Original Research Article

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

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 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.

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 cause many damages and makes fruits unfit for consumption [1, 2]. The fungus infection accelerates the maturation and deterioration of the fruits, contributing to losses that can reach up to 80% [3].

Among the methods for controlling the 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 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 [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/mLl was prepared for each extract using sterilized distilled water and 1% (v/v) of hydrophilic nonionic surfactant Tween 80 (polyoxyethylene sorbitan monooleate) as diluent. For homogenization, solutions were submitted to constant stirring in an orbital shaking incubator at 30º C 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.

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

SV	DF	Mean squares		
		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.

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.

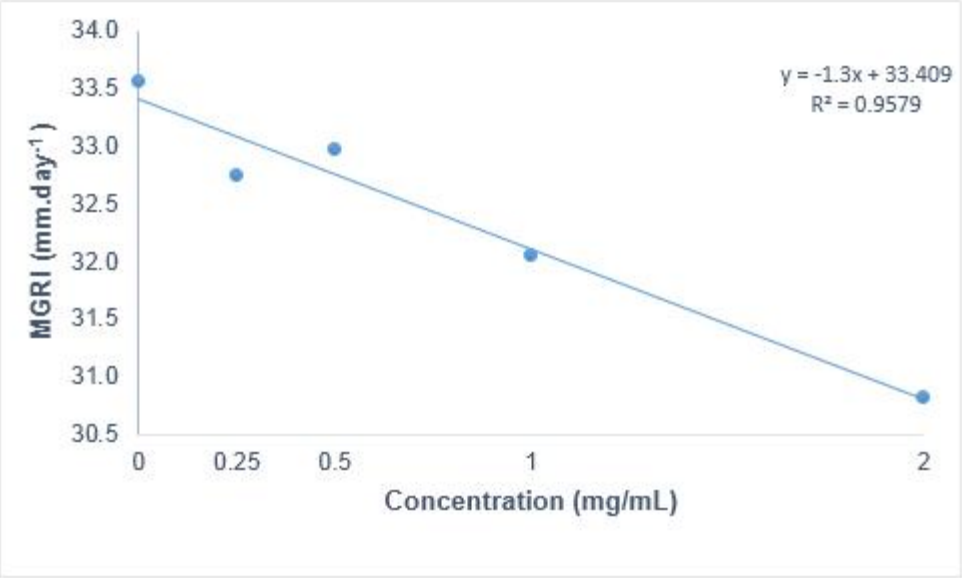

Fig. 1. Mycelial growth rate index of *Colletotrichum gloeosporioides* as a function of the different concentrations used (0, 0.25, 0.5, 1.0, 2.0 mg/mL)

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.

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

	Extracts		
Concentrations	**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.

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.

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

	Extracts		
Concentrations	**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.

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.

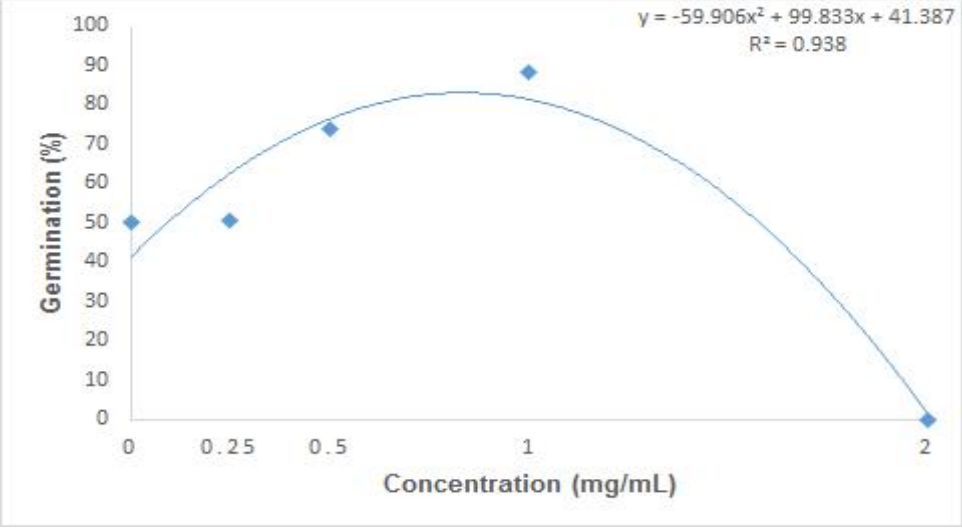

Fig. 2. Germination percentage of *Colletotrichum gloeosporioides* conidia in mango peel hexane extract as a function of the different concentrations

Studies have revealed the existence of phenolic compounds, which may have fungitoxic effect and pharmacological properties [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.

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.

REFERENCES

1. Cia P, Paschoalati SF, Benato EA. Induction of resistance in the management of post-harvest diseases. Brazilian Meeting of Induction of Resistance of Plants to Pathogens. Federal University of Lavras. Lavras. 2007; 245-269.

