

Antifungal properties of combined plants extracts against papaya anthracnose

Propriedades antifúngicas de extratos combinados de plantas contra a antracnose em mamão

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Abstract - Fungicides provide the primary means for controlling postharvest fungal decay of fruit and vegetables. Continuous use of fungicides has faced two major obstacles: increasing public concern regarding contamination of perishables with fungicidal residues, and proliferation of resistance in the pathogen populations. Recently, the exploitation of natural products to control decay and prolong storage life of perishables has received more attention. In this work, ethanolic extracts from the bacupari (*Rheedia gardneriana Planch*) seeds and beach apricot (*Labramia bojeri*) fruit were investigated for their antifungal activity against fungi *Colletotrichum gloeosporioides* causal agent of anthracnose disease of papaya fruits. When used alone, the extracts of apricot and bacupari showed no significant inhibitory potential on fungal mycelial growth. When added in a concentration of 10% (v/v) the inhibition obtained were 57% and 43%, respectively. Yet when both extracts were added to the medium at a concentration of 5% (v/v) each, the mycelial growth was completely inhibited. Taken together these results demonstrated the apricot and bacupari potential as a botanical fungicide and an alternative control measure for diseases in papaya plants.

Key words: *Colletotrichum gloeosporioides*, apricot, bacupari, disease, papaya fruit

Resumo - Fungicidas fornecem os meios primários para o controle de doenças fúngicas do pós-colheita de frutas e legumes. O uso contínuo de fungicidas enfrenta dois grandes obstáculos: a crescente preocupação pública no que se refere à contaminação de produtos perecíveis por resíduos de fungicidas, e aumento da resistência em populações do patógeno. Recentemente, a procura por produtos naturais para o controle da deterioração e prolongar a vida útil de produtos perecíveis durante o armazenamento tem recebido atenção. Neste trabalho, extrato etanólico de sementes de baquipari (*Rheedia gardneriana Planch*) e frutos abricó (*Labramia bojeri*) foram investigados quanto a sua atividade antifúngica contra *Colletotrichum gloeosporioides* causador de antracnose em mamão. Quando utilizados isoladamente, os extratos não demonstram nenhum efeito inibidor significativo sobre o crescimento micelial. Quando empregados na concentração de 10% (v/v) foram obtidas inibições de 57% e 43%, respectivamente. No entanto, quando ambos os extratos foram adicionados ao meio a 5% (v/v) de cada um, o crescimento micelial foi completamente inibido. Em conjunto, estes resultados demonstraram o potencial de abricó e bacupari como fungicidas botânicos e uma alternativa para o controle de doenças em plantas de mamão.

Palavras-chave: *Colletotrichum gloeosporioides*, abricó, baquipari, doença, mamão

Recebido para publicação em 10/09/2013; aprovado em 30/05/2014

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INTRODUÇÃO

Colletotrichum gloeosporioides (Penz.) Penz & Sacc. is a fungus that causes anthracnose disease in some tropical fruit, which leads to both low yield and poor fruit quality from the harvest to postharvest. The pathogen attacks fruits in its early stage and remains during fruit growth as a quiescent infection (ESTRADA et al., 2000). Growth of the pathogen is resumed only after harvest when the fruit starts to ripen and postharvest anthracnose develops (ALVAREZ & NISHIJIMA, 1987).

Postharvest infections also occur due to availability of inoculum in the processing environment due to a considerable amount of wounds imposed to the fruit after harvest. The typical disease symptom is characterized by sunken necrotic tissue where orange conidial masses are produced (BAILEY et al., 1992). *C. gloeosporioides* has been reported on several subtropical and temperate fruits hosts including strawberry (SMITH & BLACK 1990), citrus (BROWN et al., 1996), and papaya (SNOWDON, 1990).

The control of Anthracnose has been accomplished almost exclusively by fungicides dip or drench during the packing process (ROBS, 1996). However, the use of fungicides for extended periods may cause the emergence of strains of fungus resistant to these fungicides. Further, residues of fungicides present on the fruits may be harmful to consumers. Moreover, consumers are looking for fruit free of chemical residues. Consequently, alternative control strategies, such physical treatments and natural compounds have drawn attention.

