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## **Study of the Control of Fungus Occurring in *Schizolobium amazonicum* Seeds with the Use of Pyroligneous Extract**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author DGCM development and monitoring of the implantation process and cultivation procedures. Authors GQD and WMP conception or design, data acquisition, statistical analysis and interpretation, writing of the manuscript and coordinating the research project. Author OMY critical revision of the manuscript for intellectual content; statistical analysis. Authors MES and MACC critical revision of the manuscript for important intellectual content, statistical analysis. Authors FMSL and MPBM administrative, technical or material support and supervise the work. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Fungi are the main microorganisms present in seeds, constituting the main cause of deterioration and production losses. Among the health testing methodologies for detecting fungi in seeds, incubation tests under controlled conditions facilitate fungal growth and sporulation. Therefore, this work aimed to evaluate the efficiency of pyroligneous extract in the control of phytopathogenic fungi occurring in *Schizolobium amazonicum* seeds. Treatments consisted of exposure of seeds to pyroligneous extract for a period of five minutes at concentrations (0%, 1%, 2.5%, 5%, 7%, 10% and 12.5%). The treated seeds were placed in Petri dishes, lined with two sheets of filter paper, moistened with sterile distilled water. The experimental design was completely randomized,

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consisting of 7 treatments, with 9 seeds per plate and 10 replications per treatment, totaling 630 seeds arranged in 7 Petri dishes. The evaluation was performed 8 days later, with the aid of stereoscopic and light microscopy, where the fungal growth in each seed in the plates was verified for the incidence calculation and then the identification of these. Pyroligneous extract was found to be efficient in reducing the fungal incidence of the genera *Fusarium* sp. and *Rhizoctonia* sp., fungi considered important plant pathogens that could compromise seed quality and seedling establishment in the field. It also reduced the incidence of storage fungi capable of causing seed deterioration and consequent loss of vigor, such as *Aspergillus* spp. occurring in seed samples of *S. amazonicum*. However, *Penicillium* sp was not controlled by treatment of *S. amazonicum* seeds with pyroligneous extract up to 12.5% concentration.

**Keywords:** Wood vinegard; pinho cuiabano; seed treatment; alternative control; tropical species.

## 1. INTRODUCTION

*Schizolobium amazonicum* (Huber ex Ducke - Caesalpinioidea - Leguminosae), also popularly known as paricá or pinho cuiabano is an plant that, under natural conditions, develops within primary and secondary forests of dryland and high floodplain. In addition, it has a large dispersion capacity [1].

This plant has been widely used for planting in degraded areas, reforestation and agroforestry systems due to its rapid growth and good performance in both homogeneous and intercropping formations [2].

Thus, the demand for seeds and seedlings of this species has been increased, given their plasticity of use. However, some bottlenecks in the production and availability of seeds and vegetative propagules have been reported [3]. To obtain quality seedlings, it is necessary to control the health of the seeds and the production of the seedlings, as it can serve as a vehicle for the propagation and dissemination of pathogens [4,5].

Fungi are considered the main causative agents of plant diseases. In seeds, the importance of these organisms is related to the frequency with which some species occur associated with them, as saprophytes or as pathogens disseminated by them [6].

The incidence of fungi in seeds can cause tegument discoloration, deformation, reduction of germination, seedling diseases, necrotic spots and decay, thus decreasing their germination power and can cause problems in seedling formation in nursery, as well as being primary foci of seedlings field infection [7] and according to Carvalho et al. [8], the interference of seed-associated pathogens can promote plant

population reduction, plant debilitation and development of epidemics.

The seeds are efficient means of dissemination and introduction of pathogens in exempt areas [9,10]. The initial inoculum of epidemics may depend on the transmission of the pathogen by the seed, as well as reducing its physiological quality [11]. Damages resulting from the association of pathogenic microorganisms with seeds are not limited to direct losses of the field population, but involve other implications that can cause serious damage to the entire production system [12,13].

Alternative treatments have been studied, especially those based on plant extracts, biological control or physical treatment. The use of natural products extracted from vegetables may eventually constitute an alternative for controlling seed-associated pathogens, with the advantage of reducing costs for the producer and the absence of environmental impact caused by agrochemicals [14].

An organic product resulting from the condensation of smoke expelled in the wood carbonization process, pyroligneous extract (also called wood vinegar) has been highlighted in recent years for its great potential for pest and disease control [15]. According to Yataga [16], the pyroligneous extract consists of phenolic components, acids, neutral components, alcohols and others, most of which are water (85%) and acetic acid (5.1%). However, there is still little scientific information about its effectiveness in soil, plants, pest control and the ideal concentrations to be used [17].

