17

# Effect of papaya leaf extracts on the control of papaya anthracnose


**Jaciene Lopes de Jesus**
Embrapa Mandioca e Fruticultura

**Eliseth de Souza Viana**
Embrapa Mandioca e Fruticultura

**Vanessa de Oliveira Almeida**
Faculdade Maria Milza

**Fabiana Fumi Cerqueira Sasaki**
Embrapa Mandioca e Fruticultura

**Calila Teixeira Santos**
Universidade Estadual de Feira de Santana

**Alexsandro Branco**
Universidade Estadual de Feira de Santana



doi 10.37885/220910121

# ABSTRACT

**Objective:** The objective of this study was to evaluate whether extracts with different polarities, obtained from papaya (*Carica papaya*) leaves, have fungicide and/or fungitoxic action in the post-harvest control of anthracnose in papaya fruits. **Methods:** Hydroethanolic extract was fractionated with organic solvents of different polarities, resulting in hexane, ethyl ether, ethyl acetate, *n*-butyl alcohol and aqueous fractions. In in vitro tests, concentrations of 0, 500, 1000, 1500 and 2000 mg $L^{-1}$, diluted with 10% Tween 20, were used to evaluate fungitoxic and fungicide effects using the Mycelial Growth Inhibition percentage (MGI%) method. **Results and Discussion:** In in vivo tests, the fraction of leaf extract which had the highest MGI% was used. Severity, expressed by the average diameter of the lesion and calculation of the Area Under the Disease Progress Curve (AUDPC), and incidence of the disease were determined. Analyses of pH, soluble solids, titratable acidity, firmness and mass loss were performed to evaluate the effect of treatments on fruit quality. Aqueous extract, at a concentration of 2000 mg $mL^{-1}$, was the most efficient to control the growth of *Colletotrichum* spp. in vitro. In the in vivo test, an inhibitory effect of the extract was observed at the different concentrations tested, when associated with 10% Tween 20. The use of aqueous extract in fruits did not significantly alter their firmness and soluble solids content. **Conclusion:** Treatment of papaya fruits with Tween 20, associated or not with aqueous extract, reduced the appearance of anthracnose symptoms.

**Keywords:** *Carica Papaya*, *Colletotrichum* spp., Post-Harvest Disease, Plant Extracts.

## INTRODUCTION

Papaya (*Carica papaya* L.) is one of the main fruit crops cultivated in Brazil. One of the limiting factors to its production and commercialization is its vulnerability to a large number of diseases. Anthracnose, caused by *Colletotrichum* spp., is responsible for major losses in this crop and the control of this disease is mostly performed using synthetic fungicides in pre-harvest and post-harvest, but it can leave chemical residues in the fruits.

The main problem with the infection caused by *Colletotrichum* spp. is the presence of rounded, large, necrotic lesions, with depression in center of the tissues, which can reach different diameters from which masses of rosy-colored conidia emerge (DEMARTELAERE *et al.*, 2017). Losses resulting from contamination by pathogens still in the field compromise fruit quality and may restrict the export of fresh fruits and reduce remuneration in the domestic market (BAUTISTA-BAÑOS *et al.*, 2013).

The control of papaya diseases in post-harvest is often performed through the application of synthetic fungicides. However, the increased resistance of phytopathogens, as well as the evidence of the harmful effects of these products on the environment and health and the effects they can cause in the long term, have increased the demand for new control alternatives (RIBEIRO *et al.*, 2016).

In the literature, it can be observed that a large number of plants have already been studied in the control of post-harvest phytopathogens, and it is claimed that the antifungal properties of their extracts may be related to the presence of flavonoids, tannins, phenols, terpenoids, isothiocyanate, alkaloids, glycosides, saponins, steroids, organic acids and unsaturated sterols (MADJOS; LUCEÑO, 2019; KAD; TAMBE, 2018; POH; JIEN, 2017; ALORKPA *et al.*, 2016; IKRAM *et al.*, 2015). Some of these compounds are present in *Carica papaya* leaves, so their use can be explored and their application can be validated for the control of anthracnose in papaya fruits (POH; JIEN, 2017).

In view of the above, this study aimed to evaluate extracts obtained from leaves of papaya, cultivar 'Golden THB', with regard to their fungicide and/or fungitoxic action in the post-harvest control of anthracnose in papaya fruits.

## MATERIAL AND METHODS

### Obtaining the extract from papaya leaves and the fungus *Colletotrichum* spp.

This stage was carried out at the Phytochemistry Laboratory of the State University of Feira de Santana (Feira de Santana, BA, Brazil). Leaves of papaya cv. 'Golden THB' were collected in a commercial orchard, located in Mucuri, BA, Brazil, using the hydroethanolic


Ciências Agrárias: o avanço da ciência no Brasil - ISBN 978-65-5360-233-5- Vol. 5 - Ano 2022 - Editora Científica Digital - www.editoracientifica.com.br

extract (crude extract) obtained from leaf hydrodistillation and its fractions from the liquid--liquid partitioning process with organic solvents of increasing polarity: hexane, ethyl ether, ethyl acetate, *n*-butyl alcohol and the residual called aqueous partition. After evaporation of the solvents, the masses of each of the fractions were weighed and the yield of the fractions was calculated by the expression:

$$Yield\ (\%) = \frac{\text{Fraction mass}}{\text{Crude extract mass}} x100 \quad (1)$$

The strain of *Colletotrichum* spp., standard strain 0402, used in the experiment belongs to the collection of fungi of the Phytopathology Laboratory of Embrapa Cassava and Tropical Fruits and was obtained by isolation from infected papaya fruits.