Among the current alternative methods are the use of gamma irradiation (CIA et al., 2007), hot water and/or UV irradiation (ALVAREZ & NISHIJIMA, 1987). Besides, exhibiting fungicidal effects, these treatments can also induce resistance in fruit. CIA et al. (2007) reported that UV-C rays causes scald on papaya fruits. In addition, hot water dip treatment affects the ripening process in papaya (PAULL, 1990).

An alternative way to reduce the growth of microorganisms is the use of plant extracts. The antifungal effects of the plant extracts on different microorganisms have been described in several studies (FIORI et al., 2000; AQIL & AHMAD, 2003; JUN-YOUNG et al., 2006; MARTINS et al., 2010). However, few data have been published on the control of papaya post-harvest diseases relying on plant extracts combined. The main objective of the present work was to test the ability of combined plant extracts in controlling the postharvest development of anthracnose in papaya fruits.

MATERIAL AND METHODS

Organisms and growth conditions

Colletotrichum gloeosporioides (IB 18/85) was obtained from culture collection of the Laboratório de Bacteriologia Vegetal of Instituto Biológico de Campinas/Brazil. Fungal organism was maintained in PDA medium (potato 4 g/L, dextrose 20 g/L, agar 15 g/L).

Plant material

Plant material such as bacupari (*Rheedia gardneriana planch*) seeds, beach apricot (*Labramia bojeri*) fruit, maigoya (*Plectranthus barbatus*) leaves and peppermint (*Mentha piperita*) leaves were collected in São João da Barra, Rio de Janeiro – Brazil. collected plant material was washed and air-fried for 3 days. to obtain a crude extract, plant material were individually triturated and extracted with ethanol for 7 days. The samples were then filtered through Whatman paper (nº 1) and maintained at 4 °C for use in antifungal activity assays.

Antifungal activity

Plant extracts inhibitory activity was evaluated as described by RIBEIRO & BEDENDO (1999). Different extract concentrations (1%, 5%, 10% (v/v)) were mixed into plates of PDA performing a total volume of 20 mL/plate, just before it solidified. The control was just plain PDA. The media incorporating the antifungal substances were inoculated at the center with agar discs of the test fungi (5 mm in diameter). Three replicates of each concentration were incubated at 25 °C. After incubation for 3-7 days, the radial growth was measured. The experiment was conducted three times and the activity is expressed as inhibition activity (%) of radial growth. Combined plant extracts inhibitory activity were evaluated as described above. Concentrations of 5% from two plant extracts (1:1) were added into plates of PDA. The treatments were prepared as follows: T1- apricot 10% (v/v), T2-bacupari 10% (v/v), T3 - bacupari 5% (v/v) + apricot 5% (v/v), T4 - apricot 5% (v/v) + plantain 5% (v/v), T5 - apricot 5% (v/v) + peppermint 5% (v/v), T6-bacupari 5% (v/v) plantain + 5% (v/v), T7-bacupari 5% (v/v) + peppermint 5% (v/v).

Statistical analysis

The experiments were randomized by carrying out three repetitions and 7 treatments, using the factorial

scheme 2 x 3 + 1, the factor being two species of plants and three concentrations of crude extract, in addition to the treatment control. Data are presented as the mean ±S.D. of three independent experiments, and the differences from controls were assessed with Turkey test; statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Plant extracts have been studied against microorganisms for years, but in a more intensified way in

the last three decades. During this period, a lot of antifungal screening evaluations have been published (WILSON et al., 1997; BAUTISTA-BAÑOS et al., 2000a; BAUTISTA-BAÑOS et al., 2000b; BAUTISTA-BAÑOS et al., 2002; SOYLU et al., 2006). In this study two species without any previous report on antifungal properties were active against the microorganism *C. gloeosporioides*. Median inhibition of radial growth of *C. gloeosporioides* could be observed (Figure 1) when ethanolic extracts of apricot and bacupari were compared with their respective control (PDA).

