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Use of vegetable oils in the control of *Colletotrichum* sp. in banana fruits

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Anthracnose is a disease caused by the fungus of the genus *Colletotrichum*. It is considered an important post-harvest disease in fruits of banana (*Musa* sp.) which depreciates the commercial value of the fruit. The use of vegetable oils to control fungal growth and disease progression in plants is an important alternative to minimize the deleterious effects of toxic chemicals. Studies were carried out to evaluate the effects of the vegetable oils of murumuru (*Astrocaryum* sp.), andiroba (*Carapa guianensis*) and copaiba (*Copaifera* sp.) *in vitro* on the mycelial growth of *Colletotrichum* sp. The fungus *Colletotrichum* sp. was isolated from banana fruits. The treatments including control (mycelial growth in culture medium without the presence of oil) and vegetable oils (murumuru, andiroba and copaiba) at dosages of 0, 50, 100, 150 and 200 µL.mL^{-1} were applied. The experiment was conducted using a Completely Randomized Design (CRD), in a 3x6 factorial scheme (3 oils x 5 concentrations + 1 control), with five replications. Copaíba oil (*Copaifera* sp.) had a higher mycelial inhibition percentage (MIP) with a dose of 150 µL.mL^{-1} (65.56%). As for murumuru oil, a higher percentage of inhibition was also obtained in the concentration of 150 µL.mL^{-1} (46.44%) and the andiroba oil had a greater inhibitory effect in the concentration of 200 µL.mL-1 (34.89%). The results showed that only the copaiba oil, at all concentrations tested, had an inhibitory effect on *Colletotrichum* sp. Copaiba oil, therefore, is recommended for the control of phytopathogenic diseases caused by *Colletotrichum* sp.

Key words: Alternative control, anthracnose, crop protection, fungal diseases, *Musa* sp.

INTRODUCTION

Brazil is one of the world's largest banana (*Musa* sp.) producers, and this production plays an important role in local development, since it generates income and provides food resources to the population (Li et al., 2011). Thus, due to the increasing demand and importance of production, the consumer market imposes some requirements for high-quality fruits, and it is suggested that the harvest be carried out in order to minimize losses and with low or no toxicity inputs (Cruz et al., 2010).

It is known that the quality of agroforestry depends on aspects related to the production system and socio-environmental impacts, nutritional value and product flavor, presence of agrochemical residues, micro-organisms and insects on fruits, and visual aspects such as shape, size, color, pattern, stage of maturation and factors related to fruit appearance (Costa et al., 2013). In this scenario, the microbiological factor deserves special attention, since fungi are economically important organisms for medicine, phytopathology and bioindustry, and in addition, act as decomposers in the food chain (Leite et al., 2012).

Diseases such as panama disease, cordana leaf spot, yellow sigatoka, black sigatoka and anthracnose in fruits decrease productivity and depreciate fruits. Anthracnose, the main post-harvest disease in banana (Negreiros et al., 2013), caused by the fungus of the genus *Colletotrichum*, is responsible for large crop losses (Coelho et al., 2010). *Colletotrichum* is considered one of the most important pathogen genera that cause plant diseases (Solino et al., 2012). The symptoms of anthracnose in banana fruits are characterized to dark and depressed lesions, and as the disease progresses they are covered with pink fructification (Coelho et al., 2010).

Thus, the control of postharvest diseases should be performed alternative techniques to the use of fungicides chemicals, in order to avoid the risks of contamination of the agricultural product and the induction of resistance of the pathogen (Oliveira et al., 2019). The application of chemical fungicides in fruits to increase storage time is a major theme in the World Health Organization (WHO) (Dukare et al., 2018) due to the residual effects and risks to consumer health and the environment that these products represent (Ncama et al., 2019).

In this respect, extensive scientific research on the use of antifungal substances may help to control pre and post-harvest diseases (Kamei et al., 2014). In view of possible problems of contamination with the use of chemical substances, has been recommended the use of natural products for the control pf diseases in plants (Araujo et al., 2013). The advantage of post-harvest disease control in fruits with vegetable oils is that they present higher levels of food safety and lower risk of contamination to the environment (Fischer et al., 2018).

The use of murumuru (*Astrocaryum* sp.) oil has grown considerably in recent years, which can be explained by the investments made on discoveries for utilization of these vegetable products. The andiroba (*Carapa guianensis* Aubl.) and copaiba (*Copaifera* sp.) oils have shown a wide range of use in the Amazon region in the control of diseases (Solino et al., 2012). Therefore, the chemical composition of vegetable oils may define their potential type of use and associated biological activity (Funasaki et al., 2016).

