

Alternative methods for angular stain control in mangoes (*Mangifera indica* L.)

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Abstract

The conventional methods used for angular stain control are generally chemical methods, however the use of these products can cause high environmental impact and damage to consumer health if it is used in large quantities and undiluted and applied correctly. Based on this problem, this work aimed to evaluate in vitro alternative forms of control using *Saccharomyces* yeast (with probiotic potential), ethanolic extracts of *Mauritia flexuosa* (Buriti) and *Miconia albicans* (Cinnamon-old) plants. To evaluate four GRAS substances in angular leaf spot control caused by *Xanthomonas campestris* pv. *Mangifera indica*, during the postharvest period in mangoes. In vitro results using antagonist yeasts showed no inhibitory effect against *X. campestris*. However, the extracts of the plants *Miconia albicans* and *Mauritia flexuosa* showed a significant inhibition. Thus, as two GRAS substances, 1%, 1.5% and 3% sodium carbonate and 3% sodium bicarbonate inhibited *X. campestris* growth 100%. Given the results obtained, the plant extracts and the GRAS substances tested were effective in controlling phyto bacteria and proved to be an alternative in controlling angular leaf spot, thus avoiding economic losses during the mango postharvest phase.

Keywords: GRAS Substances; *Miconia albicans*; *Mauritia flexuosa*; Postharvest; *Xanthomonas campestris*.

Resumo

Os métodos convencionais utilizados para o controle de manchas angulares são geralmente métodos químicos, porém o uso desses produtos pode causar alto impacto ambiental e danos à saúde do consumidor se utilizado em grandes quantidades e sem diluição e aplicado corretamente. Com base neste problema, este trabalho teve como objetivo avaliar formas alternativas de controle in vitro utilizando levedura *Saccharomyces* (com potencial probiótico), extratos etanólicos de plantas *Mauritia flexuosa* (Buriti) e *Miconia albicans* (Canela-velha). Avaliar quatro substâncias GRAS no controle da mancha angular causada por *Xanthomonas campestris* pv. *Mangifera indica*, durante o período de pós-colheita em mangas. Os resultados in vitro usando leveduras antagonistas não mostraram efeito inibidor contra *X. campestris*. No entanto, os extratos das plantas *Miconia albicans* e *Mauritia flexuosa* apresentaram inibição significativa. Assim, como duas substâncias GRAS, 1%, 1,5% e 3% de carbonato de sódio e 3% de bicarbonato de sódio inibiram o crescimento de *X. campestris* em 100%. Diante dos resultados obtidos, os extratos vegetais e as substâncias GRAS testadas foram eficazes no controle de fitobactérias e mostraram-se uma alternativa no controle da mancha angular, evitando perdas econômicas na fase pós-colheita da manga.

Palavras-chave: Substâncias GRAS; *Miconia albicans*; *Mauritia flexuosa*; Pós-colheita; *Xanthomonas campestris*.

Resumen

Los métodos convencionales usados para el control de manchas angulares son generalmente métodos químicos, sin embargo, el uso de estos productos puede causar un alto impacto ambiental y dano a la salud del consumidor si se usa en grandes cantidades y sin diluir y se aplica correctamente. Con base en este problema, este trabajo tuvo como objetivo evaluar formas alternativas de control in vitro utilizando levadura *Saccharomyces* (con potencial probiótico), extractos etanólicos de plantas de *Mauritia flexuosa* (Buriti) y *Miconia albicans* (Cinnamon-old). Evaluar cuatro sustancias GRAS en el control de la mancha angular de las hojas causadas por *Xanthomonas campestris* pv. *Mangifera indica*, durante el período de poscosecha en mangos. Los resultados in vitro utilizando levaduras antagonistas no mostraron ningún efecto inhibitorio contra *X. campestris*. Sin embargo, los extractos de las plantas *Miconia albicans* y *Mauritia flexuosa* mostraron una inhibición significativa. Así, como dos sustancias GRAS, 1%, 1,5% y 3% de carbonato de sodio y 3% de bicarbonato de sodio inhibieron el 100% del crecimiento de *X. campestris*. Dados los resultados obtenidos, los extractos de plantas y las sustancias GRAS ensayadas resultaron efectivas en el control de fitobacterias y demostraron ser una alternativa en el control de la mancha angular de la hoja, evitando así pérdidas económicas durante la fase de poscosecha del mango.

