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## Metrodorea flavida (K. Krause) Extracts in Pathogen Control of Senegalia polliphyla (Dc.) Seeds

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### Authors' contributions

This work was developed with the collaboration of all authors. Authors ESM and JG prepared the practical work, performed the statistical analysis, wrote and submitted the paper. Author MDSFP collaborated in the statistical analysis, in reviewing the manuscript and translation of the paper. All authors read and approved the final manuscript.

#### Article Information

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**Original Research Article** 

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### ABSTRACT

This study aimed to determine the main pathogens present in *Senegalia polliphyla* (DC.) seeds and to evaluate the fungicidal potential of *Metrodorea flavida* (K. Krause) extracts. For this purpose, concentrations of 0.5, 1.0, 1.5 and 2.0% of the extract of *Metrodorea flavida* leaves were used, in an ethanolic or aqueous form. The experiment was carried out using the Blottertest method to assess the incidence of pathogens in seeds and after application of treatments. Thus, the effect of extracts on the incidence of pathogens and the effects of the extracts on the germination and morphological characteristics of the seedlings was analyzed. The main pathogens found in *Senegalia polliphyla* seeds were *Aspergillus flavus* and *Fusarium oxysporum*. The aqueous and ethanolic extracts reduced the infestation of pathogens associated with *Senegalia polliphyla* seeds, as well as the main fungus found: Aspergillus flavus Link. It was also found that the extracts did not influence the germination and morphological characteristics of senegalia polliphyla seedlings in all concentrations evaluated, thus being a promising alternative for pathogen control in forest seeds.

Keywords: Forest seeds; phytopathology; phyto-fungicides; Rutaceae.

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#### **1. INTRODUCTION**

The demand for wood and forest products has increased in recent years in Brazil and as a result there has been an increase in reforested areas with fast-growing species [1]. Thus, studies with native forest species have grown significantly, seeking to expand the option of species for planting in different regions of Brazil. *Senegalia polliphyla* (DC.) (Fabaceae) has a great potential in silviculture, however, there are few researches about the silviculture in general of this species, and mainly on seed technology, with seeds being the main form of propagation of native forest species in Brazil [2].

One of the main problems in the production of forest seedlings is the attack of pathogens, mainly fungi and bacteria in the seeds, preventing or interfering with germination and seedling development. Thus, to have a good quality seedling production, it is necessary that the seeds have sanitary quality, since the pathogens cause irregularities and lesions in the seedlings and may deteriorate the seeds [3]. During germination the biggest problems in the seeds are caused by fungi [4], so, the production of forest seeds is essential for the production process. however. it presents sanitarv restrictions, due to the large number of pathogens related to seeds [5].

To prevent problems with pathogens, the seeds can be treated with chemical products. However, in recent decades, society is rethinking the intensive and uncontrolled use of phytosanitary products that generate several problems to the environment, such as contamination of soil, water, animals and food intoxication of producers and farmers and removal of microorganisms responsible for the degradation of organic matter [6]. In view of these situations, the search for alternative and efficient methods for controlling pathogens in seeds that provide less impact to the environment has grown in recent years [7]. The use of plant extracts to control pathogens in seeds can be an alternative with less environmental impacts and also contribute to reduce expenses with commercial phytosanitary products [8].

In Brazil, there is a great demand for new antifungals, due to the high resistance of pathogens in forest seeds. There are several studies demonstrating that the use of extracts obtained by plant species has been efficient in controlling pathogens in forest species, due to their direct fungitoxic action [9]. Results of research with plant extracts to control pathogens have been promising among the other methods already used, but plant extracts act more satisfactorily when the mode of action of the active principles and the pathogen are known [10].

Considering the diverse flora of Brazil, the possibility of discovering new compounds with fungicidal action is significant, especially with species from the Rutaceae family, which has several secondary metabolic processes identified in plant protection. In light of the above, the present study aims to evaluate the fungicide potential of *Metrodorea flavida* (K. Krause) (Rutaceae) extracts in pathogens associated with *Senegalia polliphyla* seeds.

#### 2. MATERIAL AND METHODS

The study was conducted in the Cytogenetics and Tissue Culture Laboratory and in the Microscopy Laboratory of the State University of Mato Grosso - UNEMAT, in Alta Floresta, in the state of Mato Grosso, Brazil. The evaluated extracts were obtained from *Metrodorea flavida* leaves collected in the municipality of Alta Floresta, in the state of Mato Grosso, Brazil.

