

Plant Protection - Original Article - Edited by: Ivan Herman Fischer

### Antifungal potential of essential oils from Pectis brevipedunculata and Dizygostemon riparius in anthracnose control in mango

Lukas Allayn Diniz Corrêa<sup>1</sup>, 
 Antônia Alice Costa Rodrigues<sup>1</sup>, 
 Larisse Raquel Carvalho Dias<sup>1</sup>, 
 Erlen Keila Candido e Silva<sup>1</sup>, 
 Odair dos Santos Monteiro<sup>2</sup>,
 Leonardo de Jesus Machado Gois de Oliveira<sup>1</sup>.

<sup>1</sup> Universidade Estadual do Maranhão (UEMA), São Luis-MA, Brasil. <sup>2</sup> Universidade Federal do Maranhão, São Luis-MA, Brasil.

\*Corresponding author: aacrodrigues@outlook.com

Abstract: Essential oils have been widely studied because they are natural sources of substances that have antimicrobial properties. In fruit growing, especially in mango crop, anthracnose (*Colletotrichum gloeosporioides*) is considered the main disease because it causes series of damages in the fruit production chain and, in this sense, essential oils can be an option in this disease control. The objective of this study was to evaluate the effect of essential oils obtained from the leaves of Chá-de-moca (Pectis brevipedunculata) and Melosa (Dizygostemon riparius) in the in vitro and in vivo control of the C. gloeosporioides fungus, in seedlings and mango fruits. The in vitro bioassays were performed by testing the effect of different concentrations of essential oils (0, 1, 2, 3 and 4  $\mu$ l/mL) on the reduction of mycelial growth of the C. gloeosporioides fungus. Additionally, the anthracnose control in mango seedlings (Tommy Atkins, Constantina, Comum and Rosa cultivars) was evaluated by foliar application of essential oils at 4 µl/mL concentration. Disease control in fruits of the same mango cultivars was evaluated at 3  $\mu$ /mL concentration of essential oils. The evaluation was carried out by measuring the average diameter of the colonies, for the *in vitro* treatment, and the lesions for the *in vivo* treatments, in two diametrically opposite directions. There was a decrease in the fungus mycelial growth in all tested concentrations. There was a decrease in the disease severity from the sixth day after the oils application in Tommy Atkins cultivar seedlings, when treated with both essential oils at 4 μl/mL concentration. As for preventive treatments with fruits, it was observed that all cultivars achieved a reduction in severity from 54.83% at 3  $\mu$ l/mL concentration with the use of both essential oils. Given the results obtained, it was observed that the essential oils *P. brevipedunculata* and *D. riparius* can be a viable alternative in the anthracnose control in mango culture.

**Index terms:** *Dizygostemon riparius; Pectis brevipedunculata;* Alternative Control; Medicinal plants; *Mangifera indica* L.

Rev. Bras. Frutic., v.45, e-889 DOI: https://dx.doi.org/10.1590/0100-29452023889 Received 25 Aug, 2022 • Accepted 18 Apr, 2023 • Published Sep/Oct, 2023. Jaboticabal - SP - Brazil.



#### Potencial antifúngico de óleos essenciais de Pectis brevipedunculata e Dizygostemon riparius no controle de antracnose em manga

Resumo: Os óleos essenciais têm sido largamente estudados por serem fontes naturais de substâncias que possuem propriedades antimicrobianas. Na fruticultura, especialmente na cultura de manga, a antracnose (Colletotrichum gloeosporioides) é considerada a principal doença por causar uma série de prejuízos na cadeia produtiva dos frutos, e, nesse sentido, os óleos essenciais podem ser uma opção no controle desta doença. O objetivo deste trabalho foi avaliar o efeito dos óleos essenciais obtidos das folhas de Chá-de-moça (Pectis brevipedunculata) e Melosa (Dizygostemon riparius) no controle in vitro e in vivo do fungo C. gloeosporioides, em mudas e frutos de mangueira. Os bioensaios in vitro foram realizados testando-se o efeito de diferentes concentrações dos óleos essenciais (0; 1; 2; 3 e 4 µl/mL) sob a redução do crescimento micelial do fungo C. gloeosporioides. Adicionalmente, avaliou-se o controle da antracnose em mudas de manga (cultivares Tommy Atkins, Constantina, Comum e Rosa), pela aplicação foliar dos óleos essenciais, na concentração de 4 µl/mL. O controle da doença em frutos das mesmas cultivares de manga foi avaliado na concentração de 3 µl/mL dos óleos essenciais. A avaliação foi feita pela mensuração do diâmetro médio das colônias, para o tratamento in vitro, e das lesões, para os tratamentos in vivo, em dois sentidos diametralmente opostos. Houve diminuição do crescimento micelial do fungo em todas as concentrações testadas. Observou-se diminuição na severidade da doença a partir do sexto dia após aplicação dos óleos nas mudas de cultivar Tommy, quando tratada com ambos os óleos essenciais, na concentração de 4 µl/mL. Já para os tratamentos preventivos com frutos, foi observado que todas as cultivares obtiveram redução da severidade a partir de 54,83%, na concentração de 3 µl/mL, com uso de ambos os óleos essenciais. Diante dos resultados obtidos, foi observado que os óleos essenciais P. brevipedunculata e D. riparius podem ser uma alternativa viável no controle de antracnose na cultura de manga.

