Scientia Agraria Paranaensis – Sci. Agrar. Parana.
ISSN: 1983-1471 – Online

ANTIFUNGAL ACTIVITY OF *Cinnamomum zeylanicum* BARK NATURAL EXTRACT SUBJECTED TO XYLOPHAGOUS FUNGI

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SAP 24285 Received: 18/03/2020 Accepted: 20/07/2020
Sci. Agrar. Parana., Marechal Cândido Rondon, v. 19, n. 3, jul./sep., p. 302-306, 2020

ABSTRACT - The search for natural wood preservatives is becoming increasingly evident due to industrial preservatives presenting several environmental restrictions, such as soil, water and ecosystems contamination. Therefore, the objective of the present study is to evaluate the antifungal activity of *Cinnamomum zeylanicum* bark natural extract subjected to rotting fungi. In order to obtain the aqueous extract, two methods were performed with different concentrations, 50 and 100 g L^{-1}, using *Pycnoporus sanguineus* e *Gloeophyllum trabeum*, mycelium. In the first, we kept the *C. Zeylanicum* bark with distilled water for 1 h in water bath and, in the second, the same concentrations were used, however, this was kept in a recipient for 24 h. Then, the extract was subjected to sterilization along with the BDA medium in autoclave at 120ºC for 20 min, they were then put in petri dishes for later evaluation of the mycelium root growth compared with the control. The fungi *Pycnoporus sanguineus* presented higher fungal activity, obtaining total inhibition of all treatments and extract concentrations, however, for the fungi *Gloeophyllum trabeum*, the 100 g L^{-1} concentration warmed in water bath obtained a better result than the others. Therefore, it can be concluded that the *Cinnamomum zeylanicum* aqueous extract presented inhibitory potential. Considering this, we suggest that more studies with this thematic should be carried out, seeking to discover new alternatives for wood preservatives that are less damaging to the environment and to mankind.
Keywords: cinnamon, alternative control, fungitoxicity.

ATIVIDADE ANTIFÚNGICA DO EXTRATO NATURAL DA CASCA DE Cinnamomum zeylanicum SUBMETIDA A FUNGOS XILÓFAGOS

RESUMO - A busca por preservativos naturais de madeira está cada vez mais em evidência devido aos preservativos industriais apresentarem diversas restrições ambientais, como a contaminação do solo, da água e dos ecossistemas. Por este motivo, o objetivo do presente estudo é avaliar a atividade antifúngica do extrato natural da casca de *Cinnamomum zeylanicum* submetidas a fungos apodrecedores. Para a obtenção do extrato aquoso foram realizados dois métodos, com concentrações distintas, de 50 e 100 g L^{-1}, utilizando os micélios de *Pycnoporus sanguineus* e *Gloeophyllum trabeum*, no primeiro manteve-se a casca de *C. zeylanicum* com água destilada durante 1 h em banho-maria e no segundo, foram utilizadas as mesmas concentrações da anterior, porém, esse foi mantido em um recipiente durante 24 h. Após, o extrato foi submetido à esterilização junto com o meio BDA em autoclave a 120°C por 20 min., depois foram colocados nas placas de petri para posterior avaliação do crescimento radial dos micélios comparado com a testemunha. O fungo *Pycnoporus sanguineus* apresentou maior atividade fúngica, obtendo inibição total de todos os tratamentos e concentrações do extrato, no entanto mediante o fungo *Gloeophyllum trabeum* a concentração de 100 g L^{-1} aquecida em banho-maria conseguiu melhor resultado do que nos demais. Portanto, pode-se concluir que o extrato aquoso de *Cinnamomum zeylanicum* apresentou potencial inibitório. Em vista disso, é indicado a realização de mais estudos com essa temática, buscando encontrar novas alternativas de preservativos da madeira menos danosos ao ambiente e ao homem.
Palavras-chave: canela-da-índia, controle alternativo, fungitoxicidade.