For the preparation of ethanolic and aqueous extract, the plant material was dried in a forced air circulation oven at 65°C for 72 hours and ground in a Wiley type knife mill. Then, the ground material was weighed, and 100 grams of vegetable material powder was diluted in 500 ml of distilled water or 92.8% alcohol, thus obtaining a concentration of 20% (w/v). Subsequently, the solutions were stored in glass wrapped in aluminum foil to protect from the light, at room temperature for 72 hours. After this process, the material was filtered, stored in closed containers at 4°C and protected from light, until used. The concentrations 0.5%, 1.0%, 1.5% and 2.0% were obtained by dilution from the initial solution of 20%.

To perform the sanity test, one thousand seeds were used, of which four hundred were separated for each extract, aqueous or ethanolic, with four repetitions of 25 seeds for each treatment. One hundred seeds were also separated for the blank test and the control with fungicide (Dicarboxyde). The treatments consisted of *Metrodorea flavida* extract, in an aqueous or ethanolic form, in different concentrations: 0.5, 1.0, 1.5 and 2.0%. The

seeds were immersed in the different concentrations of ethanolic or aqueous extract, for fifteen minutes, then dried at room temperature on a sterile filter paper for 30 minutes. The Blottertest method was used to evaluate the incidence of fungi in seeds.

After the application of the treatments, the seeds remained for seven days in BOD chamber at 25°C, and 12-hour photoperiod. Subsequently, the pathogens were identified by visualizing them with the naked eye, as well as counting the number of seeds contaminated by each pathogen in each treatment. The pathogens were removed using adhesive tape and with the use of blue cotton dye, the slides were assembled. After this process, the pathogens were identified using the optical microscopic. The identification was carried out in a morphological way, with the help of specialized materials of Barnett and Hunter [11] and Menezes and Oliveira [12].

To evaluate whether the extracts could interfere with germination and seedlings, a germination test was also performed. One thousand seeds were also used, distributed in four replicates of twenty-five seeds, with four hundred seeds to evaluate each extract (aqueous or ethanolic) and one hundred seeds for control. The seeds were distributed on two sheets of germitest paper. moistened with distilled water equivalent to 2.x the mass of the non-hydrated paper and overlaid on a third sheet of germitest. Then, they were rolled, packed in plastic bags and placed in the BOD chamber at 25°C with 12-hour photoperiod for seven days [13]. In the germination test, the final germination and the following morphological characteristics of seedlings were evaluated: stem diameter (mm), root and shoot length (mm) and green weight (g).

The experimental design used for the evaluations was the factorial (type of extract X concentrations) completely randomized with four repetitions per treatment, with analysis of variance comparison of the means by the Tukey test (P = .05) and normality analysis of the data. The data that did not presented normality (incidence of pathogens) were transformed by the arcsen formula  $\sqrt{x}/100$ . The analyses were performed by the statistical package SISVAR 5.6 [14].

### **3. RESULTS AND DISCUSSION**

The contaminant fungi of *Senegalia polliphyla* seeds found in the health test are listed in Table

1. It was observed that the species *Fusarium oxysporum* (18.7%) and *Arpergillus flavus* (2.3%), were the pathogens with the highest occurrence (Table 1).

The genus *Fusarium* sp. is considered one of the most important genera to be studied, because it presents taxonomic difficulties and has a great variability in its characteristics that hinder its classification [15]. *Aspergillus flavus* deteriorates seeds and are producers of mycotoxins that are toxic to plants, animals and humans [16]. *Fusarium oxysporum* can also produce mycotoxins, causing a decrease in germination, discoloration or formation of stains, and rot of seeds [17].

The genera Aspergillus sp. and Fusarium sp. are common pathogens in seeds, occurring in several forest seed species, mainly when stored for long periods, thus affecting germination. These pathogens have already been found in seeds of: Phaseolus vulgaris (L.), Vigna Mimosa caesalpiniaefolia unguiculata (L.), (Benth.), Arachis hypogaea (L.), Araucaria angustifolia (Bertol.), Senna macranthera (DC. ex Collad.), Cedrela fissilis (Vell.), Ligustrum japonicum (Thunb.), Handroanthus serratifolius (Vahl) and Pinus insularis (Endl.) [18,19,20,21,22].

