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Efficacy of sugarcane pyroligneous extract in suppressing carpogenic germination of *Sclerotinia sclerotiorum*

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ABSTRACT

White mold, caused by the fungus *Sclerotinia sclerotiorum*, is a disease of agronomic importance that affects crops such as soybeans and beans. Considered an aggressive pathogen, it causes symptoms in different parts of the plant, and its control is difficult due to the formation of resistance structures called sclerotia, which allow it to survive in the soil for long periods. The objective of the present study was to evaluate the effectiveness of different concentrations of pyroligneous extract (PE) from sugar cane in suppressing the carpogenic germination of *S. sclerotiorum in vitro*. The concentrations tested were 0; 1,000; 2,000; 3,000; 4,000 and 5,000 ppm. The extract was incorporated into water agar medium and poured into gerbox boxes with 20 sclerotia. The total number of sclerotia with emission of stipes and apothecia, the total number of sclerotia with emission of stipes and the total number of sclerotia with apothecia were quantified. There was a significant difference in carpogenic germination between the treatments evaluated. Increasing concentrations of sugarcane PE negatively affected the carpogenic germination of sclerotia, the number of stipes per sclerotium and the number of apothecia formed per sclerotium. It was found that sugarcane PE did not inhibit the formation of stipes, but made it difficult to differentiate them into apothecium, especially at the concentration of 5,000 ppm. Sugarcane PE at concentrations of 4,000 and 5,000 ppm promoted reduction in the percentage of germinated sclerotia, as well as in the number of apothecia per sclerotium.

Keywords: white mold; pyroligneous acid; sclerotia.

INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a disease that can affect more than 400 species of plants, including monocots and dicotyledons. This fungus is distributed in different regions, whether temperate, subtropical or tropical, and can attack crops such as soybeans, beans, peas, sunflowers and potatoes (BOLAND; HALL, 1994).

Control of *S. sclerotiorum* is difficult due to the formation of resistance structures, allowing it to survive in the soil for long periods, on average six to eigth years (WILLETS; WONG, 1980). These structures, called sclerotia, play an important role in the life cycle of the fungus, under favorable conditions, and, in the presence of a susceptible host, they germinate and produce mycelium (mycelium germination), penetrating directly into the tissues at the base of the plant, or form apothecia (carpogenic germination), which emerge on the soil surface and release ascospores (primary inoculum). Both can result in infections in plants. However, the greatest epidemic potential is verified by ascospores released during carpogenic germination (LEITE, 2005; BOLTON et al., 2006).

Technologies have been studied and developed to control diseases and products from renewable sources are promising alternatives (SILVEIRA, 2010). Pyroligneous extract (PE), also called pyroligneous acid or wood vinegar, is a by-product resulting from the condensation of vapors, originating during the pyrolysis process of different plant species, such as bamboo, eucalyptus, pine, and sugar cane. It is mostly composed of water (80%) and a complex mixture of more than

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200 organic compounds, such as acetic acid, alcohols, ketones, esters, furans, phenol, guaicol, syringol, pyrocatechol and their derivatives (MAEKAWA, 2002; ENGASP, 2014; SURESH et al., 2019).

Due to its antimicrobial activity and the growing need to replace agrochemicals, the use of EP has been studied to improve productivity in the agricultural area. It is currently gaining attention in research into sustainable agriculture due to its organic properties with potential replacement for chemical fertilizers and pesticides (GREWAL et al., 2018). Research carried out by Santos Junior et al. (2013), in the in-vitro evaluation of the fungitoxicity of different concentrations of teak PE (*Tectona grandis*), on the mycelial growth of *Rhizoctonia solani*, showed that at all concentrations used the extract provided fungitoxic action on the fungus. Ribeiro et al. (2016), evaluating the effect of different natural products, including the commercially named PE Biopirol (40 and 60 $mL \cdot L^{-1}$), on the in-vitro control of *Colletotrichum gloeosporioides* in papaya (*Carica papaya*) fruits, found that the PE at both concentrations resulted in total inhibition of fungal development.