Amazon region has a considerable diversity of vegetable species with natural substances that should be investigated for applications with the most diverse purposes, and oils from native plants can be used as alternative sources in the control of plants-infesting pathogens. Thus, this work was conducted to evaluate *in vitro* action of oils from *Astrocaryum* sp., *Carapa guianenses* and *Copaifera* sp. on the mycelial growth of *Colletotrichum* sp., the causative agent of anthracnose in banana fruit, with the intent of suggesting the use of natural products for the control of phytopathogenic agents and, therefore, add value to the utilization of plant products.

MATERIALS AND METHODS

This work was carried out at the Phytopathology Laboratory of the Federal University of Acre, *Campus Floresta*, Multidisciplinary Center – CMULTI, in Cruzeiro do Sul, Acre, Brazil. The vegetable oils were acquired from producers of natural oils in the region of the production of August 2017. The accesses on genetic diversity and associated traditional knowledge present in this work were registered in the database of SisGen - National System of Genetic Heritage and Associated Traditional Knowledge under register AE76FF6.

Isolate used

Sample material with anthracnose symptoms was collected from banana plant fruits obtained from farmers in Cruzeiro do Sul – AC and was taken to the laboratory for removal of fragments containing disease. 20 fruits were examined, of which presented average of 5 infection points, and lesions with a mean diameter of 3 cm. The lesion fragments were washed in running water, subjected to 70% alcohol for one minute and 1% sodium hypochlorite for 30 seconds. Finally, the sample was washed in sterile water for isolation of *Colletotrichum* sp. The infested materials were transferred to 9 cm diameter Petri dishes with 20 mL of Potato Dextrose Agar (PDA) as growth medium. The dishes were then maintained in Biochemical Oxygen Demand (BOD) at 25°C with a photoperiod of 12 hours for seven days. After seven days of fungal culture growth, 5 mm diameter discs containing mycelia of *Colletotrichum* sp. were inoculated in the center of the Petri dishes. The phytopathogenic agent was identified at the genus level by the culture and morphological characteristics of the hyphae with the help of area

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experts and comparisons in the literature.

Bioassay

In this study, the fungitoxic effects of different concentrations (0, 50, 100, 150 and 200 µL.mL^{-1}) of the oils of *Astrocaryum* sp., *Carapa guianensis* and *Copaifera* sp. *in vitro* on the mycelium growth of *Colletotrichum* sp. in PDA medium were analyzed. The control consisted of the fungus isolate cultured only in the PDA medium (Nascimento et al., 2014). The emulsifying agent added to the oils was Tween 80 (1% v/v), which was also used in the control treatment. The effect of different concentrations of vegetable oils on fungal growth was considered after daily measurements of the diameter of the growth area on the two diametrically opposite orthogonal axes of the petri dish. The evaluations were completed when the control mycelial growth completely covered the surface of the culture medium (Ferreira et al., 2012). Based on data of the daily growth of the colony under effect of the abovementioned vegetable oils, the mycelial growth rate was calculated, according to the formula by Dias et al. (2005):

$$MGR = \Sigma \frac{(D - Da)}{N}$$

Where: MGR = mycelial growth rate, D= current mean diameter of the colony, Da= mean diameter of the colony in the previous day, N= number of days after inoculation.

Percentage of growth inhibition (PGI) of the treatments was also calculated, compared to the control, using the following equation:

$$PGI = \frac{(\text{Control diameter} - \text{treatment diameter})}{\text{Control diameter}} \times 100$$

The assay was carried out in a completely randomized design, in 3x6 factorial arrangement (3 oils x 5 concentrations + 1 control). For each oil/concentration treatment five replications were considered. The data obtained in this study were subjected to Shapiro Wilk's test to check for normality of residuals and to the Levene's test for variance homogeneity. Subsequently, to meet the parametric analysis premises, data were subjected to analysis of variance, and the means were compared by the Scott-Knott's test ($p \leq 0.05$). Subsequently, the data were submitted to Multiple Linear Regression Analysis ($p \leq 0.05$) to verify the relationship between the sources of variations (oils and concentrations) on fungal mycelial growth rate. The analyses were carried out in the R statistical program (R Core Team, 2017).

RESULTS

The results obtained for Mycelium Growth Rate (MGR) were significant ($p < 0.05$) for the different oils at the same concentrations tested, but with no significant differences ($p > 0.05$) for different concentrations considering the same vegetable oil (Figure 1). The analysis of the oils effect on mycelial growth indicated that only the copaiba oil, for all concentrations, had an inhibitory effect on the mycelial growth of *Colletotrichum* sp., when compared to the control ($p = 0.02$) (Figures 1 and 2). However, there was no difference among the means of the concentrations used for this oil, indicating that the minimum concentration of copaiba oil to cause an inhibitory effect was 50 µL.mL^{-1}.

