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Original Research Article
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Evaluation of mango peel extracts on the *in vitro C. gloeosporioides* development
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ABSTRACT
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Aims: To evaluate the *in vitro* effect of mango peel extracts using different types of solvent and concentrations on the *Colletotrichum gloeosporioides* development.
Study Design: Activities were aimed at evaluating the *in vitro* antifungal potential of mango peel extracts.
Study location and duration: The study was carried out at the Laboratory of Post-Harvest Pathology of Fruits and Vegetables - State University of Montes Claros and Laboratory of Natural Products, Department of Chemistry - Federal University of Lavras between October and December of 2018.
Methodology: 'Palmer' mango peel (*Mangifera indica*) was submitted to drying in oven and grinding. Subsequently, extracts were obtained in Soxhlet system, using methanol, ethyl acetate and hexane as solvents. The three extracts were tested *in vitro* at concentrations of 0.0; 0.25; 0.5; 1.0 and 2.0 mg/mL by adding them in culture medium against *C. gloeosporioides*, which was isolated from mango fruits with anthracnose symptoms. The effect of extracts and their respective concentrations on the mycelial growth rate and conidia production and germination was evaluated. The design was completely randomized in a 3 x 5 factorial arrangement with 5 replicates.
Results: Increased extract concentrations caused reduction in the mycelial growth rate of the pathogen (R^2 = 0.96). Both factors under study acted simultaneously in conidia production (P < 0.05), and the hexane extract presented better results for this analyzed variable. There was total germination inhibition (P < 0.05) when 1 mg/mL ethyl acetate extract and 2 mg/mL methanol and hexane extracts were used.
Conclusion: Methanol, hexane and ethyl acetate mango peel extracts had inhibitory effect on the *in vitro C. gloeosporioides* development.
11
12 *Keywords: M. indica; anthracnose; alternative control; plant extracts*.

Comment [U1]: This keyword does not reflect the contents of this topic, it is necessary to look for words that are more appropriate

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1. INTRODUCTION
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18 The mango (*Mangifera indica* L.) is a fruit tree of great economic importance in Brazil, not
19 only for its nutritional characteristics, but also for generating employment and income in
20 several regions of the country.
21

22 Mango production in Brazil is estimated at around 1 million tons [1]. However, a high
23 percentage of this fruit does not reach the consumers table. Among the main causes are the
24 lack of technology in the production chain and post-harvest diseases. Anthracnose caused
25 by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc is one of the most important post-
26 harvest disease in mango crops. Post-harvest losses caused by anthracnose cause many

damages and makes fruits unfit for consumption [2, 3]. The fungus infection accelerates the maturation and deterioration of the fruits, contributing to losses that can reach up to 80% [4].

Among the methods for controlling the disease, chemical control with the use of protective fungicides is more used [5]. However, there are several alternative control strategies, such as the use of essential oils and extracts [6, 3].

Phenolic compounds, which are considered constitutive barriers, have been associated to disease resistance in many crops, being found in stems, leaves, core, roots and fruits. Mango, mainly peel, contains several classes of polyphenols that act as natural antagonists of pathogens and potent antioxidants [7, 8]. Furthermore, these components are used in traditional medicine due to their antifungal and antibacterial properties [9]. There are several reports in the literature on the antifungal properties of plant bioactive compounds [10, 11, 12, 13]. However, further studies are important to verify the potential of *M. indica* bioactive compounds in plant disease control and the use of an alternative method of post-harvest disease control.

Thus, this work had the aim of evaluating the effect of different mango peel extracts and their concentrations on the *in vitro Colletotrichum gloeosporioides* control.

2. MATERIAL AND METHODS

2.1 Raw material

'Palmer' mangoes were manually harvested in a commercial orchard located in the municipality of Matias Cardoso-MG, at physiological maturation stage with purplish red peel color and pulp corresponding to grade 2 of the color scale [14]. Fruits were transported in plastic boxes to the Laboratory of Post-Harvest Pathology of Fruits and Vegetables, where they were sanitized with detergent, rinsed with drinking water and placed on a bench for drying.

Subsequently, fruit peel was separated from pulp using stainless steel knives, with cuts varying from 2 to 3 mm in thickness. Then, peel was weighed in a digital scale and then dried in a forced air circulation oven at 40ºC for 72 hours. After removal from the oven, mango peel was ground in a Willey-type mill. The ground vegetable material was packed in a plastic bag, stored in freezer and sent to the Laboratory of Natural Products, Department of Chemistry - Federal University of Lavras, where the experiment was carried out to obtain mango peel extracts.

Comment [U2]: Why vegetable ??? did you mean gamgo peel ?