Despite the recommended effects, there is a lack of scientific information that can support the use of pyroligneous extracts and the understanding of the mechanisms by which they work, especially regarding the protection of plants against diseases of great economic importance (ALVES et al., 2007). For Grewal et al. (2018), PE has been explored as an antimicrobial agent, but there are few studies with applications in agriculture. Souza et al. (2018), through a systematic review and a technological forecast of the existing evidence regarding the use of PE as a potential antimicrobial for humans and animals, report that the literature is also limited on this activity, but the results are promising. Furthermore, studies related to carpogenic germination are scarce, which denotes the importance of this study, which aimed to evaluate the effectiveness of different concentrations of PE from sugarcane in suppressing the carpogenic germination of *S. sclerotiorum in vitro*.

MATERIAL AND METHODS

The experiments were carried out at the Agricultural Microbiology and Phytopathology Laboratory of the Faculdade de Ciências Agrárias, at the Universidade Federal da Grande Dourados (UFGD). The sugar cane PE was supplied by the company Bioware Tecnologia, from Campinas, SP, Brazil. The inoculum of the pathogen *S. sclerotiorum* was obtained from a naturally infested area of the UFGD Agricultural Sciences Experimental Farm cultivated with safflower (*Carthamus tinctorius* L.). The experiment was carried out in a completely randomized design with six treatments and six replications. The concentrations tested were 0, 1,000, 2,000, 3,000, 4,000 and 5,000 ppm. For the control, only the agar-water culture medium was used.

To evaluate the carpogenic germination of sclerotia, there was a need to produce such structures. The sclerotia was obtained by mass production on carrot discs sterilized in an autoclave (120°C/1 atm/30 min) according to the methodology by Nasser et al. (1995). The sclerotia was superficially disinfected in a 70% alcohol solution for 1 minute and 1% sodium hypochlorite for 2 minutes, followed by washing in distilled water and autoclaved for another 1 minute. The resist structures were dried on sterile filter paper. The different volumes of sugarcane PE were removed from a stock solution with the aid of an automatic pipette, and added to the agar-water medium. Homogenization was carried out with a glass rod for 3 minutes, and then the medium was poured into previously disinfected gerbox boxes. After the medium solidified, 20 sclerotia were distributed equidistantly in each box. They were sealed with polyvinyl chloride (PVC) plastic film and incubated at 18°C with a 12-hour photoperiod. The evaluations began with the emission of stipes in the control, that is, 39 days after the implementation of the experiment.

The total number of sclerotia with emission of stipes and apothecia, the total number of sclerotia with emission of stipes and the total number of sclerotia with apothecia formed were quantified. The percentage of carpogenic germination was calculated based on the number of sclerotia with apothecia formed. Evaluations ended when sclerotia germination was stabilized.

Analysis of variance (ANOVA) was performed, followed by the Tukey's test, with the aid of the Sisvar 5.6 program (FERREIRA, 2003). Once significance was verified by the F test, regression analysis was performed for the concentration factor for each evaluation time, days after the start of incubation, with the SigmaPlot 12.5 program. The data expressed as a percentage were transformed into arcsine of $\sqrt{(x+1)} / 100$, and the others into $\sqrt{x+1}$, for analysis of variance.

RESULTS AND DISCUSSION

The evaluations began 39 days after the installation of the experiment (DAI), at which time the formation of apothecia was observed in the sclerotia of the control. From 46 days after incubation, a significant difference in carpogenic germination

was observed between the treatments evaluated. There was a linear reduction in the percentage of germinated sclerotia as the extract concentration increased (Fig. 1).

