1
2 *Original Research Article*

3 Evaluation of mango peel extracts on the *in* 4 *vitro* *Colletotrichum gloeosporioides* 5 development

6
7
8
9
10 **ABSTRACT**
11

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 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.

12
13 *Keywords: M. indica; postharvest diseases; alternative control; plant extracts; phenolic*
14 *compounds; resorcinol*.
15
16
17
18

19 1. INTRODUCTION

20
21 The mango (*Mangifera indica* L.) is a fruit tree of great economic importance in Brazil, not
22 only for its nutritional characteristics, but also for generating employment and income in
23 several regions of the country.
24
25 Much of the fruit production does not reach the consumers table and among the main
26 causes are the lack of technology in the production chain and post-harvest diseases.

UNDER PEER REVIEW

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

UNDER PEER REVIEW

78 in each procedure. Hexane, ethyl acetate and methanol were used, and the total extraction
79 time for each of these solvents was: 16 h for the first two (hexane and ethyl acetate) and 24
80 h (methanol) for the latter.
81
82 After the extraction time had elapsed, each of the three mixtures was transferred to a
83 volumetric flask with 250 mL capacity, which was taken to a rotary evaporator coupled to a
84 vacuum pump to separate the solvent from the extract. Extracts were transported in
85 styrofoam box to the Laboratory of Post-Harvesting Pathology of Fruits and Vegetables of
86 UNIMONTES to be used in the *in vitro* experiment for evaluation of mycelial growth,
87 sporulation and germination of *Colletotrichum gloeosporioides* conidia.

88 Initially, stock solution at 5 mg/mL was prepared for each extract using sterilized distilled
89 water and 1% (v/v) of hydrophilic nonionic surfactant Tween 80 (polyoxyethylene sorbitan
90 mono-oleate) as diluent. For homogenization, solutions were submitted to constant stirring in
91 an orbital shaking incubator at 30º C for 2 hours at 150 rpm.

92 **2.3 Parameters evaluated for *in vitro* studies**

93 *Colletotrichum* isolate was obtained from fruits with characteristic symptoms of anthracnose,
94 according to the indirect isolation technique [17]. Confirmation of the fungus identification
95 was performed based on its morphological characteristics through the preparation of slides
96 and observations under microscope.

97 For the mycelial sensitivity, aliquots of stock solutions were added to melting PDA (Potato-
98 Dextrose-Agar) medium so as to obtain the predetermined concentrations (0.0; 0.25; 0.5; 1.0
99 and 2.0 mg/mL). After homogenization, media were poured into identified Petri dishes,
100 where, after solidification, 5 mm *C. gloeosporioides* mycelium discs were transferred from 7
101 day-incubation cultures. Then, Petri dishes were sealed with plastic film and incubated in
102 BOD chamber at temperature of 25°C, with 12 hours photoperiod. Evaluations were
103 performed daily, measuring the growth of the mycelial diameter in two directions,
104 perpendicularly, using pachymeter in millimeters, starting 24 hours after the assembly of the
105 experiment and ending on the seventh day. MGRI (Mycelial Growth Rate Index) in mm.day^{-1}
106 was calculated using the formula [18]:

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

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

114 For germination evaluation, a conidia suspension of culture with 7 days of incubation was
115 prepared by placing 10 mL sterile distilled water on the surface of the Petri dish with the
116 fungal mycelium and gently scraping it with the aid of Drigalski loop. The suspension was
117 filtered through double-layer sterile gauze and concentration was adjusted to 2.5 x 10^5
118 conidia/mL after counting in Newbauer's chamber. Subsequently, aliquots of the stock
119 solutions of each extract were added to the melting agar medium in order to obtain the
120 predetermined concentrations. After homogenization, media were poured into identified Petri
121 dishes and when solidified, 200 µL of the conidia suspension was added to the surface of
122 the culture medium. With gentle movements, the suspension was spread over the culture
123 medium with the aid of Drigalski loop. About 100 conidia were evaluated under optical

UNDER PEER REVIEW

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.