**Termos para Indexação:** Melosa; Chá-de-moça; Controle Alternativo; Plantas medicinais; *Mangifera indica* L.

#### Introduction

Fruit growing has been reaching new levels every year and its full development evidences an increase in the demand for consumers for good appearance and quality products, combined with the lower use of pesticides. Such factors have required great attention by the producers regarding the phytosanitary care in the fruits production and post-harvest phases (CERDEÑO, 2006; LIMA et al, 2015). Among the fruit species most produced in Brazil, mango (*Mangifera indica* L.) stands out, a tropical fruit of soft taste and aroma, combined with an attractive coloration and nutritional value due to the carotenoid, carotene content and other vitamin sources (ZHENG et al., 2013).

However, every year, about 28% of this culture is lost due to problems occurring in the post-harvest. One of the major losses causes of this fruit is the susceptibility to fungal diseases, which anthracnosis stands out, caused by the *Colletotrichum gloeosporioides* Penz fungus (HU et al., 2014). This is one of the main diseases that affect mango fruit after the harvest, whose symptoms manifest in the form of dark coloring and circular format in the fruit peel, which evolve exceeding the peel reaching the pulp, resulting in reduction of the quality and shelf life (SANZANI et al., 2016). Among the main techniques used to minimize the losses caused by the development of these phytopathogens it has been used resistant cultivars, cultural control, biological control and chemical control through the application of synthetic fungicides, which still stands out as the main measure adopted (PHOULIVONG et al., 2010). However, the broad use of synthetic products has resulted in significant consequences and disadvantages, including a sharp increase in carcinogenicity, high and acute residual toxicity, degradation and environmental pollution, influence on the sensory characters of the food and side effects in humans (SANTOS et al., 2012; SHAO et al., 2015). Considering the secondary problems caused by the use of synthetic fungicides in the agroecosystems and in the environmental scenario, new differentiated segments and practices more sustainable to the environment have been studied (MORANDI and BETTIOL, 2019). These segments aim to change the priorities of the conventional agriculture systems, reducing the dependence by chemicals products and other polluting inputs to the environment, paying more attention to the use of biological products in the agricultural systems (BETTIOL, 2010).

Associated to such sustainable practices, the essential oils potential with antifungal action has been widely investigated, they are constituted of complex compounds elaborated from the secondary plants metabolites and with low toxity to humans (RAUT; KARUPPAYIL, 2014). With this, studies have evidenced the efficiency of these oils obtained from a wide range of botanical species, in the control of post-harvest diseases such as anthracnose in fruit plants (BONALDO et al., 2007; LIMA et al., 2021).

*In vitro* control of *C. gloeosporioides* with plant oils has been observed in several studies. Nobre and Marques (2021) evidenced that clove essential oil, *Caryophyllus aromaticus* L., presented efficiency in the inhibition of *C. gloeosporioides* mycelial growth from the lowest tested concentration (10<sup>-5</sup>). Sousa, Serra and Melo (2012), found prom-

ising results that corroborate the fungitoxic efficiency of essential oils and verified in their research with ten essential oils tested, that only the babaçu oils (*Attalea speciosa* Mart.), grape seed (*Vitis vinifera* L.) and almond (*Prunus dulcis L.*) did not present efficiency in the *C. gloeosporioides* fungus control. However, eucalyptus (*Eucalyptus glubulus* Labill.), copaíba (*Copaifera langsdorffii* L.), coconut (*Cocos nucifera* L.), neem (*Azadirachta indica* A. Juss), mint (*Mentha piperita* L.) and pink stick, (*Aniba rosaeodora* Ducke) oils presented excellent results inhibiting the *C. gloeosporioides* fungus mycelial growth.

In this context, highlights the importance of controlling phytopathogenic diseases with the use of sustainable natural sources, which promote greater food and nutritional security, with more effective and less toxic substances for humans and the environment, replacing synthetic chemicals (SHARMA et al., 2017; LIMA et al., 2021).

This study presents the first result of the antifungal potential of Chá-de-moça (P. brevipedunculata) and Melosa (D. riparius) typically tropical species as bioactive agents to control anthracnose in mango. P. brevipedunculata is a small size species, with small leaves and yellow flowers, rich in essential oil, but still little explored in the phytopathogenic control scope (BRITO and MARQUES, 2008). D. riparius is a newly discovered species and little described in the literature, making more studies involving this plant of paramount importance, that is currently evaluated as endangered according to the categories and criteria of the International Union for Conservation of Nature (SCATIGNA et al., 2019). Such studies thus fill in an existing gap in the scientific knowledge. Associated to this, this study aimed to evaluate the effect of Chá-de-moça (Pectis brevipedunculata (Gardner) Sch.Bip.) and Melosa (Dizygostemon riparius Scatigna & Colletta) essential oils in the control of the Colletotrichum gloeosporioides fungus in vitro and in vivo, in mango seedlings and fruits (Mangifera indica L.).

