days, only one seedling was kept in each tube. At this moment, only 24 replicates by treatment were kept for further analyses, due to mortality and lack of emerged seedlings.

Measurements of height and diameter were conducted monthly between 30 and 180 days after sowing. At the end of six months, plants were harvested to evaluate root volume, root fresh and dry mass, shoot fresh and dry mass and Dickson Quality Index (DQI). Root volume was estimated by water displacement as described by Rossiello et al. (1995). Both root and shoot dry mass were obtained after drying at 65ºC until constant mass. DQI was calculated based on equation of Dickson et al. (1960).

For statistical analyses, data were first submitted to a one-way analysis of variance at 5% probability of error. When differences among treatments were found, means were separated with the Scott-Knott test, at 5% probability of error. All tests were conducted with SISVAR Software (FERREIRA, 2019).

RESULTS AND DISCUSSION

At both, 20 or 30 days after sowing, percentage of seed germination was not responsive to any treatment with pyroligneous extract (Table 3). Statistical differences on plant height were observed only at 30 days after sowing (Table 4). T4 was the only treatment to improve this variable, compared to T1. Plant height was increased by 12.5% with use of 2.5% pyroligneous extract. Values changed statistically from 4.15 cm (T1) to 4.67 cm (T4), as seen in Figure 1.

TABLE 3 - Overall means, coefficient of variation (CV) and Pr<Fc values regarding analysis of variance of emerged seedlings (%) of *Pinus taeda* at 20 and 30 days after sowing (DAS) in response to pyroligneous extract.

	Overall mean (cm)	CV(%)	Pr<Fc
20 DAS	32.91	82.69	0.11
30 DAS	56.66	56.13	0.63

TABLE 4 - Overall means, coefficient of variation (CV) and Pr<Fc values regarding analysis of variance of height of *Pinus taeda* seedlings at 30-180 days after sowing (DAS) in response to pyroligneous extract.

	Overall mean (cm)	CV(%)	Pr<Fc
30 DAS	4.40	16.00	0.03
60 DAS	5.35	20.40	0.12
90 DAS	7.33	25.62	0.80
120 DAS	9.52	26.02	0.82
150 DAS	11.78	23.36	0.75
180 DAS	13.19	26.33	0.62

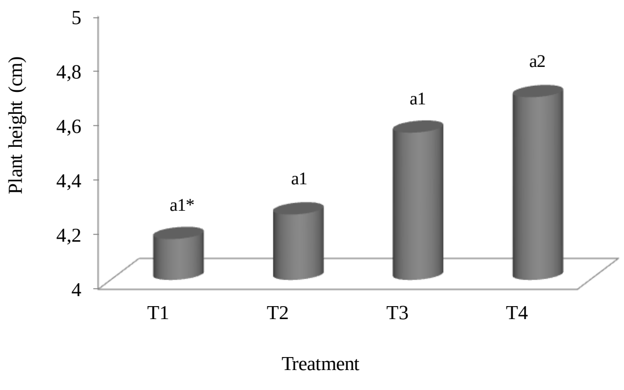

FIGURE 1 - Height of *Pinus taeda* seedlings in response to addition of pyroligneous extract (PE). T1: control, T2: 0.5% PE; T3: 1.0% PE, T4: 2.5% PE. *Means followed by different letters and numbers are different, according to the Scott-Knott test at p<0.05.

On the other hand, no significant response of diameter was recorded at any evaluation throughout the experiment (Table 5). At the end of six months, significant effects were observed only on dry root mass. Other variables related to root or shoot development, as well as Dickson Quality Index (DQI), were not affected by application of PE (Table 6).

Dry root mass was improved by 29.84% with addition of 1.0% pyroligneous extract. Plants from T3 produced an average of 0.335 g, while plants from T1 had only an average of 0.258 g mass of dry roots (Figure 2). This is the first study about application of pyroligneous extract on complete development of *Pinus* seedlings in nursery conditions. Results here presented highlight the potential of using PE to improve plant characteristics that may result in higher survival and better adaptation at the field. Information on this topic is crucial in order to develop nursery practices that are simple to perform and may contribute to evolution of silvicultural practices in Brazil and also worldwide.

The first parameter evaluated in this study was percentage of seed germination, which was not affected by PE. The same was also observed by other researchers that studied *Pinus* (PORTO et al., 2007), as well as corn and black bean (SILVA et al., 2021). Therefore, no evidence is found that PE could increase seed germination, regardless of levels of type of application.

TABLE 5 - Overall means, coefficient of variation (CV) and Pr<Fc values regarding analysis of variance of diameter of *Pinus taeda* seedlings at 30-180 days after sowing (DAS) in response to pyroligneous extract.

	Overall mean (mm)	CV (%)	Pr<Fc
30 DAS	0.93	13.13	0.90
60 DAS	0.96	14.05	0.21
90 DAS	1.33	21.08	0.54
120 DAS	1.57	17.83	0.75
150 DAS	1.89	21.41	0.84
180 DAS	2.16	19.74	0.85

TABLE 6 - Mean values of fresh and dry root mass, root volume, fresh and dry shoot mass and Dickson quality index of *Pinus taeda* seedlings at 180 days after sowing in response to pyroligneous extract.

