

Chemical profile and potential antifungal of essential oil *Schinus terebinthifolius* and its by-products

Perfil químico e potencial antifúngico do óleo essencial de *Schinus terebinthifolius* e seus subprodutos

Perfil químico y potencial antifungico del aceite esencial de *Schinus terebinthifolius* y sus subproductos

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Abstract

This study aimed to investigate the distillation time to obtain a higher yield of essential oil and by-product (extract and hydrolate) of *Schinus terebinthifolius* seeds, to determine the main chemical constituents of these products and the potential fungitoxic on the *Colletotrichum gloeosporioides*. To obtain the essential oil and by-products, we used the method of steam distillation for 2.5, 4.0, 5.5 and 7.0 hours. The determination of the chemical composition was made by GC/MS. To determine the potential fungitoxic on mycelial growth of *C. gloeosporioides* various concentrations of the oil and by-products were tested. Distillation periods did not influence the yield of aqueous extract and essential oil hydrolate. No chemical compounds have been identified in hydrolat or aqueous extract with the use of GC analysis. The essential oil at concentrations of 2%, 3% and 4% inhibited approximately 47% of the *in vitro* development of the fungus *C. gloeosporioides*.

Keywords: *Colletotrichum gloeosporioides*; Alternative control; Aqueous extract; Volatile compounds.

Resumo

Este trabalho teve como objetivo investigar o tempo de destilação para obter um maior rendimento de óleo essencial e subproduto (extrato e hidrolato) de sementes de *Schinus terebinthifolius*, determinar os principais constituintes químicos desses produtos e o potencial fungitóxico sobre o *Colletotrichum gloeosporioides*. Para a obtenção do óleo essencial e subprodutos, utilizou-se o método de destilação a vapor por 2,5, 4,0, 5,5 e 7,0 horas. A determinação da composição química foi feita por CG / EM. Para determinar o potencial fungitóxico no crescimento micelial de *C. gloeosporioides*, várias concentrações do óleo e subprodutos foram testadas. Os períodos de destilação não influenciaram o rendimento do extrato aquoso e do hidrolato de óleo essencial. Nenhum composto químico foi identificado em hidrolato ou extrato aquoso com o uso de análise de GC. O óleo essencial nas concentrações de 2%, 3% e 4% inibiu aproximadamente 47% do desenvolvimento *in vitro* do fungo *C. gloeosporioides*.

Palavras-chave: *Colletotrichum gloeosporioides*; Controle alternativo, Extrato aquoso; Compostos voláteis.

Resumen

Este estudio tuvo como objetivo investigar el tiempo de destilación para obtener un mayor rendimiento de aceite esencial y subproducto (extracto e hidrolato) de semillas de *Schinus terebinthifolius*, para determinar los principales constituyentes químicos de estos productos y el potencial fungitóxico sobre *Colletotrichum gloeosporioides*. Para obtener el aceite esencial y sus subproductos, utilizamos el método de destilación al vapor durante 2.5, 4.0, 5.5 y 7.0 horas. La determinación de la composición química se realizó mediante GC / MS. Para determinar el potencial fungitóxico sobre el crecimiento micelial de *C. gloeosporioides*, se probaron varias concentraciones del aceite y subproductos. Los períodos de destilación no influyeron en el rendimiento del extracto acuoso y del hidrolato de aceite esencial. No se han identificado compuestos químicos en hidrolato o extracto acuoso con el uso de análisis GC. El aceite esencial en concentraciones de 2%, 3% y 4% inhibió aproximadamente el 47% del desarrollo in vitro del hongo *C. gloeosporioides*.

Palabras clave: *Colletotrichum gloeosporioides*; Control alternativo; Extracto acuoso; Compuestos volátiles.

1. Introduction

Schinus terebinthifolius Raddi, is a species native to South America, belongs to the family Anacardiaceae, also known as “aroeira da praia”, pink pepper and Brazilian pepper (Souza et al., 2012). Plant typical of Brazilian’s savanna, suitable for restoration and afforestation can be found from Ceará to Rio Grande do Sul state (Jones, 1997).