Palabras clave: Sustancias GRAS; *Myconia albicans*; *Mauritia flexuosa*; Postcosecha; *Xanthomonas campestris*.

1. Introduction

Brazil is the world's seventh largest mango producer, second only to India, China, Thailand, Indonesia, Mexico and Pakistan (Fao, 2018). According to Palmieri (2018), Brazil in 2018 harvested about 57 thousand hectares of mango, where the largest production is concentrated in the regions: Northeast (73%) and Southeast (27%).

Mango is appreciated worldwide for its color and taste as well as its nutritional value. The fruit is rich in carotenoids, vitamin C, phenolic compounds, minerals, soluble fibers and has high antioxidant activity (Tonin et al., 2018). Although much appreciated for its nutritional and sensory aspects, it is highly perishable and requires care for its conservation and commercialization (Bezerra et al., 2010). About 30% of its production is lost during the storage process before reaching the end consumer (Xavier et al., 2009).

Angular spot is one of the main postharvest diseases that cause losses during this phase. It is caused by the bacterium *Xanthomonas campestris* PV *Mangifera indica*, being a disease that attacks branches, leaves, inflorescences and fruits of the hose, usually in prolonged humid periods. The symptoms in the fruits are dark lesions with yellowish halo and soggy tissue. The penetration of the bacteria occurs in the fruit through lesions and natural openings. Environmental conditions such as high temperature and humidity are favorable conditions for the disease, as well as strong winds for causing damage to the fruits (Batista and Barbosa, 2008).

The main control measures for this disease include use of resistant cultivars, crop treatments during the cultivation period, use of bactericides as copper-based compounds. However, these measures have limited effectiveness, with only a few commercially available bactericides and the additional issue that copper compounds are toxic and affect the environment (Spago et al., 2014).

Thus, there is a global trend towards safer and more environmentally friendly alternative approaches to controlling postharvest diseases (Dukare et al., 2018). Among these strategies there is the use of biological control, natural antimicrobial substances and the application of Generally Recognized As Safe (GRAS) substances has been studied and good results have been demonstrated (Spadaro and Droby, 2016).

Biological control using yeasts is a promising and efficient strategy for postharvest fruit control, based on some special advantages, such as: yeasts have fast colonization and are tolerant or compatible with abiotic stress generated by chemicals and pesticides. Besides these microorganisms are naturally present on the fruit surfaces (Ferreira et al., 2018; Spadaro and Droby 2016; Hu et al., 2017; Wisniewski et al., 2016).

Other studies have been developed and directed to the discovery of new antimicrobial agents from plant extracts and other natural products, aiming to discover compounds with activity compared to those traditionally used, but with lower

toxicity, lower environmental impact and greater efficacy against the resistance of phytopathogenic microorganisms (Bona et al., 2014). For Medeiros et al. (2013), the use of plant extracts that have direct antimicrobial action is a promising alternative for pesticide substitution, besides being an ecologically correct attitude and fitting in the integrated management of plant diseases.

The use of GRAS substance may be another viable control alternative, these chemical compounds may have antimicrobial effects and are used as food additives and are considered safe for human consumption. In addition to being used in combination with biological agents, helping biocontrol agents in their antagonistic action in diseases associated with fruits (Ferreira et al., 2018; Palou et al., 2016; Geng et al., 2011; Pimenta et al., 2010).

Given the above the objective of this work was to propose alternative methods to control the angular leaf spot caused by *Xanthomonas campestris* in vitro using biological control through yeast, natural antimicrobial substances obtained from plants and application of GRAS substances.

2. Methodology

The experimental work was carried out at the general and applied microbiology laboratory of the federal university of Tocantins, at Palmas campus (to), Brazil.

2.1 Obtainments of microorganisms

The bacterium *Xanthomonas campestris* PV *Mangifera indica* was kindly provided by the Laboratory of Biotechnology, Food and Product Analysis of Gurupi Tocantins State UFT/ TO, Brazil.

