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Evaluation of the antifungal effect of oleoresin and essential oil of *Pterodon emarginatus* Vogel (Sucupira), *Anacardium occidentale* L. (Caju) and *Anacardium humile* (cajuzinho-do-cerrado)

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Abstract* — Oil from Pterodon emarginatus Vogel (or Sucupira), Anacardium occidentale L. (Caju) and Anacardium humile (cajuzinho-do-cerrado) in natura and their fractions wereevaluated for fungitoxic activities, against two species of filamentous fungi of the genusFusarium sp and Rhizopus previously identified by their macroscopic (photographic records) and microscopic (microcultivation) characteristics in Petri dishes. Resin oil andessential oil concentrations in the range of 10 to 50% were used for qualitative and quantitative analyses. The samples were placed on 5 mm diameter paper disks and distributed on potato, dextrose, and agar (BDA) medium in Petri plates, inoculated with spores of the microorganisms, and incubated at 28°C for 10 days. Only the BDA mediumwas used on the plate as a positive control. The qualitative results showed that the oil showed good activity, but one of the fractions of the essential oil of cajuzinho-do-cerradowas highly effective against the Fusarium fungus at the concentration of 10% in 24 h. presenting halo of inhibition of 0.05 ± 0.01 mm, and 0.06 ± 0.01 mm at the concentrationof 50% in 24 h. For the Rhizopus fungus, the cashew oil was the one that showed the best performance compared to the control in all concentrations studied during 48 hours, and in the 50% concentration had a maximum growth rate ($\mu(t)$) of 7.76 ± 0.01 mm, about 41% lower than the control.

I. INTRODUCTION

One of the largest biomes in South America, known as the Brazilian savannah, possesses a large biodiversity yet is vaguely exploited for the amount of its natural resources. Its abundant vegetation has predominant characteristics, such as trees with thick and twisted trunks, as well as grasses and bushes. The Cerrado is characterized by the presence of dry winters and rainy summers, a climate classified as Aw by Kôppen (rainy tropical) (WALTER, 2006). The threat of man in this biome is characterized by expansion of agricultural frontiers that advance in the Cerrado biome and compromising the native fauna and flora, also using, indiscriminately, the water resources(RIOS et al., 2013).

The Cerrado offers its populations a wide variety of products, which can be important allies in the promotion of sustainable livelihoods, where income generation andquality of life are in line with the conservation of natural resources (SAWER, et al. 1999).Essential oils, which can be extracted from various parts of plants, have different biological activities. The Brazilian Cerrado has a wide variety of plants that produce essential oils, although many have not yet been studied (ALVES, v. 80, n. 2, p. 290-294, 2020). Fungi of the genus *Rhizopus*, considered to cause great losses of grains under storage conditions, were the main responsible for the unviability of a great part of storedseeds, because they are located preferentially in the embryo (DHINGRA, 1985), while contaminations by *Fusarium* sp. occur during the formation or maturation of the fruit (MACHADO, 1988). The importance of using healthy seeds and good sanitary quality will have a good positive result on the crop.

The control of diseases and plagues in agriculture has intensified, being basicallyperformed through the use of synthetic chemicals. However, the use of pesticides has proven inefficient, since several organisms have shown increasing resistance to such products, requiring the use of increasingly larger quantities (SANTOS et al., 2004). The production of food with a minimum degradation of natural resources has become a demand of society, and there is a growing concern of the population to consumehealthy food with a production associated with the preservation of the environment, which has made the use of chemical agents a questionable practice. Thus, several alternative methods of disease control have been studied. Such alternative methods may provide biological control of phytopathogens and induction of resistance in plants. Among these, the use of essential oils and chitosan wrapping are highlighted (STANGARLIN et al., 1999; SCHWAN ESTRADA & STANGARLIN, 2005; CAPDEVILLE et al., 2002).