#### Material and Methods Experiment location and obtainm

## Experiment location and obtainment of the phytopathogen

The experiments were conducted in the State University of Maranhão – UEMA, Campus Paulo VI, São Luís-MA. The *C. gloeosporioides* isolate was obtained from mango fruit Rosa cultivar, with anthracnose symptoms and the pathogen isolation of the symptomatic tissue was done in Petri plates containing the PDA culture medium (potato-dextrose-agar) with 250mg/L of chloramphenicol (Figure 1). The plates were incubated in the BOD incubator under 12h of light, with average temperature of 25±2°C. After the growth, it was realized morphological identification and pathogenicity test of the fungus, this was stored in test tubes, in refrigerator and was deposited in the Mycotheque "Prof. Gilson Soares da Silva" – SISBIO nº 5991355, with the MGSS–426 record where the molecular confirmation of its identification as belonging to the *C. gloeosporioides* complex based in the ITS region.



**Figure 1.** Mango fruit (Rosa cultivar) with Anthracnose symptoms collected in São Luís do Maranhão (A); *Colletotrichum gloeosporioides* colony in the PDA culture medium (B); Hyphae and conidia of *C. gloeosporioides* observed in light microscope (40x of increase) (C). Source: (São Luís, 2021).

## Collecting plant material and extracting essential oils

Essential oils were extracted from *P. Brevipedunculata* and *D. riparius* leaves following the methodology described by Brandão et al. (2020). The extractions were performed in the UFMA Chemistry laboratory. The extraction process was by hydro-

distillation with Clevenger device modified and adopted by the Brazilian Pharmacopoeia (AKISUE, 1983). The extraction time was approximately three hours. The samples of the essential oils were submitted to gas chromatography analysis coupled to mass spectrophotometer (CG-EM QP2010 Plus), Shimadzu brand, using a DB-5MS capillary column ( $30m \ge 0.25mm \ge 0.25\mu$ m), which indicated the chemical composition and majority component of each essential oil. After the extraction, these were placed in sealed ampoules, identified and kept in refrigerator at 4°C until the moment of the bioassays installation. The oleic capacity of the studied species was calculated according to Monteiro et al. (2021) through Equation 1:

> Yield =  $v_{oil(ml)} / BLU(g) \times 100$ (Equation 1)

Where: BLU is calculated by Equation 2:

 $BLU = m_{\text{plant material}} - (m_{\text{plant material}} \times \%_{\text{humidity}})$ (Equation 2)

## Essential oils effect in the C. gloeosporioides mycelial growth

The bioassays were installed in Petri plates with concentrations of 0, 1, 2, 3, and 4,  $\mu$ l/ mL of the essential oils from P. brevipedunculata and D. riparius leaves in two distinct bioassays. Predetermined concentrations of the essential oils were added to the founding culture medium (PDA Himedia<sup>®</sup>). Disks of 5 mm in diameter containing C. gloeosporioides cultures with 7 days of growth were transferred to the center of the plates with means containing the essential oils. The inoculated plates were sealed and kept in the BOD incubator under 12h of light, with average temperature of 25±2°C. The evaluation was performed by the measurement of the average diameter of the colonies in two diametrically opposite senses, with regular intervals of 24 hours for ten days. From the values obtained from the average diameter of the fungal isolate was calculated the mycelial growth speed index (MGSI), adopting the formula described by Maia et al. (2015).

$$MGSI = (D - Da) / N$$

Where: D= current average diameter of the colony; Da= average diameter of the colony in the previous day; N= number of hours or days after transfer of the mycelium disk to the plate with the treatment.

The experimental design adopted was entirely randomized, with five essential oils concentrations and six repetitions, each Petri plate constituted a repetition. The tests were performed twice to confirm the stability because it is living organisms. The data were submitted to the variance analysis (ANOVA) and Tukey test at 5% of probability, using the R Studio version software (2020).

### Essential oils in anthracnose control in Mango seedlings

Seeds of Tommy Atkins, Constantina, Comum and Rosa mango varieties were planted in 5 L pots containing soil and autoclaved tanned manure, in the 3:1 proportion, maintaining one seedling per pot, and coverage fertilization was performed at 60 days according to the recommendation for the culture (FONSECA and BORGES, 2021). The pathogen inoculation was performed 90 days after the seed germination, by the inoculation method of the fungus mycelium discs in the leaves. The seedlings were watered twice a day during the experiment until the completion of the work steps in the greenhouse (MENEZES and ASSIS, 2004). For the inoculation, the MGSS - 426 isolate of C. gloesporioides was cultivated in Petri plates in the same conditions described previously. During inoculation, 5 mm diameter discs containing fungal growth were placed on lesions performed in the foliar surface, the control was inoculated only with PDA discs, and the seedlings kept in humidity chamber. After 48 hours the P. brevipedunculata and D. riparius essential oils in concentration of 4  $\mu$ l/mL were pulverized individually in all the seedlings' leaves. Soon after the application of the treatments, the seedlings returned to the humidity chamber for another 48 hours, and then they remained in the greenhouse for the evaluation of the symptoms.

The disease severity in the inoculated leaves (lesion diameter) was evaluated at 3, 6, 10 days after the inoculation with the aid of a digital caliper. Three repetitions were performed for each treatment, each repetition composed of a seedling, where five leaves were inoculated in two points, totaling ten inoculation points per repetition. The data were submitted to the Shapiro-Wilk normality test, and the means compared by the Tukey test at 5% of probability using the R Studio version software (2020).

## Essential oils in the anthracnose control in mango fruits

Tommy Atkins, Constantina, Comum and Rosa healthy fruits were acquired in the intermediate maturation stage without the presence of lesions. Initially the fruits were washed in tap and soap water, then immersed in sodium hypochlorite solution 2% (v/v) for 1 minute, and again washed three times in sterile distilled water.