The leaves and ripe fruits of *S. terebinthifolius* are rich in essential oil (1.5-10%) (Richter et al., 2010). Studies reported high concentration of monoterpenes, along with some oil sesquiterpenes (Cole et al., 2014). The essential oil compounds contain astringent, anti-diarrheal, purgative, diuretic and febrifuges and have antimicrobial activity against gram-positive bacteria, and anti-inflammatory action. The essential oil of the plant is used through topical applications in the treatment of mycosis and candidiasis (Lima et al., 2020, Pereira et al., 2011)

Mono and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of most essential oils of vegetable origin and therefore have a high efficiency in

the control of plant pathogens. It was shown that the inhibitory action of fungal growth is often associated with the presence of mono and sesquiterpenes in the essential oil of plants (Sharifi-Rad et al., 2017).

It is known that a major problem in the production chain of the fruit are pathogenic agents that contribute significantly in reducing the production and supply of food to the population. Fungi are a major cause of disease in post-harvest fruit, while bacteria are the most important agents in deterioration of vegetables (Singh & Sharma, 2018). The fungi genus *Colletotrichum*, for example, are pathogens important in tropical and subtropical regions of the world and survive for long periods of time in soil, on plants and old bruises on fruits and leaves. Since the penetration of *Colletotrichum* sp. can occur on unripe fruits, the pathogen is able to survive as quiescent, and the symptoms manifested during fruit ripening (Cannon et al., 2012, Silva et al., 2020).

The intensive use of pesticides to control diseases, pests and weeds in agriculture, has caused many problems to the environment, such as the contamination of food, soil, water and animals, poisoning of farmers, the emergence of diseases associated with the use of pesticides, ecological imbalance and the elimination of beneficial organisms and reducing biodiversity. These and other factors led to increased research involving the use of alternative agents with the potential induction of resistance to control postharvest diseases (Aktar et al., 2009, Dantas et al., 2018).

This study aimed to investigate the time of distillation to obtain a higher yield of essential oil and by-products (extract and hydrolate) of *S. terebinthifolius* seeds, determine the main chemical constituents of these products, as well as potential antifungal thereof on the fungus that causes anthracnose tropical fruits.

2. Material and Methods

Mature seeds of *S. terebinthifolius* were collected in the city of São Cristovão, Sergipe State, Brazil. Botanical samples of material were deposited in the Herbarium of the Federal University of Sergipe under voucher n^o. 23108. After being harvested, the seeds were dried at room temperature for 48 hours to stabilize the mass. Then, the seeds were subsequently crushed in a blender semi-industrial (400W) until they reached a diameter uniform.

Extraction of essential oil and by-products

The method used was hydrodistillation in apparatus type Clevenger. 100 grams of crushed seeds were transferred in round bottom flasks with a capacity of 2L containing 1L of distilled water. Throughout the extraction process the system was protected from light and set the maximum power until the start of the distillation. Subsequently, the temperature of the heater blanket was adjusted to the boiling point of the liquid (95°C) which started counting time: 2.5, 4.0, 5.5 and 7.0 hours.

At the end of each time distilling the heating was turned off, we performed the separation of essential oil and by-products: hydrolate and aqueous extract which remained at the bottom of the distillation flask. Each fraction was quantified, transferred to amber vial 10 mL and stored in Ultrafreezer (-18 °C) to avoid degradation of its chemical constituents.

The yield of aqueous extract, hydrolate and seed oil of *S. terebinthifolius* were calculated using the following relationships volume/mass (Guimarães et al., 2008, Oliveira et al., 2011):

% Hidrolact = ((volume of hidrolact obtained (mL) / weight of plant material distillate (grams)) x 100,

% Essential oil = (volume of obtained oil (mL) / weight of plant material distillate (grams)) x 100,

% Aqueous extract = 100 % - (essential oil content + content hydrolate).

Analysis of the essential oil and by-products

The determination of the chemical composition of the seed oil of *S. terebinthifolius* and by-products (hydrolate and aqueous extract) was made in gaseous brand Varian, model 4000, coupled to an ICP mass (MS) with analyzer quadrupole and equipped with a capillary column CP- WAX52CB, 30 meters in length, diameter 0.25 mm and film thickness of 0.25 microns with helium gas carrier. The operating conditions of the gas chromatograph were: Internal pressure of the column of 21.3 psi, ratio split 1:50, gas flow in the column of 1mL.min⁻¹, injector temperature 220°C, detector temperature 260°C, programming column temperature: Start at 60°C warming to 240°C at a rate of increase of 3°C.min⁻¹ and remained at that temperature for 10 minutes totaling 70 minutes of analysis.