2.2 Obtaining extracts

Extraction was carried out in Soxhlet system, in which a volumetric flask was attached at the lower end and a cooling condenser at the upper end. About 353.16 g of the dried material were added to the extractor and approximately 1000 mL of the selected solvent were added in the round bottom volumetric flask. Three extractions were performed using a new solvent in each procedure. Hexane, ethyl acetate and methanol were used, and the total extraction time for each of these solvents was: 16 h for the first two (hexane and ethyl acetate) and 24 h (methanol) for the latter.

After the extraction time had elapsed, each of the three mixtures was transferred to a volumetric flask with 250 mL capacity, which was taken to a rotary evaporator coupled to a vacuum pump to separate the solvent from the extract. Extracts were transported in styrofoam box to the Laboratory of Post-Harvesting Pathology of Fruits and Vegetables of

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UNIMONTES to be used in the *in vitro* experiment for evaluation of mycelial growth, sporulation and germination of *Colletotrichum gloeosporioides* conidia.

Initially, stock solution at 5 mg/mL was prepared for each extract using sterilized distilled water and 1% (v/v) tween (polyoxyethylene sorbitan monooleate) as diluent. For homogenization, solutions were submitted to constant stirring in an orbital shaking incubator for 2 hours at 150 rpm.

Comment [U3]: at what temperature ?

2.3 Parameters evaluated for *in vitro* studies

Colletotrichum isolate was obtained from fruits with characteristic symptoms of anthracnose, according to the indirect isolation technique [15]. Confirmation of the fungus identification was performed based on its morphological characteristics through the preparation of slides and observations under microscope.

For the mycelial sensitivity, aliquots of stock solutions were added to melting BDA (Potato-Dextrose-Agar) medium so as to obtain the predetermined concentrations (0.0; 0.25; 0.5; 1.0 and 2.0 mg/mL). After homogenization, media were poured into identified Petri dishes, where, after solidification, 5 mm *C. gloeosporioides* mycelium discs were transferred from 7 day-incubation cultures. Then, Petri dishes were sealed with plastic film and incubated in BOD chamber at temperature of 25°C, with 12 hours photoperiod. Evaluations were performed daily, measuring the growth of the mycelial diameter in two directions, perpendicularly, using pachymeter in millimeters, starting 24 hours after the assembly of the experiment and ending on the seventh day. MGRI (Mycelial Growth Rate Index) in $mm.day^{-1}$ was calculated using the formula [16]:

Comment [U4]: you mean PDA ?

Σ MGRI = (D - Da)/N , in which D = the current mean diameter; Da = previous mean diameter and N = number of days after pricking .

After mycelial growth evaluation, 10 mL of sterilized distilled water were added to each Petri dish and with the aid of Drigalski loop the colonies were scraped to release the conidia. The conidial suspension was filtered through double-layer gauze and the solution volume was filled up to 20 mL. One drop of each suspension was added to the Newbauer chamber and in an optical microscope the spores count was performed.

For germination evaluation, a conidia suspension of culture with 7 days of incubation was prepared by placing 10 mL sterile distilled water on the surface of the Petri dish with the fungal mycelium and gently scraping it with the aid of Drigalski loop. The suspension was filtered through double-layer sterile gauze and concentration was adjusted to 2.5×10^5 conidia/mL after counting in Newbauer's chamber. Subsequently, aliquots of the stock solutions of each extract were added to the melting agar medium in order to obtain the predetermined concentrations. After homogenization, media were poured into identified Petri dishes and when solidified, 200 µL of the conidia suspension was added to the surface of the culture medium. With gentle movements, the suspension was spread over the culture medium with the aid of Drigalski loop. About 100 conidia were evaluated under optical microscope, and conidia presenting germinative tube with length greater or equal to the conidium diameter were considered germinated.

2.4 Statistical analysis

The experimental design was completely randomized, in a 3 x 5 factorial arrangement (extract x concentration), with 5 replicates, each replicate consisted of a Petri dish. Three mango shell extracts were used: methanol, hexane and ethyl acetate and the following

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122 concentrations: 0.0; 0.25; 0.5; 1.0; 2.0 mg/mL. Mycelial Growth Rate Index, sporulation and
123 germination data were transformed into $\sqrt{x + 1}$ and submitted to analysis of variance through
124 the SISVAR statistical software [17]. If significant interaction among factors was verified,
125 means were compared by means of the Tukey test at 5% probability and regression analysis
126 was used for concentrations.

127

128 3. RESULTS AND DISCUSSION

129

130 For the mycelial growth rate index (MGRI), there was no interaction between the levels of
131 the two factors (extract x concentration) by the F test at 5% probability (Table 1), indicating
132 that they acted independently.