S. cerevisiae yeasts UFT 186, UFT 5978 and UFT 5918 were obtained from the Carlos Augusto Rosa microbial culture collection from the General and Applied Microbiology and Environmental Microbiology and Biotechnology Laboratories of the Federal University of Tocantins - UFT, Brazil. Reactivation and preparation of cell concentration of Microorganisms

2.2 *Xanthomonas campestris* pv. *Mangifera indica*

X. campestris was reactivated in plates containing YMA medium (1% glucose, 0.5% peptone, 0.3% malt extract, 2% agar, 0.3% yeast extract) and incubated for up to 72h at 37° C. After this period the bacteria were replicated to the YM broth and incubated under the same growth conditions previously described (Silveira et al., 2001 with modifications). Subsequently, Gram staining was performed to observe the purity of the culture (Tortora et al., 2012).

To obtain biomass, *X. campestris* was grown in 100 mL YM broth at 37°C for up to 48h. After growth period was added to the fermenter (SOLAB) containing 3L of YM broth, rotating at 150 rpm, at 37°C for 48 h to obtain bacterial biomass.

After obtaining the biomass the bacterial cell concentration was adjusted to 1.5×10^6 cells/mL in 5mL of 0.85% saline. This adjustment was made comparing with the Mac farland Scale (0.5 corresponding to 1.5×10^8 CFU/ml) and further diluted to such concentration above.

2.3 *Saccharomyces cerevisiae* yeasts

Strains of *S. cerevisiae* UFT-186, *S. cerevisiae* UFT-5978 and *S. cerevisiae* UFT-5918 yeasts were grown in a petri dish containing YMA medium plus chloramphenicol 100 mg/L for 48h at 35°C. After this period, a loop was taken from each colony and transferred to new plates containing the same culture medium and stored under the same growth conditions to obtain biomass. Subsequently, the plates containing the yeast biomass were scraped off and added into a falcon tube containing

5 mL of 0.85% saline. To calculate the number of cells, a Neubauer Chamber was used and the concentration was adjusted to approximately 1.5×10^8 cells / mL (Silva, 2013).

2.4 Obtaining extracts of the plants *Miconia albicans* and *Mauritia flexuosa*

For plant identification, exsiccates of each species were obtained and deposited in the Tocantins Herbarium (HTO) located at the NEAMB (Center for Environmental Studies), Federal University of Tocantins, National Harbor Campus, under the following registry numbers HTO 10.952 *Mauritia flexuosa* (Buriti) and HTO 12017 *Miconia albicans* (Old Cinnamon).

The ethanolic extracts were obtained from the leaves, where they were separated and cut into smaller pieces with the aid of a sterilized stainless-steel knife. The selected leaves were dried in a circulating oven at 40 to 45°C for 48h. After cooling, the material was ground in a domestic processor and the samples stored in sterile bags under refrigeration (Oliveira et al., 2016).

To obtain the crude extracts, the Soxhlet apparatus was used, which weighed about 10 to 20 grams of the dried leaves and ground in a precision analytical balance and placed in two cellulose cartridges with 250 mL of ethyl alcohol (98 °GL). Upon completion of the process the solvent was removed by rotary evaporation at reduced pressure and a temperature of 50°C (Oliveira et al., 2016). The extracts obtained were stored in sterile vials and placed in a hood until completely evaporated, then sealed and stored under refrigeration.

2.5 Verification of growth inhibition of *Xanthomonas campestris* pv. *Mangifera indica* using antagonistic yeasts.

Agar diffusion test

Pairing Test

After reactivation and adjustment of antagonist concentrations, paired cultures were tested to verify the antagonistic potential of yeasts against phyto-bacteria *X. campestris* by observing the inhibition zone (Christensen, 1996 apud Souza, 2016 with adaptations). The experiment was divided into two types of treatment: Treatment 1: (viable yeast) and Treatment 2: (chloroform vapor inactivated yeast for 30 minutes).

In treatment 1, antagonist yeasts were streak inoculated on one side of the petri dish then the plates were incubated for 48h at 35°C. The bacteria were then streak-inoculated on the opposite side of the petri dish and incubated at 35°C for 48h. After the incubation period the inhibition of the bacteria by diffusible compounds produced by the yeast was evaluated.

For treatment 2 the yeasts were inoculated on one side of the petri dish and incubated as above, following growth the chloroform vapor inactivation was performed for 30 min. On the opposite side of the plate, the previously prepared bacterial solution was then inoculated and adjusted to a concentration of 1.5×10^6 cells/mL. After inoculation the plates were incubated again at 35°C for an additional 48h in an anaerobic environment. (Silva, 2013 with modifications).

