Evaluation of soapberry (*Sapindus saponaria L.*) leaf extract against papaya anthracnose

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ABSTRACT

Marinho, G.J.P.; Klein, D.E.; Siqueira Junior, C.L. Avaliação de extrato foliar de saboeiro (*Sapindus saponaria* L.) contra anthracnose em frutos de mamoeiro. ***Summa Phytopathologica,*** v.44, n.2, p.127-131, 2018.

Chemical fungicides provide the primary means to control fungal infection in fruits and vegetables in the postharvest. Exploitation of natural products to control decay and prolong the storage life of perishables has received more attention. In this study, hydroethanolic extracts from the leaves of soapberry (*Sapindus saponaria L.*) were investigated for their inhibitory activity against the fungus *Colletotrichum gloeosporioides*, the causal agent of anthracnose in papaya fruits. To evaluate the antifungal activity of the plant extract, the latter was incorporated into potato-dextrose-agar (PDA) medium at different concentrations (10, 50 and 100 mg mL^{-1}), and mycelial growth inhibition, spore production inhibition and morphological changes were evaluated at room temperature after 7 days. Efficacy of the extract was noted when it was used at 50 mg mL^{-1} and 100 mg mL^{-1}, resulting in a reduction in the mycelial growth ($P< 0.05$). At 50 mg mL^{-1}, the extract causes morphological changes by reducing the size of hyphae, which become shorter and more branched. At 100 mg mL^{-1} extract, damages in the hyphal structure were more severe, causing mycelial rupture. *In vivo* treatment with 100 mg mL^{-1} extract prevented the development of anthracnose symptoms in fresh papaya fruits. Soapberry leaf extract has strong inhibitory activity against *C. gloeosporioides*, preventing the mycelial growth and reducing the appearance of anthracnose symptoms. Taken together, these results demonstrated the potential of this extract as a natural fungicide, constituting an alternative measure for disease control in papaya fruits.

Keywords: postharvest; plant extract; *Colletotrichum gloeosporioides*

RESUMO

Marinho, G.J.P.; Klein, D.E.; Siqueira Junior, C.L. Evaluation of soapberry (*Sapindus saponaria* L.) leaf extract against papaya anthracnose. ***Summa Phytopathologica,*** v.44, n.2, p.127-131, 2018.

Os fungicidas químicos fornecem meios primários para o controle da infecção fúngica de frutas e legumes no período pós-colheita. A exploração de produtos naturais para controlar a deterioração e prolongar o tempo de armazenamento de alimentos perecíveis tem recebido mais atenção. Neste trabalho, extratos hidroetanólicos de folhas de saboeiro (*Sapindus saponaria L.*) foram analisados quanto a sua atividade inibitória contra o fungo *Colletotrichum gloeosporioides*, agente causador da antracnose em frutos de mamoeiro. Para avaliar a atividade antifúngica do extrato vegetal, este foi incorporado em meio de batata-dextrose-agar (BDA) à diferentes concentrações (10, 50 e 100 mg mL-1), e a inibição do crescimento micelial, inibição de produção de esporos e mudanças morfológicas foram avaliadas à temperatura ambiente após sete dias. A eficácia do extrato foi observada quando foram utilizados 50 mg mL-1 e 100 mg mL-1 do extrato, resultando em redução de crescimento micelial ($P <0.05$). À 50 mg mL-1, o extrato causa alterações morfológicas com a redução do tamanho das hifas que se tornam mais curtas e mais ramificadas. À 100 mg mL-1 de extrato, os danos na estrutura da hifa foram mais severos, causando ruptura micelial. O tratamento *in vivo* com 100 mg mL-1 de extrato impediu o desenvolvimento de sintomas de antracnose em frutos de mamão frescos. O extrato de folha de saboeiro tem uma forte atividade inibitória contra *C. gloeosporioides*, impedindo o crescimento micelial e reduzindo o aparecimento de sintomas de antracnose. Em conjunto, estes resultados demonstraram o seu potencial do extrato como fungicida natural constituindo uma medida alternativa para o controle da doença em frutos de mamoeiro.