*In vitro inhibition of Colletotrichum spp. mycelial growth*

Lyophilized extracts of papaya leaves were diluted in 10% Tween 20 solution at concentrations of 0, 500, 1000, 1500 and 2000 mg $L^{-1}$ and added to the potato-dextrose-agar (PDA) medium, and then this mixture was poured and solidified in Petri dishes, with 90 mm in diameter. At the same time, three controls were prepared, one only with the culture medium (CC), another containing culture medium and *Colletotrichum* spp. (CP) and finally the culture medium plus the commercial fungicide Cercobin® 700 WP (CN).

Discs of 5 mm in diameter, containing mycelium and spores of *Colletotrichum* spp., were removed from a pure culture aged 10 days and transferred to the center of plates containing agar mixed with the different concentrations of extracts. The plates were sealed with plastic film and kept in BOD chamber, at a temperature of 25 ºC ± 2 ºC, with photoperiod of 12 hours, until the mycelial growth of the control plate fully covered the surface of the culture medium.

The efficiency of the different concentrations of the extracts was determined by measuring the diameters of the colonies in millimeters, with a digital caliper, to obtain the Growth Inhibition Percentage (MGI%) for the inoculated fungus in comparison to the control plates CC and CP using the following formula:

$$MGI\% = \frac{D_{control\ (CP)} - D_{treatment}}{D_{control\ (CP)}} \times 100 \quad (2)$$

The experimental design used was completely randomized, in a 6 x 5 + 3 factorial scheme. Treatments consisted of the combination of six extracts (crude, hexane, ethyl ether, ethyl acetate, *n*-butyl alcohol and aqueous), at five concentrations (0, 500, 1000, 1500 and 2000 mg $L^{-1}$), and the three controls (CC, CP and CN).

The data were subjected to analysis of variance (ANOVA) to test the main effects and interactions of the treatment. The mean differences of treatment between the extracts were compared by the Scott-Knott test at 5% probability level. The differences between the concentrations of the extracts were determined by regression analyses. Statistical analyses were performed in the SISVAR program (FERREIRA, 2011).

**Evaluation of anthracnose control in papaya fruits (in vivo)**

The most efficient extract in the in vitro assays was selected for in vivo evaluation, also taking into account the nature of the solvent and yield.

Papaya fruits at maturity stage 1 (up to 15% of peel is yellow), from a commercial orchard, located in the municipality of Mucuri, BA, were artificially contaminated with the fungus *Colletotrichum spp.*, by inoculation with mycelium discs (5 mm in diameter), removed from colonies of the pathogen, grown in PDA for 10 days. Inoculation was performed by the injury method. Four holes with 1 cm depth were made on the fruit surface, using a sterile needle with 0.2-mm caliber. Inoculum discs were placed on each hole and then the fruits were placed in a humid chamber, at 25 ºC, and kept for 24 hours.

After this period, the fruits were sprayed with the aqueous extract, at concentrations of 0, 2000, 3000, 4000, 5000 and 6000 mg $L^{-1}$ of the extract selected in the in vivo test. For spraying, 50-mL spray bottles were used. The fruits were placed on shelves and remained at room temperature at 25 ºC ± 1. Fruits without inoculation of the phytopathogen, fruits with inoculation of the phytopathogen and fruits with the adjuvant Tween 20 at 10% plus inoculation of the phytopathogen were respectively the control, C1 and C2.

The mean size of the lesion was evaluated by measuring the diameter of the lesions in two diametrically opposite directions, in millimeters, using a digital caliper, at the points of inoculation, when the fruits reached maturity stage 5.

Severity was considered as the daily progress of the lesion, evaluated by measuring the diameter of the lesion every 24 h, recording the diameter of the colonies in millimeters. Measurements were interrupted on the day the fruits reached maturity stage 5. From the mean value of lesion diameter over time, the Area Under the Disease Progress Curve (AUDPC) was calculated according to the methodology of Shaner and Finney (1977), by the following formula:

$$AUDPC = \sum \left( \frac{\left(y_i + y_{(i+1)}\right)}{2 \times \left(t_{(i+1)} - t_i\right)} \right) \quad (3)$$



Ciências Agrárias: o avanço da ciência no Brasil - ISBN 978-65-5360-233-5- Vol. 5 - Ano 2022 - Editora Científica Digital - www.editoracientifica.com.br

Where $y_1$ is the diameter or incidence of a lesion at time $t_i$, in days, and $y_{i+1}$ is the diameter or incidence of the lesion at time $t_{i+1}$.