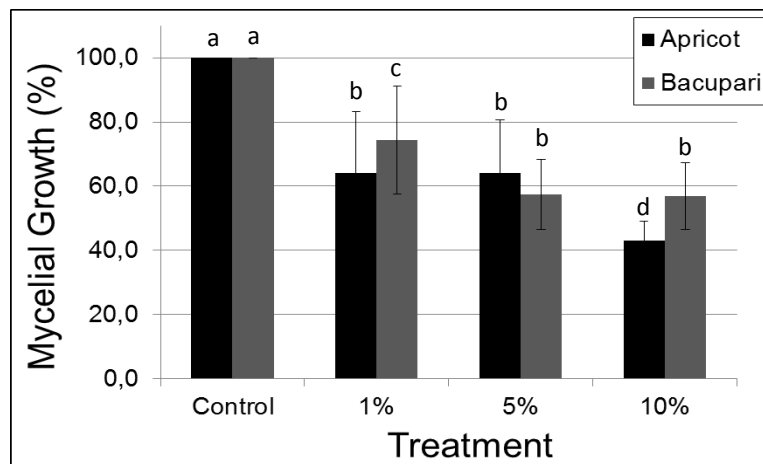


Figure 1 - Percentage inhibition of mycelial growth of *C. gloeosporioides* in different concentrations of the bacupari (gray bar) and apricot (black bar) ethanolic extracts; control refers to the fungus growth in the absence of plant extract. The values represent the means ± S.D. of three separate experiments. Data with the same letters are not significantly different according to Turkey's test ($P \leq 0,05$).

Comparison of the antifungal activity showed that apricot extract was the most active (~57%) followed by bacupari (~43%) against the tested fungi. Lower concentrations of each extract did not cause significant inhibition of radial growth of *C. gloeosporioides*. Inhibitory results are similar to those reported by RIBEIRO & BEDENDO (1999) who found relative fungitoxic activity of the garlic, pepper, castor beans and mint against *C. gloeosporioides*, and those of MILANESI et al. (2009) who demonstrated a partial inhibition in the growth of the same pathogen, with the aqueous extracts of *Baccharis trimera* and *Melia azedarach*. In the studies only various doses of individual plants and/or their derivatives have been tested against pathogens; thus, a number of plant extract were few investigated before. In

this work we also investigated, by in vitro tests, the effect of combinations of apricot and bacupari extracts and these plants extracts with other two plant species. Figure 2 shows the effect of the combination of bacupari and apricot extracts (1:1) at a total concentration of 10% (v/v) in PDA medium on the mycelial growth of the *C. gloeosporioides*. Contrasting the means of the single extracts with the means of the combination of extracts ($p < 0.05$) clearly suggested that there was synergistic effect of the combination of extracts. The mycelial growth was almost completely inhibited ($p < 0.05$) on plates containing T3 sample (combination of apricot and bacupari extracts) when compared with the control plates or with individual extracts in the same concentration (Figure 2).