This product is generally discarded in the process and released into the environment, causing pollution and waste. However, studies have shown that it has a toning effect on plants and may serve several purposes [18].

Currently the pyroligneous extract has been reported for use in various areas, and this product acts as an antimicrobial, anti-inflammatory and antioxidant agent [19]. It is a source of chemical substances used for smoking, preservation and antimicrobial protection to various foods, especially those of animal origin [20]. This antimicrobial ability in food also allows it to have excellent antifungal action against various plant pathogenic fungi, as well as excellent termiticidal activity [16,21].

Therefore, this work aimed to evaluate the efficiency of pyroligneous extract in the control of phytopathogenic fungi occurring in *S. amazonicum* seed.

## 2. MATERIALS AND METHODS

### 2.1 Local and Seed Collection

The present work was conducted at the Laboratory of Microbiology and Phytopathology, Mato Grosso State University, Campus Alta Floresta - MT, Brazil.

The seeds of *S. amazonicum* were collected from mother trees in the Leopoldo Linhares Fernandes Municipal Ecological Park, an urban forest fragment formed by Open Ombrophilous Forest. The park is located in the urban

perimeter of the municipality, which is located in the Southern Amazon Depression between latitudes 9°30'-10°8' South and longitudes 56°27'-55°30' West.

Based on the recommendations of the Seed Analysis Rules [22], 630 seeds were sampled. The "Blotter test" method was used to incubate and survey the microbiota associated with *S. amazonicum* seeds.

### 2.2 Carbonization and Extraction of Pyroligneous Extract

The pyroligneous extract was obtained from the carbonization of the wood, adopting the procedure described by Silva [23] and Fields [24]. For carbonization, wood residues of *Enterolobium contortisiliquum* species were used. To obtain the pyroligneous liquor, a surface oven was used, adapting in the chimney a hose coupled to a 30 liter container, in which the pyroligneous liquor was collected, during the wood carbonization process. After carbonization, the liquid was kept in plastic containers for a period of six months to decant tar and other impurities, in addition chemical reactions (polymerization) were ceased and the components stabilized [23]. The chemical analysis with quantification limit values (QLs) of pyroligneous liquor is presented in Table 1.

**Table 1. Chemical components found in pyroligneous liquor of *Enterolobium contortisiliquum* (Vell.)**

Parameter	QLs	Results
Total nitrogen	0.0100	572.580 mg N L <sup>-1</sup>
Total aluminum	0.0130	4.990 mg Al L <sup>-1</sup>
Total arsenic	0.0010	ND
Total barium	0.0005	ND
Total boron	0.0030	ND
Total cadmium	0.0020	ND
Total calcium	0.0410	19.540 mg Ca L <sup>-1</sup>
Total lead	0.0100	ND
Total copper	0.0013	0.312 mg Cu L <sup>-1</sup>
Total chrome	0.0020	1.254 mg Cr L <sup>-1</sup>
Total Sulphur	0.4000	79.440 mg S L <sup>-1</sup>
Total iron	0.0012	786.400 mg Fe L <sup>-1</sup>
Total phosphorus	0.0050	128.400 mg P L <sup>-1</sup>
Total magnesium	0.0050	3.749 mg Mg L <sup>-1</sup>
Total manganese	0.0004	3.445 mg Mn L <sup>-1</sup>
Total mercury	0.0010	ND
Total nickel	0.0020	ND
Total potassium	1.0000	9.000 mg K L <sup>-1</sup>
Total selenium	0.0010	ND
Total sodium	0.1000	112.000 mg Na L <sup>-1</sup>
Total zinc	0.0020	0.240 mg Zn L <sup>-1</sup>

QLS = quantification limit; ND = not detected

### 2.3 Plates Preparation and Incubation

First the seeds were washed twice with abundant sterile distilled water. Subsequently they were disinfected with pyroligneous extract in seven concentrations (0%, 1%, 2.5%, 5%, 7%, 10% and 12.5%) diluted in 100 mL<sup>-1</sup> of sterile distilled water. The seeds remained in the solution at rest for five minutes. Later they were removed from the solutions and the excess was drained for use in the implementation of the experiment. In the laminar flow, the seeds were placed on the plates equidistantly. In total, 70 sterile Petri dishes were used, lined with two autoclaved filter paper sheets, and each Petri dish received 9 seeds.