Palavras-chave: pós-colheita; extrato vegetal; *Colletotrichum gloeosporioides*

Carica papaya L. is a member of Caricaceae family, which has only four native genera. This species occurs in tropical and sub-tropical regions of American and African countries (9). According to FAOSTAT (10), papaya production represents 10% of the world production of tropical fruits. Brazil stands as the second largest producer after India. But the main factors limiting papaya exportation are postharvest diseases, particularly anthracnose caused by the fungus *Colletotrichum gloeosporioides (Penz) Sacc.* (18). In the absence of control forms, the incidence of anthracnose rises to 91% in fruits after harvest (14). In papaya, as well as in other fruits and vegetables, anthracnose results in serious yield losses and occasionally in damage to the stems and foliage. Circular sunken necrotic lesions develop in mature fruits of all sizes, and multiple lesions are often formed in individual fruits after harvest (2). Control of this disease is still usually obtained with prochloraz or propiconazole application (19), hot water dip treatment (13) and hot water dip treatment in combination with fungicides (12); more recently, treatment with gamma irradiation has been shown efficient, preventing the development of anthracnose in papaya fruits (8). However, the use of some synthetic fungicides, such as N-tricloromethiltio-4-ciclohexeno-1,2-dicarboximida and mancozeb, to control fungal diseases in food commodities is restricted due to their high and acute toxicity, long degradation periods and environmental pollution. In addition, there are concerns over the increasing efficacy loss of conventional fungicides due to pathogen resistance (26). Alternative methods such as the use of natural compounds from plant extracts may be a tool to control phytopathogenic fungi and bacteria because they constitute a rich source of bioactive chemicals (1, 3). Palhano et al. (20) suggested the use of high hydrostatic pressure combined with citral or lemongrass essential oil as an efficient way to inhibit the germination of *C. gloeosporioides* spores. Moreover, the antifungal and antibacterial effects of other plant extracts such as garlic (16), basil (15), plantain and pitomba (25) have been described. Plants of the Sapindaceae family have been described for their antimicrobial effects against some pathogens like *Sapindus mukorossi*, which has antimicrobial activity against *Helicobacter pylori* (11), and *Sapindus emarginatus,* which presents strong inhibitory activity against *Staphylococcus epidermidis*, *Proteus morganii* and *Bacillus subtilis* (17). In this study, *Sapindus saponaria* leaf extract was screened for its potential antifungal activity against the causal agent of anthracnose in papaya fruits.

MATERIALS AND METHODS

Microorganisms and Growth Conditions

The microorganisms used in these tests were obtained from 10-day cultures in PDA medium. The fungus *C. gloeosporioides* (IB 18/85), from culture collections, was kindly provided by the Bacteriology Laboratory of Plant Biology Institute of Campinas, Brazil.

Plant Material and Preparation of Crude Extracts

Leaves of soapberry (*S. saponaria*) plants were randomly collected during spring and summer in 2010 and 2012 from São João da Barra, Brazil, and were authenticated by Dr. Laura J. M. Santiago, Department of Botany, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. A voucher specimen of the plant was deposited at UNIRIO herbarium (HUNI 1375). Leaves were washed under running tap water and 10 g were ground and extracted with 30 mL water:ethanol (1:3) solution for 7 days. The crude extract was filtered through Whatman filter paper no.1 and the solvent was removed by rotary evaporation. The residual leaf extract was stored in a refrigerator at 4ºC until used in antimicrobial activity assays.

Antifungal Activity

Inhibitory activity of soapberry leaf extract was evaluated based on the methodology described by Ribeiro & Bedendo (21) with some modification. Different concentrations of the extract (10, 50, 100 mg mL^{-1}) were mixed in Petri dishes containing PDA medium at 45 °C in a total volume of 20 mL / dish. Plates containing only PDA medium (20 mL), without plant extract, were used as negative control. All plates, including control ones, were inoculated in the center of agar discs with the tested fungi (5mm diameter). Three replicates of each concentration and control were incubated at 25 ºC ± 2 during 12 h light and 12 h dark photoperiod. After incubation for 7 days, the radial growth was measured. The experiment was conducted three times and the result was expressed as mycelial growth inhibition activity (%).

Spore Production Inhibition Assay

Based on the methodology described by Siqueira-Junior et al. (24), all tested Petri dishes described above were maintained for three additional days under the same experimental conditions for spore production. At the end of the total incubation period (10 days), five agar discs (5mm diameter) of mycelia were removed from each tested plate, including control. The agar discs were immersed in 5-ml potato dextrose agar medium and stirred until spores were separated. Samples (50 μL) were examined under a light microscope to determine the number of spores/mL from each treatment, using a Neubauer hemocytometer. Fungal sporulation with regard to the concentrations of extracts, compared to control, is presented as percentage inhibition.

