ANTIFUNGAL POTENTIAL AND ACARICIDAL EFFICACY OF GURGUÉIA CHESTNUT SALINE EXTRACT (*Dipteryx lacunifera* DUCKE)

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ABSTRACT

Dipteryx lacunifera Ducke, known as the Gurgueia chestnut, is a fruit species from the Cerrado in the south and southeast of Piauí. Although it is known for its use as food, there is a lack of detailed studies on its other properties. Recent studies focus on new alternatives for controlling ixodid ticks and phytopathogenic fungi. The objective of this study was to evaluate the antifungal potential and acaricidal efficacy of the saline extract from the Gurgueia chestnut. The analysis showed that the extract inhibited only the fungus of the genus *Macrophomina* and had a fungistatic effect on the germination of *Fusarium* conidia. However, its acaricidal efficacy was 67.46%, below the 95% required for commercialization in Brazil, possibly due to the roasting process that may have affected the protein biomolecules. There was a significant reduction in oviposition (83.35%) and in larval hatching (90%). These results indicate that the saline extract from the Gurgueia chestnut represents a promising new possibility among bioproducts with antifungal activity against *Fusarium* spp., requiring further in-depth studies to explore its potential in controlling diseases in fava beans.

Keywords: Phytopathogenic control. Ixodid ticks. *Fusarium* spp. Oviposition reduction. *Dipteryx*.

1 INTRODUCTION

Dipteryx lacunifera Ducke, better known as Gurgueia chestnut, fava do morcego, or Garampara, is a fruit species belonging to the Leguminosae family, widely found in the Cerrado of southern and southeastern Piauí, specifically in the municipality of Bom Jesus, Piauí, where it is commonly seen in open markets[1]. Although the popular knowledge about this nut is well-established due to its use as food, there are still few studies in the literature that provide detailed characterizations of this nut or explore other benefits it may offer beyond its nutritional value. Carvalho[2] (2008) describes its value as an excellent nutritional source, with a chemical composition per 100 g of the fruit including: 18.6 g of proteins; 254 mg of calcium; 475 mg of phosphorus; 4.4 mg of iron; and 0.67 mg of vitamin B2. Additionally, as a nut, it is rich in lipids and proteins. However, more in-depth studies are needed, especially regarding biomolecules with potential in the agricultural sector[3].

In recent years, there has been a growing interest in researching new alternatives for controlling ixodid ticks. The use of new biomolecules that are sustainable and low-cost with acaricidal potential is considered an important alternative, offering great benefits and higher profitability to producers compared to the use of synthetic acaricides[4]. Ticks are blood-feeding ectoparasites belonging to the class Arachnida, with a worldwide geographical distribution that parasitize terrestrial vertebrates, including amphibians, reptiles, birds, and mammals. They are of great relevance as they transmit diseases to both animals and humans[5]. The direct and indirect losses attributed to ticks are largely linked to cattle infestation by *Rhipicephalus* (*Boophilus*) *microplus*[6]. In cattle farming, losses are incurred due to the cost of disease prophylaxis, control products, reduced milk and meat production, and loss of animals[7]. Various measures are employed for tick control, primarily using chemical acaricides, which is the most utilized and effective method. However, constant and incorrect use has led to the emergence of resistant tick populations, animal intoxication, and environmental impact due to the residual effects of acaricides in nature[8,9]. Additionally, there are residues of antiparasitic products in milk and meat available for human consumption, as well as direct environmental contamination[10]. With increasing resistance, many producers have erroneously increased the dosages of existing products on the market (far beyond what is indicated on the product labels) and increased the frequency of animal treatments. The impact of these measures results in increased environmental damage, health risks to the applicator, animals, and their offspring, and premature unviability of chemical products available on the market[11]. This situation has worsened with the development of resistance in these arthropods to different chemical groups in various regions of the country[12]. Consequently, the number of research studies seeking alternative methods for tick control has increased[13].

Another important biotechnological application is finding antifungal activities present in these biomolecules, particularly against phytopathogenic fungi, which mainly cause diseases in fava beans. *Phaseolus lunatus* L., belonging to the Fabaceae family and originating from Guatemala, is the second most important legume of the genus Phaseolus due to its high nutritional value, containing vitamins, proteins, and minerals that are essential elements in human nutrition, and it has become an alternative source of income and food for the population in the Northeast region. Chemical control is not usually economically and technically viable for this crop due to various environmental restrictions. Biological control has been limited in most situations, as few antagonists can establish themselves in such a competitive environment. Cultural measures, such as crop rotation, require long periods of absence of the main crop due to the efficient survival strategies of these pathogens, which substantially reduces its applicability[14].