Initially carried by dilution of the essential oil in hexane at a ratio of 1:25. For the complete removal of water from the sample was added anhydrous sodium sulfate (Na_2SO_4). For analysis we injected 0.5 μml of diluted sample.

As for determining the chemical composition of hydrosols obtained from the distillation of seeds was accomplished using a gear brand Varian Model CP- 3800, coupled with a detector of the flame ionization hydrogen (FID) and equipped with a capillary column CP- WAX52CB, 30m in length, diameter 0.25 mm and film thickness of 0.25 microns and the helium carrier gas. The operating conditions of the gas chromatograph were: Internal pressure of the column of 21.3 psi, ratio split 1:100, gas flow in the column of $1\text{mL}\cdot\text{min}^{-1}$, Injector temperature 220°C , Detector temperature 240°C , programming column: Starts at 60°C warming to 240°C at a rate of $3^\circ\text{C}\cdot\text{min}^{-1}$ and remained at that temperature for 10 min, totaling 70 min analysis.

The analysis of hidrolact carried by diluting 1 mL of the same in 1mL of hexane. For the complete removal of water from the sample, it was added a small amount of anhydrous sodium sulfate (Na_2SO_4). With the aid of a microsyringe injected with $1\mu\text{L}$ of this solution.

The identification of the compounds was performed by comparing the retention indices of Kovats obtained experimentally with the tabulated values (Adams, 2007). To calculate the retention index Kovats, a mixture of linear alkanes patterns (C_9 to C_{24}) was injected into the apparatus (0.5 μl) under the same chromatographic conditions previously mentioned. It also used database library equipment (NIST - National Institute of Standards & Technology, USA) that allows the comparison of spectral data with those contained in the library.

Potential antifungal essential oil and By-products

To determine the potential fungitoxic of the aqueous extract, hydrolate and essential oil of mastic beach various concentrations of them were tested in order to determine the Minimum Inhibitory Concentration (MIC) of these in relation to mycelial growth of *Colletotrichum gloeosporioides*.

For this reason, in aseptic chamber, the Petri dishes previously sterilized were filled with PDA culture medium (Alfenas, 2007) sterilized at 121°C and 1 atm for 15 minutes with various concentrations relative to the volume of the culture medium, an aqueous extract (5, 10, 15, 20 , 25 and 30%), hidrolact (10, 15, 20 and 25%) and essential oil (1, 2 , 3, 4 and 5%) of *S. terebinthifolius* obtained by the process described in item extraction of essential oil and

by-products. The same was plus Tween 20, which acted as a solubilizing agent. Furthermore were prepared treatments plus various concentrations of Tween 20 for determining the possible fungicidal action of the detergent when added to the culture medium. As positive control we used the fungicide Viper 700[®] [tiophanate - methyl (Benzimidazole)].

After solidification of the culture medium was placed a disk of PDA with approximately 0.5 cm in diameter containing structures colony pathogen in the center of each plate. The plates were placed in BOD at 26 ± 1 °C under a photoperiod of 12 hours of light for seven days or until the colony treatment control reached the edges of the plate taking its entire surface. After that, with the aid of a caliper measurements were taken perpendicular to the axis diameter of the colony on each plate to determine the final diameter of the colony. Each treatment consisted of five Petri plates, each plate considered the experimental unit.

Statistical analysis

The experiment conducted was completely randomized in a 3 x 4 factorial design referring to the three plant materials (aqueous extract, hydrolate, essential oil) and four times distillation (2.5, 4.0, 5.5, 7.0 hours) with three replications, totaling 12 treatments and 36 installments. Data were subjected to analysis of variance using the statistical R program and means were compared by Tukey test at 5 % significance (Bhering, 2017, R Core Tem, 2018).

The entire experiment was conducted in a completely randomized design with 23 treatments and five repetitions each totaling 115 experimental units. Data were subjected to analysis of variance, Tukey test at 5% probability and regression analysis statistical R program.

3. Results and Discussion

Obtaining by-products of *S. terebinthifolius* seeds

After hydrodistillation harvested seed can be observed at the times for no influence on the yield of aqueous extract of hydrolate and essential oil (Table 1).