Influence of Soapberry Extract on Mycelial Morphology

Morphological changes in the mycelium, caused by the action of extracts, were evaluated based on a modification of the method described by Chen & Die (7). Briefly, mycelial discs (5mm diameter) from 10-day-old cultures of *C. gloeosporioides* were placed in baffled shaker flask (50 mL) containing potato dextrose broth (PDB) in addition to soapberry extract (10, 50, 100 mg mL^{-1}); then, they were incubated at room temperature in a orbital shaker incubator (ES-20, Biosan). Negative control contained only potato dextrose broth and mycelial disc. After 7 days, any changes in the morphology of the mycelia were observed under a light microscope. All treatments were performed in triplicate.

Effect of Soapberry Leaf Extract on Inoculated Fruits

Papaya anthracnose caused by *C. gloeosporioides* was used as a model system for *in situ* studies following the methodology described by Siqueira-Junior et al. (24). Papaya fruits in the stage of more yellow than green coloration were purchased in the local market. Sixty papaya fruits were surface sterilized with 70% ethanol bath for 5 min, and each fruit was injured (0.5cm depth and 0.2cm diameter) at three sites on the surface. Thirty injured fruits were then dipped in 100 mg mL^{-1} soapberry leaf extract solution for 5 min. Control treatment consisted of dipping thirty inoculated fruits in extraction solution (water:ethanol (1:3)) for 5 min. Fruits were air dried for 15 min after treatments and then inoculated with 20 uL *C. gloeosporioides* spore suspension (2 x 10^4 spores). All fruits were incubated for 7 days at 28 ºC. At the end of the incubation period, lesions produced as anthracnose symptoms were analyzed.

Statistical Analysis

Analysis of variance (ANOVA) was carried out with the statistical software ASSISTAT for Microsoft Windows version 7.7 beta (Federal University of Campina Grande) (22), and statistical significance was set to the level of $P< 0.05$. Replicate averages with similar results were analyzed together. Averages and standard errors were calculated and reported for each experiment. When averages were significantly different, a comparison was performed by using Tukey's test ($P< 0.05$).

RESULTS AND DISCUSSION

This study was conducted to assess the antifungal efficacy of soapberry leaves against *C. gloeosporioides*. Results revealed that the crude extract of soapberry leaves has inhibitory efficacy against *C. gloeosporioides* in a concentration dependent manner ($p < 0.05$). Concentrations lower than 50 mg mL^{-1} did not affect the growth of fungi, but fungal mycelial *in vitro* growth was significantly inhibited (70%) by 50 mg mL^{-1} and such inhibition was slightly higher (80%) in the presence of 100 mg mL^{-1} soapberry extract after a 7-day incubation period (Figure 1). In previous studies, the biocontrol efficacy of *Ricinus cummunis*, *Rheedia gardneriana* Planch and *Labramia bojeri* was evaluated against this pathogen (23, 24). Based on these data, soapberry leaf extract can be suggested to have a stronger activity, compared with these previously analyzed plants, and could be employed in plant defense against fungal pathogens.

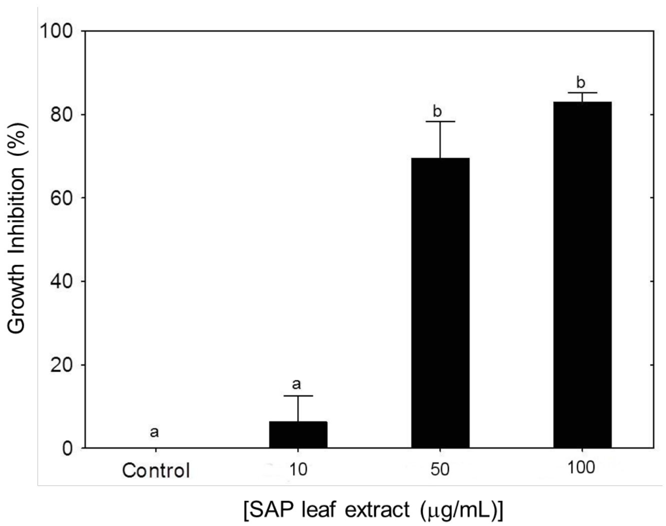

Figure 1. Percentage of mycelial growth inhibition of *Colletotrichum gloeosporioides* at different concentrations of soapberry hydroethanolic extracts. Mycelial growth was measured 7 days after inoculation on PDA medium. Control refers to the fungus growth without plant extract in PDA medium. The values represent the means ± S.D. of three separate experiments. Data with the same letters are not significantly different according to T test ($P \leq 0.05$).

Antifungal activity against *C. gloeosporioides* has been described for other plants. Peppermint oil showed 100% activity against *C. gloeosporioides* at a concentration of 100 μL (in Petri dishes containing PDA medium with 20 mL total volume), while rosemary and tea tree oils at the same concentration showed 80% activity against fungal growth (3).