The positive control consisted only of yeast in culture medium without the presence of bacteria and the negative control only of phyto-bacteria. The treatments were performed in triplicate and compared with the positive and negative control.

Diffusion test for "spot"

To perform this test, the recommendations set forth in the CLSI (Clinical Laboratory Standards Manual Institute)/ANVISA (National Health Surveillance Agency) standards were amended. This experiment was divided into two types of treatments, treatment 1: (viable yeast) and treatment 2: (chloroform vapor inactivated yeast for 30 minutes). For treatment 1, after adjusting the cellular concentration of the microorganisms, the suspensions were sown according to the methodology proposed by Souza (2016) where the antagonists were spread with a sterile swab and then inoculated. 50 µL of phyto-bacterium was placed in the center of the plate, after this procedure the plates were incubated at 35°C for 48h.

For treatment 2, antagonists were spread throughout the petri dish and incubated at 35°C for 48h. After culture growth, antagonists were inactivated. Then the *X. campestris* bacteria was inoculated as a spot (50 µL of the solution containing cell concentration of 1.5×10^6 CFU/mL) and the plates were incubated for an additional 48h at 35°C in an anaerobic environment. Positive control consisted only of antagonists and negative only of phyto-bacteria inoculated in culture medium. This test was performed in triplicate and the average diameter of the halos presented was calculated at the end.

2.6 Verification of phyto-bacterial inhibition *X. campestris* using extract of the plants *Miconia albicans* and *Mauritia flexuosa*.

Antimicrobial assays were performed in triplicate by the diffusion method (Clisi, 2009) per well in petri dishes containing 50 mL of YMA agar medium for bacteria. Inoculum solutions were prepared and adjusted. As a negative control, 10% dimethyl sulfoxide (DMSO) solution was used, and 10 µg/mL of copper sulfate solutions for positive control.

Using sterile Swab, the solutions containing the inoculum containing *X. campestris* were sown on the surface of the plates containing YMA culture medium and then 5 mm diameter wells were drilled. These wells were filled with 50 µL of each ethanolic extract obtained from *M. albicans* and *M. flexuosa* diluted in 10% DMSO at concentrations (200, 100 and 50 mg/mL) and with positive and negative controls. After 48h of incubation at 37°C the phyto-bacterial inhibition halos were measured using a digital caliper (Oliveira et al., 2017).

The results of the experiments were conducted in a completely randomized design with two replications per triplicate treatment. The averages of the halos obtained were subjected to analysis of variance and compared to each other using Tukey tests at 5% of significance. Statistical analysis was performed using AgroEstat-System for Statistical Analysis of Agronomic Testing (Barbosa & Maldonado junior, 2015).

2.7 Verification of inhibition of phyto-bacterium *X. campestris* for GRAS substances

Four substances listed by the Food and Drug Administration FDA (1999) were selected for the study: sodium bicarbonate, sodium carbonate, calcium carbonate and potassium chloride. These substances were used in the study because they are already used as food additives and studies have already shown their possible fungistatic and fungicidal effects (Janisiewicz and Conway, 2010).

The bacterium *X. Campestris* was previously reactivated in YMA culture medium and incubated at 37°C for about 48h. Petri dishes containing YMA culture medium supplemented with 1, 3 and 5% of each substance were prepared for the test, except potassium chloride whose concentration was 0.1%, 0.5% and 1%. This is due to the establishment of the maximum limit of supplementation of this substance in foods that is up to 1% (FDA, 1999).

After the phyto-bacteria growth period, cell concentration adjustment was performed obtaining about 1.5×10^8 CFU.mL⁻¹ (Clisi, 2003). It was then inoculated with a sterile swab on plates containing YMA medium plus GRAS at the indicated concentrations. As a positive control, the bacteria were inoculated with a swab in YMA plates without the presence of GRAS substances. The negative control consisted only of plaques with the substances without the inoculum of the bacteria. The plates were incubated in anaerobiosis at 37°C for a period of 48 hours. Phyto-bacterial growth was evaluated and compared with the positive control during the incubation period; each assay was performed from three replicates.