After the assepsia, the fruits remained in room temperature until they were completely dry. After the drying, the fruits were dived, for 1 minute, in concentration of 3  $\mu$ l/mL of P. brevipedunculata or D. riparius essential oils, following an entirely randomized design, with factorial arrangement composed of three treatments (control, P. brevipedunculata essential oil and D. riparius essential oil) and cultivars evaluated (Tommy Atkins, Constantina, Comum and Rosa), with four repetitions per treatment, each repetition composed of a fruit with two inoculation points. The experiments were performed twice. The controls were immersed only in distilled water. Tween 80® 1% was used to solubilize the oil in the water for the treatments application.

After the period of 1 hour after immersion in the essential oils, the fruits in room temperature were inoculated with *C. gloeosporioides*, performing holes with five point awl (5mm in diameter) in the depth of approximately 2mm in two locations (apex / base sense) of the fruits being subsequently placed on the lesions, discs (5mm in diameter) containing fungus mycelium. The fruits were placed in plastic trays, and submitted to humidity chamber for 72 hours in room temperature. After this period, the humidity chamber was removed and the fruits remained in plastic containers for six days for the evaluation of the symptoms.

The lesions diameters were determined daily for six days after the inoculation. In the last evaluation day, a transversal cut was performed at 4 cm in the fruits to visually evaluate the lesion depth in the pulp. The data were submitted to the variance analysis (ANOVA) and Tukey test at 5% of probability, using the R Studio version software (2020).

#### **Results and Discussion**

#### Identification of the majority components of the *P. brevipedunculata* and *D. riparius* essential oils

The yield in the extraction of the essential oils was of 1.5% for both studied species, similar to the observed in other studies (BRANDÃO et al., 2020; OLIVEIRA et al., 2011).

The oil from *P. brevipedunculata* leaves presented the citral substance, a monoterpenic aldehyde derived from the mixture of the geranial and neral isomers, as the majority components, representing approximately 76% of the plant oil *in natura*. Results similar to the Oliveira and Berbert, (2011) that obtained the citral (neral and geranial) as the majority component of *P. brevipedunculata*. It is known that this compound interferes in the biological processes that involve the electron transfer and reacts with compounds containing nitrogen (for example, proteins and nucleic acid) inhibiting the growth of phytopathogenic fungi (GUPTA, 2008).

In the *D. riparius* essential oil, the fenchil and fenchol acetate compounds were observed as the majority components. Menezes et al. (2018) evidenced the same majority compounds in the *D. riparius* essential oil indicating an effective action against the *Aedes aegypti* larvae. However, there are no reports in the literature on studies performed for the phytopathogen control with *D. riparius* oil, however the fenchil acetate compound can be observed as the majority compound in the oils of other plant species with proven biofungicidal potential (XAVIER et al., 2021).

According to Compagnone et al. (2010), the composition and cytotoxic activity of maravuvuia leaves (*Croton matourensis* Aubl.) essen-

tial oil were successful in inhibiting the fungus *Candida albicans* var. *stellatoidea*, having fenchil acetate (25.3%) as the majority constituent, as well as metileugenol (14.2%), isoelemicin (11.3%) and elemicin (7.6%).

## Effect of *P. brevipedunculata* and *D. riparius* essential oils on *C. gloeosporioides* mycelial growth

The essential oils tested proved to be efficient in the fungistatic control of *C. gloeosporioides* from the first day evaluated. There was reduction in the colonies diameter in the presence of *P. brevipedunculata* and *D. riparius* evidencing a relationship "dependent dose" with the concentrations of the essential oils (Table 1).

*C. gloeosporioides* fungus presented lower mycelial growth in the concentration of  $4\mu$ l/mL, showing greater sensitivity in the *P. brevipedunculata* oil. The mycelial growth speed index (MGSI), showed that the increase of the oils concentration promotes a gradual and significant decline in the mycelial growth (Table 1).

**Table 1**. Mean diameters and mycelial growth speed index (MGSI) of *C. gloeosporioides* colonies in the PDA culture medium with concentrations of 0 to 4 uL/mL of *Dizygostemon riparius* and *Pectis brevipedunculata* essential oils. Source: (São Luís, 2021)

ESSENCIAL OIL (µl/mL)	COLONY DIAMETER (MM)				
	Dizygostemon riparius	MGSI	Pectis brevipedunculata	MGSI	
0	72.8± 6.1 a	7.2±0.6	72.8± 6.1 a	7.2±0.6	
1	68.8± 4.3 b	6.5±0.4	64.9± 1.8 b	6.9±0.1	
2	61.9± 4.1 b	6.5±0.4	58.3± 0.5 c	6.4±0.0	
3	57.8± 3.0 b	6.3±0.3	50.5± 0.4 c	6.1±0.0	
4	41.8± 6.1 c	5.9±0.6	39.9± 0.1 d	5.7±0.0	

\*Averages followed by the same letter in the columns do not differ among themselves by the Tukey test at 5 % of probability.

Several authors, such as Pedroso et al. (2009), Ali et al. (2016) and Pastana et al. (2016), evidenced the antifungal potential of the essential oils in the *C. gloeosporioides in vitro*, due biofungicidal action present, derived from the secondary metabolism observed in the *P. brevipedunculata* oil.