II. MATERIALS AND METHODS

Essential Oil Prospecting

To verify the potential of essential oils as fungicides, a selection of plants presents in the Cerrado biome, in the southern region of Tocantins in the city of Gurupi, was carried out. Among them, *Pterodon emarginatus Vogel* (or Sucupira), *Anacardium occidentale L.* (Caju) and *Anacardium humile* (cajuzinho-do-cerrado).

The seeds and cashew nuts were ground manually to increase the surface area of contact, seed-water, and then submitted to extraction. The seeds were weighed 200g, 1000 mL of water were added and placed in a round bottom flask and left to boil for 4 hours, controlling the temperature in approximately 100ºC. The extraction of the essential oil was performed in the CEMAF Laboratory of the Federal University of Tocantins in October 2020. The extraction method chosen was maceration, using acetone to obtain the acetonic extract.

Bioassays for fungicidal activity of essential oils

The fungal species evaluated in this study belonged to the mycoteca of the Bioactive Compounds Laboratory of the UFT, Gurupi Campus and were kept in Petri plates containing agar, potato dextrose (BDA) medium, stored at 4°C, with replication every 2 months and reactivated to perform the bioassays along with the essential oils of the species *Pterodon emarginatus Vogel* (or Sucupira), *Anacardium occidentale L.* (Caju) and Anacardium humile (cajuzinho-do-cerrado) as fungicide agents. (Caju) and *Anacardium humile* (cajuzinho-do-cerrado) as fungicide agents.

For this experiment pure oil was used at concentrations: 10, 20, 30 and 50%. As a control, BDA (Potato-Dextrose-Agar) medium without the addition of supplements wasused. Aliquots of oil were added to the melted BDA medium poured into Petri dishes. Inthe center of each plate, after medium solidification, a mycelium disk of *Fusarium sp* and*Rhizopus* fungi grown on BDA medium was placed individually, previously identified by their macroscopic (photographic records) and microscopic (microcultivation) characteristics on Petri plates, and the plates were incubated at 22 ± 2°C under a 12-hour photoperiod. The area of the irregular polygons formed by mycelial growth was calculated using Pick's theorem (Murty & Thaim, 2007), according to Equation

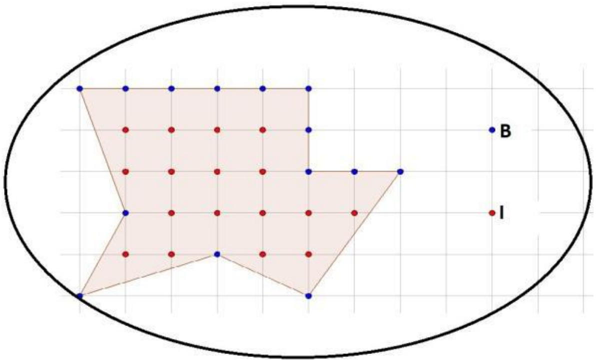
$$1.Pick(P) = \frac{1}{2}B + I - 1 \quad (1)$$

Where:

Pick(P) is the area of the irregular polygon formed by mycelial growth;

B is the number of points formed by the border image of a checkered grid overlaid on theplate containing the growing fungus;

I is the number of points inside this mesh.



The mycelial diameter of the irregularly formed colonies were calculated usingEquation 2.

$$D = 2\sqrt{\frac{A}{\Pi}} \qquad (2)$$

Where:

D is the diameter of the colony;

A is the area formed by the mycelial growth of the fungus;

Π is the value of the PI constant.

The experiment was performed in 3 repetitions for each concentration of essential oil in each strain and the specific growth rate was evaluated for each fungus in their respective media according to Equation 3, in an entirely randomized manner.

$$\mu(t) = \frac{Dd}{Ddt} \qquad (3)$$

Where:

$\mu(t)$ is the specific growth velocity (h^{-1}); t is the time (h) and D is the colony diameter (mm). The results of the specific growth velocities of each fungus and medium were subjected to analysis of variance (ANOVA) and Tukey's test at the 5% significance level for comparison of means. The radial and kinetic growth profile of each fungus was evaluated for the concentrations of essential oil in the culture medium.