	Overall mean	CV(%)	Pr<Fc
Fresh root mass (g)	1.635	42.36	0.73
Dry root mass (g)	0.271	49.88	0.05
Root volume (mL)	1.722	46.30	0.74
Fresh shoot mass (g)	1.610	53.18	0.88
Dry shoot mass (g)	0.547	55.19	0.76
Dickson quality index	0.098	45.31	0.41

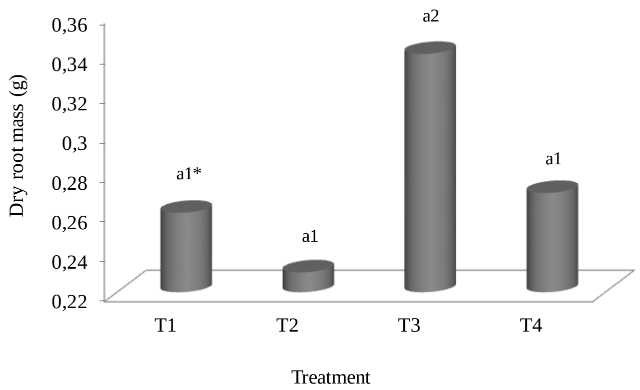

FIGURE 2 - Dry root mass of *Pinus taeda* seedlings in response to addition of pyroligneous extract. T1: control; T2: 0.5% PE; T3: 1.0% PE, T4: 2.5% PE. *Means followed by different letters and numbers are different, according to the Scott-Knott test at $p<0.05$.

Regarding growth development of forestry species in response to PE, only two studies have been previously carried on in Brazil with *Pinus elliottii* and *Eucalyptus grandis* x *E. urophylla* clones (SOUZA-SILVA et al., 2006; PORTO et al., 2007). Souza-Silva et al. (2006) applied PE at 0.1%, 0.5%, 1.0% and 2.0% three times per week, for 45 days. However, they found no evidence of beneficial effects of PE on *Eucalyptus* seedlings regarding height, diameter, shoot or root mass. Porto el al. (2007) evaluated early development of *Pinus seedlings* in field conditions, using a combination of PE applied in soil and also leaf-sprayed. They observed that seedling length, with a mixture of soil + composte, was of 19.72 cm. Addition of PE increased this number to 30.02, a 52% improvement in seedling length. Application of PE at other crops, such as *Zea mays*, has resulted in increments of 12 cm on plant height at early development (SILVA et al., 2021). We also detected improvements in plant height, but only at 30 days after sowing.

In the current study, another parameter that was improved by pyroligneous extract was root dry mass. Porto et al. (2007) also verified that a mixture of soil + composte without PE resulted in root length of 5.19 cm. With 1% PE added, this value changed to 8.44 cm. This information corroborates findings from this current research about potential benefits of PE to root development.

Root mass is a key factor for survival and growth in field conditions. Carneiro et al. (2007) reported that *Pinus taeda* plants with an average of 0.16 g of root dry mass, at the end of the nursery cycle, had an average survival of 82% when transplanted to the field. Plants that produced approximately 0.54 g of root presented 98.9% survival when transferred to the field. Hence, methods that can enhance root production before field transplanting are extremely important to avoid losses due to mortality and, therefore, reduce the need of replacing dead plants, what would cause delays and growth heterogeneity in reforestation sites.

Furthermore, plant roots contribute to C immobilization and sequestration, and their exudates are substrates that improve diversity and activity of many bacteria and fungi; both facts are highly related to soil quality (GREGORY, 2021). Therefore, use of PE can contribute, at indirect ways, to these aspects, because of its action on root development.

It is important to emphasize that this is a pioneer study regarding full nursery cycle in *Pinus taeda* exposed to PE addition in the substrate, and further combinations of levels and types of application should be tested. Pyroligneous extract has been applied in different ways, such as on seeds (SILVA et al., 2021; OFOE et al., 2022), in the substrate or soil (GUERREIRO et al., 2021; SILVA et al., 2021; ZENG et al., 2022) leaf-sprayed (PORTO et al., 2007), or also incorporated in irrigation water (SOUZA-SILVA et al., 2006). Its properties may range from soil conditioner to fertilizer, due to type of application, but these aspects are not explored for any forest species studied so far. Hence, we encourage further research on this topic to better comprehend benefits and promising effects of PE on *Pinus taeda*, as well as other species.

Potential experiments could also test addition of PE combined to reduced levels of fertilizers, given the increasingly high costs of these products. Once that PE is source of several plant nutrients, and acts stimulating root absorption, it may produce beneficial effects regarding reduced fertilizer inputs. We must emphasize that PE can be produced anywhere worldwide, because it is a by-product of wood charcoal, and may be easily commercialized and tested in several forest, as well as agricultural species. Research on this topic may significantly contribute to more sustainable silvicultural practices, improving plant development at nursery, as well as in field conditions.

CONCLUSION

Addition of 1.0% pyroligneous extract to substrate is recommended to improve root development of *Pinus taeda* seedlings at nursery.

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