Table 1. Yield of by-products obtained from the hydrodistillation *S. terebinthifolius* Raddi.

| Distillation time (hours) | Aqueous Extract | Hydrolate | Essencial oil |
|---------------------------|-----------------|-----------|---------------|
| 2.5 | 89.64aA | 7.60aB | 2.76aC |
| 4.0 | 90.14aA | 7.20aB | 2.66aC |
| 5.5 | 89.74aA | 7.90aB | 2.36aC |
| 7.0 | 90.04aA | 7.10aB | 2.86aC |
| C.V. | 0.34 | 4.18 | 11.61 |

*Values followed by the same lower-case letter in the column do not differ by Tukey test at 5% significance. Values followed by the same capital letter in the line do not differ by Tukey test at 5% significance. Source: Author's own compilation (2020).

As observed in this experiment both the extraction of essential oil as by-products during a longer period showed no significant increase when compared with shorter extraction. The knowledge of the influence of time on yield contributes to make the process less costly, faster and less destructive with respect to the compounds present in the plant.

In addition, during all time of extraction, the aqueous extract showed a significantly higher value than other products, followed by hidrolact and the essential oil showed the lowest yield among the three income products assessed. This is because the extract is formed from the water used during the distillation process, while the hidrolact and oil are obtained from the condemnation of water evaporated by the plant material.

Large quantities of essential oil in the seeds of *S. terebinthifolius* were expected, due to them being considered reserve organs of the plant and present a cellular structure adapted to the accumulation of nutrients and solutes that are used in the development of the embryo when it starts the process of germination. Fact reported in which essential oils can be produced by a variety of existing structures in the different plant organs presenting yield, chemical composition and physical and chemical characteristics distinct (Dhifi et al., 2016).

Seeds of *S. terebinthifolius* showed hydrolate yields varying between 7.16 and 7.66 % at all time points were not statistically different from each other, while the amount of aqueous extract obtained from the seeds was about 90% for all distillation periods.

The different times of distillation does not result in significant differences in the levels of essential oil in seeds of *S. terebinthifolius*, with 2.86 % as higher oil seeds. It was also observed when extracted seed oil *S. terebinthifolius*, with a yield of 4.65%, confirming the high essential oil yield in seeds (Barbosa et al., 2007).

A small variation in the levels of by-products of the seeds was not significant. Thus the use of 2.5 hours distillation has become most suitable for exposing the plant material to high temperatures for a shorter period of time thus reducing the chemical degradation of some

components of these products are more sensitive to heat. Essential oils, because they are mostly formed by substances from the class of terpenes, are volatile and lipophilic compounds and at high temperatures may decompose forming isoprene (Turek and Stintzing, 2013).

Sesquiterpenes as major components found during the steam distillation of chamomile (*Chamomilla recutita* (L.) Raeuchert) while during the steam distillation was found caryophyllene as the major component of this plant. This fact indicates that the synthesis of caryophyllene may have been stimulated by exposure for a greater period to heat the distillation (Borsato et al., 2008). Working with rosemary (*Rosmarinus officinalis* Linn) obtained as major compounds α - pinene, β - myrcene, camphor and eucalyptol after being employed different extraction times (30, 60, 90 and 120 minutes), whereas the highest periods (90 and 120) stimulated the concentration of α -pinene and β -myrcene, as camphor and eucalyptol to be more soluble in water are extracted in the early stages with a tendency as the volatilization time of distillation (Prins et al., 2006).

The contents present in the essential oil of *S. terebinthifolius* obtained after a few hours of distillation indicate that the volatile compounds existing in this plant are of easy volatilization, possibly due to their low molecular weight and consequent low boiling temperature. Several authors (Cavalcanti et al., 2015) observed that the essential oil of *S. terebinthifolius* has several monoterpenes as major components that give the oil density and molecular weight lower to those found in the essential oil of other plants.

Determining the chemical composition of by-products S. terebinthifolius seeds

The chromatographic analysis did not identify chemical compounds in aqueous extract and hidrolact mastic, possibly because they are in quantities below the detection limit of the GC / FID or they are volatilized, since essential oils of the same species studied were found in many compounds (Santos et al., 2010).

From the information in Table 2, were identified 16 major compounds, totaling more than 91% of the chemical composition of the essential oil of mastic after various periods of distillation.

Table 2. Percentage of volatile compounds identified in the essential oil extracted from *Schinus terebinthifolius* seeds distillate for different periods.