Copaiba oil had a higher fungitoxic effect on the MGR of *Colletotrichum* sp. (*F-statistic* = 10.39; *DF* = 58; R-square = 0.52 and $p < 0.01$) with an estimated reduction of the mycelial growth rate of 0.9 cm/day to each microliter (µL) of oil added to the culture medium ($p = 0.02$). In fact, the reduced MGR achieved with the use of *Copaifera* sp. oil, compared to the other oils tested, demonstrates its potential fungitoxic effect, which makes it a promising alternative product to control diseases caused by fungi of the genus *Colletotrichum*.

The mean inhibition percentage of mycelium growth of *Colletotrichum* sp. (Table 1) indicates that all oils used in the study had some percentage of mycelium growth inhibition, but some of them with higher efficiency than the others, indicating promising results especially with the use of copaiba oil.

With respect to the mycelial inhibition percentage (MIP), the copaiba (*Copaifera* sp.) oil exhibited a higher effect with the dose of 150 µL.mL^{-1} (65.56%) (Figure 3). Considering the same concentration value, it was also found greater effects for the murumuru oil (46.44%), and the andiroba oil exhibited a greater inhibitory effect at the concentration of 200 µL.mL^{-1}. Of the oils studied, copaiba (*Copaifera* sp.) oil was the most effective, with over 50% of inhibitory effects for all concentrations tested.

DISCUSSION

In this study, only the copaiba oil showed a fungitoxic effect for all concentrations tested, inhibiting more than 50% of *in vitro* mycelial growth of *Colletotrichum* sp. isolated from banana plant fruits. Our results are in accordance with the work done by Solino et al. (2012), they observed that copaiba oil reduced the mycelial growth of *Colletotrichum gloeosporioides in vitro* and *in vivo* experiment. The application of natural defensive agents based on vegetable oil can be done by the method of spraying on the surface (Mamarabadi et al., 2018) or by immersion of the fruits (Solino et al., 2012).

The chemical composition of the oils has a direct influence on the inhibitory effects on the phytopathogen, since the possible antifungal action of the oils can be attributed to the presence of biologically active chemical substances (Pieri et al., 2009). Some chemical compounds have an excellent fungicidal potential for the control of phytopathogenic fungi, such as alcohol, aldehydes, derivatives of fatty acids, terpenes and phenols can be found in vegetable oils (Ncama et al., 2019). Thus, acting alone or synergistically, these molecules contribute to the biological activity exerted by vegetable oils (Calvo-Garrido et al., 2014).

The chemical composition of the oil can define its potential type of use and indicate possible biological activities. The andiroba and murumuru oils, for example, have a high content of fatty acids (Funasaki et al., 2016), and the lipid profile of andiroba can be represented in

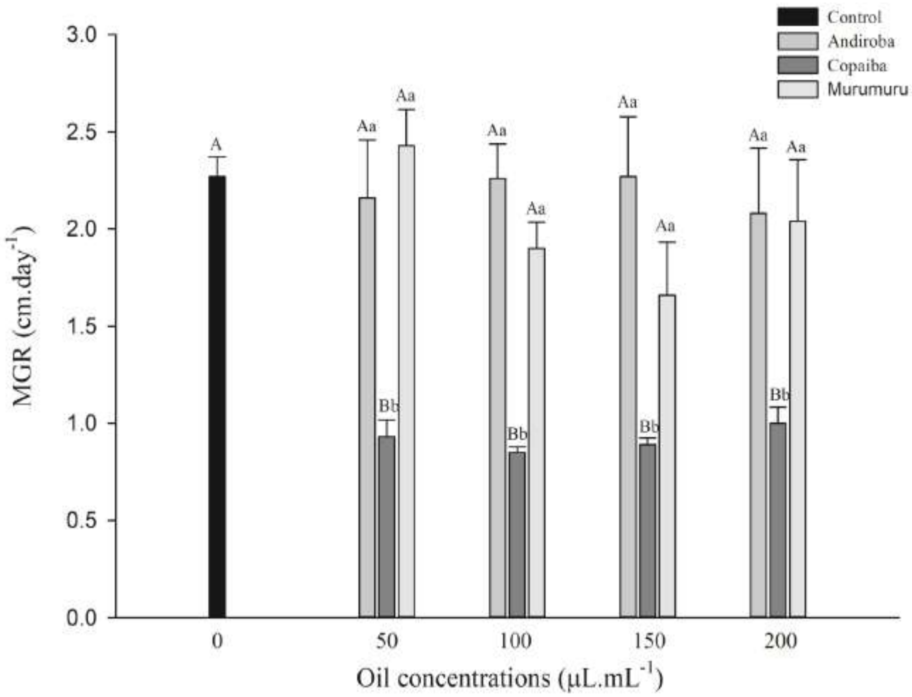

Figure 1. *In vitro* effects of vegetable oils on mycelium growth of *Colletotrichum* sp. * MGR: Micelial Growth Rate. Means followed by same uppercase letter refer to the comparison between different concentrations of the same oil, and lowercase letters for different oils for each concentration at 95% significance level by the Scott-Knott's test.