3. Results and Discussion

3.1 Agar diffusion test

Pairing test and diffusion test for spot

In order to verify the production of antagonist substances by paired culture and spot diffusion, the two tests were not efficient in inhibiting phyto-bacteria development, either treatments 1: (viable yeasts) or treatment 2: (chloroform vapor inactivated yeast) for 30 minutes).

The results obtained from paired culture and spot diffusion tests showed that the yeast did not have any antagonistic potential *in vitro* against the bacterium *X. campestris*. After these experiments it was concluded that these yeasts did not present desirable characteristics as biocontrollers for this phyto-bacteria.

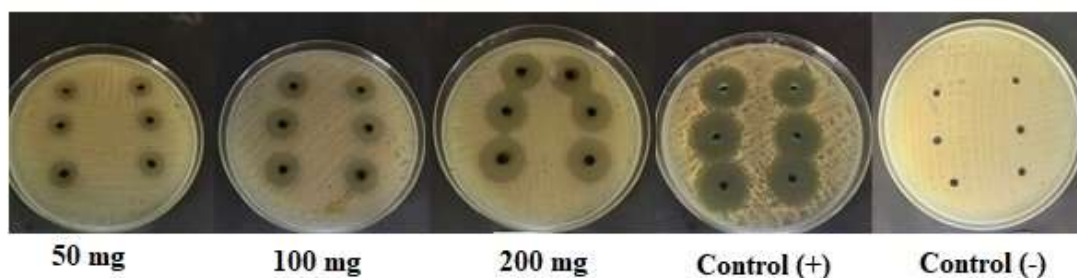
In the literature, no methodology similar to that used in this study was found, based on the use of antagonist yeasts to control *X. campestris*. Only similar studies were found for the control of *filamentous fungi*. Like the study developed by Yinsheng (2016) that obtained similar results using *S. cerevisiae* UFT-186, *S. cerevisiae* UFT-5978 and *S. cerevisiae* UFT-5918 yeasts for the control of *P. roqueforti* growth by the diffusion test. By spotting that viable yeasts did not control fungal growth when compared to the positive control, only less sporulation and change in fungal color was observed, assuming that there was a competition for space between the yeast and the fungus studied. In the treatment using inactivated yeasts, no significant difference was observed in relation to the control, when comparing the color and the growth of the pathogen.

Silva (2013) used *S. boulardii* and *S. cerevisiae* yeasts to verify the antifungal capacity on *Aspergillus parasiticus* growth, and observed that viable yeasts were able to reduce 46.3% and 45.8% respectively compared to control. Inactivated yeasts decreased by 24.9% and 22.6%. This inhibition was not observed in this experiment.

3.2 Verification of inhibition of phyto-bacteria x. *Campestris* using extract of the plants *miconia albicans* and *mauritia flexuosa*

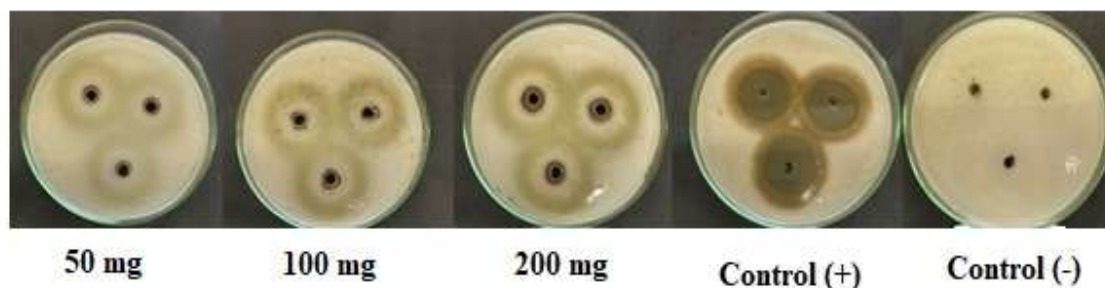
In order to verify phyto-bacterial inhibition, a well diffusion test was performed using the ethanolic extracts of the leaves of the plants *M. albicans* (Old Cinnamon) and *M. flexuosa* (Buriti). The results of the tests are shown in figures 1 and 2. The analysis of variance showed a significant effect on the control of phyto-bacterial growth in relation to plant extracts of the studied species and the concentrations analyzed when compared to the positive control. As the interaction was significant, we proceeded to the results (Table 1).