In studies in C. gloeosporioides control, Schwan-Estrada et al. (2000) reported a positive action in the mycelial growth inhibition and sporulation of several phytopathogenic fungi due to the antifungal potential in the in vitro treatments with raw extract and the marjoram (Origanum majorana L.) essential oil that present the same majority components present in the *P. brevipedunculata* oil. Linde et al. (2010) observed that the same majority compound (citral) presented satisfactory results in the control of phytopathogenic fungi including the C. gloeosporioides fungus, where they found the complete inhibition of C. gloeosporioides at a concentration of 3  $\mu$ L/L.

# Effect of *P. brevipedunculata* and *D. riparius* essential oils on severity reduction of *C. gloeosporioides* in mango seedlings

For the tests with seedlings kept in the greenhouse the results show that there was no statistical difference in the treatments with both essential oils three days after the inoculation (data not shown). However, in the case of the different cultivars at the sixth and tenth day, there was significant statistical difference, highlighting the Tommy Atkins cultivar that presented greater severity when compared to the other cultivars (Table 2).

In the evaluation at the sixth day after the inoculation, it was possible to evidence that the *P. brevipedunculata* oil differed statistically from the control in the anthracnose control in the Tommy Atkins cultivar showing reduction of the disease severity (Table 2).

10.1± 0.8 Ba

13.7± 1.8 Bab

10.1± 1.8 Ba

19.4± 4.4 Aa

10.0± 0.7 Ba

brevipedunculata and Dizigostemon riparius essential oils.							
		DIAMETER OF LESIONS (MM)					
	COLIIVAR	Control	Dizygostemon riparius	Pectis brevipedunculata			
	Tommy	15.1± 3.0 Aa	12.6± 1.4 Aab	9.5± 2.0 Bb			
Six days	Constantina	11.7± 3.2 Ba	9.5± 1.0 Ba	9.7± 1.7 Ba			
	Comum	9.8± 0.2 Bb	9.7± 1.1 Bb	18.1± 4.2 Aa			

9.5± 0.2 Ba

16.1±2.8 Aa

12.8± 3.1 Ba

10.1± 0.2 Bb

9.6± 0.1 Ba

**Table 2.** Mean diameters (mm) of *Colletotrichum gloeosporioides* lesions in Tommy Atkins, Constantina, Comum and Rosa seedlings at the sixth and tenth day treated with 4uL/mL of *Pectis brevipedunculata* and *Dizigostemon riparius* essential oils.

\* Averages followed by the same letter do not differ statistically among themselves, capital in the column and lowercase in the line, by the Tukey test at 5% of probability.

However, the application of *P. brevipedunculata* essential oil resulted in the development of anthracnose lesions superior to the control treatment in Comum mango seedlings. The majority compound of this oil is citral, the same evaluated by Moura (2010) that, in researches with lemon grass essential oil (*Cymbopogon citratus* L.) in the anthracnose control in yellow passion fruit, observed that there was an increase in the severity of *C. gloeoesporioides* in fruits in 79.3% compared to the control.

Rosa

Tommy

Constantina

Comum Rosa

Ten days

Constantina and Rosa mango cultivars did not present statistical differences among the essential oils treatments and the control. Corroborating with this research, Fonseca et al. (2019) found little effectiveness of essential oils in the curative control of anthracnose in the Rosa cultivar, probably explained by the low translocation capacity in the interior of the infected tissues of the plant; however, the level of severity of the disease remained stable, preventing it from evolving causing more damage.

As for the severity evaluations, at the tenth day after the inoculation it was observed that the *D. riparius* essential oil acted significantly in the reduction of the severity caused by the *C. gloeosporioides* fungus when compared to the control in Tommy Atkins mango (Table 2). For the mango seedlings Constantina and

Rosa there was no statistical difference. Just as at the sixth day, the severity averages in the Comum cultivar remained higher when compared to the control.

8.3± 0.4 Ba

10.0± 1.4 Ab

10.1± 0.9 Aa

9.7 ± 1.1 ABb

8.4± 0.4 Ba

During the evaluations was evidenced a sharp leaf fall from the fourth day in both treatments with essential oils, being of greater intensity in the treatments with the *D. riparius* oil. According to Brandão et al. (2020), no other essential oil with high fenchil and fenchol acetate content was previously found in the literature. This can be a factor that justifies the high phytotoxicity caused in seedlings and fruits, when treated with *D. riparius* essential oil. This essential oil is presented with low polarity and with high hydrophobic character, which tied to high waxiness in leaves and fruits hinders the diffusion of the essential oil.

## Effect of *P. brevipedunculata* and *D. riparius* essential oils on the severity of Colletotrichum gloeosporioides in mango fruits

The concentration of 3  $\mu$ l/mL of the essential oils was chosen to be applied in mango fruits, since higher concentrations caused phytotoxicity in the seedlings and fruits. It was observed that both essential oils reduced the severity caused by *C. gloeosporioides*, with significant differences among the treatments in all cultivars evaluated

(Figure 2). These results corroborate with other studies in which the use of essential oils decreased the post-harvest deterioration in several fruits, such as banana (*Musa spp.*) (VILAPLANA et al., 2018), pepper (*Capsicum* annuum L.) (HONG et al., 2015) and strawberries (*Fragaria vesca*)

(CAMPOS-REGUENA et al., 2017). According to Ben-Jabeur (2015), these results are possibly related to the stress caused by substances present in the essential oils, which stimulates the production of phenolic and peroxidases that will act in the defense against the phytopathogen.