In this context, the objective of this study was to evaluate the antifungal potential and acaricidal efficacy of the saline extract from the Gurgueia chestnut (*Dipteryx lacunifera* Ducke).

2 MATERIAL & METHODS

The plant material used was the roasted nut of the Gurgueia chestnut tree (*Dipteryx lacunifera* Ducke) SISGEN AFF149E, obtained from local open markets in the region. The nuts were briefly washed with distilled water and left to dry at 35ºC for 3 days, then broken down to form some flour. 10% (w/v) of the powder was used and diluted in a 0.15 M NaCl saline solution, maintained under magnetic stirring for 120 minutes at room temperature. Subsequently, this content was centrifuged at 8000 rpm for 15 minutes, and the supernatants were designated as saline solution.

For the antifungal activity, fungi from the Microorganism Culture Collection of the Phytopathology Laboratory at the Federal University of Piauí – CPCE Campus were initially selected and used. The selected isolates were from the genera *Colletotrichum*, *Fusarium*, and *Macrophomina*, originating from fava bean seeds produced in regions near the municipality of Bom Jesus - PI. The isolates were cultivated in Potato-Dextrose-Agar (PDA) medium maintained at room temperature. The fungal inoculum of each isolate was adjusted to a concentration of 10^6 CFU/mL using a Neubauer chamber count. The microbiological screening to evaluate the antifungal potency of the crude extracts at 10% was based on the liquid medium microdilution technique [15,16,17,18].

In the analysis of the extract's interference on conidial germination, the extract was tested for its inhibitory effect on the germination of fungal isolates' conidia. For this purpose, aliquots of 0.5 mL of the extract solution were mixed with 0.5 mL of conidial suspension (approximately 5 x 10^6 CFU/mL), obtained from cultures grown for 7 days on PDA, and homogenized for 30 seconds. The system was immediately incubated at 28°C. Samples of this mixture were taken at 24 hours for analysis. The number of germinated conidia was determined using a Neubauer chamber, counting the total number of conidia, and subsequently calculating the percentage inhibition of conidial germination compared to the control experiment. Each analysis was performed using a common optical microscope (Zeiss® model Primo Star)[19,20].

For the *in vitro* evaluation of the bioactivity of the saline extract, the bioacaricide test was used, through the immersion test of engorged ixodid females, according to Drummond et al.[21, 22] (1971, 1973). Bioactivity was determined by calculating the product's efficacy (PE) using the formulas described by Drummond et al.[22] (1973). The results were interpreted considering a minimum efficacy value of 95%, as per relevant legislation for the commercialization of acaricides in the country[23]. For the in vitro evaluation of the bioactivity of the extracts on unfed ixodid larvae, the "sandwich" technique recommended by Shaw[24] (1966) and adapted by Leite[25] (1988) was used.

RESULTS & DISCUSSION

It was possible to observe the inhibition profiles of microbial growth produced by the saline extract on fungi of the genera *Colletotrichum*, *Fusarium*, and *Macrophomina*. Through the analysis of the results, it was observed that the saline extract exhibited inhibitory activity only against the fungus of the genus *Macrophomina*. It was also noted that the extract showed a greater inhibitory effect on conidial germination of the Fusarium isolate, with statistically significant results compared to the control (Figure 1).

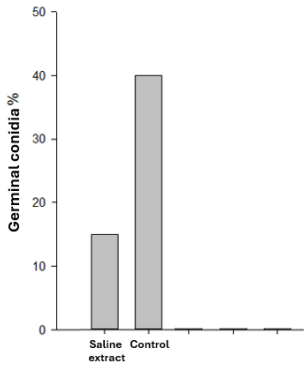

Figure 1 Percentage of germinated *Fusarium* conidia in the control and in the presence of saline solution.

The percentage obtained for the *in vitro* efficacy of the saline extract from the Gurgueia chestnut tree (*Dipteryx lacunifera* Ducke) was 67.46%, therefore demonstrating acaricidal activity below 95%, which is under the relevant legislation for its commercialization as an acaricide in Brazil. This low index is probably due to the roasting process of the nuts, which, being at high temperatures, may have affected the integrity, potentiality, and denaturation of the protein biomolecules present in the nuts. In Table 1, we can observe a significant reduction in oviposition (83.35%) and especially in larval hatching (90%).

Table 1 Effects of the extract from the roasted seed of the Gurguéia chestnut tree (*Dipteryx lacunifera* Ducke) on engorged females of *Rhipicephalus* (*Boophilus*) *microplus*.