Analyzing the effect of *Metrodorea flavida* extracts in the incidence of pathogens in *Senegalia polliphyla* seeds (Table 2). It was verified that there was no interaction between the factors analyzed (concentration X type of extract), so the two extracts were analyzed separately for the total incidence of pathogens and the main genera or species identified (Tables 3 and 4).

The treatments with the extracts decreased the incidence of pathogens attack in the seeds, with no statistical difference between the four extracts evaluated (Table 2). There are several studies that prove the efficiency of plant extracts in the control of pathogens, however, the majority of research is caried out with medicinal and aromatic plants and only few of them are performed with forest species. For example, it was analyzed the effects of melon extracts (Momordica charantia L.) and Allamanda blanchetti L. on the incidence of pathogens in seeds of Enterolobium contortisiliquum (Vell.), Manihot glazioviiv (Muell.) and Caesalpinia pulcherrima (L), which showed a fungitoxic action, inhibiting the rate of pathogens on the

seeds of these species [23,24,25]. Extracts of *Momordica charantia* also caused a significant decrease in pathogens of the genera *Fusarium* sp., *Cladosporium* sp. Link, *Curvularia* sp. Boedijn and *Alternaria* sp. Nees Von Esenb.Ex Fries, influencing the high germination index of *Pterogyne nitens* (Tul.) seeds [26].

Other plants commonly studied that present fungicide action in seed pathogens are garlic (*Allium sativum* (L.)), ginger (*Zingiber officinale* Roscoe) and rue (*Ruta graveolens* L.), effectively in controlling mainly *Aspergillus* sp. and *Fusarium* sp. [8,17,27,10].

A forest species that has been studied as a possible fungitoxic is *Corymbia citriodora* Hook, mainly through its essential oil, being efficient in controlling the development of pathogens and inhibiting its growth [8]. The pathogen with the highest incidence in *Senegalia polliphyla* seeds was *Fusarium oxysporum* and the effect of the extracts on its occurrence are presented in Table 3.

All concentrations used for both aqueous and ethanolic extract decreased the incidence of *Fusarium oxysporum* in *Sengalia polliphyla* seeds (Table 3). Again, it is noted that there was no significant difference between the four concentrations analyzed, and none of them reached the control values. However, there was a significant reduction compared to the blank test. Since the concentrations used in this study are considered low, higher concentrations may cause more significant results.

Plant extracts have already been shown to be efficient in inhibiting pathogenic fungi in seeds, mainly the genus *Fusarium* sp. Ethanolic extract from all parts of *Senna alata* (L.) Roxb. is able to control the pathogen *Fusarium oxysporium* [25]. The ethanolic extract of *Allium sativum* has an inhibitory action on *Fusarium oxysporum* [27] and the ethanolic extract of *Pachira aquatica* (Aubl.) seeds is able to inhibit the development of the pathogen *Fusarium* [28].

Analyzing the effects of extracts on germination and morphological characteristics, it is possible to note that both aqueous and ethanolic extracts of *Metrodorea flavida*, did not interfere in the germination or development of *Senegalia polliphyla* seedlings (Table 4).

Extracts of plant species may have different influences on seed germination. The aqueous extract of *Sorghum bicolor* (L.) did not interfere in the germination of *Glycine Max* (L.) plants [29], similar to the results observed in this study (Table 4). Ethanolic extracts, when compared to aqueous extracts, drastically reduced the germination of *Brachiaria decumbens* (Trin.) seeds [30].

Genera	Incidence (%)
Fusarium oxysporum Schltdl.	18.7
Aspergillus flavus Link.	2.3
Penicillium sp. Link	0.8
Rhizoctonia DC.	0.6
Trichoderma Persoon ex Gray	0.6
Fusarium moniliforme J.Sheld.	0.1

Table 1. Contaminant fungi and their incidence (%) in Senegalia polliphyla seeds

# Table 2. Effect of Metrodorea flavida extract on pathogen incidence (%) in Senegalia polliphyla seeds

Concentration (%)	Ethanolic	Aqueous	
0 (T1)	0.94 c	0.94 c	
0,5 (T2)	0.39 b	0.54 bc	
1,0 (T3)	0.30 b	0.38 b	
1,5 (T4)	0.39 b	0.39 b	
2,0 (T5)	0.43 b	0.39 b	
Control (T6)	0.0 a	0.00 a	