INTRODUCTION

Due to its biological nature, wood is susceptible to attacks from xylophagous organisms, commonly known as "rotting". Among them, we have fungi, bacteria, termites, beetles and borers, these are important agents that can drastically reduce wood useful life (SUNDARARAJ et al., 2015). Because of the great frequency of cases, fungi are considered the main wood deteriorating agents (STANGERLIN et al., 2011). The *Basidiomyceta* phylum is the main decomposer of lignocellulosic materials, causing the seizure of around 80 Tg of carbon, annually, in wood biomass, which makes it fundamental to the global carbon cycle (HISCOX et al., 2018). These are related in different ways with the wood, being able to cause stains or total decomposition, classified as staining, rotting and molding (MOTTA et al., 2013).

Fungi are among the organisms that indirectly feed from wood, decomposing it to use it as energy source,

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considered the most responsible for wood damage (MORESCHI, 2013). Among those responsible for wood rotting, the basidiomycetes class stands out, in which we find the brown rot fungi, which mainly degrade cell wall polysaccharides (cellulose and polyoses), and white rot fungi, which attack polysaccharides and well as lignin (BARI et al., 2015).

Due to losses caused by biological decomposition, it is necessary for the wood production industry to employ preservative substances, this being the most efficient alternative to avoid attacks by xylophagous organisms (BROCCO et al., 2017). Thus, most of the commercialized low-durability wood are treated with preservatives that are damaging to human beings and to the environment, this type of preservative is used due to its high protective potential (LEBOW, 2010).

Because of the disadvantages of using industrial preservatives, emerges the need to use botanical extracts that are able to increase wood durability, avoiding its degradation with an ecological product (KIRKER et al., 2013). There is a large number of medicinal plants that present antifungal properties in its extracts, depending on a number of factors inherent to the plants. The product's degree of efficiency is related to the species involved, the type of disease to be controlled and the processes used to obtained and manipulate the extract (SILVA et al., 2005).

The *Cinnamomum zeylanicum* species, commomly known as cinnamon, belongs to the Lauraceae family and is originated from the Asian continent, its bark and leaves are usually used in cosmetics, manufacturing of liquor and cooking, due to its aromatic and spice properties (SILVA et al., 2012); besides presenting antibacterial and antifungal activity (PAWAR & THAKER, 2006). Considering this, the present study aimed to evaluate the use of *Cinnamomum zeylanicum* bark as a natural preservative, as well as its antifungal activity when subjected to xylophagous fungi attacks.

MATERIAL AND METHODS

The experiment was carried out at the Biodeterioration Laboratory of the Forest Engineering Department, at the Federal University of Santa Maria. During the execution of the present study, we obtained *Cinnamomum zeylanicum* bark commercially, with the intent of preparing the aqueous extract, as well as colonies of rotting fungi, *Pycnoporus sanguineus* and *Gloeophyllum trabeum*, white and brown rot, respectively. The aqueous extract was obtained by two treatment methods, in the first, the *C. Zeylanicum* bark was kept in distilled water for 1 h in water bath and, in the second, the same concentrations were used, however, they were kept in a recipient for 24 h.

The experiment was composed of one control, besides two different concentrations of the aqueous extract (50 g L^{-1} and 100 g L^{-1}) for each fungi. In all methods, potato dextrose agar (PDA) were added and autoclaved at 120ºC for 20 min., to later incorporate them in 5 petri dishes, per treatment. Then, each dish received a mycelial disk with 8.5 mm diameter, stored in a acclimatized BOD, with a constant temperature of 25ºC, and incubated for 6 days, until the control filled the first dish.

The mycelial growth index (MGI) of the *Pycnoporus sanguineus* and *Gloeophyllum trabeum* fungi was evaluated through daily measurements, which started 24 h after inoculation, thus averages of two diametrically opposed measures of the fungi colony were done, so that it was possible to obtain MGI. This was calculated by the modified Maguire formula and adapted by Oliveira (1992). The experimental outline used was entirely randomized, with 5 repetitions in a 2x3 factorial scheme, being 2 extract obtainment methods and 2 concentrations. Therefore, MGI was calculated and subjected to the Tukey average differentiation test, at 5% error probability, performing regression analysis, with the aid of the ASSISTAT 7.5 Version Beta (SILVA, 2008).