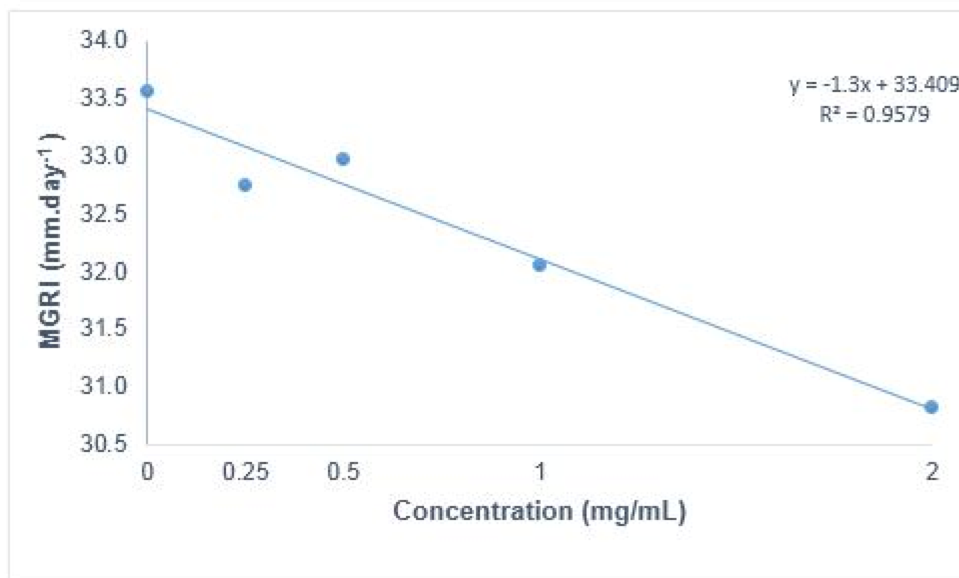
Table 1. Summary of the analysis of variance (Mean Squares) for variables mycelial growth rate index (MGRI), sporulation (SPO) and germination (GERM)

SV	Mean squares			
	DF	MGRI	SPO	GERM
Extract (E)	2	0.03^{ns}	$1.15 \times 10^{6*}$	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

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

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.

UNDER PEER REVIEW



163
164 **Fig. 1. Mycelial growth rate index of *Colletotrichum gloeosporioides* as a function of**
165 **the different concentrations used (0, 0.25, 0.5, 1.0, 2.0 mg/mL)**
166
167 With regard to *C. gloeosporioides* sporulation, there was interaction between the two factors
168 studied (extract x concentration) by the F test at 5% probability (Table 1), thus, both
169 simultaneously acted on the variable under study. Significant difference ($P < 0.05$) among
170 mango peel extracts at concentrations of 0.25; 0.5; 1.0 and 2.0 mg/mL (Table 2) was
171 observed by the Tukey test. In each of these concentrations, hexane extract provided lower
172 spore production when compared to methanol and ethyl acetate extracts, thus presenting
173 fungitoxic effect. At concentrations 0.25; 0.5 and 2.0 mg/mL, an increase in spore production
174 was observed with the use of the methanol mango peel extract in comparison with other
175 extracts, showing that this treatment induced *C. gloeosporioides* sporulation.
176
177 **Table 2. Effect of mango peel extracts (EME: methanol extract; EAC: ethyl acetate**
178 **extract; EHE: hexane extract) on *Colletotrichum gloeosporioides* sporulation**
179 **(spores/mL) as a function of each concentration (mg/mL) used**
180