After plating, the plates were sealed with clear plastic film and kept in a growth chamber (type B.O.D.) under controlled temperature of 25 ± 2°C and photoperiod of 12 hours for 7 days [22].

### 2.4 Fungi Culturing and Evaluation

After the incubation period, the seeds were analyzed for the percentage of fungal colonies present per seed of each replicate. For this procedure, stereoscopic and optical microscopes were used, allowing the visualization of morphological structures (spores, mycelia and hyphae) and their identification. For the more detailed examination, slides were prepared to identify the fungal species through the visualization of morphological structures (spores, mycelia and hyphae), comparing with the specialized literature [25,26].

### 2.5 Experimental Design and Statistical Analysis

The experimental design was completely randomized with 7 treatments (pyroligneous liquor concentrations) and 10 replications.

The collected data on fungal growth were submitted to variance analysis and the qualitative means compared by Tukey test at 5% probability. As for the quantitative analyzes, they were verified for the best polynomial regression that could explain mathematically the occurred phenomenon.

The data were worked using Excel software and the statistical program Sisvar.

## 3. RESULTS AND DISCUSSION

### 3.1 Fungi Incidence

The results regarding the composition and incidence of mycoflora of seeds of *S. amazonicum* not treated with pyroligneous extract (control treatment) are shown in Fig. 1. The fungi of the genera *Penicillium* sp.; *Cladosporium* sp., *Fusarium* sp., *Rhizoctonia* sp., *Paecilomyces* sp., *Geotrichum* sp., *Aspergillus* sp., *Rhizopus* sp., *Trichoderma* sp., *Colletotrichum* sp. and *Verticillium* sp. were found in petri dishes and identified properly, using microscopes and consulting specialized literature. Similar mycoflora was also detected by Santos et al. [27], studying the sanitary quality of seeds of other forest species.

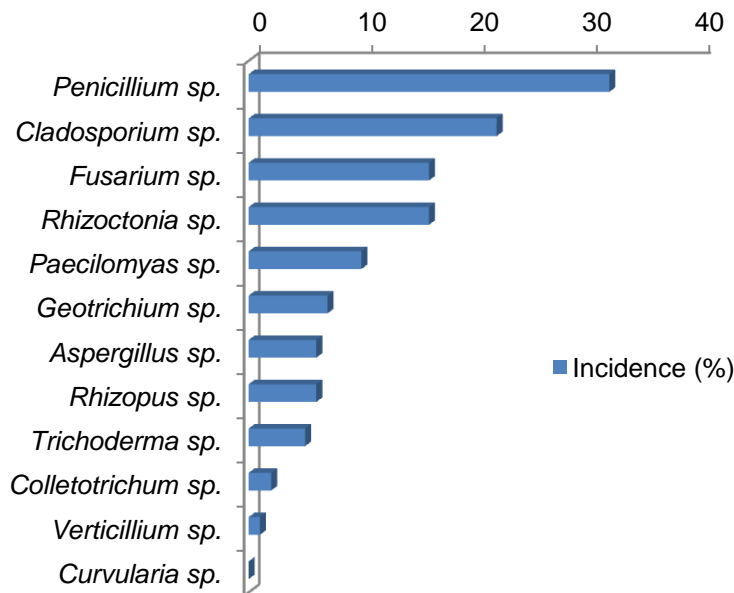
Also in Fig. 1 it is possible to verify the percentage of incidence of each fungus, present in the seeds not treated with the pyroligneous extract: *Penicillium* sp. (32%); *Cladosporium* sp. (22%), *Fusarium* sp. (16%) and *Rhizoctonia* sp. (16%) other fungi that occurred less frequently were *Paecilomyces* sp. (10%), *Geotrichum* sp. (7%), *Aspergillus* sp. (6%), *Rhizopus* sp. (6%), *Trichoderma* sp. (5%), *Colletotrichum* sp. (2%) and *Verticillium* sp. (1%).

It is noteworthy that the fungus *Cladosporium* sp., although of secondary importance, had an incidence above 22%. *Penicillium* sp., considered the main storage fungus, occurred in almost one third of the samples, probably due to seed storage time.