Assays	Oviposition reduction (%)	Larvae hatching (%)	Hatching reduction (%)	Product efficacy (%)
Gurgueia chestnut tree	83,35	10	90	67,46
Positive control (Cypermethrin)	64,44	0	100	100
Negative control (Distilled water)	0	90	0	N/A

3 CONCLUSION

The saline extract obtained from the roasted nuts of the Gurgueia Chestnut tree exhibited antifungal activity against *Macrophomina* associated with fava bean seeds. Additionally, *Fusarium* experienced a fungistatic effect on conidial germination. From these results, it can be deduced that this extract represents a new and promising possibility among bioproducts with antifungal activity against *Fusarium* spp. However, further, more in-depth studies are necessary to explore the potential control of diseases caused by this pathogen in fava beans. Although the saline extract showed a low product efficacy index, there was a significant reduction in oviposition and especially in larval hatching.

REFERENCES

1 RIBEIRO, F. S. C., SOUZA, V. A. B., LOPES, A. C. 2012. Revista Brasileira de Fruticultura. 34. 190-199.
2 CARVALHO, M. G. 2008. Revista Ciência Agronômica. 39 (4). 517-523.
3 VERA, R., JUNIOR, M. S., NAVES, R. V., SOUZA, E. R. B., FERNANDES, E. P., CALIARI, M., LEANDRO, W, M. 2009. Revista Brasileira de Fruticultura. 31. 112-118.
4 NEPOMOCENO, T. A. R., PIETROBON, A. J. 2018. Revista Cientifica. Semana Acadêmica.
5 MASSARD, C. L., FONSECA, A. H. 2004. A Hora Veterinária. 135 (1). 15-23.
6 ANDREOTTI, R, GARCIA, M. V., KOLLER, W. W. 2019. Carrapatos na cadeira produtiva de bovinos. Brasília, DF: Embrapa Gado de Corte, 240.
7 ALMAZAN, C. et al. 2018. Frontiers in Bioscience. 23. 1535-1551.
8 FURLONG, J. 1993. Cadernos Técnicos da Escola de Veterinária da UFMG. 8. 46-61.
9 FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2004.25–77.
10 MOLENTO, B. M. 2004. DBO Saúde Animal. 18-22.
11 BAHIENSE, T. C., BITTENCOURT, V. R. E. P. 2004. Annals of the New York Academy of Sciences. 1026. 319-322.
12 MENDES, M. C., SILVA, M. X., BRACCO, J. E. 2001. Revista Brasileira de Parasitologia Veterinaria. 10 (2). 61-65.
13 BARCI, L. A. G., ALMEIDA, J. E. M., NOGUEIRA, A. H. C., ZAPPELINI, L. O., PRADO, A. P. 2009. Revista Brasileira Parasitologia Veterinária. 18 (1). 7-13.
14 LIMA G. S. A., ASSUNÇÃO I. P., VALLE L. A. C. 2005. Controle genético de doenças radiculares. *In:* MICHEREFF S. J., ANDRADE, D. E. G. T., MENEZES, M. (Org). Ecologia e manejo de patógenos radiculares em solos tropicais. Recife: Imprensa da UFRPE. 247-278.
15 CLEELAND, R., SQUIRES, E. 1991. Evaluation of new antimicrobials in vitro and experimental animal infection *In:* LORIAN, V. Antibiotics in laboratory medicine. 3. ed. Baltimore: Williams and Wilkiam.739-787.
16 ELOFF, J. N. 1998. Planta Médica. 64 (8). 711-713.
17 HADACEK, F., GREGER, H. 2000. Phytochemical Analysis. 11 (3). 137-147.
18 KONEMAN, E. W. et al. 1993. 1ª edição, p. 345-347.
19 RANA, B. K. SINGH, U. P., TANEJA, V. 1997. Journal of Ethnopharmacology. 57 (1). 29-34.
20 SHARMA, N., TRIPARTHI, A. 2008. Microbiological Research. 163 (3). 337-344.
21 DRUMMOND, R. O. et al. 1971. Journal of Economic Entomology. 64. 686-688.
22 DRUMOND R. O. et al. 1973. Journal of Economic Entomology, 66, 130- 133, 1973.
23 BRASIL, Ministério da Agricultura. Portaria n.90 de 04 de dez. de 1989. Normas para produção, controle e utilização de produtos antiparasitários. Diário Oficial, 22 de janeiro de 1990, sec. 1, col. 2.
24 SHAW, R. D. 1966. Bulletin Entomological Research. 56. 389-404.
25 LEITE, R.C. 1988. *Boophilus microplus* (Canestrini, 1887) - Susceptibilidade, uso atual e retrospectivo de carrapaticidas em propriedades das regiões fisiográficas da Baixada do Grande Rio e Rio de Janeiro. Uma abordagem epidemiológica. Tese de doutorado,Universidade Federal Rural do Rio de Janeiro, 151.

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