Palhano et al. (20) demonstrated that lemongrass and citral essential oils cause inhibition of *C. gloeosporioides* spore germination. Compared to control, soapberry leaf extract strongly inhibited *C. gloeosporioides* spore production (Figure 2).

Comparison of the data obtained in this study with previously published results suggests that soapberry extracts could be an effective biological agent to prevent *C. gloeosporioides* infection in papaya fruits. Ribeiro & Bedendo (21) demonstrated that garlic aqueous extracts have a significant inhibitory effect (68%) against mycelial growth of the same fungi but did not inhibit spore production. In addition, these authors showed that peppermint, castor bean and pepper extracts have a strong inhibitory effect on conidial production; similarly, our results showed an effective inhibition of mycelial growth and spore production

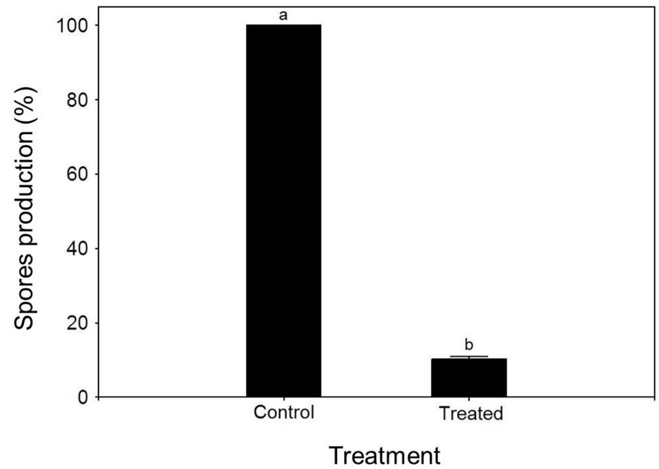

Figure 2. Inhibition of *Colletotrichum gloeosporioides* sporulation in PDA medium by supplementation with soapberry hydroethanolic extract. The amount of produced spores was measured 10 days after inoculation on PDA medium. Control refers to the fungus sporulation in the absence of plant extract in PDA medium. As a reference, 100 spores / mL were counted in the control sample by using a Neubauer hemocytometer. The values represent the means ± S.D. of three separate experiments. Data with the same letters are not significantly different according to T test ($P \leq 0.05$).

for the tested extract.

Influence of soapberry extract on the mycelia of *C. gloeosporioides* was observed under a microscope after incubating the culture for 7 days at 28 ºC (Figure 3). Mycelia treated with the extract showed an altered morphology: it was shorter and slightly more branched (Figure 3B) than control (Figure 3A). In addition, the growth of treated mycelia is slower than that of untreated mycelia. These data are similar to those described by Chen & Dai (7), who showed morphological changes in the mycelia of *Colletotrichum lagenarium*, causing anthracnose in cucumber, treated with *Cinnamomum camphora* extract.

In papaya, as well as in mango, the postharvest phase of anthracnose caused by *C. gloeosporioides* is the most devastating and economically significant phase, which is directly linked to the field phase, in which the infection occurs in developing fruits and remains quiescent in the form of appresoria until the onset of ripening (4, 6). There were significant differences among treatments ($P < 0.05$) for fruits inoculated after treatment application in the *in situ* experiment, compared to control fruits. Soapberry extract has a protective effect on papaya fruits when applied after artificial *C. gloeosporioides* inoculation (Figure 4).

Anthracnose incidence was significantly ($P<0.05$) lower in treated papaya fruits. Untreated fruits (control) showed lesions characteristic of anthracnose, which reached an average size of 4 cm^2, compared to fruits treated with soapberry extract, the lesions of which were restricted to the inoculation site (0.1 cm^2).

These data confirm the analysis of *in vitro* experiments, suggesting that the inhibition of mycelial growth and spore production is involved in reducing anthracnose symptoms in treated fruits, which shows the antifungal potential of soapberry extract.

Our group reported similar results when castor bean oil was applied to papaya fruits after artificial spore inoculation (24). Soapberry extract acts by protecting the papaya fruit due to the presence of substances that inhibit and prevent the penetration of *C. gloeosporioides* germ tube.