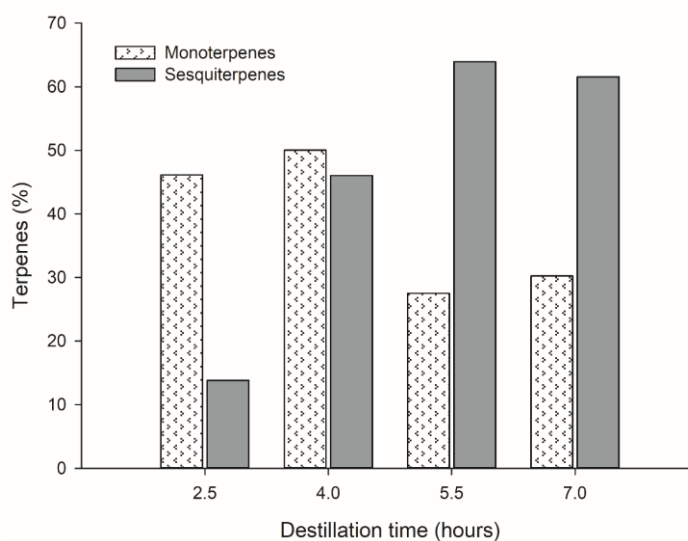
| Compound | R.T ^a | Distillation time (hours) | | | |
|-------------------------|------------------|---------------------------|--------|--------|--------|
| | | 2.5 | 4.0 | 5.5 | 7.0 |
| ρ -Menth-1-en-9-ol | 4.393 | 35.340 | 29.128 | 8.277 | 9.381 |
| β -pinene | 4.527 | 2.559 | 2.712 | 1.289 | 1.372 |
| α -thujene | 4.586 | 1.993 | 1.893 | 1.262 | 1.443 |
| Canphene | 5.210 | 2.131 | 5.110 | 4.785 | 5.268 |
| α – fenchene | 5.401 | 2.438 | 8.201 | 8.463 | 9.467 |
| Terpinen-4-ol acetate | 7.131 | 1.289 | 1.014 | 0.619 | 0.619 |
| Bornyl acetate | 16.646 | 0.328 | 1.533 | 1.697 | 1.820 |
| Caryophyllene | 17.218 | 0.571 | 1.851 | 2.091 | 1.965 |
| Terpinen-4-ol | 17.540 | 0.204 | 0.880 | 1.387 | 1.339 |
| α -terpineol | 20.970 | 0.179 | 1.073 | 1.410 | 1.352 |
| germacrene – D | 21.193 | 2.055 | 6.943 | 7.922 | 7.917 |
| δ -cadinene | 23.028 | 1.005 | 1.005 | 1.094 | 1.010 |
| Hedycaryol | 33.754 | 3.175 | 11.626 | 18.634 | 18.837 |
| α -gurjunene | 36.320 | 2.326 | 8.343 | 12.133 | 11.747 |
| α -eudesmol | 37.825 | 1.936 | 6.757 | 9.177 | 8.310 |
| β -eudesmol | 38.018 | 2.417 | 7.987 | 11.151 | 9.946 |
| Others | | 40.054 | 3.944 | 8.609 | 8.207 |

^aRetention index calculated using the Van den dool and Kratz (1963) (40) equation for a homologous series of n-alkanes (nC9–nC18). Source: Author's own compilation (2020).

The essential oil showed a fairly uniform composition in all periods of distillation varying the amount of each component after a certain time of distillation. After 2.5 hours of distillation the oil showed the compound ρ -Menth-1-en-9-yl as whereas the other major essential oils contained: ρ -Menth-1-en-9-ol, camphene, germacrene-D, hedicariol, α -gurjunene, α -eudesmol and β -eudesmol as major compounds.

It is observed in Figure 1, along the distillation there was a decrease in the amount of monoterpenes found in essential oils, against an increase in the amount of sesquiterpenes with increasing time of distillation thereof. The change in the composition of the oil shows that the increased exposure of the oil to heat can degrade chain compounds with a lower number of carbons and stimulate the synthesis of other compounds with a higher number of carbons.

Figure 1. Content of mono- and sesquiterpenes in the essential oil extracted from *Schinus terebinthifolius* seeds distilled for different periods.



Source: Author's own compilation (2020).

The various periods of distillation altered concentrations of certain compounds in the essential oil of mastic, significantly increasing the amount of compounds with retention times greater, for example, camphene, α -fenchene, germacrene-D, δ -cadinene, hedicariol, α -gurjunene, α -eudesmol and β -eudesmol, oils distilled for 4.0, 5.5 and 7.0 hours when compared to oil distilled for 2.5 hours.