Incidence was evaluated with the naked eye, based on the presence or not of the lesion at the inoculation point, until the fruit reached maturity stage 5, and calculated based on the number of symptomatic fruits (ALI *et al.*, 2015) according to equation 4:

$$I = \frac{NF_{infected}}{NF_{total}} \times 100 \quad (4)$$

Where $NF_{infected}$ corresponds to the number of fruits with symptoms and $NF_{total}$ corresponds to the total number of fruits.

The experiment was conducted using a completely randomized design, in a 5 x 6 + 3 factorial scheme, with five concentrations of extracts, six days of evaluation and three controls (control, control 1 and control 2), with 4 fruits per replicates and two experimental replicates.

Lesion size data as well as AUDPC data were transformed to $\sqrt{x}$. Analysis of variance (ANOVA) was applied and, in case of significance at 5% probability level by the F test, treatment means were compared using the Scott-Knott test. Statistical analyses were performed in the SISVAR program (FERREIRA, 2011).

### Physicochemical characterization of fruits inoculated with *Colletotrichum spp.*

Characterization of the fruits was performed when they reached maturity stage 5, which occurred around 6 days after inoculation. Analyses of moisture, pH, soluble solids (ºBrix) and titratable acidity (% citric acid) were performed according to Instituto Adolfo Lutz (2008). Firmness of the fruit, with peel and without peel, was obtained with an analog penetrometer, with a 7.9-mm-diameter tip, and expressed in kgf. Mass loss was obtained by weighing the fruit on the day of treatment application ($M_i$) and on the day of evaluation ($M_f$) by the following formula:

$$` \quad Mass\ loss = \frac{(M_i - M_f)}{M_i} \times 100 \quad (5)$$

## RESULTS AND DISCUSSION

### Yield of the obtained extracts and in vitro inhibition of *Colletotrichum spp.* mycelial growth

It was observed that the most polar solvent (aqueous) showed the highest yield (57.2%), followed by ethyl ether (15.7%). The lowest yield was obtained for *n*-butyl (7.2%) (Table 1).

This result shows that papaya leaves had in their composition predominance of compounds with more polar characteristics.

The only study on phytochemical screening of papaya leaves found in the literature was conducted by Baskaran *et al.* (2012). These authors evaluated the solvents ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane and water and observed the presence of alkaloids, carbohydrates, saponins, glycosides, proteins and amino acids, phytosterols, phenolic compounds, flavonoids, terpenoids and tannins. Possibly, the extracts obtained in the present study have a composition similar to those of the extracts obtained by the aforementioned authors. Also in this study, the results of the phytochemical screening performed on the aqueous extract indicated only the presence of alkaloids.

**Table 1.** Yield in obtaining the fractions, after liquid-liquid partitioning performed from the hydroethanolic extract of papaya leaves.

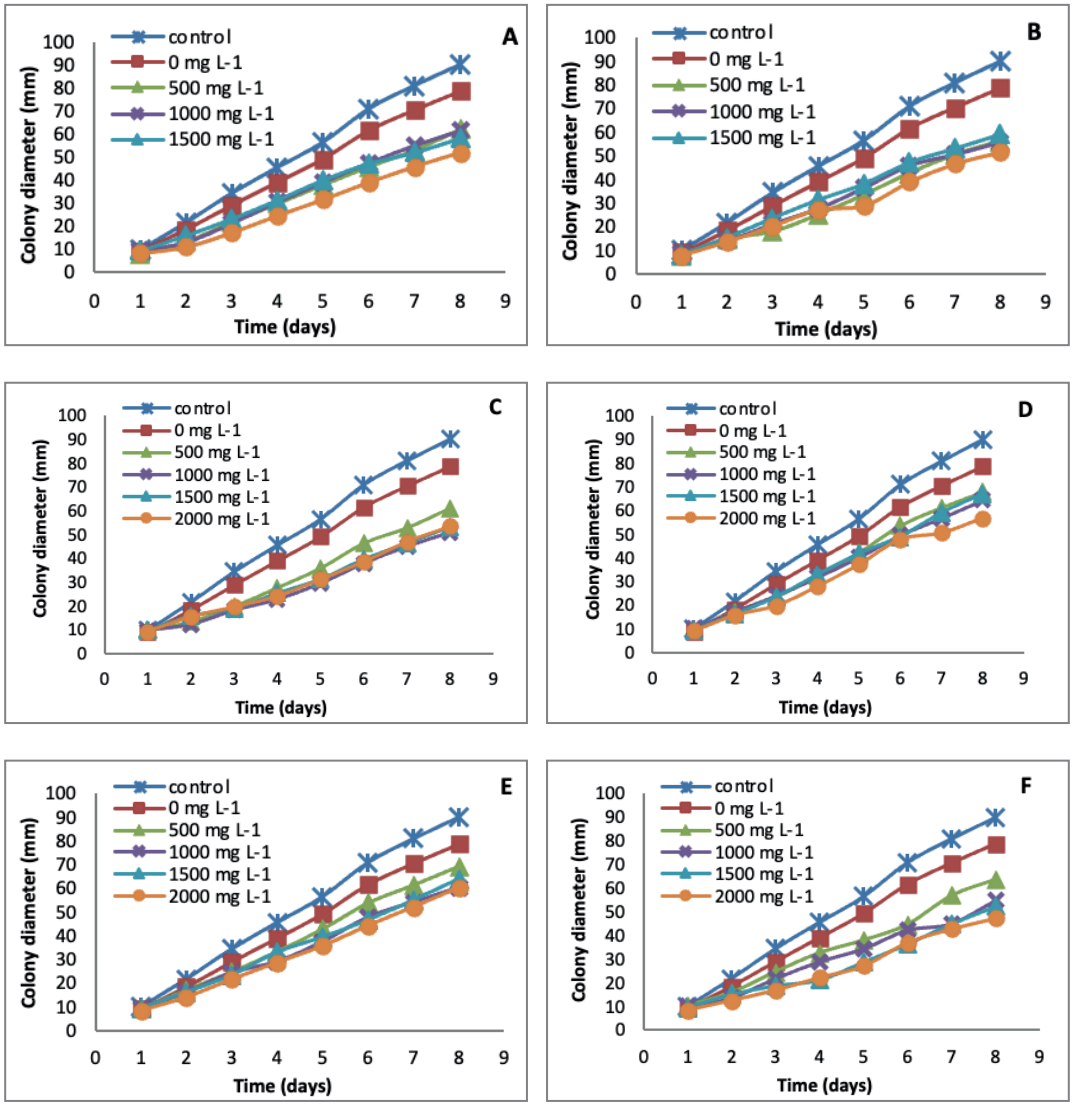
| Treatment | Yield (%) |
|---|---|
| *n*-butyl | 7.2 |
| Ethyl ether | 15.7 |
| Hexane | 10.8 |
| Ethyl acetate | 9.0 |
| Aqueous | 57.2 |
| Crude | 100 |