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

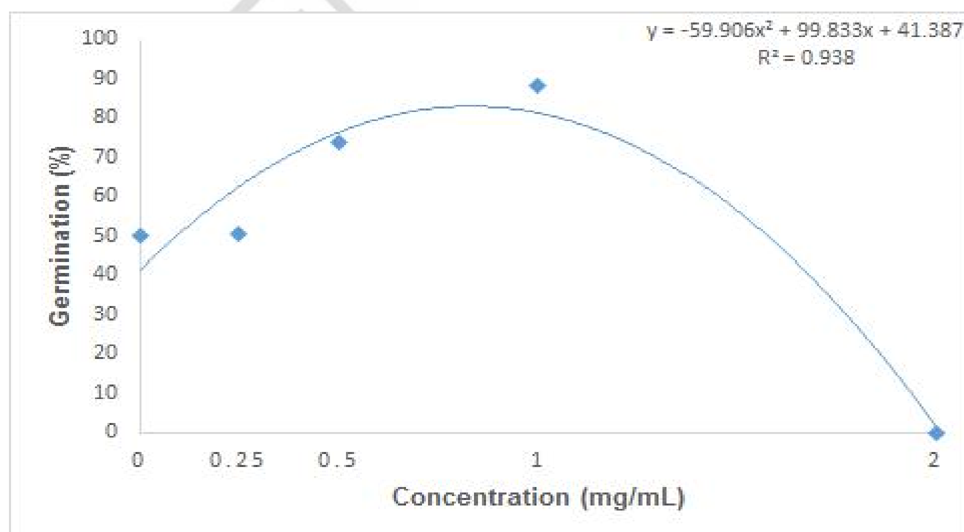
181 *Means followed by the same letter in row do not differ from each other by the Tukey test at 5%*
182 *probability.*
183
184 Significant interaction between factors (extract x concentration) by the F test at 5%
185 probability for the percentage of conidia germination was verified (Table 1). For 0.0 and 0.5
186 mg/mL concentrations, there was no significant difference among extracts (Table 3) by the

187 Tukey test (P < 0.05). Mango peel hexane extract contributes to lower the germination
188 percentage of *C. gloeosporioides* conidia when used at concentrations of 0.25 and 2 mg/mL.
189 This effect was observed at concentration of 1 mg/mL, as it increases the germination
190 percentage in contrast to methanol and ethyl acetate extracts. For the highest concentration
191 used in this study, the peel extract obtained with ethyl acetate differed from the others,
192 because it was not able to totally inhibit conidial germination. However, at concentration of 1
193 mg/mL, total germination inhibition was observed when this treatment was used.

194 **Table 3. Effect of mango peel extracts (EME: methanol extract, EAC: ethyl acetate**
195 **extract, EHE: hexane extract) on the germination percentage of *Colletotrichum***
196 ***gloeosporioides* conidia as a function of each concentration (mg/mL) used**
197

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

198 *Means followed by the same letter in row do not differ from each other by the Tukey test at 5%*
199 *probability.*
200
201 For germination, a quadratic model was the best fit (R^2 = 0.938) for the regression analysis
202 of the dose of mango peel hexane extract (Fig. 2). For the other extracts, third-order
203 polynomial models were the most adequate to describe the phenomenon. Albíter-Hernández
204 [23] found reduction in the conidia germination percentage (7%) for one of *C.*
205 *gloeosporioides* isolates using crude mango leaf extract (*Mangifera indica*). High sensitivity
206 in the germination of this phytopathogen was also confirmed by Reis [24] who evaluated the
207 efficacy of natural products in the *in vitro* anthracnose control in papaya and observed that
208 clove and cinnamon extracts at concentrations of 7.5% were able to partially inhibit *C.*
209 *gloeosporioides* germination.



210

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

213
214 Studies have revealed the existence of phenolic compounds, which may have fungitoxic
215 effect and pharmacological properties [25, 6, 14, 26]. Research suggests that the resistance
216 of green mango to *C. gloeosporioides* is due to a constitutive defense system composed of
217 antifungal resorcinols, gallotannins and chitinases [15, 27]. Few studies have been
218 published regarding the effect of mango peel extracts on post-harvest disease fungi. Thus,
219 the potential of using mango peels as a natural source of polyphenols combined with
220 extraction using different solvents maximizes the use of these substances in a pathogenic
221 system.

222 4. CONCLUSION

223
224 Methanol, hexane and ethyl acetate mango peel extracts inhibit the *in vitro C.*
225 *gloeosporioides* development. The increase in concentrations reduced mycelial growth of
226 the pathogen. The hexane extract provides greater reduction in spore production in contrast
227 to the others extracts. In germination of conidia, the effect of each extract depends on the
228 concentration used. Methanolic and hexane extracts of mango peel totally inhibit germination
229 only at the highest concentration.