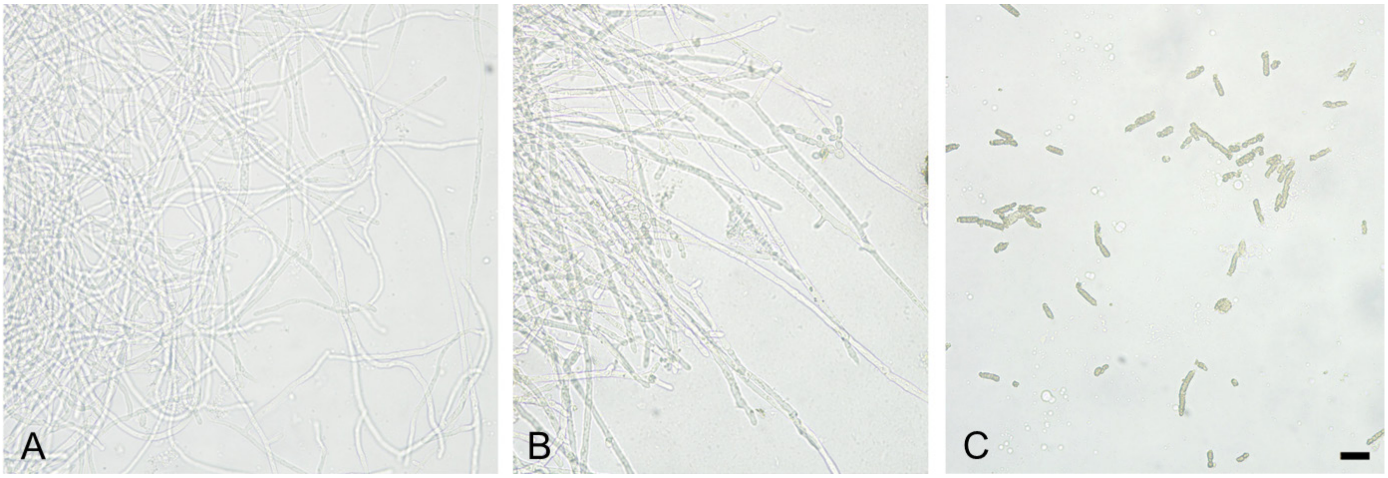

Figure 3. Microscopic observation of the influence of *Sapindus saponaria* extract on the mycelium morphology after incubating the culture for 7 days at 28 ºC, compared with control. (A) control; (B) mycelium treated with 50 mg mL^{-1} extract; (C) mycelium treated with 100 mg mL^{-1} extract. The result is a representative picture of one among three independent experiments. Bar means 20 µm.

Control **Treated**

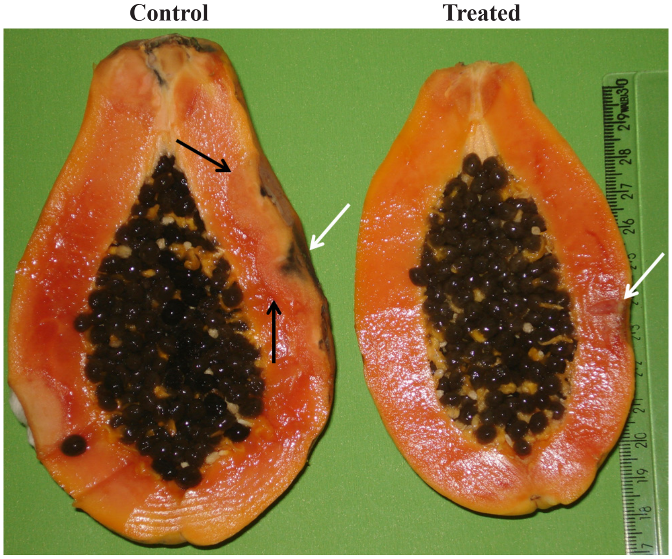

Figure 4. Effect of soapberry leaf extract on anthracnose incidence in naturally infected papaya fruits. The fruits were treated with 100 mg mL^{-1} extract. As control, fruits were treated with the extraction solution without extract. Black arrows mean anthracnose symptoms in control fruits. White arrows mean the inoculation sites. The result is a representative picture of one (n=30) among three independent experiments.

Moreover, this plant extract could be responsible for inducing defensive mechanisms in the fruit, such as induction of physical barriers and production of defensive plant compounds. Protective action against *C. gloeosporioides* in papaya fruits was also observed by Bautista-Baños et al. (5), who reported the potential of treatment with chitosan against anthracnose when fruits were treated before artificial inoculation. In the present study, we propose that one or more active compounds in the extract can directly affect the mycelia of *C. gloeosporioides*, resulting in disease control due to the inhibition of mycelial growth and spore production. Whether those active compounds could kill the pathogen or induce resistance as elicitors, which would induce some resistant reactions to affect the mycelia, will be the subject of our future study.

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