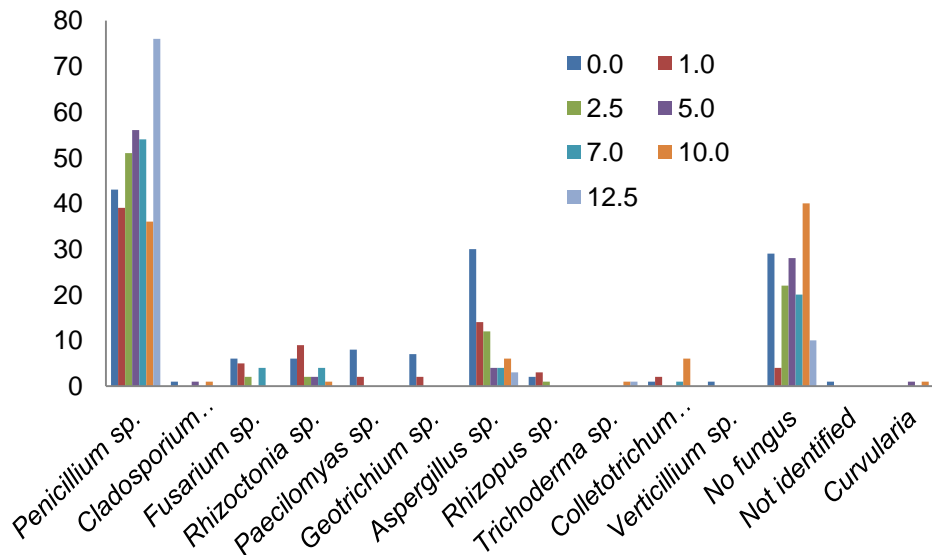
For Bedendo [28], among the fungal pathogens that attack seeds, deuteromycetes predominate, mainly the species belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Diplodia* and *Cladosporium*, these fungi are favored when the moisture content of the seed is around 25%, as also verified in the results of this study.

### 3.2 Effect of Pyroligneous Extract Treatment

Regarding the effect of pyroligneous extract treatment on seeds, it was found in Figs. 2 and 3, that no significant difference was found in the incidence of fungi compared to the untreated seeds presented in the previous figure. It is also possible to observe that the concentrations did not interfere in the incidence of the general fungal microbiota on *S. amazonicum* seeds.



**Fig. 1. Incidence of fungi (%) in *Schizolobium amazonicum* seeds without previous treatment with pyroligneous extract**



**Fig. 2. Incidence (%) of pathogenic fungi after each previous treatment of *Schizolobium amazonicum* seeds with pyroligneous extract. The colors indicate the concentration of pyroligneous extract (%) used for seed treatment**

It is noteworthy that *Curvularia* sp. was verified by attacking these treated seeds, even in only two samples. This organism, considered secondary, was probably infesting some seeds used in the experiment.

However, other pathogens had their incidence significantly reduced, such as *Fusarium* sp.,

*Rhizoctonia* sp. and *Aspergillus* sp. Similarly, [29], using pyroligneous extract of *Tectona grandis*, reported control of *Ceratocystis fimbriata*. According to Kumar et al. [30], pyroligneous oak extract effectively inhibited the germination of *Coleosporium plectranthi* urediniospores. Loo et al. [31] also reported efficient management of several pathogens that

attacked *Rhizophora apiculata* with the use of pyroligneous extract. Also, Furtado et al. [32] found that pyroligneous extract totally inhibited the mycelial growth of *Botrytis cinerea*, *Cylindrocladium clavatum* and *Rhizoctonia solani* in *Eucalyptus* sp. Macedo et al. [33] reported that this product showed efficient inhibitory capacity on mycelial growth of *Pestalotia* sp.

Such behavior is attributed to the composition of the pyroligneous extract, which presents numerous compounds [15], which may have negative effects on the mycelium advancement on the culture medium, compounds that are not found in the P.A. alcohol, base of the hydroalcoholic solution.

Thus, according to these results and also others published in the literature, it is possible to verify that the product has potential for use. In addition, research in other countries and field observations show that pyroligneous extract also repels certain types of pests and prevents certain diseases, including reducing pesticide dosage [30,34].

This information, together with the results of this research may make the use of pyroligneous extract promising for future research, allowing this product to have potential as a phytosanitary management tool, with agroecological bias.



