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Clove Extract (*Syzygium aromaticum*) in Germination and Sanity of Catingueira Seeds (*Poincianella pyramidalis* (Tul.) L.P. Queiroz)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EPA, LGC and GJCS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NAA managed the analyses of the study. Author MJHL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research aimed to evaluate the effects of Clove extract (*Syzygium aromaticum*) on germination and sanity of Catingueira seeds (*Poincianella pyramidalis*). Five treatments with Clove extract of India were applied at the following concentrations T1 (Control) = 40 mL of sterile H_2O, T2= 10 mL extract + 30 mL of sterile H_2O, T3= 20 mL extract + 20 mL of sterile H_2O, T4= 30 mL extract + 10 mL of sterile H_2O and T5= 40 mL of extract. *P. pyramidalis* seeds were immersed in due concentrations for 30 seconds, then placed in sterilized Petri dishes and taken to isolation chapel. The sanity test lasted seven days, after this period, the associated microorganisms were evaluated in the seeds. For germination tests, the same treatments used for the health test were used. At the end of the experiment the percentage of germinated seeds, the Germination Speed Index (IVG),

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Mean Germination Time (TMG), Mean Germination Speed (VMG) and the length of part area and root were calculated. Clove extract (*Syzygium aromaticum*) showed no toxic effect on germination and development of *Poincianella pyramidalis*. It was indicated the concentration of 25 and 50% of the extract, because it provided a higher germination percentage, IVG, VMG and TMG to seeds of the species *Poincianella pyramidalis*. Further studies with immersion time of the seeds higher than those of this research are suggested.

Keywords: Forest pathology; propragative material; sanitary quality.

1. INTRODUCTION

Seeds are one of the main sources of propagules since they come from high-quality genetic material, however they are susceptible to the attack of pathogens throughout their life cycle.

Studies have shown that both the seeds collected in the tree top as well as the seeds collected in the soil under the canopy of the "mother plant" bring prisoners in their structure several inoculates [1] if they do not undergo sanitary processes, they can in some cases be transmitted to the plant themselves and result in damage from seed degradation, transmission of pathogens.

The constitution of the seeds, for example, high levels of proteins, carbohydrates and minerals [2] provides the necessary conditions for colonization, permanence and consequently the development of the pathogens. Inin this way the sanitary measures become basic and strategic actions for the control of fungi and the production of quality seedlings.

Sanitary control of propagative material can be performed by means of chemical treatments [3] physical, such as thermotherapy, by osmotic conditioning [4,5], through biological means, through products produced from the isolation of fungi [6] and through the activity of secondary metabolites? Compounds produced by the plants [7,8,9].

The use of secondary metabolites products in the control of fungi associated with various species, as well as the affect of these products on seed germination has become increasingly relevant since the use of synthetic products when uncontrolled use can result in various effects on the environment. Examples of these damages are the release of undesirable compounds in the soil and the development of plant resistance to inoculum [10].

The use of secondary compounds is still incipient, but positive results have already been reported in several studies, such as the work of Verturoso et al. [11], which evaluated the efficiency of garlic aqueous extracts (*Allium sativum* L.), cinnamon (*Cinnamomum zeylanicum* Breym) and Clove (*Syzygium aromaticum* (L.) Merr & Perry), on the development of *Fusarium solani*, among other works [12,13,14]. These compounds act through their elicitor activity, triggering the synthesis of phytoalexins, which are toxic to the pathogen, inhibit mycelial growth and germination of spores [15]. In addition to reducing the incidence of pathogens, these secondary metabolites? compounds can act on seed germination by positively affecting it.

Among the main fungi that attack the seeds a re those of the genus Aspergillus. Their presence is reported in several studies, considered as one of the main fungi of seed storage that are potentially harmful, because in addition to causing the deterioration of propagative material, fungi belonging to this genus can survive at low temperatures and thus have their microflora preserved along the seeds throughout the storage period [16].

Thus, the importance of seed collection at the appropriate time is supported reinforced with all stages actions related to the process of seed technology performed correctly, such as the collection in matrices that present good genetic quality, exact drying, and processing and storage within the standards required for each seed group so that the effect of fungi attack is minimized.

The use of plant extracts to control seed-associated fungi is an alternative to reduce the use of chemicals. Therefore, this research has as main proposal to evaluate the use of clove extract in the germination and health of seeds of Catingueira (*Poincianella pyramidalis* (Tul.) LP Queiroz.) And to provide subsidies for future work in this field. same line of research.