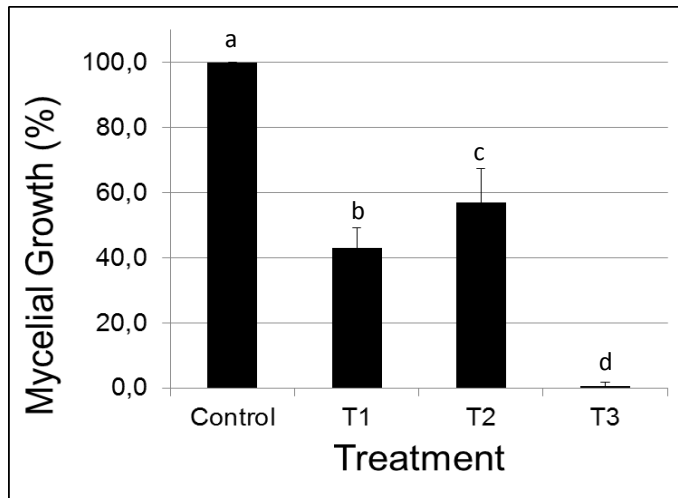


Figure 2 - Inhibition of *C. gloeosporioides* growth in PDA medium by supplementation with: T1- apricot extract (10 % (v/v)); T2- bacupari extract (10% (v/v)); T3- combined apricot and bacupari extracts (5% + 5% (v/v)); control refers to the fungus growth in the absence of plant extract. The values represent the means \pm S.D. in triplicates obtained from three independent experiments. Data with the same letters are not significantly different according to Turkey's test ($P \leq 0,05$).

Apricot extract was also tested in combination with extracts of plantain (T4) and peppermint (T5). When combined, the extracts strongly inhibit mycelial growth of *C. gloeosporioides* (Figure 3). SIQUEIRA-JUNIOR et al. (2011) demonstrated that the ethanolic extract of plantain inhibits growth in 90% of the same fungus at a

concentration of 10% in PDA medium. The results of the present study agree with these earlier findings, demonstrating that this potential can be elevated when added to the extract of apricot, using only half of the concentration (5%).

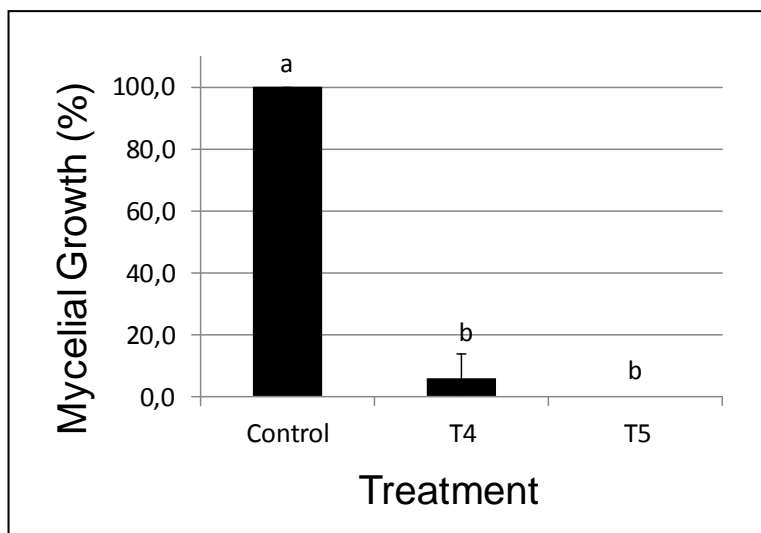


Figure 3 - Inhibition of *C. gloeosporioides* growth in PDA medium by supplementation with: T4- combined apricot and plantain extracts (5% + 5% (v/v)); T5 – combined apricot and peppermint extracts (5% + 5% (v/v)); control refers to the fungus growth in the absence of plant extract. The values represent the means \pm S.D. in triplicates obtained from three independent experiments. Data with the same letters are not significantly different according to Turkey's test ($P \leq 0,05$).

Similar results were observed when plantain and bacupari extracts (T6), or peppermint and bacupari extracts (T7) were combined (Figure 4), demonstrating that the active compounds present in these extracts are acting synergistically increase the inhibitory effect against fungal development.

It is clear from the results that the use of combined extracts has inhibitory effects against *C. gloeosporioides* in vitro development. This increased inhibitory potential could be explained because the presence of different active compounds in each plant. Actually, plants utilize a

vast array of diverse secondary metabolites to defend themselves against abiotic and biotic stress (WEBER, 2002). These metabolites can be constitutively present in the plant as preformed chemical barriers as active compounds. Then, in the extract combination, one plant compound could activate or inactivate others on another plant extract, increasing its effects against fungal growth. For this reason, these plant extracts could be used as an effective botanical to control the fungus. It will avoid environmental pollution as well as will minimize higher production costs.