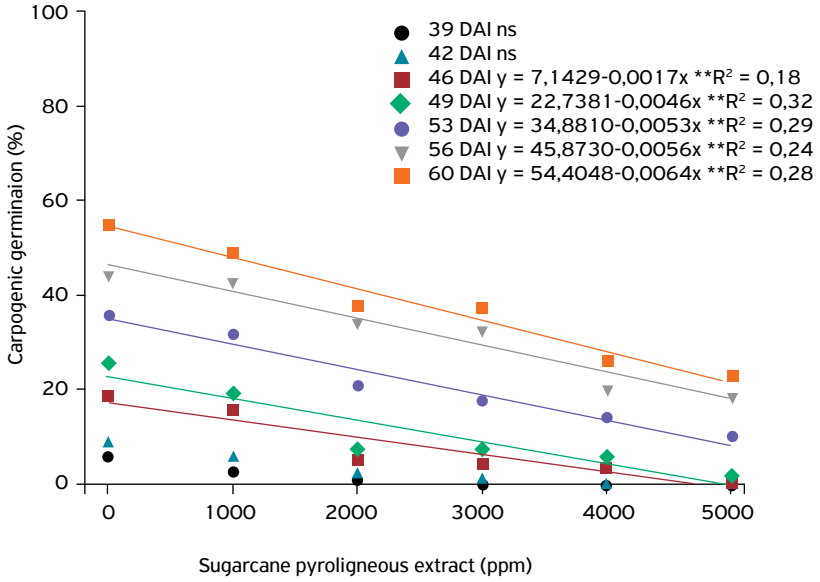

Figure 1. Percentage of germinated sclerotia with apothecia formation under the effect of different concentrations of the pyroligneous extract of sugar cane (*Saccharum officinarum*).
Source: Elaborated by the authors.

Although no studies were found in the literature on the use of PE to inhibit carpogenic germination, some results are observed with plant extracts. However, according to Zanella et al. (2018), most studies refer to the effects of vegetable extracts and oils on the mycelial growth of the pathogen. Therefore, studies on the carpogenic germination of sclerotia are scarce.

Silva et al. (2011), evaluating the carpogenic germination of *S. sclerotiorum* under different residues of cultivated plants and their extracts, found results similar to this study. The authors found that, regardless of whether by residues or extracts and their partitions, the suppression of carpogenic germination was permanent, with a fungicidal nature. They also found that all extracts from plant residues with their different partitions had a negative influence on carpogenic germination.

Zanella et al. (2015) evaluated the carpogenic germination of *S. sclerotiorum* sclerotia under different extracts and oils from plant species and found that, when subjected to extracts from *Geophila repens, Palicourea crocea*, and *Schinus terebinthifolius* and under the ethyl acetate and chloroform fractions of *Annona cacans*, a carpogenic germination was lower, as well as the number of apothecia formed in the presence of *Annona cacans* extract.

Similar pattern to the percentage of germination was observed for the number of stipes formed by sclerotia (Fig. 2). As sugarcane PE concentrations were increased, there was a gradual reduction in the number of stems formed. Since the first evaluation, 39 days after incubation, it was found that the concentrations negatively affected the formation of primordia, and the sclerotia subjected to the concentration of 5,000 ppm did not show the development of stipes. In the following evaluations, at 42, 46 and 49 DAI, with increasing extract concentrations, the reduction in the number of stems formed by sclerotia was linear. At 53 days after incubation, the lowest concentrations (1,000 and 2,000 ppm) had no effect on reducing the formation of stipes in sclerotia, however, in treatments of 3,000, 4,000 and 5,000 ppm, the formation of stipes was lower. From 56 and 60 DAI, there was no significant effect between treatments.

The loss in the fungistatic capacity of PE, observed over time, can be explained by the fact that plant extracts, in general, are chemically unstable in the presence of air, light and high temperatures, leading to rapid evaporation and degradation of the components. Furthermore, they do not have synthetic molecules and are unstable, with low residual power, requiring several applications to achieve satisfactory control (AZEVEDO et al., 2013). The present study was carried out under laboratory conditions (in vitro). The experimental units containing sugarcane PE and sclerotia were subjected to a 12-hour photoperiod, which may have caused the photodegradation of the molecules and the gradual decrease in the inhibitory power of the extract.

