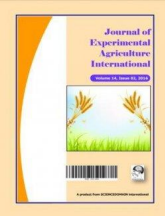

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Evaluation of Mango Peel Extracts on the *in vitro* *Colletotrichum gloeosporioides* Development

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LSS, EHM and GPM designed the study, performed the experiment and wrote the first draft of the manuscript. Authors DFO and VACC contributed to obtain extracts. Author FPM assisted in the statistical analysis. Authors RCFR and MPSC assisted in the isolation and identification of the fungus. Author MLMR assisted in the assembly and evaluation of the experiment. All authors read and approved the final manuscript.

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ABSTRACT

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

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Products, Department of Chemistry - Federal University of Lavras during October and December 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.

Keywords: M. indica; postharvest diseases; alternative control; plant extracts; phenolic compounds; resorcinol.

1. INTRODUCTION

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

Much of the fruit production does not reach the consumers Table and among the main causes are the lack of technology in the production chain and post-harvest diseases. Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc is one of the most important post-harvest disease in mango crops. Post-harvest losses caused by anthracnose causes many damages and makes fruits unfit for consumption [1,2]. The fungus infection accelerates the maturation and deterioration of the fruits, contributes to 80% losses to fruits [3].

Among the methods for controlling this disease, chemical control with the use of protective fungicides is more used [4]. However, there are several alternative control strategies, such as the use of essential oils and extracts [5,2].

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 [6,7]. Furthermore, these components are used in traditional medicine due to their antifungal and antibacterial properties [8]. There are several reports in the literature on the antifungal properties of plant bioactive compounds [9,10,11,12].

Several compounds have already been identified in the phenolic fractions of mango peel extracts, such as: gallic acid, protocatechuic acid, gentisic acid, syringic acid, quercetin, mangiferin pentoside, methyl gallate and maclurin hexoside [13,14]. Antifungal resorcinols were isolated and identified in mango peel and suggested as the cause of resistance of the unripe fruit to the attack of *C. gloeosporioides* [15]. 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 of varied 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 [16]. 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, 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.

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 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) of hydrophilic nonionic surfactant Tween 80 (polyoxyethylene sorbitan mono-oleate) as diluent. For homogenization, solutions were submitted to constant stirring in an orbital shaking incubator at 30ºC for 2 hours at 150 rpm.

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 [17]. 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 PDA (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 [18]:

> 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 concentrations: 0.0; 0.25; 0.5; 1.0; 2.0 mg/mL. Mycelial Growth Rate Index, sporulation and germination data were transformed into $\sqrt{x + 1}$ and submitted to analysis of variance through the SISVAR statistical software [19]. If significant interaction among factors was verified, means were compared by means of the Tukey test at 5% probability and regression analysis was used for concentrations.

3. RESULTS AND DISCUSSION

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

There was significant difference ($P < 0.05$) for concentrations under study and the linear model was the best fit to describe the behavior of the mycelial growth rate index as a function of the different concentrations (Fig. 1). Increased extract concentrations caused reduction in the mycelial growth rate of the pathogen. Lins [20] evaluated the mycelial growth of *Lasiodiplodia theobromae* using aqueous mango peel extract in PDA (Potato-Dextrose-Agar) culture medium and found significant results at 50% and 75% concentrations. In addition, in the study above, control of peduncular rot was verified with mango peel extract through a satisfactory result in the reduction of the area under the disease progress curve (AUDPC). In investigating the use of extracts of agroindustrial residues for the control of phytopathogenic fungi, Malaguetta [21] obtained partial *in vitro* inhibition of the mycelial growth of *Colletotrichum dematium* using ethanol mango bagasse extract at concentrations of 500 and 2000 ppm. In the study conducted by Roja [22], mango peel extract inhibited the radial growth of *C. gloeosporioides*, *S. sclerotiorum* by 50% and *F. oxysporum* by 33.33%, thus suggesting that the presence of polyphenols in mango peels is an attractive alternative source for bioactive compounds, such as antioxidants and antifungal molecules.

With regard to *C. gloeosporioides* sporulation, there was interaction between the two factors studied (extract x concentration) by the F test at 5% probability (Table 1.), thus, both simultaneously acted on the variable under study. Significant difference ($P < 0.05$) among mango peel extracts at concentrations of 0.25; 0.5; 1.0 and 2.0 mg/mL (Table 2.) was observed by the Tukey test. In each of these concentrations, hexane extract provided lower spore production when compared to methanol and ethyl acetate extracts, thus presenting fungitoxic effect. At concentrations 0.25; 0.5 and 2.0 mg/mL, an increase in spore production was observed with the use of the methanol mango peel extract in comparison with other extracts, showing that this treatment induced *C. gloeosporioides* sporulation.