**Figure 2.** Mean diameter of lesions (mm) of *C. gloeosporioides* in mango fruits Tommy Atkins, Constantina, Comum and Rosa treated with 3uL/mL of *Pectis brevipedunculata* and *Dizigostemon riparius* essential oils six days after inoculation. Source: (São Luís, 2021). Averages followed by the same letter do not differ significantly among themselves (Tukey at 5% of probability). The bars represent average standard error.

The tests with *D. riparius* essential oil presented the lowest severity averages in all the treatments evaluated (Figure 2). Studies such as the ones of Fonseca et al. (2019) observed also in mango culture that noni (Morinda citrofolia Linn) essential oil presented itself efficient in the preventive control of the severity from the concentration of 2 %. It is worth noting that, after the end of the evaluation period of 6 days, the fruits were cut to the middle for a visual evaluation of the lesion depth and both treatments with the essential oils were effective reducing significantly the lesion in the fruit pulp. However, the best results were observed in the Tommy Atkins mango fruits treated with D. riparius essential oil, which presented a significant reduction in the lesion depth when compared to the other treatments (Figure 3). The control mango fruits were the ones that presented greater lesion depth and greater damage in the fruit pulp, visually differing from the treatments with both essential oils.

Studies conducted by Guimarães (2016) observed that lemon grass essential oil (*C. citratus*) in 'Palmer' mangos inoculated with *C. gloeosporioides* had lower lesion diameters from the treatment of 2% of the essential oil, besides lower fresh mass losses and lower damage in the fruit surface at the end of the storage period in room temperature.



**Figure 3**. Cross-sectional cut of Tommy Atkins mango cultivar evidencing the reduction of the anthracnose severity in the pulp and fruit surface with the use of *Dizigostemon riparius* and *Pectis brevipedunculata* essential oil. Source: (São Luís, 2021).

Santos et al. (2018) observed that the effect of a cassava starch coating associated with clove essential oil for papaya fruits coating, presented efficient result for the anthracnose injury growth control. Evidencing thus that the essential oils when incorporated to other substances can maintain their antifungal effect, not only in pure or diluted state, but in also having an effective action when combined with other substances for the formation of bio products.

#### Conclusion

*P. brevipedunculata* and *D. riparius* essential oils inhibited the development of *Colletotrichum gloeosporioides in vitro* and in Tommy Atkins, Constantina, Comum and Rosa mango fruits. Mango leaves of these same varieties did not present any lower development of the pathogen lesions when treated with the same essential oils, with the exception of the Tommy Atkins variety treated with *Pectis brevipedunculata*.

#### References

- ALI, A.; HEI, G.K.; KEAT, Y.W. Efficacy of ginger oil and extract combined with gum arabic on anthracnose and quality of papaya fruit during cold storage. Journal of Food Science and Technology, Mysore, v.3, n.1, p.1-10, 2016.
- AKISUE, G. Aparelho extrator de óleo essencial: modificação do aparelho de Clevenger. **Revista Brasileira de Farmacognosia**, São Paulo, v.1, n.2, p.247-52, 1986.