2. METHODOLOGY

The experiment was carried out at the Forest Pathology Laboratory, forest engineering course, the Center for Health and Rural Technology of the Federal University of Campina Grande, Patos-PB Campus. Catingueira seeds (*P. pyramidalis*) were collected in August 2018 in the district of Iara-Ceará.

The sanity test was performed using the filter paper method "BlotterTest" where 500 seeds were used for the experiment, which underwent a pre-selection, in order to identy seeds free from visible damage.

Five treatments with Clove extract (*Syzygiuma aromaticum*) were applied at the following concentrations T1 (Control) = 40 mL of sterile H_2O, T2= 10 mL extract + 30 mL of sterile H_2O, T3= 20 mL extract + 20 mL of sterile H_2O, T4= 30 mL extract + 10 of sterile H_2O and T5= 40 mL extract. In order to determine the concentrations to use in these treatments, the volume of sterile water to cover a sample of 100 *P. pyramidalis* seeds (T1) and proportional to the other treatments were determined. The hydroalcoholic extract of Clove (*S. aromaticum*) was obtained according to Henry's methodology [17].

P. pyramidalis seeds were immersed in due concentrations for 30 seconds, then placed in sterilize Petri dishes and taken to an isolation facility, previously disinfected. On each plate, 3 sheets of sterile filter paper and moistened with sterile water were arranged. At the end of the process, the plates were placed in the incubation chamber for fungi at room temperature.

The sanity test lasted seven days, after this period, the associated microorganisms were evaluated in the seeds with the aid of a stereomicroscope. Slides with fungal structures were prepared and analyzed with the aid of an optical microscope, as described by Barnett and Hunter [18] when it was not possible to identify the organisms using the sanity test.

For germination tests, the same treatments used for the health test were used. Subsequently, the seeds were sown seded in 15 cm x 10 cm x 4.5 cm plastic trays previously sterilized with 700 GL alcohol, containing sterile sand substrate moistened with distilled water. The germination test was conducted in a germination camera at room temperature and was closed on the 17th day when the germination process stabilization was observed.

At the end of the experiment, the percentage of germinated seeds, the Germination Speed Index (IVG), Mean Germination Time (TMG) and the Mean Germination Speed (VMG) and the length of part area and root were calculated with assistance graduated rule.

The statistical design used was randomized (IHD) with 5 treatments, 4 repetitions and 25 seeds in each repetition, where the results as a percentage were transformed into arc sen $\sqrt{x}/100$ and the means were compared by the Tukey test to one significance level with the aid of the Statistical Program Sisvar [19].

3. RESULTS AND DISCUSSION

The germination process of *P. pyramidalis* seeds was affected by the different concentrations of *S. aromaticum* (Table 1). Statistical significant differences were verified for the variables: Germination (%), Germination Speed Index (IVG), Mean Germination Time (TMG) and Mean Germination Speed (VMG) except for shoot and root length.

All treatments resulted in an increase in germination of *P. pyramidalis* seeds, therefore, *S. aromaticum* extract exerted a stimulating effect on seed germination. The best results were obtained when 25 and 50% concentrations of *S. aromaticum* extract were used, which resulted in an average germination percentage of 83 and 85%, respectively.

Results similar to this study were reported by Medeiros et al. [20]. These authors evaluated the aqueous extract of São Caetano melon (*Momordica charantia* L.) and Alamanda (*Allamanda blanchetti* A. DC.) in physiological quality and the reduction of the incidence of fungi associated with monkfish seeds (*Enterolobium contortisiliquum* (Vell.) Morong.) The extract of *M. charantia* when used in concentrations of 500 and 1000 ppmprovided the increase in germination and first count. Bressan et al. [12], evaluated the potential of essential oils in germination and in the control of *Rhizoctonia* sp. *In vitro* germination of seeds of Angico-Vermelho (*Rigid parapiptadenia* (Benth) Brenan) and reported that the oil of Melaleuca (*Melaleuca alternifolia*) provided the highest percentage of germination among the different types of

essential oils tested and concomitantly reduced the development of *Rhizoctonia* sp.

Research conducted showed the positive effect of secondary products on the increase in germination percentage and sanitary quality of seeds [21,22,23,24]. The products of secondary order have an advantage over for their implementation in the sanitary control of seeds because they are sustainable, cause less damage to the environment, and in addition to being less costly, are easy to acquire because they come from natural products.