2. Borges IV, Cavalcanti LS, Neto AF, Almeida JRG, Rolim LA, Lima MAG. Application of coatings with black jurema extract in the control of anthracnose in mango fruits. Ibero-American Journal of Post-Harvest Technology. 2016; 17 (2): 205-216.

3. Bill M, Sivakumar D, Korsten L, Thompson AK. The efficacy of combined application of edible coatings and thyme oil in inducing resistance components in avocado (*Persea americana* Mill.) against anthracnose during post-harvest storage. Crop Prot. 2014;64:159-167. https://doi.org/10.1016/j.cropro.2014. 06.015

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

5. Lemos LMC, Coutinho PH, Salomão LCC, Siqueira DL, Cecon PR. Control of anthracnose in post-harvest 'ubá' mango with the use of alternative products. Revista Brasileira de Fruticultura. 2013; 35 (4): 962-970.

6. Ajila CM, Aalami M, Leelavathi K, Rao UP. Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. Innovative Food Science & Emerging Technologies. 2010;*11*(1):219-224.

7. Souza MEAO. Antioxidant potential of mango peel extracts (*Mangifera indica* L.) of the Tommy Atkins variety obtained by low and high pressure methods and sizing of a column for supercritical extraction. Thesis (Doctoral Degree in Food Engineering). Federal University of Santa Catarina, Florianópolis. 2015.
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8. Batool N, Ilyas N, Shabir S, Saeed M, Mazhar R, Amjid MW. A mini-review of therapeutic potential of *Mangifera indica* L. Pakistan journal of pharmaceutical sciences. (2018);*31:*(4).

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

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

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

12. Mbunde MVN, Mabiki F, Innocent E, Andersson PG. Antifungal activity of single and combined extracts of medicinal plants from Southern Highlands of Tanzania. Journal of Pharmacognosy and Phytochemistry. 2019;8(1):181-187.

13. Ajila CM, Rao LJ, Rao UP. Characterization of bioactive compounds from raw and ripe *Mangifera indica* L. peel extracts. Food and Chemical Toxicology. 2010;*48*(12):3406-3411.

14. Barreto JC, Trevisan MT, Hull WE, Erben G, de Brito ES, Pfundstein B, Wurtele G, Spiegelhalder B, Owen RW.Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). Journal of agricultural and food chemistry. 2008;56(14): 5599-5610.

15. Karunanayake LC, Adikaram N, Kumarihamy BMM, Bandara BMR, Abayasekara C. Role of antifungal gallotannins, resorcinols and chitinases in the constitutive defence of immature mango (*Mangifera indica* L.) against *Colletotrichum gloeosporioides.* J. Phytopathol. 2011;*159*(*10*):657–664. https://doi.org/10.1111/j.1439-0434.2011.01818.x.

16. Deutsche Gesellschaft Für Technische Zusammenarbeit. Manual de exportación: frutas tropicales y hortalizas. Eschborn, GTZ. 1992; 34.

17. Alfenas AC, Ferreira FA, Mafia RG, Gonçalves RC. Isolation of phytopathogenic fungi. In: Alfenas, A.C .; Mafia, R.G. Methods in Phytopathology. Viçosa: Editora UFV. 2007; 53-91.

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

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

20. Lins SRO, Oliveira SMA, Alexandre ER, Santos AMG, Oliveira TAS. Alternative control of peduncular rot in mango. Summa Phytopathologica. 2011; 37(3):121-126.

21. Malaguetta H. Agroindustrial waste extracts for the control of phytopathogenic fungi. Dissertation (Master of Science). University of São Paulo, Piracicaba. 2016.

22. Rojas R, Alvarez-Pérez OB, Contreras-Esquivel JC, Vicente A, Flores A, Sandoval J, Aguilar CN. Valorisation of Mango Peels: Extraction of Pectin and Antioxidant and Antifungal Polyphenols. Waste and Biomass Valorization. 2018; 1-10.

23. Hernández-Albíter RC, Barrera-Necha LL, Bautista-Baños S, Bravo-Luna L. Antifungal Potential of Crude Plant Extracts on Conidial Germination of Two Isolates of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Revista Mexicana de Fitopatología. 2007; 25(2).

24. Reis HFD, Bacchi LMA, Scalon SDPQ, Flores, JKP. In vitro antimicrobial activity and alternative control of anthracnose in papaya. Arquivos do Instituto Biológico. 2018; *85*.

25. Olasehinde GI, Sholotan KJ, Openibo JO, Taiwo OS, Bello OA, Ajayi JB. Phytochemical and Antimicrobial Properties of *Mangifera indica* Leaf Extracts. Covenant Journal of Physical and Life Sciences.2018.

26. Berardini N, Carle R, Schieber A. Characterization of gallotannins and benzophenone derivatives from mango (*Mangifera indica* L. cv. “Tommy Atkins”) peels, pulp and kernels by highperformance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun. Mass Spectrom. 2004; 18: 2208–2216.

27. Adikaram NKB, Karunanayake LC, Sinniah GD, Abayasekara CL, Komala VS, Yakandawala DMD. A review of the role for natural defences in the management of *Colletotrichum* rotting of ripe mangoes. In XI International Mango Symposium. 2015; 229-232.