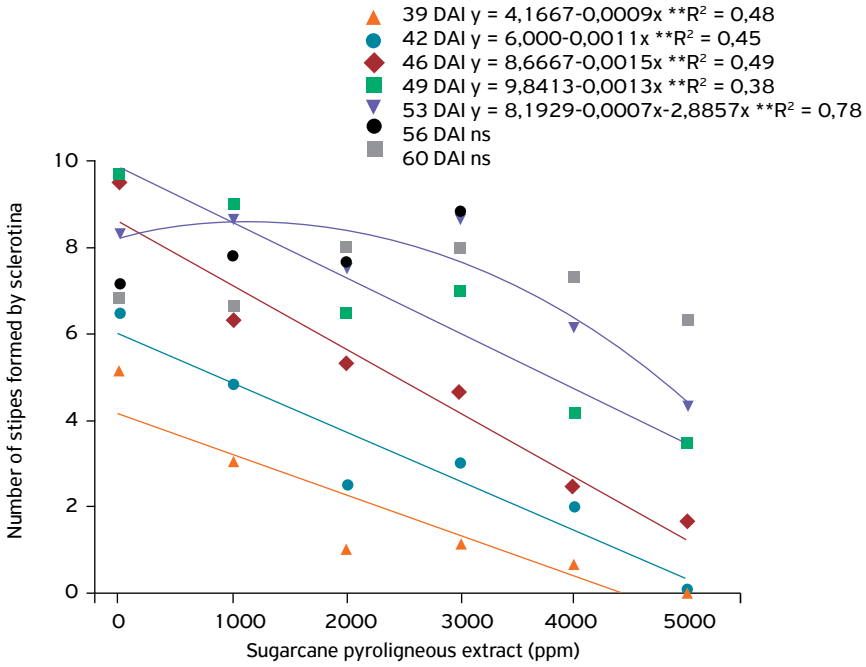

Figure 2. Number of stipes formed by sclerotia under the effect of different concentrations of pyroligneous extract of sugar cane (*Saccharum officinarum*).
Source: Elaborated by the authors.

Regarding the average number of apothecia formed by sclerotia, there was no significant difference between treatments in the first evaluation (39 DAI). In the other evaluations carried out, with increasing concentrations, the reduction was linear in the formation of apothecia by sclerotia. At 42, 46 and 49 days after incubation, there was no development of apothecia at concentrations of 4,000 and 5,000 ppm (Fig. 3).

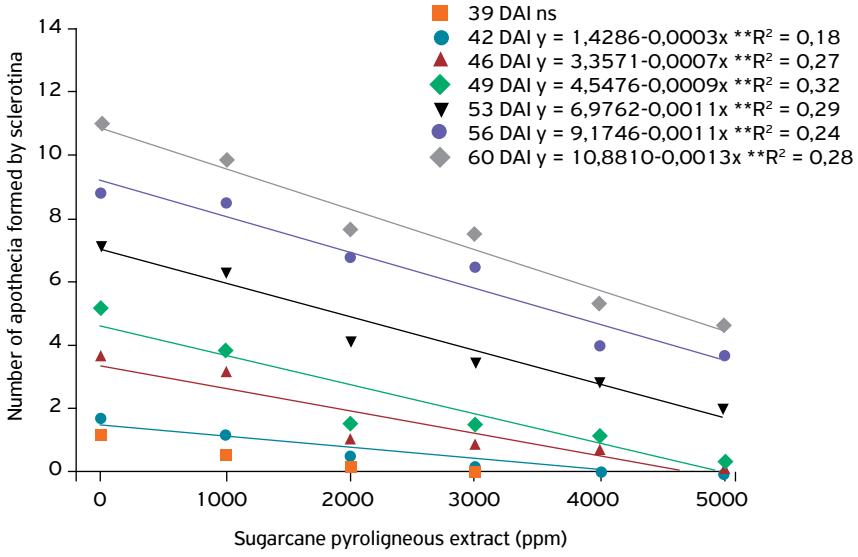

Figure 3. Number of apothecia formed by sclerotia under the effect of different concentrations of pyroligneous extract from sugar cane (*Saccharum officinarum*).
Source: Elaborated by the authors.

In this study, sugarcane PE did not inhibit the formation of stipes, but made it difficult to differentiate them into apothecium, especially at the concentration of 5,000 ppm. It was found that in some experimental units, in which the sclerotia was subjected to the concentration of 5,000 ppm, the development of branching stipes occurred (Fig. 4). Although PE did not inhibit the formation of stipes at the highest concentration, it did not allow, for the most part, the development of apothecia, and, when their formation occurred, they soon entered senescence (Figs. 4 and 5).