133 **Table 1. Summary of the analysis of variance (Mean Squares) for variables mycelial**
134 **growth rate index (MGRI), sporulation (SPO) and germination (GERM)**

135

SV	Mean squares			
	DF	MGRI	SPO	GERM
Extract (E)	2	0.03^{ns}	$1.15 \times 10^{6*}$	1.69^{ns}
Concentration (C)	4	0.12^{*}	$4.67 \times 10^{5*}$	121.31^{*}
E x C	8	0.02^{ns}	$1.55 \times 10^{5*}$	33.29^{*}
Residue	60	0.02	8.21×10^{3}	0.96
CV(%)		2.44	11.90	15.41

136 *(ns): Not significant; (*) Significant at 5% by the test F.*

137

138 There was significant difference ($P < 0.05$) for concentrations under study and the linear
139 model was the best fit to describe the behavior of the mycelial growth rate index as a
140 function of the different concentrations (Fig. 1). Increased extract concentrations caused
141 reduction in the mycelial growth rate of the pathogen. Lins et al. [18] evaluated the mycelial
142 growth of *Lasiodiplodia theobromae* using aqueous mango peel extract in BDA (Potato-
143 Dextrose-Agar) culture medium and found significant results at 50% and 75%
144 concentrations. In addition, in the study above, control of peduncular rot was verified with
145 mango peel extract through a satisfactory result in the reduction of the area under the
146 disease progress curve (AUDPC). In investigating the use of extracts of agroindustrial
147 residues for the control of phytopathogenic fungi, Malaguetta [19] obtained partial *in vitro*
148 inhibition of the mycelial growth of *Colletotrichum dematium* using ethanol mango bagasse
149 extract at concentrations of 500 and 2000 ppm. In the study conducted by Rojas et al. [20],
150 mango peel extract inhibited the radial growth of *C. gloeosporioides*, *S. sclerotiorum* by 50%
151 and *F. oxysporum* by 33.33%, thus suggesting that the presence of polyphenols in mango
152 peels is an attractive alternative source for bioactive compounds, such as antioxidants and
153 antifungal molecules.
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Comment [U5]: PDA ???

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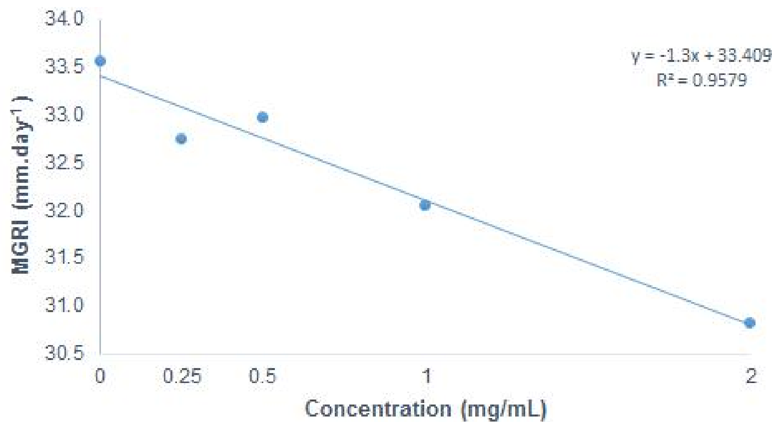

156
157 **Fig. 1. Mycelial growth rate index of *Colletotrichum gloeosporioides* as a function of**
158 **the different concentrations used (0, 0.25, 0.5, 1.0, 2.0 mg/mL)**
159
160 With regard to *C. gloeosporioides* sporulation, there was interaction between the two factors
161 studied (extract x concentration) by the F test at 5% probability (Table 1), thus, both
162 simultaneously acted on the variable under study. Significant difference ($P < 0.05$) among
163 mango peel extracts at concentrations of 0.25; 0.5; 1.0 and 2.0 mg/mL (Table 2) was
164 observed by the Tukey test. In each of these concentrations, hexane extract provided lower
165 spore production when compared to methanol and ethyl acetate extracts, thus presenting
166 fungitoxic effect. At concentrations 0.25; 0.5 and 2.0 mg/mL, an increase in spore production
167 was observed with the use of the methanol mango peel extract in comparison with other
168 extracts, showing that this treatment induced *C. gloeosporioides* sporulation.
169
170 **Table 2. Effect of mango peel extracts (EME: methanol extract; EAC: ethyl acetate**
171 **extract; EHE: hexane extract) on *Colletotrichum gloeosporioides* sporulation**
172 **(spores/mL) as a function of each concentration (mg/mL) used**
173

Concentrations	Extracts		
	EME	EAC	EHE
0.0	906.02 a	906.02 a	906.02 a
0.25	752.16 a	431.02 b	261.19 c
0.5	1164.54 a	798.38 b	513.73 c
1.0	866.33 a	831.65 a	404.75 b
2.0	1282.19 a	775.79 b	621.69 c

174 *Means followed by the same letter in row do not differ from each other by the Tukey test at 5%*
175 *probability.*
176
177 Significant interaction between factors (extract x concentration) by the F test at 5%
178 probability for the percentage of conidia germination was verified (Table 1). For 0.0 and 0.5
179 mg/mL concentrations, there was no significant difference among extracts (Table 3) by the
180 Tukey test ($P < 0.05$). Mango peel hexane extract contributes to lower the germination
181 percentage of *C. glooesporioides* conidia when used at concentrations of 0.25 and 2 mg/mL.