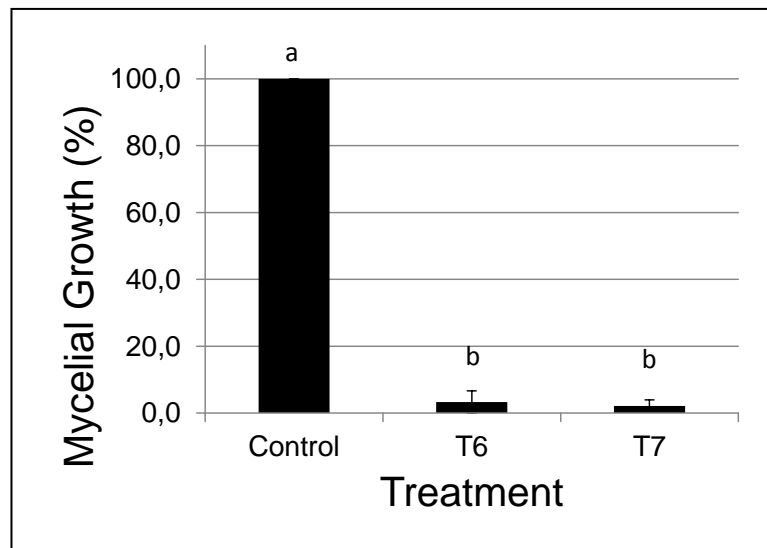


Figure 4 - Inhibition of *C. gloeosporioides* growth in PDA medium by supplementation with: T6- combined bacupari and plantain extracts (5% + 5% (v/v)); T7 – combined apricot and peppermint extracts (5% + 5% (v/v)); control refers to the fungus growth in the absence of plant extract. The values represent the means \pm S.D. in triplicates obtained from three independent experiments. Data with the same letters are not significantly different according to Turkey's test ($P \leq 0,05$).

CONCLUSIONS

The findings of this study demonstrated that the biological control activity of apricot and bacupari extracts against anthracnose of papaya was significantly enhanced by the combination of these extracts or the combination of the both individual extracts with plantain and peppermint extracts. Therefore, the discoveries of these potential plant antifungal agents are encouraging in replace the current commercial antifungal drugs that induce many problems to environment. Although this study showed that the effectiveness of combined extract for reduction of *C. gloeosporioides* growth could be improved by a treatment with bacupari, apricot, plantain and peppermint under laboratory conditions, a full-scale commercial evaluation is needed to recommend this treatment to the packinghouse.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Maria Ligia R. Macedo for her support. This research was supported by the Núcleo de Pesquisas em Biocontrole de Doenças e Proteção em Sistemas Agrícolas – NuPSA (NuPSA/UNIRIO – Brazil).

REFERENCES

- ALVAREZ, A. M.; NISHIJIMA, W. T. Postharvest diseases of papaya. *Plant Disease*, v. 71, p.681-686, 1987.
- AQIL, F.; AHMAD, I. Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World Journal of Microbiology & Biotechnology*, v.19, p.653-657, 2003.
- BAILEY, J. A.; O'CONNELL, R. J.; PRING, R. J.; NASH, C. Infection strategies of *Colletotrichum* species.