It was also observed that there was a significant reduction in the concentrations of p -Menth-1-en-9-yl when comparing the oil obtained from distillation of 2.5 hours for a period of 4.0, 5.5 and 7.0 hours (Table 2). This fact was also observed when essential oil extracted from seeds and leaves mastic beach and observed increase in the amount of certain compounds and reduce the amount of other during the rising period distillation (Cavalcanti et al., 2015).

The phytochemical profile of the essential oil derived from this study is different from those shown by other authors that have used similar methods of extraction (Pilati et al., 2018, Cole et al., 2014), where noted, for example, the presence of α -phellandrene, β -phellandrene, elemol, α -cadiol, sabinene, δ -3-carene and sylvestrene, while in this study these compounds were not observed, indicating that the genetic characteristics of the plant and abiotic factors significantly interfere with the chemical composition of the essential oil of *S. terebinthifolius* seeds.

A large variation in the chemical composition of the essential oils of plants directly linked to genetic and environmental factors (Duarte et al., 2010) and, who also found variation in the chemical composition of the essential oil of *Hyptis suaveolens* L. Poit in different growing conditions (Martins et al., 2006).

Thus, it was found that exposure over a long period of distillation for obtaining the essential oil at elevated temperatures can alter the concentrations of their major compounds in the same way that genetics and environmental factors can alter the concentration and composition of the essential oils of *S. terebinthifolius* seeds.

Fungitoxic potential by-products of S. terebinthifolius

In the tests *in vitro* for the control of the fungus *C. gloeosporioides* by-products from the distillation of *S. terebinthifolius* seeds, the plates treated with different concentrations of the essential oil statistical differences when compared with the control, and the fungicide treatment had the highest fungus control when compared with others (Table 3).

Table 3. Mean diameter of colonies of *Colletotrichum gloeosporioides* on PDA culture medium containing aqueous extract, essential oil and hidrolact extracted from *Schinus terebinthifolius* seeds.

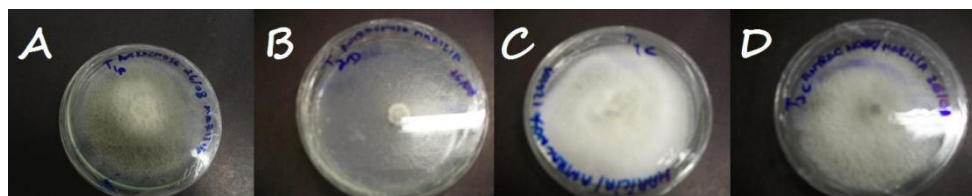
| Treatment | Diameter |
|-------------------|----------|
| Fungus | 8.44f |
| Fungicide | 1.67a |
| Fungicide + Tween | 1.49a |
| Tween 0.1% | 6.69d |
| Tween 0.2% | 5.64c |
| Tween 0.3% | 7.42de |
| Tween 0.4% | 6.78d |
| Tween 0.5% | 7.05d |
| Extract 5% | 8.74f |
| Extract 10% | 8.86f |
| Extract 15% | 8.46f |
| Extract 20% | 8.73f |
| Extract 25% | 9.0f |
| Extract 30% | 8.34ef |
| Hydrolate 10% | 8.8f |
| Hydrolate 15% | 8.28ef |
| Hydrolate 20% | 8.64f |
| Hydrolate 25% | 8.5f |
| Oil 1.0% | 5.69c |

| | |
|----------|--------|
| Oil 2.0% | 4.81bc |
| Oil 3.0% | 4.77bc |
| Oil 4.0% | 4.11b |
| Oil 5.0% | 4.77b |
| C.V. | 5.86 |

*Values followed by the same lower-case letter in the column do not differ by Tukey test at 5% significance Source: Author's own compilation (2020).

The concentration 5, 10, 15, 20, 25 and 30% aqueous extracts, 10, 15, 20 and 25% hidrolact incorporated into the PDA culture medium did not show efficiency in combating *in vitro* development of the fungus *C. gloeosporioides*. The fungus was developed very well in these treatments and had a cottony aspect when compared to plates fungicide Viper 700[®] which showed no fungal growth (Figure 2).