RESULTS AND DISCUSSION

In the results obtained using the *Pycnoporus sanguineus* fungi, the variance analysis was not significant in the mycelial growth index, when analyzing the *Cynnamomum zeylanicum* extract, warmed and kept in a recipient for 24 h. In Table 1, we can observe that there was no difference among the used concentrations when analyzing mycelial growth rate.

TABLE 1 - Concentration of the average mycelial growth index values of the *Cinnamomum zeylanicum* aqueous extracts subjected to the *Pycnoporus sanguineus* fungi, where (A) = warmed and (B) = water bath.

Aqueous extract concentrations (g L^{-1})	MGI
50 (A)	0.00 a*
50 (B)	0.00 a
100 (A)	0.00 a
100 (B)	0.00 a
Control	61.47 b

*Equal letters in the column do not differ among each other, by the Tukey test, at 5% error probability.

Based on the average MGI values of the *Pycnoporus sanguineus*, all concentrations using agueous *Cynnamomum zeylanicum* extract were efficient, with total inhibition, thus, completely stopping mycelial growth in 100% of the tested experimental units. Therefore, we can say that, for this fungi species, cinnamon brings inhibition benefits to its growth, seen as it presented better MGI results.

Mycelial growth inhibition in the different methods and *Cinnamomum zeylanicum* aqueous extract

concentrations was observed visually after the experiment execution, as shown in Figure 1. Therefore, it was verified that all the tested extractive concentrations have a high growth inhibition value for the *Pycnoporus sanguineous* fungi, since the control petri dish was completely filled, while the fungi did not develop in the others.

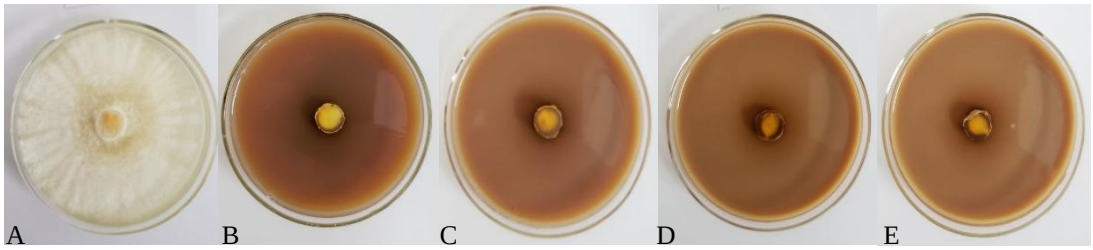

FIGURE 1 - Mycelial growth evaluation, using *Pycnoporus sanguineus* fungi, where: (A) = control, (B) = 50 g L^{-1} - warmed, (C) = 50 g L^{-1} - water bath, (D) = 100 g L^{-1} - warmed, (E) = 100 g L^{-1} – water bath. Source: the authors.

In the results obtained using *Gloeophyllum trabeum* fungi, with the same concentrations, it was possible to verify that, when the extract is warmed, we are able to obtain satisfactory results for mycelial growth inhibition (Table 2). It can also be observed that the highest degree of toxicity was reached with the warmed extract, so that, when the control presented average MGI of 66.58, differing from the others, at the same time, the 100 g L^{-1} dosage had a growth of only 8.13. When subjected to the 50 g L^{-1}, the value increased to an average of 21.02.

TABLE 2 - Average mycelial growth index values (MGI) of the *Cinnamomum zeylanicum aqueous* extract subjected to *Gloeophyllum trabeum* fungi, where (A) = warmed and (B) = water bath.

Aqueous extract concentration (g L^{-1})	MGI
50 (A)	21.02 a*
50 (B)	39.24 b
100 (A)	8.13 c
100 (B)	41.29 b
Control	66.58 e

*Equal letters in the column do not differ among each other, by the Tukey test, at 5% error probability.

Mycelial growth inhibition in the different methods and *Cinnamomum zeylanicum aqueous* extract concentrations, in contact with the *Gloeophyllum trabeum* fungi, can be observed visually, as shown in Figure 2. Based on the presented image, we can observe that while the control presented the fungi in 100% of the filled dish, the treatments containing the extract had reduced growth. It is noted that the 100 g L^{-1} concentration presented the best results in inhibiting the growth of the *G. trabeum* fungi with the *C. zeylanicum* aqueous extract.