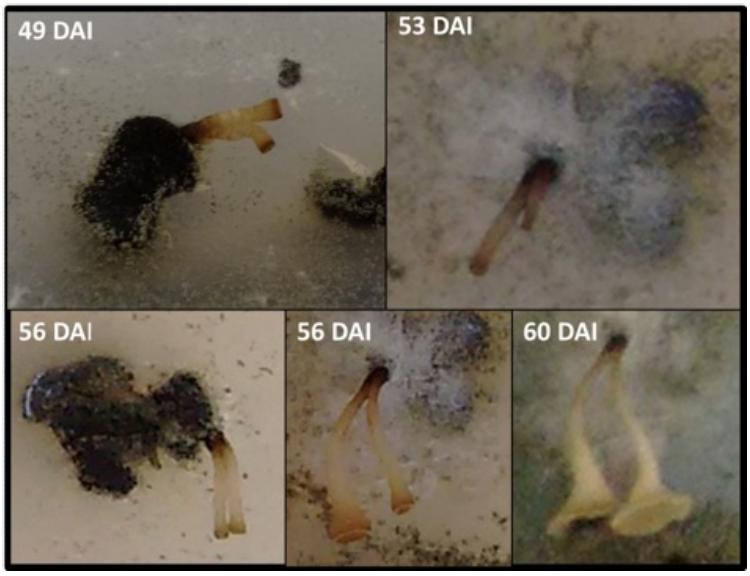

Figure 4. Stipes with branches in sclerotia subjected to the concentration of 5,000 ppm of pyroligneous extract of sugar cane in the different evaluation periods after the installation of the experiment (DAI).
Source: Elaborated by the authors.

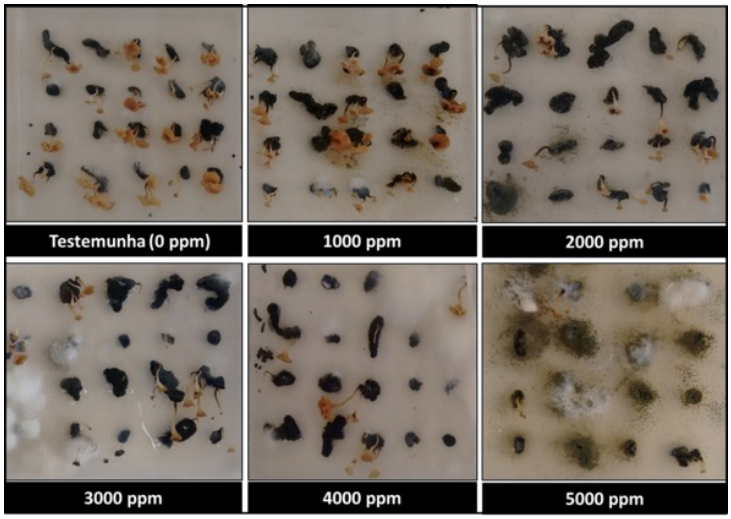

Figure 5. Carpogenic germination of *Sclerotina sclerotiorum* sclerotia 56 days after the installation of the experiment, subjected to different concentrations of sugarcane pyroligneous extract.
Source: Elaborated by the authors.

This effect on the differentiation of structures into apothecia was also observed by Huang and Blackshaw (1995) when evaluating the effect of herbicides on carpogenic germination. The authors found that the herbicide atrazine, despite promoting the emission of stems, caused the formation of abnormal apothecia. The stipe did not differentiate into apothecium in the discoid form, but it underwent branching into secondary stipes. These gave rise to abnormal apothecia, of a globose and filamentous shape, which, according to microscopy carried out, showed few asci with ascospores. Radke and Grau (1986) reported that herbicides from the triazine group also led to the development of abnormal apothecia and that, in most cases, there was no expansion of the apothecia in its discoid form, preventing the spread of the fungus. Oliveira (2005) also found that the use of herbicide, although not influencing the formation of stipes, did not allow the development of apothecia and ascospores, hindering the spread of the fungus.