Figure 1. Growth inhibition of phyto-bacteria *X. campestris* in YMA medium with *Mauritian flexuosa* ethanolic extract at 50, 100, 200 mg/mL concentrations, with positive control, the plates were incubated at 37°C for 48 hours in anaerobiosis.



Source: Authors (2021).

Figure 2. Growth inhibition of phytopathogen *Xanthomonas campestris* in YMA medium in ethanolic extract of *Miconia albicans* at concentrations of 50, 100, 200 mg/mL, with positive control, plates were incubated at 37 °C for 48 hours in anaerobiosis.



Source: Authors (2021).

Table 1. Growth inhibition means of *X. campestris* by the ethanolic extracts of *Mauritia flexuosa* and *Miconia albicans*

EXTRACT (mg/L)	50 (mg)	100 (mg)	200 (mg)	C+
<i>M. flexuosa</i>	13,18 mm ^{bb}	17,75 mm ^{ab}	24,11 mm ^{aA}	22,62 mm ^{bA}
<i>M. albicans</i>	17,05 mm ^{ac}	19,91 mm ^{abC}	22,59 mm ^{aB}	30,07 mm ^{aA}

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ statistically at 5% significance by Tukey test. Source: Authors.

When comparing the ethanolic extracts of the two species within each concentration, there was no significant difference in the concentrations of 100 and 200 mg/L, however in the concentrations of 50 mg/L was significant difference. When compared to the concentrations of the extracts of each species, we observed that for the ethanolic extract of the species *M. flexuosa* and *M. albicans* the concentration of 200 mg/L had the highest value followed by 100 and 50 mg/L (Table 1). The concentration of 200 mg/L of the ethanolic extract of *M. flexuosa* showed a reduction zone bigger of growth of *X. campestris*.

Studies by Kalpana et al. (2016) when testing four leaf extracts of *Euphorbia heterophylla* (wild peanut) and *Tamilnadia uliginosa* revealed that in vitro antibacterial tests showed that the extracts significantly inhibited the growth of *X. axonopodis* pv. *cit*. The best result was obtained for *Tamilnadia uliginosa* Retz ethanolic extract at 1,000 ppm showed an inhibition zone 74% over *X. campestris* pv. *citri*.

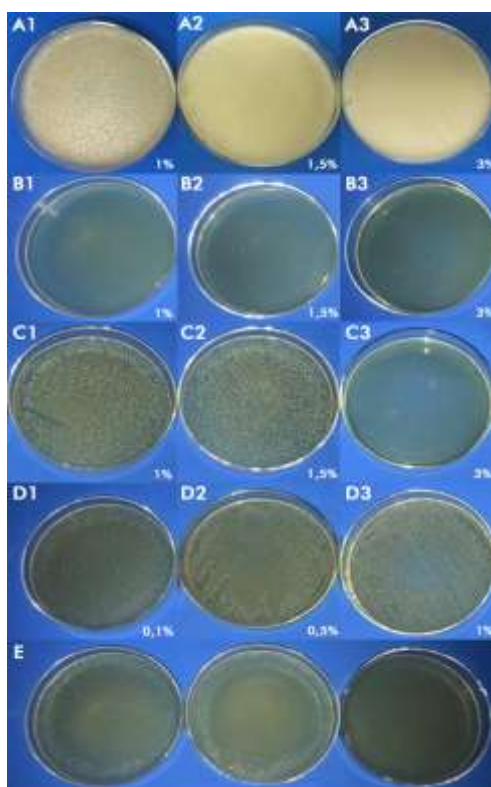
Vieira (2018) by extracting fungi from *Antarctic algae* produced 66 extracts, which were performed antimicrobial tests against three species of *Xanthomonas*, the author observed that 18 extracts had significant inhibitory activity in at least one of the species of *Xanthomonas* to which it was tested. Among the 18 extracts, 9 were positive against *X. euvesicatoria*, 9 were positive against *X. axonopodis* pv. *passiflorae* and 16 showed activity against *X. citri* subsp. *citri*, all with inhibition greater than or equal to 90% in vitro.