It was found that all extracts had a significant inhibitory effect on the mycelial growth of *C. gloeosporioides* compared to the control ($p<0.05$). Overall, the mycelial growth of the fungus was dependent on the concentration of the extracts. There was a positive linear relationship between the concentrations and diameters of the lesions for all extracts (Figure 1).

The percentages of inhibition of mycelial growth for the *n*-butyl, ethyl ether, hexane, ethyl acetate, aqueous and crude extracts at 2000 mg $L^{-1}$ after 8 days were 33.1%, 40.6%, 42.7%, 36.9%, 47.4% and 42.6%, respectively. Thus, the aqueous extract, at a concentration of 2,000 mg $L^{-1}$, was the most effective with 47.4% inhibition.

Lower results were reported by Bautista-Baños *et al.* (2002), who evaluated the fungicide effect of papaya leaf aqueous extract on the in vitro growth of *Colletotrichum gloeosporioides* and obtained 27.5% inhibition of mycelial growth on the seventh day of evaluation.

**Figure 1.** Mycelial growth (mm) of the fungus *Colletotrichum* spp. in PDA culture medium, with addition of the crude extracts of papaya leaf (A) and its fractions, hexane (B), ethyl ether (C), ethyl acetate (D), *n*-butyl (E) and aqueous (F).



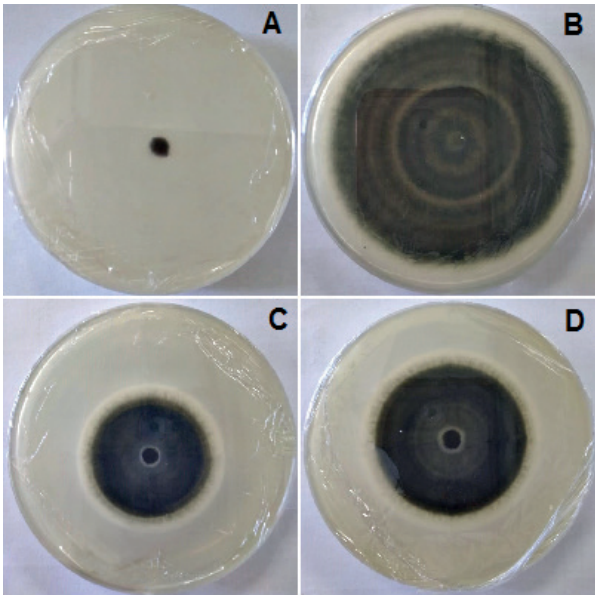

Bautista-Baños *et al.* (2003) evaluated the in vitro fungicide effect of chitosan and aqueous extracts of papaya leaves and seeds on the development of *Colletotrichum gloeosporioides* and observed that the extracts alone had no fungicide effect, while the combination of chitosan at 2.5% with the tested extract showed fungistatic effect. Chavez-Quintal *et al.* (2011) evaluated in vitro the antifungal activity of ethanol extracts of papaya leaves, at a concentration of 20 mg $mL^{-1}$, and observed 21.84% inhibition of *C. gloeosporioides* mycelial growth, obtaining a lower result than that for aqueous extract observed in the present study.