Fig. 3. Incidence of phytopathogenic fungi in *Schizolobium amazonicum* seeds

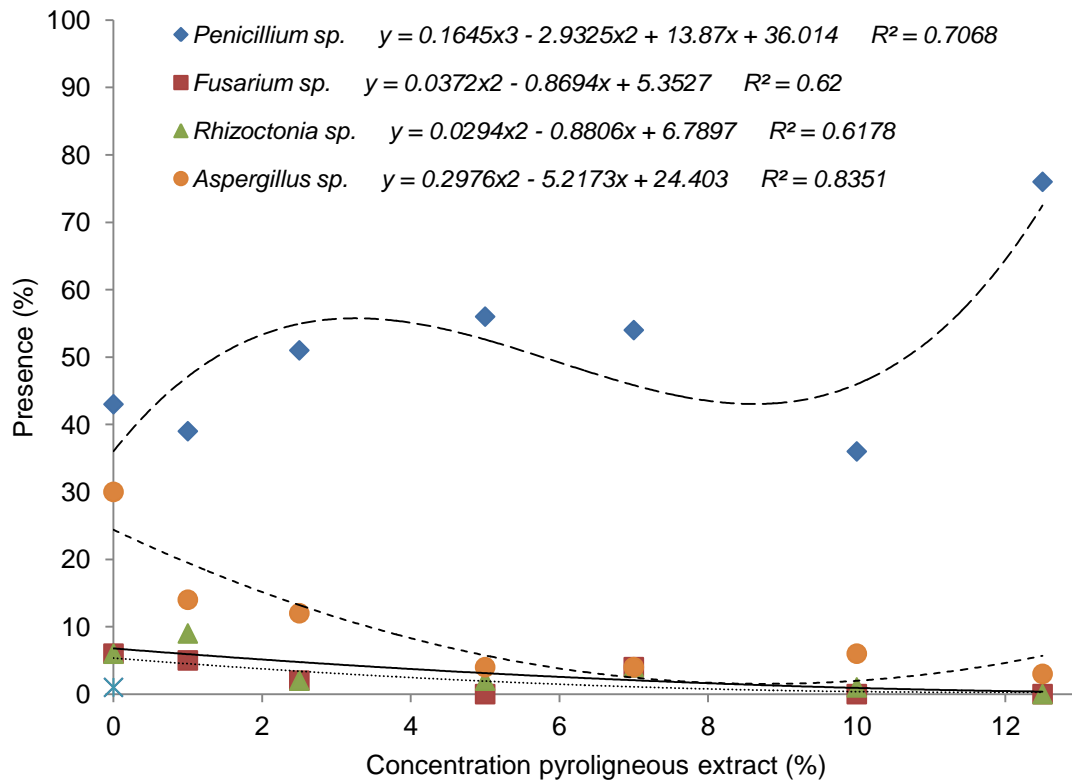


Fig. 4. Presence (%) of different pathogens on *Schizolobium amazonicum* seeds previously treated with increasing concentrations of pyroligneous extract

The control of *Rhizoctonia* sp. and *Fusarium* sp. [35] is very important for the establishment of field crops. These pathogens can cause much damage and have the ability to infect a wide range of hosts [36]. Its control is highly desirable and is not always achieved even with the use of agrochemicals. Reis et al. [37] warn about the importance of controlling this pathogen in common bean seeds and the potential damage if it is inefficient.

Regarding the treatment with the different concentrations of pyroligneous extract (1%, 2.5%, 5%, 7%, 10% and 12.5%) in relation to the control, the pathogens *Fusarium* sp., *Rhizoctonia* sp. and *Aspergillus* sp. (Fig. 4). It is possible to verify that the used concentrations were efficient in the control of these fungi, whose remaining percentages after treatment were all less than 10%.

Studies developed with crude extract and essential oil, obtained from medicinal plants, have indicated their potential for controlling phytopathogens [38], both for their direct fungitoxic action, inhibiting mycelial growth and germination. spores and phytoalexins induction [39].

Although this research has studied the efficiency of pyroligneous extract on a single species, with only a few concentrations, it was sought to analyze the efficiency of this process, so that without many laboratory apparatus or complex techniques, the practice could be viable and reproducible to small farmer.

And the results proved the potential use of pyroligneous extract for some fungal species that occur frequently in seeds of forest species.

These results reinforce what the literature has already shown, that the product is recognized for being repellent and insecticide against insects that attack several species that are commercially exploited in the fields [16]. In addition, it has been found to be an antimicrobial agent for wood fungus and as a weed control agent [40,41].

#### 4. CONCLUSION

Pyroligneous extract is efficient in reducing the incidence of important soil phytopathogens, commonly present and disseminated by seeds,

represented by the genus *Fusarium* sp. and *Rhizoctonia* sp.

The extract has potential to control storage fungi such as *Aspergillus* sp. occurring in the seed samples of *Schizolobium amazonicum*.

*Penicillium* sp. is not controlled by the treatment of *S. amazonicum* seeds with pyroligneous extract up to 12.5% concentration.

Further studies are needed to confirm the potential use of pyroligneous extract in alternative control.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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