- In: Bailey, J. A.; Jeger.(eds). *Colletotrichum: Biology, Pathology and Control*. Wallingford: CAB International; 1992. p. 88-120.
- BAUTISTA- BAÑOS, S.; BARRERA-NECHA, L. L.; BRAVO-LUNA, L.; BERMUDEZ-TORRES, K. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloeosporioides* of papaya and mango fruit after storage. *Mexican Journal of. Phytopathology*, v.20, p.8-12, 2002.
- BAUTISTA- BAÑOS, S.; HERNANDEZ-LOPEZ, M.; DIAZ-PEREZ, J. C.; CANO-OCCHOA, C. F. Evaluation of the fungicidal properties of plant extracts to reduce *Rhizopus stolonifer* of 'ciruela' (*Spondias purpurea* L.) during storage. *Postharvest Biology and Technology*, v.18, p.67-73, 2000b.
- BAUTISTA-BAÑOS, S.; HERNANDEZ-LOPEZ, M.; Barrera-Necha, L. L.; Antifungal screening of plants of the state of Morelos, Mexico against four fungal postharvest pathogens of fruits and vegetables. *Mexican Journal of Phytopathology*, v.18, p.36-41, 2000a.
- BROWN, A. E.; SREENIVASAPRASAD, S.; TIMMER, L. W. Molecular characterization of slow-growing-orange and key lime anthracnose strains of *Colletotrichum* from citrus as *C. acutatum*. *Phytopathology*, v.86, p.523-527, 1996.
- CIA, P.; PASCHOLATI, S. F.; BENATO, E. A.; CAMILI, E. C.; SANTOS, C. A. Effects of gamma and UV-C irradiation on the postharvest control of papaya anthracnose. *Postharvest Biology and Technology*, v.43, p.366-373, 2007.
- ESTRADA, A. B.; DODD, J. C.; JEFFRIES, P. Effect of humidity and temperature on conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. *Plant Pathology*, v.49, p.608-618, 2000.
- FIORI, A. C. G.; Schwan-Estrada, K. R. F.; Stangarlin, J. R.; Vida, J. B.; Scapim, C. A.; Cruz, M. E.S.; Pascholati, S. F. Antifungal Activity of Leaf Extracts and Essential Oils of some Medicinal Plants against *Didymella bryoniae*. *Journal of Phytopathology*, v.148, p.483-487, 2000.
- JUN-YOUNG, C.; CHOI, G. J.; LEE, S. W.; JANG, K. S.; LIM, H. K.; LIM, C. H.; LEE, S. O.; CHO, K. Y.; KIM, J. C. Antifungal activity against *Colletotrichum* spp. of cucurminoids isolated from *Curcuma longa* L. rhizomes. *Journal of Microbiology and Biotechnology*, v.16, p.280-285, 2006.
- Martins, J. N.; Oliveira, S. P. A.; Santos, D. C.; Oliveira, E. N. A.; Castellon, R. E. R. Evaluation of capacity antifungal oily extract of purple garlic (*Allium sativum* L.). *Revista Verde*, v.5, p.211-226, 2010.
- MILANESI, P. M.; BLUME, E.; MUNIZ, M. F. B.; BRAND, S. C.; MANZONI, C. G.; WEBER, M. N. D. Fungitoxic action of plant extract on the mycelial growth of *Colletotrichum gloeosporioides*. *Revista da FZVA*, v.16, p.01-13, 2009.
- PAULL, R. E. Postharvest heat treatments and fruit ripening. *Postharvest News Information*, v.1, p.355-363, 1990.
- RIBEIRO, L. F.; BEDENDO, I. P. Efeito inibitório de extratos vegetais sobre *Colletotrichum gloeosporioides* – agente causal da podridão de frutos de mamoeiro. *Scientia Agricola*, v.56, p.1267-1271, 1999.
- ROBS, C. F. Enfermidades de pós-colheita do mamão. In: Mendes, Dantas, Morales, Mamão No Brasil. Cruz das Almas, BA: EUFBA/EMBRAPA-CNPMPF; 1996.
- Siqueira-Junior, C. L.; Moraes, T. C.; Martins, J. A. B.; Freire, M. G. M. Controle da antracnose em mamão por extratos vegetais. *Perspectivas online*, v.1, p.99-105, 2011.
- SMITH, B. J.; BLACK, L. L. Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. *Plant Disease*, v.74, p.69-76, 1990.
- SNOWDON, A. L. *Color Atlas of Post-harvest Diseases and Disorders of Fruit and Vegetables*, 1st ed. Boca Raton, FL: CRC Press Inc, 1990.
- SOYLU, E. M.; SOYLU, S.; KURT, S. Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*. *Mycopathologia*, v.161, p.119-128, 2006.
- Weber, H. Fatty-acid derived signals in plants. *Trends in plant science*, v.7, p.217-224, 2002.
- WILSON, C. L.; SOLAR, J. M.; GHAOUTH, A.; Wisniewski, M. E. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease*, v.81, p.204-210, 1997.