Figure 1 shows that, for the control treatment, the mean inhibition was equal to 12.7% for all extracts tested, showing that 10% Tween 20, which is present in the positive control and other extract concentrations, has some inhibitory effect on *Colletotrichum* spp.

Figure 2 shows the mycelial growth of the fungus *Colletotrichum* spp. in vitro at eight days of evaluation. The results of the most efficient extract (aqueous; Figure 2C) and the least efficient (*n*-butyl; Figure 2D) were presented. It is observed that in the negative control plate,

using the commercial fungicide Cercobin® 700 WP (Figure 2A), there was no growth of the fungus (Figure 2A) but, in the positive control, the fungus occupied the whole plate (Figure 2B).

**Figure 2.** Mycelial growth of *Colletotrichum* spp. in PDA culture medium for 8 days of mycelial growth. A- Negative control: fungus grown in PDA with addition of the fungicide Cercobin® 700 WP, B- Positive control: fungus grown in PDA; C- Fungus grown in PDA with addition of 2000 mg $L^{-1}$ of aqueous extract; D: fungus grown in *n*-butyl extract at 2000 mg $L^{-1}$.




## Evaluation of anthracnose control in papaya fruits

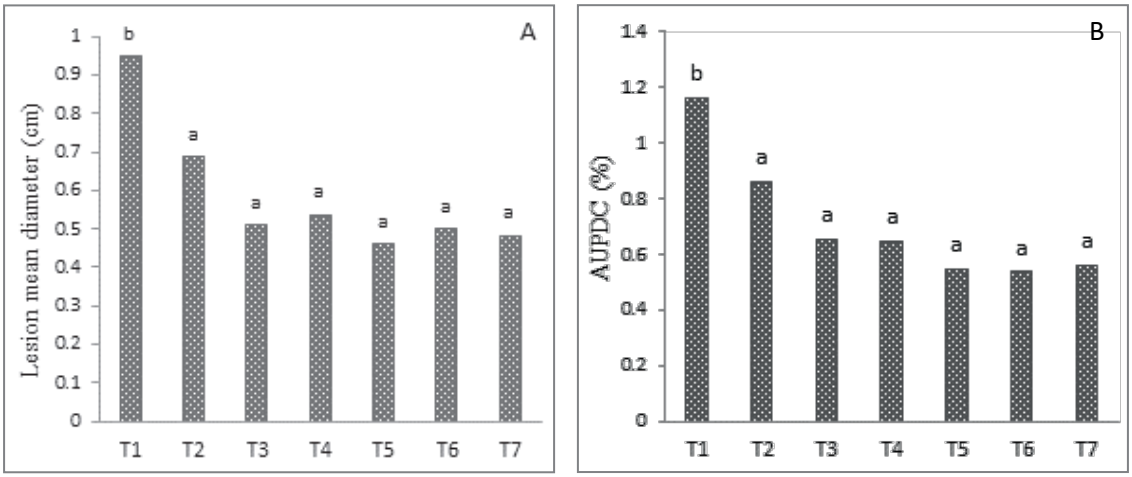
For the in vivo study, the extract and concentrations were selected from the results observed in the in vitro assay, considering the efficiency of mycelial growth inhibition, associated with the highest yield. Therefore, it was decided to use the aqueous extract, with concentrations higher than that which was the most effective in the in vitro test, with 2000 mg $L^{-1}$ being the lowest concentration tested.

It can be observed in Figure 3A that the different concentrations of extract tested reduced the mean diameter of *C. gloeosporioides* lesions in the fruits, when compared to T1 (Control 1); however, no significant difference was observed when cultivated in the presence of 10% Tween 20 (Control 2) (Figure 3A). Thus, it is not possible to attribute the inhibitory effect exclusively to the extract, and this result suggests that the surfactant used also contributed to reduce the growth of the fungus under study, a result also observed in the in vitro tests (Figure 1).

Martinez *et al.* (2020) evaluated the severity of anthracnose in fruits of papayas *cv.* 'Havaí', using the fraction extracted with dichloromethane and the compound 7-desmethylsuberosin isolated from the fractions extracted with *n*-hexane and dichloromethane, both obtained from the extract of 'Pau-Rainha' (*Brosimum rubescens* Taub.) sawdust. The lesion sizes for dichloromethane and 7-desmethylsuberosin at a concentration of 200 mg $mL^{-1}$ were 25 and 24 mm, respectively, measured 7 days after inoculation with *C. gloeosporioides*.

When evaluating the progress of the disease (AUDPC), it was observed that the treatments differed from treatment 1 (control 1), but there was no difference between them (Figure 3B). The aqueous extract, at any of the concentrations used, had an effect on disease control as well as the treatment with the surfactant Tween 20 at 10% (control 2). Thus, the inhibitory result can be attributed both to the surfactant and to the aqueous extract of papaya leaves.

**Figure 3.** Lesion mean diameter (A) and Area Under Disease Progress Curve - AUDPC (B) in papaya fruits, at 6 days after inoculation with *Colletotrichum* spp.