- BETTIOL, W. Conversão de sistemas de produção: uma visão global. *In:* VENZON, M.; PAULA JÚNIOR,
  T.J.; PALLINI, A. Controle alternativo de pragas e doenças na agricultura orgânica.Viçosa:
  Empresa de Pesquisa Agropecuária de Minas Gerais Zona da Mata, 2010. cap.1, p.1-24.
- BEN-JABEUR, M.; GHABRI, E.; MYRIAM, M.; HAMADA, W. Thyme essential oil as a defense inducer of tomato against gray mold and *Fusarium wilt*. **Plant Physiology and Biochemistry**, New Delhi, v.94, p.35-40, 2015.
- BONALDO, S.M.; SCHWAN-ESTRADA, K.R.F.; STANGARLIN, J.R.; CRUZ, M.E.S.; FIORI-TUTIDA, A.C.G. Contribuição ao estudo das atividades antifúngica e elicitora de fitoalexinas em sorgo e soja por eucalipto (*Eucalyptus citriodora*). **Summa Phytopathologica**, São Paulo, v.33, n.4, p.383-7, 2007.
- BRANDÃO, C.M.; CAVALCANTE, K.S.B.; TELES, R.M.; MARQUES, G.E.C.; MONTEIRO, O.S.; ANDRADE, E.H.A.; MAIA, J.G.S. Composition and Larvicidal Activity of the Oil of *Dizygostemon riparius* (Plantaginaceae), a New Aromatic Species Occurring in Maranhão, Brazil. Chemistry & Biodiversity, Zurich, v.17, n.11, p.1-11, 2020.
- BRITO, T.C.R.; MARQUES, A.M. **Comparação das técnicas hidrodestilação e microextração em fase sólida na obtenção do óleo essencial de Pectis brevipedunculata**. Rio de Janeiro, 2008. Disponível em: <u>http://www.sigma.uff.br/UFRJ/SIGMA</u>. Acesso em: 04 jun. 2020.
- CAMPOS-REGUENA, V.H. Thermoplastic atarch/clay nanocomposites loaded with essential oil constituents as packaging for strawberries *in vitro* antimicrobial synergy over *Botrytis cinerea*. **Postharvest Biology and Technology**, Amsterdam, v.129, p.29-36, 2017.
- CERDEÑO, V.J.M. Hábitos de compra y consumo de frutas y hortalizas Resultados del Observatório del Consumo y la Distribución Alimentaria. **Revista Distribución y Consumo**, Melchior de Palau, v.16, n.88, p.5-28, 2006.
- COMPAGNONE, R.S.; CHAVES, K.; MATEU, E.; ORSINI G.; ARVELO, F.; SUÁREZ, A.I. Composition, and cytotoxic activity of essencial oils from *Croton matourensis* and *Croton micans* from Venezuela. **Records of Natural Products**, Kocaeli, v.4, n.2, p.101-8, 2010.
- FONSECA, A.C.C.; ROTILI, E.A.; FERREIRA, T.P.S.; MOURÃO, D.S.C.; DIAS, B.L. Potencial do óleo essencial de noni no controle preventivo e curativo da antracnose da mangueira. Journal of Biotechnology and Biodiversity, Gurupi, v.7, n.3, p.356–62, 2019.
- FONSECA, N.; BORGES, A.L. Calagem e adubação para a mangueira. *In*: BORGES, A.L. **Recomendações** de calagem e adubação para abacaxi, acerola, banana, citros, mamão, mandioca, manga e maracujá. 2.ed. Brasília, DF: Embrapa, 2021.
- GUIMARÃES, J.E.R. **Produtos naturais no controle da antracnose e na qualidade pós-colheita de mangas 'palmer'**. 2016. 123 f. Tese (Doutorado Agronomia) Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, 2016.
- GUPTA, C.; GARG, A.P.; UNIYAL, R.C.; KUMARI, A. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. African Journal of Microbiology Research, Lagos, v.2, n.9, p.247-51, 2008.
- HU, M.; YANG, D.; HUBER, D.J.; JIANG, Y.; LI, M.; GAO, Z.; ZHANG, Z. Reduction of postharvest anthracnose and enhancement of disease resistance in ripening mango fruit by nitric oxide treatment. **Postharvest Biology and Technology**, Amsterdam, v.97, n.1, p.115-22, 2014.
- HONG, J.K. Application of volatile antifungal plant essential oils for controlling pepper fruit anthracnose by *Colletotrichum gloeosporioides*. **Plant Pathology Journal**, Fasialabad, v.31, n.3, p.269-77, 2015.
- LIMA, N.B.; LIMA, W.G.; TOVAR-PEDRAZA, J.M.; MICHEREFF, S.J.; CÂMARA, M.P.S. Comparative epidemiology of *Colletotrichum* species from mango in northeastern Brazil. **European Journal of Plant Pathology**, Dordrecht, v.141, n.4, p.679-88, 2015.

- LIMA, T.S.; MELO, Y.N.C. da S.; SILVA, J.L. da; COSTA, J.F. de O.; LIMA, G.S. de A.; ASSUNÇÃO, I.P. Efficiency of essential oils to control *Colletotrichum theobromicola in vitro*. **Revista AgroAmbiente On-Line**, Boa Vista, v.15, n.1, p.1-10, 2021.
- LINDE, J.H.; COMBRINCK, S.; REGNIER, T.J.C.; VIRIJEVIC, S. Chemical composition and antifungal activity of the essential oils of *Lippia rehmnii* from South Africa. **South African Journal of Botany**, Pretoria,v.76, n.1, p.37-42, 2010.
- MAIA, L.C.; CARVALHO JÚNIOR, A.A.; CAVALCANTI, L.H.; GUGLIOTTA, A.M.; DRECHSLER-SANTOS, E.R.; SANTIAGO, A.L.M.A.; CÁCERES, M.E.S.; GIBERTONI, T.B.; APTROOT, A.; GIACHINI, A.J. Diversity of Brazilian Fungi. **Rodriguésia**, Rio de Janeiro, v.66, n.4, p.1033-45, 2015.
- MENEZES, M; ASSIS, S.M.P. Guia prático para fungos fitopatogênicos. 2.ed. Recife: Imprensa Universitária, 2004.
- MENEZES, E.L.; PINTO, T.J.S.; BRANDÃO, C.M.; CAVALCANTE, K.S.B.; TELES, R.M. Estudo químico do óleo essencial de espécie vegetal encontrada em são benedito do Rio Preto -MA e sua aplicação como larvicida contra o *Aedes aegypti*. **Cb de Química**, v.2, n.1, p.1-7, 2018.
- MONTEIRO, O.S.; FERNANDES, Y.; MATOS, J.; LIMA, C.; TARDINI, A.; VIERA, F.; MAIA, J.; LONGATO, G.; ROCHA, C. Essential oils obtained from aerial eugenia punicifolia parts: chemical composition and antiproliferative potential evidenced through cell cycle arrest. **Journal Of the Brazilian Chemical Society**, São Paulo, v.33, p.917-21, 2021.
- MORANDI, M.A.B.; BETTIOL, W. Controle biológico de doenças de plantas no Brasil. *In:* BETTIOL, W.; MORANDI, M.A.B. (ed.). **Biocontrole de doenças de plantas**: usos e perspectivas. Jaguariúna: Embrapa Meio Ambiente, 2019. v.3, p.7-14.
- MOURA, G.S. **Conservação pós-colheita e controle da antracnose em frutos de maracujá-amarelo por derivados de capim-limão**. 2010. 75 f. Dissertação (Mestrado) - Universidade Estadual de Maringá, Maringá, 2010.
- NOBRE, J.O.S.; MARQUES, M.L. da S. Alternative methods for *in vitro* control of *Colletotrichum gloeosporioides* causal agent of anthracnose in "dedo-de-moça" pepper. **Research, Society and Development**, Itabira, v.10, n.3, p.1-7, 2021.
- OLIVEIRA, M.T.R.; BERBERT, P.A. Efeito da temperatura do ar de secagem sobre o teor e a composição química do óleo essencial de *Pectis brevipedunculata*. **Química Nova**, São Paulo, v.34, n.7, p.1200-4, 2011.
- PASTANA, R.F.; VIEIRA, G.H.C; MACHADO, P.P. Uso da própolis no controle "*in vitro*" do fungo *Colletotrichum gloeosporioides* causador da antracnose em berinjela. **Revista de Agricultura Neotropical**, Cassilandia, v.3, n.1, p.12–15, 2016.
- PEDROSO, D.; MENEZES, V.; JUNGES, E.; MULLER, J.; GIRARDI, L.; DILL, A.; MUNIZ, M.; BLUME, E. Potencial inibitório *in vitro* de *Alternaria solani* sob efeito de extratos botânicos. **Revista Brasileira de Agroecologia**, Porto Alegre, v.4, n.2, p.4260-3, 2009.
- PHOULIVONG, S.; CAI, L.; CHEN, H.; MCKENZIE, E.H.; ABDELSALAM, K.; CHUKEATIROTE, E.; HYDE, K.D. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. **Fungal Diversity**, Heildelberg, v.44, n.1, p.33-43, 2010.
- RAUT, J.S.; KARUPPAYIL, S.M. A status review on the medicinal properties of essential oils. **Industrial Crops and Products**, Amsterdam, v.62, p.250-64, 2014.
- SANTOS, N.S.T.; AGUIAR, A.J.A.A.; OLIVEIRA, C.E.V.; SALES, C.V.; SILVA, S.M.; SILVA, R.S.; STAMFORD, T.C.M.; SOUZA, E.L. Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* Lessential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). Food Microbiology, London, v.32, n.2, p.345-53, 2012.