Figure 2. In vitro development of *Colletotrichum gloeosporioides* when subjected to treatments: A = control, B = fungicide, C = hydrolate 10%, D = aqueous extract 5%.



Source: Author's own compilation (2020).

The low efficiency presented by hydrolate and aqueous extract of *S. terebinthifolius* on the fungicidal activity may be due to the absence of bioactive substances or the presence of these low concentrations.

Other plant species were also not effective in controlling fungus, where aqueous extracts obtained from leaves and fruits of *Melia azedarach* L. incorporated into the PDA culture medium did not affect significantly the mycelial growth of the fungus *C. gloeosporioides* (Damas, 2009).

The negative effect of the aqueous extract was also found in *Rosmarinus officinalis* L., *Cordia verbenacea* DC., *Solanum sisymbriifolium* Lam., *Phyllanthus corcovadensis* Müll. Arg., *Eclipta alba* Hassk. and *Curcuma longa* L., on the mycelial growth of the fungus *C. gloeosporioides* (Silva et al., 2009).

Failure to inhibit the development of the fungus may have been stimulated by a change in the chemical composition of the extracts after prolonged heating. In a study on the use of aqueous extracts and decoctions in the *in vitro* control of *C. gloeosporioides* and *Glomerella*

cingulata (Rozwalka et al., 2008).also found that some substances can also have the opposite effect, stimulating the development of the fungus. The superior effect of the essential oil when compared to extracts probably happens due to the high concentrations of the active compounds in oils (Sharifi-Rad et al., 2017).

Table 3 shows that the treatments with 1, 2, 3, 4 and 5% essential oil showed a significant reduction in the size of the colonies compared with the control. The figures indicated that the essential oil of *S. terebinthifolius* presents inhibition average 46.97% on the growth of the fungus *C. gloeosporioides*. The effect of oil on fungal growth was due to fungitoxic action thereof, which increased with increasing the oil concentration in the culture medium. The maximum value of the reduction of growth of the fungus presented in this paper is given by the fact that the concentration of the constituents of the oil has no effect on their potential antifungal from 2% concentration of essential oil in between. This fact is due to the mechanism of action of oil, which acts in a metabolic pathway specific for fungal growth (Nazzaro et al., 2017). As the volatile nature of essential oils can cause a drop in potential fungitoxic because its active components evaporate and cease to act on the growth of fungus.

The reduction in the fungitoxic action of essential oils to their volatile nature as well as the instability of these constituents to light, temperature and humidity, which can modify the atmosphere inside the plates and thus decrease the action of essential oil on the fungi (Chouhan et al., 2017). Without forgetting that the fungicidal action of essential oils is often associated with synergism between the compounds of major and minor oil (Sampaio et al., 2020).

Thus even with the increase of the concentration of essential oil in the culture, medium synergism between its components and its consequent fungitoxic action may be limited by the mechanism of action of the essential oil on the fungus.

Despite the good performance of the essential oil in the control of *C. gloeosporioides*. The treatment of lowest mycelial growth was containing the fungicide Viper 700[®] in the composition of the culture medium. This action was consistent with the nature of the fungicide class benzimidazole class which is widely used to control species of the genus *Colletotrichum*.

4. Final Considerations

- The time of hydrodistillation has no influence on the yield of essential oil and by-products,

- Seeds of *S. terebinthifolius* showed high amounts of essential oil,
- Distillation lasting 2.5 hours is the optimal period for obtaining the by-products of *S. terebinthifolius*,
- The chromatographic analysis did not detect the compounds present in the aqueous extract and hydrolate,
- The essential oil of *S. terebinthifolius* presents a volatile profile consisting mostly of ρ -Menth-1-en-9-ol, α -thujene, β -pinene, camphene, α -fenchene acetate, terpinen-4-ol acetate, bornyl, caryophyllene, terpinen-4-ol, α -terpineol, germacrene-D, δ -cadinene, hedycaryol, α -gurjunene, α -eudesmol, β -eudesmol,
- The aqueous extract and hydrolate at all concentrations tested did not show potential fungitoxic *in vitro* development of *C. gloeosporioides*,
- Essential oil of *S. terebinthifolius* at concentrations of 2%, 3 % and 4 % inhibited approximately 47% of development of the fungus *C. gloeosporioides in vitro*,
- The essential oil of *S. terebinthifolius* has great potential in the control of diseases caused by *C. gloeosporioides* during post-harvest guavas.

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