T1: control 1 - papaya inoculated with *Colletotrichum* spp., T2: control 2 - papaya inoculated with *Colletotrichum* spp. with addition of 10% Tween 20, T3: Extract at concentration of 2000 mg $L^{-1}$, T4: Extract at concentration of 3000 mg $L^{-1}$, T5: Extract at concentration of 4000 mg $L^{-1}$, T6: Extract at concentration of 5000 mg $L^{-1}$, T7: Extract at concentration of 6000 mg $L^{-1}$. Columns with equal letters do not differ by Scott-Knott test at 5% probability level

Tween 20 is a non-ionic, non-toxic surfactant consisting of esters of polyoxyethylene sorbitol fatty acid and widely used in the food industry, due to its low cost and easy availability (BASAK; GUHA, 2017). In this study, the use of this surfactant was necessary because not all the extracts used are water-soluble. Thus, it was used in all treatments, as it was necessary to standardize them. Some studies have also reported the inhibitory effect of this surfactant on fungi. Domingues *et al.* (2000) stated that Tween can alter the morphology and surface of the cell wall of fungi. Santos (2013) evaluated the activity of the surfactant Tween 20 on the growth of the standard strain of *Cryptococcus neoformans* and observed that its growth was significantly inhibited at the concentrations from 25 to 100%, and the inhibition was attributed to the toxicity that the surfactant causes in the medium.

The incidence of anthracnose was practically null in the first three days, and there was a marked increase in the symptoms of the disease in the two days preceding the point of consumption of papaya, but the symptoms did not manifest in all holes.

These active infections occur when papaya fruits have already started or completed the maturation process, progressing as the environmental conditions favor the growth of the pathogen. Jarvis (1994) also states that normal physiological changes in fruits can initiate the transition from the latency phase to the active phase, promoting the development of the disease.

Fruits treated with the extracts showed reduction in the incidence of anthracnose when compared to those of the control treatments, T1 (control with the phytopathogen *Colletotrichum* spp.) and T2 (control with the adjuvant Tween 20 at 10%), which showed 100% incidence of fungi at the end of the experiment (Table 2). The absolute control treatment (fruit without inoculation) did not show lesion. The lowest incidence of disease was observed for treatments T4 and T7. The highest mean incidence of 90% disease was observed in the treatment T6.

Efri *et al.* (2019) obtained positive results when evaluating the effect of papaya leaf extracts at concentrations of 20, 30, 40 and 50% in papaya fruits, because at six days of evaluation, the concentration of 30% reduced the incidence of anthracnose by 22.5%.

Contrary to the result observed in the present study, Bautista-Baños *et al.* (2002) evaluated the incidence of anthracnose in papaya fruits treated with aqueous extract of papaya leaves, at a concentration of 200,000 mg $L^{-1}$ and obtained total inhibition of the disease, a result that can be attributed to the high concentration used, which is much higher than those tested in the present study.

**Table 2.** Incidence of anthracnose in papaya fruits inoculated with *Colletotrichum* spp. and treated with aqueous extract of papaya seeds and leaves diluted in 10% Tween 20.

| Treatment | Incidence (%) |
|---|---|
| T1- control 1: Papaya +*Colletotrichum* spp. | 100 |
| T2- control 2: Papaya + *Colletotrichum* spp. + 10% Tween 20 | 100 |
| T3- Extract at concentration of 2000 mg $L^{-1}$ | 80 |
| T4- Extract at concentration of 3000 mg $L^{-1}$ | 70 |
| T5- Extract at concentration of 4000 mg $L^{-1}$ | 80 |
| T6- Extract at concentration of 5000 mg $L^{-1}$ | 90 |
| T7- Extract at concentration of 6000 mg $L^{-1}$ | 70 |
| Mean | 84.3 |
| CV (%) | 28.2 |

The physical, chemical and physicochemical evaluations of the fruits revealed that the different treatments tested had a significant effect on pH, titratable acidity and mass loss (Table 3). There was no significant difference between treatments regarding soluble solids content (10.2 ºBrix to 10.9 ºBrix) and fruit firmness (0.9 kgf to 1.3 kgf). Bautista-Baños *et al.* (2013) obtained lower values of soluble solids compared to the results of the present study (between 8.3 ºBrix and 9.4 ºBrix) in papaya fruits, 'Maradol' variety, coated with chitosan and in combination of chitosan with plant extract. This can be attributed to the different varieties evaluated in the two studies.

**Table 3.** Physical, chemical and physicochemical characteristics of papaya fruits, at maturity stage 5, subjected to treatments with different concentrations of aqueous extract of papaya leaves.