- SANTOS, B.J.R; REIS, R.C.; ALMEIDA, J.M.; BATISTA, D.V.S.; SASAKI, F.F.C. Efeito do óleo essencial de cravo-da-índia e do revestimento à base de fécula de mandioca no controle da antracnose e nas características físico-químicas do mamão. *In*: JORNADA CIENTÍFICA – EMBRAPA MANDIOCA E FRUTICULTURA, 12., 2018. **Anais** [...]. Cruz das Almas: EMBRAPA, 2018. p.68.
- SANZANI, S.M.; REVERBERI, M.; GEISEN, R. Mycotoxins in harvested fruits and vegetables: Insights in producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. **Postharvest Biology and Technology**, Amsterdam, v.122, n.2 p.95-105, 2016.
- SCHWAN-ESTRADA, K.R.F.; STANGARLIN, J.R.; CRUZ, M.E. da S. Uso de plantas medicinais no controle de doenças de plantas. Uso de extratos vegetais no controle de fungos fitopatogênicos. Revista Floresta, Curitiba, v.30, n.1, p.129-37, 2000.
- SCATIGNA A.V.; BRANDÃO C.M.; COLLETTA G.D.; TELES R.DE M.; CAVALCANTE K.S.B.; SOUZA V.C.; SIMÕES A.O. *Dizygostemon riparius* (Plantaginaceae, Gratioleae), a new species from Maranhão, northeastern Brazil. **Willdenowia**, Berlin, v.49, n.2, p.177-86, 2019.
- SHAO, X.; CAO, B.; XU, F.; XIE, S.; YU, D.; WANG, H. Effect of postharvest application of chitosan combined with clove oil against citrus green mold. Postharvest Biology and Technology, Amsterdam, v.99, p.37-43, 2015.
- SHARMA, K.; MAHATO, N.; CHO, M.H.; LEE, Y.R. Converting citrus wastes into valueadded products: Economic and environmently friendly approaches. **Nutrition**, new York, v.34, p.29-46, 2017.
- SOUSA, R.M; SERRA, I.M.R.S.; MELO, T.A.Efeito de óleos essenciais como alternativa no controle de *Colletotrichum gloeosporioides*, em pimenta. **Summa Phytopathologica**, São Paulo, v.38, n.1, p.42-7, 2012.
- VILAPLANA, R.; PAZMINO, L.; VALENCIA-CHAMORRO, S. Control of anthracnose, caused by *Colletotrichum musae*, on postharvest organic banana by thyme oil. **Postharvest Biology and Technology**, Amsterdam, v.138, p.56–63, 2018.
- XAVIER, L.D.; BINHARA, R.C.; FERREIRA, F.D. Evaluation of the antifungal activity of the essential oil of *Sygygium Cumini*. **Brazilian Journal of Development**, Curitiba, v.7, n.5, p.380-90, 2021.
- ZHENG, M.SHI, J.; SHI, J.; WANG, Q.; LI, Y. Antimicrobial effects of volatiles produced by two antagonistic *Bacillus strains* on the anthracnose pathogen in postharvest mangos. **Biological Control**, Amsterdam, v.65, n.2, p.200-6, 2013.