Comment [U6]: *gloeosporioides*

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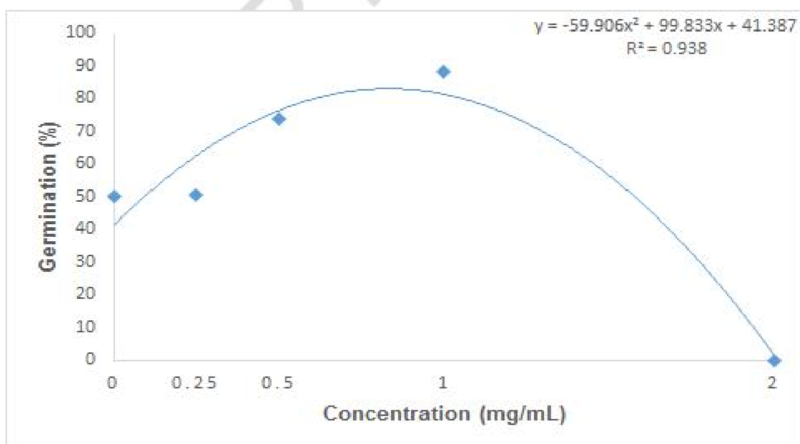
182 This effect was observed at concentration of 1 mg/mL, as it increases the germination
183 percentage in contrast to methanol and ethyl acetate extracts. For the highest concentration
184 used in this study, the peel extract obtained with ethyl acetate differed from the others,
185 because it was not able to totally inhibit conidial germination. However, at concentration of 1
186 mg/mL, total germination inhibition was observed when this treatment was used.

187 **Table 3. Effect of mango peel extracts (EME: methanol extract, EAC: ethyl acetate**
188 **extract, EHE: hexane extract) on the germination percentage of *Colletotrichum***
189 ***gloeosporioides* conidia as a function of each concentration (mg/mL) used**
190

Concentrations	Extracts		
	EME	EAC	EHE
0.0	50.18 a	50.18 a	50.18 a
0.25	95.40 a	73.95 b	50.60 c
0.5	91.06 a	88.56 a	73.88 a
1.0	13.64 a	0.00 a	88.40 b
2.0	0.00 a	29.03 b	0.00 a

191 *Means followed by the same letter in row do not differ from each other by the Tukey test at 5%*
192 *probability.*
193
194 For germination, a quadratic model was the best fit (R^2 = 0.938) for the regression analysis
195 of the dose of mango peel hexane extract (Fig. 2). For the other extracts, third-order
196 polynomial models were the most adequate to describe the phenomenon. Albíter-Hernández
197 [21] found reduction in the conidia germination percentage (7%) for one of *C.*
198 *gloeosporioides* isolates using crude mango leaf extract (*Mangifera indica*). High sensitivity
199 in the germination of this phytopathogen was also confirmed by Reis et al. [22] who
200 evaluated the efficacy of natural products in the *in vitro* anthracnose control in papaya and
201 observed that clove and cinnamon extracts at concentrations of 7.5% were able to partially
202 inhibit *C. gloeosporioides* germination.



203

204 **Fig. 2. Germination percentage of *Colletotrichum gloeosporioides* conidia in mango**
205 **peel hexane extract as a function of the different concentrations**

Studies have revealed the existence of phenolic compounds, which may have fungitoxic effect and pharmacological properties [23, 7, 24, 25]. Research suggests that the resistance of green mango to *C. gloeosporioides* is due to a constitutive defense system composed of antifungal resorcinols, gallotannins and chitinases [25, 26]. Few studies have been published regarding the effect of mango peel extracts on post-harvest disease fungi. Thus, the potential of using mango peels as a natural source of polyphenols combined with extraction using different solvents maximizes the use of these substances in a pathogenic system.

4. CONCLUSION

Methanol, hexane and ethyl acetate mango peel extracts inhibit the *in vitro C. gloeosporioides* development.

The increase in concentrations reduced mycelial growth of the pathogen.

The hexane extract provides greater reduction in spore production in contrast to the others extracts.

In germination of conidia, the effect of each extract depends on the concentration used. Methanolic and hexane extracts of mango peel totally inhibit germination only at the highest concentration.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest related to this study.

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