| Treatment* | pH | SS (ºBrix) | TA (% citric acid) | Firmness (kgf) | Mass loss (%) |
|---|---|---|---|---|---|
| T0- Control | 5.7$^{b}$ | 10.6$^{ns}$ | 0.066$^{b}$ | 1.1$^{ns}$ | 5.0$^{b}$ |
| T1- Papaya +*Colletotrichum* spp. | 5.7$^{b}$ | 10.2$^{ns}$ | 0.064$^{b}$ | 1.1$^{ns}$ | 5.1$^{b}$ |
| T2- Papaya + *Colletotrichum* spp. + 10% Tween 20 | 5.7$^{b}$ | 10.8$^{ns}$ | 0.066$^{b}$ | 1.2$^{ns}$ | 5.3$^{b}$ |
| T3 - Extract at concentration of 2000 mg $L^{-1}$ | 5.8$^{a}$ | 10.7$^{ns}$ | 0.054$^{c}$ | 1.1$^{ns}$ | 5.3$^{b}$ |
| T4 - Extract at concentration of 3000 mg $L^{-1}$ | 5.8$^{a}$ | 10.4$^{ns}$ | 0.048$^{c}$ | 1.0$^{ns}$ | 5.9$^{a}$ |
| T5 - Extract at concentration of 4000 mg $L^{-1}$ | 5.9$^{a}$ | 10.6$^{ns}$ | 0.054$^{c}$ | 0.9$^{ns}$ | 5.7$^{a}$ |
| T6 - Extract at concentration of 5000 mg $L^{-1}$ | 5.8$^{a}$ | 10.9$^{ns}$ | 0.068$^{b}$ | 1.1$^{ns}$ | 5.6$^{a}$ |
| T7 - Extract at concentration of 6000 mg $L^{-1}$ | 5.3$^{c}$ | 10.5$^{ns}$ | 0.094$^{a}$ | 1.3$^{ns}$ | 5.8$^{a}$ |
| Mean | 5.7 | 10.6 | 0.064 | 1.1 | 5.4 |
| CV (%) | 1.3 | 4.8 | 11.28 | 27.1 | 8.8 |

Equal letters in the same column did not differ statistically by Scott-Knott test at 5% probability level, SS: Soluble Solids, TA: Titratable acidity, ns: not significant, control: fruit not inoculated.

Papaya fruits treated with higher concentration of aqueous extract, T7, had lower pH (5.3) and higher percentage of acidity (0.094%). These observed values may be a consequence of the acceleration of some metabolic reactions that occur during fruit ripening, with consequent production of galacturonic acid. This inference is in agreement with Costa and Balbino (2002), who state that the increase in titratable acidity in papaya pulp can be attributed to a possible synthesis of galacturonic acid during ripening, resulting from the process of hydrolysis of pectin by the enzymes pectin methylesterase and polygalacturonase.

The values of pH and titratable acidity obtained by Batista *et al.* (2020) for 'Golden THB' papaya fruits coated with clove essential oil at 0.175 mL $L^{-1}$ were close to those obtained in the present study, 5.59 and 0.08%, respectively. Silva *et al.* (2017) also obtained similar values for titratable acidity, between 0.08 and 0.09%, in commercial fruits of the same variety destined for the domestic market and export.

It was observed that leaf extract concentrations equal to 3000 mg $L^{-1}$ or higher promoted greater mass loss in the fruits at the end of the evaluation period (Table 3). According to Blum *et al.* (2008), papaya fruits have on their surface waxes that reduce water loss, which leads to the inference that the natural protective layer of the fruits had its composition altered when exposed to the high concentrations of the extract. Another fact to be considered is that papaya has a low-density epidermis, which contributes to the mass loss by transpiration (Demartelaere *et al.*, 2015).

Silva *et al.* (2017), evaluating papaya fruits of the 'Golden THB' variety, without treatment, kept at ambient temperature, observed fresh mass loss of about 3.9%. This value is lower than that obtained in the present study, because the time of evaluation was only five days.

## CONCLUSIONS

The aqueous fraction of papaya leaf extract at a concentration of 2000 mg L-1 was the most efficient for the in vitro control.

Tween 20, associated or not with aqueous extract, reduces the appearance of anthracnose symptoms in fresh papaya fruits.

The different concentrations of aqueous extract applied in papaya fruits do not interfere in their physicochemical quality.

**Acknowledgments**

To the Brazilian Agricultural Research Corporation (Embrapa) for the financial support to the project and to the Bello Fruit company for the donation of papaya fruits.

## REFERENCE

ALI, A.; WEE PHENG, T.; MUSTAFA, M. A. Application of lemongrass oil in vapour phase for the effective control of anthracnose of 'Sekaki' papaya. **Journal of applied microbiology**, v. 118, n. 6, p. 1456-1464, 2015.

ALORKPA, E. J. et al. Phytochemical screening, antimicrobial and antioxidant properties of assorted *Carica papaya* leaves in Ghana. . **J Med Plant Stud**, 4(6): 193–198, 2016.

BASAK, S.; GUHA, P. Betel leaf (*Piper betle* L.) essential oil microemulsion: Characterization and antifungal activity on growth, and apparent lag time of *Aspergillus flavus* in tomato paste. **LWT**, v. 75, p. 616-623, 2017.

BASKARAN, C. et al. The efficacy of *Carica papaya* leaf extract on some bacterial and a fungal strain by well diffusion method. **Asian Pacific Journal of Tropical Disease**, v. 2, p. S658-S662, 2012.

BATISTA, D. V. S. et al. Edible coatings in post-harvest papaya: impact on physical–chemical and sensory characteristics. **Journal of Food Science and Technology**, v. 57, n. 1, p. 274-281, 2020.