According to Palou et al. (2016) extracts obtained from medicinal or exotic plants from Africa, Asia or South American countries such as Brazil are compounds that may have antimicrobial activity and also some ability to retard maturation and prolong the life of fruit shelf and other treated products. Typically, these compounds are secondary metabolism products produced by the plant for its own protection against pests and pathogens and have been used for their significant activities against the most important phytopathogens for postharvest disease control. (Ruiz et al., 2016; Nicosia et al., 2016; Obagwu and Korsten, 2003; Mekbib et al., 2009 and Barrera Necha et al., 2003).

3.3 Verification of inhibition of phyto bacteria *X. campestris* for GRAS substances

The sensitivity test for GRAS substances (sodium bicarbonate, sodium carbonate, calcium carbonate and potassium chloride) showed that sodium bicarbonate at 3% and sodium carbonate at 1%, 1.5% and 3% were able to inhibit *X. campestris* growth by 100% (Figure 3). The other GRAS substances tested did not achieve satisfactory bacterial growth reduction.

Figure 3. Effect of different GRAS substances at different concentrations on growth of *Xanthomonas campestris* after 48 h incubation at 37°C. (A1, A2, A3) Calcium carbonate and their concentrations (1%, 1.5% and 3%); (B1, B2, B3) Sodium carbonate and their concentrations (1%, 1.5% and 3%); (C1, C2, C3) Sodium bicarbonate and their concentrations (1%, 1.5% and 3%); (D1, D2, D3) Potassium chloride and their concentrations (0.1%, 0.5% and 1%) and (E) Positive control.



Source: Authors (2021).

No similar research was found using GRAS substances to control bacteria of the species *Xanthomonas* or any other species. However, the literature shows the same substances being used to control other microorganisms. Like the research developed by Yinsheng (2016), in which the author tested *P. roqueforti* fungus for growth sensitivity in medium containing GRAS substances, and observed that sodium bicarbonate and sodium carbonate inhibited 100% growth of *P. roqueforti* at concentrations of 1%, 3% and 5%, similar results to those presented in this paper.

Ferreira et al. (2018) also analyzed the susceptibility of *Colletotrichum gloeosporioides* to GRAS substances (sodium carbonate, calcium carbonate, sodium bicarbonate, calcium chloride, potassium chloride), and observed that only carbonate and sodium bicarbonate were able to significantly inhibit phytopathogen growth at all concentrations tested. On the other hand, calcium chloride, calcium carbonate and potassium chloride did not inhibit fungal growth at any of the evaluated concentrations, corroborating the results obtained in this work and demonstrating that these substances have antimicrobial power.

Sodium bicarbonate and sodium carbonate showed good efficiency in controlling growth *X. Campestris*, being sodium carbonate effective in all concentrations used. However, sodium bicarbonate proved to be the best strategy for the control of *X. campestris*, as it is already a substance studied for the control of several phytopathogenic fungi and has the purpose of increasing the fruit preservation period, besides being easily accessible and inexpensive when compared to other GRAS substances (Bru et al., 2013; Lai et al., 2015; Fallanaj et al., 2016).

Alternative methods for reducing postharvest diseases such as biological control, GRAS substances and plant extracts that have antimicrobial properties have been evaluated against several important postharvest fruit pathogens in temperate, subtropical and tropical climate (Palou et al., 2016). With this research work, it was possible to identify probable antimicrobial compounds with potential for the development of non-polluting chemical alternatives suitable for use in the control of angular spot caused by *X. campestris* in mangoes.

4. Conclusion

Yeasts evaluated as antagonists were not efficient in controlling phyto-bacteria *X. campestris*. However, the extracts of the plants *Miconia albicans* and *Mauritia flexuosa* showed good control efficiency, and the extract obtained from *M. flexuosa* presented the best phyto-bacterial inhibition. Regarding the GRAS substances applied, only sodium carbonate and sodium bicarbonate were able to inhibit *X. campestris*. Sodium carbonate obtained results in the three concentrations tested and sodium bicarbonate only in the highest concentration of 3%. The results obtained from *Miconia albicans* and *Mauritia flexuosa* plant extracts and GRAS substances showed promise in controlling the angular leaf spot produced by *X. campestris*. However, more future studies need to be carried out to elucidate the antibacterial action mechanisms performed by the GRAS substances and the extracts of the tested plants.

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