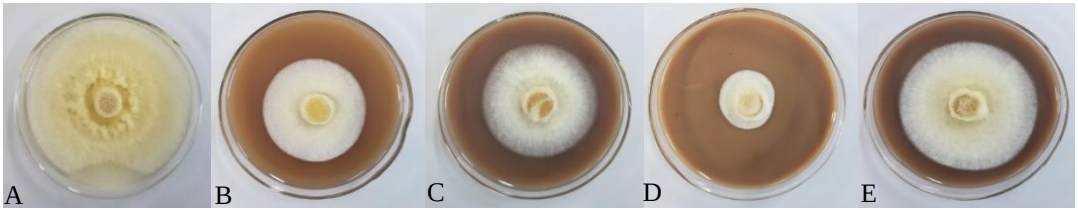

FIGURE 2 - Mycelial growth evaluation, using *Gloeophyllum trabeum* fungi, where: (A) = control, (B) = 50 g L^{-1} - warmed, (C) = 50 g L^{-1} - water bath, (D) = 100 g L^{-1} - warmed, (E) = 100 g L^{-1} - water bath. Source: the authors.

In a similar study, Talgatti et al. (2020) reported that aqueous extracts from *Hovenia dulcis* and *Ateleia glazioviana* wood, bark and leaves, with different concentrations, presented fungitoxic potential in relation to *Pycnoporus sanguineus* and *Gloeophyllum trabeum* fungi, respectively, representatives of white and brown rot. Furthermore, Viegas et al. (2005) verified the *in vitro* action of garlic and cinnamon essential oils on the development of the *Aspergillus flavus* fungi, which attack agricultural plants and cause mold in grains. With the results from the study, the authors concluded that there was higher toxicity in the cinnamon essential oil, inhibiting up to 100% of growth, when using a concentration of 50 mg L^{-1} of *Cinnamomum zeylanicum* extract, demonstrating that there is an inhibitory substance in cinnamon.

According to Araújo et al. (2009), the aqueous extract from cinnamon bark provided a reduction in the *Penicillium roqueforti* and *Rhizoporus stolonifer* mycelial growth speed index. The cinnamaldehyde is the compound

with the main fungal activity in the cinnamon extracts, the other chemical components have an additive or synergistic component to the total fungitoxic activity (JHAM et al., 2005). Antifungal activity using *Cinnamomum zeylanicum* essential oil in different concentrations were efficient and able to inhibit mycelial growth of fungi that attack the agricultural environment, such as *Apergillus*, *Penicillium*, *Cladosporium* and *Furasium* (SIMIC et al., 2004).

According to Huller et al. (2019), a significant reduction can be observed in the mycelial growth of *Gloeophylum trabeum* and *Pycnoporus sanguineus* fungi from rue aqueous extract, despite manipulating it with only 15% of the vegetable, even so, there was MGI inhibition. Similarly, Nascimento et al. (2013) concluded that rue extract at 10000 mg L^{-1} concentration generated an inhibitory effect of 30% in *Cercospora calendulae*, making it possible to inhibit its development, seen as it presented the best MGI result.

According to Silveira et al. (2017), the use of 40 and 50 g L^{-1} concentrations of tannin are able to inhibit fugal activity for *Pycnoporus sanguineus* fungi in 89.12 and 93.60%, respectively, when compared with control. Thus, in light of what was exposed in this study, it is possible to observe that natural extracts can be highly toxic for xylophagous agents, being able to reduce or even inhibit its growth.

CONCLUSIONS

The *Cinnamomum zeylanicum* aqueous extract was efficient in *in vitro* control of *Pycnoporus sanguineus* and *Gloeophyllum trabeum* fungi development, in all tested concentrations, showing that there is a substance with inhibitory potential in its composition.

In light of the positive results, the performance of more studies with this theme is suggested, aiming to find new wood preservative alternatives, less damaging to the environment and humans.

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