BAUTISTA-BAÑOS, S. et al. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloeosporioides* of papaya and mango fruit after storage. **Revista Mexicana de Fitopatología**, v. 20, n. 1, p. 8-12, 2002.

BAUTISTA-BAÑOS, S. et al. Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. **Crop protection**, v. 22, n. 9, p. 1087-1092, 2003.

BAUTISTA-BAÑOS, S. et al. A review of the management alternatives for controlling fungi on papaya fruit during the postharvest supply chain. **Crop Protection**, v. 49, p. 8-20, 2013.

BIRTH, G. S. et al. An optical method for estimating papayas maturity. **Journal of the American Society for Horticultural Science**, v. 109, n. 1, p. 62-66, 1984.

BLUM, J. et al. Uso de cera na conservação pós-colheita do caqui cv. Giombo. **Revista Brasileira de Fruticultura**, v. 30, n. 3, p. 830-833, 2008.

CHÁVEZ-QUINTAL, P. et al. Antifungal activity in ethanolic extracts of *Carica papaya* L. cv. Maradol leaves and seeds. **Indian journal of microbiology**, v. 51, n. 1, p. 54-60, 2011

COSTA, A.F.S.; BALBINO, J. M. S. Características da fruta para exportação e normas de qualidade. In: FOLEGATTI, M. I. S.; MATSUURA, F. C. A. U. (Eds.). **Mamão**: pós–colheita. Brasília, DF: Embrapa Informação Tecnológica, 2002.

DEMARTELAERE, A. C. F. et al. Elicitors on the control of anthracnose and post-harvest quality in papaya fruits. **Pesquisa Agropecuária Tropical**, v. 47, n. 2, p. 211-217, 2017.

DOMINGUES, F. C. et al. The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* Rut C-30. **Enzyme and Microbial Technology**, v. 26, n. 5-6, p. 394-401, 2000.

DRAETTA, I. D. S. et al. Transformações bioquímicas do mamão (*Carica papaya* L.) durante a maturação. **Coletânea do Instituto de tecnologia de Alimentos, Campinas**, v. 6, n. 2, p. 395-408, 1975.

EFRI, E. et al. The Influence of Papaya Leaf Extract to Anthracnose Disease in Papaya Fruit. **Annual Research & Review in Biology**, v. 34, n. 3, 2019.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011.

IKRAM, E. H. K. et al. Phytochemicals of papaya and its traditional health and culinary uses–A review. **Journal of Food Composition and Analysis**, v. 41, p. 201-211, 2015.

IAL-INSTITUTO ADOLFO LUTZ. Normas Analíticas do Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. 2008.

JARVIS, W. R. Latent infections in the pre-and postharvest environment. **HortScience**, v. 29, n. 7, p. 749-751, 1994.

KAD, D. R.; TAMBE, V. S. Phytochemical screening and evaluation of platelet stimulating activity of *Carica papaya* leaf ethanolic extract. **Plant Sci Open Acc J**, v. 1, n. 1, p. 1-5, 2018.

MADJOS, G. G.; LUCEÑO, A. M. Comparative cytotoxic properties of two varieties of *Carica papaya* leaf extracts from Mindanao, Philippines using Brine Shrimp Lethality Assay. **Bulletin of Environment, Pharmacology and Life Sciences**, v. 8, n. 2, p. 113-118, 2019.

MARTINEZ, J. et al. Controlling anthracnose by means of extracts, and their major constituents, from *Brosimum rubescens* Taub. **Biotechnology Reports**, v. 25, p. e00405, 2020.

SILVA, K. S. et al. Patogenicidade causada pelo fungo *Colletotrichum gloeosporioides* (Penz) em diferentes espécies frutíferas. **Revista Brasileira de Fruticultura**, v. 28, n. 1, p. 131-133, 2006.

POH, K. B.; JIEN, P. W. Characterization of Phytochemical Compounds and Antimicrobial Activity of Crude Alkaloid from Papaya (*Carica papaya* L. var. Eksotika) Leaf Extract. **Pertanika Journal of Tropical Agricultural Science**, v. 40, n. 4, 2017.

RIBEIRO, J. G.; SERRA, I. M. R. S.; ARAÚJO, M. U. P. Uso de produtos naturais no controle de antracnose causado por *Colletotrichum gloeosporioides* em mamão. **Summa Phytopathologica**, v. 42, n. 2, p. 160-164, 2016.

SANTOS, P. S.; PIRES, M. F. C. Avaliação in vitro da atividade antifúngica de óleos essenciais sobre *Cryptococcus neoformans*. **BEPA. Boletim Epidemiológico Paulista (Online)**, v. 10, n. 113, p. 17-18, 2013.

SHANER, G.; FINNEY, R. E. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. **Phytopathology**, v. 67, n. 8, p. 1051-1056, 1977.

SILVA, W. et al. Dimensionamento amostral para frutos de mamoeiro 'Golden THB' destinados ao mercado nacional e à exportação. **Revista Agro@ mbiente On-line**, v. 11, n. 2, p. 128-136, 2017.