230

231 ACKNOWLEDGEMENTS

232
233 The authors acknowledge the financial support from CAPES, CNPq and FAPEMIG.
234
235
236
237

238 COMPETING INTERESTS

239
240 The authors declare that they have no conflict of interest related to this study.
241
242

243 REFERENCES

244
245 1. Cia P, Paschoalati SF, Benato EA. Induction of resistance in the management of post-
246 harvest diseases. Brazilian Meeting of Induction of Resistance of Plants to Pathogens.
247 Federal University of Lavras. Lavras. 2007; 245-269.
248
249 2. Borges IV, Cavalcanti LS, Neto AF, Almeida JRG, Rolim LA, Lima MAG. Application of
250 coatings with black jurema extract in the control of anthracnose in mango fruits. Ibero-
251 American Journal of Post-Harvest Technology. 2016; 17 (2): 205-216.
252
253 3. Bill M, Sivakumar D, Korsten L, Thompson AK. The efficacy of combined application of
254 edible coatings and thyme oil in inducing resistance components in avocado (*Persea*
255 *americana* Mill.) against anthracnose during post-harvest storage. Crop Prot. 2014;64:159-
256 167. https://doi.org/10.1016/j.cropro.2014. 06.015

257 4. AGROFIT. Phytosanitary pesticide systems. Acessed 01 February 2019. Available:
258 http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons.

UNDER PEER REVIEW

259 5. Lemos LMC, Coutinho PH, Salomão LCC, Siqueira DL, Cecon PR. Control of
260 anthracnose in post-harvest 'ubá' mango with the use of alternative products. Revista
261 Brasileira de Fruticultura. 2013; 35 (4): 962-970.
262
263 6. Ajila CM, Aalami M, Leelavathi K, Rao UP. Mango peel powder: A potential source of
264 antioxidant and dietary fiber in macaroni preparations. Innovative Food Science & Emerging
265 Technologies. 2010;*11*(1):219-224.
266
267 7. Souza MEAO. Antioxidant potential of mango peel extracts (*Mangifera indica* L.) of the
268 Tommy Atkins variety obtained by low and high pressure methods and sizing of a column for
269 supercritical extraction. Thesis (Doctoral Degree in Food Engineering). Federal University of
270 Santa Catarina, Florianópolis. 2015.
271 .
272 8. Batool N, Ilyas N, Shabir S, Saeed M, Mazhar R, Amjid MW. A mini-review of therapeutic
273 potential of *Mangifera indica* L. Pakistan journal of pharmaceutical sciences. (2018);*31:*(4).

274 9. Chaves MRV, de Oliveira GM, Neto MJ, Neves FMDL, Barbosa IML. Potential fungicide of
275 medicinal plants of the Cerrado of the east coast of the state of Mato Grosso do Sul. Revista
276 Saúde e Meio Ambiente. 2018; 6 (1): 71-80.

277 10. Mahato TK, Sharma K. Study of medicinal herbs and its antibacterial activity: a
278 review. Journal of Drug Delivery and Therapeutics. 2018;8(5-s):47-54.

279 11. Asael RGH, Guevara-González RG, de Jesús RGS, Angélica FPA. Antifungal activity of
280 Mexican endemic plants on agricultural phytopathogens: a review. In 2018 XIV International
281 Engineering Congress (CONIIN). 2018;1-11.