For the variable IVG, the best result was obtained when 25% of the extract was used that enabled IVG of 5.36, but none of the concentrations tested reduced the IVG for the seeds of *P. pyramidalis*, because all the concentrations used allowed to express greater vigor than that expressed by the seeds present of the control treatment.

For the variable VMG the best results were obtained when 25 and 50% of the extract of *S. aromaticum* was used, at this same level of comparison to the TMG variable which was also positively affected when the concentrations of 25 and 50% of the extract were used, because it promoted the germination of *P. pyramidalis* seeds in a shorter time in relation to the control.

It was noticed that, when using 75% concentration of the extract, MGT was statistically equal to the control and the increase in the concentration of the extract to 100%, significantly affected the variable mentioned above because it resulted in lower VMG and? the higher TMG among the treatments evaluated.

In general, it is expected that the methods used in the control of pathogenic organisms or to overcome the dormancy of seeds of certain groups of species, are able to enable the seeds, the highest germination percentage, linked to the highest vigor expression through the IVG variable, higher germination speed (VMG) and that the IVG and VMG are expressed in a lower possible TMG among the treatments tested because a slow germination predisposes plants to weather-imposed as possible stresses, less possibility of competition for resources due to lower growth.

Table 2 shows the results for the microflora of fungi found associated with *P. pyramidalis* seeds treated with different concentrations of Clove extract (*S. aromaticum*).

The microflora found in *P. pyramidalis* seeds is composed of fungi of the genera *Aspergilus niger* and *A. glaucous,* which were in higher percentages of incidence in the different concentrations of ginger extract used in this experiment than by fungi of the genera *A. flavus, Dispora, Trichoderma, Alternaria* and *Botrytis* that were in lower percentages.

The microflora found associated with *P. pyramidalis* seeds is in accordance with the results found by Costa et al. [25] where fungi of the genus *A. niger, A. glaucous and A. flavus* were found in a higher percentage of incidence associated with seeds of (ingazeira) *Lonchocarpus sericeus* (Poir.) DC than or compared to Nascimento et al. [26] reported that *A. niger* were also found in higher percentages of incidence in four tree species of the Caatinga biome, compared to work by Medeiros et. al. [20] also showed similar results to those of this study where *A. niger* and *A. flavus* were found associated with the seeds of (monkfish) *Enterolobium contortisiliquum* (Vell.) Morong. in higher percentages in different seed lots evaluated, compared to.

Table 1. Average germination percentage (%) germination speed index (IVG), average germination speed (VMG) and average germination time (TMG) of *Poincianella pyramidalis* seeds treated with *Syzygium aromaticum* extract

Treatments	Germination (%)	IVG	VMG	TMG
Control	65 b	2,96 b	0,14 ab	6,93 ab
25% of extract	83 a	5,36 a	0,17 a	5,81 a
50% of extract	85 a	4,63 ab	0,16 a	6,14 a
75% of extract	80 ab	4,21 ab	0,15 ab	6,68 ab
100% of extract	79 ab	3,94 ab	0,13 b	8,04 b

**Different letters in the same column indicate significant differences among the different treatments according to Fischer's least significant difference test (p ≤ 0.05)*

Table 2. Incidence of fungi in *Poincianella pyramidalis* seeds treated with *Syzygium aromaticum* extract

Treatments	*A. niger*	*A. glaucous*	*A. flavus*	*Dispora*	*Trichoderma*	*Alternaria*	*Botrytis*
Control	67,0 b	33,0 a	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a
25% Clove extract	42,0 b	48,0 ab	4,0 a	3,0 a	4,0 a	0,0 a	0,0 a
50% Clove extract	50,0 b	41,0 ab	3,0 a	0,0 a	3,0 a	3,0 a	1,0 a
75% Clove extract	0,0 a	86,0 b	0,0 a	0,0 a	4,0 a	10,0 a	0,0 a
100% Clove extract	60,0 b	36,0 ab	0,0 a	0,0 a	0,0 a	3,0 a	0,0 a