Means followed by the same lowercase letter in the column, do not differ statistically by Tukey test (P = .05). Concentration: (T1) Blank test, (T2) Treatment with 5% concentration, (T3) Treatment with 10% concentration, (T4) Treatment with 15% concentration, (T5) Treatment with 20% concentration and (T6) Control with Dicarboxyde Fungicide. Data was transformed by the arcsen formula  $\sqrt{x}/100$ 

Concentration (%)	Ethanolic	Aqueous			
0.0 (T1)	52.00* c	52.00 c			
0.5 (T2)	21.00 b	23.00 b			
1.0 (T3)	18.00 b	15.00 b			
1.5 (T4)	18.00 b	16.00 b			
2.0 (T5)	19.00 b	15.00 b			
Control (T6)	0.00 a	0.00 a			
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 Table 3. Effect of Metrodorea flavida ethanolic and aqueous extracts on Fusarium oxysporum incidence in Senegalia polliphyla seeds

\*Incidence (%) of pathogens

Means followed by the same lowercase letter in the column, do not differ statistically by Tukey test (P = .05). Concentration: (T1) Blank test, (T2) Treatment with 5% concentration, (T3) Treatment with 10% concentration, (T4) Treatment with 15% concentration, (T5) Treatment with 20% concentration and (T6) Control with Dicarboxyde Fungicide

# Table 4. Morphological characteristics of Senegalia polliphyla seedlings submitted to aqueous and ethanolic extracts of Metrodorea flavida

Aqueous extracts								
Concentration	Germination	Root collar	Root	Aerial part	Green weight			
(%)	(%)	(mm)	(cm)	(cm)	(g)			
0.0 (T1)	63.00 a	1.45 a	4.53 a	4.08 a	0.33 a			
0.5 (T2)	58.00 a	1.07 a	4.26 a	3.02 a	0.29 a			
1.0 (T3)	58.00 a	1.13 a	4.40 a	3.76 a	0.30 a			
1.5 (T4)	54.00 a	1.04 a	4.41 a	3.58 a	0.31 a			
2.0 (T5)	56.00 a	1.06 a	3.74 a	3.84 a	0.28 a			
Control (T6)	48.00 a	1.06 a	2.86 a	4.03 a	0.28 a			
Ethanolic extracts								
Concentration	Germination	Root collar	Root	Aerial part	Green weight			
(%)	(%)	(mm)	(cm)	(cm)	(g)			
0.0 (T1)	63.0 a	1.45 a	4.53 a	5.08 a	0.33 a			
0.5 (T2)	68.00 a	1.08 a	3.65 a	4.64 a	0.34 a			
1.0 (T3)	65.00 a	1.16 a	3.60 a	4.01 a	5.33 a			
1.5 (T4)	63.00 a	1.05 a	3.68 a	4.01 a	0.29 a			
2.0 (T5)	68.00 a	1.07 a	3.64 a	3.79 a	0.29 a			
Control (T6)	58.00 a	1.06 a	3.86 a	4.34 a	0.28 a			

Means followed by the same lowercase letter in the column, do not differ statistically by Tukey test (*P* = .05). Concentration: (T1) Blank test, (T2) Treatment with 5% concentration, (T3) Treatment with concentration 10%, (T4) Treatment with concentration 15%, (T5) Treatment with concentration 20%o and (T6) Control with Dicarboxyde Fungicide

*Cinnamomum zeylanicum* Bl. extract reduced the germination and vigor of castor bean seeds (*Ricinus communis* L.), while *Zingiber officinale* extract did not affect the quality of castor bean seeds [31].

Thus, it is noted that studies focusing on the influence of plant extracts and their concentrations on germination and morphological characteristics, are extremely necessary to verify their possible use. Some substances from plant extracts can be toxic at high concentrations and beneficial or stimulants at low concentrations and may also be influenced by the part of the plant used in the preparation of the extract [32,33,34].

Thus, it was observed that the extracts of *Metrodorea flavida* did not influence the germination or morphological characteristics of *Senegalia polliphyla* seedlings, and provided a decrease in pathogen infestation even at low concentrations, presenting a potential use as an alternative form of pathogen control in forest seeds.

#### 4. CONCLUSION

The main pathogens found in *Senegalia polliphyla* seeds were *Aspergillus flavus* and *Fusarium oxysporum*.

Ethanolic and aqueous extracts showed good results in the inhibition of pathogens in *Senegalia polliphyla* seeds, even at low concentrations.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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