III. RESULTS AND DISCUSSION

The effect of the concentrations of the essential oils in this study (sucupira, caju and Cajuzinho-do-Cerrado) on the mycelial growth of the Fusarium fungus was evaluated from the experiments in Petri dishes and the kinetics of the specific microbial growth speed was obtained. The tested concentrations are determinant factors in the growth, causing significant changes in the diameter of the colonies.

The results of the specific growth speed relative to the *Fusarium* microorganism within 96 hours are shown in Table 1, presented with the respective concentrations of the oils in the BDA culture medium, to be compared with the WITNESS, without the addition of oils. Significant differences (p-value<0.05) were detected by ANOVA and the comparison between the means of maximum growth rates by Tukey's test. Sucupira oil after 96 hours of observation showed significant difference only at 50% concentration compared to the control. The cashew and cajuzinho-do-Cerrado oils showed a significant reduction in the specific growth speed of the *Fusarium* fungus at the concentration of 50% immediately after 24 hours of inoculation, and also at the concentrations of 10, 20, 30 and 50% after 96 hours. This data is important to help establish the minimum growth time of the fungus after fungicide application.

Table 1 - Maximum specific speed of growth ($\mu(t)$) for Fusarium species at the different concentrations of the essential oils evaluated.

	Sucupira			Cashew			Cajuzinho-do-Cerrado		
Concentration	24h	48h	96h	24h	48h	96h	24h	48h	96h
10%	0.196^a	0.13^a	0.08^a	0.28^a	0.16^a	0.10^a	0.05^a	0.15^a	0.09^a
20%	0.218^b	0.13^a	0.08^a	0.28^a	0.16^a	0.10^a	0.13^b	0.16^b	0.10^b
30%	0.063^c	0.13^a	0.08^a	0.25^b	0.16^b	0.09^b	0.14^b	0.16^b	0.09^a
50%	0.229^d	0.12^a	0.07^b	0.28^a	0.15^a	0.10^a	0.06^a	0.16^b	0.09^a
Witness	0.072^e	0.04^b	0.09^a	0.16^c	0.16^a	0.17^c	0.10^c	0.10^c	0.10^b

The same letters in the column do not differ significantly by Tukey's test at 5% significance level.

The *Rhizopus* fungus reached maximum growth in 48 hours, totally colonizing the Petri dishes. The cashew essential oil had the best performance already in the concentration of 10% with significant differences (p-value <0.05) in relation to the control. COUTINHO et al. (1999) evaluated in their studies with hydroalcoholic extracts of cashew (*Anacardium occidentale L.*) and mastic (*Astronium urundeuva* Engl.) the fungicide efficiency in comparison to the chemical fungicides Benomyl® and Captan®, finding a higher efficiency for the chemical fungicides alone or in mixture, for the controlof the microflora associated to the bean seeds. They also concluded that the vegetal extracts only exercised a partial control of the fungus found; however with interference in the process of seed germination.

Table 2 - Maximum Specific Speed of Growth (μ(t)) for the Rhizopus fungus atdifferent concentrations of the essential oils evaluated in 48 hours.

Species	Oil Concentration in BDA medium			
	10%	20%	30%	50%
Sucupira	14.33^{a}	14.45^{a}	13.12^{a}	13.29^{a}
Caju	9.20^{b}	8.05^{b}	10.03^{b}	7.76^{b}
Cajuzinho-do-Cerrado	14.33^{a}	14.45^{a}	14.45^{c}	13.10^{a}
witness	12.74^{c}	12.59^{c}	13.11^{a}	13.11^{a}

The same letters in the column do not differ significantly by Tukey's test at 5%significance level.

IV. CONCLUSION

The essential oils Sucupira, Caju, and Cajuzinho-do-Cerrado have the potential toinhibit the development "in vitro" of *Fusarium* and *Rhizopus* fungi, being important to reduce the production losses by fungal activities in stored grains, enabling new alternatives to mitigate the ecological impacts and suggesting a new path for food production.

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