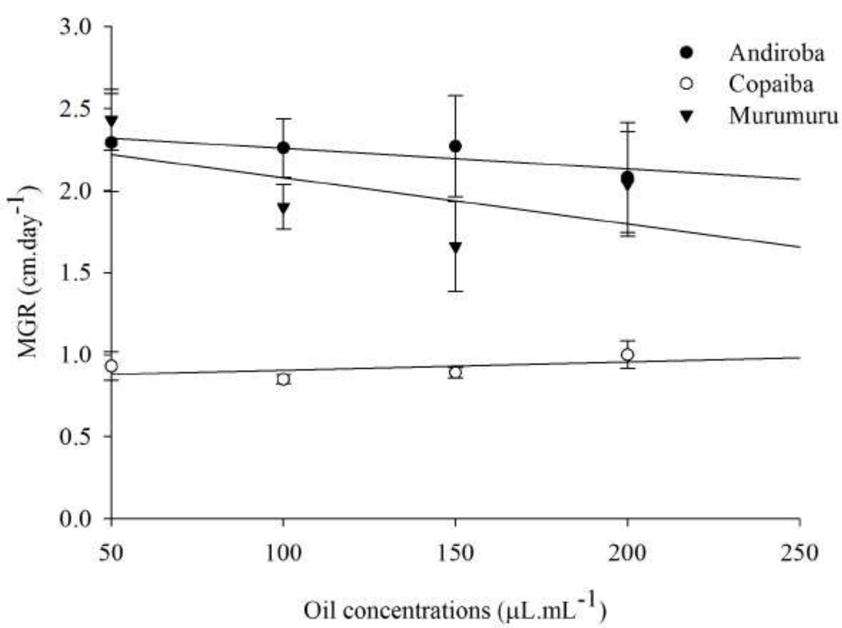

Figure 2. Effect of different concentrations of vegetable oils on the mycelial growth of *Colletotrichum* sp., which causes anthracnose in banana fruits.

more than 97% by oleic, palmitic, stearic, linoleic and arachidonic acids (Milhomem-Paixão et al., 2016). Copaíba oil presents diterpene acids, including copalic acid, kaurenoic acid, alepterolic acid and polyaltic acid in

Table 1. Mean mycelial inhibition percentage (MIP) of *Colletotrichum* sp*.* with different concentrations of oils from andiroba, copaiba and murumuru. Cruzeiro do Sul, Acre, Brazil. August of 2017.

Concentrations (µL.mL^{-1})	Percentage of inhibition of vegetable oils		
	Andiroba	Copaiba	Murumuru
50	17.6±6.5	61.11±6.1	23.56±4.3
100	12.67±6.4	62.89±1.1	39.11±3.9
150	25.5±5.9	65.56±1.1	46.44±6.2
200	34.89±6.3	63.56±1.7	37.78±6.8