Significant interaction between factors (extract x concentration) by the F test at 5% probability for the percentage of conidia germination was verified (Table 1.). For 0.0 and 0.5 mg/mL concentrations, there was no significant difference among extracts (Table 3.) by the Tukey test ($P < 0.05$). Mango peel hexane extract contributes to lower the germination percentage of *C. gloeosporioides* conidia when used at concentrations of 0.25 and 2 mg/mL. This effect was observed at concentration of 1 mg/mL, as it increases the germination percentage in contrast to methanol and ethyl acetate extracts. For the highest concentration used in this study, the peel extract obtained with ethyl acetate differed from the others, because it was not able to totally inhibit conidial germination. However, at concentration of 1 mg/mL, total germination inhibition was observed when this treatment was used.

For germination, a quadratic model was the best fit ($R^2 = 0.938$) for the regression analysis of the dose of mango peel hexane extract (Fig. 2). For the other extracts, third-order polynomial models were the most adequate to describe the phenomenon. Albíter-Hernández [23] found reduction in the conidia germination percentage (7%) for one of *C. gloeosporioides* isolates using crude mango leaf extract (*Mangifera indica*). High sensitivity in the germination of this phytopathogen was also confirmed by Reis [24] who evaluated the efficacy of natural products in the *in vitro* anthracnose control in papaya and

observed that clove and cinnamon extracts at concentrations of 7.5% were able to partially inhibit *C. gloeosporioides* germination.

Studies have revealed the existence of phenolic compounds, which may have fungitoxic effect and pharmacological properties [25,6,14,26]. 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 [15,27]. 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.

Table 1. Summary of the analysis of variance (mean squares) for variables mycelial growth rate index (MGRI), sporulation (SPO) and germination (GERM)

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

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

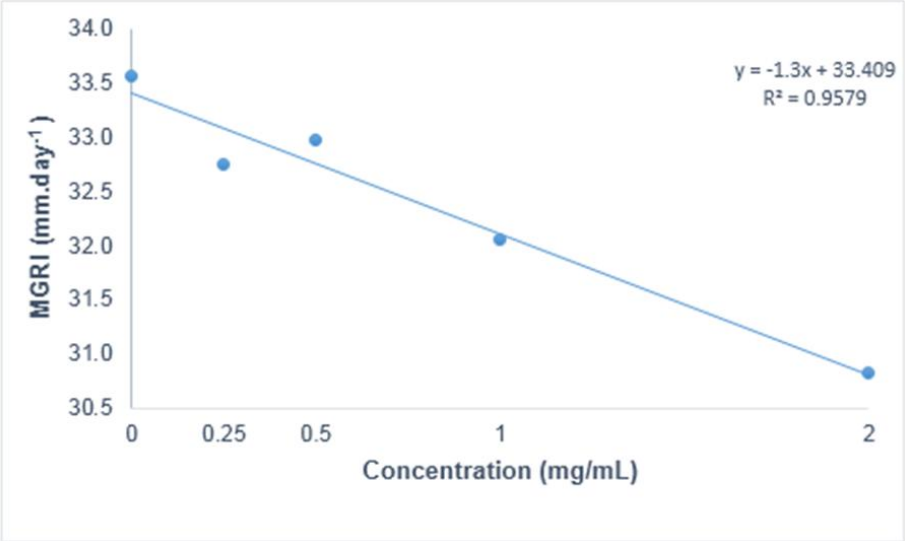

Fig. 1 Mycelial growth rate index of *Colletotrichum gloeosporioides* as a function of the different concentrations used (0, 0.25, 0.5, 1.0, 2.0 mg/mL)

Table 2. Effect of mango peel extracts (EME: Methanol Extract; EAC: Ethyl Acetate Extract; EHE: Hexane Extract) on *Colletotrichum gloeosporioides* sporulation (spores/mL) as a function of each concentration (mg/mL) used

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

Means followed by the same letter in row do not differ from each other by the Tukey test at 5% probability.

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

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

Means followed by the same letter in row do not differ from each other by the Tukey test at 5% probability

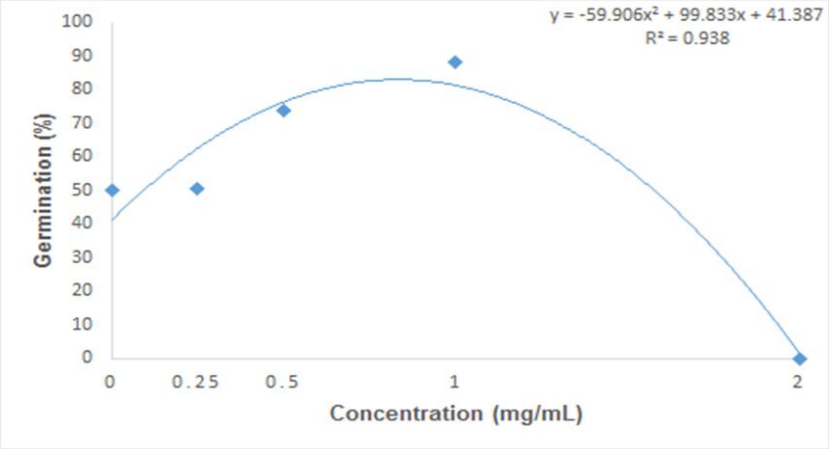

Fig. 2 Germination percentage of *Colletotrichum gloeosporioides* conidia in mango peel hexane extract as a function of the different concentrations

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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