Vrisman et al. (2014), evaluating the inhibitory effect of herbicides and fungicides on the carpogenic germination of sclerotia of the fungus *S. sclerotiorum*, observed that both herbicides and fungicides provided reduction in germination. However, fungicide treatments showed a reduced number of apothecia per sclerotia and greater formation of non-viable stems.

In the present study, PE at the concentration of 5,000 ppm did not inhibit the formation of stipes, but, when formed, they were unviable. Costa and Costa (2004), in determining the effect of fungicides on the carpogenic germination of *S.*

sclerotiorum sclerotia in the soil, under controlled conditions, found that, among the fungicides evaluated, vinclozolin was the most efficient product, showing 100% inhibition on formation of stipes and apothecia. The fungicide fluazinan only allowed the formation of non-viable stipes, resulting in the absence of apothecia. Therefore, it is important to have knowledge about the effect of products that have a fungicidal nature on the viability of *S. sclerotiorum* sclerotia, since the fungus is aggressive, with several forms of infection. The efficiency of these products in the biological cycle of the fungus can influence the reduction of inoculum density in the soil, inhibiting both mycelogenic and carpogenic germination of sclerotia. These observations, when found under field conditions, are interesting in the sense that they can contribute to the reduction of the source of inoculum in the form of ascospores (COSTA; COSTA, 2004).

According to Saigusa (2002), the activating or inhibitory effect of PE on living organisms depends on its concentration. For Yatagai et al. (2002), it is the components of wood vinegar that are responsible for the differences in germicidal activities. According to the literature, the chemical composition of PE varies according to the raw material used, the temperature to which the material is subjected in the manufacturing process and the exposure time (MORALES et al., 2019). For Grewal et al. (2018), the usefulness and benefits of PE in agriculture are also attributed to its chemical composition, and the antimicrobial property is due to the presence of high concentrations of phenolic compounds and organic acids. Studies carried out to verify the antifungal action of PE from *Eucalyptus globulus* report that the acetic acid and phenolic compounds present in the extract promote the inhibition of fungal growth (BAIMARK et al., 2008). MA et al. (2011) also suggest that phenols and organic acids are the active components for microbial inactivity.

However, research is needed to understand the mechanisms of action and impact of PE on different stages of plant growth and crop quality (GREWAL et al., 2018). According to Morales et al. (2019), in pyroligneous extracts there are no compounds toxic to humans (polycyclic aromatic hydrocarbons – PAHs) nor significant risks in their use. Therefore, they can be used as alternatives in agriculture, mainly as a biopesticide and organic fertilizer. This view is supported by Campos (2018), who explains that, when PE production occurs in the recommended temperature range (maximum 150°C), PAH levels remain within the safety limit (sum of 8 PAHs – 0.49 mg/L), without danger of contamination.

PE from sugarcane should be considered as an alternative to be studied at the field level in the management of *S. sclerotiorum*, as it interferes with the carpogenic germination of the fungus, hindering and modifying the development of stems and, consequently, reducing the formation of apothecia. Additional studies should investigate the effect on plants, as well as application times and doses.

CONCLUSIONS

In the carpogenic germination of , sugarcane PE at concentrations of 4,000 and 5,000 ppm promoted reduction in the percentage of germinated sclerotia, as well as in the number of apothecia per sclerotium.

AUTHORS' CONTRIBUTIONS

Conceptualization: Smaniotto, S.P.; Gavassoni, W.; Bacchi, L. **Formal analysis:** Smaniotto, S.P.; Gavassoni, W.; Bacchi, L. **Investigation:** Smaniotto, S.P.; Gavassoni, W. **Methodology:** Smaniotto, S.P.; Gavassoni, W.; Bacchi, L. **Supervision:** Gavassoni, W.; Bacchi, L. **Validation:** Smaniotto, S.P.; Gavassoni, W.; Bacchi, L. **Visualization:** Smaniotto, S.P.; Gavassoni, W.; Bacchi, L. **Writing – original draft:** Smaniotto, S.P. **Writing – review & editing:** Gavassoni, W.; Bacchi, L.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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