**Means followed by the same letter in the columns are the same as each other by the Tukey test ($p<0.05$)*

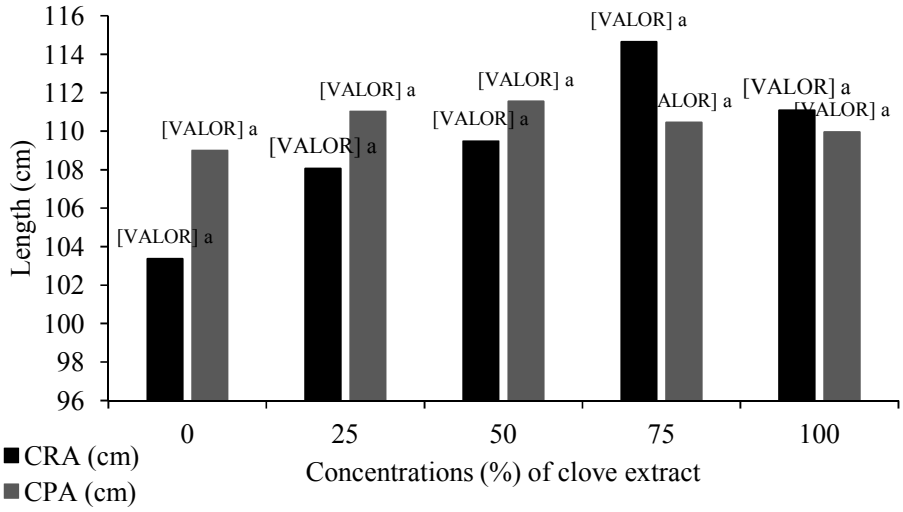

Fig. 1. Root and aerial shoot growth of seedlings from *Poincianella pyramidalis* seeds treated with *Syzygium aromaticum* extract

The results found in this study differfrom the results shown in the research by Souza et al. [27], and also in the research carried out by Bressan et al. [12] where fungi of the genus *Aspergilus* were lower in percentage of incidence, compared to *Aspergilus* is commonly found in studies evaluating the health of forest seeds and in agricultural seeds [28,29]. These fungi can contaminate seeds at various stages, i.e. from the pre-harvest of seeds to storage and are responsible for causing damage such as reduction of vigor and deterioration of propagative material [30].

The different concentrations of Clove extract used in this study were not able to reduce the incidence of *A. niger,* which is one of the fungi found in the highest percentage of incidence colonizing the seeds of *P. pyramidalis*. Comparing the treatment in which 75% of the Clove extract was used for *A. glaucus* with the treatment in which 100% of the extract was used, it is possible to perceive that there was a reduction in the incidence *A. glaucus*, but there were no statistical differences between this treatment and the control treatment.

For *A. flavus, Dispora, Trichoderma, Alternaria* and *Botrytis*, association was found with the seeds of *P. pyramidalis* and there was however no effect of the different concentrations used because the percentage of incidence of these fungi was low.

The positive effect of the substances used to treat the seeds may depend on the concentration of the substances, the time that these substances remain in contact with the seeds and

according to Machado [31], the location of the pathogens in seeds, because a group of pathogens can manifest themselves in the forms of contamination (when present in external structures and without activity) or infection (when infiltrated into the internal structures of the seeds). Since the fungus infiltrates the seed the activity of the substances used to treat the seeds becomes ineffective because phytopathogenic agents are already infiltrated in the form of mycelia.

Seed asepsis is due to the elimination of fungi associated with the external structures of seeds, such as saprophyte fungi [28], thus, it is necessary to emphasize that the conservation of the physiological quality of the seeds starts prior to storage, and is done with care in the choices of genetic matrices for seed collection, which must be of good sanitary quality, and the drying and processing activities suitable for quality materials and the production of posterior seedlings is performed with seeds that present ideal sanitary and physiological patterns.

There were no statistical differences between the different concentrations of *S. aromaticum* extract on the growth of shoot and root of *P. pyramidalis* seedlings (Fig. 1).

The morphology of *P. pyramidalis* seedlings had been poorly differentiated when treated with *S. aromaticum* extract, however none of the evaluated treatments reduced or inhibited the growth of the root system and shoot in *P. pyramidalis* seedlings. All evaluated treatments provided upper root and shoot growth in relation to the control, which further property the use of S. aromaticum extracts in *Poincianella pyramidalis* plant development and as an alternative method in seed sanity.

4. CONCLUSION

Clove extract (*Syzygium aromaticum*) showed no toxic effect on germination and development of *Poincianella pyramidalis*. It is indicated that the concentration of 25 and 50% of the extract, provides a higher germination percentage, IVG, VMG and TMG to seeds of the species *Poincianella pyramidalis*. Further studies with immersion time of the seeds higher than those of this research are suggested.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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