282 12. Mbunde MVN, Mabiki F, Innocent E, Andersson PG. Antifungal activity of single and
283 combined extracts of medicinal plants from Southern Highlands of Tanzania. Journal of
284 Pharmacognosy and Phytochemistry. 2019;8(1):181-187.
285
286 13. Ajila CM, Rao LJ, Rao UP. Characterization of bioactive compounds from raw and ripe
287 *Mangifera indica* L. peel extracts. Food and Chemical Toxicology. 2010;*48*(12):3406-3411.
288
289 14. Barreto JC, Trevisan MT, Hull WE, Erben G, de Brito ES, Pfundstein B, Wurtele G,
290 Spiegelhalder B, Owen RW.Characterization and quantitation of polyphenolic compounds in
291 bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). Journal of agricultural and
292 food chemistry. 2008;56(14): 5599-5610.
293
294 15. Karunanayake LC, Adikaram N, Kumarihamy BMM, Bandara BMR, Abayasekara C.
295 Role of antifungal gallotannins, resorcinols and chitinases in the constitutive defence of
296 immature mango (*Mangifera indica* L.) against *Colletotrichum gloeosporioides.* J.
297 Phytopathol. 2011;*159*(*10*):657–664. https://doi.org/10.1111/j.1439-0434.2011.01818.x.
298
299 16. Deutsche Gesellschaft Für Technische Zusammenarbeit. Manual de exportación: frutas
300 tropicales y hortalizas. Eschborn, GTZ. 1992; 34.
301
302 17. Alfenas AC, Ferreira FA, Mafia RG, Gonçalves RC. Isolation of phytopathogenic fungi.
303 In: Alfenas, A.C .; Mafia, R.G. Methods in Phytopathology. Viçosa: Editora UFV. 2007; 53-
304 91.

305 18. Oliveira J.A. Effect of fungicidal tipping on seeds in control of cucumber seedlings
306 (*Cucumis sativas* L.) and pepper (*Capsicum annanum* L.). Dissertation (Master in
307 Phytosanitary) - School of Agriculture of Lavras, Lavras. 1991; 111.
UNDER PEER REVIEW

308 19. Ferreira DF.SISVAR.Universidade Federal de Lavras-MG. Versão 5.3. 2010.

309 20. Lins SRO, Oliveira SMA, Alexandre ER, Santos AMG, Oliveira TAS. Alternative control
310 of peduncular rot in mango. Summa Phytopathologica. 2011; 37(3):121-126.
311
312 21. Malaguetta H. Agroindustrial waste extracts for the control of phytopathogenic fungi.
313 Dissertation (Master of Science). University of São Paulo, Piracicaba. 2016.
314
315 22. Rojas R, Alvarez-Pérez OB, Contreras-Esquivel JC, Vicente A, Flores A, Sandoval J,
316 Aguilar CN. Valorisation of Mango Peels: Extraction of Pectin and Antioxidant and Antifungal
317 Polyphenols. Waste and Biomass Valorization. 2018; 1-10.
318
319 23. Hernández-Albíter RC, Barrera-Necha LL, Bautista-Baños S, Bravo-Luna L. Antifungal
320 Potential of Crude Plant Extracts on Conidial Germination of Two Isolates of *Colletotrichum*
321 *gloeosporioides* (Penz.) Penz. and Sacc. Revista Mexicana de Fitopatología. 2007; 25(2).
322
323 24. Reis HFD, Bacchi LMA, Scalon SDPQ, Flores, JKP. In vitro antimicrobial activity and
324 alternative control of anthracnose in papaya. Arquivos do Instituto Biológico. 2018; *85*.
325
326 25. Olasehinde GI, Sholotan KJ, Openibo JO, Taiwo OS, Bello OA, Ajayi JB. Phytochemical
327 and Antimicrobial Properties of *Mangifera indica* Leaf Extracts. Covenant Journal of Physical
328 and Life Sciences.2018.
329

330 26. Berardini N, Carle R, Schieber A. Characterization of gallotannins and benzophenone
331 derivatives from mango (*Mangifera indica* L. cv. “Tommy Atkins”) peels, pulp and kernels by
332 highperformance liquid chromatography/electrospray ionization mass spectrometry. Rapid
333 Commun. Mass Spectrom. 2004; 18: 2208–2216.
334
335 27. Adikaram NKB, Karunanayake LC, Sinniah GD, Abayasekara CL, Komala VS,
336 Yakandawala DMD. A review of the role for natural defences in the management of
337 *Colletotrichum* rotting of ripe mangoes. In XI International Mango Symposium. 2015; 229-
338 232.

339

UNDER PEER REVIEW