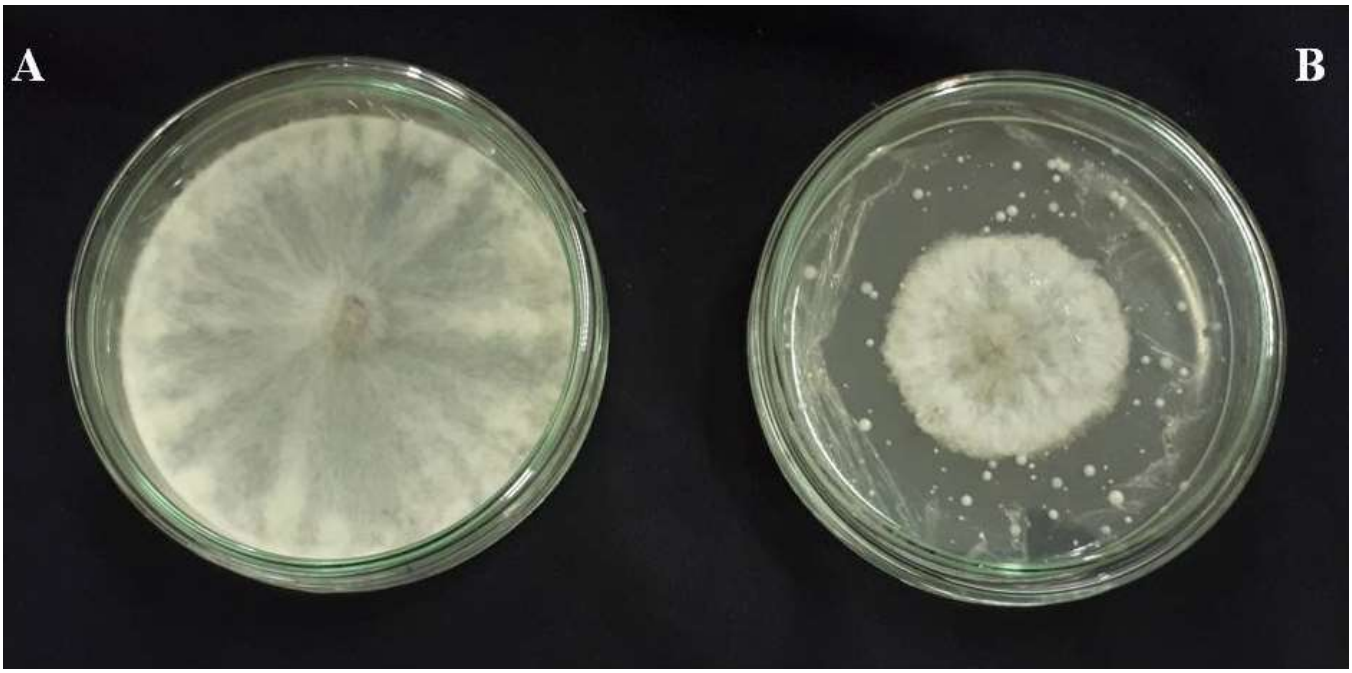

Figure 3. Effect of vegetable oil on the mycelial growth of *Colletotrichum* sp., isolated from banana fruits. A: Control treatment. B: Copaiba oil (150 µL.mL^{-1}).

its composition and which are considered biologically active (Trindade et al., 2018). With regard to the murumuru oil, no inhibitory effect on *Colletotrichum* sp. growth was found for the concentrations used. However, promising results were obtained in studies with other species of phytopathogenic fungi (Nascimento et al., 2014). The chemical substances present in vegetable oils have potential for the control of phytopathogenic fungi, and in this case the characteristics of the cell wall of the fungi and their interaction with the molecular structure of the applied compounds should also be considered (Avis and Bélanger, 2001). The ability to destabilize fungal structures and interfere with cell wall, plasma and mitochondrial functions, and depends on the specific interaction between fungus and vegetable oil used (Ncama et al., 2019).

The chemical components of plant oils cause morphological changes, increased fluidity in the membranes of fungal cells, alterations in protein conformation or enzymatic activity, followed by destruction of organelles (Knechtle et al., 2014; Shokri, 2016), and changes in production of reactive oxygen species (ROS) (Nazzaro et al., 2017). The andiroba oil did not provide a fungitoxic effect on the mycelial growth of *Colletotrichum* sp. when compared to the control. Other researches with this oil also reported low inhibition of the mycelial growth with fungi of other species (Machado et al., 2013). However, Nascimento et al. (2019), working with the oil of two species of the genus *Carapa*, verified antifungal activity, suggesting that qualitative and quantitative differences in the chemical components of vegetable oils may also indicate their fungitoxic potential.

Investigations on the biological activity of plant oils and extracts along with biological control and induced resistance are technologies with a potential control of fungi that attack plants. The easy access and low toxicity potential of vegetable oils suggests that they should be recommended as alternative products for the control of these diseases (Fernandes and Bonaldo, 2011). It is worth noting that the mechanisms of action of vegetable oils on the mycelial growth of phytopathogenic fungi are relatively unknown. In this work, we identified inhibition of the mycelial growth of *Colletotrichum* sp. after subjecting it to different concentrations of vegetable oils. However, the mechanism of action still requires further investigations to evidence and substantiate the actual synergetic or isolated action of compounds.

Conclusion

Out of vegetable oils used in this work, copaiba oil showed promising results for the control of mycelial growth of *Colletotrichum* sp. at concentrations of 50 $\mu L.mL^{-1}$ and over and inhibition rates higher than 50%. These results show its potential use in the control of anthracnose in banana plant fruits. Further studies should be conducted in order to substantiate soundly the mechanism of action of the substances contained in these vegetable oils, and also to identify the active ingredient of each chemical component present in oil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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